

Role of Immunohisto Chemistry and its Correlation with Histopathological Grading and Severity of Psoriatic Disorder

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Received: 29-12-2022 / Revised: 13-01-2023 / Accepted: 10-02-2023

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Conflict of interest: Nil

Abstract

Introduction: Skin diseases are the leading cause of non-fatal burden of diseases in India. Psoriasis is one of the main dermatological disorder with psychological distress. Psoriasis is a chronic, disfiguring, inflammatory dermatosis in which both genetic and environmental influences have a critical role. Psoriasis is a complex T-cell mediated disorder characterized by presence of CD4+ and CD8+ T cells in dermis and epidermis, T cell activation is followed by cytokine release leads to cascade of reaction including abnormal keratinocyte proliferation, neutrophil migration, potentiation of Th1 cell response and angiogenesis. Vascular alteration observed in lesions are considered to be most important feature of the disease.

Angiogenesis plays an important role by promoting inflammation through recruitment of inflammatory cells. Angiogenesis in skin lesions and activation of endothelial cells via cytokines form a bridge between altered keratinocytes and immunological component of the disease. The study was done to represent the importance of angiogenesis in disease process and its implication in the disease severity.

Aims and Objectives: 1) To study the histomorphological features of psoriasis. 2) To assess the expression of monoclonal antibody CD34 with reference to severity of lesion.

Material and Methods: This retrospective study includes 50 cases received from the dermatology outpatient clinic in Government Mohan kumaramangalam Medical College, salem from November 2018 to June 2020. Assessment of severity of the clinically confirmed cases was done using Psoriasis Assessment severity index (PASI) Score method. Punch biopsy was taken from the lesional area, the biopsy tissue was processed and stained with hematoxylin and eosin stain. After staining, the slides were examined under light microscope and various histological parameters were studied and observations were recorded. Followed by immunohistochemistry using CD34 was done. Using CD34 staining, Micro vessel Density (MVD) was evaluated with hot spot method. The score is calculated. Finally, the intensity of staining was compared and correlated with PASI score.

Observation and Results: In the present study, Severity of psoriasis was assessed using PASI score. Out of 50 cases, 33 patients (66%) had score < 10 and 17 patients (34%) had PASI score > 10. In this study 66% of cases was graded as mild psoriasis and 34% were graded as moderate / severe type psoriasis. All the patients with severe degree of CD34 staining had moderate/severe degree of psoriasis. And two patients with moderate/severe degree of psoriasis had moderate (2+)

degree of CD34 staining. Patients with mild degree of CD34 staining had a mild degree of psoriasis.

Conclusion: With respect to Micro vessel density patients with PASI score > 10 had high intensity of CD34 staining as compared with patients with PASI < 10. Thus, signifying angiogenesis as most important factor contributing the clinical progression and severity of the lesion.

Keywords: Psoriasis Assessment Severity Index Score, Microvessel Density, Histomorphology.

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Introduction

Psoriasis is a disease of overactive immunity in genetically susceptible individual. The pattern is polygenetic rather than single gene mutation. A decade of genome wide linkage scan established PSORS1 as strongest susceptible loci. This locus is responsible for 50% of genetic component of psoriasis. Psoriasis is a T cell mediated disorder with both CD4+ and CD8+ T cells in papillary dermis and epidermis. CD8+ cells are predominantly in epidermis while CD4+ T cells in dermis. CD8+ cell in epidermis believed to be a key event in pathogenesis [1]. Recent data shows lesion of psoriasis exhibit clonal expression of epidermal lymphocytes and few dendritic cells. These Activated Dendritic cells present antigen to T cell result in activation of T cell that develop into memory cell which circulates in body. Followed by activation of these T cells an array of cytokines GM-CSF, Epidermal growth factor, IL-8, IL-12, 23, 17, 1, 16, Fractalin, TNF Alpha and others are produced. Activation of T cell and cytokine release causes cascade of response including increased proliferation of keratinocyte, migration of neutrophils, potentiation of Th-1 cell response, angiogenesis, increased expression of adhesion molecule and epidermal hyperplasia [1].

Angiogenesis is a physiologic process where there will be growth of new blood vessels from the Preexisting vessel. Angiogenesis is not only a cofactor but inducer of psoriasis. The superficial microvascular changes are due to pro angiogenic mediators such as

TNF, VEGF, HIF, IL-8 or angiopoietin [2,3]. The pro angiogenic role is due to TH-1 cytokine IL-17 [4,5]. This process occurs due abnormal balance between angiogenic and anti-angiogenic stimuli where thrombospondin (TSP1), an endogenous inhibitor of angiogenesis expression is reduced in psoriatic skin [6-8].

Endothelial cells are stained by a variety of endothelial markers. out of which, an excellent marker is CD34, a surface antigen. CD34 is a cell surface 110-120 KD monomeric transmembrane glycoprotein. It is a pan endothelial marker of endothelial cells. This angiogenic marker has a major role in the assessment of microvessel density than other markers such as CD31, which is present both in endothelial cells and in macrophages

Materials and Methods

This retrospective study was conducted at Government Mohan Kumaramangalam Medical College, Salem from November 2018 to June 2020 and include totally 50 cases. A total of 50 patients who are attending the outpatient clinic in dermatology department with scaly lesion and diagnosed clinically as psoriasis were included for this study.

The diagnosed cases were then analysed and graded using the PASI Score as follows. PASI (Psoriasis Assessment Severity Index) score is used for assessment of extensive psoriasis. Four sites of affection (head, trunk, upper limb, lower limb) are scored separately

scored by using three parameters, Erythema, Induration and Desquamation. Each of which is graded on a severity scale of 0 – 4 (0- nil, 1- mild, 2- moderate, 3- severe, 4- very severe). The area-wise percentage involvement of the involved sites are calculated as 1 = less than 10%, 2= 10-29 %, 3 = 30 – 49 %, 4= 50- 69%, 5= 70-89% and 6= more than 90 %.

PASI Score = Head + Trunk + Upper Limb + Lower Limb

Punch biopsy was taken after getting consent and application of local anaesthesia using disposable punch of size 4mm. The biopsy tissue was transferred to container with 10% neutral buffered formalin with great care. The tissue was further processed using tissue processor and embedded in paraffin. Embedded paraffin blocks were sectioned with microtome into 3-5micron thickness and stained with haematoxylin and eosin stain. After staining, the slides were examined under light microscope and various histological parameters were studied and observations were recorded. Followed by immunohistochemistry using CD34 was also performed for selected cases and evaluated.

CD34 – (Micro vessel Density MVD) staining was evaluated with capillary counting method in the highly Vascularized

areas also known as hot spots, under 40x field. CD 34 showed membranous and cytoplasmic staining. Vessels in selected fields were counted at 40X. Single or clusters of endothelial cells, with or without lumen, were considered to be individual vessels. The score is calculated as follows:

- 0 None
- 1+ Mild (4-10 capillaries)
- 2 + Moderate (11-20 capillaries)
- 3 + Severe (21-28 capillaries).

Observation and Results

The present study entitled “Clinicopathological study of psoriasis with reference to Immunohistochemistry in GMKMCH, Salem” was conducted on 50 skin biopsy samples in the Department of Pathology, Government Mohan Kumaramangalam Medical College, Salem. Histopathological findings of each skin biopsy were studied and Immunohistochemistry was done in selected cases. The observations of present study were as follows.

Out of 50 patient’s majority (34%) of them belongs to 41-60yrs and the least number of patients were in < 20yrs with prevalence of 16%. Youngest patient was 14yrs old and oldest patient was 80 years old.

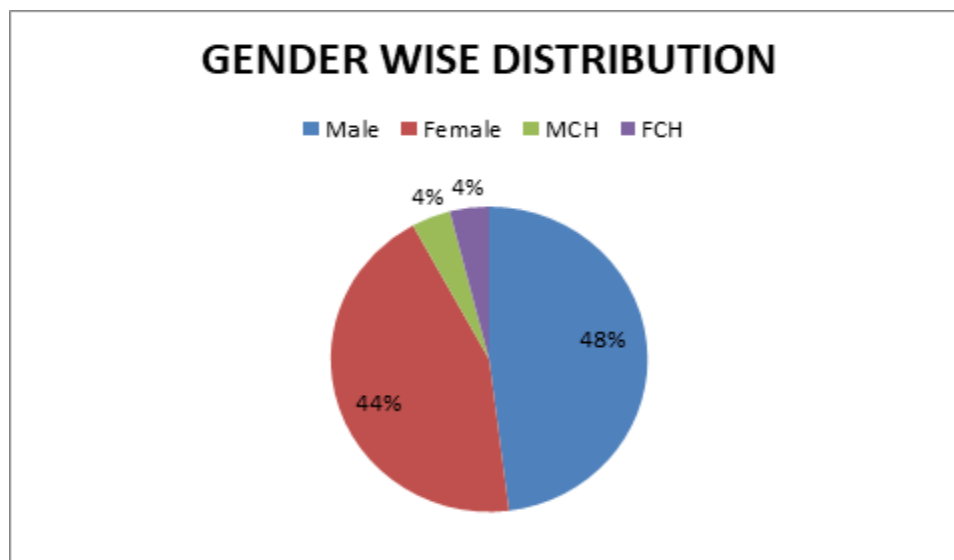


Chart 1: Pie Chart Illustrating the Gender Distribution of Cases.

Out of 50 cases that were studied, 24(48%) were males and 22(44%) were females. (chart no:1) Pediatric cases (8%) were four in number. In the present study, lower limb (66%) were the most common site involved, followed by upper limb involving 82%. Other sites involved were trunk, head & neck in 34% and 26% cases respectively. Scalp was the least commonly involved site in about 4 % of cases. In the present study majority (80%) of patients presented with plaque, erythema in 72%, scales in 68%, papule in 20% and hypo pigmented lesions in 22%.

Severity of psoriasis was assessed using PASI score. According to PASI score, 33 patients (66%) had score < 10 and 17 patients (34%) had PASI score > 10.(Table no:1 & 2)

Table 1: Table illustrating the distribution of severity of cases.

Grade	No of Patients	%
Mild	33	66.0
Moderate/severe	17	34.0

Table 2: Illustrating the distribution of PASI Score

PASI Score	No of Patients	%
>10	17	34
<10	33	66

In the present study out of 50 patients, 46 (92%) patients showed parakeratosis, 48 (96%) showed acanthosis, 45 (90%) showed hyperkeratosis, 41 (82%) showed hypo granular layer, 43 (86%) showed supra papillary thinning, 41 (82%) showed elongated rete ridges, 33 (66%) showed Munro micro abscess, 28 (56%) showed spongiform pustules of kogoj. Dermis showed inflammatory infiltrates in 48 (96%) of cases and 40 (80%) showed dermal capillary dilatation.

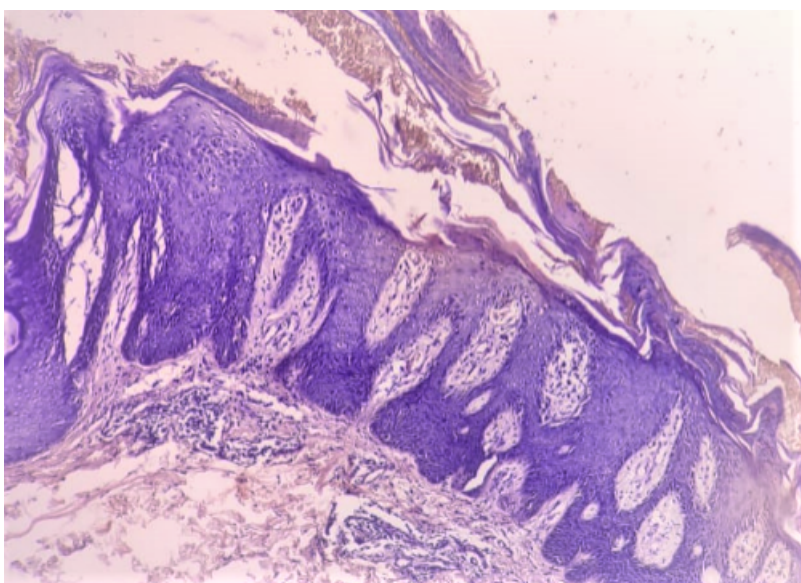


Figure 1: Epidermis showing acanthosis, parakeratosis, regular elongated rete ridges, supra papillary thinning. (10X)

Immunohistochemistry CD34 staining was done in 10 selected cases. The results were interrupted.

Table 3: Assessment of Microvessel Density with Cd34 And Its Relation with PASI Score

Cd34 Staining	No of Cases (N= 10)	PASI Score /Severity
0	0	-
1 + (mild)	2	< 10 (mild)
2 + (moderate)	3	< 10 (mild) = 1
		>10 (moderate/severe) = 2
3 + (severe)	5	>10 (moderate / severe) = 5

In the present study among 10 cases 2 showed mild degree of staining, 3 showed moderate degree of staining and 5 showed severe degree of staining. (Table no:3) All the patients with severe degree of CD34 staining had moderate/severe degree of psoriasis. And two patients with moderate/severe degree of psoriasis had moderate (2+) degree of CD34 staining. Patients with mild degree of CD34 staining had a mild degree of psoriasis.

Discussion

Psoriasis is a chronic immunological mediated disorder with many serious disabilities. Histologically these lesions shows acanthosis, parakeratosis, munro micro abscess, spongiform pustules, supra papillary thinning and dilated capillaries in dermal papillae [10-12]. Apart from epithelial proliferation as a main event in disease, the pathogenesis is mainly due to imbalance in immunological response and angiogenesis. T lymphocytes are found to play a significant role in pathogenesis of the disease.

This disease is associated with severe social stigma and profound morbidity. Thus treatment should be aimed not only at reducing epidermal proliferation, but also should stop the inflammatory process and angiogenesis. Although several treatment with immuno-modulators are in use but they

are found to have their own side effects and little benefits to the patients. Angiogenesis which is both a consequence and contributing factor [13-15].

In the present study, histopathological findings from skin biopsies of all 50 patients of psoriasis showed acanthosis in 96%, parakeratosis in 92%, hyperkeratosis in 90%, hypo granular layer in 82%, suprapapillary thinning in 86%, elongated rete ridges in 82%, Munro micro abscess in 66%, spongiform pustules of kogoj in 56%, dermal inflammatory infiltrates in 96% and dermal capillary vessel dilatation in 80% patients.

In a study done by kalpana kumar *et al* the findings of acanthosis, hypo granular layer, supra papillary thinning and dermal inflammation are in concordance with present study. Findings of hyperkeratosis, elongated rete ridges and Munro microabscess are consistent with study done by Raghuveer *et al*. Majority of findings of the present study are in concordance with the study done by Dr. Medha yadhav *et al*. (Table no: 3)

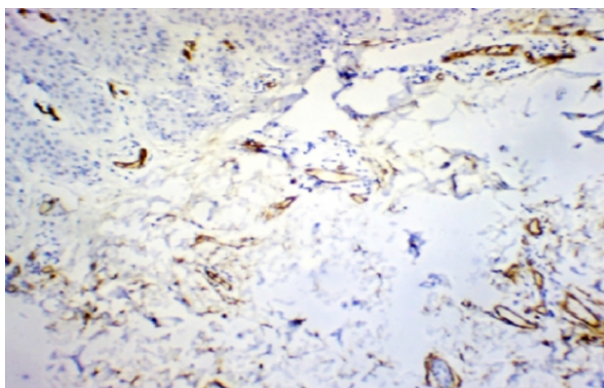
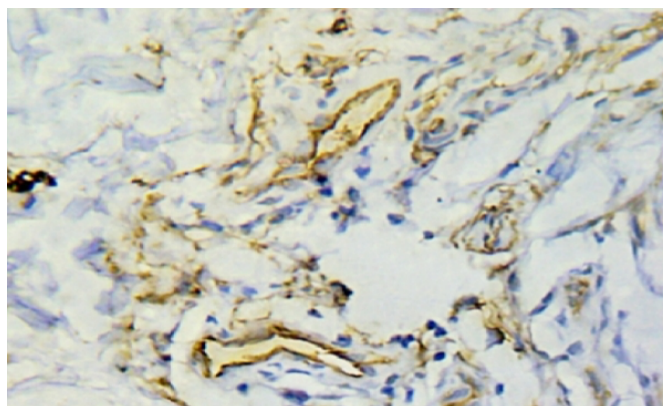
In contrast to the present study where spongiform pustules of kogoj was reported in 56% of cases, but the observations made by kalpana kumar *et al*, Pandi GA *et al* and Raghuveer *et al* reported spongiform pustules in 20%, 11.9% and 30% of the cases.

Table 3: Comparative Analysis of Histopathological Appearance in Cases Of Psoriasis.

Histopathological Appearance	Raghuveer <i>et al</i> (2015) n=100	Narayankar <i>et al</i> (2015) n=42	Kalpana kumara M.K <i>et al</i> (2017) n=50	Dr. Medha yadhav <i>et al</i> (2020) n=53	Present study n=50
Parakeratosis	77 (77%)	42 (100%)	50 (100%)	51 (96.2%)	46 (92%)
Acanthosis	75 (75%)	41(97.61%)	49 (98%)	50 (94.3%)	48 (96%)
Hyperkeratosis	89 (89%)	10 (23.8%)	41 (82%)	46 (86.8%)	45 (90%)
Hypo Granular Layer	57 (57%)	39 (92.8%)	42 (84%)	50 (94.3%)	41 (82%)
Suprapapillary Thinning	-	40 (95.2%)	43 (86%)	25 (47.2%)	43 (86%)
Elongated Rete Ridges	75 (75%)	36 (85.71%)	48 (96%)	48 (90.6%)	41 (82%)
Munro Microabcess	58 (58%)	35 (83.33%)	22 (44%)	31 (58.5%)	33 (66%)
Spongiform Pustules	30 (30%)	5 (11.9%)	10 (20%)	-	28 (56%)
Dermal Capillary Dilatation	90 (90%)	41 (97.6%)	50 (100%)	42 (79.2%)	40 (80%)
Dermal Inflammation	98 (98%)	42 (100%)	50 (100%)	51 (96.2%)	48 (96%)

Comparative analysis of microvessel density

In present study among the 10 cases, 50% cases showed a CD34 with severe intensity of staining having PASI score > 10. In a similar study done by Lakshna *et al* (2017), Guijiao BI *et al* (2000) showed increase in CD34 staining with majority of cases correlating with severity of disease. (figure 2, 3 & 4).

**Figure 2: Showing cd34 staining in dermal vessels. (40X)****Figure 3: IHC showing CD34 (2+) staining in dermis 40X.**

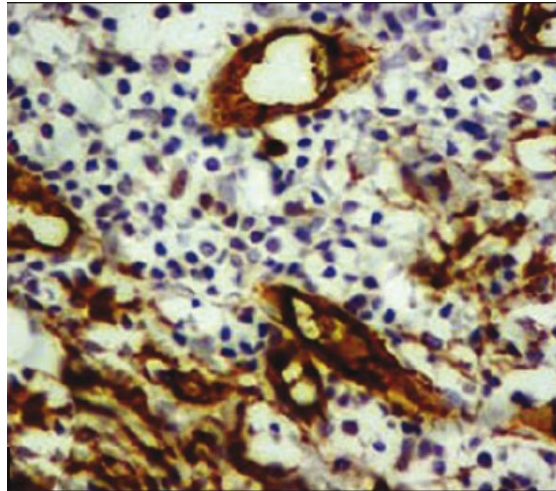


Figure 4 : IHC showing CD34 (3+) staining (40X).

Conclusion

Among the 50 cases, 24 were male, 22 were female patients. Most of the cases belongs to age group of 41 – 60 years of age. Most cases had mild degree of severity of psoriasis with PASI score being less than 10. Among the histomorphological presentation, parakeratosis, acanthosis, hyperkeratosis, dermal inflammatory infiltrates, hypo granular layer, supra papillary thinning, dermal capillary dilatation, elongated rete ridges were consistent findings in majority of cases. Though Munro micro abscess, spongiform pustules of kogoj were found in significant number of cases.

With respect to Micro vessel density patients with PASI score greater than 10 had high intensity of staining as compared with patients with PASI less than 10. Thus, signifying angiogenesis as most important factor in contributing the clinical progression and severity of the lesion. So that in future, multidisciplinary treatment like chemotherapy combined with targeted therapy with anti CD34 could be considered.

This approach would provide better therapeutic response and thereby, improving the survival of the patient.

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