A study of vascular endothelial growth factor& CD31 as a prognostic marker in Psoriasis

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Abstract

Aim and Background: In the present study was investigate that the Vascular endothelial growth factor& CD31 as a prognostic marker in Psoriasis patients. Psoriasis is a very common, chronic inflammatory skin disease and related to various comorbidities. It was found out that both keratinocytes and the T lymphocytes secrete angiogenic factors which are responsible for proliferation of blood vessels in psoriasis. **Materials and Methods:** This case control study was conducted at Arunai Medical College and Hospital, Tamil Nadu, this study was conducted after approval of institutional ethical clearance in the year of 2021. In our study Immunohistochemical staining was performed on 30 newly diagnosed cases and 30 control from the normal healthy skin and graded according to the staining pattern. All the patients were selected based on the Inclusion and Exclusion criteria. **Results:** The maximum number of psoriasis affected patients were in the age group of 18 to 30 years with male predominance**.** Among control VEGF positivity was very less. 90% of control showed negative staining for VEGF, only 10% of control showed [1+] or mild staining. In our study, expression of VEGF was significantly higher among the cases compared to controls with a statistical significant difference (p value<0.01). Expression of CD31 in cases were significantly higher in cases than control which is statistically significant [p=<0.01]. **Conclusion:** In the present study showed that the VEGF and CD31 may be considered as prognostic markers and development of targeted anti-angiogenic therapy might be beneficial to minimize the progression of disease to more severe stages.

Key Words: Vascular Endothelial Growth factor, CD31, Psoriasis

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1. Introduction

Psoriasis is a chronic, non-communicable, recurrent inflammatory skin disease having characteristic features such as dysregulation, proliferation of keratinocytes and dermal blood vessels and inflammatory cell infiltration in the skin. It is associated with various disease conditions but initially it affects skin, nails and joints. Psoriasis patients are more prone to develop cardiovascular and non-communicable diseases[1].

Psoriasis is a commonly encountered skin disorder worldwide. Psoriasis can affect any group but commonly affect between 50-69years of age group[2] with no gender predominance and can affect both men and women equally[3]. Prevalence of the disease varies in different populations . In a study, it was observed that prevalence of psoriasis in different countries varies between 0.09% to 11.4% [4], thus psoriasis is becoming a serious global issue. In a study done at a tertiary health care center in north India, psoriasis patients accounted for 2.3% of the total dermatology outpatient department.[5].

Pathogenesis of Psoriasis is very complex and it involves several factors such as genetic, immunologic and environmental. There are various other factors which plays vital role in pathogenesis such as Vascular endothelial growth factors (VEGF),T cells, cytokines, Langerhans cells, macrophages . In various studies it has been postulated that initially T cells get activated which results in secretion of cytokines, inflammatory cells and keratinocytes. Neo- vacularization is considered as hallmark of chronic inflammation, which plays a vital role in the pathogenesis of psoriasis. Vascular endothelial growth factor is a proangiogenic factor which gets activated and expressed in dermal papillae EC (endothelial cells) of psoriatic lesion probably due to the immune reponse initated by T helpercells.

Psoriasis is diagnosed clinically in presence of certain features such as well demarcated erythematous plaques covered by silvery scales which are commonly seen on the elbows, knees, lower back and scalp. Auspitz sign is one of the important clinical feature for the diagnosis of psoriasis, which is characterized by painful and pruritic lesion which bleeds on scraping. On the basis of morphological pattern psoriatic lesions are classified as plaque psoriasis, guttate psoriasis, pustular psoriasis, inverse psoriasis, nail psoriasis and erythrodermic psoriasis. Among all other morphological pattern of psoriasis, plaque psoriasis is most commonly encountered pattern and constitutes about 90%of the cases of psoriatic lesion, and is commonly known as psoriasis vulgaris[6] . Arthritis is one of the common manifestation associated with psoriasis, In a study it was observed that in 85% of the patients arthritis were diagnosed after the onset of the psoriatic lesion whereas 15% of the patients were diagnosed with psoriatic arthritis before or at the time of onset of skin disease [7]. In about 16% of the patients suffering from chronic psoriatic arthritis develops arthritis mutilans[8] .

Histopathological diagnosis of psoriasis is given on the basis of certain characteristic features such as parakeratosis , presence of munro microabscess in the stratum corneum layer of the epidermis, elongation of rete ridges , presence of mitotic figures in the basal layer of keratinocytes and proliferation of dermal blood vessels.

As psoriatic lesions are visible, it affects psychological and social aspects of life [9].

Hence, this study is designed to understand the role of angiogenesis and proangiogenic mediators like VEGF and CD31 in pathogenesis of psoriasis and also to understand new strategies to treat psoriasis with therapeutics that halt angiogenesis responsible for psoriatic lesions.

2. Materials and Methods

This case control study was conducted at Arunai Medical College and Hospital, Tamil Nadu, India over a period of 19 months from January 2021 to July 2022. The ethical committee approval was obtained in December 2021.

2a. Sample collection

This study includes 60 skin punch biopsy sample from the clinically diagnosed case of

psoriasis. Out of which 30 skin punch biopsy samples were obtained from the clinically diagnosed psoriatic lesion and considered as cases and 30 skin punch biopsy from the on-lesional skin from the same patient and considered as control. All the samples were taken from the outpatient department of Dermatology at Arunai Medical College and Hospital. All the patients and their attenders were informed regarding the significance of the study followed which consent was obtained.

Biopsy was obtained by 6mm disposable skin biopsy punches, Separate punch was used for both lesional and non-lesional skin. The biopsies were performed under local anaesthesia and aseptic condition. Biopsy sites were closed by suture.

All the biopsy samples were received by Department of Pathology, Arunai Medical College and Hospital. Samples in formalin were processed and by routine histopathological procedure , slides were prepared and stained by routine hematoxylin and eosin(H&E) stain for further evaluation.

Among sixty sample of skin punch biopsy, 30 samples from the lesional skin were analyzed for characteristic histopathologic features and diagnosed as Psoriais, whereas in the 30 samples from non-lesional skin normal histology was given.

2b. Inclusion Criteria

All newly diagnosed cases of psoriasis, those who never received any topical or systemic therapy. Patients between age group of 18 to 70 years either male or female were included in this study.

2c. Exclusion Criteria

Old clinically diagnosed cases of psoriasis who received or undergoing any topical or systemic therapy were excluded from the study.

2d. Immunohistochemical Study

All slides with histopathological diagnosis of psoriasis and of normal skin were evaluated and then paraffin blocks were subjected to immunohistochemical study.

2e. Immunonohistochemical Evaluation

Immunohistochemical reactivity for the slides were examined under light microscope. The intensity of immunostaining for VEGF was evaluated on the basis of staining of the epidermis. VEGF shows cytoplasmic staining in immunohistochemistry.

Intensity of Staining was divided in three groups:

1. Staining positivity in Basal layer of epidermis only -1+
2. Staining positivity in lower half of the epidermis -2+
3. Staining positivity in Whole epidermis -3+

The evaluation of intensity of immunostaining by CD 31 was based on Microvessel Density (MVD). It was performed by counting of blood capillaries. The blood capillaries were counted in three highly vascularized areas (hot spots) selected by light microscope under 40X magnification. CD31 shows both membranous and cytoplasmic staining . Single endothelial cell or clusters of endothelial cells, either with or without lumen, were considered to be individual vessels .It shows cytoplasmic and nuclearstaining.

Intensity of staining was divided in to three groups:

1. Mild (4-10capillaries)
2. Moderate (11-20capillaries)
3. Severe (21-28 capillaries)

PASI Score

Psoriasis area and severity index Score (PASI) was assessed for all the 30 cases in this current study to assess the severity of the disease.

2f. Statistical Analysis

All the data`s were analyzed by statistical package for the social sciences (SPSS) Software Version 21. The data were computed and differences in quantitative variables between groups were assessed by means of the unpaired t-test. Pearson's coefficient of correlation was computed to assess the relationship between the variables. Chi-square test was computed to assess differences in categoric variables between groups. A p-value of < 0.01 was considered

significant.

3. Results

In the present study among the 30 cases age group was divided in to 3 groups: Group 1- (18-30)years, Group 2- (31-50)years and Group 3- (>50) years. **Table.1** shows that the maximum number of psoriasis affected patients were in the age group of 18 to 30 years,(13/30) psoriais patients were diagnosed in this age group and constitute (43.3%) of the total cases. In age group between (31-50) years, total 10 cases were diagnosed as psoriasis and constitutes(33.3%) of the total cases. Out of 30 cases, 7 cases (23%) were diagnosed at the age of >50 years .

Table 1: Distribution of Cases by age

Age group		Frequency	Percent
	18 TO 30	13	43.3
	31 TO 50	10	33.3
	> 50	7	23.3

Distribution of cases on gender basis

Among the cases, 17 cases (56.7%) were male and 13 cases(43.3%) were female. .The male to female ratio 1.30:1 was noted in this study. **(Table.2)**

Table 2: Distribution of cases by gender

Gender		Frequency	Percent
	MALE	17	56.7
	FEMALE	13	43.3

Intensity of VEGF immunoreactivity in Cases

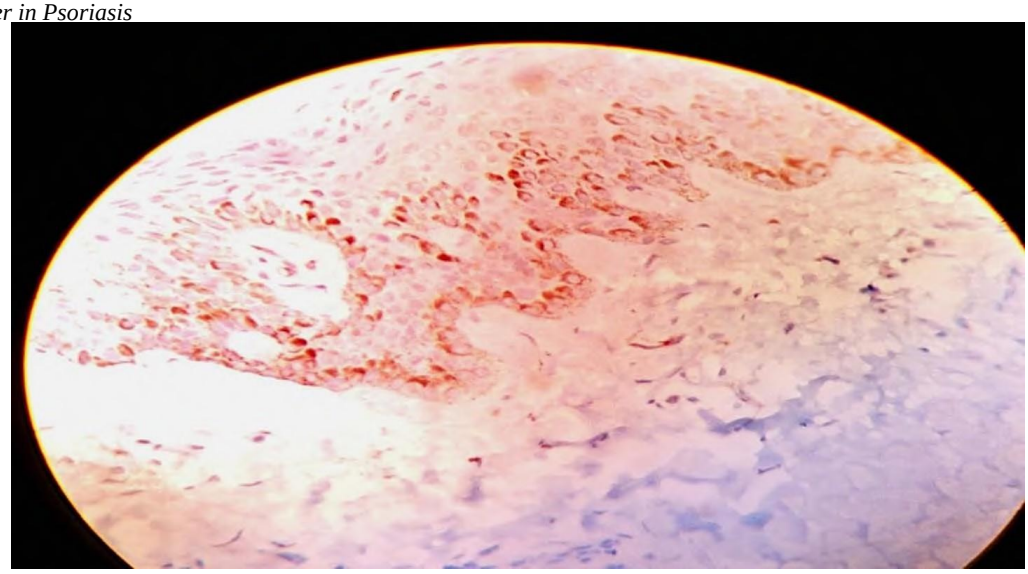
In the present study all the 30 cases showed positivity for VEGF of varied intensity. 9 cases (30%) showed (2+) VEGF positivity limited to lower half of the epidermis and 21 cases(70%) showed (3+) full thickness epidermalstaining.

Table.3 VEGF immunoreactivity in cases

Immunoreactivity	**Frequency**	**Percent**
2+	**9**	**30.0**
3+	**21**	**70.0**

Figure 1. Shows whole thickness staining positivity (IHC VEGF 10X)

Figure 2: Shows staining positivity in lower half of epidermis (IHC VEGF 10X)



Intensity of CD31 immunoreactivity in Cases

Among 30 cases stained with CD31 21/30 (70%) cases showed 2+ positivity (11-20 capillaries) and 9/30(30%) cases showed 3+ positivity (21-38 capillaries).**(Table.4)**

Table 4: Intensity of CD 31 immunoreactivity among cases

Immunoreactivity		Frequency	Percent
	2+	21	70.0
	3+	9	30.0

Intensity of CD 31 Immunoreactivity among control

Among 30 control stained by CD31, 27/30 (90%) showed no immunoreactivity where as 3/30 (10%) showed 1+ or weak staining. **(Table.5)**

Table 5: Intensity of CD 31 immunoreactivity among control

Immunoreactivity	Frequency	Percent
NEGATIVE	27	90.0
1+	3	10.0

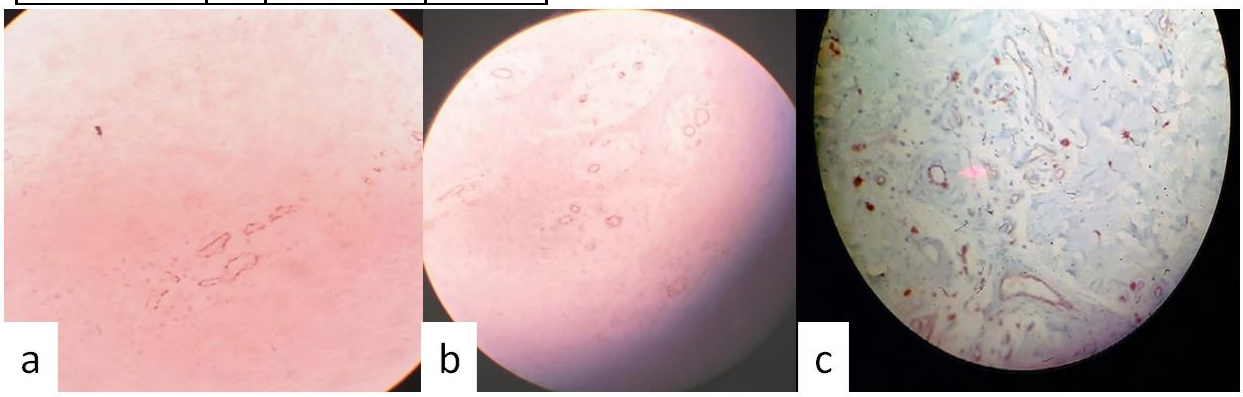


Figure 3: a) 33 Staining positivity (mild) of dermal capillaries in psoriasis (IHC CD31 40X); b) 34 Staining positivity (moderate)of dermal capillaries in psoriasis (IHC CD31 40X); c) 35 staining positivity (severe) of dermal capillaries in psoriasis (IHC CD31 40X)

4. Discussion

Psoriasis is very common and oldest known disease worldwide with complex pathogenesis [123] and it is characterized by certain features such as alteration in the keratinocyte proliferation and differentiation, immune cells

mediated inflammation and dysregulation of angiogenesis as well as vascular remodeling. In various studies, it was found that Th1 type of cell, Th17 cell, keratinocytes, antigen presenting cell, natural killer cell, macrophages, Langerhans cells, and various cytokines has important role in the patho- mechanism of psoriasis. Proliferation of blood vessels (angiogenesis) occurs only in the early stages of psoriatic lesion and disappears when the lesion resolves [10]. In psoriatic lesions neo-vascularization was found in the superficial dermis . Capillaries present in the papillary dermis shows elongation, dilatation, increased tortuosity [11].In psoriatic lesion, first the morphological changes becomes evident then hyperplasia of the epidermis takes place [126]. microvascular changes which takes place in the psoriasis has correlation with the increased cutaneous blood flow and these changes are also observed in the perilesional areas of psoriasis.

Thus it is postulated that neo-vascularization (angiogenesis) is not just a cofactor but it leads to development of psoriatic lesion. In various studies ,it was observed that proangiogenic cytokines like hypoxia inducible factor, tumour necrosis factor, Interleukin 8,VEGF and angiopoietins show increased expression in the psoriatic lesion[12].

There are various methods which are used for evaluation of blood vessels and its formation in health and disease .Electron microscopy is used for investigating cellular ultrastructure. In psoriatic lesion ultrastructural evaluation revealed that the capillaries are of venous type and in normal skin arterial type of capillaries are found out [13]. The Venous capillary present in the psoriatic lesion changes into arterial type after the lesion resolves .It was also found out in the ultra structural evaluation that the EC(endothelial cells) shows enlarged Golgi bodies and Weibel Palade bodies in the psoriatic lesion . In autoradiography as well as in the immunohistochemistry, increased proliferation of EC (endothelial cells) in psoriasis was made out and proved [14].

conducted by Liew SC et al., there was no significant correlation between PASI score and VEGF (p=0.232) [15].

In this present study, correlation between CD 31 and PASI score was significant (r=0.952; p= 0.000.In a similar study by Lakshna et al and Guijiao BI et al., indicates that CD 34 expression was increased in vascular endothelial cells in dermis of psoriatic skin lesions and might be related to severity of psoriasis . CD 34 might also be involved in adhesion and migration of inflammatorycells.[16]

In the present study, VEGF showed positivity in all the cases with various intensities. Among the cases 70% showed [3+] positivity in whole epidermis and 30% of the cases showed [2+] positivity upto the lower half of epidermis. None of the cases showed [1+] or only basal layer staining and negative for VEGF staining . Among control VEGF positivity was very less. 90% of control showed negative staining for VEGF, only 10% of control showed [1+] or mild staining. In our study, expression of VEGF was significantly higher among the cases compared to controls with a statistical significant difference (p value<0.01). Similar results were observed in the following studies.

According to a study conducted by Lakshna Sankar et al the intensity of VEGF expression was higher in cases compared to controls (p=0.016) [16]. Simonetti O et al, observed that there was diffuse VEGF (13.15±6.6) immunohistochemical expression in epidermis of psoriatic skin lesions compared to epidermis of normal skin [17]. In another study done by Rashed HE et al., there was strong VEGF expression in epidermis (mean 46.1±19.66) and a moderate expression in vessels and inflammatory infiltrates (mean 19±5.4 and 8±2.16). VEGF expression was significantly higher in skin of psoriasis cases compared to normal healthy controls. In a similar study conducted by Kim YG et al., the expression of VEGF was significantly enhanced in skin of psoriasis cases when compared to controls[19].

In reference to all the above studies which correlate with the finding in our study and support that VEGF promotes endothelial cell survival, new blood vessel formation and thus

plays a significant role in pathogenic basis of psoriasis.

In our study, CD 31 positivity was observed in all the cases of psoriasis with variable intensities. Among 30 cases of psoriasis stained by CD 31, 70% of the cases showed [2+] or moderate positivity(11-20capillaries) and 30% showed [3+]or severe positivity(21-38 capillaries) . In control CD 31 expression was mild positive or negative .Among 30 control 90 % showed negative staining whereas 10% showed mild positivity to CD 31 . Expression of CD31 in cases were significantly higher n cases than control which is statistically significant[p=<0.01].

Similar results were obtained in other study where CD 31 expression was higher in cases than control .In a study done by Nitika et al. they observed that CD 31 expression was higher in cases when compared to controls[20] . A study conducted by Barton SP et al., showed that there is higher endothelial cell volume and volume of lumen in the skin of psoriatic lesions compared to the non lesional skin of psoriasis and healthy skin of controls [21]. Both study showed increase in microvasculature in psoriasis as compared to their respective controls. Results observed by other studies support our observation which shows that in psoriasis cases there is vascular proliferation, tortuosity and elongation of vessels followed by inflammation which is reflected by increased microvessel density.

Thus the present study supports the role of VEGF and CD31 with increased MVD in psoriasis than normal skin. Hence, CD 31 may be considered as a prognostic marker for antiangiogenic therapy in the early lesions of psoriasis to minimize the progression of disease to severe stages. Our study comes to conclusion that psoriatic lesions exhibit potent angiogenic activity and show increased MVD along with other histomorphological parameters such as parakeratosis and Munro'smicroabscesses.

5. Conclusion

In the present study we concluded that vascular endothelial growth factor plays vital role in pathogenesis of psoriasis. Thus, VEGF and CD31 may be considered as prognostic markers and development of targeted anti-angiogenic therapy might be beneficial to minimize the progression of disease to more severe stages.

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