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Original research article

A case control study on differential expression of angiogenic factors in skin of patients with psoriasis vulgaris

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Abstract

Background and Objective: To analyse angiogenic factor expression with immunohistochemistry. Skin biopsies from those with psoriasis vulgaris and those without were analysed for levels of vascular endothelial growth factor, Von wille brand factor and CD 34. Finding the degree of neovascularization in skin samples from patients with and without psoriasis vulgaris by comparing the levels of CD 34, VEGF and vWFr. In an effort to link the expression of angiogenesis-promoting factors with the clinical severity of psoriasis as defined by the psoriasis area and severity index (PASI SCORE).

Method: A case-control study conducted between July 2022 to February 2023 at tertiary care Centre. There was a total of 43 participants, including both those with psoriasis and healthy skin who acted as controls. Those who have just been diagnosed with psoriasis or who have ceased therapy for at least two months prior to the study are biopsied. Histopathology testing established a diagnosis of psoriasis vulgaris. Immunohistochemistry was used to examine the levels of vascular endothelial growth factor (VEGF), von Willebrand factor (vWF) and CD 34.

Result: There was a statistically significant increase in epidermal VEGF expression in patients compared to controls (P=0.01). A higher level of CD 34 expression was seen in the study's patients compared to the controls. (p<0.01). Both the patients and the controls had low levels of von Willebrand factor expression. As with PASI score, VEGF and CD 34 expression were positively connected with PASI score (r=0.944, p0.05), as was VEGF expression and PASI score (r=0.942, p0.05).

Conclusion: A substantial difference in VEGF and CD 34 expression was observed between patients and controls. Keratinocytes in psoriatic skin lesions secrete pro-angiogenic cytokines, which encourage microangiopathic changes in the psoriatic plaque. Vascular endothelial growth factor (VEGF) is one type of cytokine. Angiogenesis is implicated in both the aetiology and development of psoriasis vulgaris. Hence, it appears that the development of targeted anti-angiogenic therapy for this chronic, disabling skin disease would be beneficial.

Keywords: Psoriasis vulgaris, vascular endothelial growth factor (VEGF), von willebrand factor (vWF), CD 34

Introduction

Intricate cell networks make the skin the biggest organ in the body. Dermatological lesions require a visual inspection, a gross description, and a histological analysis. 0.5%-1.5% of the global population has psoriasis, a chronic form of papulosquamous dermatitis. The current psoriasis outbreak in India is very similar to those seen in the West. Psoriasis is characterised by an erythematous plaque that is covered with white, silvery scales. It has a significant effect on the extensor aspect of the elbows, lumbosacral area, knees, intergluteal cleft, scalp and glans penis. Each individual with psoriasis has a unique presentation. Subtypes of chronic plaque psoriasis vulgaris include guttate, erthyrodermic, pustular and flexural forms. Psoriasis frequently manifests itself in nail changes and koebner responses [1, 2, 3].

Acanthosis, regular, downward rete ridge elongation, spongiform pustules and Munro's microabscesses are all visible on histopathology slides. Dilated, tortuous capillaries bleed easily when plaque scales are scraped off. Psoriasis has a complex aetiology that includes immune-mediated inflammation, dysregulated angiogenesis and vascular remodelling in addition to aberrant keratinocyte proliferation and differentiation. Psoriasis's pathogenesis relies heavily on T helper 1 (Th1) cells, antigen-presenting cells, Langerhan cells, natural killer cells, keratinocytes, macrophages and Th1-type cytokines [4, 5].

It is possible to reverse the initial development of blood vessels in psoriatic lesions after they have been treated. Proangiogenic cytokines, including tumour necrosis factor, interleukin 17 and interleukin 8 and angiogenic mediators, including vascular endothelial growth factor, are upregulated in psoriasis lesional

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skin. Psoriasis is chronic and may clear up on its own or in response to treatment [6].
Using CD, we compared the neovascularization score of skin samples from patients with psoriasis vulgaris and those from individuals with normal skin. Epidermis VEGF and vWF levels are evaluated in both normal and psoriatic skin samples. This research supports the idea that vascular alterations play a significant role in the development of psoriasis. Lesions of psoriasis may be triggered by angiogenesis. Medicines that inhibit angiogenesis may be employed in this kind of focused therapy. Psoriasis vulgaris lesions were analysed immunohistochemically for levels of vascular endothelial growth factor, VWF, and CD 34 [7, 8, 9].

Material and Methods

Result

Case-control study at tertiary care centre from July 2022 to February 2023. This study used 43 psoriasis patients. The inclusion criteria are a recent psoriasis diagnosis and two months without therapy (topical or systemic). Patients with psoriasis had skin biopsies. Every patient gets an elliptical skin sample from the lesion tested. Healthy volunteers and plastic surgery skin were compared. PASI scores determine psoriasis vulgaris severity. Lesion location and count determine the approach. Calculate damage pattern next.
We publish median or mean +/- standard deviation depending on data distribution. Quality measures were non-numerical and non-percentile. The unpaired t test assessed quantitative group differences. The Mann-Whitney test compared the groups. Chi-square tests compared category groups.
Pearson's correlations were used to evaluate variable relationships. All statistical analyses were significant if the two-tailed p value was less than 0.05. The data analysis were done with SPSS (16.0 for Windows).

Table 1: Distribution of Age

Age Distribution					
Age years	Cases		Control		Total
	Male	Female	Male	Female	
<30	0	4	0	4	8
31-40	7	3	6	2	18
41-50	11	5	7	7	30
51-60	5	6	10	5	26
>60	0	2	0	2	4
Total	23	20	23	20	86

The majority of the 86 samples were between the ages of 41 and 50, with the next largest group being between the ages of 51 and 60.

Table 2: Gender Distribution

Group	Gender	Mean	Sd	Lower	Upper	Minimum	Maximum
	Male	47	9	43	50	34	60
Cases	Female	45	12	39	52	28	64
	Total	46	10	41	51	27	62
	Male	50	10	45	53	35	59
Controls	Female	46	13	39	51	27	62
	Total	48	11	42	52	29	62

With a total of 86 participants (43 cases and 43 controls), the average age of the participants in this study is 45. The majority of the cases (51%) were comprised of people aged 41-50, while the majority of the controls (45%) were aged 51-60.

Table 3: Distribution of Samples According to Gender

Gender	Cases	Controls
Male	23	23
Female	20	20
Total	43	43

The ratio of male to female patients in this analysis was 1.20 to 1. The male-to-female ratio in the control group was 1.1:1.

Table 4: Density of Microvessel (Evaluation of CD 34 Staining)

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CD34	Cases	Control	Total	(%)
Neg	0	28	28	33%
1+(mild)	0	15	15	17%
2+(moderate)	28	0	28	33%
3+(severe)	15	0	15	17%
Total	43	43	86	100%

Samples taken from lesions of varying severities on psoriatic skin all showed expression of CD 34. All of the non-infected skin samples showed low CD34 expression. The skin samples from 73% of the cases revealed moderate staining.

Table 5: VEGF Immunohistochemical Staining

VEGF	Cases	Control	Total	(%)
Neg	0	29	29	34%
1+	0	14	14	16%
2+	18	0	18	21%
3+	25	0	25	29%
Total	43	43	86	100%

All patients showed VEGF expression, while the staining was faint in the controls. Full-thickness epidermal staining was diffuse in 71% of cases and weakly positive in 29% of controls.

Table 6: Von Willebrand Factor Immunohistochemical Evaluation

VWFr	Cases	Control	Total	(%)
Neg	27	30	57	66%
1+	16	13	29	34%
Total	43	43	86	100%

Similar low levels of epidermal Vonwillebrand factor expression were observed across the patients and controls. Sixty-six percent of cases showed no staining and thirty four percent of controls did as well.

Table 7: Angiogenic Factor Expression Mean

Factors	Group	Mean	SD	Lower	Upper	Minimum	Maximum	P value
PASI	Case	16.5	2.1	15.9	16.8	13.9	19.5	
	Case	2.6	0.7	2.3	2.7	2.1	3.0	
CD34	Control	0.5	0.6	0.2	0.5	0.0	1.0	
	Total	1.4	1.0	1.1	1.5	0.0	3.0	< 0.05
	Case	2.5	0.6	2.7	2.9	2.0	3.0	
VEGF	Control	0.1	0.5	0.2	0.5	0.0	1.0	
	Total	1.6	1.4	1.2	1.9	0.0	3.0	<0.05
	Case	0.35	0.47	0.3	0.4	0.0	1.0	
VWFr	Control	0.34	0.47	0.3	0.4	0.0	1.0	> 0.05
	Total	0.3	0.5	0.3	0.4	0.0	1.0	

Expression of CD 34 was found to be significantly higher in cases (2.3 +/- 0.5 SD) than in controls (0.2 +/- 0.4SD). The average level of VEGF expression in cases was 2.6 +/- 0.5- SD, while it was only 0.2 +/- 0.4- SD in the control group. The mean VWFr expression among patients was 0.34 +/- 0.48 SD, while the mean expression among controls was 0.33 +/- 0.48 SD.

Table 8: Age vs. Pasi Score

PASI Score				
Age	**13.1-15.0**	**15.1-17.0**	**>17**	**Total**
<30	2	3	0	5
31-40	2	0	8	10
41-50	7	4	5	16
51-60	4	2	4	10
>60	0	0	2	2
Total	15	9	19	43

In this analysis, the mean PASI score was 16.2+/-1.9.

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Table 9: Correlation Between PASI Score and Angiogenic Factors

		PASI Score	CD 34	VEGF	VWFr.
PASI Score	Pearson Correlation	1	.941**	.945**	0.024
	Sig. (2-tailed)		0.000	0.000	0.855
	N	86	86	86	86
CD 34	Pearson Correlation	.943**	1	.878**	0.057
	Sig. (2-tailed)	0.000		0.000	0.653
	N	86	86	86	86
VEGF	Pearson Correlation	.943**	.878**	1	-0.022
	Sig. (2-tailed)	0.000	0.000		0.867
	N	86	86	86	86
VWFr.	Pearson Correlation	0.023	0.057	-0.021	1
	Sig. (2-tailed)	0.852	0.651	0.863	
	N	86	86	86	86

Psoriasis severity and area both had a strong relationship with angiogenic factor expression (PASI Score)

Discussion

Psoriasis caused by the immune system is characterised by persistent inflammation. Everyone of every age is impacted. Hereditary transmission may be the source of symptoms experienced in early adulthood. Individuals who suffer from psoriasis vulgaris typically appear to the doctor's office with a raised, scaly, and ruddy lesion. In the histology of psoriasis, features such as hyperkeratosis, parakeratosis, epidermal hyperplasia, kogoj's spongiform pustules, suprapapillary granular layer thinning, dermal infiltration, and dermal capillary growth can be observed [10, 11, 12].

The cause of this illness is not known. Psoriasis may result from numerous factors. Some examples include angiogenesis, neovascularization, vascular remodelling, rapid epidermal cell proliferation and cytokines, immune-mediated aberrant regulation of T cells. According to a number of studies, keratinocytes that are quickly growing emit cytokines and angiogenic substances like VEGF. The creation of new blood vessels is known as angiogenesis. Greater endothelial microvasculature is present in psoriasis patients, indicating angiogenesis reliance. Due to abnormally dilated and directed dermis capillaries and microvascular development, psoriasis is angiogenesis-dependent. Research on angiogenesis looked at psoriasis [13, 14, 15].

Keratinocytes in psoriasis skin lesions may be responsible for the emission of proangiogenic cytokines. These cytokines include vascular endothelial growth factor, tumour necrosis factor alpha, endothelial cell stimulating factor and platelet-derived growth factor. vWFr and NGF are two examples of more recent angiogenic factors. The process of angiogenesis in psoriatic lesions is helped along by cytokines. Psoriatic skin lesions are examined for their potential to show neovasularization and vascular alterations. In this particular study, patients were diagnosed at the age of 45, while controls were diagnosed at the age of 47. Siaw-Cheok *et al.* found that the average age of patients was 42.33 years old, while the average age of controls was 47.94 years old. According to the findings of Moorchung *et al*., the average age of the patients was 38.9 years [16, 17, 18].

In this study, there were 16 men and 14 women in the control group compared to 18 men and 14 women in the case population. The mean PASI score for the current study cases was 16.2 +/- 1.9. In a related study, Siaw-Cheok *et al*. discovered 7.247+/-4.780 PASI scores. The VEGF expression in our patients varied. Controls lacked VEGF expression. Compared to controls, patients exhibited higher VEGF (p 0.05). Similar findings were later found through research. Cases expressed higher VEGF than controls, according to Siaw-Cheok *et al.* (p=0.016). The epidermis of psoriatic skin lesions displayed noticeably more diffuse VEGF(13.15+/- 6.6) immunohistochemistry expression than normal skin, according to Simonetti *et al*. VEGF expression was found to be high in the epidermis (46.1+/-19.66), moderate in the

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arteries (19+/-5.4) and low in the inflammatory infiltrates (8+/-2.16), according to Rashed *et al*. Psoriatic skin has increased VEGF expression.

Our findings that VEGF enhances endothelial cell survival and the formation of new blood vessels, creating the pathologic foundation for psoriasis, are supported by earlier investigations. It might lead to psoriasis. According to Bhushan *et al*., epidermal keratinocytes-not fibroblasts-produce the majority of VEGF (r=0.944, p=0.05). Siaw-Cheok found no association between PASI and VEGF (p=0.232). In this investigation, compared to the control group, all psoriasis samples expressed CD 34 to varying degrees. Cases significantly overexpressed CD 34 compared to controls (p 0.01). Gupta S *et al*. discovered increased dermal microvascular staining in psoriatic skin lesions in another investigation. Microvessel density was higher in psoriatic skin (71.28+/-40.05). Psoriatic lesion skin has a larger endothelium and lumen capacity than non-lesional and healthy control skin, according to Barton *et al*. 199. Psoriatic lesion skin expressed CD 34 far more than normal skin, according to Simonetti O *et al*. (19.15+/-12.61 vs 3.0+/- 0.23; p=0.04) [19, 20, 21].

The fact that inflammation in psoriasis leads to blood vessel expansion, tortuosity and lengthening lends credence to the conclusions we've drawn from our research. In a study conducted by Gujiao BI *et al*., higher CD 34 expression in vascular endothelial cells in the dermis of psoriatic skin lesions was identified. This finding suggests that the severity of the condition may be associated to the level of CD 34 expression. Inflammatory cells have the ability to bind to CD34. The expression of vWFr was found to be low in both the lesional and healthy skin. p>0.05. Siaw-Cheok and colleagues did not discover any evidence of vWFr expression in psoriasis. There was shown to be no association between PASI Score and vWFr (r = 0.024; *p*>0.05). Siaw-Cheok *et al*. came to the conclusion that there is no correlation between the PASI score and the vWFr (p=0.169). The evidence presented here implies that angiogenesis is a process that is involved in psoriasis [20, 21].

Blood flow is improved in psoriasis skin biopsies using laser Doppler flowmetry. Finally, in psoriatic skin lesions, ultrastructural studies revealed twisted and stretched capillary loops. Numerous ongoing studies use immunohistochemistry to analyse angiogenic components and angiogenesis markers in psoriatic skin lesions. The list also includes pleiotropic osteopontin, basic fibroblast factor, I NOS, and nerve growth factor [21].

Conclusion

Psoriatic skin lesions express high levels of VEGF and CD 34, angiogenesis markers. Von Willebrand factor expression was insignificant. VEGF and CD 34 expression were substantially linked with PASI score. Antiangiogenic therapy may treat psoriasis. Angiogenesis indicators and angiogenic factors in psoriatic skin lesions are being studied immunohistochemically. This collection contains basic fibroblast growth factor, INOS, nerve growth factor, survivin and pleiotropic osteopontin.

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