"STUDY OF DERMAL VASCULAR CHANGES IN PSORIASIS – AN OBSERVATIONAL STUDY"

By

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"STUDY OF DERMAL VASCULAR CHANGES IN PSORIASIS - ANOBSERVATIONAL STUDY."**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

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Chairman,
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LIST OF ABBREVIATIONS USED

S.No	Abbreviation	Expansion
1.	Th	T-helper cells
2.	CD	Cluster of Differentiation
3.	IHC	Immunohistochemistry
4.	MVD	Micro Vascular Desnsity
5.	MVC	Micro Vascular Calibre
6.	Lef 1	Lymphoid enhancer-binding factor 1
7.	PSORS	Psoriasis
8.	HLA	Human leukocyte antigen
9.	IL	Interleukin
10.	TNF-ALPHA	Tumor necrosis factor alpha
11.	IFN	Interferon
12.	M-PROTEIN	Monoclonal protein
13.	VEGF	Vascular endothelial growth factor
14.	HIF	Hypoxia-inducible factor
15.	P-63	Tumor protein p63
16.	110.kDa	110 kilodaltons (a unit of molecular weight)

ABSTRACT

TITLE: STUDY OF DERMAL VASCULAR CHANGES IN PSORIASIS - AN OBSERVATIONAL STUDY

BACKGROUND and OBJECTIVES: Psoriasis is a skin disease characterized by the proliferation and differentiation of abnormal keratinocytes, immune-mediated inflammation and vascular remodeling. However, little is known about the role played by dermal microvasculature in psoriasis despite extensive studies. The aim of this research was therefore to evaluate dermal vascular changes in psoriasis using histopathological evaluation with morphometry through CD34 immunohistochemical staining.

METHODOLOGY: One-year observational study was conducted at KLE's Dr. Prabhakar Kore Hospital and MRC Belagavi. A total of 70 samples were studied, including 35 histopathologically confirmed psoriasis cases and 35 controls from normal skin tissues. The samples underwent CD34 immunohistochemical staining to assess microvascular density (MVD) and microvascular calibre (MVC).

RESULTS: The study revealed significant differences in MVD and MVC between psoriasis cases and controls. Psoriasis samples exhibited higher MVD and MVC compared to controls. Histopathological features such as parakeratosis, hypogranulosis, perivascular lymphocytic infiltrate, and dilated tortuous vessels were significantly correlated with increased MVD and MVC in psoriasis. The mean MVD in psoriasis cases was 13.74±3.07 vessels/400x, while in controls it was 3.39±0.39 vessels/400x. The mean MVC in psoriasis cases was 10.48±2.20 μm, compared to 4.48±1.08 μm in controls.

CONCLUSION: The findings indicate that psoriasis is associated with significant angiogenesis and vascular remodeling, as evidenced by increased MVD and MVC. The study highlights the potential of CD34 immunohistochemical staining as a diagnostic tool for assessing vascular changes in psoriasis and underscores the importance of targeting angiogenesis in therapeutic strategies.

KEYWORDS: Psoriasis, Dermal Vascular Changes, CD34 Immunohistochemistry, Angiogenesis, Microvacular density, Microvascular calibre.

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INTRODUCTION

Skin being the body's largest organ, make up for 12-15% of an adult's body weight and spanning an area of 1-2 m^2. Due to its extensive distribution, external exposure, and vascular complexity, the skin is susceptible to a variety of external and internal pathological disorders. These conditions often necessitate direct visual inspection, gross description, and histopathological analysis of skin lesions for accurate diagnosis.

Psoriasis is a chronic papulosquamous dermatitis, affecting approximately 0.5% to 1.5% of the general population[1]. This condition is characterized by increased epidermal proliferation, abnormal differentiation, and inflammation involving both the epidermis and dermis[2]. Clinically, psoriasis typically present as distinct erythematous plaques with silvery-white scales, most commonly affecting the extensor surfaces of the elbows, the lumbosacral region, the knees, the intergluteal cleft, the scalp, and the glans penis.

The etiology of psoriasis is multifactorial, involving genetic, immunological, and environmental factors. The pathogenesis of psoriais is complex, involving abnormal differentiation and proliferation of keratinocytes, immune system-driven inflammation, angiogenesis and vascular changes[3]. Key players in its pathogenesis include Th1 and Th17 cells, antigen-presenting cells, Langerhans cells, natural killer cells, keratinocytes, macrophages, and various Th1 cytokines[4].

Histopathologically, psoriasis is characterized by thickening of the epidermis (acanthosis) and elongation of the rete ridges. It also features kajog pustules and the formation of Munro's microabscess. Additionally, there is evidence of edema and

tortuous dilated capillaries in the papillary dermis, which can cause bleeding upon gentle skin scraping[5].

Neovascularization is observed in the early stages of psoriatic lesions and tends to subside as the disease clears[6]. Numerous anti-angiogenesis therapies have been proposed, with some proving successful in treating psoriasis[7,8]. CD34, a glycosylated transmembrane protein, serves as an immunological marker for measuring angiogenesis, thus providing a means to assess microvasculature[9,10].

However, there are limited studies that examine the dermal vascular changes in psoriasis through histopathological evaluation and morphometric analysis of dermal vessels using CD34 immunohistochemical (IHC) staining. Further research in this area is essential to enhance our understanding and treatment of psoriasis.

OBJECTIVE OF THE STUDY

To study the dermal vascular changes in psoriasis using CD34 IHC stain.

REVIEW OF LITERATURE

SKIN

Skin is the largest organ of the body, and it constitutes approximately 15% of an adult's body weight.[11]. It comprises numerous cell types that provide mechanical support, photoprotection, immunosurveillance, nutrition metabolism, and healing[12].

EMBRYOLOGY

The development of the skin involves the integration of two primary embryological components: the prospective epidermis, originates from the surface of the early gastrula, and the prospective mesoderm, which interacts with the inner surface of the epidermis during the process of gastrulation[13]. The mesoderm gives rise to the dermis and is essential for developing epidermal structures, such as hair follicles[14]. The development of the epidermis is regulated through interactions between the Notch and Wnt signaling pathways. It also involves β-catenin, Lef1, and Notch peptides [15].

By third gestational week, the skin is composed of a single layer of undifferentiated ectodermal cells filled with glycogen[16]. By the fifth week, these cells differentiate into two distinct layers: outer surface periderm and the inner basal stratum germinativum[17]. By the tenth week, a new layer called the stratum intermedium emerges. This layer forms between the periderm and the stratum germinativum. By the nineteenth week, the periderm flattens and multiple layers of intermediate cells develop. Keratohyaline granules are observed with the stratum intermedium around 23 weeks. Near the stratum corneum, keratinocytes are nearly fully grown, while periderm cells shed.

ANATOMY AND HISTOLOGY OF THE SKIN

A thorough comprehension of histology (Fig. 1) is essential for the diagnosis of skin diseases. The skin comprises two primary layers, the epidermis and the dermis. These layers are distinct in both histological and anatomical aspects, and are functionally linked. Beneath the dermis lies subcutaneous adipose tissue.

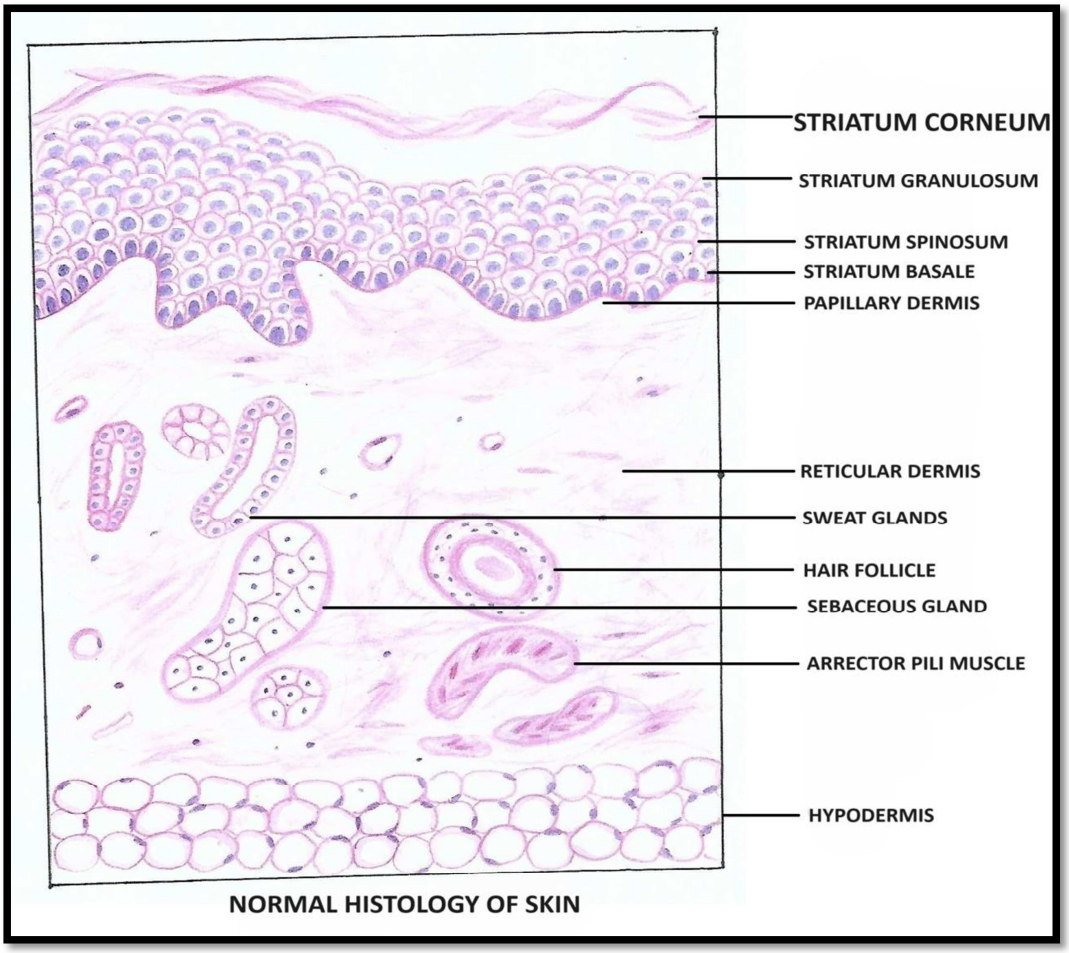


Figure 1: Normal histology of skin

EPIDERMIS

The epidermis is made up of stratified squamous epithelial cells referred to as keratinocytes. The epidermis also contains Langerhans cells. It includes neuroendocrine cells (Merkel cells) and melanocytes as well. Additionally, unmyelinated axons are present in the epidermis.. The epidermis consists of five layers (Fig. 2)[18]:

1. Stratum basale
2. Stratum spinosum
3. Stratum granulosum
4. Stratum lucidum
5. Stratum corneum

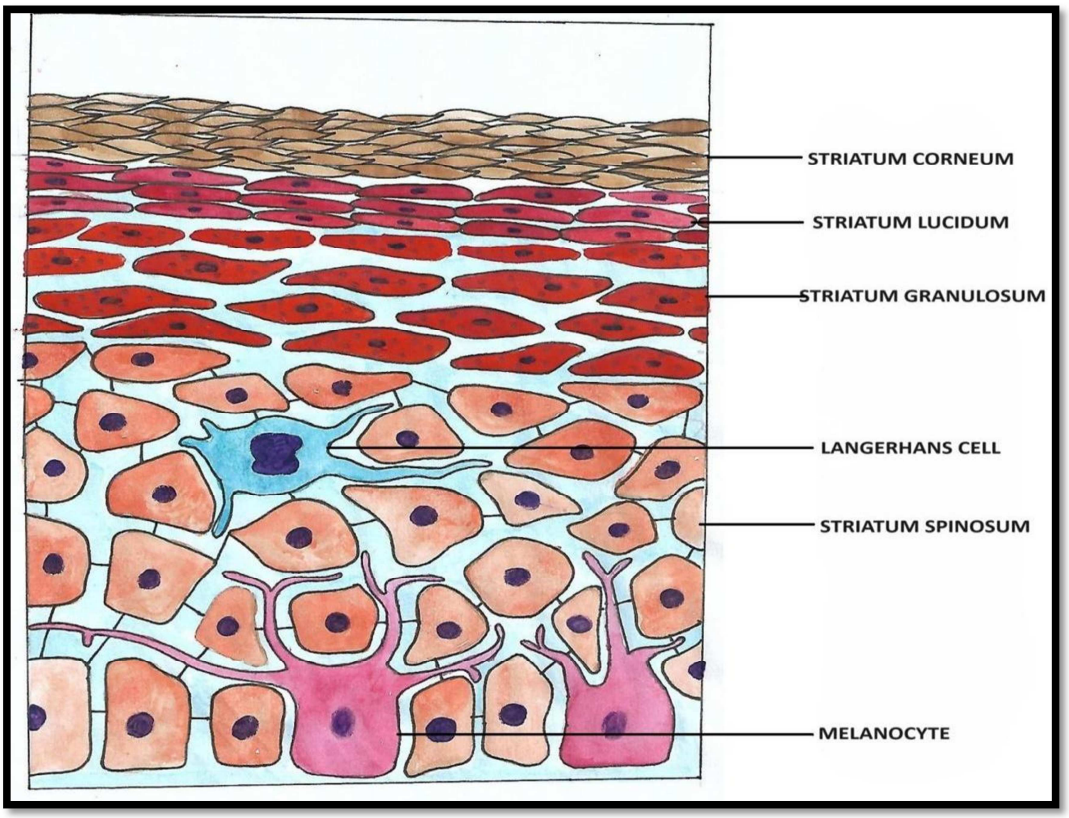


Figure 2: Layers of Epidermis

STRATUM BASALE

The stratum basale, also known as the basal cell layer, is the lowest part of the epidermis. This layer consists of a single row of columnar cells characterized by round to oval nuclei and basophilic cytoplasm. Melanin pigmentation is provided by adjacent melanocytes[18]. The basal layer is supported by a basement membrane, which divides the epidermis and dermis. This layer aids in regenerating other epidermal layers through continuous mitotic division. Desmosomes connect basal cells to upper squamous cells, while hemidesmosomes connect basal cells to the underlying basement membrane.

STRATUM SPINOSUM

The stratum spinosum, or prickle cell layer, is directly above the basal layer and comprises polyhedral keratinocytes in four to six rows. This layer contains cells with large, pale-stained nuclei and prominent nucleoli, indicating active synthesis of protein. Within their cytoplasm, intermediate filaments known as cytokeratins aggregate to form tonofibrils, that provide tensile strength and help resist abrasion to the epidermis[19].

STRATUM GRANULOSUM

The stratum granulosum, also known as the granular cell layer, comprises three to five layers of flattened keratinocytes and located just above the stratum spinosum. This layer is characterized by the presence of dense keratohyaline granules within its cells. As keratinocytes in the granular layer moves towards the skin's surface, they gradually lose their nuclei and cytoplasm[18].

STRATUM LUCIDUM

The stratum lucidum is composed of several layers of closely packed, flattened cells. These cells lack nuclei and organelles. This layer is most prominent on the palms and soles, where keratin filaments are densely packed within the cells[18].

STRATUM CORNEUM

The stratum corneum, representing the outermost layer of the epidermis, consists of dead cells that are flattened, devoid of nuclei, and filled with soft keratin. These surface keratinized cells are continuously shed or desquamated. They are regenerated by new cells from the basal layer[18].

VARIATIONS IN EPIDERMIS IN VARIOUS SITES

Human skin can be classified into two types: glabrous (non-hairy) skin and hair-bearing skin. This glabrous skin is characterized by a thick stratum corneum, contains encapsulated sensory organs, and lacks hair follicles and sebaceous glands. In contrast, hair-bearing skin consist of hair follicles and sebaceous glands but does not contain encapsulated sensory organs. The size, shape, and density of hair follicles differ depending on the body site. Hair-bearing skin contains eccrine sweat glands, apocrine glands, and sebaceous glands influencing skin surface lipid composition. Elastic fibers in the dermis vary in size and organization, and cutaneous blood supply differs between distensible and rigid skin[20].

The dermis in the lower back is exceptionally thick and contains wide, parallel bundles of collagen fibers. In the nasal skin, there are prominent sebaceous glands within the dermis that open directly onto the skin surface. The skin on the feet, palms, and the ventral sides of fingers and toes features a thicker stratum corneum. It also has

an unique epidermal ridge pattern. Additionally, the dermis in these area is fairly dense[20].

The skin of the scalp contains a large number of terminal hair follicles, with many of their bulbs situated within the subcutaneous fat layer. The axilla skin contains apocrine glands in the lower dermis. The areola's skin has numerous smooth muscle fibers, occasionally with lactiferous ducts. The lip's outer skin contains skeletal muscle fibers and keratinizing stratified squamous epithelium. The auricular cartilage of the ear is covered by a layer of skin that includes vellus hairs and a comparatively thin dermis[20].

DERMIS

The dermis, situated just beneath the epidermis, consists of dense connective tissue fibers. This layer includes cellular components such as fibroblasts and dermal dendritic cells, as well as components like macrophages and mast cells[18]. Collagen and elastic fibers constitute the extracellular components found in the dermis. Papillary dermis is a pale-stained thin layer of connective tissue located just beneath the epidermis. Dermal papillae are structures formed when the papillary dermis projects into the basement membrane of the epidermis. The reticular dermis, which accounts for the majority of the dermis, primarily consists of dense connective tissue. The dermal connective tissue is richly supplied with blood vessels, lymphatic vessels, and nerves.

The dermis contains various skin structures, including hair follicles, sebaceous glands, apocrine glands, and eccrine sweat glands.

PSORIASIS:

"Psoriasis" term originates from the Greek word "psora," which means "itch." Psoriasis can present with various clinical symptoms and is characterized by chronic, recurring lesions[21]. It affects 1-3% of the global population and causes significant morbidity. Psoriasis can affect individuals of any age, with an incidence appearing to be equal between men and women[22].

GENETIC BASIS OF PSORIASIS:

Genetic predisposition plays a crucial role in the manifestation of psoriasis. Multiple genes are implicated in the complex molecular genetics of psoriasis. Psoriasis susceptibility loci have been associated with seven main loci. Multiple studies have shown that the main susceptibility locus, PSORS1, on chromosome 6p21, is present in most populations[23]. Furthermore, chromosomes 1p (PSORS7)[24] and 1q (PSORS4)[25] have been linked to an increased susceptibility to psoriasis. Similarly, chromosomes 3q (PSORS5)[26] and 4q (PSORS3)[27] have also been associated with the condition. Additionally, chromosomes 17q (PSORS2)[28] and 19p (PSORS6)[29] contribute to the heightened risk of developing psoriasis. There is a proven familial clustering of patients with psoriasis[30], with childhood psoriasis showing a higher familial incidence than adult-onset psoriasis. Certain HLA antigens, such as HLA A1, B17, and Cw6[31], are linked to psoriasis vulgaris. In South India, associations with DR7 and HLA Bw57 have been observed[32], while in North India, the predominant allele with a higher frequency is HLA Cw0602[33].

TRIGGER FACTORS:

Multiple modifiable risk factors can increase an individual's likelihood of developing psoriasis. These factors can also exacerbate existing cases of the condition. Smoking, alcohol consumption, and obesity are known to increase the risk of psoriasis. Additionally, dietary habits, various infections, medications, and stress also play crucial roles. The exact mechanisms that aggravate psoriatic skin lesions remain unknown[34].

Infections caused by bacteria and viruses have been recognized as triggers that can initiate or exacerbate psoriasis. Additionally, certain medications, such as beta blockers and antimalarial drugs, along with lithium, tetracyclines, and non-steroidal anti-inflammatory drugs (NSAIDs), have been associated with the development or worsening of psoriasis. The withdrawal of steroids has also been identified as a factor contributing to psoriasis exacerbation. Psychological stress can also trigger psoriasis.

CLINICAL FEATURES OF PSORIASIS:

- Well-defined erythematous plaques: These can be dispersed throughout the trunk and limbs, varying in size and number[35].
- Silvery white scales: The affected skin area is often covered with silvery white scales (Fig. 3)[35].
- Severe erythema: The affected skin may exhibit significant redness, especially in flexures. Erythrodermic psoriasis, affecting more than 90% of the body surface, frequently presents with widespread erythema.
- Burning or itching sensation: Patients often experience a burning or itching sensation in the affected areas. Pustules are frequently observed in

palmoplantar pustulosis, a chronic condition characterized by deep-seated yellowish-sterile pustules.

- Nail changes: Psoriasis can cause nail thickening, pitting, discoloration, and detachment from the nail bed[35].

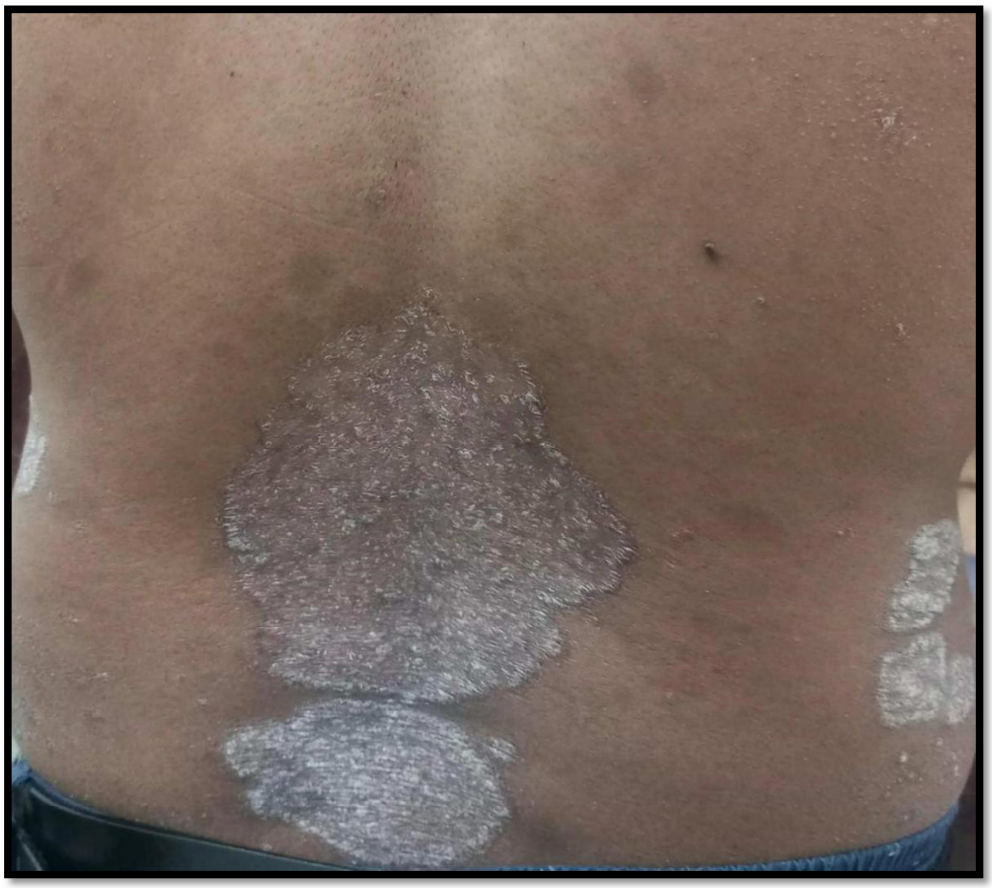


Figure 3: Clinical presentation of psoriasis showing well demarcated plaques with silvery scales.

PATHOGENESIS OF PSORIASIS:

Until the late 1970s, psoriasis was primarily considered to be caused by defective epidermal keratinocytes[36]. Further clinical and fundamental research has revealed that the activation of lymphocytes is crucial in the development of psoriasis. In psoriasis cases, effective treatment with cyclosporine A, a medication known for its immunosuppressive effects by inhibiting proliferation of T-cells and cytokine production, provided the first clinical evidence. This evidence highlighted the significant role that T-lymphocytes play in psoriasis pathogenesis[37].

Primary pathogenic aspects of psoriasis, in order of occurrence, are:

A. Endothelial activation and vascular changes

B. Lymphocyte recruitment

C. Keratinocyte-lymphocyte interactions

D. Amplification of inflammatory mechanisms

E. Keratinocyte proliferation[38]

The initial two features are linked to the typical process of wound healing. The infiltration of inflammatory cells is driven by various cytokines, immunological, and inflammatory modulators released by keratinocytes[39]. Due to the similarity between the amino acid sequences of streptococcal M-protein and keratin 17, an epitope on keratin 17 might become a target for autoreactive lymphocytes in psoriasis [40]. CD4+ and CD8+ T-lymphocytes are observed in the papillary dermis of patients with psoriatic lesions. They are also found in the epidermis, contributing to the inflammatory processes characteristic of psoriasis.

When lymphocytes are activated, they initiate the production of various cytokines. These include interleukin-2 (IL-2), which is crucial for T-cell proliferation and differentiation. Additionally, activated lymphocytes produce tumor necrosis factor-alpha (TNF-α) and gamma-interferon (IFN-γ), which play significant roles in immune responses and inflammation regulation[41]. Specifically, they induce keratinocytes to secrete interleukin-8 (IL-8). IL-8 is known for its potent chemoattractant properties, drawing T-lymphocytes and neutrophils to the site of inflammation. This recruitment contributes to the formation of Munro's microabscesses.

Recent studies suggest that infections involving foreign antigens initiate a cascade of events beginning with the activation of dendritic cells and macrophages. These immune cells respond by releasing IL-23 and IL-1β. They also release other pro-inflammatory cytokines, which collectively stimulate dermal T cells to produce significant levels of IL-17. This IL-17 production enhances traditional acquired immune responses. Keratinocytes are activated by IL-17, IL-22, and TNF, leading to hyperproliferation[42].

ANGIOGENESIS:

Angiogenesis is the formation of new blood vessels from existing capillaries, primarily occurs during embryonic development and is relatively rare in adult tissues. In psoriasis, angiogenesis functions both as a cofactor and an inducer of the disease. Alterations in the superficial microvessels of psoriatic skin lesions promote angiogenesis. Increased levels of proangiogenic cytokines such as angiopoietins, VEGF, TNF-α, hypoxia-inducible factor (HIF), and IL-8 are enhanced in the psoriatic lesional skin[43].

The development of psoriatic skin lesions is initiated by neoangiogenesis in the superficial dermis. Capillaries shows significant dilation, increased tortuosity, permeability, and elongation in the paillary dermis[44]. These morphological alterations occur before the appearance of epidermal hyperplasia[45]. In the early stages of psoriatic lesions, microvascular changes are linked to increased blood flow in the surrounding perilesional skin[46]. The growth of superficial dermal microvessels observed in psoriatic skin lesions is likely driven by angiogenesis.

HISTOPATHOLOGICAL FEATURES OF PSORIASIS (Fig. 4):

In early psoriatic lesions, the blood vessels in the papillary dermis become elongated and dilated, accompanied by edema and infiltration of lymphocytes around these vessels. These vessels appear tortuous and dilated, with neutrophils present within their lumens. Inflammatory infiltrate also extends into the lower epidermis, leading to the development of spongiosis. As the condition progresses, localized changes in the epidermis, such as parakeratosis and Munro's microabscesses, indicate abnormal keratinization and inflammatory responses. These are often accompanied by hypogranulosis and acanthosis, reflecting altered maturation and thickening of the skin layers. Additionally, elongation of the rete ridges, thinning of the suprapapillary plate, and the presence of spongiform pustules of Kogoj denote further structural and cellular abnormalities in the affected areas.[47].

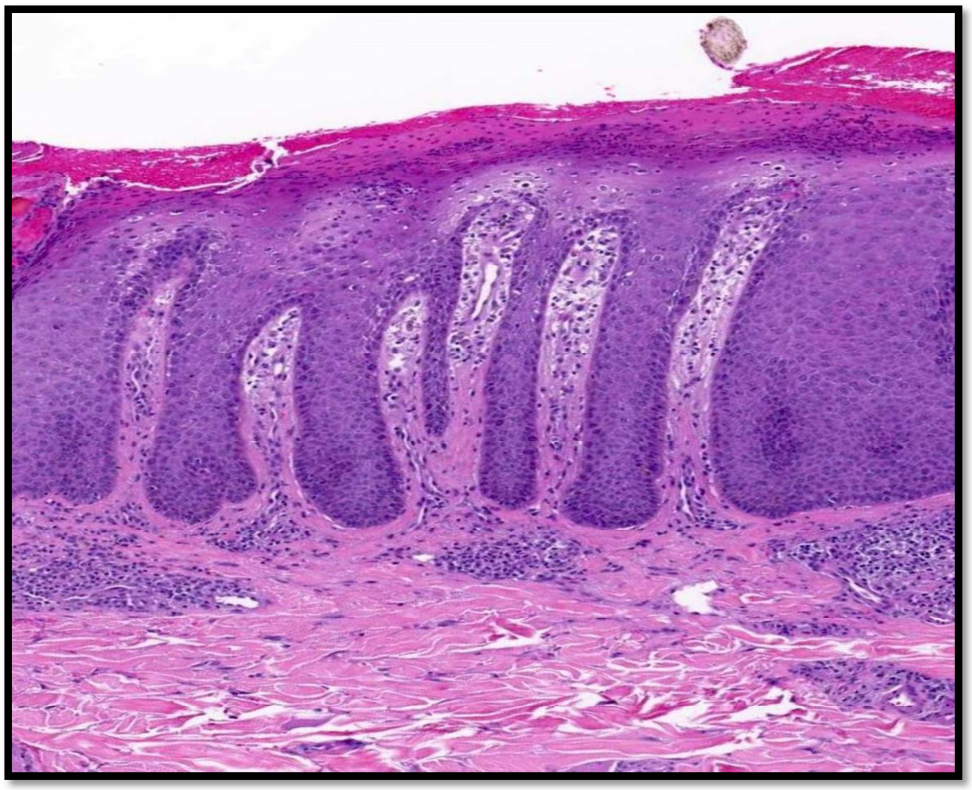


Figure 4: Psoriasis showing parakeratosis, elongation of the rete ridges, dilatation of blood vessels in the papillary dermis and lymphocytic infiltration around the vessels H & E.

ROLE OF IMMUNOHISTOCHEMISTRY IN PSORIASIS:

Dermatologists and pathologists frequently conduct studies to identify additional criteria that are crucial for understanding the progression of psoriasis and evaluating the effectiveness of treatment strategies using immunohistochemistry. The expression of IHC markers involved in cell proliferation, apoptosis, vascularization and inflammation varies according to the type of psoriasis and the severity of the disease. Among the significant immunohistochemical markers investigated are CD3, CD34, CD68, VEGF, Ki-67, P63, and S100[48-52].

CD34:

CD34 is a 110-kDa transmembrane glycoprotein that is predominantly located in stem cells and endothelial cells. It functions as a molecular "Teflon," preventing the adhesion of mast cells, eosinophils, and dendritic cell precursors, and thereby facilitating the opening of arterial lumens [53]. The CD34 immunomarker stains blood vessel endothelium. The intensity of CD34 staining is assessed by counting capillaries in the 3-5 most heavily vascularized locations selected at a magnification of less than 40X. Individual blood vessels are characterized as single endothelial cells or clusters of endothelial cells, which may or may not contain lumens. They are classified as moderate, mild, or severe based on the number of vessels stained[54]. Normal skin sections are used as an internal control to study vascular changes.

ROLE OF CD34 IMMUNOHISTOCHEMISTRY IN PSORIASIS:

CD34 is a marker of angiogenesis and is suggested to play both a cofactor and an inducer role in the development of psoriasis. Several studies have shown that CD34 expression is significantly elevated in lesions of psoriasis compared to normal skin. Emerging immunodeficient principles have led to novel therapeutic approaches for psoriasis, targeting both chemical mediators and immune cells to induce clinical changes in psoriatic plaques. Tumor Necrosis Factor (TNF) is a key cytokine involved in the pathogenesis of psoriasis. It significantly influences hyperproliferation of keratinocytes, regulates endothelial cells, and is essential for the recruitment and memory T cell function[55].

The CD34 immunomarker is utilized to stain the endothelium of blood vessels, enabling the determination of the Microvessel Density (MVD) index for the quantification of angiogenesis. Microvessels in the dermis are observed by scanning

sections at low power (× 100) magnification to identify the area with the highest number of distinctly CD34-immunostained microvessels, referred to as the "hotspot." The counting of microvessels and measurement of their calibre are conducted under high-power magnification (× 400) across five fields within the hotspot area. This approach aims to identify the sections of the papillary dermis with the most significant vascularization. The number of vessels in these high-density locations is counted at greater magnification (× 400), and the mean number is then calculated and graded[56].

MATERIALS AND METHODS

This is a hospital based observational study in which 35 cases of histopathologically diagnosed psoriasis cases were studied. This study was done for a period of one year, from 1st January 2023 to 31st December 2023 at KLE'S Dr. Prabhakar kore Hospital and MRC, Belagavi.

Sample Size: 70: 35 psoriasis cases and 35 controls:

(Skin tissue taken from the surgical margins of amputated limbs and skin from a mastectomy were used as controls because they had no particular pathology and no skin disease.)

Sampling technique: Non random sampling.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human Subjects Research

Inclusion Criteria:

Clinically newly diagnosed cases with confirmed histological findings of psoriasis in the age group between 18-70 years.

Exclusion Criteria:

- Clinically newly diagnosed cases of psoriasis in the age group less than 18 years and above 70 years of age.
- Cases previously diagnosed with psoriasis and currently receiving treatment.
- Other inflammatory skin diseases

Consent was taken from all the cases and the clinical data was collected from the dermatology department. The specimens fixed in 10% formalin were received at Department of Pathology.

Gross description of the tissue size was obtained. Also, the presence or absence of epidermis and hair, its color, and any alterations to the epidermal surface were mentioned. The tissue was obtained, then bisected. It was processed and embedded in paraffin blocks. Consecutive ribbon sections of 3-4 micrometers thick were prepared from the embedded tissue and affixed on glass slide. These sections underwent hematoxylin and eosin staining. Subsequently, the slides were mounted and accurately labeled. Finally, the slides were meticulously examined under a microscope.

All the cases reported histopathologically as psoriasis and the controls were stained with CD34 immunohistochemical marker to study the dermal vascular density and micro vascular calibre in the papillary dermis.

In this study, the CD34 immunomarker was used to stain the blood vessel endothelium. The MVD index for the quantification of angiogenesis was determined. The dermal microvessels were initially observed at low power magnification (X100). The area with the highest concentration of distinctly CD34 immunostained microvessels, referred to as the "hotspot," was identified. Microvessel counting and calibre measurements were then performed under high power magnification (X400) across five fields within the hotspot area. At a higher magnification (X400), the number of vessels was counted in five high-density locations, and the mean was then graded.

The scoring pattern(Table 1) is tabulated as follows[55]

Table 1: Scoring and grading pattern of MVD

MVD (number of vessels in 5 highly vascularized areas in x400)	GRADE
Less than 4 vessels/400x	0
4-10 vessels/400x	1+
11-20 vessels/400x	2+
>21vessels/400x	3+

Morphometric analysis of the CD34 immunostained sections was performed using a computerized digital photomicrograph system (JENOPTIK GRYPHAX V 1.1.10.6). The image analysis software was precisely calibrated with the standard scale. For each sample, morphometric analysis was performed on five high power fields (X400) in the hotspot area. In determining the mean microvessel caliber (MVC), both circular and elliptical microvessels were measured, using the minor axis of elliptical vessels as their caliber. The software was utilized to measure the microvessel caliber, which was subsequently graded as follows. The scoring pattern(Table 2) is given here[56].

Table 2: Scoring and grading pattern of MVC

Mean MVC (sum of diameter of all vessels in micrometer and taken its average)	GRADE
Less than 5 micrometer	1
5.1-10 micrometer	2
More than 10.1 micrometer	3

The correlation between various clinicopathologic parameters along with histopathological features as well as IHC were studied and correlated with age, gender, MVD, and MVC.

Statistical Analysis

Statistical analysis was done using Microsoft Excel and SPSS V23 software. Categorical variables were reported using frequency and percentage along with graphs and charts. The grading of MVD and MVC in psoriasis cases in relation to control was assessed using the chi square test. A "p" value of less than 0.05 was considered statistically significant.

OBSERVATION AND RESULTS

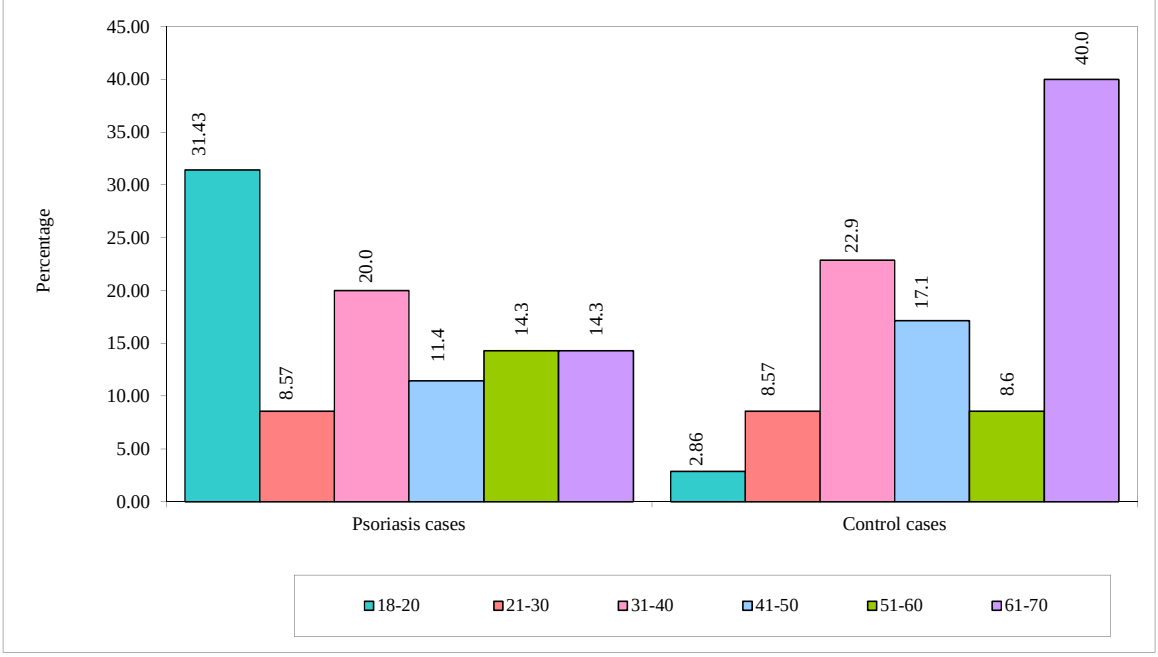
In our study 70 samples were collected and analyzed, comprising 35 psoriasis cases and 35 control samples of normal and healthy skin. Histopathological evaluation, along with immunohistochemical evaluations, was performed on all samples. The data were collected, and entered into a Microsoft Excel spreadsheet for further analysis as described below.

Table 3: Age distribution in study group (n= 35 cases & 35 controls)

Age	Psoriasis (n=35)	%	Controls (n=35)	%
18-20	11	31.43	1	2.86
21-30	3	8.57	3	8.57
31-40	7	20.00	8	22.86
41-50	4	11.43	6	17.14
51-60	5	14.29	3	8.57
61-70	5	14.29	14	40.00
Total	35	100.00	35	100.00

*mean ± SD = 43.6714 ± 17.2337

Graph 1: Age distribution in study group.

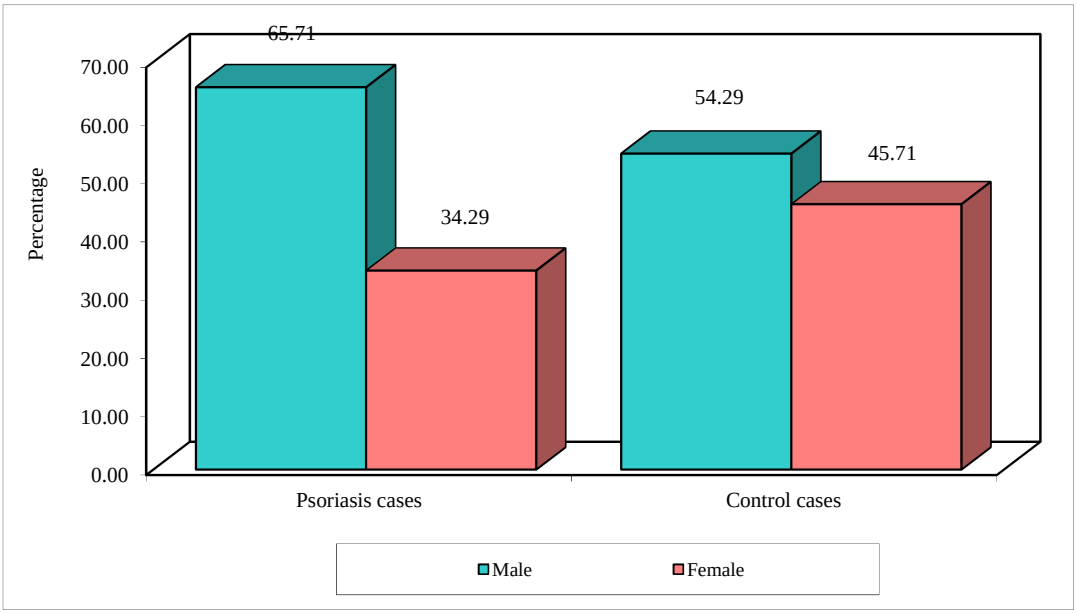


Among the cases maximum number of psoriasis patients belonged to 18 to 20 years of age (11 cases, 31.43%) and majority of the controls belonged to 61 – 70 years(14 cases, 27.14%). The mean ± standared deviation (SD) of the study population was 43.6714 ± 17.2337. According to the aforementioned age, psoriasis peaks between the second and fifth decades of life. Distribution is shown in Table no: 3 and in Graph no:1

Table 4: Gender distribution in study group (n= 35 cases + 35 controls)

Gender	Psoriasis (n=35)	%	Control (n=35)	%
Male	23	65.71	19	54.29
Female	12	34.29	16	45.71
Total	35	100.00	35	100.00

Graph 2: Gender distribution in study group.

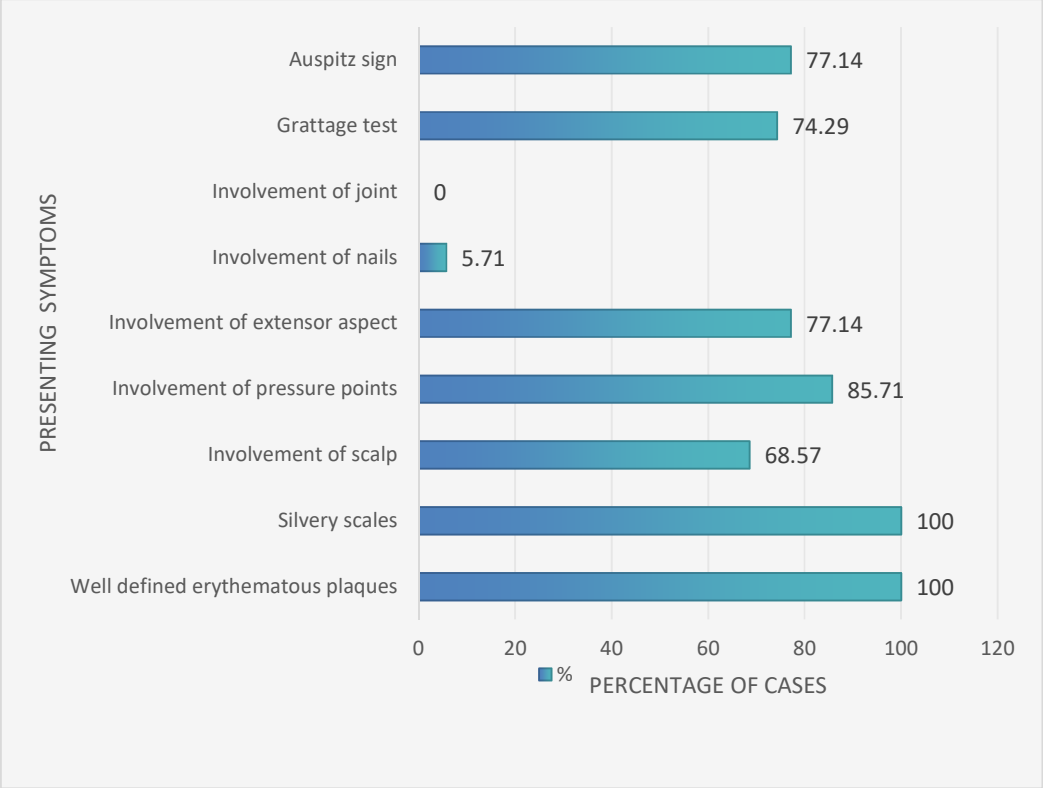


In the study, the majority of psoriasis cases were male, with 23 cases (65.71%), resulting in a male to female ratio of 1.916:1, showing a male predominance. Similarly, most control samples were also male, with 19 cases (54.29%), leading to a male to female ratio of 1.187:1. These variables revealed that there is a male predominance in psoriasis. Distribution is shown in Table no:4 and in Graph no:2

Table 5: Presenting symptoms of psoriasis cases.

Presenting symptom	Number of patients (n=35)	%
Well defined erythematous plaques	35	100
Silvery scales	35	100
Involvement of scalp	24	68.57
Involvement of pressure points	30	85.71
Involvement of extensor aspect	27	77.14
Involvement of nails	02	5.71
Involvement of joint	00	0
Grattage test	26	74.29
Auspitz sign	27	77.14

Graph 3: Presenting symptoms of psoriasis cases



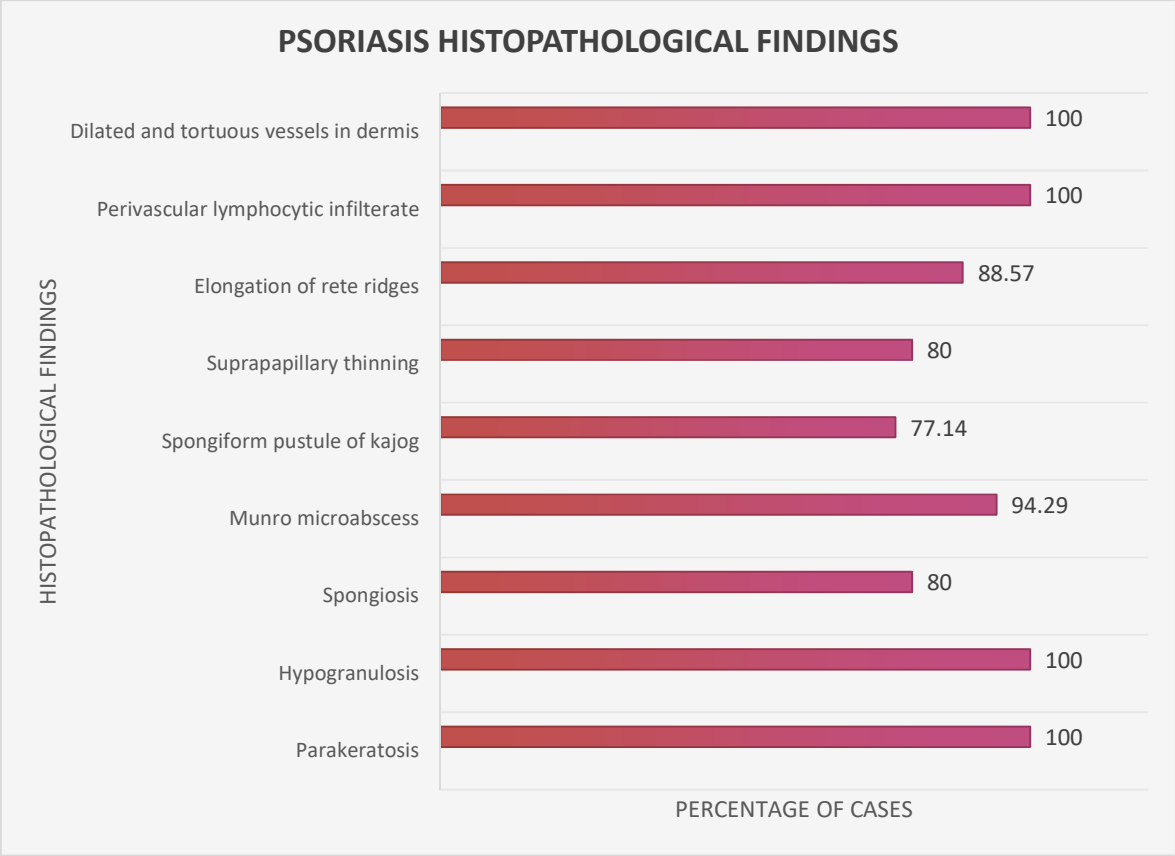
Clinical presentation: Well defined erythematous plaques and Silvery scales were seen in all cases (100%) of psoriasis. Involvement of pressure points, extensor aspect of body and involvement of scalp were seen in, 30 (85.71%), 27 (77.14%) and 24 (68.57%) cases respectively. Involvement of nails was seen only in 2 cases (5.71%). Grattage test was positive in 26 (74.29%) cases. Auspitz sign came positive in 27 (77.14%) cases. None of the psoriasis cases showed involvement of joints. The above clinical scenario revealed that Auspitz sign, involvement of the extensor aspect, and pressure points, in addition to well-defined erythematous plaques and silvery scales, are extremely reliable presenting symptoms of psoriasis for clinical diagnosis. Distribution is mentioned in Table no:5 and in Graph no: 3

Table 6: Histopathological pattern of Psoriasis

Histo-pathological features	Psoriasis (n=35)	%	Control (n=35)	%	Chi-square	p-value
Parakeratosis	35	100.00	0	0.00	70.0000	0.0001*
Hypogranulosis	35	100.00	0	0.00	70.0000	0.0001*
Spongiosis	28	80.00	0	0.00	46.6670	0.0001*
Munro microabscess	33	94.29	0	0.00	62.4320	0.0001*
Spongiform pustule of kajog	27	77.14	0	0.00	43.9530	0.0001*
Suprapapillary thinning	28	80.00	0	0.00	46.6670	0.0001*
Elongation of rete ridges	31	88.57	0	0.00	55.6410	0.0001*
Perivascular lymphocytic infiltrate	35	100.00	0	0.00	70.0000	0.0001*
Dilated and tortuous vessels in dermis	35	100.00	0	0.00	70.0000	0.0001*

*$p<0.05$

Graph 4: Histopathological pattern of Psoriasis



Based on histopathological findings, 35 cases of Psoriasis (100%) showed parakeratosis(Fig:6,9), hypogranulosis(Fig:8), perivascular lymphocytic infiltrate (Fig:10)and dilated and tortuous blood vessels(Fig:8).None of the controls showed parakeratosis, hypogranulosis, perivascular lymphocytic infiltrate and dilated and tortuous blood vessels. These histopathological findings were statistically (p value = 0.001) significant for psoriasis. 33 cases of Psoriasis (94.29%) cases showed munro microabscess and, 0 control showed munro micro abscess(Fig:9) which was statistically significant (p value = 0.001). 31 cases of Psoriasis (88.57%) showed elongation of rete ridges(Fig:6,7) and whereas 0 control showed elongation of rete ridges also showed statistical significance (p value = 0.001). 28 cases of Psoriasis (80%) showed spongiosis(Fig:7) and suprapapillary thinning(Fig:6,7) and, 0 control

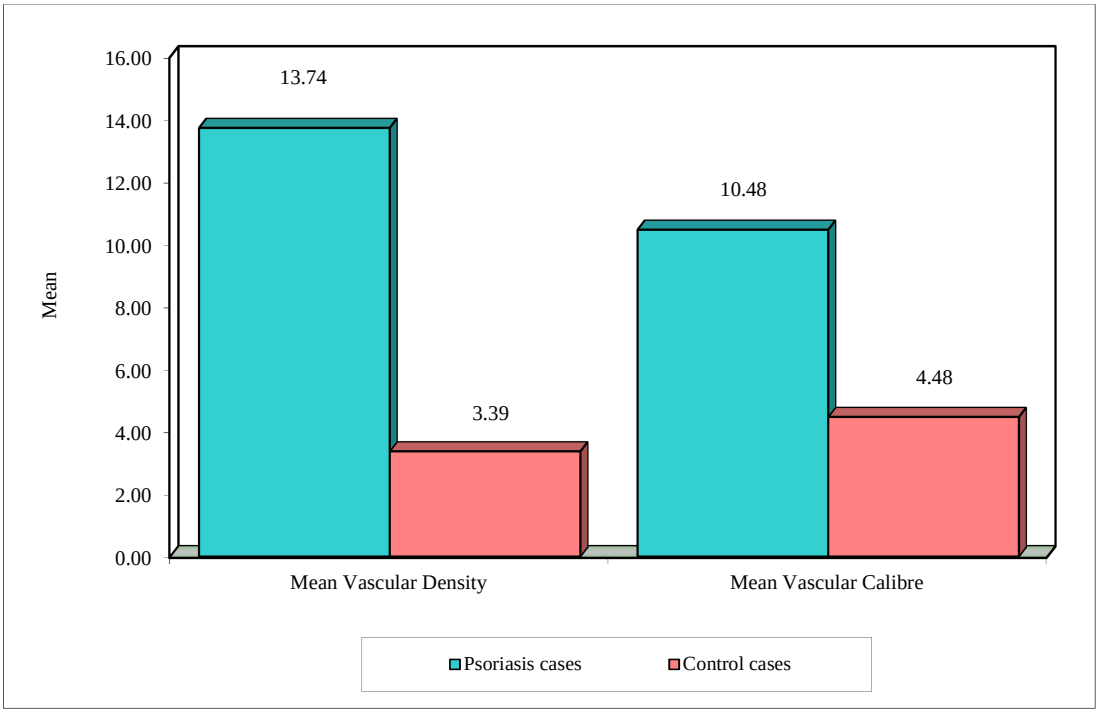
showed spongiosis and suprapapillary thinning which were statistically significant (p value = 0.001). 27 cases of Psoriasis (77.14%) cases showed spongiform pustule of kajog and 0 control showed spongiform pustule of kajog which was statistically highly significant (p value = 0.001). All the histological findings distribution is shown in Table no:6 and Graph no:4

Table 7: Comparison of MVD and MVC in the study group

Variable	Cases	Mean	SD	SE	t-value	P-value
Mean Vascular Density	Psoriasis cases (n=35)	13.74	3.07	0.52	19.7699	0.0001*
	Control cases (n=35)	3.39	0.39	0.07		
Mean Vascular Calibre	Psoriasis cases (n=35)	10.48	2.20	0.37	14.4978	0.0001*
	Control cases (n=35)	4.48	1.08	0.18		

*p<0.05

Graph 5: Comparison of MVD and MVC in the study group



Comparison of psoriasis cases and control cases for MVD and MVC performed by independent t test,

MVD of psoriasis cases revealed a mean value of 13.74±3.07SD and controls with a mean value of 3.39±0.39SD which was statistically highly significant (p value = 0.001).

MVC in psoriasis cases showed a mean value of 10.48±2.20 SD and controls with a mean value of 4.48±1.08SD which was statistically highly significant (p value = 0.001).

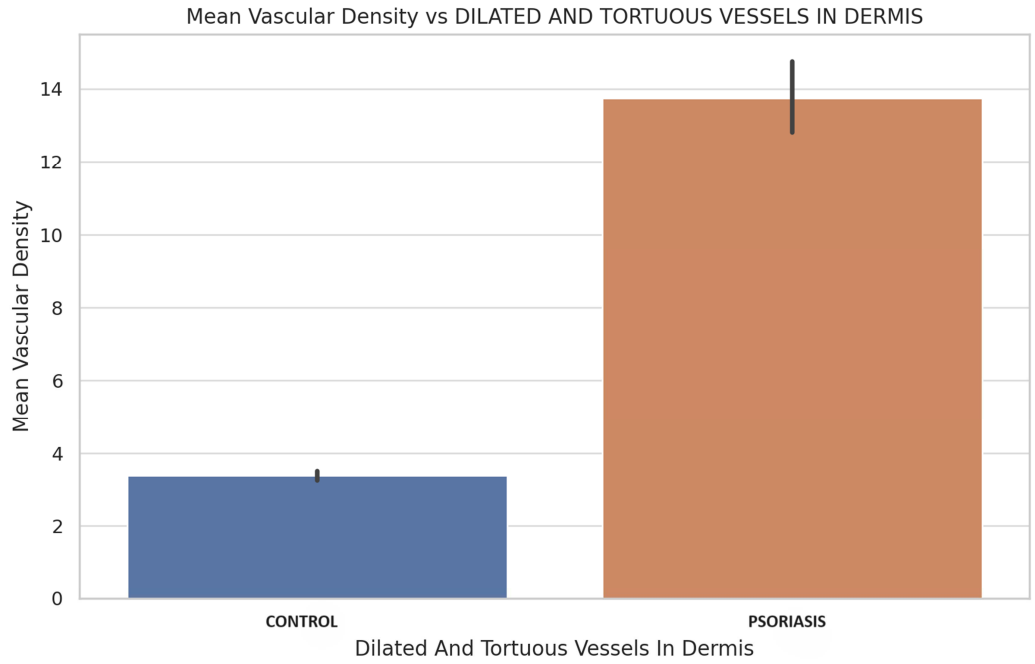
The above study revealed that the values (Table 7 and Graph 5) of MVD and MVC were highly reliable to explain the vascular changes present in cases of psoriasis when compared with normal skin.

Table 8: Comparison of Histopathological features VS MVD

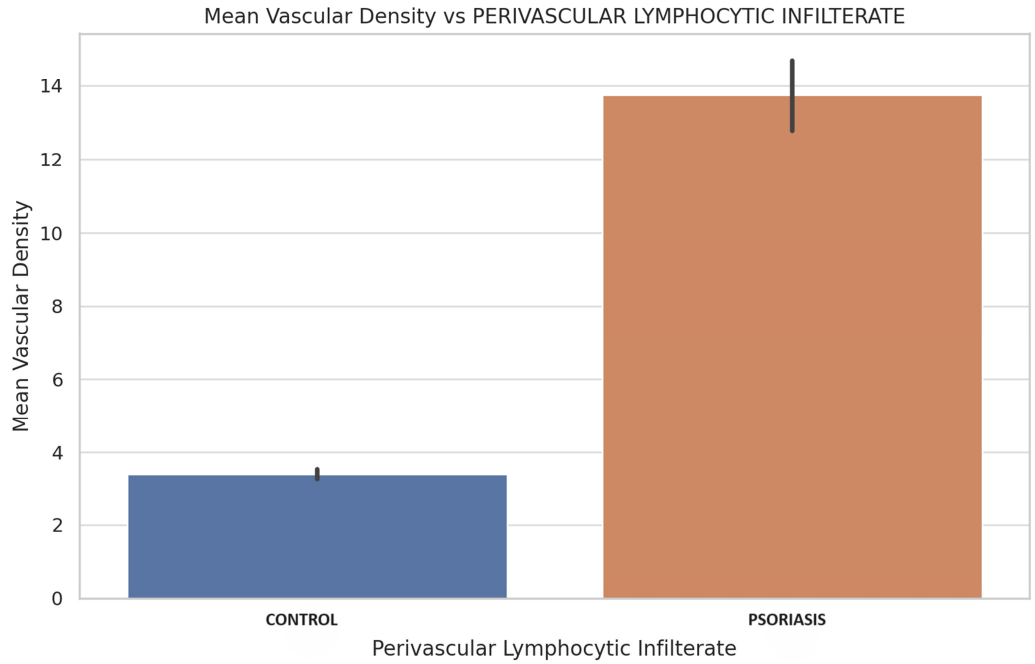
HISTOPATHOLOGICAL FEATURES	Psoriasis (n=35)	Control (n=35)	Chi Square value	P value
PARAKERATOSIS	35	0	66.06	0.0001*
HYPOGRANULOSIS	35	0	66.06	0.0001*
SPONGIOSIS	28	0	43.39	0.0001*
MUNRO MICROABSCESS	33	0	58.71	0.0001*
SPONGIFORM PUSTULE OF KAJOG	27	0	40.76	0.0001*
SUPRAPAPILLARY THINNING	28	0	43.39	0.0001*
ELONGATION OF RETE RIDGES	31	0	52.11	0.0001*
PERIVASCULAR LYMPHOCYTIC INFILTRATE	35	0	66.06	0.0001*
DILATED AND TORTUOUS VESSELS IN DERMIS	35	0	66.06	0.0001*

*$p<0.05$

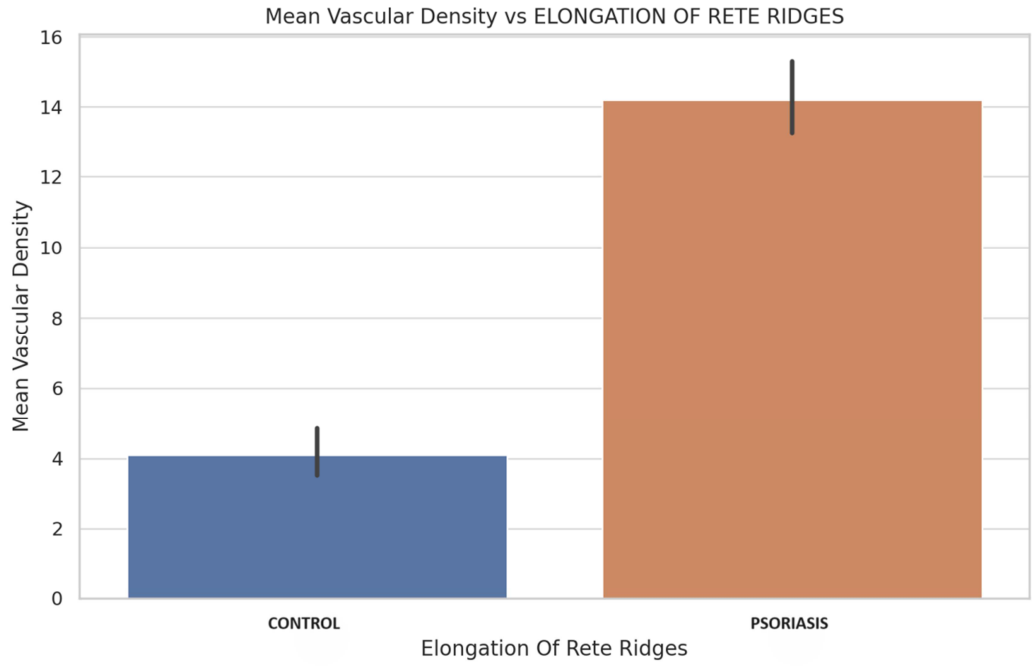
Graph 6: Comparison of MVD with Dilated and tortous vessels in dermis



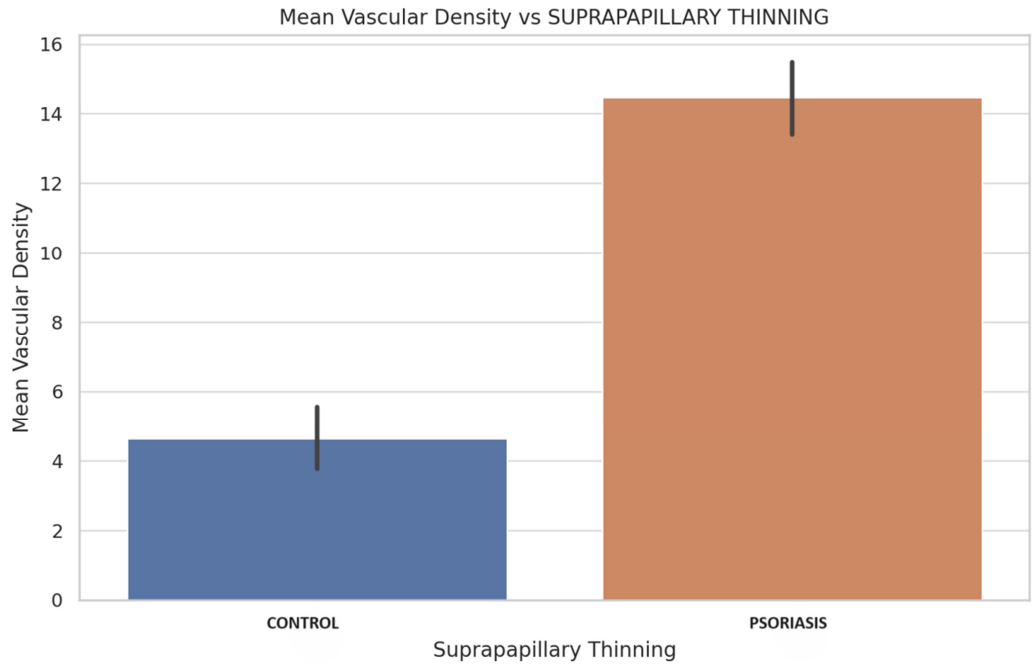
Graph 7 : Comparison of MVD with Perivascular lymphocytic infiltrate



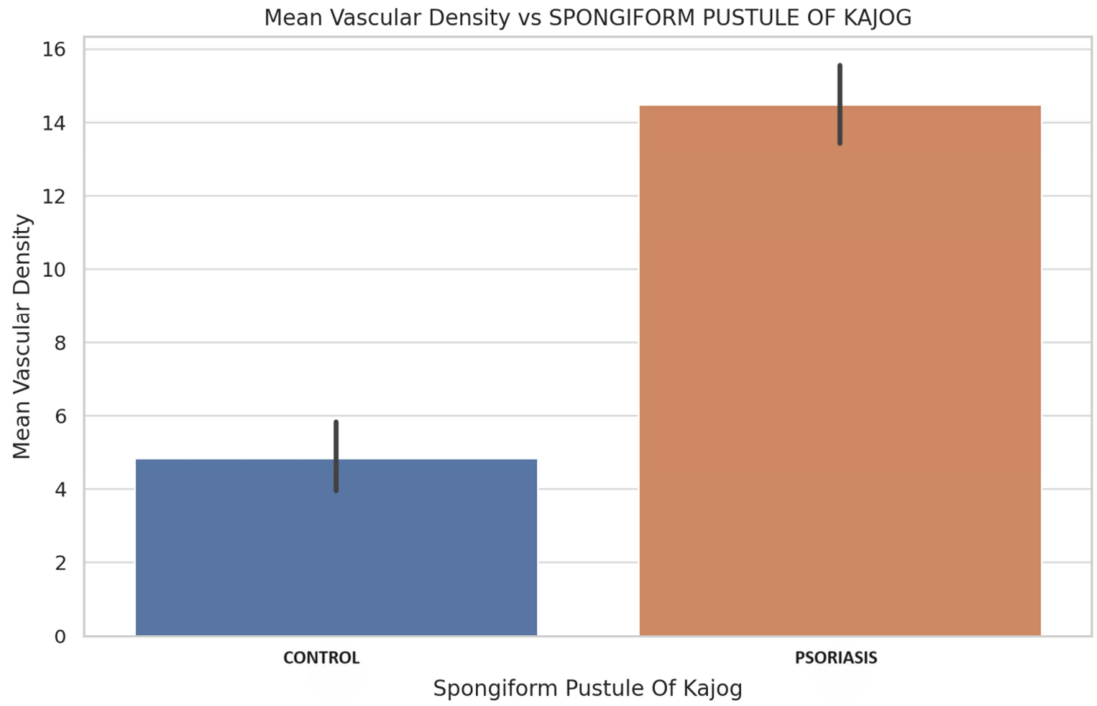
Graph 8 : Comparison of MVD with Elongation of rete ridges



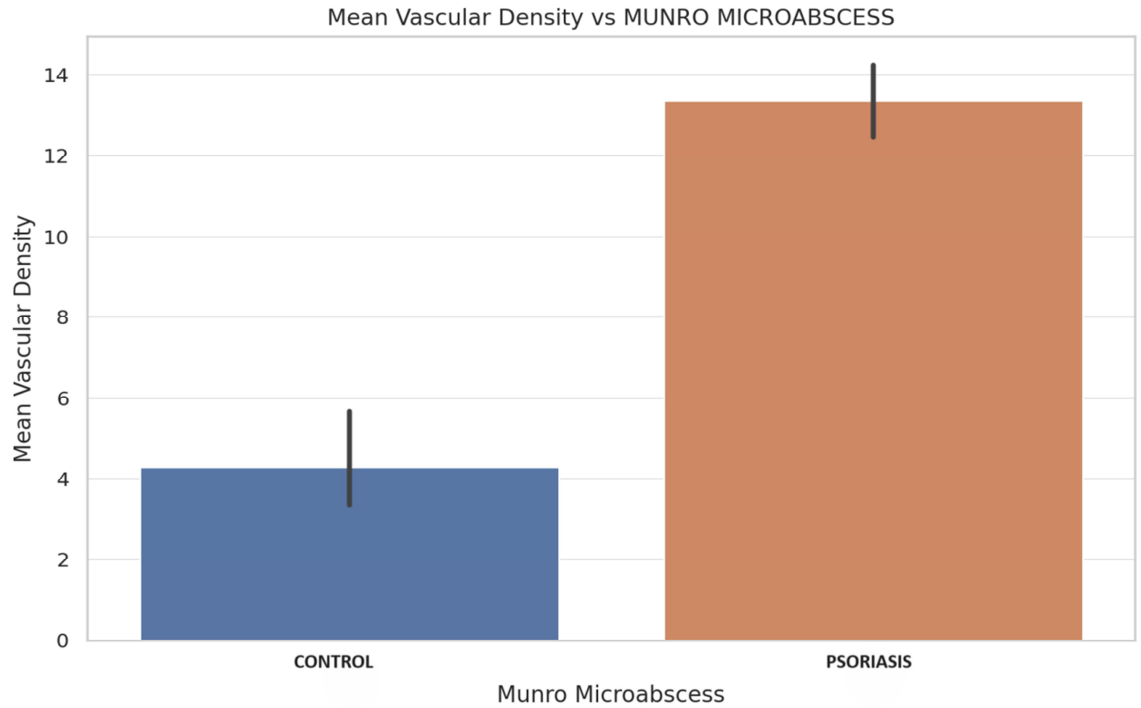
Graph 9 : Comparison of MVD with Suprapapillary thinning



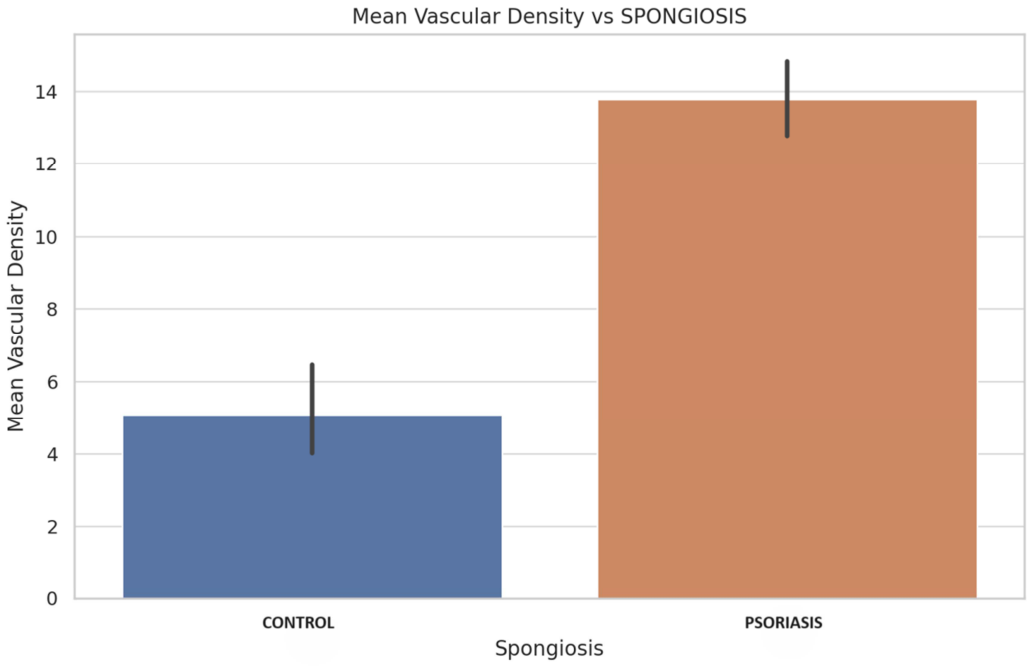
Graph 10 : Comparison of MVD with Spongiform pustule of kajog



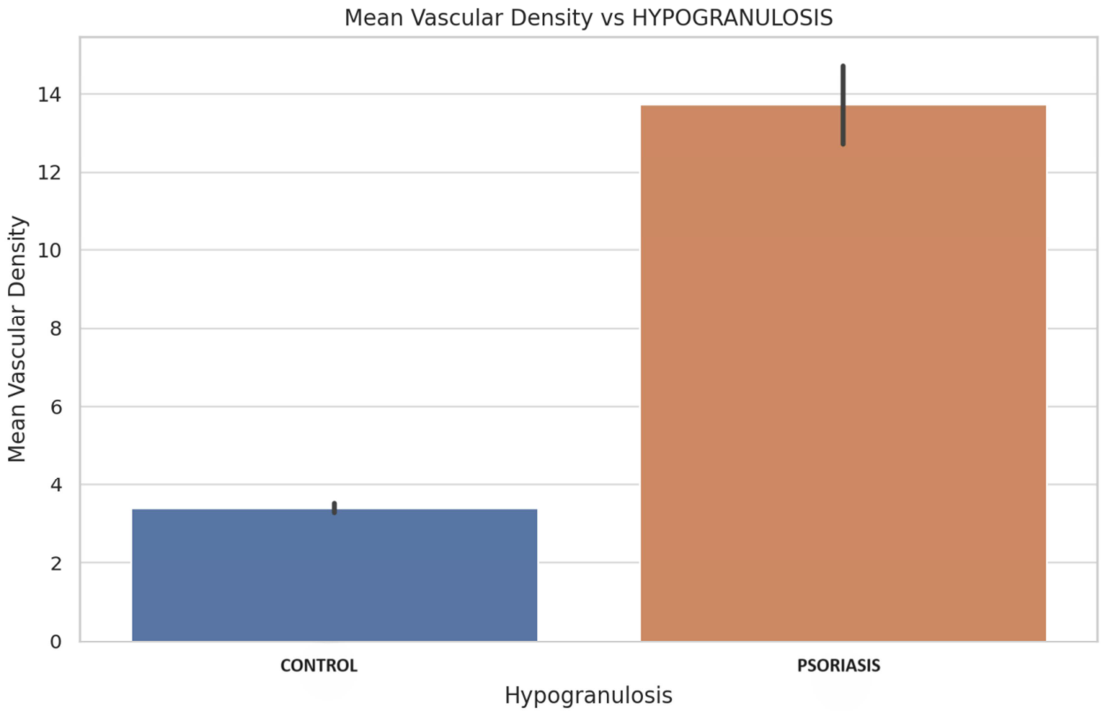
Graph 11: Comparison of MVD with Munro micro abscess



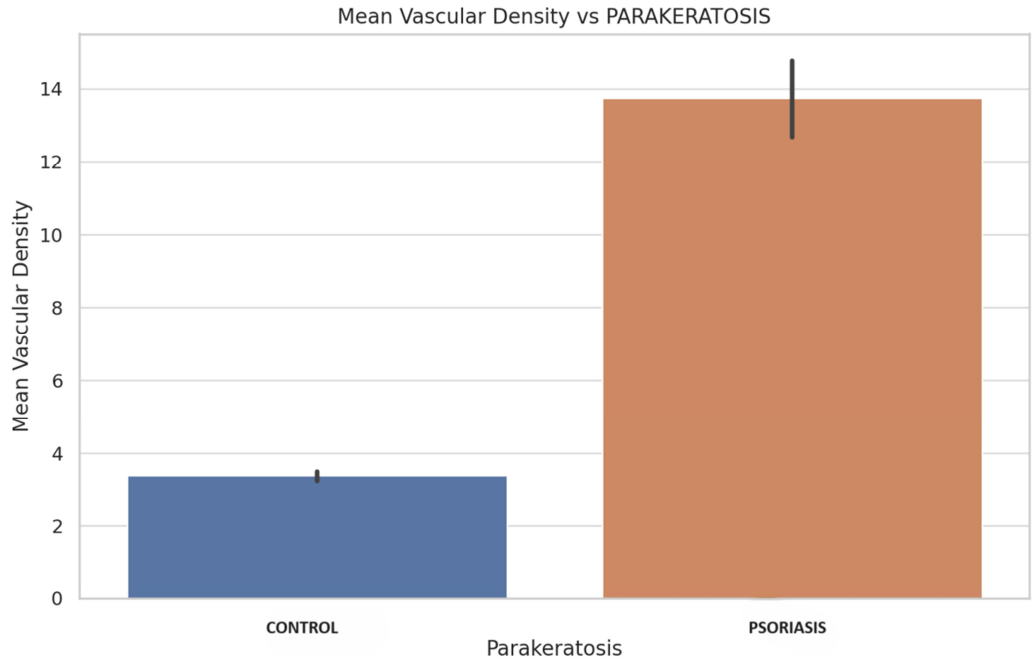
Graph 12 : Comparison of MVD with Spongiosis



Graph 13 : Comparison of MVD with Hypogranulosis



Graph 14 : Comparison of MVD with Parakeratosis

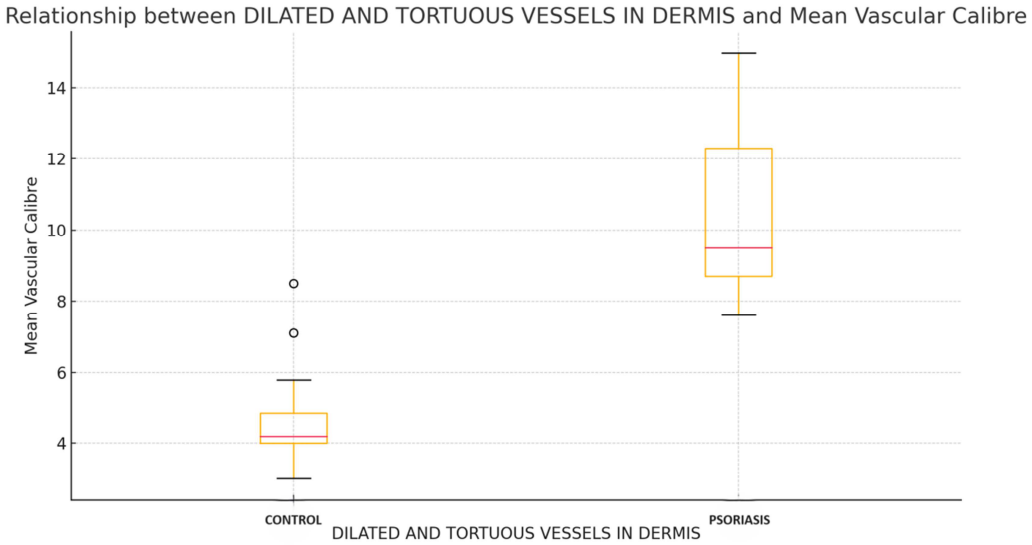


With a chi-square value of 66.06, parakeratosis, hypogranulosis, perivascular lymphocytic infiltrate, and dilated tortuous blood vessels have the largest correlation with mean vascular density. With a chi-square value of 40.76, kajog pustules has the lowest correlation among the relevant traits. According to these results, there is a substantial correlation between mean vascular density and all of the features; however, the correlation is lowest for Kajog pustule. These findings were mentioned in Table 8 and Graphs 6-14.

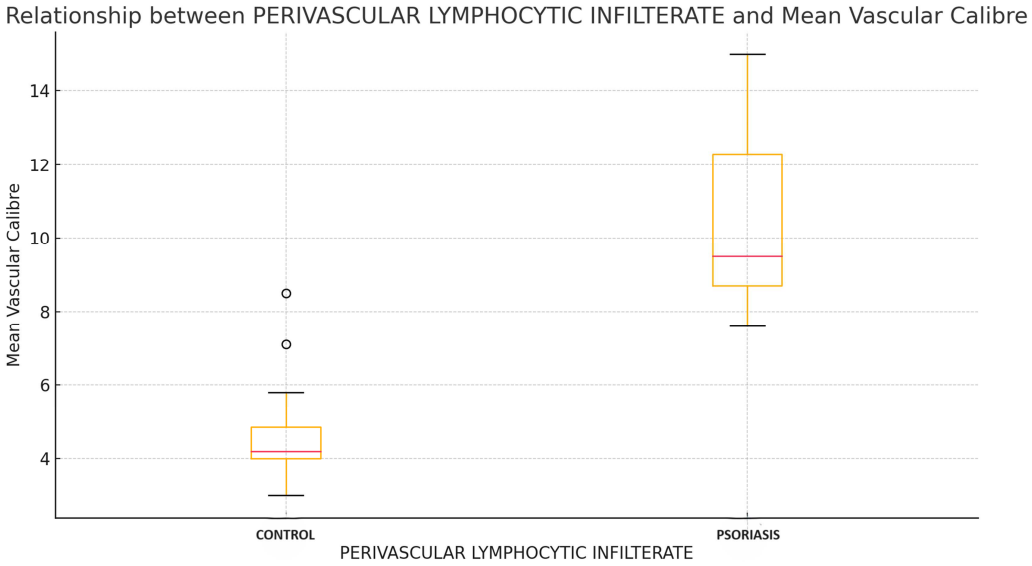
Table 9: Comparison of Histopathological features VS MVC

HISTOPATHOLOGICAL FEATURES	Psoriasis (n=35)	Control (n=35)	Chi Square value	P value
PARAKERATOSIS	35	0	68.00	0.00675
HYPOGRANULOSIS	35	0	68.00	0.00675
SPONGIOSIS	28	0	63.75	0.01678
MUNRO MICROABSCESS	33	0	63.31	0.01836
SPONGIFORM PUSTULE OF KAJOG	27	0	63.67	0.01707
SUPRAPAPILLARY THINNING	28	0	63.75	0.01678
ELONGATION OF RETE RIDGES	31	0	61.89	0.02443
PERIVASCULAR LYMPHOCYTIC INFILTRATE	35	0	68.00	0.00675
DILATED AND TORTUOUS VESSELS IN DERMIS	35	0	68.00	0.00675

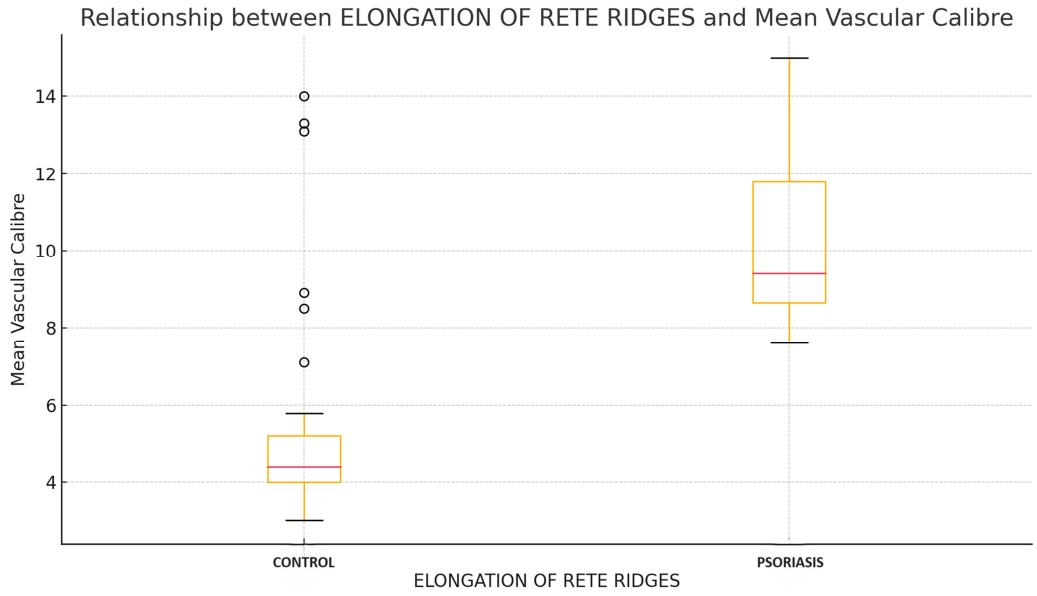
Graph 15 : Comparison of MVC with Dilated and tortuous vessels in dermis



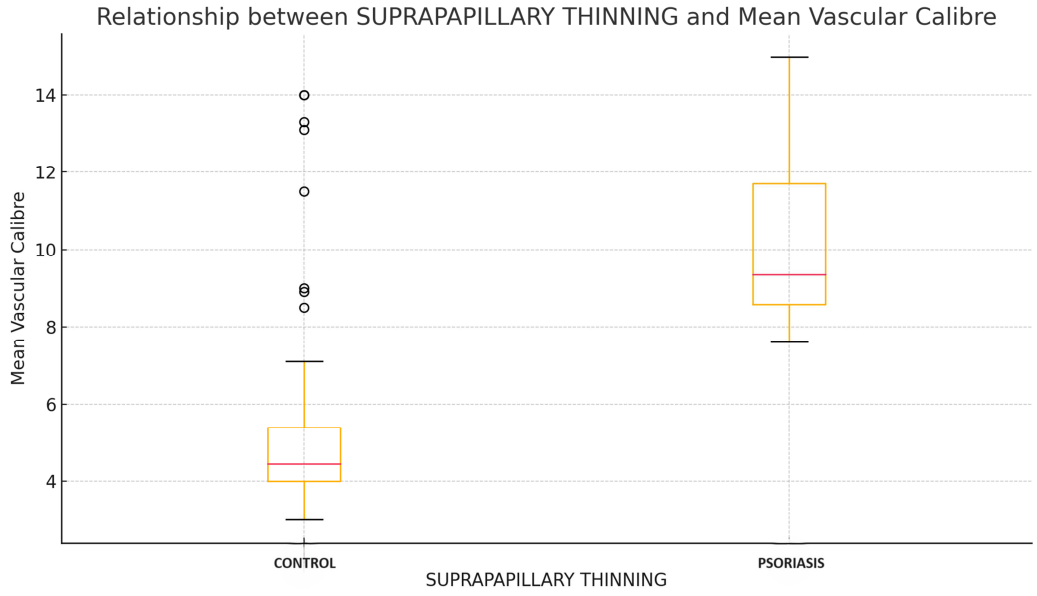
Graph 16 : Comparison of MVC with Perivascular lymphocytic Iinfiltrate



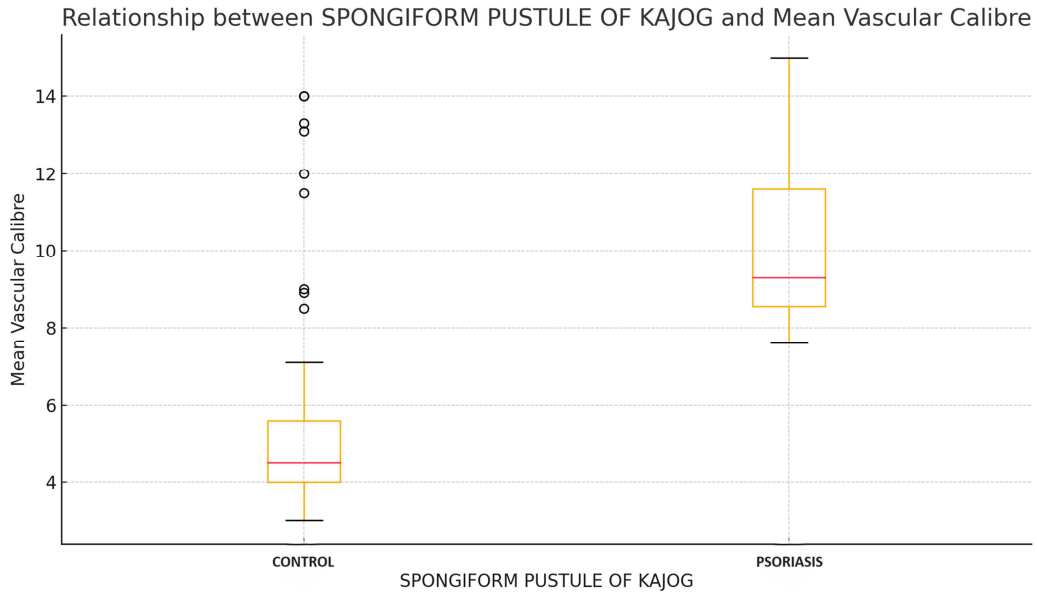
Graph 17 : Comparison of MVC with Elongation of rete ridges



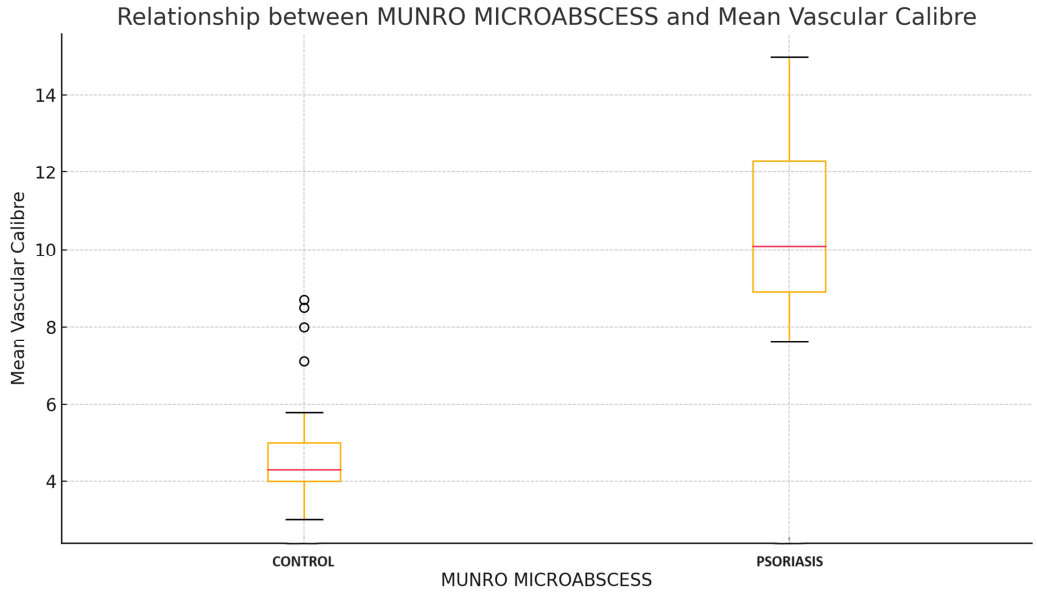
Graph 18 : Comparison of MVC with Suprapillary thinning



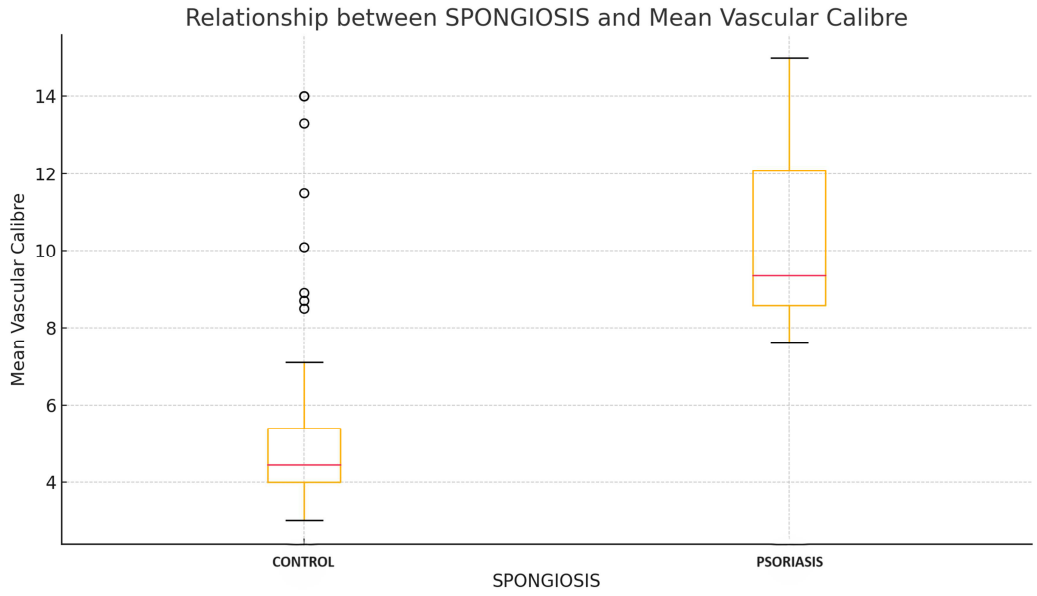
Graph 19 : Comparison of MVC with Spongiform pustule of kajog



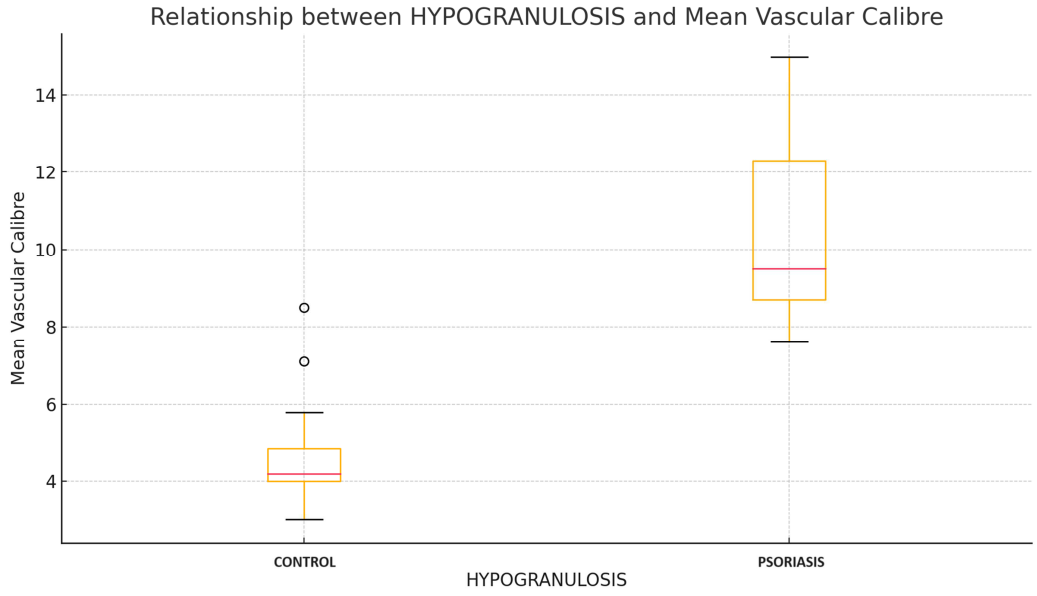
Graph 20 : Comparison of MVC with Munro microabscess



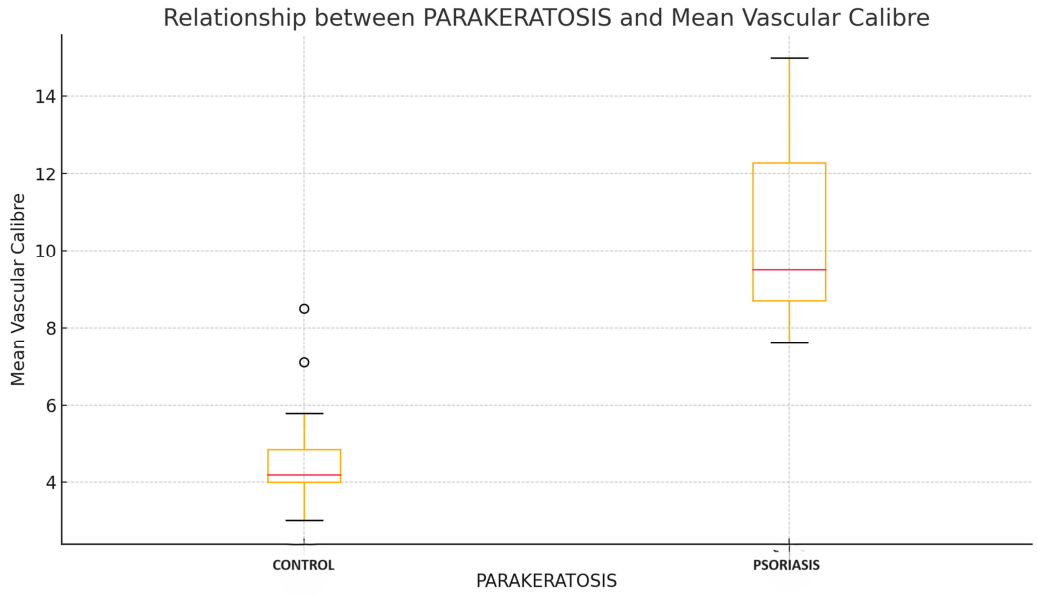
Graph 21 : Comparison of MVC with Spongiosis



Graph 22 : Comparison of MVC with Hypogranulosis



Graph 23 : Comparison of MVC with Parakeratosis



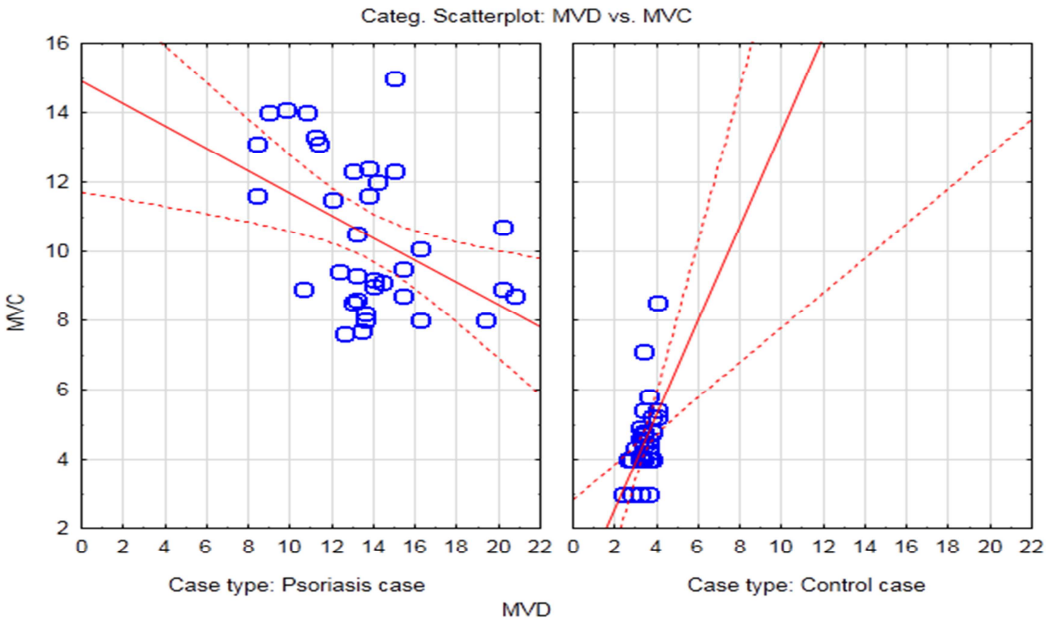
Because of their extremely low p-values (0.00675), we may conclude that the features parakeratosis, hypogranulosis, perivascular lymphocytic infiltrate, and dilated tortuous blood vessels have the strongest correlations with mean vascular calibre. On the other hand, out of all the traits, elongation of rete ridges has the lowest significant correlation with mean vascular calibre. Despite this, it is still significant because its p-value is less than 0.05. These findings were mentioned in Table 9 and Graphs 15-23**.**

Table 10 : Correlation between MVD with MVC in control and psoriasis patients

Samples	Variables	Correlation between MVD with		
		r-value	t-value	P-value
Psoriasis cases	MVC	-0.4492	-2.8886	0.0068*
Control cases	MVC	0.4868	3.2013	0.0030*

*p<0.05

Graph 24 : Correlation between MVD with MVC in control and psoriasis patients



From the above-obtained variables of MVD and MVC, in psoriasis, we could find out that when MVD value increases, MVC reduces (Karl Pearson's correlation coefficient 'r'-0.4492), whereas in control cases, it revealed that when MVD value increases, MVC also increases ('r' 0.4868). It also showed the statistical significance

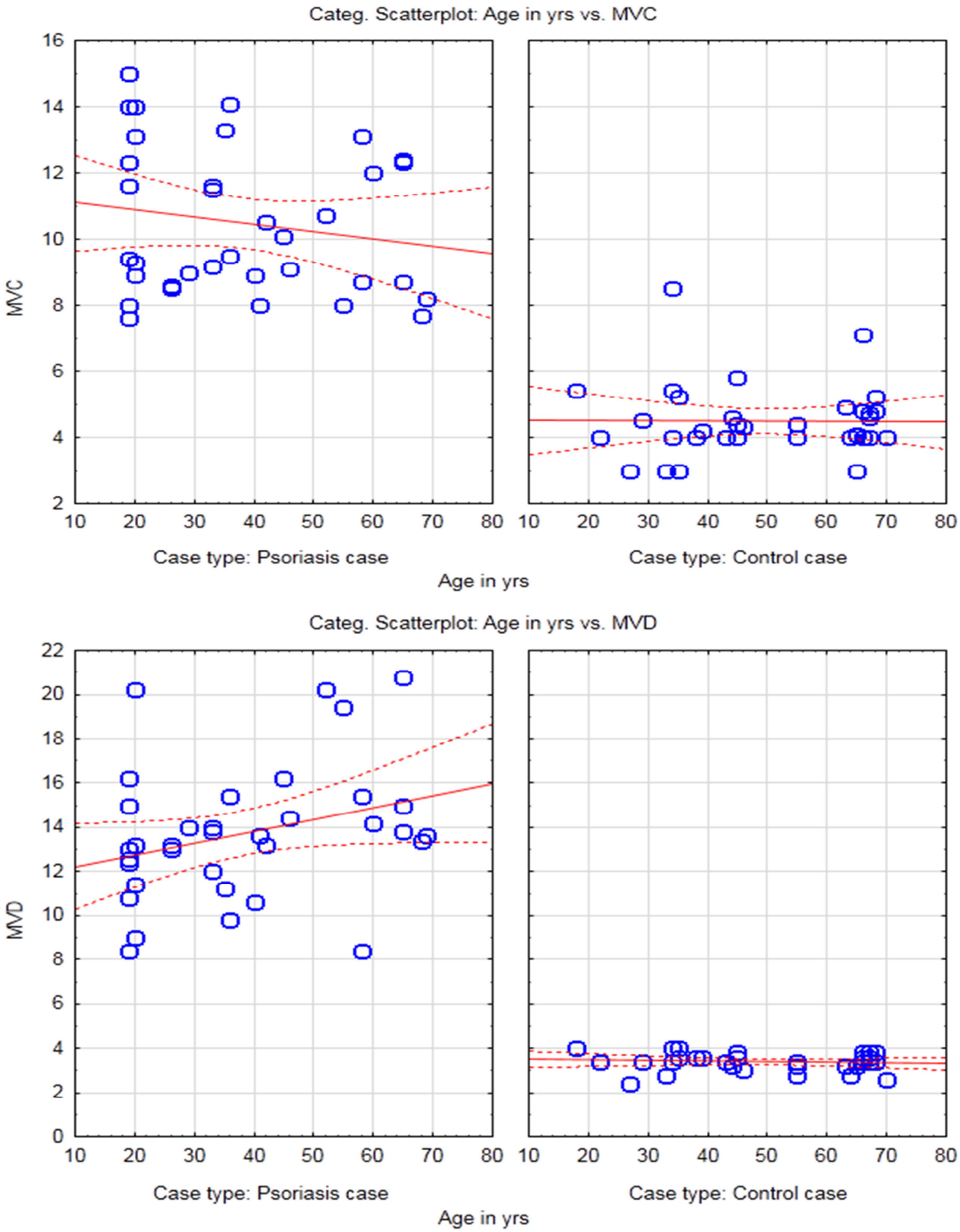
of the correlation between MVD with MVC in psoriasis cases (P <0.05) and controls (P <0.05). Correlation between MVD with MVC shown in Table no:10 and in Graph no:24.

Table 11 : Correlation between age in years with MVD and MVC in control and psoriasis patients

Samples	Variables	Correlation between age in yrs with		
		r-value	t-value	P-value
Psoriasis cases	MVD	0.3032	1.8281	0.0766
	MVC	-0.1695	-0.9879	0.3304
Control cases	MVD	-0.1123	-0.6494	0.5206
	MVC	-0.0089	-0.0509	0.9597

*p<0.05

Graph 25 : Correlation between age in years with MVD and MVC in control and psoriasis patients



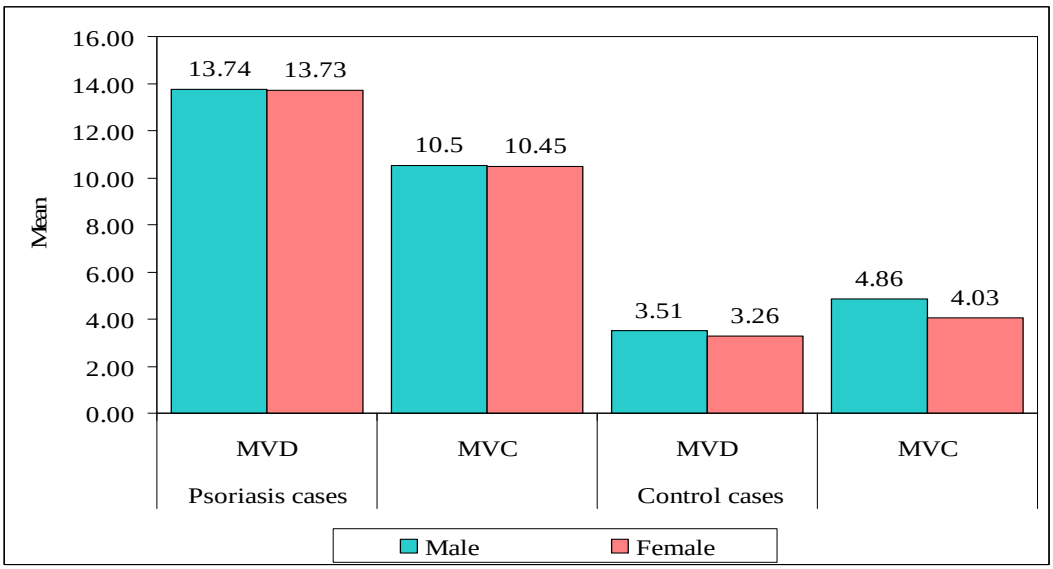
In psoriasis cases, MVD increases with age (‘r’-0.03032) and MVC decreases with age (‘r’-0.1695). Whereas in controls MVD ('r'-0.1123) and MVC ('r'-0.0089) reduces with increase in age. It also revealed that there is no statistical significance to the correlation between age in years with MVD and MVC in psoriasis cases and controls (P > 0.05). Correlation between age in years with MVD and MVC is shown in Table no:11 and in Graph: 25.

Table 12 : Correlation of gender with MVD and MVC in control and psoriasis cases

Samples	Variables	Male		Female		t-value	p-value
		Mean	Std.Dev.	Mean	Std.Dev.		
Psoriasis cases	MVD	13.74	2.87	13.73	3.56	0.0052	0.9959
	MVC	10.50	2.17	10.45	2.34	0.0630	0.9502
Control cases	MVD	3.51	0.36	3.26	0.38	1.9232	0.0631
	MVC	4.86	1.24	4.03	0.64	2.4248	0.0210*

*p<0.05

Graph 26 : Correlation of gender with MVD and MVC in control and psoriasis cases



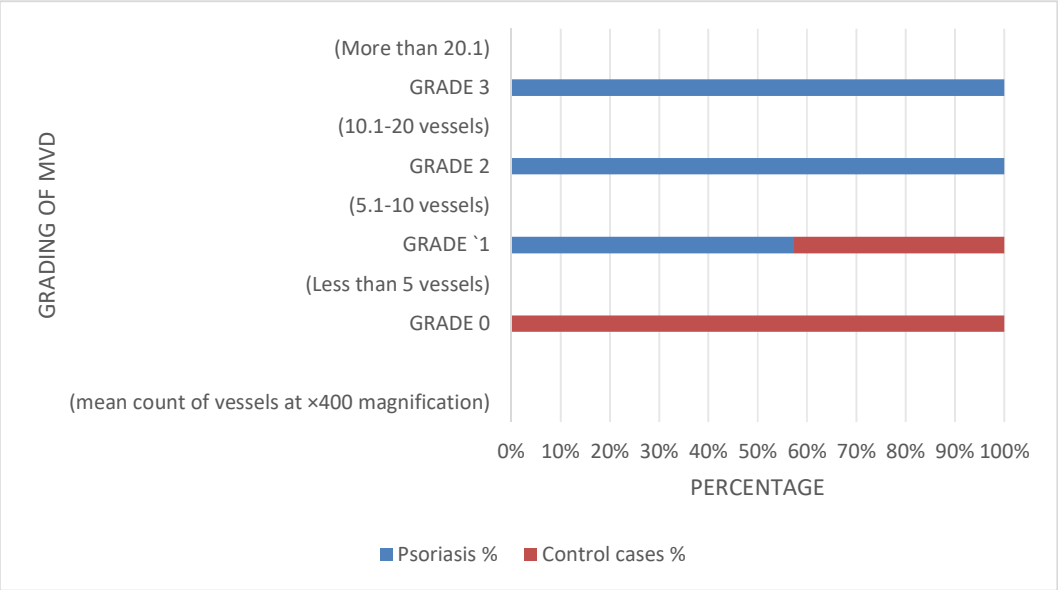
From the above variables, it was revealed that there is no statistical significance to the correlation between gender with MVD and gender with MVC in psoriasis cases as well as in control, with a P value > 0.05. Correlation of gender with MVD and MVC is shown in Table no:12 and in Graph no: 26.

Table 13 : MVD distribution based on grading in the study group.

MVD GRADING (mean count of vessels at ×400 magnification)	Psoriasis (n=35)	%	Control (n=35)	%	Chi-square	p-value
GRADE 0 (Fig:12) (Less than 4 vessels)	0	0.00	32	91.43	63.142	0.0001*
GRADE 1(Fig:13,14) (4-9 vessels)	4	11.43	3	8.57		
GRADE 2 (Fig:15) (10-19 vessels)	28	80.00	0	0.00		
GRADE 3 (Fig:16) (> 20vessels)	3	8.57	0	0.00		
Total	35	100.00	35	100.00		

*p<0.05

Graph 27 : MVD distribution based on grading in the study group.



We observed that MVD (assessed by grading) in 80% of psoriasis cases was in grade 2, with a mean of 13.74 ± 3.07 vessels/400x. Whereas the control shows 91.43% in grade 0, and with a mean of 3.39 ± 0.39 vessels/400x. A statistically significant p-value of 0.0001 was obtained when comparing the cases with the control group.

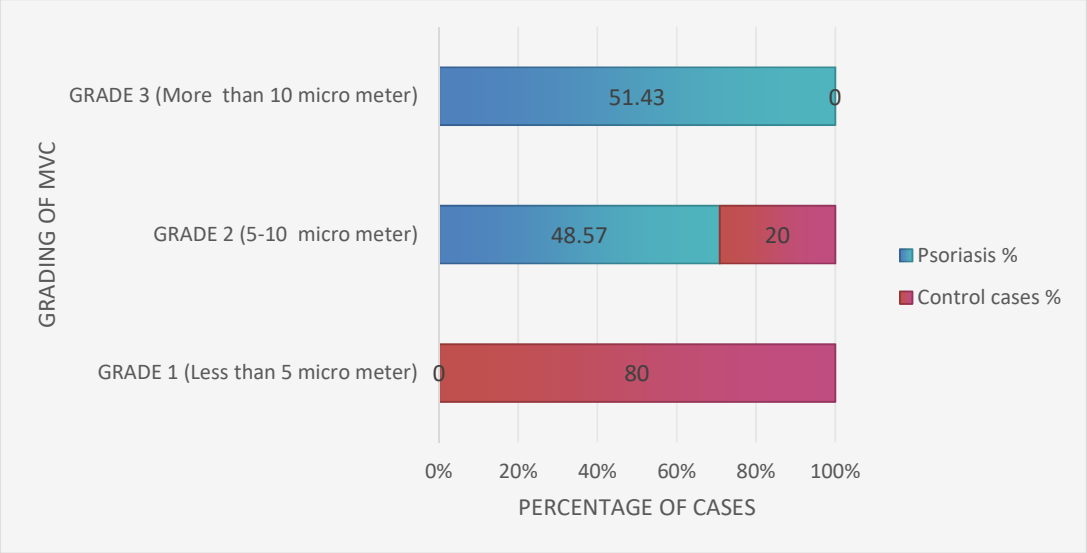
When compared to controls, the largest number of psoriasis cases occurred in Grade 2 (10–19 vessels/HPF), indicating that MVD was higher as a result of microvascular proliferation in the psoriasis pathogenesis. MVD distribution based on grading in the study group shown in Table no:13 and in Graph: 27

Table 14 : MVC distribution based on grading in the study group.

Grading of MVC	Psoriasis (n=35)	%	Control (n=35)	%	Chi-square	p-value
GRADE 1(Fig:18) <5 micro meter	0	0	28	80	50.166	0.0001*
GRADE 2(Fig:19) 5-10 micro meter	17	48.57	7	20		
GRADE 3(Fig:20) >10 micro meter	18	51.43	0	0		
Total	35	100	35	100		

*p<0.05

Graph 28 : MVC distribution based on grading in the study group.



We observed MVC in psoriasis cases were, grades 2 and 3 (48.57% and 51.43% respectively) with a mean of 10.48 μm ± SD 2.20μm. Whereas the control shows 80% of cases in grade 1 with a mean of 4.48 μm ± SD 1.08 μm. A statistically significant p-value of 0.0001 was obtained when comparing the psoriasis cases with the control group.

When compared to controls, the psoriasis cases revealed that the mean MVC was more than 5 μm (Grades 2 and 3), whereas the controls were below 5 μm (Grade 1), which explained the dilated and tortuous blood vessels in the histopathological findings of psoriasis. MVC distribution based on grading in the study group shown in Table no:14 and in Graph:28

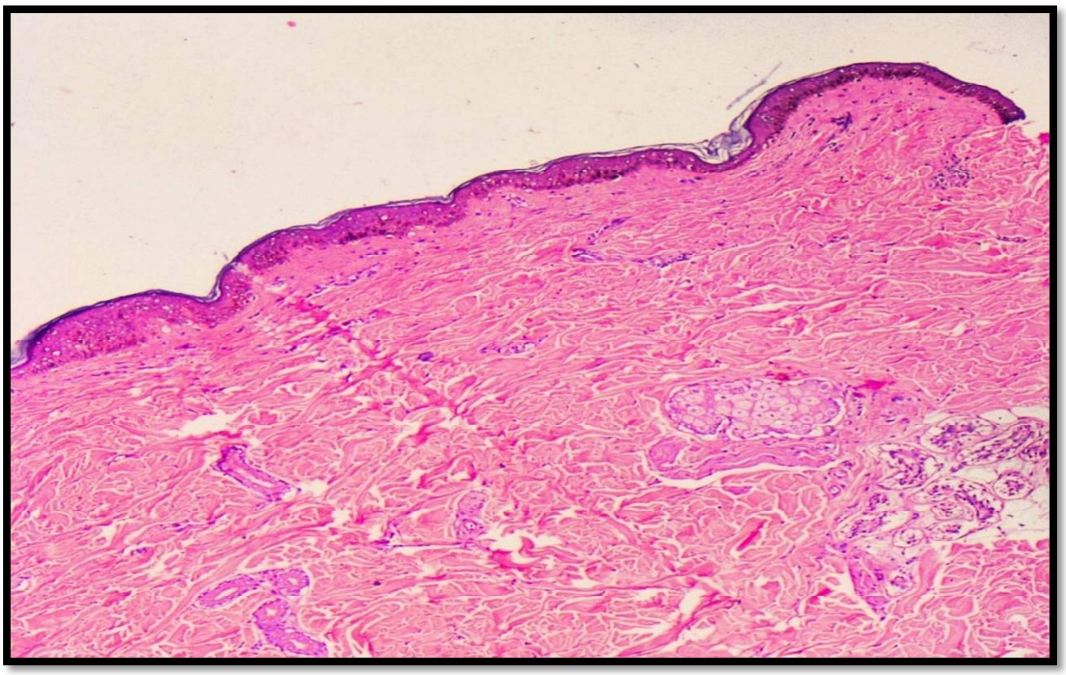


Figure 5 : Control: Normal Skin (H&E; 4X)

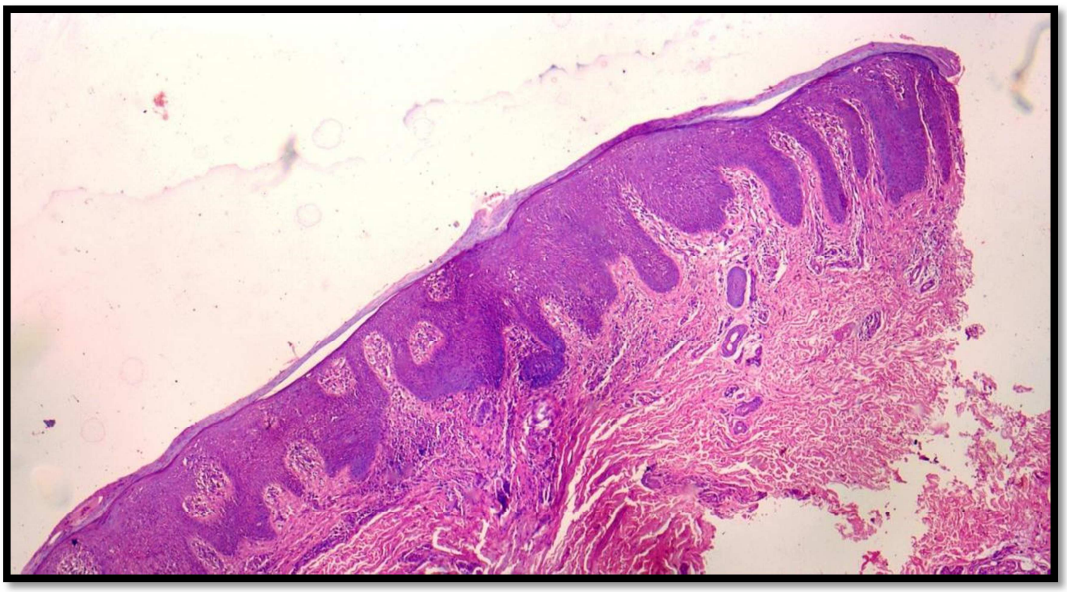


Figure 6 : Psoriasis: Showing Elongation of rete ridges, Suprapapillary thinning, inflammatory infiltrate in dermis- (H&E; 4X)

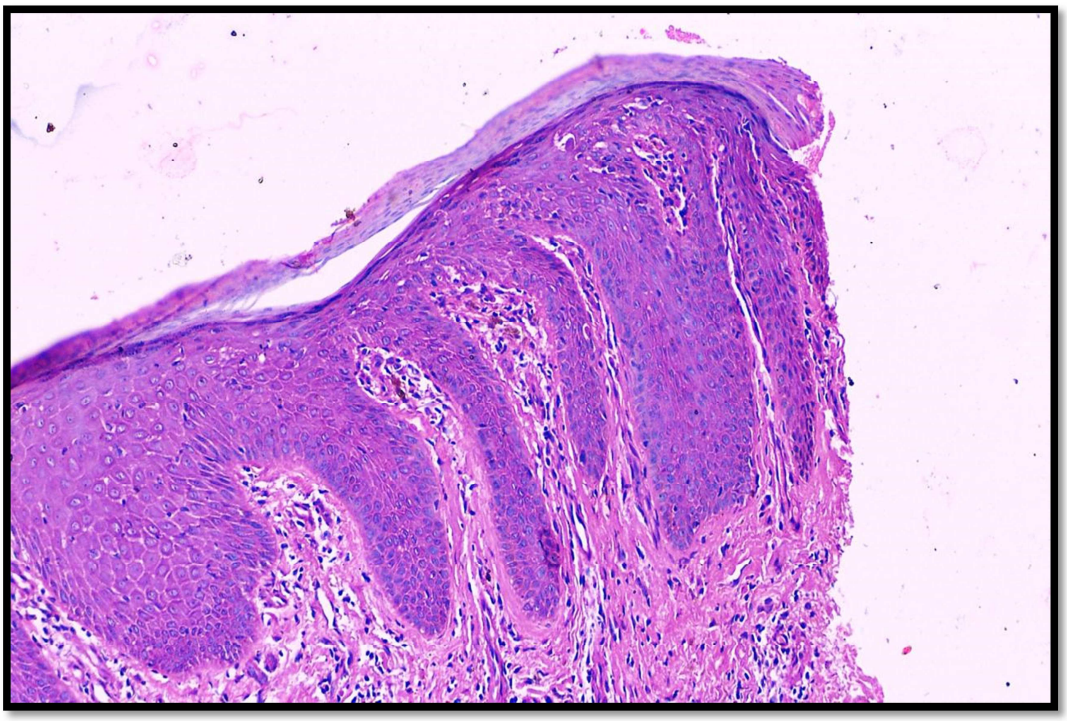


Figure 7 : Psoriasis: Showing Spongiosis, Parakeratosis, Elongation of Rete Ridges, Suprapaillary Thinning - (H&E; 10X)

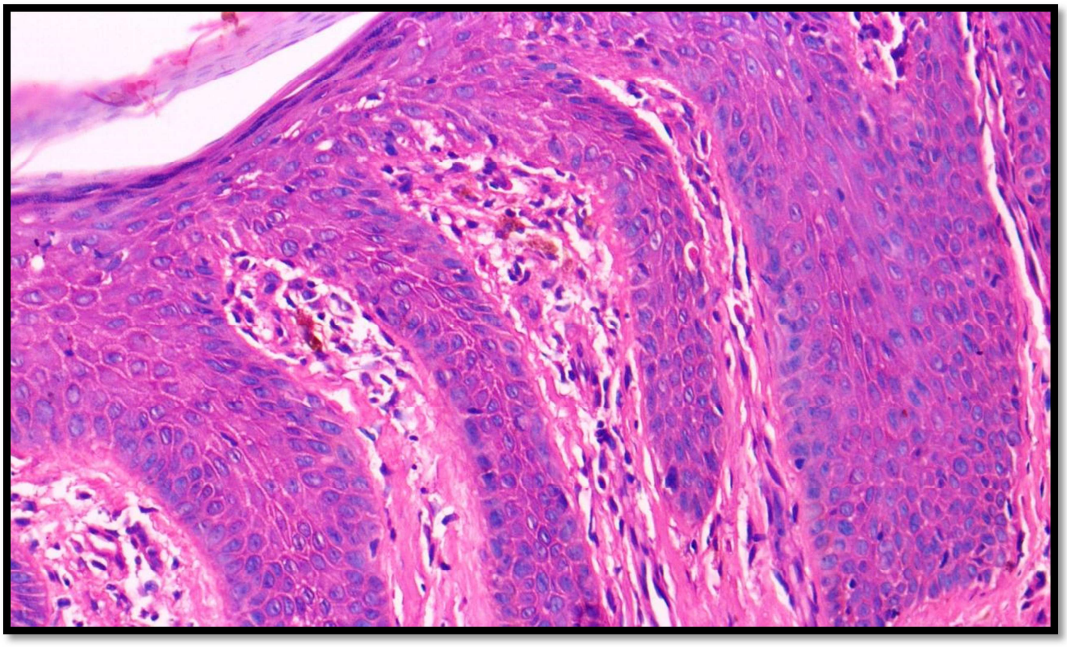


Figure 8 : Psoriasis: Showing Hypogranulosis, and Dilated tortuous blood vessels - (H&E; 40X)

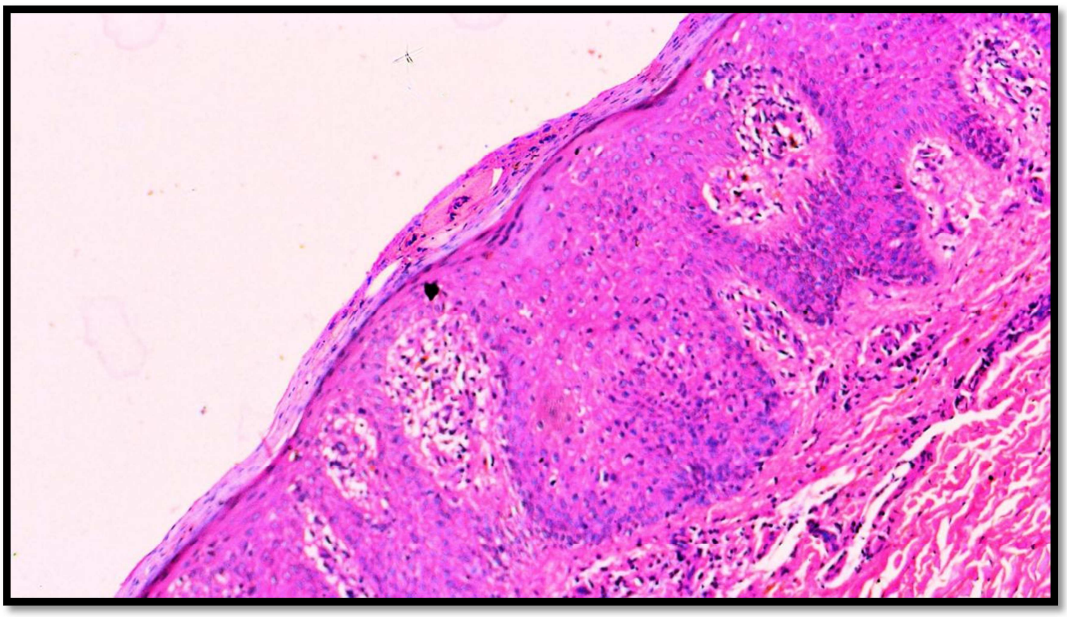


Figure 9 : Psoriasis: Showing Munro micro abscess, Parakeratosis and exocytosis of inflamatory infiltrate - (H&E; 20X)

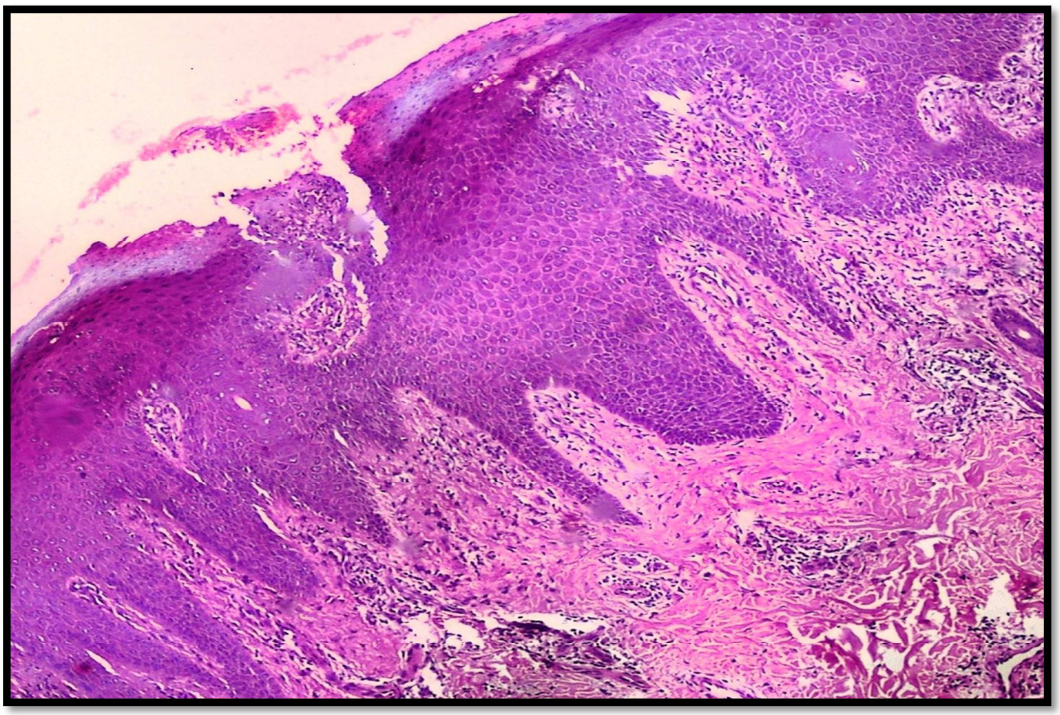


Figure 10 : Pustular psoriasis: Showing pustules in epidermis and dermal perivascular inflammation - (H&E; 40X)

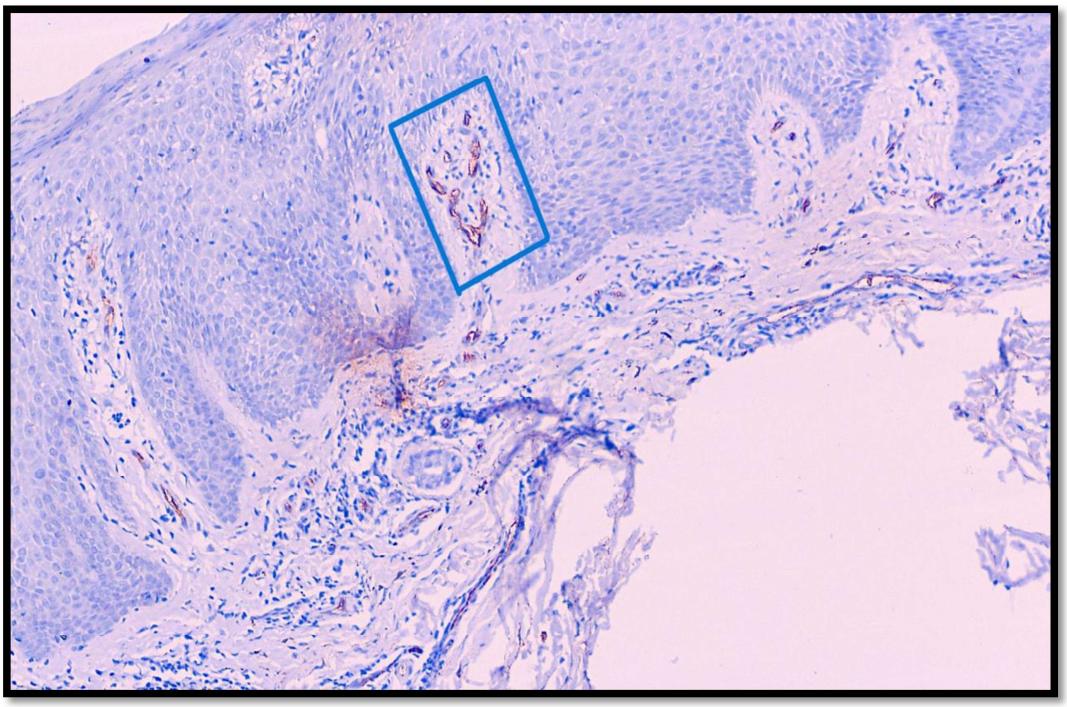


Figure 11 : Psoriasis: Finding HOTSPOT (CD34; 40X)

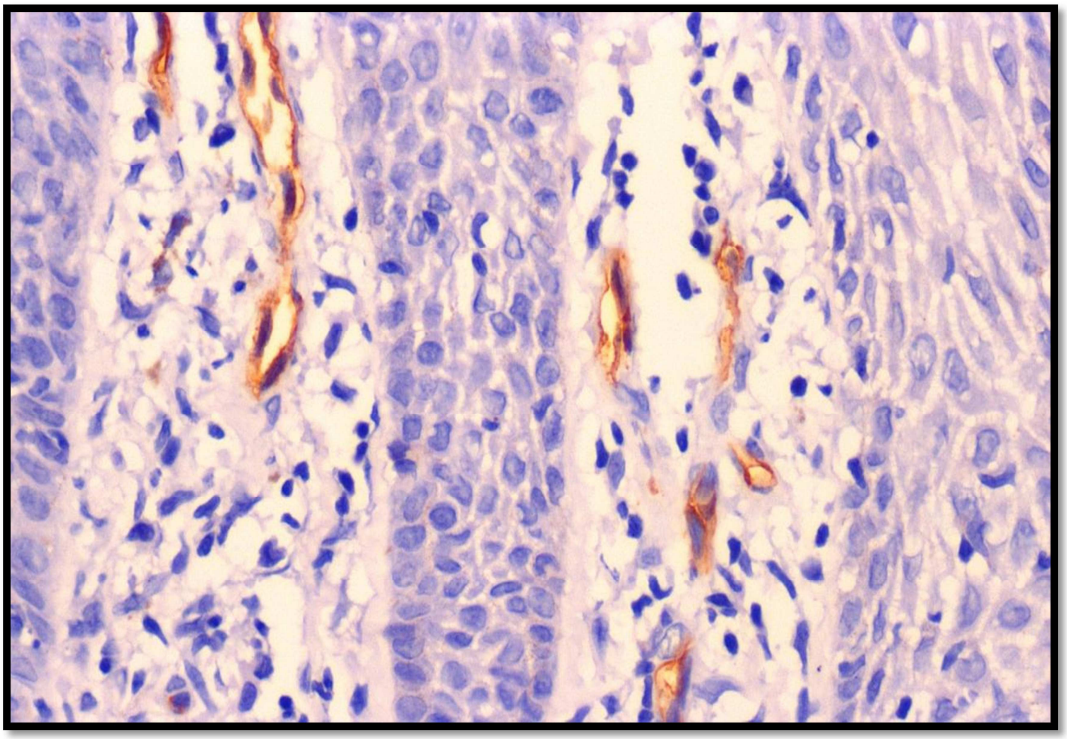


Figure 12 : Counting number of vessels on high power to assess mean MVD CD 34 IHC (400X)

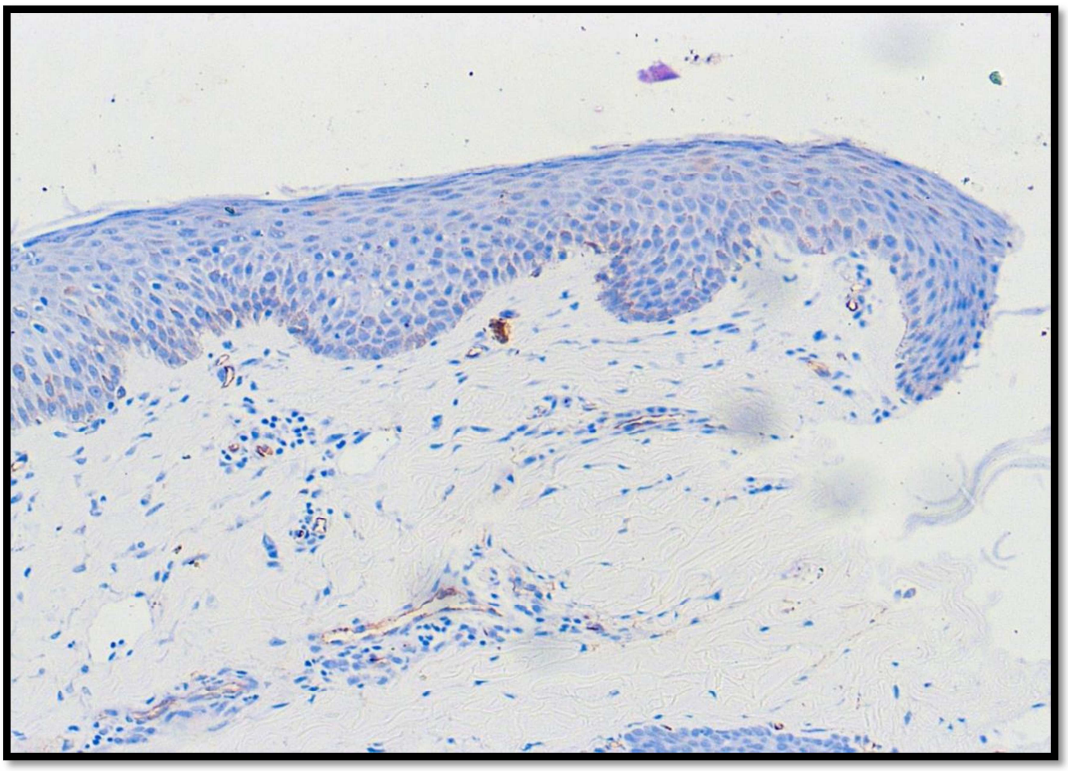


Figure 13 : Control: Microvascular density Grade 0 (CD 34; 40x)

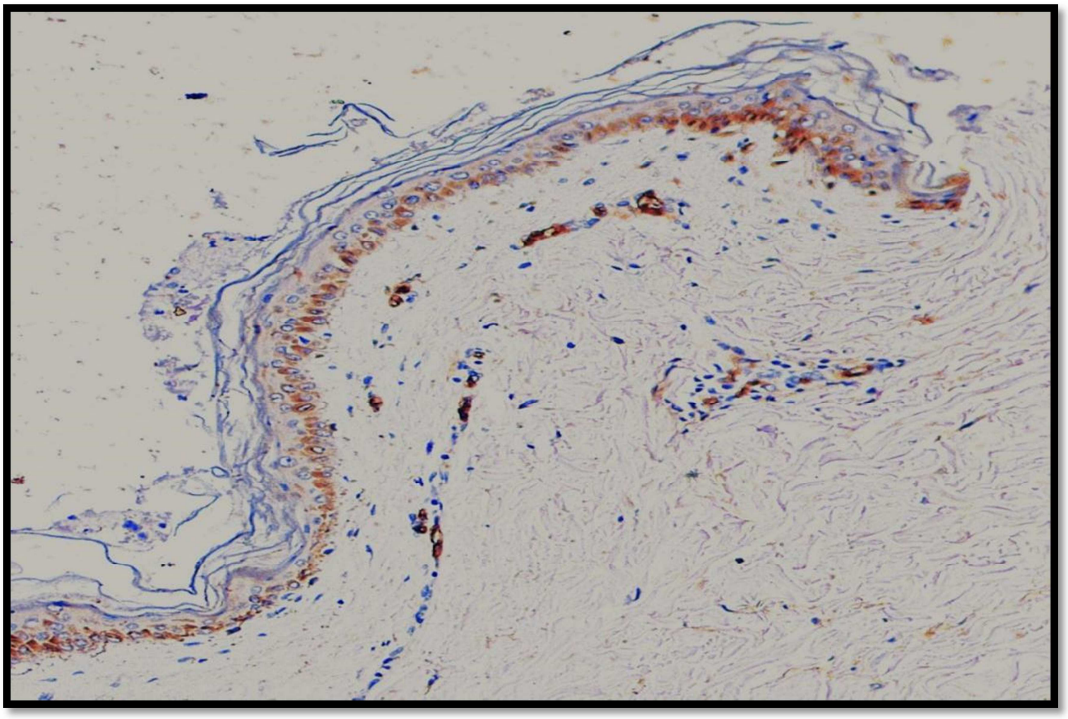


Figure 14 : Control: Microvascular density Grade 1- (CD 34; 40X)

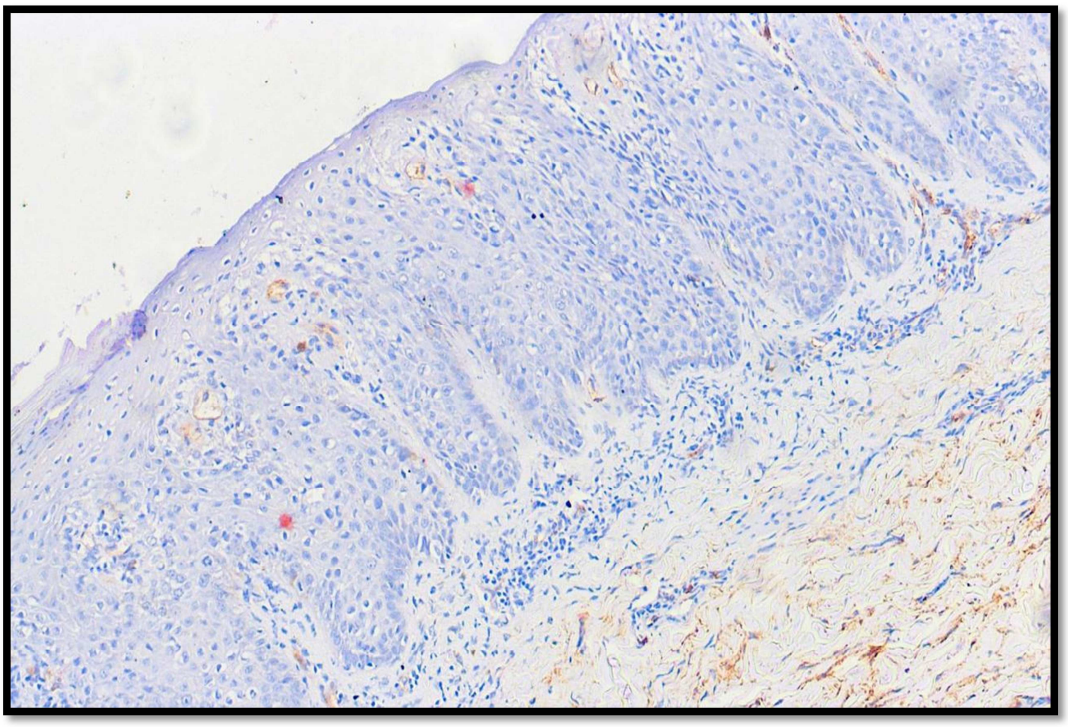


Figure 15 : Psoriasis: Microvascular density Grade 1 - (CD 34; 40X)

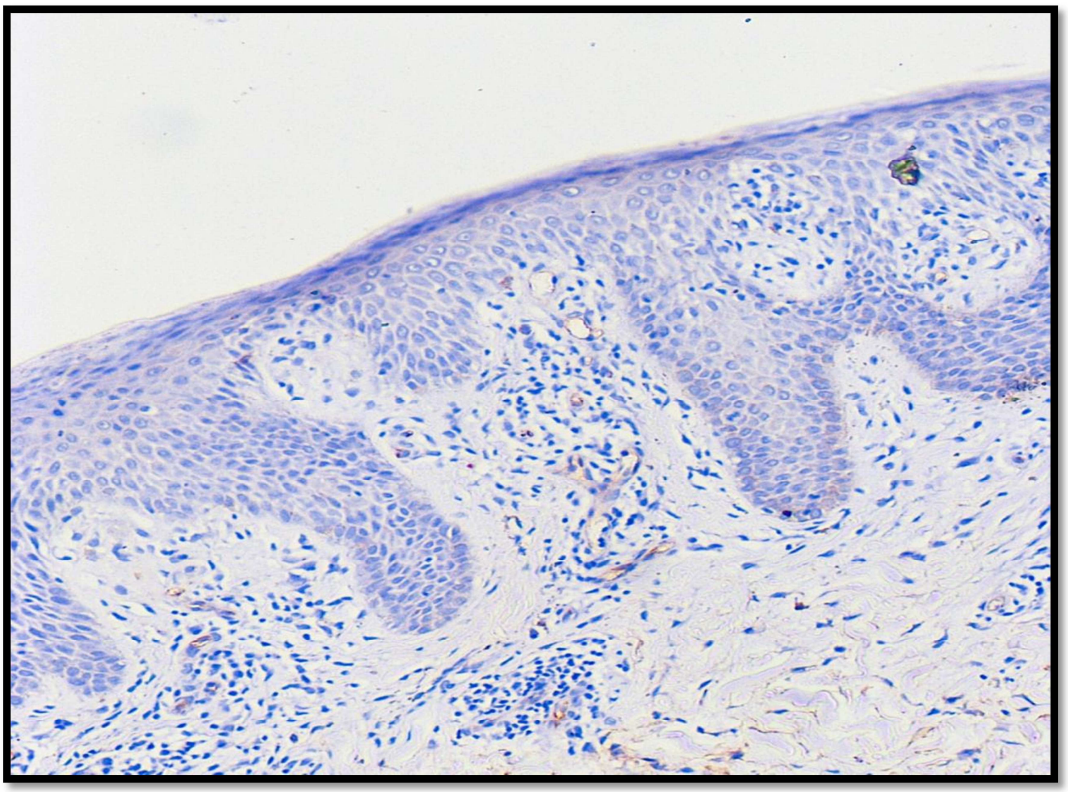


Figure 16 : Psoriasis: Microvascular density Grade 2 - (CD 34; 40X)

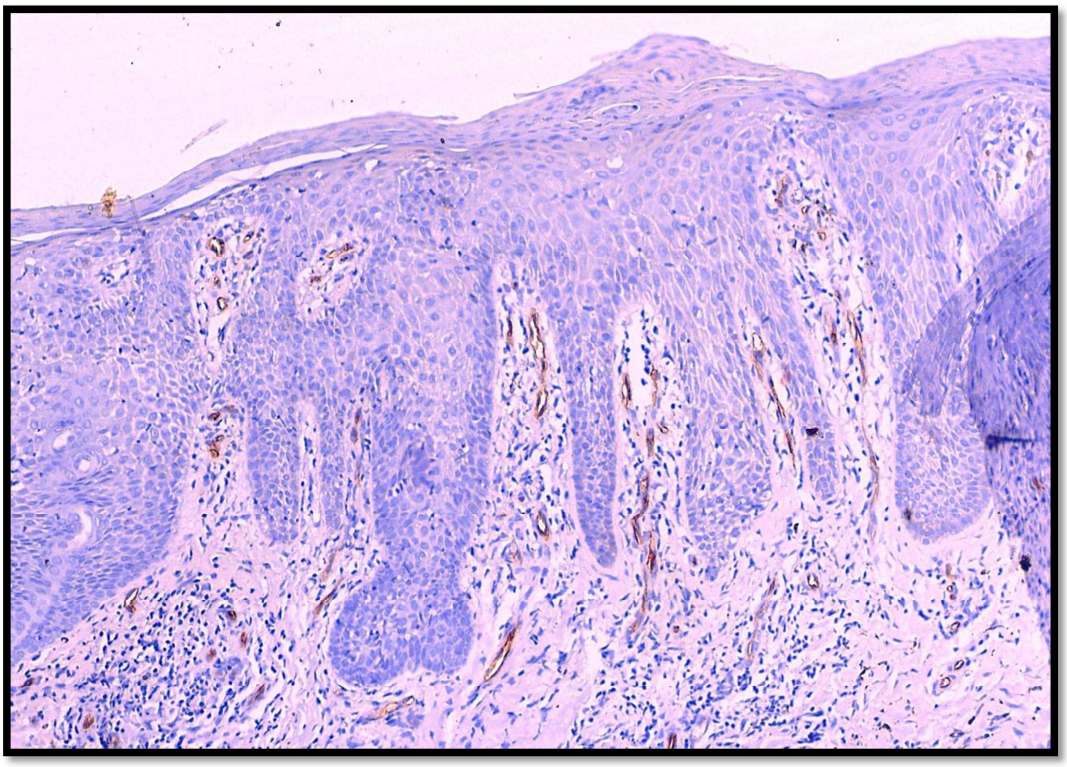


Figure 17 :Psoriasis Microvascular density Grade 3- (CD 34; 40X)

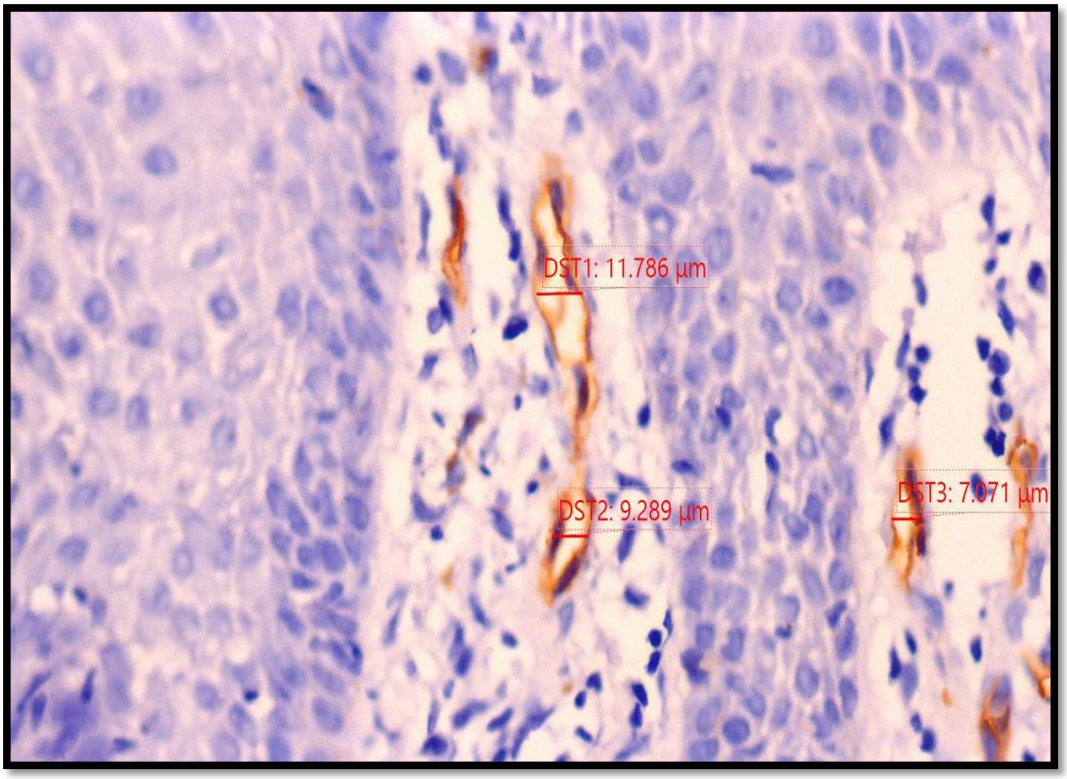


Figure 18 : Measuring Micro Vascular Calibre (CD 34 IHC; 40X)

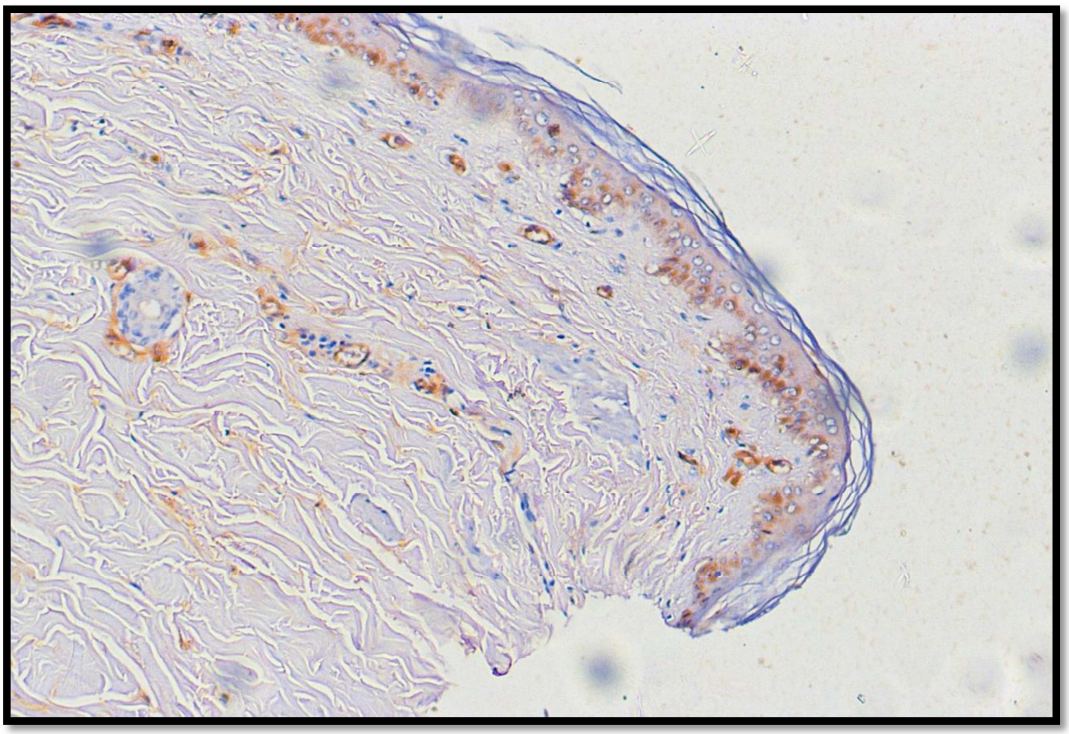


Figure 19 : Control: Microvascular calibre Grade 1 - (CD 34; 40X)

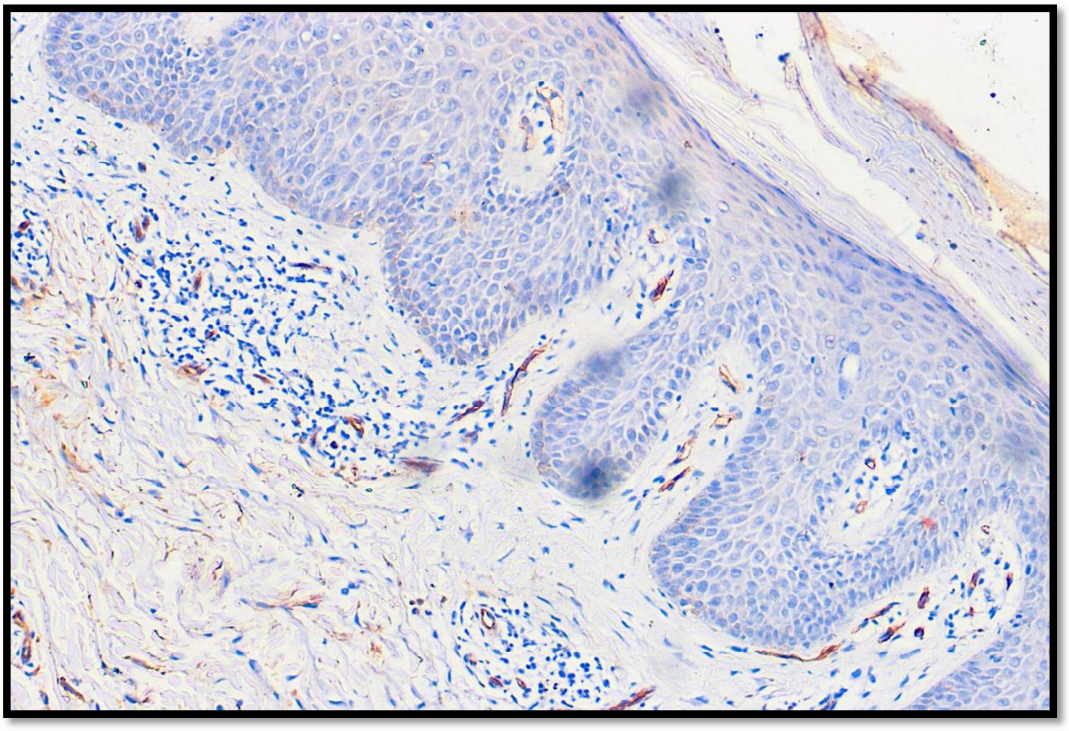


Figure 20 : Psoriasis: Microvascular calibre Grade 2 - (CD 34; 40X)

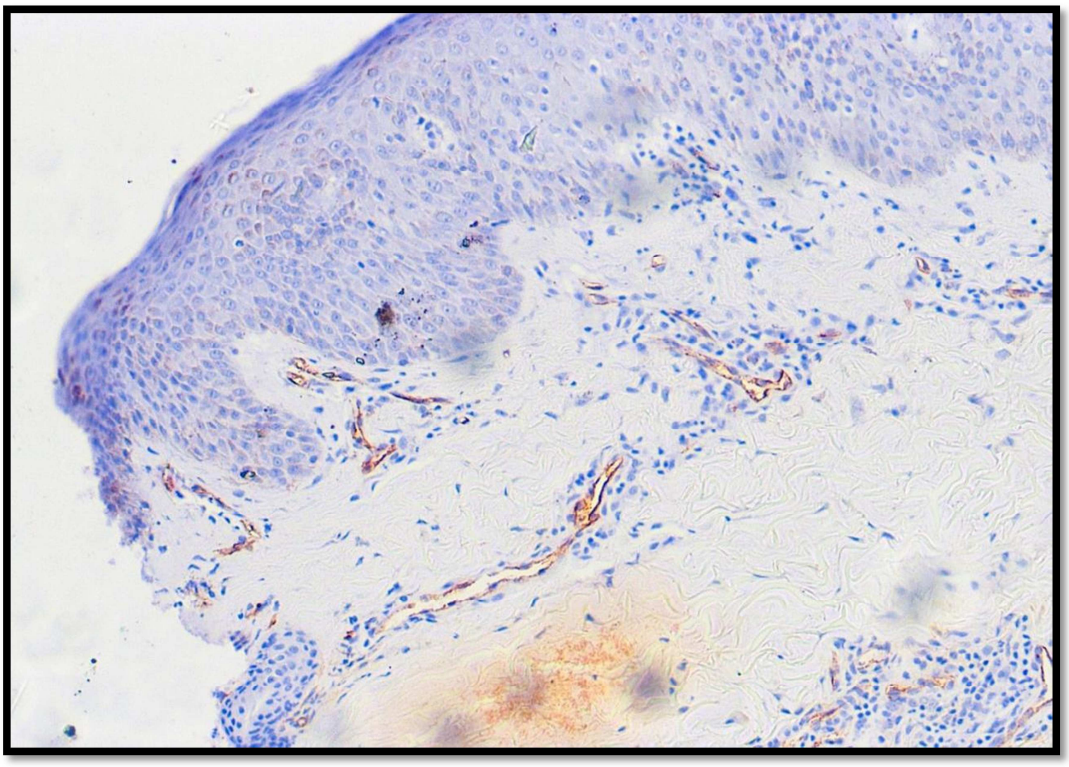


Figure 21 : Psoriasis: Microvascular calibre Grade 3 - (CD 34; 40X)

DISCUSSION

Psoriasis is a chronic autoimmune inflammatory skin disease that can affect individuals of all age groups. Clinically, it manifests as chronic plaque-type lesions with well-defined erythematous plaques covered by silvery scales. Histopathologically, psoriasis is characterized by parakeratosis, spongiosis, Munro's microabscesses, spongiform pustules of Kogoj, elongation of the rete ridges, suprapapillary thinning, inflammatory infiltrates around the vessels in the dermis, and dilated, tortuous vessels in the dermis.

The pathophysiology has not yet been determined. Several factors are implicated in the pathogenesis of psoriasis. These include angiogenesis, neovascularization, vascular remodeling, interactions between proliferating epidermal cells and various cytokines, and the immune-mediated dysregulation of T lymphocytes. Studies have shown that proliferating keratinocytes secrete certain cytokines and angiogenic factors, such as VEGF. The process of creating new blood vessels from preexisting vascular channels is known as angiogenesis. The endothelial microvasculature is significantly increased in psoriasis, supporting the theory that psoriasis is dependent on angiogenesis. The importance of angiogenesis in psoriasis is highlighted by the expansion of microvessels in the dermis, along with the abnormal dilation and orientation of capillaries.

Proangiogenic cytokines, including vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNF-α), endothelial cell stimulating factor, and platelet-derived growth factor (PDGF), are believed to be produced by keratinocytes in psoriatic skin lesions. Newer angiogenic factors include nerve growth factor (NGF) and von Willebrand factor (vWFr). When psoriatic skin lesions are present, these

cytokines promote angiogenesis. This study examines the the dermal vascular changes in psoriasis using CD34 IHC stain.

In this study, the mean age of the study group was 43.67 years with a standard deviation of 17.23. The highest number of psoriasis patients were in the 18–20 age group (31.43%), whereas the majority of control participants were in the 61–70 age group (27.14%). Khandpur et al[57]. reported that most psoriasis cases (66.8%) fell within the 21–50 age range, while Fry[58] indicated that two-thirds of cases occurred before the age of 30, which is consistent with the findings of the present study (Table: 15).

Psoriasis being a disease of male predominance in our study, In this study, a majority of psoriasis cases were male, accounting for 65.71% of the total, resulting in a male-to-female ratio of 1.916:1, indicating a male predominance. According to Dogra and Yadav[59], psoriasis is reported to be twice as common in males compared to females. Rakhesh et al[60], Kaur et al[61] and Khandpur et al[57]. also stated that male predominance was seen in psoriasis (Table: 15).

Table 15 : Age and Gender distribution in various studies

	Khandpur *et al* (n=30)%	***Kaur et al* (n=30)%**	**Present study (n=35)%**
AGE	40.03 ± 2.83	38.12 ±12.34	43.6714 ± 17.2337
GENDER	61%	53.3%	65.71%

Patients of Psoriasis clinically presents with well defined erythematous plaques, silvery scales or and positive auspitz sign. Out of 35 cases of Psoriasis well defined erythematous plaques and silvery scales are seen in all cases (100%) of psoriasis and auspitz sign was seen in 27(77.14%) cases of Psoriasis. Comparable to Meier and Seth's[62] study, ours study also revealed that well-defined erythematous and scaly plaques were clinically significant for psoriasis. Maize et al[63]. have reported that parakeratosis in the cornified layer of skin histologically coincides with the silvery scale of psoriasis. According to Hellgren et al[64]., Auspitz's sign is a pathognomic for psoriasis. In our investigation, the Auspitz sign was determined to be clinically significant for psoriasis. Patients who were receiving treatment were not included in the study since this indicator is only seen in patients who are not receiving treatment.

In our study, all 35 cases of psoriasis showed parakeratosis. A study by *Gordon* and Johnson et al[65]., and Mehta et al[66]. showed similar features. Also, all 35 psoriasis cases (100%) showed hypogranulosis, which was similar to the study by Mehta et al[66]. They also stated that hypogranulosis should be considered one of the essential histopathological criteria of psoriasis. The keratohyalin granules found in the keratinocytes of the granular layer are packed with proteins rich in cysteines and histidines that seem to hold the keratin filaments together. Thus, the primary role of granules of keratohyalin is to bind filaments of intermediate keratin. Cells release lamellar bodies, which contain lipids and proteins, into the extracellular space at the boundary between this layer and the stratum corneum. The hydrophobic lipid envelope that gives skin its barrier qualities is formed as a result. The granular cells of the stratum corneum turn into non-viable corneocytes as a result of these cells simultaneously losing their nuclei and organelles. This layer is lacking in psoriasis due to a loss of differentiation.

All cases of psoriasis showed dilated and tortuous vessels in the dermis. A study by *Gordon* et al[65]. and Leelamma J.P. et al[67]. Showed the presence of dilated and tortuous vessels in the dermis in about 75% and 87.5% of cases, respectively, which is almost similar to the present study. Since Psoriasis is a chronic inflammatory disorder all 35 cases of psoriasis showed perivascular lymphocytic infiltrate, study by Lal S et al[68]. and Leelamma J.P. et al[67]. Showed the presence perivascular lymphocytic infiltrate in all 100% of cases, which is similar to the present study.

Out of 35 psoriasis cases, 33 cases (94.29%) showed the presence of Munro microabscess. A similar study done by Leelamma J.P. et al[67]. and Gordon[65] also showed the presence of munro microabscess in about 87.5% and 75% of cases, respectively, which is almost similar to the present study. Munro microabscess should be considered one of the essential histopathological criteria of psoriasis. 31 cases (88.57%) cases showed elongation of rete ridges. A similar study done by Mehta et al[66] also showed elongation of rete ridges in about 93.10%, which is almost similar to the present study. 28 psoriasis cases (80%) showed the presence of suprapapillary thinning in patients with psoriasis. This, like the study done by Lal S et al[68] (88%), Mehta et al[66]. (65.51%). 28 cases (80%). Also 28 psoriasis cases (80%) showed spongiosis. A Study done by Bai S et al[69]. Also showed 91.67% cases with spongiosis which is similar to the present study. 27 cases (77.14%) showed a spongiform pustule of kajog, A similar study done by Bai S et al[69]. and Gordon et al[65] showed the presence of a spongiform pustule of kajog only in 46.67% and 31% respectively which is a lower value compared to the present study(Table: 16).

Table 16 : Comparison of Histoogical features of Psoriasis in various studies

Histo-pathological features.	Mehta et al (n=58)%	Leelamma J.P. et al (n=40)%	Gordon et al (n=100)%	Lal et al (n=25)%	present study (n=35)%
PARAKERATOSIS	**93.10**	**100**	**97**	**92**	**100**
HYPOGRANULOSIS	**87.93**	**97.5**	**75**	**72**	**100**
SPONGIOSIS	**-**	**-**	**-**	**-**	**80**
MUNRO MICROABSCESS	**-**	**87.5**	**75**	**20**	**94.29**
SPONGIFORM PUSTULE OF KAJOG	**-**	**12.5**	**31**	**-**	**77.14**
SUPRAPAPILLARY THINNING	**65.51**	**100**	**98**	**88**	**80**
ELONGATION OF RETE RIDGES	**93.10**	**100**	**100**	**100**	**88.57**
PERIVASCULAR LYMPHOCYTIC INFILTRATE	**-**	**100**	**95**	**100**	**100**
DILATED VESSELS IN DERMIS	**91.37**	**95**	**96**	**-**	**100**

When the histopathological results of the psoriasis cases in this study were compared to the control cases, all the results were statistically significant (p value 0.0001) and consistent with the information found in standard histopathology textbooks on dermatopathology.

In psoriatic skin, Cremer et al[70]. found a marked rise in vascular density in areas of epidermal hyperplasia, indicating a clear link between angiogenesis and epidermal thickening. Additionally, they discovered that dilated blood arteries in the psoriatic lesions' dermal papillae play a substantial role in the elevated MVD seen in these regions. Henno et al[71]. emphasized the correlation between regions of parakeratosis and elevated vascular density, pointing out that angiogenesis contributes to the high cellular turnover found in psoriatic plaques. A Study done by Bhushan et al[72]., areas of psoriatic lesions with a high concentration of inflammatory cells, suggesting that inflammation promotes angiogenesis in psoriasis. The current study's findings, along with all of the previous research', were comparable to the histological characteristics of psoriasis with MVD.

Nickoloff et al[73]. claim that increased microvascular calibre improves blood flow by supplying nutrients and oxygen necessary for keratinocyte hyperproliferation. Also emphasized were capillary loops that are expanding and commonly seen in the papillary dermis. This trait is indicative of higher microvascular caliber and is diagnostic of psoriatic lesions. Increased microvascular calibre may speed up keratinocyte turnover, which would maintain nuclei in the stratum corneum, according to research by Krueger et al[74]. It was also shown that when microvascular permeability and calibre are raised, inflammatory cells are attracted to and migrate into the dermis more easily. The results of our investigation showed that, despite being statistically significant, from other histopathological findings rete ridge elongation had the least significant connection with mean vascular calibre. Study done by Rudikoff et al[75]., revealed enhanced capillary loops at the tips of rete ridges provide a rich blood supply supporting epidermal proliferation leading to elongation of rete ridges in clinically well-formed psoriasis cases. When compared to the

histological characteristics of psoriasis with MVC, the current study's results and those of all previous research were similar. We observed that MVD (assessed by grading) in 80% of psoriasis cases was in grade 2, and MVD ranges from 8–20 vessels/400x, with a mean of 13.74 ± 3.07 vessels/400x. Whereas the control shows 91.43% in grade 0, and MVD ranges from 2–4 vessels/400x with a mean of 3.39 ± 0.39 vessels/400x. In the study done by R. Sujatha et al[76]. and Rajan et al[77]., MVD was observed in psoriasis cases with a mean of 18.68 ± 5.20 vessels/400x and 15.30 ± 3.80 vessels/400x, respectively. Whereas the control shows a mean of 4.88 ± 2.26 vessels/400x and 5.15 ± 1.46, respectively (Table: 17), which is almost similar to the present study. We obtained a statistically significant p value when comparing with controls.

Table 17 : Distribution of MVD in psoriasis cases in various studies

R. Sujatha et al **(n=25)%**	**Rajan** et al **(n=20)%**	**present study (n=35)%**
18.68 ± 5.20	**15.30 ± 3.80**	**13.74 ± 3.07**

Table 18 : Distribution of MVC in psoriasis cases in various studies

Hern S et al (n=25)%	**Boruah et al (n=50)%**	**Present study (n=35)%**
6-17µm	**8.65-18.90 µm**	**7.60–14.10µm**

We observed MVC in psoriasis grades 2 and 3 at 48.57% and 51.43%, respectively, with a mean of 10.48 µm ± SD 2.20µm. Whereas the control shows 80% of cases in grade 1 with a mean of 4.48 µm ± SD 1.08 µm. In the study conducted by Hern S et al[78]., MVC in psoriasis cases ranged from 6 to 17 µm. Similarly, Boruah et al[79]. reported MVC values ranging from 8.65 to 18.90 µm, with an average of 11.82 µm, which is closely comparable to the findings of the present study. (Table: 18). We

obtained statistically significant p value on comparing with psoriasis cases and controls.

In our study, we found an inverse relationship between MVD and MVC, with an increase in MVD corresponding to a decrease in MVC. Research by Vasudevan B et al[56]. Demonstrated that inflammatory infiltration significantly increased MVC. Their findings suggest that in the context of inflammation, the microvascular caliber and MVD show a weaker association, implying that the dilation of existing blood vessels plays a more crucial role than the formation of new vessels.

Research on the relationship between MVD, MVC, and age and sex in various clinical circumstances conducted by Swift et al[80]., Pili et al[81]., and Marinho et al[82]. found that aging delays the formation of new vessels from pre-existing vasculature. Compared to control patients, MVD increased and MVC decreased with age in our research of psoriasis cases. This is not showing any statistical correlation in the present study. Those studies also clarify that in order for the formation of new vessels to proceed normally, endothelial cell activation, basement membrane degradation, migration, and proliferation are necessary. That is, age and sex is not an independent factor to explain MVD and MVC. Chronicity, severity, and the amount of keratinocytes under stress in psoriasis are also necessary features.

The blood vascular system has developed to regulate tissue homeostasis, according to the aforementioned results and statistical analysis. The quiescent contact between tissues is maintained by the vasculature under normal circumstances. Upon meeting an inflammatory insult, the vasculature undergoes modifications that aid the endothelium in actively regulating blood flow, permeability, leukocyte infiltration, and tissue edema. These modifications ultimately function to eliminate the original stimulus.

SUMMARY

- A total of 70 cases were included in our study. Amoung these, 35 psoriasis patients and 35 control subjects were studied to achieve a balanced and statistically valid comparison.
- Maximum psoriasis patients were within the 18-20 group (11 cases or 31.43%).
- Male preponderance was noted, (M: F=1.916:1).
- From clinical presentation, well defined erythematous plaques (100%), Silvery scales (100%), Auspitz sign (77.14%) are the main features.
- Histopathological evaluation revealed parakeratosis (100%), hypogranulosis (100%), perivascular lymphocytic infiltrate and dilated (100%) and tortuous blood vessels (100%) along with munro microabscess (94.29%) as the main histopathological features for the diagnosis of psoriasis.
- CD 34 IHC marker was used to study the vascular changes in psoriasis cases and control cases. Psoriasis cases had a mean MVD of 13.74 ± 3.07 vessels/40x and mean MVC of 10.48 μm ± SD 2.20μm, while control cases revealed a MVD of 3.39 ± 0.39 vessels/40x and MVC of 4.48 μm ± 1.08 μm.
- On grading MVD and MVC, maximum number of cases of psoriasis revealed MVD of grade 2 (80%), which is more than 10 vessels/40X. In maximum number of psoriasis cases MVC was grade 2 (48.57%) and 3 (51.43%), which is more than 5 μm and 10 μm respectively.
- Maximum number of controls revealed MVD of grade 0 (91.43%), which is less than 5 vessels/40X, and MVC was grade 1 (80%), which is less than 5 μm.

- MVD and MVC were found to be highly reliable to assess the vascular changes present in cases of psoriasis when compared with normal skin (P value: <0.05).

- In psoriasis cases, MVD shows a negative correlation with MVC, indicating that an increase in MVD is associated with a decrease in MVC. Conversely, in control cases, an increase in MVD corresponds to an increase in MVC. This relationship is statistically significant ($P < 0.05$).

- The analysis revealed that parakeratosis, hypogranulosis, perivascular lymphocytic infiltrate, and dilated tortuous blood vessels have the highest correlation with mean vascular density, with a chi-square value of 66.06. Conversely, Kajog pustules exhibit the lowest correlation among the features, with a chi-square value of 40.76. Despite this, all above features in psoriasis cases demonstrate statistical significance with p-values less than 0.05.

- Furthermore, parakeratosis, hypogranulosis, perivascular lymphocytic infiltrate, and dilated tortuous blood vessels show the strongest associations with mean vascular calibre, indicated by extremely low p-values (0.00675). Although elongation of rete ridges has the lowest significant correlation with mean vascular calibre, it remains statistically significant with a p-value below 0.05.

- There is no statistically significant relation of MVD and MVC with gender and age.

CONCLUSION

According to our research, psoriasis is dependent on angiogenesis because of the aberrant microvascular dilatation and increased endothelial microvasculature. The dilated and tortuous blood vessels in the histopathological findings of psoriasis can be explained by the vascular proliferation, which was studied by CD34 expression. Psoriasis cases show a negative correlation between MVD and MVC, while normal skin show a positive correlation between the two with increasing age.

LIMITATIONS OF THE STUDY

- Exclusion of patients with psoriasis who were already receiving treatment may have overlooked the effect of those medications on changes in cutaneous vascular changes.
- Compared to the psoriasis group, the control group included a higher proportion of older people (61–70 years old), which could introduce age-related bias. The results could be distorted by aging-related changes in vascular density and caliber. Hence inclusion of age matched controls would reduce the bias.
- Assessment of vascular changes by histopathology and immunohistochemistry is the main emphasis of this study. It may be difficult to determine the physiological significance of the reported changes in MVD and MVC without functional tests (such as blood flow measurements).

FUTURE SCOPE OF THE STUDY

- Using larger samples could identify the very early stages of psoriasis and thoroughly examine the level of inflammation along with MVD and MVC. This will help to determine whether the disease is chronic and identify the angiogenic pathways that could lead to the development of targeted anti-angiogenic therapy, which could be helpful for this debilitating chronic condition.
- Blood flow studies can be conducted using dermoscopy, a non-invasive imaging technique, to visualize and analyze the microcirculation within the skin. This method will allow for detailed observation of blood vessel morphology and flow patterns, providing valuable insights into the vascular characteristics of the study subjects

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ANNEXURE I - PROFORMA

CASE ☐ **CONTROL** ☐

PATIENT HISTORY

Name:

Age:

Date :

IP no.:

BRIEF CLINICAL HISTORY :

- ✓ Duration:
  - **i.** Onset
  - **ii.** Previous episode
  - **iii.** Changes in severity
  - **iv.** Seasonal variation
- ✓ Site of the lesion:
- ✓ Size of the lesion:
- ✓ Aggravating and relieving factor
- ✓ Associated symptoms

PAST HISTORY:

- ✓ Treatment history:

FAMILY HISTORY:

Anyone in the family having same symptoms?

PERSONAL HISTORY:

- ✓ Allergic history
- ✓ Occupation
- ✓ Smoking
- ✓ Alcohol
- ✓ Anxiety
- ✓ Exposure to irritants
- ✓ Medication
- ✓ Other significant history

GENERAL EXAMINATION :

- Morphology of lesions:

	YES	NO
1. Well defined erythematous plaques		
2. Silvery scales		
3. Involvement of scalp		
4. Involvement of pressure points		
5. Involvement of extensor aspect		
6. Involvement of nails		
7. Involvement of joint		

- BED SIDE TEST

BED SIDE TEST	POSITIVE	NEGATIVE
1. GRATTAGE TEST		
2. AUSPITZ SIGN		

CLINICAL DIAGNOSIS : _____

HISTOPATHOLOGICAL FINDINGS:

FINDINGS	YES	NO
1. PARAKERATOSIS		
2. ACANTHOSIS		
3. SPONGIOSIS		
4. ELONGATION OF RETE RIDGES		
5. KAJOG PUSTULES		
6. SUPRAPAPILLARY THINNING		
7. MUNRO'S MICRO ABSCESS		
8. DILATION AND TORTUOSITY OF CAPILLARY LOOPS IN DERMAL PAPILLAE		
9. LYMPHOCYTIC INFILTERATE IN THE UPPER DERMIS		

HISTOPATHOLOGICAL DIAGNOSIS: _____

DERMATOVASCULAR CHANGES (Morphometric Analysis):

	1st field	2nd field	3rd field	4th field	5th field	Mean value
Micro Vascular Density						
Micro Vascular Calibre						

ANNEXURE II - INFORMED CONSENT FORM

"STUDY OF VASCULAR CHANGES IN DERMIS IN PSORIASIS - AN OBSERVATIONAL STUDY"

Name of Student/Principal Investigator: BN0121011

Name of Guide/Co Investigators:

Objective: To study vascular changes in dermis in Psoriasis with the help of CD34 IHC staining procedure.

Introduction: Numerous diseases are linked to an insufficient or over-active vessels. So mainly vascular remodelling and inflammatory cells can be seen in psoriasis.

Clinically different lesions may show similar histological patterns. Therefore, though histopathology is considered the gold standard in dermatological diagnosis, in addition to routine staining procedure, special stains or immunohistochemical (IHC) stains may be required sometimes.

Explanation of procedure: Collecting detailed history of psoriasis attending the skin OPD and the skin punch biopsy received at the histopathological laboratory will be studied. Formalin fixed and paraffin embedded sections of 3-4 microns thick will be prepared and stained with hematoxylin and eosin, reticulin stain (where-ever possible) and CD34 IHC stain.After IHC stain with CD34, morphometric analysis of the blood vessel such as MVD, MVC will be done, using image analysis software (Gryphax) in psoriasis will be studied. Observations of cases will be compared with that of control samples. Results will be statistically analysed in the form of percentage comparing psoriasis and control cases. P value also will be calculated to note the statistical significance

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact: "BN0121011, **********, **********"gmail.com" If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"STUDY OF VASCULAR CHANGES IN DERMIS IN PSORIASIS - AN OBSERVATIONAL STUDY"**. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:**BN0121011**

Signature of the investigator:

ANNEXURE III

HEMATOXYLIN AND EOSIN STAIN

REAGENTS

1. Erhlich's Haematoxylin solution
2. Eosin Y solution 1%
3. 1% acid alcohol solution

HEMATOXYLIN AND EOSIN STAIN – PROCEDURE

1. Deparaffinise the tissue sections in xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
2. Subject the tissue section to water through reducing grades of alcohol (90% alcohol for 5 mins + 70% alcohol for 5 mins)
3. Keep it in hematoxylin for 8 to 10 minutes
4. Rinse it in tap water for 2 mins
5. Differentiate with 1% acid alcohol for 10 sec
6. For bluing - place in tap water for about 10 minutes
7. Counter stain by eosin 1-2 minutes
8. Rinse in water
9. Dehydration increasing grades of alcohol (70% alcohol for 30 sec + 90% alcohol for 30 sec)
10. Clearing is done by Xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
11. Mount it with Dibutylphalate Polystyrene Xylene (DPX).

ANNEXURE IV

IMMUNOHISTOCHEMISTRY

This is a two step indirect technique based on detection of antigens in the cells and tissues. It is a two step process:

1. Specific epitopes binds the primary antibody
2. The following step is calorimetric reaction to detect the antigen antibody binding

REAGENTS IN IMMUNOHISTOCHEMISTRY

i. Peroxide block which is 3% hydrogen peroxide in water

ii. Power block : Is a highly effective universal protein blocking agent. It consists of casein and propriety additives in PBS with 15Mm sodium azide.

iii. Chromogen : DAB – 3,3'- diaminobenzidine

iv. Liquid DAB substrate buffer contains peroxide and stabilizers

v. Superenhancer reagent

vi. Poly HRP reagent

vii. Mayer's haematoxylin is used for counterstaining

viii. Buffer solutions.

BUFFER SOLUTIONS

TRIS BUFFER (pH 7.6)

- ✓ TRIS buffer salt : 0.605 gm

- ✓ Sodium chloride : 8gm
- ✓ Distilled water : 1000 ml
- ✓ 1N Hydrochloric acid : 3ml

CITRATE BUFFER : (pH 6.0)

- ✓ Trisodium citrate : 2.94gm
- ✓ Distilled water : 1000ml
- ✓ 1N Hydrochloric acid : 5ml

TRIS EDTA : (pH 9.0)

- ✓ TRIS buffer salt : 6.05gm
- ✓ Disodium EDTA : 0.744gm
- ✓ Distilled water : 1000ml

IMMUNOHISTOCHEMICAL (IHC) STAINING – PROCEDURE

1. Sections are deparaffinised in xylene for 30 minutes.
2. Sections are washed in absolute alcohol for five minutes with two changes followed by tap water wash for ten minutes.
3. Sections are rinsed in distilled water for five minutes.
4. Antigen retrieval is done by immersing slides in appropriate buffer solutions in microwave – medium mode for fifteen minutes and high mode 10 minutes.
5. Cool to room temperature and then the slides are washed in distilled water.

6. The slides are washed in TBS buffer for five minutes with two changes. Sections are treated with peroxide block for ten minutes followed by wash in TBS buffer for five minutes with two changes.

7. Treat with power block for ten minutes.

8. Then drain the slides and cover the sections with primary antibody (supplied by biogenex) for one hour.

9. This is followed by TBS buffer wash for five minutes with two changes. Cover the sections with superenhancer for 30 minutes.

10. Wash in TBS buffer for five minutes with two changes. Cover the sections with poly HRP reagent for thirty minutes.

11. Then again wash the slides with TBS buffer for five minutes with two changes.

12. Sections are treated with DAB chromogen with substrate buffer for five to eight minutes.

13. Wash in TBS buffer with two changes, each wash for five minutes. Wash the slides under tap water for five minutes.

14. Counterstain the slides using Mayer's Haematoxylin for one minute. Wash under tap water for five minutes.

15. Slides are air dried and mount with cover slips using DPX mountant.

ANNEXURE - V

KEY TO MASTER CHART

Grade 0	:	Less than 4 vessels/400x
Grade 1	:	4-10 vessels/400x
Grade 2	:	11-20 vessels/400x
Grade 3	:	More than 20vessels/400x

ANNEXURE - VI - MASTER CHART

CASE NO	Age	SEX	Well defined erythematous plaques	Silvery scales	Involvement of scalp	Involvement of pressure points	Involvement of extensor aspect	Involvement of nails	Involvement of joint	Grattage test	Auspitz sign	PARAKERATOSIS	HYPOGRANULOSIS	SPONGIOSIS	MUNRO MICROABSCESS	SPONGIFORM PUSTULE OF KAIOG	SUPRAPAPILLARY THINNING	ELONGATION OF RETE RIDGES	PERIVASCULAR LYMPHOCYTIC INFILTERATE	DILATED AND TORTUOUS VESSELS IN DERMIS	OTHER FINDINGS	DIAGNOSIS	1	2	3	4	5	SUM	MEAN VASCULAR DENSITY	GRADING	MEAN VASCULAR CALIBRE
1	69	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	19	13	15	10	11	68	13.6	2	8.2
2	20	F	YES	YES	NO	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	SUBCORNEAL PUSTULES	PUSTULAR PSORIASIS	14	12	8	12	11	57	11.4	2	13.1
3	20	F	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	13	15	16	8	14	66	13.2	2	9.3
4	65	M	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	ORTHOKERATOSIS	CHRONIC PLAQUE PSORIASIS	21	16	25	18	24	104	20.8	3	8.7
5	36	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	14	10	11	6	8	49	9.8	1	14.1
6	26	F	YES	YES	YES	YES	YES	NO	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	15	12	14	16	8	65	13	2	8.5
7	65	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	13	12	19	15	16	75	15	2	12.3
8	19	M	YES	YES	YES	NO	NO	NO	NO	NO	NO	YES	YES	NO	YES	NO	NO	NO	YES	YES	NIL	EARLY PSORIASIS	12	7	11	12	12	54	10.8	2	14
9	42	M	YES	YES	NO	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	13	15	13	9	16	66	13.2	2	10.5
10	41	F	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	SUBCORNEAL PUSTULES	PUSTULAR PSORIASIS	14	12	15	12	15	68	13.6	2	8
11	58	F	YES	YES	NO	YES	NO	NO	NO	NO	NO	YES	YES	YES	YES	NO	NO	NO	YES	YES	NIL	EARLY PSORIASIS	11	8	7	6	10	42	8.4	1	13.1
12	19	F	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	14	18	19	15	9	75	15	2	15
13	33	F	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	15	13	10	14	18	70	14	2	9.2
14	33	M	YES	YES	NO	YES	NO	NO	NO	NO	NO	YES	YES	NO	YES	NO	NO	YES	YES	YES	NIL	EARLY PSORIASIS	12	9	13	12	14	60	12	2	11.5
15	19	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	14	11	14	15	9	63	12.6	2	7.6
16	68	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	14	16	8	15	14	67	13.4	2	7.7
17	20	F	YES	YES	NO	YES	YES	NO	NO	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	24	19	16	19	23	101	20.2	3	8.9
18	19	M	YES	YES	YES	NO	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	18	14	11	7	15	65	13	2	12.3
19	65	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	16	15	15	15	8	69	13.8	2	12.4
20	46	F	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	16	15	16	13	12	72	14.4	2	9.1
21	20	M	YES	YES	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	YES	NO	NO	YES	YES	YES	NIL	EARLY PSORIASIS	12	6	9	11	7	45	9	1	14
22	19	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	16	13	7	12	14	62	12.4	2	9.4
23	40	M	YES	YES	NO	YES	NO	NO	NO	NO	NO	YES	YES	YES	YES	NO	NO	NO	YES	YES	NIL	EARLY PSORIASIS	14	13	12	8	6	53	10.6	2	8.9
24	55	F	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	ORTHOKERATOSIS	CHRONIC PLAQUE PSORIASIS	18	23	15	18	23	97	19.4	2	8
25	33	F	YES	YES	NO	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	17	11	16	7	18	69	13.8	2	11.6

26	60	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	NIL	PSORIASIS	17	15	16	15	8	71	14.2	2	12
27	36	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	19	18	15	12	13	77	15.4	2	9.5
28	19	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	15	18	15	16	17	81	16.2	2	8
29	35	M	YES	YES	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	YES	NO	NO	NO	YES	YES	NIL	EARLY PSORIASIS	15	13	13	7	8	56	11.2	2	13.3
30	58	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	18	19	17	14	9	77	15.4	2	8.7
31	29	M	YES	YES	YES	YES	YES	NO	NO	NO	NO	YES	YES	YES	YES	NO	NO	YES	YES	YES	NIL	PSORIASIS	16	17	13	16	8	70	14	2	9
32	19	F	YES	YES	NO	YES	NO	NO	NO	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	8	12	5	9	8	42	8.4	1	11.6
33	52	M	YES	YES	YES	NO	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	20	24	18	22	17	101	20.2	3	10.7
34	45	M	YES	YES	NO	YES	YES	NO	NO	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	16	18	16	15	16	81	16.2	2	10.1
35	26	M	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NIL	PSORIASIS	15	12	11	15	13	66	13.2	2	8.6
36	55	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	2	4	4	3	16	3.2	0	4
37	63	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	5	3	2	2	16	3.2	0	4.9
38	67	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	4	4	3	4	17	3.4	0	4
39	22	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	3	3	4	17	3.4	0	4
40	45	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	5	3	4	4	18	3.6	0	5.8
41	55	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	3	5	1	4	17	3.4	0	4.4
42	44	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	2	4	3	4	16	3.2	0	4.6
43	64	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	3	3	2	2	14	2.8	0	4
44	67	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	5	3	1	4	4	17	3.4	0	4.7
45	70	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	2	2	2	13	2.6	0	4
46	55	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	2	4	1	14	2.8	0	4
47	67	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	3	6	3	19	3.8	0	4
48	33	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	2	4	3	1	14	2.8	0	3
49	18	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	5	4	4	4	20	4	1	5.4
50	38	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	6	3	3	4	2	18	3.6	0	4
51	34	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	5	2	5	2	17	3.4	0	4
52	68	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	1	3	3	4	6	17	3.4	0	4.8

53	66	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	3	5	3	4	17	3.4	0	7.1
54	45	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	4	4	4	5	19	3.8	0	4
55	66	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	2	3	6	4	18	3.6	0	4
56	43	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	2	4	5	4	17	3.4	0	4
57	68	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	5	4	4	4	19	3.8	0	5.2
58	35	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	5	4	2	18	3.6	0	3
59	27	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	2	4	2	1	12	2.4	0	3
60	29	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	3	3	4	3	17	3.4	0	4.5
61	46	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	1	3	4	3	15	3	0	4.3
62	35	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	5	4	4	3	20	4	1	5.2
63	39	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	4	5	3	2	18	3.6	0	4.2
64	65	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	3	3	3	16	3.2	0	3
65	67	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	3	3	5	4	18	3.6	0	5
66	34	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	2	4	2	6	3	17	3.4	0	5.4
67	65	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	2	4	2	5	16	3.2	0	4.1
68	66	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	5	4	4	3	3	19	3.8	0	4.8
69	45	F	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	4	3	3	4	4	18	3.6	0	4.4
70	34	M	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NIL	NORMAL SKIN	3	4	5	4	4	20	4	1	8.5