## **ORIGINAL ARTICLE**

# Use Immunohistochemistry to Determine the Severity of CD34 Expression in Psoriasis that has been Histopathologically Diagnosed

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## ABSTRACT

**Objective:** The goal of this study is to determine the degree of CD4 expression in histopathologically confirmed cases of psoriasis using immunohistochemistry.

Study Design: Cross-sectional/ Descriptive study

**Place and Duration:** Pathology Department of North West School of Medicine (NWSM), Peshawar and Avicenna Medical and Dental College, Lahore for duration of six months April 2021 to September 2021.

**Methods:** There were 80 patients of both genders with age 18-70 years were presented in this study. Informed written consent was taken from all the patients for detailed demographics including age, sex and body mass index. Psoriasis was diagnosed by taking skin biopsy of all the patients. Histopathology slides were made from paraffin-embedded sections of the whole skin biopsy. When examined in the 40x magnification field, the cases were classified as having light staining (4-10 capillaries), moderate staining (11-20 capillaries), or strong staining (21-28 capillaries). On the basis of histopathology, psoriasis was determined to be present when the following features were observed: hyperkeratosis, acanthosis, a munro's abscess, the extension of rete ridges, and abnormalities of the dermal vasculature. SPSS 24.0 version was used to analyze.

**Results:** Majority of the patients in or study were males 55 (68.8%) and the rest were females 25 (31.2%) with mean age 35.51+19.61 years. Mean BMI of the patients was 24.23+11.34 kg/m<sup>2</sup>. Frequency of moderate staining of CD34 expression was high found in 50 (62.5%) cases, mild staining in 17 (21.3%) patients and 21-28 capillaries (strong staining) in 13 (16.3%) patients. According to histopathological findings hyperkeratosis, acanthosis, a munro's abscess, the extension of rete ridges, and abnormalities of the dermal vasculature majority had moderate (11-20 capillaries) staining of CD34 expression.

**Conclusion:** We concluded in this study that the mild staining expression of CD34 was majority seen among patients of psoriasis. Psoriasis's enhanced cutaneous vasculature has been demonstrated by this crucial angiogenesis marker. As a result, it can be used to better understand the disease's histiogenesis. **Keywords:** CD34 expression, Histopathology, Immunohistochemistry, Psoriasis

## INTRODUTION

Psoriasis is a chronic inflammatory cutaneous illness with a complex but unexplained aetiology that affects approximately 2% of the world's population [1-4]. It is the most common chronic inflammatory cutaneous disorder in the world. Among the symptoms of this condition include cutaneous inflammation, hyperproliferation, and inadequate differentiation of epidermal keratinocvtes [5]. Histopathologically, psoriasiform dermatitis has epidermal proliferation with regular blood vessels, which is characteristic of the condition. Psoriasis, on the other hand, is the most well-known manifestation of psoriasiform dermatitis, and other cutaneous illnesses can reveal psoriasiform epidermal hyperplasia, which can lead to uncertainty in histopathologic diagnosis [6]. A clear distinction between psoriasis and psoriatic arthritis is critical for diagnosing, prognosizing, and treating psoriatic arthritis. The use of immunohistochemistry techniques may be able to achieve this differentiation [6].

Non-psoriasis psoriasiform dermatoses (NPPD) are conditions that clinically and histopathologically resemble psoriasis but do not cause it. Seborrheic dermatitis, pityriasis rosea, pityriasis rubra pilaris (PRP), and lichen

simplex chronicus are just a few of the conditions that might occur. [7] The dermatopathologist is frequently forced to make a general diagnosis despite the presence of characteristic histopathological features such as Munro's tortuous, microabscesses and dilated capillaries (psoriasis); alternating horizontal and vertical parakeratosis (pityriasis rubra pilaris); mounds of parakeratosis with extravasation of erythrocytes (pityriasis rose The Ki-67 antigen is a labile nuclear protein complex ranging in size from 345 to 395 kD. Cell cycle regulation is regulated by this protein, which is the most extensively used proliferation immunohistochemistry (IHC) marker. According research, its expression is elevated in psoriatic lesions as compared to non-lesional skin in the disease. [8] However, there is minimal information available on the comparative expression of this marker in different kinds of psoriasis when compared to NPPD in the literature.

Several conditions, such as infection, inflammation, damage, and autoimmune disorders[9], can worsen psoriasis. These variables include:

It has been shown that the epidermis contains the majority of cytotoxic CD8+ T cells, whereas the dermis of people with psoriasis has the majority of CD4+ T cells [10,

11]. It was previously discovered that the severity of the expression of markers on 14 distinct blood samples in Egypt was determined in an earlier study. Researchers looked into a number of different markers, including osteopontine (OPN), Ki67, and CD34. As a result of the findings, CD34 staining was mild in 21.4 percent of patients, moderate in 50 percent, and strong in 28.6 percent of the cases investigated.

In addition to plaque psoriasis, there are various other forms of psoriasis to consider, such as guttate psoriasis, pustular psoriasis, erythrodermic psoriasis, flexural psoriasis, and erythrodermatosis. In spite of this, plaque psoriasis is the most common kind of psoriasis, accounting for more than 80 percent of all cases. On the other hand, guttate psoriasis affects roughly 10% of patients, while erythrodermic psoriasis and pustular psoriasis impact less than 3% of patients, respectively[11, 12]. No analogous study has ever been conducted in Pakistan, despite the fact that psoriasis is a fairly common skin ailment in that country. Anxiety, sadness, and other psychological disorders may arise as a result of the long-term nature of psoriasis therapy. It is also essential to understand how dermal angiogenesis contributes to the course of the disease. Current immunohistochemical markers can be used to better understand the histiogenesis of psoriasis since they highlight the increased dermal vasculature that occurs in the disease and is recorded as mild, moderate, and strong staining, according to the severity of the staining. As a result, it will assist in the development of personalised drug therapy against angiogenesis, allowing for better patient management and speedier treatment of individuals who require it.

## MATERIAL AND METHODS

This cross-sectional/ descriptive study was conducted at Pathology Department of North West School of Medicine (NWSM), Peshawar and Avicenna Medical and Dental College, Lahore for duration of six months April 2021 to September 2021. The study was comprised of 80 patients. Informed written consent was taken from all the patients for detailed demographics including age, sex and body mass index. Patients <18 years of age and those did not give any written consent were excluded from this study.

Age of the patients was 18-70 years. The tissue processor at Fukushima University Medical Center was used to treat skin biopsy specimens that had been fixed in 10% formalin and then cut into sections in preparation for paraffin sectioning (SAKURA TISSUE TEK-R TEC5 MODEL 220-240). Following the embedding in paraffin, rotatory microtome sections of 4-5 micrometres thickness were cut using a rotatory microtome (SAKURA ACCU-CUT MODEL SRM 200 CW). Hematoxylin and eosin were used to stain the slides in order to examine the morphology. For the purpose of ensuring the accuracy of the diagnosis, two specialised histopathologists performed microscopic investigations. In this study, the immune-histochemical approach of Avidin-Biotin-Peroxidase was used in conjunction with DAB chromogen to examine the cells. After endogenous peroxidase activity was suppressed with 0.6 percent hydrogen peroxide, sections were incubated for 60 minutes at room temperature with prediluted CD34 antibodies for 60 minutes. Negative and positive CD34 controls were utilised. CD34 exhibited brown cytoplasmic and membranous staining when seen under a light microscope from Olympus. Two consultant histopathologists examined the entire section, and immunoreactivity for CD34 was detected by counting capillaries in the three most vascularized regions under a 40x magnification field and graded as follows: mild (4-10 capillaries), moderate (11-20 capillaries), and severe (21-30 capillaries) CD34 intensity was measured using these criteria, which were taken from an Egyptian study.

On the basis of histopathology, psoriasis was determined to be present when the following features were observed: hyperkeratosis, acanthosis, a munro's abscess, the extension of rete ridges, and abnormalities of the dermal vasculature. SPSS 24.0 version was used to analyze.

### RESULTS

Majority of the patients in or study were males 55 (68.8%) and the rest were females 25 (31.2%) with mean age 35.51+19.61 years. Mean BMI of the patients was 24.23+11.34 kg/m<sup>2</sup>.(table 1)

Variables	Frequency	Percentage		
Mean age (years)	35.51+19.61			
Mean BMI (kg/m <sup>2</sup> )	24.23+11.34			
Gender				
Male	55	68.8		
Female	25	31.2		

Frequency of moderate staining of CD34 expression was high found in 50 (62.5%) cases, mild staining in 17 (21.3%) patients and 21-28 capillaries (strong staining) in 13 (16.3%) patients.(table 2)

Table 2: Prevalence of CD34 e	xpression among enrolled cases
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Variables	Frequency	Percentage
CD34 Expression		
Mild	17	21.3
Moderate	50	62.5
Severe	13	16.3

Table 3: Outcomes according to histopathological findings

CD34 Expression	Frequency	Percentage		
Hyperkeratosis				
Mild	5	6.3		
Moderate	75	93.7		
Severe	0	0		
Acanthosis				
Mild	0	0		
Moderate	80	100		
Severe	0	0		
Munro Abscess				
Mild	17	21.3		
Moderate	40	50		
Severe	23	28.7		
Elongation of rete ridges				
Mild	18	22.5		
Moderate	48	60		
Severe	14	17.5		
Dermal Vasculature				
Mild	16	20		
Moderate	49	61.3		
Severe	15	18.7		

According to histopathological findings hyperkeratosis, acanthosis, a munro's abscess, the extension of rete ridges, and abnormalities of the dermal vasculature majority had moderate (11-20 capillaries) staining of CD34 expression.(table 3)

#### DISCUSSION

In accordance with the World Health Organization, psoriasis is a complicated multifactorial condition caused by a combination of genetical, environmental, and immunological variables that affects between 1.3 and 2.2 percent of the world's population. A clinically diverse disorder, it can be divided into several distinct clinical subtypes, including chronic plaque psoriasis (also called psoriasis vulgaris), guttate psoriasis, and generalised or locally localised (pustular)psoriasis, among others. Clinical indicators include well-demarcated, symmetrical erythematous plaques on the skin surface with adherent silvery scales adhering to the skin surface (85-90 percent of cases). It is most usually seen as a chronic plaque type (85%-90 percent of cases). The scalp, elbows, knees, and pre-sacral area of the back are some of the most commonly affected areas of the body. There is a high incidence of nail involvement, and approximately 30 percent of persons with psoriasis have been diagnosed with psoriatic arthritis [13]. Dermatologically, psoriasis is a chronic inflammatory illness that affects the skin and joints in particular. It manifests itself at a bimodal age of onset (16 to 22 years and 57 to 60 years) and affects both sexes in similar proportion. [14]

In this descriptive study 80 patient of both genders with ages 18-70 were included. In our study male were majority 55 (68.8%) and the rest were females 25 (31.2%) with mean age 35.51+19.61 years. Mean BMI of the patients was 24.23+11.34 kg/m<sup>2</sup>. Results of our study were comparable to the previous researches.[15,16] Frequency of moderate staining of CD34 expression was high found in 50 (62.5%) cases, mild staining in 17 (21.3%) patients and 21-28 capillaries (strong staining) in 13 (16.3%) patients. This was comparable to the previous study.[17] Tadini et al. were the first to discover P53 expression in the nuclei of psoriasis skin cells [18]. A few years following these findings, a different study [18] used similar antibodies, but found no P53-positive cells in skin biopsies from psoriasis sufferers. According to another study, the number of P53positive cells in the lesions of the disease was much more numerous than in the normal skin and controls [19]. For example, Moorchung et al. [20] discovered a modest connection between P53 immunostaining in epidermal cells and lesional psoriatic skin. P53 is overexpressed in psoriatic epidermis keratinocytes, according to other studies [21]. P53 nuclear staining was found in 43.3 percent of 30 Egyptian individuals with psoriasis plaques, according to a study. [22]

The condition has severe emotional and psychosocial consequences for its victims, in addition to its clinical manifestations. It can cause stigmatisation, low selfesteem, and increased stress, all of which can have a negative impact on social functioning and interpersonal interactions. Even though it has a significant negative impact on quality of life, psoriasis is underdiagnosed and undertreated because suitable facilities are not readily

available at the primary care level [23]. Better understanding of disease diagnosis and the availability of more effective treatment choices are required in order to address this issue successfully .. According to histopathological findings hyperkeratosis, acanthosis, a munro's abscess, the extension of rete ridges, and abnormalities of the dermal vasculature majority had moderate (11-20 capillaries) staining of CD34 expression.[17] When Gupta et al. [24] examined skin biopsies from patients with psoriasis, they discovered considerably higher CD34 positivity on standard microscopy, as well as significantly greater microvessel length density in psoriasis than in psoriasiform lesions. It was also shown that the density of microvessels was larger in patients with psoriasis, however this was not statistically significant (P > 0.05). Amin and Azim [25] conducted a study that produced their findings.

### CONCLUSION

We concluded in this study that the mild staining expression of CD34 was majority seen among patients of psoriasis. Psoriasis's enhanced cutaneous vasculature has been demonstrated by this crucial angiogenesis marker. As a result, it can be used to better understand the disease's histiogenesis.

#### REFERENCES

- 1 Nemati H, Khodarahmi R, Sadeghi M, Ebrahimi A, Rezaei M, Vaisi-Raygani A. Antioxidant status in patients with psoriasis. Cell Biochem Funct. 2014; 32(3): 268–73.
- 2 Ayala-Fontánez N, Soler DC, McCormick TS. Current knowledge on psoriasis and autoimmune diseases. Psoriasis (Auckl). 2016; 6: 7–32.
- 3 MacDonald A, Burden AD. Psoriasis: advances in pathophysiology and management. Postgrad Med J. 2007; 83(985): 690–7.
- 4 Kurd SK, Richardson SK, Gelfand JM. Update on the epidemiology and systemic treatment of psoriasis. Expert Rev Clin Immunol. 2007; 3(2): 171–85.
- 5 McKay IA, Leigh IM. Altered keratinocyte growth and differentiation in psoriasis. Clin Dermatol. 1995; 13(2): 105–14.
- 6 Sezer E, Böer-Auer A, Cetin E, Tokat F, Durmaz E, Sahin S, et al. Diagnostic utility of Ki-67 and Cyclin D1 immunostaining in differentiation of psoriasis vs. other psoriasiform dermatitis. Dermatol Pract Concept. 2015; 5(3): 7–13
- 7 Sehgal VN, Dogra S, Srivastava G, Aggarwal AK. Psoriasiform dermatoses. Indian J Dermatol Venereol Leprol. 2008;74(2):94-9.
- 8 Amin MM, Azim ZA. Immunohistochemical study of osteopontin, Ki-67, and CD34 of psoriasis in Mansoura, Egypt. Indian J Pathol Microbiol. 2012;55(1):56-60
- 9 Nickoloff BJ, Xin H, Nestle FO, Qin JZ. The cytokine and chemokine network in psoriasis. Clin Dermatol. 2007;25(6):568-73.
- 10 Res PC,Piskin G,de Boer OJ,van der Loos CM,Teeling P,Bos JD.Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. PloS One. 2010;5(11):e14108
- 11 Huynh N,Cervantes-Castaneda RA, Bhat P, Gallagher MJ, Foster CS. Biologic response modifier therapy for psoriatic ocular inflammatory disease.Ocul Immunol Inflamm. 2008;16(3):89-93
- 12 Papp KA, GriffithsCE, Gordon K, Lebwohl M, Szapary PO, Wasfi Y et al.Long-term safety of ustekinumab in patients

with moderate-to-severe psoriasis: final results from five years of follow-up.Br J Dermatol. 2013  $\,$ 

- 13 Ramezani M, Hashemi BS, Khazaei S, Rezaei M, Ebrahimi A, Sadeghi M. Diagnostic value of immunohistochemistry staining of Bcl-2, CD34, CD20 and CD3 for distinction between discoid lupus erythematosus and lichen planus in the skin. Indian J Pathol Microbiol. 2017; 60(2): 172–6
- 14 Mrowietz U, de Jong EM, Kragballe K, Langley R, Nast A, Puig L, et al.A consensus report on appropriate treatment optimization and transitioning in the management of moderate-to-severe plaque psoriasis.J Eur Acad Dermatol Venereol. 2014; 28(4):438-53.
- 15 Ramezani M, Shamshiri A, Zavattaro E, et al. Immunohistochemical expression of P53, Ki-67, and CD34 in psoriasis and psoriasiform dermatitis. Biomedicine (Taipei). 2019;9(4):26.
- 16 Mirzayans R, Andrais B, Kumar P, Murray D. Significance of Wild-Type p53 Signaling in Suppressing Apoptosis in Response to Chemical Genotoxic Agents: Impact on Chemotherapy Outcome. Int J Mol Sci. 2017; 18(5). pii: E928
- 17 JavedS, AhmedM, KaziF, Sohaill, KhanMA.Histopathological and immunohistochemicalstudy of CD34 in skin biopsies of patients presenting with psoriasis. Pak J Pathol. 2019: 30(1): 24-28

- 18 Tadini G, Cerri A, Crosti L, Cattoretti G, Berti E. P53 and oncogene expression in psoriasis. Acta Derm Venereol (Stockh).
- 19 Moles JP, Theillet C, Basset-Seguin N, Guilhou JJ. Mutation of the tumor suppressor gene TP53 is not detected in psoriatic skin. J Invest Dermatol.
- 20 Baran W, Szepietowski JC, Szybejko-Machaj G. Expression of p53 protein in psoriasis. Acta Dermatovenerol Alp Pannonica Adriat. 2005; 14(3): 79–83.
- 21 Moorchung N, Vasudevan B, Dinesh Kumar S, Muralidhar A. Expression of apoptosis regulating proteins p53 and bcl-2 in psoriasis. Indian J Pathol Microbiol. 2015; 58(4): 423–6
- 22 El-Domyati M, Barakat M, Abllel-Razek R. Expression of apoptosis regulating proteins, P53 and bcl-2, in psoriasis. J Egypt wom Dermatol Soc. 2006; 3: 46–51.
- 23 EL-Adel R, Abdel Hameed M, El-Shaer M, Imam A, Abdel Hafez N. Immunohistochemical Study of Protein P53 In Egyptian Psoriasis. Rep Opinion. 2011; 3(1): 65–84
- 24 Gupta S, Kaur M, Gupta R, Singh S, Pant L, Singh PP. Dermal vasculature in psoriasis and psoriasiform dermatitis: a morphometric study. Indian J Dermatol. 2011; 56(6): 647–9.
- 25 Amin MM, Azim ZA. Immunohistochemical study of osteopontin, Ki-67, and CD34 of psoriasis in Mansoura. Egypt. Indian J Pathol Microbiol. 2012; 55(1): 56– 60.