EXPRESSION OF ANGIOGENIC MARKERS: VASCULAR ENDOTHELIAL GROWTH FACTOR AND CD-34 IN PSORIATIC SKIN LESIONS

By

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DOCTOR OF MEDICINE
IN
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Under the guidance of

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April 2015

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"The man on top of the mountain didn't fall there"-Vince Lombardi

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Date: Signature of the Candidate

Place: Kolar **Dr. PRIYA T RAJAN**

Dedicated with

REVERENCE

to

My Parents

LIST OF ABBREVIATIONS

Sl No	Abbreviation	Expansion
1	AML	Acute Myeloid Leukaemia
2	APC	Antigen Presenting Cell
3	BFU	Blast Forming Unit
4	BFU-E	Blast Forming Unit-Erythroid
5	BM	Basement Membrane
6	CFU	Colony Forming Unit
7	CFU-GEMM	Colony Forming Unit-Granulocyte Erythroid Monocyte Megakaryocyte
8	CFU-GM	Colony Forming Unit-Granulocyte Monocyte
9	CM	Cultured Media
10	CNS	Central nervous system
11	COX-2	Cyclo-oxygenase 2
12	CsA	Cyclosporin A
13	DAB	Di-amino-benzidine
14	DFSP	Dermatofibrosarcoma protuberans
15	DPX	Distyrene Plasticizer Xylene
16	EC	Endothelial cell
17	ECM	Extra-cellular matrix
18	EDTA	Ethylene diamine tetra-acetic acid
19	FGF	Fibroblast Growth Factor
20	GM-CSF	Granulocyte-monocyte Colony Stimulating Factor
21	GRO-α	Growth Related Oncogene-α
22	H&E	Hematoxylin and Eosin
23	HIF	Hypoxia Inducible factor

24	HPF	High Power Field
25	HRP	Horse Radish Peroxidase
26	ICAM-1	Intracellular Cell Adhesion Molecule-1
27	IFN	Interferon
28	IHC	Immunohistochemistry
29	ILVEN	Inflammatory Linear Verrucous Epidermal Nevus
30	IP-10	Interferon γ induced Protein
31	LFA-1	Leukocyte Function Associated Antigen
32	MA	Monoclonal antibody
33	MCP-1	Monocyte Chemoattractant Protein-1
34	MGS	Melanocyte Growth Stimulating
35	MHC	Major Histo-Compatibility
36	mRNA	messenger Ribonucleic acid
37	MVD	Micro-vessel Density
38	NFAT	Nuclear Factor of Activated T cells
39	NGF	Nerve Growth Factor
40	NK	Natural Killer
41	PAI-2	Plasminogen Activator Inhibitor-2
42	PAL-E	Pathologische anatomie leiden Endothelium
43	PASI	Psoriasis Area and Severity Index
44	PDGF	Platelet Derived Growth Factor
45	PUVA	Phototherapy with Ultra-Violet A
46	SUP	Selective Ultraviolet Phototerapy
47	TARC	Thymus and Activation-Regulated Chemokine
48	TBS	TRIS Buffered Saline
49	TCR	T-Cell Receptor

50	TGF-a	Tumour Growth Factor-a
51	TGF-b	Tumour Growth Factor-b
52	TNF	Tumour Necrosis Factor
53	UVA/B/C	Ultra-Violet A/B/C
54	VCAM	Vascular Cell Adhesion Molecule
55	VEGF	Vascular Endothelial Growth Factor
56	VEGF-R	Vascular Endothelial Growth Factor-Receptor
57	VIP	Vasoactive Inhibitory Peptide
58	VLA	Very late antigen

ABSTRACT

Background

Psoriasis is a common skin disorder that affects one to two per cent of the population world over and significantly impairs quality of life**.** Psoriasis treatment is long-term as it is a chronic disease. Moreover, patients are often resistant to treatment and are frequently prone to relapses. Hence, there is a need for new treatment modalities for psoriasis. It has been suggested by various studies that psoriasis was dependent on angiogenesis and that vascular proliferation would be a good target for the development of drugs for the treatment of psoriasis. Identifying the role of the angiogenic factor VEGF in psoriasis could aid in understanding the disease pathogenesis as well as the development of a novel targeted approach for therapy.

Objectives

1. To study the expression of VEGF and CD34 in psoriatic skin lesions by immunohistochemical examination when compared to normal skin.

2. To compare the expression of VEGF and CD34 in psoriatic skin lesions with the histological grade.

Materials and methods

This study was conducted on patients presenting to the Department of Dermatology, R.L. Jalappa Hospital and Research Centre. 49 patients clinically diagnosed with psoriasis were selected and 5 mm punch biopsies were obtained from the skin lesions. 20 control biopsies were obtained from healthy volunteers (10) and autopsy specimens within 12 hours of death (10). The biopsies were subjected for histopathological examination for confirmation of diagnosis. Histological grade was

given to each lesion. Immunohistochemical evaluation was done for the psoriatic biopsies for the expression of Vascular Endothelial Growth Factor (VEGF) and micro-vessel density assessment using CD34 and compared with the controls.

Interpretation and Results

The most common age-group among the patients was 31-40 years (30.6%). The sex-ratio of the psoriatic patients was 1.45:1. Family history was known in only 23 of the total cases, out of which 17% (4) had one/ more members of the family suffering from psoriasis. The most common histological features seen included acanthosis (100%), parakeratosis (81.6%) and dermal inflammation (98%). The mean percentage expression of VEGF of total epidermal cells of 49 cases was 49.80 +/- 21.16% and that of the 20 controls was 3.95 +/- 3.94%. The difference was statistically significant with $p < 0.00001$. Mean micro-vessel density was 15.30 +/- 3.81 per HPF among cases. The mean micro-vessel density among controls was 5.16 +/-1.46 per HPF. The difference between the two values were statistically significant, with a p value less than 0.00001. Among the cases, it was seen that there is a positive correlation between VEGF expression by keratinocytes and the micro-vessel density in the papillary dermis ($r = 0.664$). The correlation is significant with p value less than 0.01. There was a positive correlation between the VEGF expression by keratinocytes and the histopathological grade ($r = 0.292$). However, the correlation was not significant with $p>0.05$. There was a positive correlation between the micro-vessel density and the histopathological grade ($r = 0.226$). The correlation was not significant with $p>0.05$.

Conclusion:

The results of this study showed that VEGF expression in keratinocytes is raised when compared to healthy skin. Also, the microvasculature in papillary dermis is increased when compared to healthy skin. There was a significant positive correlation between the degree of expression of VEGF by the epidermis and the micro-vessel density in the dermis. When compared to the histopathological grade, it was seen that there was no significant correlation with the VEGF expression or the micro-vessel density. Hence, it can be concluded that VEGF plays a significant role in the pathogenesis of psoriasis by way of its angiogenic activity in the lesions

Key words: Psoriasis, VEGF, immunohistochemistry, micro-vessel density.

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Introduction

Psoriasis is an inflammatory and chronic proliferative disorder of the skin. Psoriasis is manifested clinically as well-circumscribed papules and plaques which are erythematous and are covered with silvery scales. The lesions are most typically located in the extensor surfaces and scalp[1]. Various environmental and systemic factors influence the disease course. Its course is unpredictable, with spontaneous improvements and exacerbations of lesions without a known cause. Genetic predisposition as well as an immune system dysfunction is believed to be at the core of the disease process.

Psoriasis is a common skin disorder that affects one to two per cent of the population world over[1].In India, the prevalence varies from 0.44% to 2.8%. However, the data obtained is limited to that from tertiary care centers and found to be quite insufficient[2].

Psoriasis treatment is long-term as it is a chronic disease**.** Various treatment options are available. The main modalities include topical, phototherapy and systemic agents. They are used alone or in combination. However, patients are often resistant to treatment and are frequently prone to relapses. Hence, there is a need for new treatment modalities for psoriasis.

Studies first conducted by Folkman have shown that there is increased angiogenesis in psoriatic skin lesions[3]. Angiogenesis is mediated by various cytokines and inflammatory mediators. VEGF[4], CD-31[5]and Nerve Growth Factor (NGF)[6]are cytokines which have been established as markers of angiogenesis. By investigating the expression of these angiogenesis growth factors in psoriasis patients and healthy controls, the association of these angiogenesis growth factors with the pathogenesis of psoriasis can be established. This finding will be of great importance in the understanding of the nature and progression of the disease and also for therapeutic

purposes.Anti-VEGF therapy is currently under different stages of clinical trials for various malignancies. Targeted anti-VEGF therapy has been proven to reduce angiogenesis in cancers and play a role in preventing growth and progression of inflammatory lesions and tumours[7].

The study of the expression of VEGF and CD-34 in psoriatic skin lesions as compared to normal skin may help in the understanding of pathogenesis of psoriasis and may also aid in the development of targeted therapy.

Objectives of the study

1. To study the expression of VEGF and CD-34 in psoriatic skin lesions by immunohistochemical examination when compared to normal skin.

2. To compare the expression of VEGF and CD-34 in psoriatic skin lesions with the histological grade.

Review of literature:

Psoriasis

Psoriasis is a chronic inflammatory and proliferative disease involving skin and small joints. Psoriasis significantly impairs quality of life. In a majority of the patients, psoriasis manifests clinically by the age of thirty years. Psoriatic patients tend to develop a chronic and severe course of the disease.[1]

Epidemiology

Psoriasis is known to affect around 2% of the world population. Both sexes are equally affected. There is a bimodal age distribution among psoriatics, with one peak at the age-group of 15-20 years, and another peak at the age-group of 55-60 years[1].

Two types of psoriasis have been described based on the age of onset and inheritance pattern[8].

Type 1 psoriasis comprises of patients with age of onset below 40 years. This forms 65% of the psoriatic patients.

Type 2 psoriasis affects patients over 40 years of age, with no positive family history. Type 2 affects 35% of the population.

Prevalence of psoriasis in India

In India, the prevalence of psoriasis varies from 0.44 to 2.8%[2]. Psoriasis is found to be twice as more common in males than females as per most studies conducted in the country. Most of the patients are in their twenties or thirties at the time of presentation. These studies are limited by the absence of standardand accepted diagnostic criteria. Furthermore, there isn't any reliable information on time trends of the disease[2].Literature on the Indian scenario of patients affected by psoriasis is insufficient and the data is limited to that obtained from tertiary care centers alone.

Clinical features of psoriasis[8]:

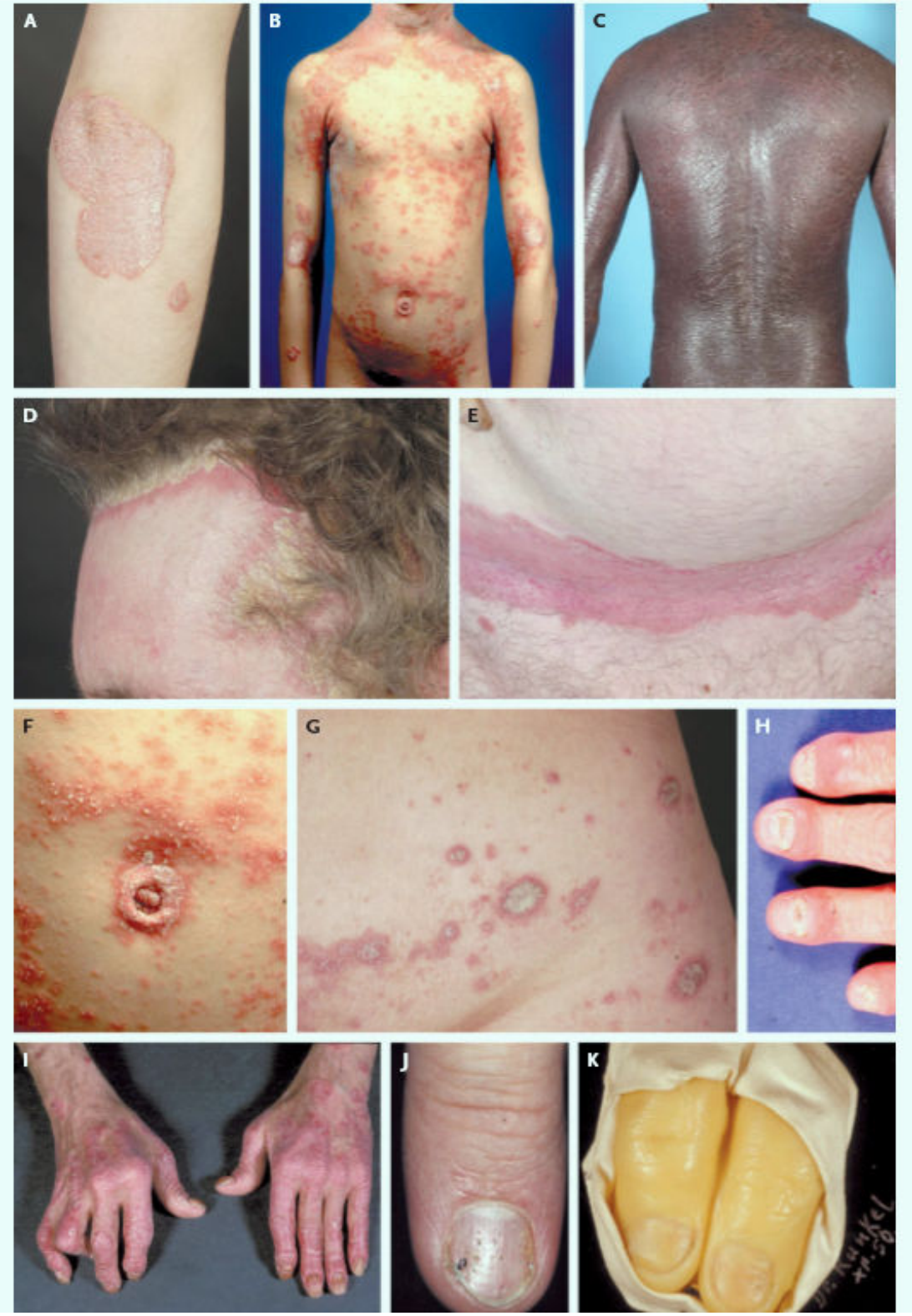
There are several psoriasis phenotypes. The most common clinical variant is psoriasis vulgaris, which affects approximately 85 to 90% of all patients with the disease.Psoriasis vulgaris typically consists of well-circumscribed erythematous plaques with a silvery white scale. When the scale is removed, characteristic bleeding points develop (Auspitz's sign). Pruritis is occasionally present. There is a predilection for the extensor surfaces of extremities, including the elbows, knees, sacral region, scalp and nails. Almost 50% of psoriasis patients develop nail changes, such as pitting, discoloration, subungual hyperkeratosis, and onycholysis. Scarring alopecia is rare. Oral lesions and lesions of the lips are uncommon. Penile lesions may be seen in uncircumcised men. Lesions are seen to develop at sites of trauma. In 5% or more of psoriatics, a sero-negative arthritis develops. This arthritis is either an oligoarthritis of distal interphalangeal joints or a polyarthritis of small joints in the hands, feet, and spine, which can lead to severe deformation. Arthritis often improves with cutaneous improvement.

Clinical variants:

Other clinical variants of psoriasis are guttate, erythrodermic and pustular. Each form can coexist or interchange with other variants. In erythrodermic psoriasis, skin of the affected individual is inflamed. Guttate psoriasis usually presents as sudden generalized appearance of multiple small red lesions on the trunk and proximal limbs. It is the most frequent subtype in children and young adults. Inverse or seborrheic psoriasis usually present as erythematous lesions in the intertriginous areas without scaling. The sides of the nose, mouth, and eyes or center of the chest might be affected. This variant often coexists with chronic plaque psoriasis. Pustular psoriasis is

characterized by localized or generalized sterile pustules in scaly and erythematous skin. Rare clinical variants include photosensitive psoriasis, a nevoid form, psoriasis spinulosa,follicular psoriasis, erythema gyratumrepens-like psoriasis, rupoid psoriasis, congenital erythrodermic psoriasis, annular veruccous psoriasis and linear psoriasis.

Figure 1: Clinical Features of Psoriasis.



A: The typical psoriatic lesion is a sharply demarcated erythematous plaque covered by silvery white scales, of- ten appearing on the elbow.

B: Initial eruptions of psoriasis may exhibit a guttate distribution pattern.

C: In a dark-skinned patient, erythrodermic psoriasis, a clinical subtype of the disease, affects the entire body surface.

D: Scalp involvement in a patient with psoriasis, and the lesions typically extend a short distance beyond the region covered by terminal hair.

E: Inverse psoriasis, which is located at intertriginous areas and usually shows only scant scaling.

F: Sterile pustules, which may be seen in many patients with psoriasis vulgaris

G: Generalized pustular psoriasis may start from coalescing disseminated pustules on deeply erythematous skin.

H: Shows the fingers of a patient with severe onychodystrophy.

I: Psoriatic arthritis

J: Nail involvement in a patient with psoriasis, and mild cases are characterized by small pits and yellowish discoloration of the nail plate.

K: Psoriatic nail pits were recreated in a wax-model moulage manufactured approximately 100 years ago.

Image source: Schön MP, Boehncke WH. Psoriasis. N Engl J Med 2005;352:1899-912.

Associated conditions:

Psoriasis has also been reported in association with vitiligo, benign migratory glossitis, minor hair shaft abnormalities, gout, gliadin antibodies, diabetes, ankylosing spondylitis, inflammatory linear verrucous epidermal nevus (ILVEN) and inflammatory bowel disease.

A family history of psoriasis as well as an association with HLA-Cw6 are often present in those with early onset. Psoriasis runs a chronic course, although spontaneous or treatment-induced remission has been known to occur. Psoriasis can have a significant effect on the quality of life in those with the disease.

Inheritance and genetic predisposition

Numerous family studies have provided compelling evidence of a genetic predisposition to psoriasis, although the inheritance pattern is still uncertain. As many as 71% of patients with childhood psoriasis have a positive family history[8].

One locus in the major- histocompatibility-complex (MHC) region on chromosome 6 has been replicated in several populations. This locus, termed psoriasis susceptibility 1 (PSORS1), is considered the most important susceptibility locus. Other susceptibility loci are located on chromosomes 17q25 (PSORS2), 4q34 (PSORS3), 1q (PSORS4), 3q21 (PSORS5), 19p13 (PSORS6) and 1p (PSORS7).

Most recently, an additional gene locus for psoriasis susceptibility has been discovered on chromosome 17q25. This locus, a runt-related transcription factor 1 (RUNX1) binding-site variant, encodes for a gene involved in the development of blood cells, including those of the immune system.

Subtypes of psoriasis[9]:

Type I psoriasis

Type I psoriasis, also called early-onset type psoriasis, is seen to occur before the age of 40 (usually at 16-22 years of age). Majority of patients with a positive family history demonstrate positivity to human lymphocyte antigen-Cw6 (HLA-Cw6). Cw6-positive patients typically present with a guttate psoriasis. These patients tend to develop extensive plaques and a more severe disease which worsens following throat infections. Koebner's phenomenon is more often present in Cw6-positive patients. These patients benefit from UV-phototherapy. Type I psoriasis has an irregular course and tends to generalize.

Type II psoriasis

The onset of type II psoriasis occurs after the age of 40 (usually at 57-60 years). This late- onset type presents with a minor hereditary association and no family history. Compared to the early-onset type, type II psoriasis is considered to be mild.

Trigger factors[9]

Psoriasis has been known to be associated with several triggering factors. Trauma to the skin by sunburns, surgery or from scratching can lead to development of psoriatic plaques in upto40% of the cases. This is also known as Koebner's phenomenon. It has also been shown that psychological stress, probably by neuro-immunological mechanisms, can cause exacerbation of psoriatic lesions.

Drugs triggering psoriasis include beta-blockers, anti-malarials, lithium and angiotensin-converting enzyme inhibitors[10]. Certain bacterial, viral and yeast infections have also known to be associated with the pathogenesis of psoriasis, in particular, Group A beta hemolytic

streptococcal infections[1]. Obesity, smoking and alcohol consumption have been significantly associated with increased frequency of psoriasis.

Clinical assessment of psoriasis

The current gold standard for assessment of extensive psoriasis has been the Psoriasis Area and Severity Index (PASI).The PASI is a measure of the average redness, thickness, and scaliness of the lesions (each graded on a 0–4 scale), weighted by the area of involvement[11].

The Psoriasis Area and Severity Index (PASI) is a widely used tool for the measurement of the severity of psoriasis. The PASI combines the assessment of the severity of lesions and the area affected, into a single score within the range of 0 to 72. The body is divided into four sections: head (10% of the body area), arms (20%), trunk (30%) and legs (40%). Each of these areas is scored separately, and the four scores are then combined. For each section, the percentage of the area of skin involved is estimated and then transformed into a grade from 0 to 6. The PASI is the most validated objective method to measure the severity of psoriasis and has a high intra-rater reliability and a good inter-observer correlation when used by trained assessors. The PASI system is sensitive to changes and reflects disease improvement or deterioration, although the sensitivity to change for small areas of involvement is poor. PASI 75 is a widely used concept, meaning the percentage of patients achieving a 75% improvement in PASI from baseline to the primary endpoint, usually 12 to 16 weeks of treatment. Achieving a 75% improvement in the PASI is considered to be successful treatment. PASI 50 (50% improvement) and PASI 90 (90% improvement) are sometimes also used.

Histopathology of psoriasis:

Histologically, chronic psoriasis plaques are characterized by typical changes in the epidermis and in the dermis[1,12]. The earliest changes, seen within 24 hours duration, consist of dilatation and congestion of vessels in the papillary dermis and a mild, perivascular, lymphocytic infiltrate. It is seen associated with some adjacent edema. There is also exocytosis of lymphocytes into the epidermis overlying the vessels and this is usually associated with mild spongiosis. The epidermis is usually normal[13].

Typical epidermal findings in well-developed lesions include hyper-proliferation of keratinocytes, leading to epidermal thickening and elongated rete ridges that form fingerlike protrusions into the dermis. This is termed as acanthosis. The granular layer of the epidermis, the site of origin of terminal keratinocyte differentiation, is strongly reduced or missing. The normally anuclear layer of cornified keratinocytes contains foci with nucleated keratinocytes is also termed as parakeratosis.

Within the dermis, inflammatory infiltrate composed of lymphocytes, macrophages, mast cells and neutrophils is observed. A subset of macrophages that are spindle shaped, situated along the basement membrane, has been described as a typical feature. These cells are positive for CD11c. Plasma cells and eosinophils are usually absent, but eosinophil cationic protein has been identified, particularly in the upper third of the epidermis in psoriasis. Elongated and dilated blood vessels in the dermal papillae represent a further histological hallmark of psoriatic skin lesions.Neutrophils localize to the dermis, migrate focally into the epidermis, and form Munro microabscesses, which become translocated upward within the epidermal stratum.[14]Similar collections in the spinous layer (spongiform pustules of Kogoj) are less common. In resolved or treated psoriatic lesions, there is progressive diminution in the inflammatory infiltrate, a

reduction in the degree of epidermal hyperplasia and restoration of granular layer. Vessels in the papillary dermis are still dilated, although by now there is an increase in fibroblasts in this region with mild fibrosis. After 10-14 weeks of treatment, the histological appearances return to normal.

Figure 2: Micro-photograph showing hyperkeratosis in a case of psoriasis

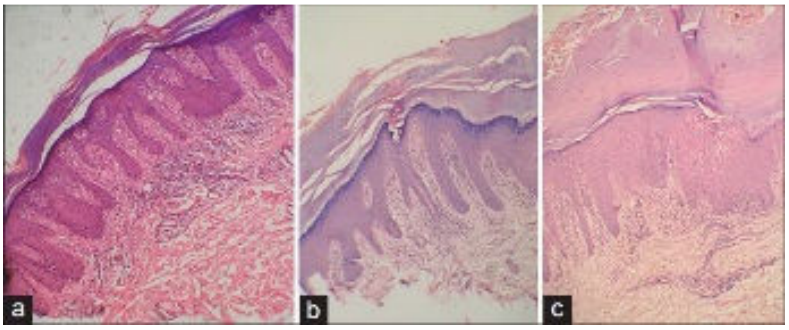


Figure 3: Micro-photograph showing acanthosis with supra-papillary thining in a case of psoriasis

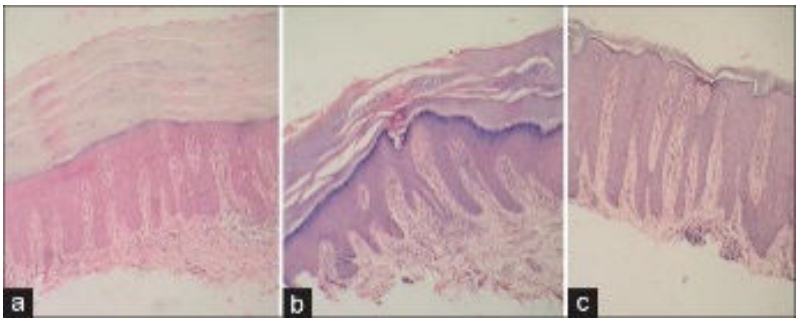


Figure 4: Micro-photograph showing dermal inflammation in a case of psoriasis

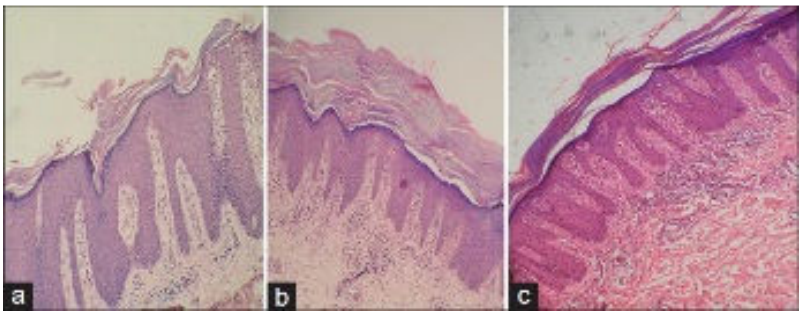


Figure 5: Histopathological Alterations in Psoriatic Skin.

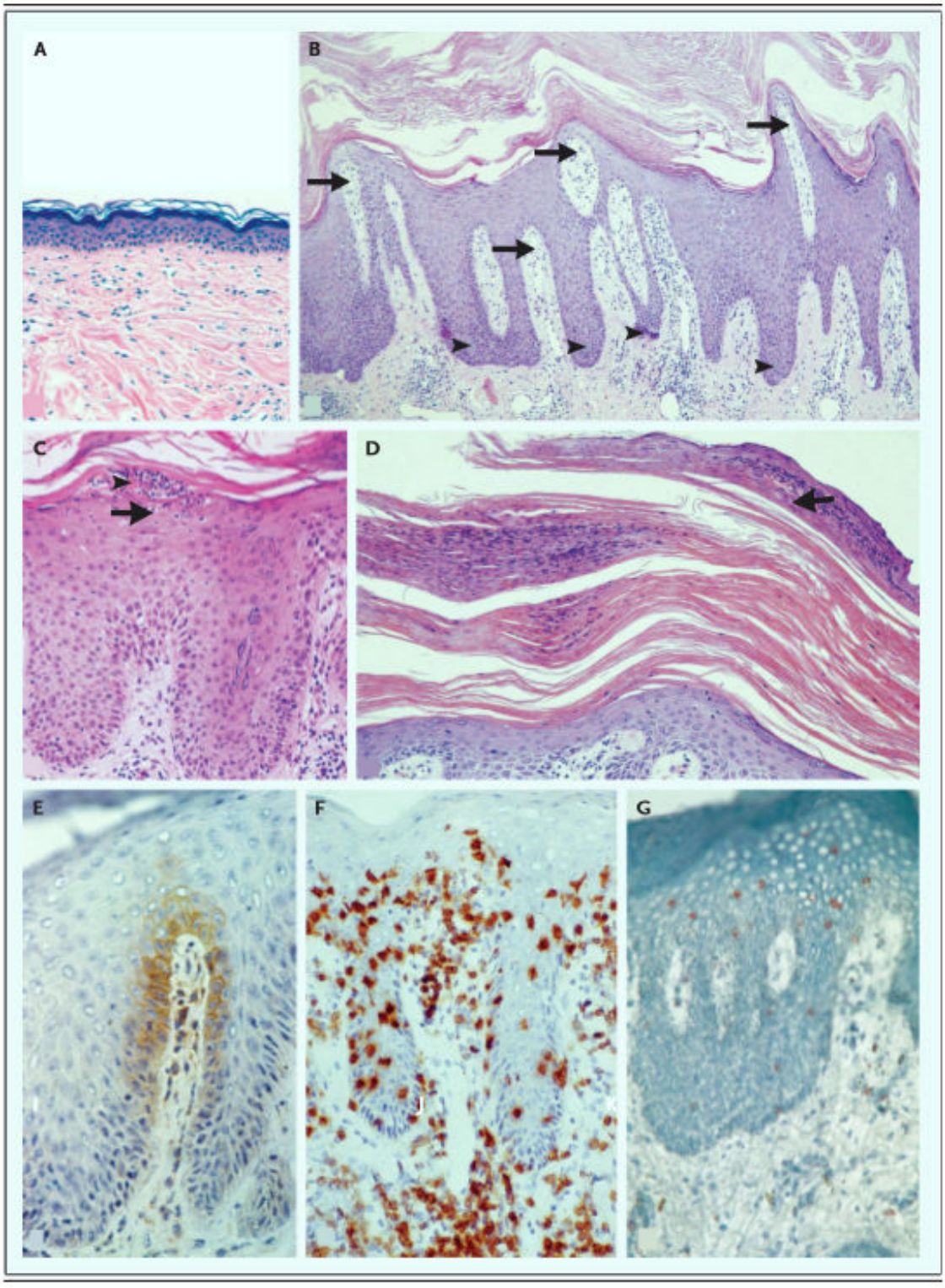


Figure 5: Histopathological Alterations in Psoriatic Skin.

A: Histology of normal skin

B: The epidermis in psoriatic skin is characterized by dramatic histopathological alterations, including profound acanthosis (thickening of the viable cell layers), elongation of epidermal rete ridges (arrowheads), marked hyperkeratosis (thickening of the cornified layer), loss of the granular layer, and parakeratosis (nuclei in the stratum corneum). In addition, dermal blood vessels are increased in number and size (by both angiogenesis and dilatation); they are contorted and reach up to locations directly underneath the epidermis (arrows). A mixed leukocytic infiltrate is seen in both dermis and epidermis.

C: As a histopathological hallmark of psoriatic lesions, neutrophilic granulocytes transmigrate through the epidermis (arrow) and form the telltale Munro microabscesses underneath the stratum corneum (arrowhead).

D: As the lesions progress, these microabscesses are transported to the upper layers of the stratum corneum, where they slough off (arrow).

E: Focal expression of intercellular adhesion molecule 1 (ICAM-1) in psoriatic epidermis indicates the activation of keratinocytes.

F: Immunostaining of CD3, a T-cell receptor–associated antigen, shows that abundant T lymphocytes are present in psoriatic skin within both the dermis and the epidermis.

G: CD103, an adhesion receptor that binds to epidermal E-cadherin, is expressed almost exclusively by intraepidermal T cells.

Image source: Schön MP, Boehncke WH. Psoriasis. N Engl J Med 2005;352:1899-912.

TABLE 1: Psoriasiform lesions and their differentiating features[13]:

Disease	**Histopathological features**
Psoriasis	Progressive psoriasiform epidermal hyperplasia, initially mild, mitoses in basal keratinocytes; dilated vessels in dermal papillae; parakeratosis, initially focal and containing neutrophils, later confluent with few neutrophils; thinning of the suprapapillary epidermis
Pustular psoriasis	Spongiform postulation overshadows epidermal hyperplasia; except in lesions of some duration when both are present
Reiter's symdrome	Closely resembles pustular psoriasis; the overlying, thick scale crust often detatches during processing
Pityriasisrubrapilaris	Alternating orthokeratosis and parakeratosis, vertically and horizontally; follicular plugging with parafollicularparakeratosis; mild to moderate epidermal hyperplasia; no neutrophil exocytosis
Parapsoriasis	Variable epidermal hyperplasia; the superficial perivascular or band-like infiltrate involves the papillary dermis; some exocytosis/epidermotropism; probably represents early cutaneous lymphoma
Lichen simplex chronicus	Conspicuous psoriasiform hyperplasia, sometimes irregular; prominent granular layer with patchy parakeratosis; thick suprapapillary epidermal plates; thick collagen with vertical streaks

	in papillary dermis; variable inflammatory infiltrate and plump fibroblasts
Chronic spongioticdermatitides	Progressive psoriasiform hyperplasia, usually with diminishing spongiosis eventually merging with picture of lichen simplex chronicus; chronic nummular lesions “untidy” with mild exocytosis; eosinophils may be present in nummukar and allergic contact lesions; cronic seborrheic dermatitis may mimic psoriasis but no neutrophils, less hyperplasia and sometimes perifollicularparakeratosis
Erythroderma	Variable psoriasiform hyperplasia, usually focal spongiosis, no distinguishing features
Mycosis fungoides	Epidermotropism; papillary dermal infiltrate of lymphocytes with variable cytological atypia
Chronic candidiasis and dermatophytosis	Psoriasiform hyperplasia, not as regular or marked as psoriasis; spongiform pustules or neutrophils in parakeratotic scale; fungal elements may be sparse in candidiasis
ILVEN: Inflammatory Linear Verrucous Epidermal Nevus	Papillatedpsoriasiform hyperplasia with foci of hyperkeratosis overlying hypogranulosis; often focal mild spongiosis; may have alternating orthokeratosis and parakeratosis in a horizontal direction.
Norwegian scabies	Marked orthokeratosis and scale crust; numerous mites, larvae and ova in the keratinous layer.
Bowen’s disease	Full thickness atypia of keratinocytes but basal layer sometimes spared; cells sometimes pale staining

Clear cell acanthoma	Pallor of keratinocytes but no atypia; abundant glycogen; some exocytosis of inflammatory cells
Lamellar icthyosis	Mild psoriasiform hyperplasia with a thick compact or laminated orthokeratin layer overlying a prominent granular layer.
Pityriasisrosea (herald patch)	Mild psoriasiform hyperplasia; spongiosis and exocytosis of lymphocytes leading to 'mini Pautrier simulants'; focal parakeratosis
Pellagra AcrodermatitisenteropathicaGlucagonoma syndrome	Mild to moderate psoriasiform hyperplasia; the upper epidermis shows pallor and ballooning progressing sometimes to necrosis, vesiculation or postulation (not in pellagra); confluent parakeratosis overlying these changes; many cases of pellagra show mild, even non-specific changes.
Secondary syphilis	Superficial and deep dermal infiltrate which often includes plasma cells; may have lichenoid changes or granuloma formation in late stages.

Histological grading of psoriasis:

Histological grading of psoriatic lesions was proposed by Trojak[15] in 1992. Since then it has been cited in various studies and aids in assessing the degree of histological changes in the given lesion. The schema offers a method of grading change that can be especially useful in studies of therapeutic agents.

TABLE 2: Trojaks' histopathological grading system for psoriatic lesions

No.	Microscopic criteria	Value/Criteria
1	Regular elongation of the rete ridge	1
2	Club shaped rete ridges	2
3	Elongation and edema of dermal papillae	1
4	Perivascular mononuclear infiltrate in the upper dermis of papillae	1
5	Absent granular layer: a. Focal b. Total	1 2
6	Parakeratosis : a. Focal b. Total	1 2
7	Suprapapillary plate thinning	2
8	Mitosis above basal cell layer	2
9	Munro microabscesses	3
10	Spongiform pustule	3
	TOTAL:	19

Pathogenesis of psoriasis

Dysregulation of T cell- keratinocyte interaction:

The primary pathogenic mechanism for psoriasis is still unknown. Keratinocytes, fibroblasts, antigen-presenting cells, T cells, and endothelial cells are known to play a role. It is likely, however, that abnormal regulation of T cell-keratinocyte interaction with a complex cytokine network is involved[10].

Hypothesizing that the primary defect resides in keratinocytes, the defective epidermal keratinocytes could be activated by physical or chemical injury increasing the synthesis and release of cytokines. This results in antigen-independent activation of T lymphocytes, which, in turn, releases additional cytokines stimulating inflammation and proliferation of keratinocytes and T lymphocytes.

Studies have demonstrated that cytokines secreted by psoriatic epidermal cells potentiate T lymphocyte activation to a greater extent than in normal epidermal cells. It is also postulated that only psoriatic keratinocytes respond to activated T cell messages with hyperproliferation, because of their specific receptors or signal-transducing mechanisms[16].

Only fibroblasts derived from either psoriatic lesional or nonlesional skin have been demonstrated to induce hyperproliferation of normal keratinocytes in a skin-equivalent system in vitro[17].

Hyperproliferation of keratinocytes

The cell cycle time of hyperproliferating psoriatic keratinocytes is short, and while maturation and shedding of keratinocytes takes 26 days in normal epidermis it occurs in 4 days in psoriatic epidermis[10]. Growth factors, coming from various cell types, are believed to control the increased proliferation.

Inflammation

T cells bind to the endothelium, and LFA-1 /ICAM and VLA/VCAM interact (LFA-1 = leukocyte function associated antigen, ICAM = intercellular adhesion molecule, VLA = very late antigen, VCAM = vascular cell adhesion molecule).

Diapedesis, migration through the vessel wall, occurs after binding. Activated T cells and secretions of other inflammatory cells, such as local macrophages, dendritic cells, vascular endothelial cells and keratinocytes, induce the keratinocyte changes (hyperproliferation) and the expression of adhesion molecules by endothelial cells. Activated keratinocytes produce growth factors stimulating neutrophil influx, vascular alterations, and keratinocyte hyperplasia[16,17].

Superantigens derived from bacteria can form a bridge between the APC and the T cell by binding to MHC class II and the TCR (an antigen-independent mechanism of T cell activation in psoriasis). Defined by the TCR rearrangements, the T cell population involved in psoriasis seems to be, however, clonally expanded to such an extent that a common psoriatic antigen may be causing effects in different patients and in different tissues affected by psoriasis[18].

Angiogenesis

The vasculature of the skin in adults remains dormant by the dominance of angiogenesis inhibitors over angiogenic stimuli. Pathological angiogenesis occurs in tumours and in chronic inflammatory processes like rheumatoid arthritis and psoriasis.

It was suggested by Folkman that psoriasis was angiogenesis-dependent and that vaso-proliferation would be a good target for the development drugs for the treatment of psoriasis[3]. Lesional psoriatic skin has dilated and elongated superficial capillaries passing into the dermal papillae with multiple vascular segments in the papillary tip. This represents increased tortuosity and coiling of the apical segment of the capillary loop.

Barton et al., in their study, demonstrated higher endothelial volume and luminal volume in the lesional psoriatic skin compared to uninvolved skin of patients with psoriasis as well as control subjects[19].

Morphometric analysis in a study conducted by Gupta et al[20] in 2011 revealed that length density of micro-vessels in psoriasis was significantly higher when compared to psoriasiform lesions. Micro-vessel density was also more in psoriasis than psoriasiform lesions. The study concluded that, in psoriasis there was significant vascular proliferation in response to inflammation, as indicated by tortuosity and elongation of vessels.

In a developing psoriatic plaque, endothelial cells swell and become activated showing prominent Golgi apparatus and Weibel-Palade bodies[21]. Activated endothelial cells migrate, sprout, and lay down a BM with pericytes for structural support to form novel vessel networks[22]. Although angiogenesis may not be the primary event in the pathogenesis of psoriasis, understanding the pathways leading to angioproliferation may help in finding novel antipsoriatic

drugs. In fact, vitamin D, retinoids, and cyclosporin all possess anti-angiogenic activity as well as anti-proliferative and anti-inflammatory effects[23].

Several pro-angiogenic factors like VEGFR3, VEGF-A, isoform VEGF121 are upregulated in psoriatic skin when compared to normal skin. Keratinocytees in lesional skin are a major source of pro-angiogenic cytokines[24].

Cytokine mediators

Several cytokines form a complex and multi-dimensional network in psoriasis pathobiology, none of which alone can be considered to be the causative mechanism[25].

Cytokines are essential in the various steps of psoriasis pathobiology. They influence keratinocyte proliferation, induce neutrophil and T cell chemotaxis, keep T cells in type 1 differentiation, enhance angiogenesis and upregulate adhesion molecules on endothelial cells, and stimulate the release of other chemokines. In psoriatic lesional skin, a large number of cytokines are reported to be up- or downregulated.

TNF-α production is an early event in cutaneous inflammation and is increased in psoriasis as well[31]. TNF-α stimulates the keratinocytes to produce IL-8, ICAM-1, TGF-α, β-defensins (antimicrobial peptides), and PAI2 (plasminogen activator inhibitor-2), which is thought to protect cells from apoptosis. In addition, TNF-α upregulates CD40 and MHC-II proteins on keratinocytes[17]. Macrophages also enhance their pro-inflammatory cytokine and chemokine production when stimulated by TNF-α.

Endothelial cells express adhesion molecules and increase production of VEGF leading to increased angiogenesis and erythema. TNF-α is produced by stimulated Langerhans cells, macrophages, monocytes, T cells, and keratinocytes[17,26].

IFN-γ induces the expression of the adhesion molecule ICAM-1 on keratinocytes and endothelial cells, influencing the trafficking of T lymphocytes into lesional epidermis[12,17]. IFN-γ and IL-2 activated keratinocytes secrete IL-1, IL-6, IL-8, IFN-γ, TNF-α, and TGF-α and chemokines MIG and IP-10, which influence both themselves and other cell types including T lymphocytes[10,17]. IFN-γ also stimulates TNF-α release from dermal macrophages or monocytes[17]. Findings suggest an altered response by psoriatic keratinocytes to γ-IFN, which could contribute to both the increased proliferation and impaired differentiation of epidermal cells in psoriasis. IFN-γ stimulates APC activity and upregulates a number of TNF-α receptors[25].

GM-CSF, produced by various cell types including keratinocytes, in response to IL-1 or TNF-α among others, increases keratinocyte proliferation and activates neutrophils[25,27]. It also stimulates migration and proliferation of endothelial cells[28]. Therefore, psoriasis is exacerbated with GM-CSF therapy[29].

IL-1 family consists of IL-1α, IL-1β, and IL-1-receptor-antagonist, which are produced mainly by keratinocytes in skin. IL-1 is a direct keratinocyte mitogen itself, and mediates angiogenesis, activates T lymphocytes, and induces other cytokines (i.e., TNF-α, IL-2, IFN-γ, TGF-α, IL-6, IL-8, GM-CSF[25,27,30]. IL-2 is a growth factor and chemoattractant for T cells and induces T cell cytotoxicity as well as stimulating NK cell activity[25]. IL-6 acts as a major mediator of the host response to injury and infection. IL-6 is produced by keratinocytes, fibroblasts, endothelial cells, and by T cells. Psoriatic keratinocytes seem to be more sensitive to the growth-promoting effect of IL-6 than normal ones[25].IL-8 is the main chemotactic signal for neutrophils to migrate into the

epidermis and for T cells.It also enhances the activation and proliferation of T lymphocytes and is reported to stimulate angiogenesis[25,26].

bFGF has mitogenic and angiogenic properties and is found not only basally but alsosuprabasally in psoriasis[31]. NGF (nerve growth factor) induces chemokine expression in keratinocytes[32], and keratinocytes in lesional and nonlesional psoriatic tissue, in turn, express high levels of NGF. NGF stimulates keratinocyte and endothelial cell proliferation and adherence molecule expression[28]. Endothelin-1, produced in part by endothelial cells, is mitogenic to keratinocytes and a chemoattractant to neutrophils[25].

Several chemokines are known to be upregulated in psoriatic skin: activated dermal endothelial cells can synthesize TARC, MIG, and IP-10, which are chemoattractants to T cells. MIG and IP-10 are also synthesized by epidermal keratinocytes in response to IFN-γ[30]. The synthesis of MGS/GROα is stimulated by TNF-α and IL-1 in vitro, and it attracts neutrophils to the psoriatic epidermis[25]. MCP-1 is a monocyte chemoattractant stimulated by IL-1, TNF-α, IFN-γ, and TGF-α.

Psoriasis and VEGF

VEGF, along with other angiogenic cytokines, regulates vascular growth and remodeling in psoriastic lesions[30,33]. Leukocytes show increased adhesion to selectins and VCAM expressed on new vessels in skin, and therefore VEGF may be the link between angiogenesis and cell-mediated inflammation in psoriasis[34]. VEGF together with bFGF enhances Ang2 (angiopoietin) and Tie 2 (endothelial-specific receptor) expression in dermal microvascular endothelial cell cultures. Ang2, in turn, destabilizes vessels by blocking Tie2 signaling and acts with VEGF to initiate angiogenesis[31].

VEGF was first described as vascular permeability factor and constitutes a homodiemric glycoprotein of 40-45kDa in its active form. The receptors for VEGF, VEGF-R1 and VEGF-R2 are primarily expressed by endothelial cells. VEGF binding to these receptors causes receptor activation and intra-cellular signal transduction. VEGF signaling often represents a critical rate limiting step in physiological angiogenesis. VEGF promotes thegrowth of endothelial cells derived from arteries, veins and lymphatics and is known to be a survival factor for endothelial cells.

VEGF family is divided into VEGF-A, VEGF-B, VEGF-C and VEGF-D and PIGF (Placeneta Growth Factor)[35]. The VEGF-A subfamily had four isoforms: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$. VEGF-A regulates angiogenesis and lymphangiogenesis in embryonic, postnatal development, wound healing and skeletal growth. VEGF-B is found predominantly in myocardium and muscle tissues. It also plays a role in embryonic development as well as vasculogeesis. VEGF-C and VEGF-D are lymphangiogenic in nature. PIGF is expressed during wound healing, ischemia, inflammation and in tumorigenesis.

VEGF also has the ability to induce vascular leakage and hence has a significant role in inflammation and other pathologies.

VEGF also contributes to keratinocyte proliferation and epidermal barrier homeostasis. VEGF receptors are both detectable and functional in keratinocytes. Physiological production of VEGF has been shown to contribute to normal proliferation, differentiation and function of the epidermis. Consequently VEGF over-expression in psoriasis may contribute to the epidermal changes in this disease[36].

Young et al[37]provided the first genetic evidence that an angiogenic constitution could determine psoriasis susceptibility based on the analysis of single nucleotide polymorphisms of the VEGF gene in psoriatic and healthy individuals. The authors demonstrated for the first time that the distinct small nucleotide polymorphisms of the VEGF gene occur more frequently in subset of psoriasis patients and they suggest that these haplotypes could contribute to the elevated VEGF levels in observed patients. Because of the increasing evidence that VEGF mediated activation of vascular endothelium plays a major role in the pathogenesis of psoriasis, these findings further suggest that the individual "angiogenetic constitution" determines disease susceptibility. Additional emerging evidence for a role of VEGF in the etiology of psoriasis comes from recent genetic analyses showing an association between VEGF promoter polymorphisms and the development of psoriatic symptoms.

A major role of VEGF in the pathogenesis of skin inflammation in psoriasis was further confirmed by several experimental models: Detmar et al. observed a chronic inflammatory skin disease resembling human psoriasis in a transgenic mouse model using the keratin 14 promoter to selectively target expression of murine $VEGF_{164}$ to basal epidermal keratinocytes[38]. These VEGF transgenic mice showed severe cutaneous inflammation and were characterized by a significant increase in the density of dermal blood vessels, predominantly capillaries that were tortuous and displayed increased branching. Moreover, blood vessels in the skin of VEGF transgenic mice were hyperpermeable for circulating plasma proteins. Interestingly, VEGF over-expressing mice were also characterized by greatly enhanced rolling and adhesion of peripheral blood mononuclear cells on cutaneous post-capillary venules, suggesting that VEGF, in addition to its pro-angiogenic activity, might also contribute to the recruitment of leukocytes to inflamed

skin. Moreover, VEGF transgenic mice showed the characteristic Koebner phenomenon, with induction of chronic psoriasis- like lesions by unspecific skin irritation. As a surprising finding, VEGF transgenic mice also showed a significantly increased density of cutaneous mast cells, mostly located around dermal blood vessels. These findings suggest that VEGF might act indirectly on mast cells by inducing the release of mast cells- activating factors by endothelial cells.

Besides K14- VEGF transgenic mice, another genetically engineered mouse model of psoriasis that targets the vascular endothelium includes the Tie2 transgenic mouse model. Tie2 transgenic mice were constructed using a driver transgene, pTek-tTA, which localizes Tie2, the receptor for angiopoietin-1 and angiopoietin-2, to vascular endothelium as well as to keratinocytes in the epidermis and hair follicles. Like K14-VEGF mice, type 2 transgenic mice also develop a vasculocentric cutaneous inflammatory disease with vascular hyperplasia, increased VEGF protein expression, epidermal acanthosis and psoriasis- like skin inflammation.

Leukocyte traffic into the dermis in psoriasis is enhanced by local VEGF production – VEGF itself promotes vascular permeability and is chemoattractant for monocytes and macrophages that express the VEGF receptor flt-1.

Diaz et al showed in their study that retinoids, which are used in the systemic treatment of psoriasis, block production of VEGF through a direct effect on the VEGF gene[39].In a study conducted, a significant overexpression of VEGFR3 (expressed by lymphatic endothelial cells) and the VEGF-A isoform VEGF121 (responsible for increasing vascular permeability) was observed in the non-lesional psoriatic skin as compared to that of normal volunteers[23].

It has been shown that sera from patients with psoriasis have enhanced VEGF levels. Moreover, serum-VEGF levels correlate with disease severity. In addition, single nucleotide polymorphisms of the VEGF gene strongly correlated with psoriasis pathogenesis, suggesting that VEGF represents a modifier gene in the aetiology of psoriasis[40].

VEGF is also being currently evaluated as a prognostic marker in malignancies of the breast, ovary, colon, rectum, oesophagus, stomach and head and neck tumours[7].

Treatment[41]:

The traditional approach of the psoriasis treatment involves a start with less aggressive therapeutic modalities (such as topical therapy, phototherapy) and, if the answer to this is unsatisfactory, a move to a more aggressive therapy (systemic, biological preparations). Local treatment is the most common method, being usually the first choice, especially for small and medium-sized forms of psoriasis.

There are several categories of local treatments: -

Keratolytic agents: salicylic acid, prepared at various concentrations from 2 to 10%. It can be used alone, but more often in combination with a dermatocorticoid; urea, lactic acid.

Oxidation reduction agents: vegetable tars (anthralin, cignolin), wood tars, coal tars or bituminous rock tars.

Topical corticosteroids: For more than four decades the topical corticosteroids were used generally in the treatment of skin diseases and specifically in the psoriatic disease. The topical corticoids remain the main treatment of psoriasis, despite the introduction of new topical non-steroidal.

Vitamin D Analogs: calcipotriol, the most used product, especially in combination with topical corticoids; maxacalcitolol and tacalcitol.

Topical Retinoids: topical tazarotene

Phototherapy remains one of the key therapeutic options for patients with moderate to severe psoriasis. Currently are in use different treatment schemes that use phototherapy sources. These are: PUVA therapy, broadband UVB, narrowband UVB, selective phototherapy (selective ultraviolet phototerapy SUP). It has to be mentioned also the heliomarine cure.

In the systemic treatment of psoriasis there are used multiple agents, providing a good control of the disease to most of patients and also improving the life quality indices. Often it is used a combined therapeutic scheme to increase the efficiency of medications.

The following systemic agents have been described in detail:

Methotrexate, used for patients with psoriasis vulgaris who have more than 10-20% body surface area affected, pustular psoriasis, erythrodermic psoriasis, arthropatic psoriasis and, not least, for patients with psoriasis vulgaris resistant to other therapeutic modalities (local or phototherapy)

Cyclosporine is an immunosuppressive agent with the same indications as methotrexate.

Aromatic retinoids (etetrinat and acitretin) designate the compounds whose action reproduce the biological effects of vitamin A. They are used mainly in the treatment of psoriasis vulgaris and pustular psoriasis.

The biological medication represents an edge of the medical technology and research, being used in the treatment of psoriasis. The preparations currently used in the treatment of psoriasis are:

TNF alpha targeting agents: etanerceptum, infliximabum, adalimumabum.

In order to reduce the toxicity of the systemic therapies, there are used combinations of various medications or the so-called rotation therapy or combination therapy, in which the systemic medication is subsequently replaced by another. The phototherapy is often associated with systemic and topical therapy. Rarely are made associations between two systemic therapies. The use of combination therapy is more effective than monotherapy; more, the toxicity of individual treatment may be decreased, because the doses are often lower.

Anti- angiogenic therapies

As angiogenesis is closely linked with the clinical manifestations of psoriasis anti-angiogenic therapies may represent promising treatment approaches. Established systemic therapies for psoriasis, such as methotrexate and cyclosporine, TNF antagonists or inhibitors of T-cell migration, interfere with both immune activation and pro-angiogenic mediators in psoriasis[40].

Drugs having anti- angiogenic actions include the following. SU-5416 blocks the action of VEGF receptor signaling is under Phase I/II clinical trial for Kaposi's sarcoma, advanced malignancies, in Phase II for von-Hippel Lindau disease, Phase III trials for metastatic colorectal cancer. SU-6668 acts by blocking VEGF, FGF and PDGF receptor signaling. SU-6668 is in Phase I clinical trials against advanced tumours[7].

VEGF as a target for psoriasis treatment

Therapies targeting VEGF signaling might be beneficial in the treatment of psoriasis. Indeed, there is some clinical evidence of complete remission of psoriasis following anti-VEGF treatment. Akmanet al[42] reported a patient with psoriasis who had complete remission of psoriasis during bevacizumab (monoclonal antibody against VEGF) therapy for colon cancer. A

dramatic improvement in the patients' psoriasis occurred 45 days after the first infusion. During a follow-up of 3 months, no relapse of the disease was observed despite no further treatment for psoriasis. This report supports the potential benefit of blocking VEGF in psoriasis. However, further studies are required before any definitive conclusions can be made about the usefulness of bevacizumab or other anti-VEGF drugs in the treatment of psoriasis patients.

Still, there is an increasing body of experimental data demonstrating the benefit of anti-VEGF approaches in the treatment of psoriasis. Recently, Schonthaleret al[43] used the anti-VEGF antibody G6-31, which potently inhibits both human and murine VEGF, as a therapeutic approach in a mouse model of chronic psoriasis- like skin inflammation (double knockout for c-Jun and JunB). These mice showed a dramatic decrease in disease activity and anti-VEGF treatment reversed the inflammatory skin phenotype. Furthermore, blocking of VEGF inhibited the development of cutaneous inflammation and these mice showed a pronounced reduction of the inflammatory cell infiltrate within the dermis and normal epidermal differentiation. These findings indicate that inhibition of the angiogenic growth factor VEGF is able to reverse a psoriasis-like skin phenotype including the epidermal abnormalities.

Furthermore, there is evidence that standard treatments used in the management of psoriasis patients might target directly or indirectly the VEGF pathway. As an example, anti-TNFα treatment reduces VEGF and thereby reduces blood supply at the site of the tissue damage and psoriatic inflammation. It has also been reported that cyclosporine A (CsA) which inhibits the activity of transcription factors of the nuclear factor of activated T cells (NFAT) family inhibits migration of primary endothelial cells and angiogenesis induced VEGF. This effect appears to be mediated through inhibition of cyclooxygenase (cox-2) which is activated by VEGF in primary

endothelial cells. This finding suggests that CsA might mediate beneficial effects in diseases such as psoriasis and rheumatoid arthritis through its effects on the vasculature.

CD-34

CD-34 is a commonly used marker of hematopoietic progenitor cells and endothelial cells. It is an intercellular adhesion protein and cell surface glycoprotein. The CD-34 ligand is CD62L, which is also known as L-selectin.

Structure: The CD-34 antigen is a single-chain trans-membrane glycoprotein, 105 to 120 kilodaltons (kd). The antigen is associated with human hematopoietic progenitor cells and is a differentiation stage-specific leucocyte antigen.The CD-34 antigen is present on immature hematopoietic precursor cells and all hematopoietic colony-forming cells in bone marrow and blood, including unipotent (CFU-GM, BFU-E) and pluripotent progenitors (CFU-GEMM, CFU-Mix, and CFU-Blast).Terminal deoxynucleotidyltransferase-positive B- and T-lymphoid precursors in normal bone marrow are CD-34^{+}.The CD-34 antigen is present on early myeloid cells that express the CD33 antigen but lack the CD14 and CD15 antigens and on early erythroid cells that express the CD71 antigen and dimly express the CD45 antigen. CD-34 may mediate attachment of hematopoietic stem cells to bone marrow extracellular matrix or directly to stromal cells, although its specific function is still unknown. CD-34 staining defines adult hematopoietic stem cells, but CD-34+ cells can also differentiate into neural cells[43]Normal peripheral blood lymphocytes, monocytes, granulocytes, and platelets do not express the CD-34 antigen. CD-34 antigen density is highest on early hematopoietic progenitor cells and decreases as cells mature. The antigen is absent on fully differentiated hematopoietic cells.Most CD-34^{+} cells reciprocally express either the CD45RO or CD45RA antigens, with the $CD45RO^{+}$ population being the more primitive. Approximately 60% of acute B-lymphoid leukemias and acute myeloid leukemias

(AMLs) and 1% to 5% of acute T-lymphoid leukemias express the CD-34 antigen. The antigen is not expressed on chronic lymphoid leukemias or lymphomas.

The CD-34 antigen is also found on capillary endothelial cells and approximately 1% of human thymocytes.Normal histological structures that are CD-34+ include the following: endothelium of blood vessels (including alveolar wall capillaries and glomeruli, but not hepatic sinusoids or splenic sinusoids)[44]. Other cells are dendritic interstitial cells, dermal dendrocytes, endometrial stroma, endoneurium, fibroblasts, fibrocytes, follicle cells, interstitial cells of Cajal (20%), mast cells, megakaryocytes, neural stem cells in CNS[43], osteoblasts, perivascular stroma, umbilical cord blood[50] and vascular adventitial fibroblastic cells in stomach.

CD 34 plays a role in dermatopathology and in diagnoses of soft tissue tumours. It aids to distinguish CD-34+ dermal neoplasms such as Kaposi's sarcoma, dermatofibrosarcoma protuberans / DSFP (both CD-34+) and epithelioid sarcoma (often CD-34+) from dematofibroma (CD-34-). It also helps to distinguish solitary fibrous tumor (CD-34+) from desmoplastic mesothelioma (CD-34-). Other examples include distinguishing hemangiopericytoma (CD-34+) from endometrial stromal sarcoma (CD-34-).CD-34 was strongly expressed by micro-vessels within benign as well as malignant tissues. Studies have shown that assessment of micro-vessel density using CD-34 has helped to accurately predict the survival and outcome in prostatic carcinomas[46], and in hepato-cellular carcinomas[45].

In one study done to assess expression of angiogenic markers in gastric carcinomas, morphometry was used. The markers that were compared included CD-34, CD-31 and vWF. Microvascular density was determined using the stereological parameter of "length density" and the "hot spots". It was observed that CD-34-stained preparations were the easiest to assess. The

number of labeled vessels, especially micro-vessels, was also the highest in the case of the above reaction. The results achieved in angiogenesis evaluation using various endothelial markers were not directly comparable. The vascular network density was significantly associated with tumor stage. Such an association was most clearly seen in CD-34 reactions[47].CD-34 used in a panel with CD-31 and Factor VIII has also been used to mark Kaposi's sarcoma and angiosarcomas. Other studies have also indicated that CD-34 and CD-31 can be used as markers for myeloid progenitor cells that recognize different subsets of myeloid leukemia infiltrates (granular sarcomas)[47].

MVD assessment:

A large body of studies on immunohistochemical staining of micro-vessels and subsequent assessment of micro-vessel density shows an association of the degree of intratumoral neovascularization with metastatic disease and poor prognosis in a variety of human cancers. Anti-Factor VIII antibody was one of the first used for micro-vessel staining and the most popular in the published clinicopathological studies. However, new antibodies with a higher specificity, such as anti-CD-31, anti-CD-34, and PAL-E, have recently been introduced.

Materials and methods

We have studied a total of 49 punch biopsies from cases which were newly diagnosed with psoriasis from the department of dermatology at R.L.Jalappa Hospital and Research Centre between January 2007 to July 2014.20 punch biopsies were taken for the control group. 10 were from healthy volunteers, 10 were from fresh autopsy specimens, within 12 hours of death.

Study Duration:

The study was conducted from January 2013 to July 2014, for a period of 18 months.

Study Design:

Laboratory observation and descriptive study.

Prior to the study ethical clearance was obtained from the institutional ethical board.

Inclusion Criteria:

1. All patients who visited the Dermatology OPD at RL Jalappa Hospital and Research Center with skin manifestations of psoriasis and were newly diagnosed with psoriasis.
2. Skin biopsy from histopathologically proven cases of psoriasis.

Exclusion Criteria:

Clinically suspected cases of psoriasis, but not confirmed by histopathology.

Data collection and processing:

DEMOGRAPHIC AND CLINICAL DETAILS

The following details of the patients were noted:

1. The age and sex of the patient
2. The selected site for punch biopsy.
3. Treatment history of the patient, wherever available.

The punch biopsy includes the full thickness skin and subcutaneous fat and was done by using round punch biopsy needle with a 5mm internal diameter. The skin was swabbed with an antiseptic and then injected with a local anaesthetic prior to obtaining the biopsy. Tissue was fixed in 10% formalin and processed further.

Control specimens:

5 mm Punch biopsies were obtained from healthy volunteers(10) as well as fresh autopsy specimens(10) after obtaining a written consent from the relatives. The biopsies were matched with the psoriatic patient specimens with respect to the age group.

Microscopic Examination

1. Skin biopsy specimens were routinely processed and stained with H&E staining.
2. H & E sections were examined and reviewed for histopathological diagnosis of psoriasis and histopathologicalgrading was done based on Trojak's grading system for psoriatic lesions.

Histological grading technique

Psoriasis can easily be confused for other psoriasiformdermatoses. The diagnostic features on histopathology include the presence of psoriasiformacanthosis with regular elongation and club shaped rete ridges with suprapapillary thinning, Munro's microabscess, or the presence of Spongiform pustule of Kogoj.

TABLE 3: Trojak'shistopathological grading system for psoriatic lesions:

No	Microscopic criteria	Max Score
1	Regular elongation of the rete ridge	1
2	Club shaped rete ridges	2
3	Elongation and edema of dermal papillae	1
4	Perivascular mononuclear infiltrate in the upper dermis of papillae	1
5	Absent granular layer: a. Focal b. Total	1 2
6	Parakeratosis : a. Focal b. Total	1 2
7	Suprapapillary plate thinning	2
8	Mitosis above basal cell layer	2
9	Munro microabscesses	3
10	Spongiform pustule of Kogoj	3
	Total	**19**

Immunohistochemical technique (IHC)

The immunohistochemistry (IHC) was performed on 4-μm thick sections from 10% formalin-fixed paraffin-embedded tissues, using non –biotin polymer based HRP detection system.

TABLE 4: Antibodies used in the current study

Antigen	Clone	Species	Producer	Dilution	Control	Stain
CD-34	QBEND/10	Mouse	Biogenex	Ready to use	Tonsil	Endothelium
VEGF	Polyclonal	Mouse	Biogenex	Ready to use	Placenta	Keratinocytes

The VEGF polyclonal antibody used was of the subtype $VEGF_{165}$, which has the highest expression by keratinocytes.

The IHC procedure includes the following steps:

1. Sections are cut 3-5mm thickness, floated on to organo-saline coated slide and left on hot plate at 60^{o} over night
2. **Deparaffinization**using Xylene I and II for 15 min each
3. **Dexylenisation**using absolute alcohol I and II for1 min each
4. **Dealcoholisation**using 90% and 70% alcohol for 1 min each
5. Washing with distilled water.
6. **Antigen Retrieval technique:**

   VEGF polyclonal antibody: Microwave power 10 for 6 minutes in TRIS EDTA buffer of pH-9.0.

CD-34: Microwave power 10 in TRIS EDTA buffer of pH-9.0 for 2 cycles of 6 minutes each.

7. Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH- 7.6) - 5minutes x 2 times-wash.
8. **Peroxidase block**- 30minutes to block endogenous peroxidase enzyme. TBS buffer for 5 minutes washing for 3 times.
9. **Power block**- 30 minutes to block non- specific reaction with other tissue Antigen.
10. VEGF: Cover sections with targeted primary antibody for 40 minutes.

CD-34: Cover sections with targeted primary antibody for 1 hour.

11. TBS buffer- 5min x 3 times.
12. **Super Enhancer**- 45 minutes to enhance the reaction between primary and secondary antibodies.
13. TBS buffer- 5min x 3 times
14. Super sensitive poly- HRP (secondary antibody) for 30 min
15. TBS buffer- 5min x 3 times
16. **Color development** with working color development solution (DAB) for 5-8 min

All the slides were examined for colour development

17. TBS wash- 5min x 3 times
18. Counter stain with Harris Haematoxylin for 30 sec
19. Tap water wash for 5 minutes.
20. Dehydrate and clear.
21. Mount with DPX.

Micro-vessel Density (MVD) quantification:

Quantification of angiogenesis was done for punch biopsies from both cases and controls using the following method:

The Micro-Vessel Density (MVD) index was evaluated as described by Behrem et al[48,49]. The method used to evaluate micro-vessel density of CD-34 stained slides was as follows.

First, the slide was screened at low power (40×) to identify areas of the highest vascularization in the papillary dermis, which were also known as hot spots.

Second, vessels were counted in three such high density areas at high power magnification (400×).

Areas with a dense hemorrhagic infiltrate were excluded. Vessels of a caliber larger than approximately 8 red blood cells were excluded from the count.

Vessel quantification was performed on x400 field. Single endothelial cells or a cluster of endothelial cells positive for CD-34 are regarded as a distinct single countable micro-vessel. The total count of micro-vessels are defined as micro-vessels per high power field. The average counts of three fields were taken. The micro-vessel count was restricted to the papillay dermis, which is the known site for increased microvasculature in psoriasis. The values were taken as final after a mutual consensus was obtained between the two observers and was entered as the MVD/HPF for each individual lesion. The micro-vessel density was also categorized into mild, moderate and strong positivity. This was based on the number of blood vessels seen per high power field[50]:

Mild [≤10/HPF]	Moderate [11-20/HPF]	Strong [>20/HPF]

The microscope used was Olympus CH20i with a field of view number 18 and high power area of 0.152 mm^2.

High power field area was calculated as follows:

Diameter of microscopic field[49] = Field of view number / Initial magnification of high power objective

= 18/40 =0.45mm

High power field area = $\prod r^2$

= $0.152mm^2$

The average micro-vessel density calculated per high power field was then converted to MVD/mm^2 for each biopsy assessed.

Grading of VEGF staining:

Punch biopsies from cases and controls are taken for immunohistochemical evaluation. Immunostaining for VEGF was semi-quantitatively scored as done in studies conducted by Soo et al[51,52]:

0 : Negative

1 : Weak (<10% expression of cells)

2 : Moderate (10-20% expression of cells)

3 : Strong (Expression in >50%)

A cell showing granular positivity within the cytoplasm is considered to be positive for VEGF. Supra-basillar layers of the epidermis alone are assessed for VEGF expression.

The mean percentage of positive cells for the expression was determined in atleast 5 areas at 400x magnification. Biopsies with less than 10 percent positive stained cells were defined as negative.

Statistical Analysis

The demographic data was analyzed using descriptive statistics. The VEGF and micro-vessel density values were compared using independent t test to compare the mean values. p value of less than 0.05 was considered significant.

The correlation between the VEGF expression by keratinocytes and micro-vessel density among cases and controls individually was found using Pearsons correlation. p value of less than 0.05 was considered to be statistically significant. SPSS software was used to make the necessary calculations.

RESULTS

The current study deals with histopathological and immunohistochemical evaluation of psoriatic skin biopsy specimens. Punch biopsies obtained from psoriatic patients from January 2007 to July 2014 were selected. All cases which satisfied the inclusion criteria were included in the study. A total of 49 cases and 20 controls were studied.

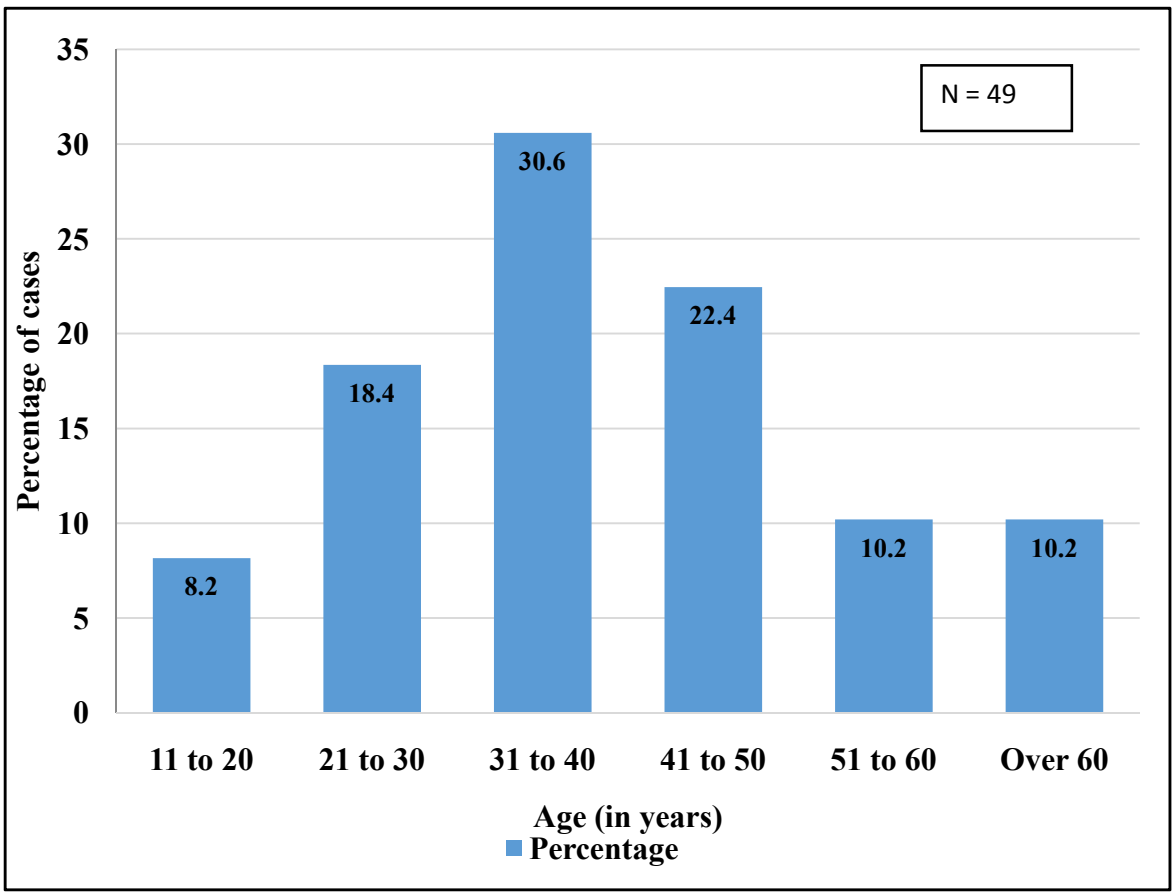
AGE DISTRIBUTION

In the present study, age group ranged from 15 to 70 years with mean age of 39.67 +/- 14.94 years. Majority of the patients belonged to 31-40 years which constituted 15 cases (30.6 %), followed by 11 cases (22.45 %) where patients belonged to age group 41-50 yrs.

TABLE 5: Age-wise distribution of psoriatic patients

Age Group (Years)	Number	Percentage
≤10	0	0
11-20	4	8.2%
21-30	9	18.4%
31-40	15	30.6%
41-50	11	22.4%
51-60	5	10.2%
>60	5	10.2%
Total	49	100%

FIGURE 6: Age-wise distribution of psoriatic patients



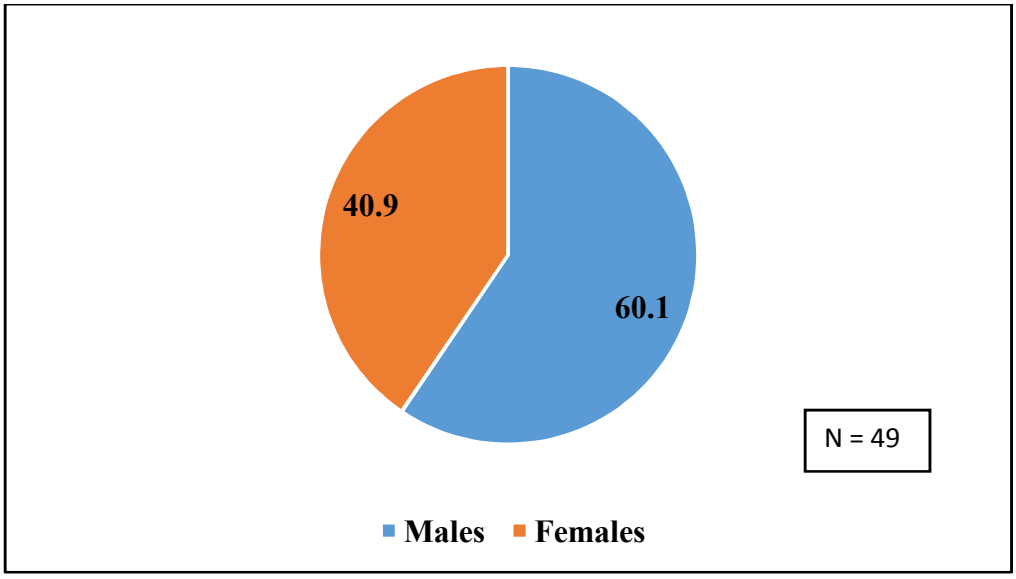
SEX DISTRIBUTION

The study included 49 patients, out of which, 29 were males (59.1%) and 20 were females (40.9%). The Male: Female ratio was 1.45:1.

TABLE 6: Sex Distribution of psoriatic patients

Sex	Number	Percentage
Male	29	59.1%
Female	20	40.9%
Total	49	100%

FIGURE 7: Sex distribution of psoriatic patients



Positive family history among psoriatic patients:

Family history was known in only 23 of the total cases, out of which 17% (4) had one/ more members of the family suffering from psoriasis.

TABLE 7: Frequency of histological features in skin biopsy

Histological Feature	No. of cases	Percentage
Epidermal changes		
Acanthosis	49	100%
Parakeratosis	40	81.6%
Suprapapillary thinning	37	75.5%
Spongiform pustule of Kogoj	29	59.1%
Munro microabscesses	31	63.3%
Hypogranulosis	27	55.1%
Dermal changes		
Papillary edema	24	49%
Dermal inflammation	48	98%

The most common features seen on histopathological examination of psoriatic lesions in decreasing order of frequency acanthosis (100%), dermal inflammation (98%), parakeratosis (81.6%).

FIGURE 8: Frequency of histological features in skin biopsy specimens

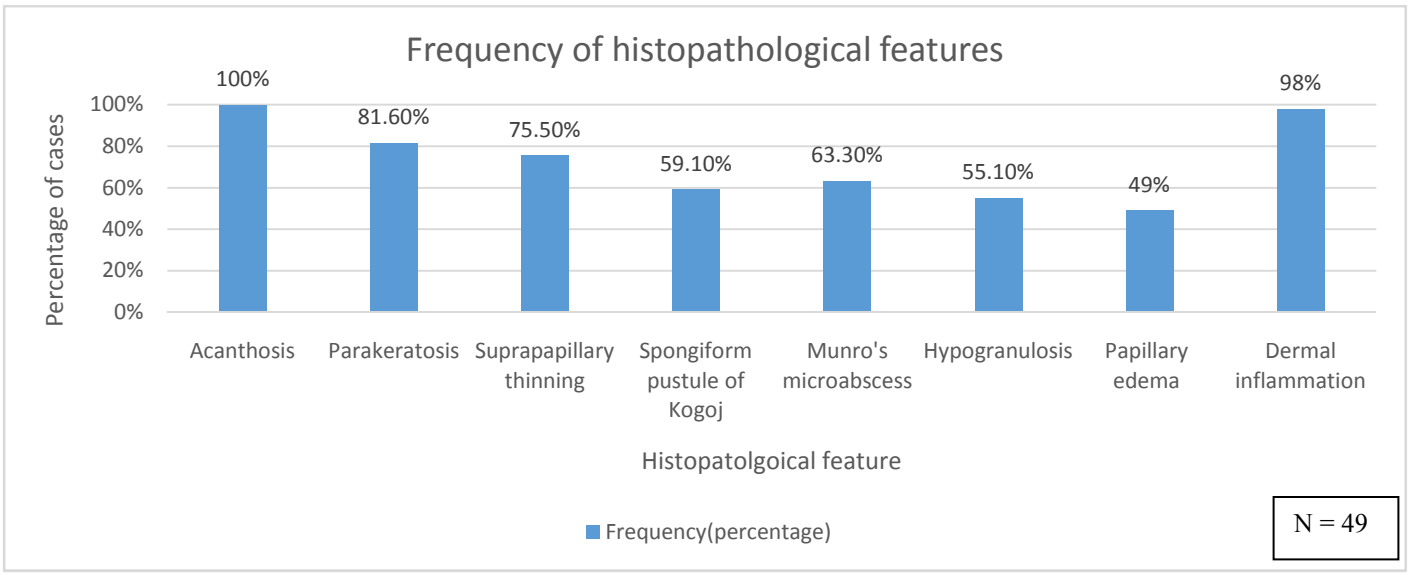
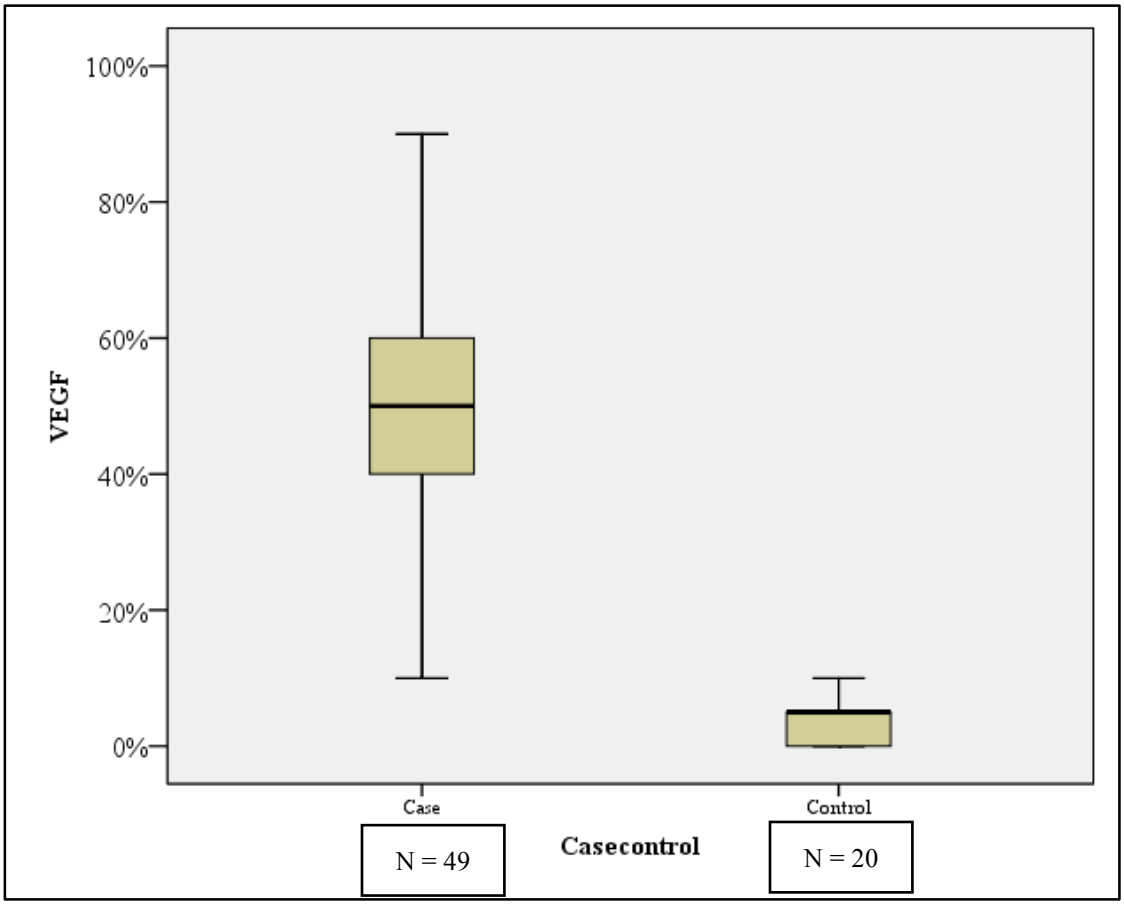


TABLE 8: VEGF expression by keratinocytes in Cases & Controls

		N	Mean (% of keratinocytes)	Std. Deviation(%)	Std. Error Mean	p Value
VEGF	Cases	49	49.80	21.16	3.02	
	Controls	20	3.95	3.94	0.90	
						<0.000001

The mean percentage expression of VEGF of total epidermal cells of 49 cases was 49.80 +/- 21.16% and that of the 20 controls was 3.95 +/- 3.94%. The difference in the values were statistically significant with a p value less than 0.000001.

FIGURE 9: Box-plot of VGEF expression (as percentage of positive cells among keratinocytes) of cases and controls



MICRO-VESSEL DENSITY ASSESSMENT

The current study showed a mean MVD among psoriatic patients to be 15.30 +/- 3.81 per HPF or 100.67+/- 25.04 per mm^2. The mean value among healthy controls was 5.16 +/-1.46 per HPF or 33.93 +/- 9.625 per mm^2. There was a significant reduction in MVD among controls (p value< 0.00001).

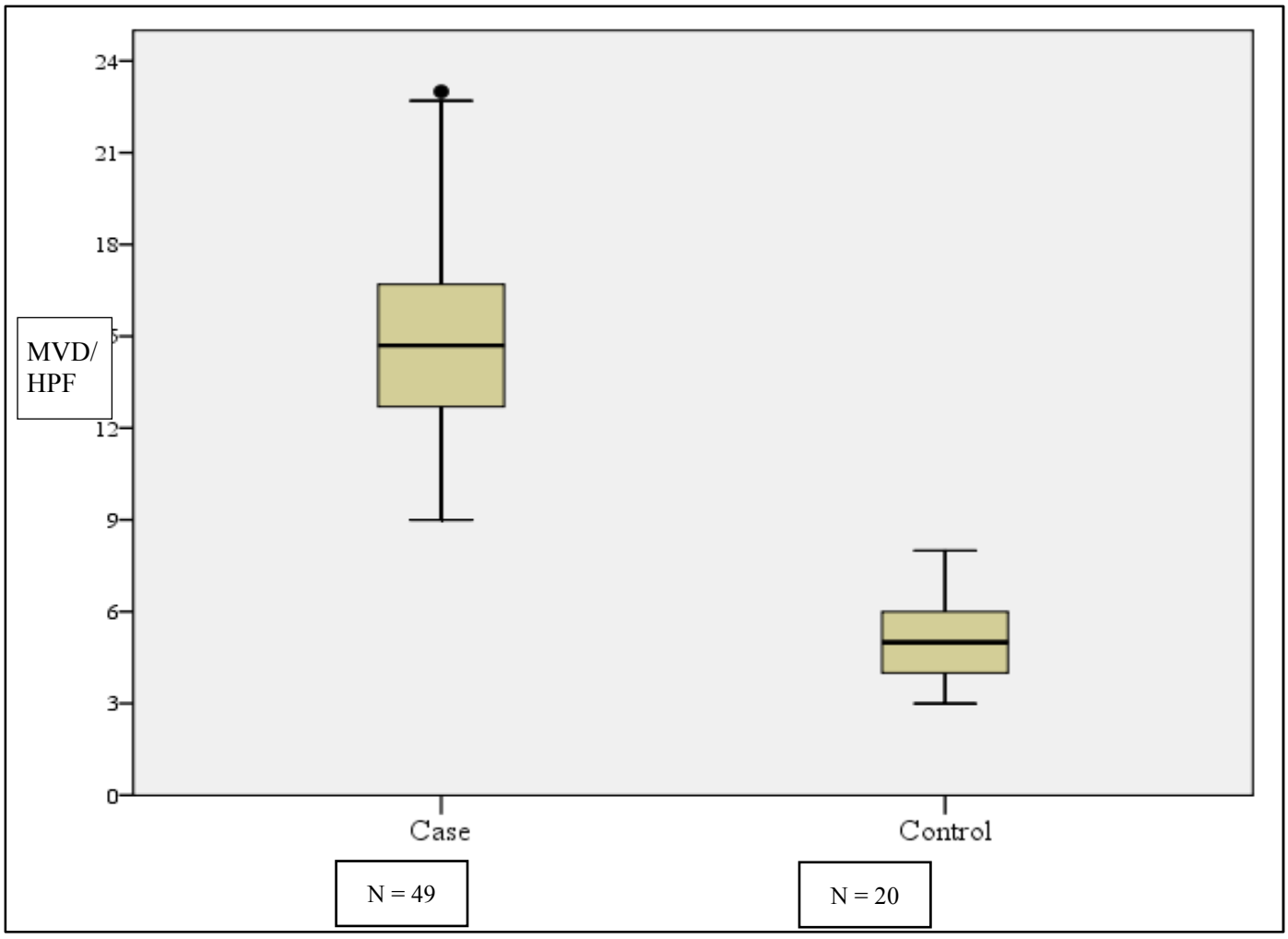
TABLE 9: Comparison of mean values of micro-vessel density among cases and controls

		Total number	Mean (per HPF)	Std. Deviation	Std. Error Mean	p value
MVD	**Case**	49	15.302	3.8061	0.5437	
	Control	20	5.158	1.4630	0.3356	
						<0.00001

TABLE 10:Categorical Distribution of cases and controls based on Micro-vessel density

	Cases		Controls	
Category	**No of cases**	**Percentage of cases**	**No of cases**	**Percentage of controls**
Mild [≤10/HPF]	1	2%	20	100%
Moderate [11-20/HPF]	37	75.5%	0	0%
Strong [>20/HPF]	11	22.5%	0	0%
Total	**49**	**100%**	**20**	**100%**

FIGURE 10: Box-plot graph depicting micro-vessel density of cases and controls



The micro-vessel density of the cases ranges from 9/HPF to 23/HPF. The values at the first, second, third and fourth quartile are 9/HPF, 13/HPF, 16.5/HPF and 23/HPF respectively.

The mean value is 15.30 +/- 3.81 per HPF.

The micro-vessel density of controls ranges from 3/HPF to 8/HPF. The values at the first, second, third and fourth quartile are 3/HPF, 4/HPF, 6/HPF and 8/HPF respectively. The mean value is 5.16 +/-1.46 per HPF.

CORRELATION BETWEEN MICRO-VESSEL DENSITY AND VEGF EXPRESSION

TABLE 11: Correlation between Micro-vessel density and VEGF expression of cases

		Micro-vessel density	VEGF expression
Micro-vessel density	Pearson Correlation	1	.664**
	Sig. (2-tailed)		.000
	N	49	49
**** Correlation is significant at the 0.01 level (2-tailed).**			

Among the cases, it was seen that there is a positive correlation between VEGF expression by keratinocytes and the micro-vessel density in the papillary dermis (r = 0.664). The correlation is significant with p value less than 0.01.

TABLE 12: Correlation between Micro-vessel density and VEGF expression of controls

		Micro-vessel density	VEGF expression
Micro-vessel density	Pearson Correlation	1	.657**
	Sig. (2-tailed)		.002
	N	20	20
**** Correlation is significant at the 0.01 level (2-tailed).**			

Among the controls, it was seen that there is a positive correlation between VEGF expression by keratinocytes and the micro-vessel density in the papillary dermis (r = 0.657). The correlation is significant with p value less than 0.01.

FIGURE 11: Scatter plot depicting the correlation between the micro-vessel density and VEGF expression of the cases.

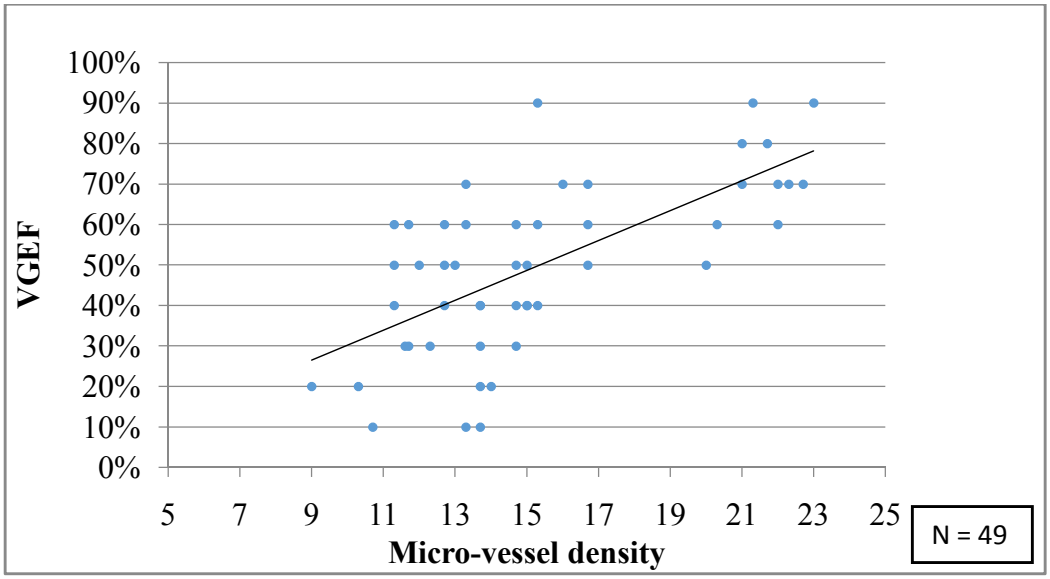


FIGURE 12: Scatter plot depicting the correlation between the micro-vessel density and VEGF expression of the controls.

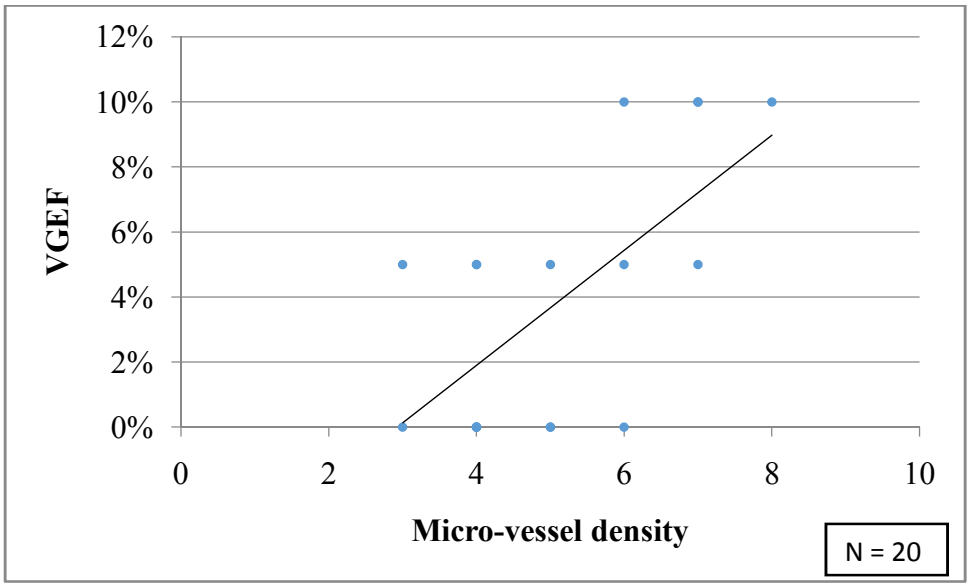


TABLE 13: Comparison of histopathological grade with VEGF expression of cases

		Histopathological grade	VEGF expression
VEGF Expression	Pearson Correlation	.154	1
	Sig. (2-tailed)	.292	
	N	49	49

There was a positive correlation between the VEGF expression by keratinocytes and the histopathological grade (r = 0.292). However, the correlation was not significant with p>0.05.

TABLE 14: Comparison of histopathological grade with micro-vessel density of cases

		Histopathology grade	Micro-vessel density
Micro-vessel density	Pearson Correlation	.226	1
	Sig. (2-tailed)	.118	
	N	49	49

There was a positive correlation between the micro-vessel density and the histopathological grade (r = 0.226). However, the correlation was not significant with p > 0.05.

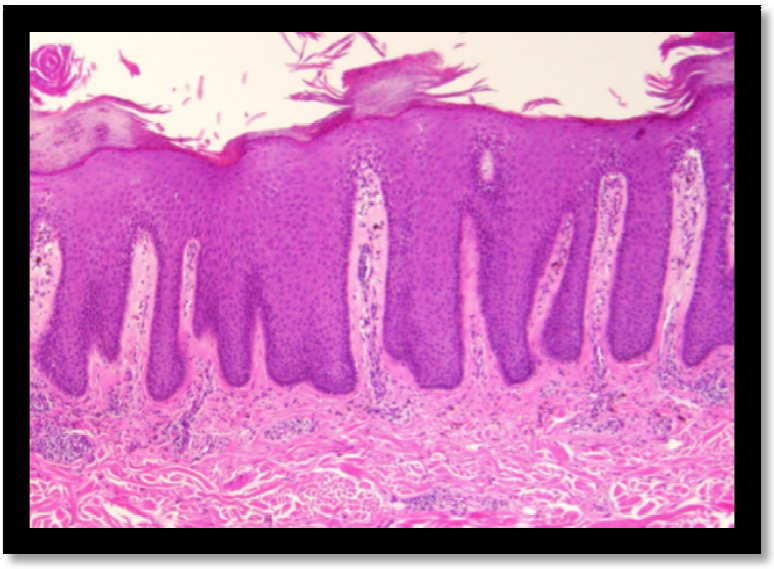


FIGURE 13: Microphotograph showing regular acanthosis with supra-papillary thinning in a case of psoriasis. 100x. (B/676/13)

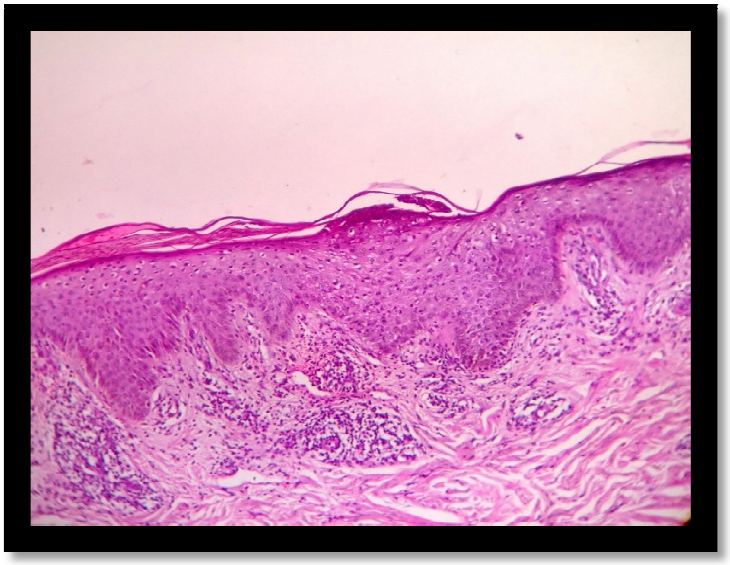


FIGURE 14: Microphotograph showing dermal peri-vascular inflammation in a case of psoriasis. 100x. (B/676/13)

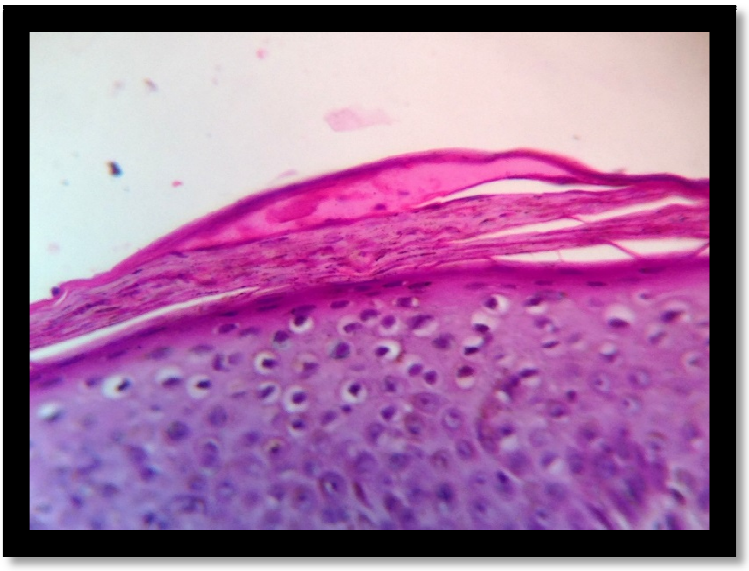


FIGURE 15: Microphotograph showing parakeratosis in a case of psoriasis. 400x. (B/914/13)

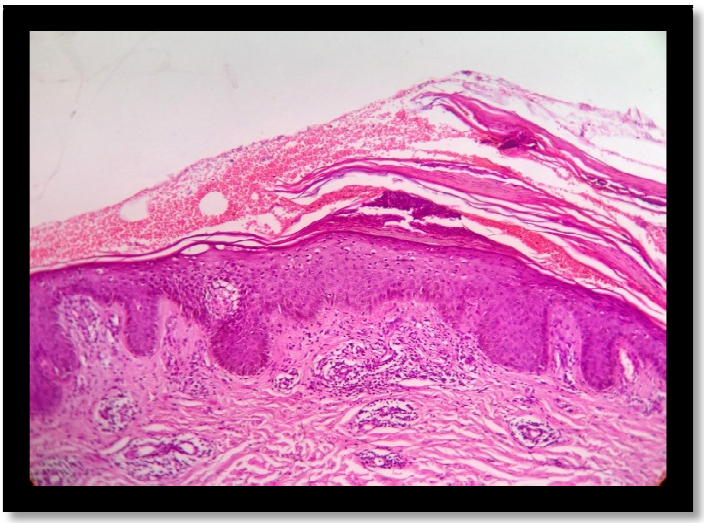


FIGURE 16: Microphotograph showing Munro's micro-abscess in a case of psoriasis. 100x. (B/1015/12)

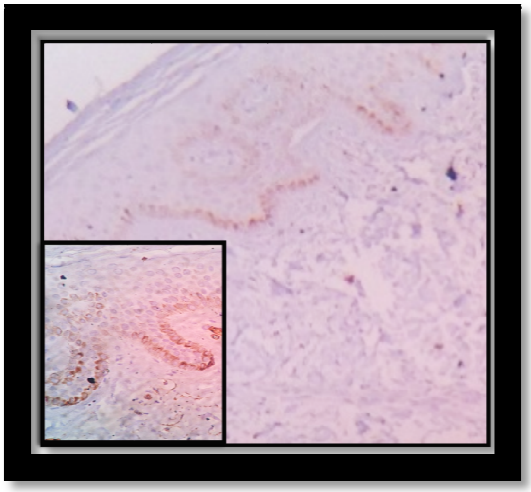


FIGURE 17: Negative expression (<10%) of VEGF by Keratinocytes in control specimen on IHC. (100x) (B/1112/14)
Inset: 400x magnification of same image.

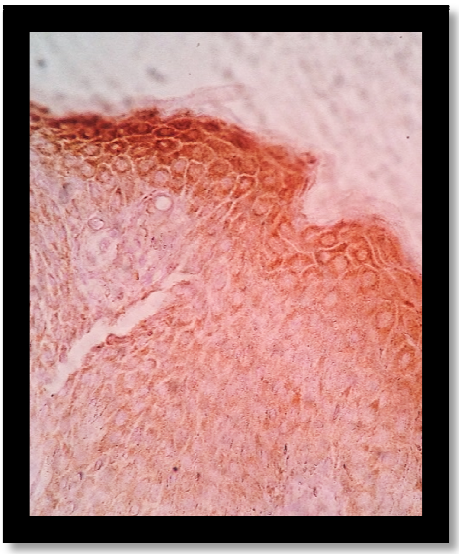


FIGURE 18: Strong positive expression of VEGF by keratinocytes in a lesional biopsy on IHC. 400x (B/1107/07)

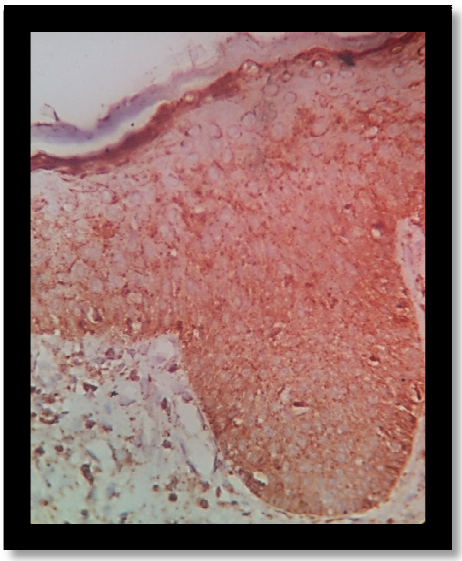


FIGURE 19: Moderate positive expression of VEGF by keratinocytes in a lesional biopsy on IHC. 400x (B/676/13)

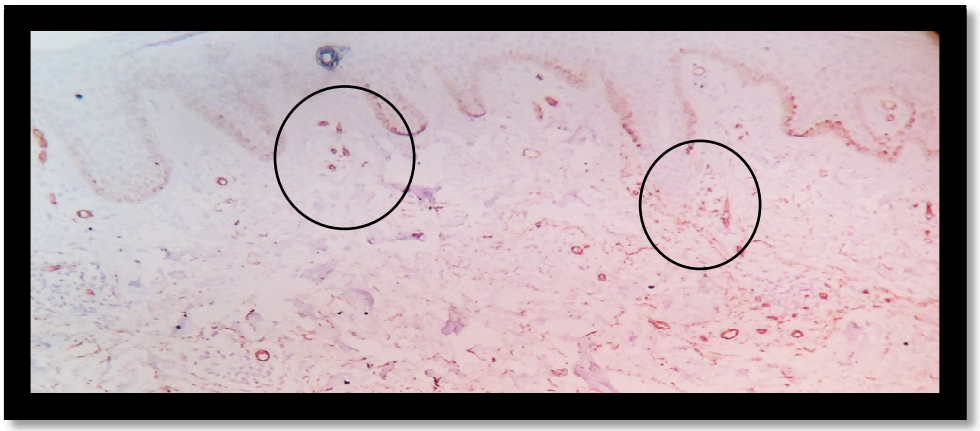


FIGURE 20: Selection of Hot-spot after CD-34 immunostaining of biopsy under 100x. (B/1336/13)

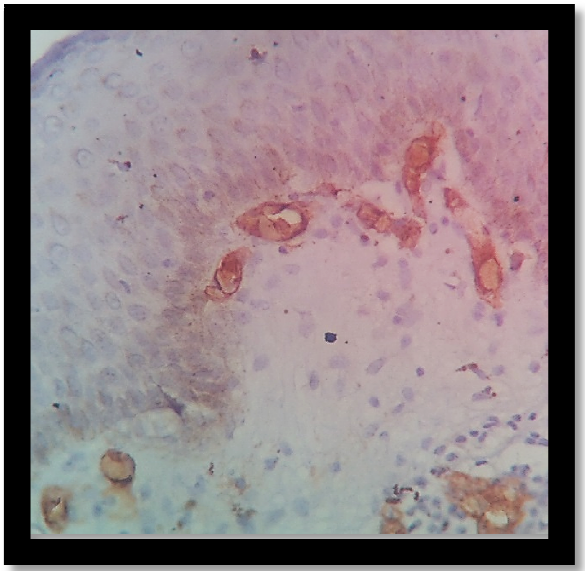


FIGURE 21: Moderate degree of micro-vessel density in a cases of psoriasis. 400x. (B/1194/12)

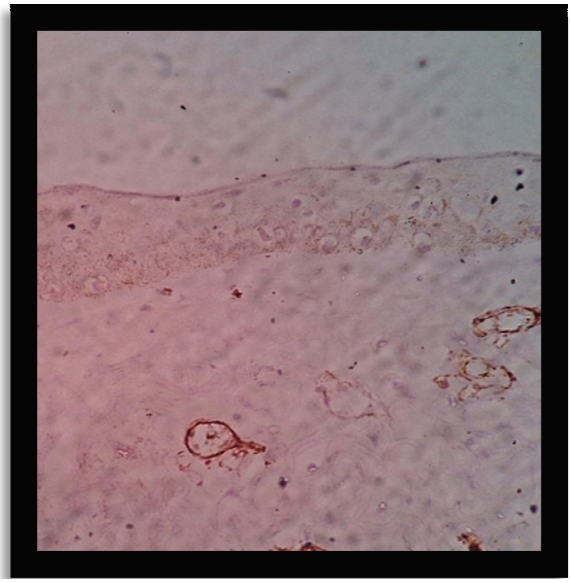


FIGURE 22: Micro-vessel density in a healthy control specimen. CD34 immuno-staining. 400x .(B/1020/13)

DISCUSSION

Psoriasis is a chronic disease that significantly impacts daily life and carries a substantial burden. Psoriasis dramatically affects quality of life, with approximately 50% of patients known to report frustration, helplessness and embarrassment with their psoriasis. Psoriasis also impairs working ability[1]. Psoriasis is considered to be a heterogenous disease that waxes and wanes over a patient's lifetime. Patients experiencea wide range of symptoms and disease severities with episodic flares and few spontaneousremissions. It is known to be a complex genetic disease that often passed from one generation to the next, although themechanism of inheritance is unknown[53].Numerous studies have supported the concept that interactions between dendritic cells, T cells, keratinocytes, neutrophils, and the cytokines released from immune cells are likely to contribute to the initiation and propagation of the cutaneous inflammation that is characteristic of psoriasis.

Histologic studies and electron microscopy have established that alterations in the blood vessel formation of the skin are prominent in psoriasis[13].

Pathological angiogenesis within the dermis has been known to occur in other chronic inflammatory processes like rheumatoid arthritis and also in tumours.

It was first suggested by Folkman that psoriasis was dependant on angiogenesis and that vascular proliferation would be a good target for the development of drugs for the treatment of psoriasis[3].

Various studies focused on identification of pro-angiogenic mediators in psoriatic skin. These included VEGF, HIFs, angiopoietins, TNF, TGF-α, IL-8 and IL-17[40].

A major role of VEGF in the pathogenesis of psoriasis was confirmed by several experimental models[38].There is also increasing data demonstrating the benefit of anti-VEGF approaches in the treatment of psoriasis[42].

Methods to quantify dermal angiogenesis involve assessment by light microscopy and estimation of micro vessel density on tissue sections probed with endothelial markers by immunohistochemistry (IHC)[48].By investigating the expression of VEGF in psoriasis patients and healthy controls, its association with the pathogenesis of psoriasis can be established. This finding will be of great importance in the understanding of the nature and progression of the disease and also for therapeutic purposes.In India, the prevalence of psoriasis varies from 0.44 to 2.8%[2]. These studies are limited by the absence of standardaccepted diagnostic criteria. Furthermore, there isn't any reliable information on time trends of the disease[2]. Literature on the Indian scenario of patients affected by psoriasis is insufficient and the data is limited to that obtained from tertiary care centers alone.

TABLE 15: Age distribution of psoriatic patients: Comparison with other studies conducted

Study conducted	No of cases studied	Highest incidence seen in age-group	Percentage of total cases
Present study	49	31-40yrs	30.6%
D'costa and Bharambe BM(2010)[54]	61	30-40yrs	42%
Sinniah et al(2010)[55]	531	40-60yrs	17.2%

Psoriasis is known to be more common in the younger age group. In most cases studied, the patients were between 20-40 years of age.

Dogra and Yadav[2] reported in a study published in 2010 on the epidemiology of psoriasis in India that the highest incidence of psoriasis was in the age group of 20-39 years.

D'costa and Bharambe BM(2010)[54] reported that majority of the psoriatic patients were in the age group of 30-40 years (61 cases).

One study which included a total of 515 patients with psoriasis had a mean age of onset was 30.48 years, range 1-75 years. 263 patients (51.1%) were in group I, that is age of onset below 30 years of age, and 252 (48.9%) were in group II(i.e age of onset above 30 years of age). Mean age of onset in group I was 19.67 years. Mean age of onset in group II was 41.85 years.[56]

In the present study, the highest incidence of cases was in the age group of 31-40 years (30.6%). This finding is in concordance with other studies world-over.

There is a bimodal age distribution among the affected individuals, with a peak at the age-group of 15-20 years, and another at the age-group of 55-60 years[1]. Two types of psoriasis have been described based on the age of onset and the inheritance pattern[8].Type 1 psoriasis comprises of 65% of the patients, with an age of onset below 40 years.The majority of patients have a positive family history and demonstrate positivity to human lymphocyte antigen-Cw6 (HLA-Cw6). Cw6-positive patients typically present with guttate psoriasis. These patients tend to develop more extensive plaques and a more severe disease which worsens during or after throat infections. Koebner's phenomenon is more often present in Cw6-positive patients, and they benefit from UV-phototherapy. Type I psoriasis has an irregular course and tends to generalize. Type 2 psoriasis affects 35% of patients, with an age of onset of over 40 years.They usually don't have a positive family history. Compared to the early-onset type, type II psoriasis is considered to be mild.

TABLE 16: Sex distribution among psoriatic patients: Comparison with other studies

	Number of cases	**Year of publication of study**	**Male: female ratio**
Present study	49	2014	1.45: 1
Kaur et al[57]	782	1986	2.3: 1
Alexander et al[58]	61	2001	7.7: 1
Karumbaiahet al[59]	22	2013	2.33: 1
Bedi et al [60]	530	1995	2.46: 1
Kaur et al [61]	782	1997	2.83: 1
Ejaz et al [56]	515	2009	3.33: 1

Psoriasis seems to be more common in males world-over in the various studies conducted.

In the present study, male: female ratio was 1.45:1, which is in concordance with other studies. However, it has been mentioned in various texts that the sex ratio of the prevalence of psoriasis is balanced world over. However, in recent years, several reports have documented that men receive more treatment that women, and different hypotheses were made. Various National registries, like the European registry and the PsoReg, the national registry for systemic treatment of psoriasis in Sweden, observed a predominance of men (PsoReg: 59%). One study[62]showed that men had more severe psoriasis than women according to the Psoriasis Area and Severity Index(PASI), regardless of the age of enrolment, and throughout the study period. This study concluded that more men were enrolled for systemic treatment of psoriasis world over because of the differing disease activity between the sexes, although as many women as men suffer from the disease.

Family history of psoriasis:

Farber et al[63]reported familial occurrence in 36% of their patients suffering form psoriasis.

Bedi[64]reported positive family history of psoriasis in 14% of their patients. While Kaur *et al*.[61] reported family history in only 2% of their patients.

In the current study, family history was known in only 23 of the total cases, out of which 4(17%) had one/ more members of the family suffering from psoriasis.

Numerous family studies have provided compelling evidence of a genetic predisposition to psoriasis, although the inheritance pattern is still unclear. As many as 71 % of patients with childhood or Type 1 psoriasis have a positive family history[8].One locus in the major-histocompatibility-complex (MHC) region on chromosome 6 has been replicated in several

populations. This locus, termed psoriasis susceptibility 1 (PSORS1), is considered the most important susceptibility locus. Other susceptibility loci are located on chromosomes 17q25 (PSORS2), 4q34 (PSORS3), 1q (PSORS4), 3q21 (PSORS5), 19p13 (PSORS6) and 1p (PSORS7). Most recently, an additional gene locus for psoriasis susceptibility has been discovered on chromosome 17q25. This locus, a runt-related transcription factor 1 (RUNX1) binding-site variant, encodes for a gene involved in the development of blood cells, including those of the immune system.

Histopathological features seen on H& E:

Histopathological features commonly seen in psoriatic skin biopsy specimens include hyperkeratosis, parakeratosis, acanthosis, supra-papillary thinning of the epidermis, Munro's micro-abscess, spongiform pustule of Kogoj and dermal inflammation.

TABLE 17: Frequency of histopathological features seen on psoriatic biopsies

Study	**Number of cases**	**Most common histological features seen(% of cases)**
Present study(2015)	49	Acanthosis (100%) Dermal inflammation (98%) Parakeratosis(82%)
Karumbaiah et al(2012)	22	Hyperkeratosis(77%) Parakeratosis (73%) Acanthosis (73%)
Younas M and Haque A[65](2004)	14	Hyperkeratosis (100%) Acanthosis (78.5%) Parakeratosis (71%)
Gordon M and Johnson WC[66](1967)	100	Parakeratosis (97%) Munro's microabscess (75%) Acanthosis (75%)

Many studies as described above have shown that hyperkeratosis, acanthosis and parakeratosis are among the most common histopathological features seen in psoriasis, followed by suprapapillary thinning of the epidermis, Munro's micro-abscess, spongiform pustule of Kogoj and dermal inflammatory infiltration.

Gordon M and Johnson WC[66]observed 100 psoriatic lesions and also reported the presence of features such as vacuolization, disruption and hydropic degeneration of the basal cells above the

tips of the dermal papillae. These findings have not been validated in other studies and are not of diagnostic significance.

The most common features seen in the current study on histopathological examination of psoriatic lesions in decreasing order of incidence are acanthosis(100%), dermal inflammation (98%), parakeratosis (81.6%).

A study showed that the site of the biopsy in the psoriasis lesion, the location of the lesion, and the moment of the course of the disease during which the biopsy was taken, had no influence on the histopathological signs observed in the patient[67].

The percentage incidence of the various histopathological features in the lesions in the 110 cases examined were as follows: Parakeratosis, hyperkeratosis, acanthosis and supra-basillar mitosis were seen in all (100%) of the cases. Munro's microabscess and spongiform pustule of Kogoj was seen in 16% and 21% of the cases respectively.

However, there are contradictory reports by Burks and Montgomery[68] who observed biopsies from various lesions in all stages of development and involution form different individuals. They studied 1 or more specimens obtained from over 225 patients with psoriasis and concluded that histological changes varied considerably in different stages of the lesion or disease process.

Early histological changes are predominantly vascular dilatations with tortuosity of venules and lymphatics in the dermal papillae and an associated lymphohistiocytic inflammatory infiltrate. Although these vascular and inflammatory changes are amongst the most consistent features of psoriasis, similar changes are seen in other dermatoses, such as seborrhea, nummular dermatitis, and pityriasisrubrapilaris. Elongation of the rete ridges is found in several "psoriasiform" dermatoses; however, in active psoriasis it is a constant feature and the acanthosis is regular, whereas in other conditions the ridges are of uneven lengths.

The expanded tip or “club shape” of the rete ridges is characteristic of psoriatic epidermis and, when present, helps to differentiate it from seborrhea and nummular dermatitis, where the ridge tips are squared-off.

Partial or complete absence of the stratum granulosum that correlates with the presence of the parakeratosis often occurs in a cyclical fashion. Uniform absence of the granular cell layer with confluent parakeratosis is reported in only 15-33% of the specimens; however, partial presence of these two features is noted in over 50% of specimens. The remainder show orthokeratosis and a prominent granular cell layer.

Thinning of the suprapapillary plate, with a stratum Malpighii only two to four cell layers thick, is a feature seen in fully developed plaques and is not a mark of other psoriasiform conditions. The presence of this microscopic criterion strongly supports the diagnosis of psoriasis vulgaris.

One study concluded that suprapapillary thinning and the absence of granular cell layer, could be added to the list of essential histopathological criteria for psoriasis, in addition to Munro micorabscess and Kogoj's abscess[69].

Several authors stress on the importance of suprabasilar mitosis as part of the microscopic picture. These are not seen in the normal epidermis, but are occasionally a feature of other “psoriasiform” disorders. Studies have shown a direct correlation between mitotic counts, the extent of change in the stratum granulosum and the presence of parakeratosis and, hence, with disease activity.

The two microscopic criteria that are variably present but are considered typical of psoriasis are the Munro microabscess and the spongiform pustule of Kogoj. In one study, out of 50 psoriatic biopsies taken, 45 (90%) of them showed Munro’s microabscesses[70]. It is known that VEGF is overproduced in psoriatic lesions, and participates in the process leading to vessel dilatation and

hyperpermeability in the lesions. This may increase extravasation of nutrients to the keratinocytes and cause rapid turnover in the epidermis, and possible extravasation of leucocytes, resulting in epidermis microabscess formation.Morphologically a few Langerhans cells (CD1a+), helper/inducer T cells (CD4+, CD5+), and macrophages (CD14+) were found among the neutrophils within the microabcsess.

The keratinocytes surrounding the microabscess were seen to produce IL-8 and some epidermal cells also expressed IL-6 and IL-8 in psoriatic lesions. These cytokines cause both chemotaxis and the proliferation of epidermal cells. Hence, it was suggested that these interleukins might contribute to the microabscess formation of psoriatic lesions[71].

Spongiform pustules are also seen in other conditions. Two of these: Reiter's syndrome and geographic tongue, are thought to be closely related to psoriasis. Lesions such as candidiasis as well as dermatophytosis may show neutrophils in the stratum corneum. However, there is usually accompanying orthokeratosis, or basket weave keratin in these cases and the organism is identifiable by the use of special stains. Necrolytic migratory erythema may show spongiform pustules but is often associated with vacuolization of the surface epidermis, a feature not commonly seen in psoriatic biopsies[72].

Histological grading of psoriatic lesions was proposed by Trojak[15] in 1992. Since then it has been cited in various studies and aids in assessing the degree of histological changes in a psoriatic lesion. This grading system offers a method of grading change that can be useful in studies of therapeutic agents. Various microscopic features considered include regular elongation and club-shaped appearance of Rete ridges, elongation and edema of dermal papillae, perivascular inflammmtory infiltration, hypogranulosis and parakeratosis, suprapapillary thinning of epidermis, supra-basal mitosis, Munro's microabscess and spongiform pustule of

Kogoj. If all microscopic criteria are present maximally, a score total of nineteen is possible for a given specimen. A "perfect" score is rarely achieved, and most specimens show a varying numerical degree of psoriasiform change. This system offers a numerical comparison of treatment and control biopsies. It offers an objective basis for satisfactory reporting of a diagnostic specimen. It is however, of little value in a statistically defined clinical study.

Epidermal thickness is also often recorded in psoriatic biopsy specimens, as an indication of disease activity. It is measured from the base of the stratum corneum to the tip of the rete ridges. An average of six measurements obtained with a standard ocular micrometer is taken[15].

Table 18: Expression of VEGF by keratinocytes in psoriasis: Comparison with other studies

Study	Mean VEGF expression: Cases:Percentage of total epidermal cells	Mean VEGF expression: Controls: Percentage of total epidermal cells
Current study	49.80 +/- 21.16%	3.95 +/- 3.94%
Salem et al[52]	46.4% +/- 19.7%	1.2% +/- 0.4%
Swapnil et al[73]	68.8%	

In a study conducted by Swapnil A et al[73]on 25 patients, VEGF expression was strong in lesional skin and ranged from 5-100% of the total epidermal cells with a mean percentage of 68.8%.

In a study conducted by Salem et al[52] published in 2014 on 20 psoriatic patients showed strong cytoplasmic expression of VEGF throughout the epidermis. The mean value of expression was 46.4% +/- 19.7% of total epidermal cells. There was a significant increase in expression when compared to healthy skin (10 cases) which showed faint VEGF expression with a mean value of 1.2% +/- 0.4% of total epidermal cells.

The results of the current study were as follows: mean percentage expression of VEGF of total epidermal cells of 49 cases was 49.80 +/- 21.16% (SEM = 3.02) and that of the 20 controls was 3.95 +/- 3.94% (SEM = 0.90%). The difference in the values were statistically significant with a p value less than 0.000001. This is in agreement with the above mentioned studies.

Vascular endothelial growth factor (VEGF) is an endothelial-specific growth factor, a potent angiogenic as well as a hyperpermeability factor. Psoriasis is known to be angioegenesis-dependant. Several other angiogenic factors have been identified in psoriatic epidermis,

including IL-8, TNF-a, transforming growth factor-a, endothelial cells-stimulating angiogenesis factor, thymidine phosphorylase.In psoriatic skin, the VEGF receptors, VEGFR-1 and VEGFR-2, are detectable and functional in the keratinocytes.Keratinocyte cell-derived VEGF probably actively contributes to the pathogenesis of psoriasis by the induction of permeability in dermal vessels, its chemotactic action on inflammatory cells and endothelial cells, and its angiogenic effect. It may also contribute to keratinocyte proliferation and epidermal barrier homeostasis.

Tissue levels of VEGF were assessed by culture of isolated keratinocytes and fibroblasts from punch biopsy specimens of psoriatic patients. These levels were compared to the value of VEGF tissue levels of healthy volunteers. VEGF (28.1 +/- 6.9 ng/mg wet weight) levels were significantly raised in involved skin as compared with normal control skin (6.5 +/- 0.4 ng/mg wet weight; P =0.0001)[74].

Another study done by Mohammad et al on 40 psoriatic patients concluded that on immunohistochemical staining, there was overexpression of VEGF in keratinocytes of psoriatic lesions and there was strong VEGF staining of dermal papillae when compared to normal skin[75].

Sera from patients with psoriasis have enhanced VEGF levels. Moreover, serum-VEGF levels have been seen to correlate with disease severity[76]. Andrys C et al[77]demonstrated that the serum levels of VEGF (329.4 +/- 125.5 microg/ml) in psoriatic patients were significantly higher than those measured in healthy blood donors (VEGF 236.4 +/- 55.9 pg/ml).A study was conducted by Neilsenet al[78] in 2002 on 16 psoriatic patients whose serum VEGF levels were compared with healthy volunteers (13). It was seen that the median serum level was 51 pg/ml, ranging from (36-95.5 pg/ml). The serum VEGF levels in healthy volunteers ranged from 27-33 pg/ml, median being 33pg/ml.

Also, single nucleotide polymorphisms of the VEGF gene strongly correlated with psoriasis pathogenesis[37]. VEGF overexpression selectively in basal keratinocytes (K14VEGF) in transgenic mice resulted in a chronic skin inflammation with increase tortuous capillaries, elevated expression of VEGFR-1 and -2, increased mast cells in the upper dermis, leukocyte rolling and adhesion[38]. Moreover, transgenic mice treated with the VEGF antagonist VEGF-trap remained healthy.

Table 19: Micro-vessel density assessment: Comparison with other studies

Study	**Number of cases**	**Mean MVD (per HPF)**
Present study	CASES: 49 CONTROLS:20	15.30 +/- 3.81 5.16 +/-1.46
Swapnil et al[73]	CASES: 25 CONTROLS:10	11.52 +/- 3.177 5.38+/-2.309

Table 20: Comparison with other studies based on degree of micro-vessel density:

	Number of cases(n)	**Mild [4-10/HPF] (% of cases)**	**Moderate [11-20/HPF] (% of cases)**	**Strong [21-28/HPF] (% of cases)**
Present study:				
Cases	49	1(2%)	37(75.5%)	11(22.5%)
Controls	20	20(100%)	0	0
Amin and Azim[79]:				
Cases	14	3(21.4%)	7(50%)	4(28.6%)
Controls	10	10(100%)	0	0

The current study showed a mean MVD among psoriatic patients to be 15.30 +/- 3.81 per HPF or 100.67+/- 25.04 per mm^2. The mean value among healthy controls was 5.16 +/-1.46 per HPFor 33.93 +/- 9.625 per mm^2. There was a significant reduction in MVD among controls (p value< 0.00001). This was in accordance with the expected value as per conclusions from previous studies.

Other studies who assessed the micro-vessel density of psoriatic lesions in comparison to normal healthy skin by identical methods include:

The value of MVD in 50 psoriatic lesions assessed by morphometry showed values ranging from 87 to 329 per mm^2, mean value being 185+/- 57 per mm^2 in a study by Boruahet al[80]. The higher value of micro-vessel density in comparison to our study can be due to the usage of morphometry for the vessel count.

Psoriasis begins with angiogenesis in the superficial dermal microvasculature. Dermal papillary capillaries increase in tortuosity, dilatation and permeability, and show prominent elongation[40]. These changes occur prior to visible epidermal hyperplasia. Electron microscopy shows ultrastructural changes of the capillary loops in the dermal papillae. The capillary loops exhibit characteristic features of venous capillaries such as a single or multilayered basement membrane and bridged fenestrations of the endothelium in psoriasis plaques. Following successful therapy, venous capillary loops return to normal arterial capillaries, which precedes normalization of the epidermal structure. Increased proliferation of endothelial cells of psoriasis plaques is demonstrated by autoradiography and immunohistochemistry[76].In psoriatic skin, the superficial microvasculature shows enhanced levels of integrin $\alpha_v\beta3$ compared with healthy skin[76].

The other pro-angiogenic factors that are implicated in the pathogenesis of psoriasis include VEGF, HIFs, angiopoietins, TNF, TGF-α, IL-8 and IL-17[40].

VEGF expression and Micro-vessel density analysis:

VEGF has been known to have paracrine actions which include, increased vascular permeability, influx of inflammatory cells, and angiogenesis through induction of endothelial-cell specific gene expression, and potentially recruitment of marrow-derived endothelial progenitor cells into the site of inflammation[81].Experimental evidence of correlation between VEGF expression among keratinocytes and dermal micro-vessel density has limited literature and documentation.

A study which compared expression of VEGF with micro-vessel density in psoriatic skin was reported by Swapnilet al[73] in 2010. It was concluded that VEGF is increased in psoriatic plaques and may play an important role in the pathogenesis of the disease, contributing to the vascular dilatation, increased vascular permeability and epidermal hyperplasia seen in psoriasis. It was also suggested that the blockade of the action of VEGF may be of importance in the development of novel selective therapeutic strategies for the treatment of psoriasis.

In our study, there was a positive correlation between the micro-vessel density and the expression of VEGF in both cases ($r=0.664$) and controls ($r=0.657$). The correlation was significant with $p<0.01$.

Evidence at a genetic level for VEGF-mediated angiogenic activity in the pathogenesis of psoriasis was shown by the association between VEGF promoter polymorphisms and the development of psoriatic symptoms. VEGF transgenic mice showed cutaneous inflammation and were characterized by a significant increase in the density of dermal blood vessels, predominantly of capillaries that were tortuous and displayed increased branching[35].

Several treatment modalities have been used to target angiogenesis in psoriasis. Phototherapy and photochemotherapy inhibits angiogenesis and induces apoptosis of endothelial cells.

Retinoids and cyclosporine-A (drugs widely used in the systemic treatment of psoriasis) have been shown to down-regulate VEGF synthesis or inhibit in vitro and in vivo VEGF-induced angiogenesis.

Cyclosporin-A is also known to have the action of inhibiting migration of primary endothelial cells and angiogenesis induced by VEGF; the effect which appears to be mediated through the inhibition of cyclooxygenase (Cox)-2. The transcription of Cox-2 is activated by VEGF in primary endothelial cells. It has been reported that Infliximab reduces VEGF and thereby reduces blood supply at the site of tissue damage, improving psoriatic lesions.

Avramidis et al[82] found that the mean VEGF expression in the endothelial cells of the dermis was 30.82 ± 0.65% of total endothelial cells in psoriatic skin, and they reported reduction in VEGF expression by 81.6% at week 10, under etanercept treatment.

It has been found that there was an improvement of psoriasis after selective photothermolysis of superficial capillaries in the psoriatic lesion (laser therapy) and with immunohistochemical study there was reduced expression of VEGF in the psoriatic lesion after laser therapy.

Recently, angiogenesis can be blocked by ablation of VEGF by monoclonal antibodies (MA) to VEGF or its receptor, as well as soluble decoy receptors and small molecule inhibitors of the tyrosine kinase activity of the VEGFRs. Another approach is the generation of a mutant heterodimeric VEGF-molecule to block the receptor binding sites, inhibiting signal transduction. Small molecules inhibiting the tyrosine kinase domain of VEGFR as SU5416 (Sugen) have been shown to effectively and selectively inhibit angiogenesis in vitro and in vivo and are in the process of clinical testing. Clinical studies with antibodies, scavengers, gene therapy or blocking molecules for VEGF or its receptors or VEGF-conjugated toxins as well combinations of the above with conventional therapy have been reported with increasing success.

TABLE 21: Correlation of Histopathological grade with VEGF expression and micro-vessel density:

Correlation with histopathological grade	**r Value**	**p Value**
VEGF expression	0.154	>0.05
Micro-vessel density	0.226	>0.05

The results of this study show thatexpression of VEGF by keratinocytes or with the micro-vessel densitydid not correlate significantly with histopathological grade in cases of psoriasis.

Psoriasis is known to be a dynamic process, both clinically and histologically. Microscopic diversity occurs among psoriatic patients with clinically similar lesions, between lesions of an individual, and even within single plaques. Other studies demonstrating the role of histopathological features on the disease severity or activity showed that the features did not vary depending on the moment(ie during remission or active disease) of the course of the disease during which the biopsy was taken[67]. A study conducted on clinically severe forms of psoriasis showed that histological grade does not correlate with disease severity[83].

Thus, the role of histopathological examination in psoriatic patients is limited to help confirm the diagnosis, and to differentiate it from other psoriasiformdermatoses.

SUMMARY

A laboratory observation and descriptive study to evaluate histopathological features of psoriasis along with immunohistochemical evaluation of VEGF expression by keratinocytes and correlation with micro-vessel density was undertaken from January 2012 to June 2014. The following are the salient observations noted:

1. Most psoriatic patients in the study fell within the age-group of 31-40 years (30.6%).
2. The sex-wise distribution of 49 psoriatic patients was 29 males (59.1%) and 20 females (40.9%). The Male: Female ratio was 1.45:1
3. Family history was known in only 23 of the total cases, out of which 17% (4) had one/ more members of the family suffering from psoriasis.
4. Out of the cases studied, the most common and persistent histopathological features included acanthosis (100%), dermal inflammation (98%) and parakeratosis (81.6%).
5. The mean percentage expression of VEGF of total epidermal cells of 49 cases was 49.80 +/- 21.16% and that of the 20 controls was 3.95 +/- 3.94%. The difference between the two values were statistically significant, with a p value less than 0.000001.
6. Mean micro-vessel density was 15.30 +/- 3.81 per HPF among cases. The mean micro-vessel density among controls was 5.16 +/-1.46 per HPF. The difference between the two values were statistically significant, with a p value less than 0.00001.
7. Among the cases, it was seen that there is a positive correlation between VEGF expression by keratinocytes and the micro-vessel density in the papillary dermis (r = 0.664). The correlation is significant with p value less than 0.01.

8. Among the controls, it was seen that there is a positive correlation between VEGF expression by keratinocytes and the micro-vessel density in the papillary dermis ($r = 0.657$). The correlation is significant with p value less than 0.01.

9. There was a positive correlation between the VEGF expression by keratinocytes and the histopathological grade ($r = 0.292$). However, the correlation was not significant with $p>0.05$.

10. There was a positive correlation between the micro-vessel density and the histopathological grade ($r = 0.226$). However, the correlation was not significant with $p>0.05$.

CONCLUSION

The results of this study showed that VEGF expression in keratinocytes is raised in psoriasis when compared to healthy skin. Also, the microvasculature in papillary dermis is increased when compared to healthy skin. There was a significant positive correlation between the degree of expression of VEGF by the epidermis and the micro-vessel density in the dermis. When compared to the histopathological grade, it was seen that there was no significant correlation with the VEGF expression or the micro-vessel density. Hence, it can be concluded that VEGF plays a significant role in the pathogenesis of psoriasis by way of its angiogenic activity in the lesions.

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ANNEXURE-1

Proforma for "EXPRESSION OF ANGIOGENIC MARKERS: VASCULAR ENDOTHELIAL GROWTH FACTOR AND CD-34 IN PSORIATIC SKIN LESIONS"

Proforma number:

- Patient name:
- Hospital number:
- Age: Sex:
- Family history: Occupation:
- Duration of complaints: Treatment history:
- Clinical diagnosis:
- Site of punch biopsy taken:
- Recent lesion: YES/NO
- Biopsy number: IHC number:

Histo-pathological report:

Histopathological assessment of psoriatic lesions:

No	Microscopic criteria	Max Score	LesionScore
1	Regular elongation of the rete ridge	1	
2	Club shaped rete ridges	2	
3	Elongation and edema of dermal papillae	1	
4	Perivascular mononuclear infiltrate in the upper dermis of papillae	1	
5	Absent granular layer: FocalTotal	1 2	
6	Parakeratosis: Focal Total	1 2	
7	Suprapaillary plate thinning	2	
8	Mitosis above basal cell layer	2	
9	Munro microabscesses	3	
10	Spongiform pustule	3	
	Total	**19**	

IHC report:

- **VEGF:**
- **CD-34: Micro-vessel density:**

Key to the master chart

Sl No	Serial number
Pt Name	Patient name
Hosp No	Hospital number
Biopsy No	Biopsy number
F/h	Family history
HP grade	Histopathology grade
MVD-1	Micro-vessel density- area 1
MVD-2	Micro-vessel density- area 2
MVD-3	Micro-vessel density- area 3
Mean MVD	Mean Micro-vessel density
VEGF Exp	VEGF Expression

Sl no	Pt name	Hosp no	Biopsy no	Age	Sex	Site	F/h	Hp grade	MVD-1	MVD-2	MVD-3	Mean MVD	VEGF exp
1	Krishnappa	375466	07-1107	70	M	Back	UK	13	15	15	14	14.7	40%
2	Abdul	394473	07-1173	48	M	Thigh	UK	17	14	13	13	13.3	60%
3	Surendra	307654	08-1087	24	M	Fore-arm	UK	13	22	24	20	22	70%
4	Meenamma	309889	08-1115	48	F	Arm	UK	7	23	24	20	22.3	70%
5	Sapna	414683	08-368	18	F	Leg	UK	7	9	10	16	11.6	30%
6	Nagaraj Rao	425197	08-553	24	M	Fore-arm	UK	7	9	14	17	13.3	70%
7	Lakshmamma	419982	08-889	50	F	Leg	UK	10	15	6	16	12.3	30%
8	Ramaswamy	534651	09-1619	39	M	Leg	UK	8	16	16	12	14.7	30%
9	Mohan R	558196	09-2671	22	M	Back	UK	8	18	15	13	15.3	40%
10	Renuka	721316	11-1532	30	F	arm	UK	9	12	18	15	15	40%
11	Munivenkatappa	633140	11-1548	48	M	Fore-arm	UK	8	13	10	18	13.7	40%
12	Raju C	731574	11-1758	64	M	leg	UK	8	15	16	15	15.3	90%
13	Noor Pasha	732081	11-1780	70	M	Fore-arm	UK	14	16	12	10	12.7	40%
14	Manjula	754665	11-2492	40	F	Back	UK	9	15	13	16	14.7	60%
15	Mangamma	690789	11-278	34	F	Back	UK	11	18	12	16	15.3	60%
16	Ramesh T	689897	11-32	21	M	Arm	UK	10	16	16	12	14.7	50%
17	Syed Fairoz	665980	11-33	32	M	Back	UK	9	24	20	22	22	60%
18	Shivaprasad	804289	12-1015	15	M	Fore-arm	UK	9	11	10	10	10.3	20%
19	Narayanaswamy	734234	12-1194	45	M	Leg	UK	7	14	11	7	10.7	10%
20	Muniyappa	817153	12-1334	45	M	Back	UK	7	22	20	21	21	80%
21	Gurappa Shetty	682369	12-2286	60	M	Back	UK	10	18	15	17	16.7	70%
22	Syed Hussain	871724	12-2491	17	M	Arm	UK	8	22	20	21	21	70%
23	Syed Halim	871723	12-2492	60	M	arm	UK	14	18	15	17	16.7	60%
24	Sulochana	796157	12-813	30	F	Fore-arm	n	7	12	10	12	11.3	40%
25	Gnana Bharathi	914883	13-1031	21	F	Leg	n	12	15	15	9	13.7	10%
26	Lakshmi	916378	13-1072	22	F	Fore-arm	n	11	15	15	11	13.7	20%
27	Shalini	919494	13-1194	40	F	Leg	n	13	14	12	8	11.3	50%
28	Sharada	923055	13-1247	38	F	Leg	n	9	14	14	10	12.7	60%
29	Venkatesh Prasad	926139	13-1336	16	M	Back	n	11	26	22	21	23	90%
30	Krishnappa	903367	13-1351	62	M	Arm	n	9	16	16	18	16.7	50%
31	Narayanswamy	943973	13-1774	48	M	Leg	y	15	15	16	14	15	40%
32	Naresh Kumar	951974	13-1928	25	M	Fore-arm	n	14	20	20	24	21.3	90%
33	Gopi	953874	13-1977	38	M	Back	y	13	23	20	25	22.7	70%
34	Chandrakala	791648	13-1990	28	F	arm	n	7	12	13	16	13.7	30%
35	Nagarathnamma	959059	13-2110	32	F	Back	y	7	15	14	11	13.3	10%
36	Kanakamma	880273	13-278	50	F	Fore-arm	n	7	18	12	15	15	50%
37	Anjaneya Reddy	883132	13-280	31	M	Foot	n	7	10	13	16	13	50%

38	Jayammaaakkj	583136	13-32	45	f	Arm	n	7	12	12	10	11.3	60%
39	Narayanamma	894618	13-676	56	F	Fore-arm	n	9	12	12	14	12.7	50%
40	Mangamma	901733	13-710	45	F	Arm	n	13	23	21	21	21.7	80%
41	Venkatesh K	910262	13-904	39	M	Leg	n	9	10	14	12	12	50%
42	Sandhya	910585	13-914	22	F	Back	n	7	12	14	16	14	20%
43	Venkatappa	900897	14-132	45	M	Leg	y	10	21	20	19	20	50%
44	Nagarathnamma	987677	14-170	39	F	Leg	n	7	18	13	17	16	70%
45	Padmanabhan	985344	14-203	37	M	Arm	n	14	14	10	17	13.7	40%
46	Eshwar Reddy	995066	14-471	55	M	Arm	n	14	10	14	11	11.7	30%
47	Kemparaju	999895	14-674	38	M	Leg	UK	7	10	9	8	9	20%
48	Venkatamuniyappa	1005911	14-726	62	M	Fore-arm	UK	8	15	10	10	11.7	60%
49	Ramakka	900546	14-904	56	F	Arm	UK	14	21	20	20	20.3	60%
50	Muniswamy		A/11/14	67	M	Back	N/A	N/A	4	3	2	3	5
51	Surendra		A/12/14	55	M	Fore-arm	N/A	N/A	6	10	8	8	10
52	Krishna		A/22/13	43	M	Back	N/A	N/A	7	5	6	6	0
53	Ramya		A/23/14	27	F	Back	N/A	N/A	3	4	5	4	5
54	Sumithramma		A/28/14	82	F	Abdomen	N/A	N/A	5	7	9	7	10
55	Ramesh		A/35/13	27	M	Abdomen	N/A	N/A	7	7	7	7	5
56	Amin		A/37/13	34	M	Back	N/A	N/A	4	5	6	6	10
57	Pavithramma		A/44/13	54	F	Fore-arm	N/A	N/A	3	4	2	4	0
58	Naresha		A/55/13	65	M	Fore-arm	N/A	N/A	5	4	6	5	0
59	Ramaswamy		A/54/13	69	M	Back	N/A	N/A	4	3	2	4	0
60	Ganesha	100034	B/1114/14	38	M	Back	N/A	N/A	4	4	4	4	0
61	Muniyappa	945876	B/113/14	55	M	Back	N/A	N/A	5	5	5	5	0
62	Sundaram	945987	B/167/13	52	M	Abdomen	N/A	N/A	4	3	5	4	0
63	Srinivas	900342	B/208/13	45	M	Abdomen	N/A	N/A	3	3	3	3	0
64	Narayanappa	100320	B/209/14	45	M	Back	N/A	N/A	7	8	6	7	10
65	Ramappa	875698	B/342/13	60	M	Back	N/A	N/A	3	4	5	4	5
66	Farhan	109890	B/768/13	17	M	Back	N/A	N/A	6	5	7	6	5
67	Rukkumani	998567	B/905/13	21	F	Back	N/A	N/A	5	5	5	5	5
68	Jyothiakshmi	998706	B/987/13	22	F	Abdomen	N/A	N/A	6	6	6	6	5
69	Subramanian	980674	B/119/13	62	M	Abdomen	N/A	N/A	7	7	7	7	5