

Study of Dermal Vascular Changes in Inflammatory Skin Diseases

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Abstract: The skin is the largest organ in the body. A multitude of diseases are linked to an insufficient or overactive dermal vasculature. Vessels change their phenotype, i.e. vascular remodelling in inflammatory conditions. The aim of this study was to analyse the dermal vascular alterations in various inflammatory skin diseases & to assess their utility in establishing the differential diagnosis. A unicenter study of 55 (fifty five) skin biopsies received from patients of various non tumorous common inflammatory skin diseases such as, psoriasis, lichen planus, erythema multiform, blistering (bullous) lesions and 10 control samples were studied. Morphometric analysis was performed on CD 34 immunostained sections using image analysis software (Leica Q Win V3 software analysis system). Mean vascular density (MVD) and mean vascular calibre (MVC) was calculated. The MVD was maximum in psoriasis, followed by lichen planus, bullous lesions and erythema multiform. MVD in psoriasis was significantly higher than the controls. MVC was highest in psoriasis followed by erythema multiform, lichen planus and bullous lesions. The MVC in psoriasis, erythema multiform, lichen planus, bullous lesions were significantly higher than the controls. Elongated, tortuous blood vessels were more prominent in psoriasis. The endothelial changes noted were swelling in the form of nuclear hypertrophy, increased cytoplasm and endothelial gaps. Angiogenesis was increased in inflammatory skin diseases and it was highest in psoriasis. Therefore, therapeutic intervention at the level of vasoproliferation may prove to be useful.

Keywords: Psoriasis, Lichen Planus, bullous lesions, Mean vascular density, Mean vascular calibre.

INTRODUCTION

Skin is the largest organ in the body and consists of two main layers, the epidermis and dermis [1]. The epidermis is nonvascular while dermis is vascular [2]. Owing to its size, superficial location and architectural complexity, diseases of the skin are very common. The pathological changes may arise in the epidermis, dermis and / or the subcutaneous tissue [3]. The blood vessels to the skin are derived from a number of arterial plexuses. Capillary loops arising from this plexus pass into each dermal papilla. The resting cutaneous blood vessels contain a continuous basement membrane. The endothelium lining the blood vessels is in direct contact with the blood and circulating cells, plays a major role in control of

inflammation and angiogenesis [4]. A multitude of diseases are linked to an insufficient or overactive vasculature. Among them are many inflammatory diseases. Acute inflammatory dermatoses include Urticaria, Acute eczematous dermatitis, Erythema Multiforme (EM). Chronic inflammatory dermatoses are Psoriasis, Seborrheic dermatitis, Lichen Planus (LP), Blistering (bullous) diseases and other less common inflammations. Superficial perivascular infiltrate is seen in urticaria, acute eczematous dermatitis, EM, seborrheic dermatitis. Along with blood vessels, the lymph vessels are also present in superficial and deep plexuses. However, it is very difficult to differentiate between them on routine tissue sections. Therefore, the immunohistochemistry (IHC)

staining with endothelial antibodies is an important method, to differentiate between blood vessels and lymph vessels. The endothelial antibodies are CD (cluster of differentiation) 31, CD34, Factor VIII, FLI-1(Friend Leukaemia integration 1) and D2-40 (podoplanin) [5]. CD34 is strongly expressed in endothelial cells of capillaries, arteries, veins, arterioles and venues while D2-40 labels endothelial cells of only lymphatic[6]. Morphometric analysis gives a quantitative dimension to histopathology. The exact number of blood vessels can be measured by special image analysis software, after IHC staining with CD34 antibody [7].

Aim was to study dermal vascular alterations in inflammatory skin diseases and to assess the utility of the dermal vascular alterations in the differential diagnosis of inflammatory skin diseases.

MATERIALS AND METHODS

This was a retrospective and prospective unicenter study, carried out in the tertiary care hospital over a period of 1 year duration, from September 2015 to September 2016. The study included 55 (fifty five) skin biopsies received from patients of various non tumorous common inflammatory skin diseases such as, psoriasis, lichen planus, Erythema Multiforme, blistering (bullous) lesions and 10 control samples. The

skin tissue received in other conditions like lipoma, breast tissue in mastectomy, skin biopsies which lack specific pathology with no skin disease served as control. Institute Ethics Committee Clearance was obtained before the start of study. Routine H & E and CD-34 IHC staining was done. Morphometric analysis was performed on CD 34 immunostained sections using image analysis software (Leica Q Win V3 software analysis system). The measuring scale of image analysis software was properly calibrated with standard scale, as per instructions given in the software manual. Micro vessels in dermis were observed by scanning sections at low power (x100) magnification. The area with greatest number of distinctly CD34 immunostained microvessel (“hot spot”) was selected. Micro vessel counting and calibre measurement were evaluated under high power magnification (x400) in 5 fields within the hot spot. The area of per field measuring frame is 46591.49 μm², which is constant. 106 μm²= 1 mm². Thus 46591.49 μm² = 0.046 mm². The number of micro vessels in 5 fields were counted and divided by the area (0.046 mm²) to get the micro vessel density (MVD). The micro vessels which appeared circular and elliptical in the section were used to get mean micro vessel calibre (MVC). For the elliptical vessel, the minor axis was considered as its caliber. The MVC was measured using the software.

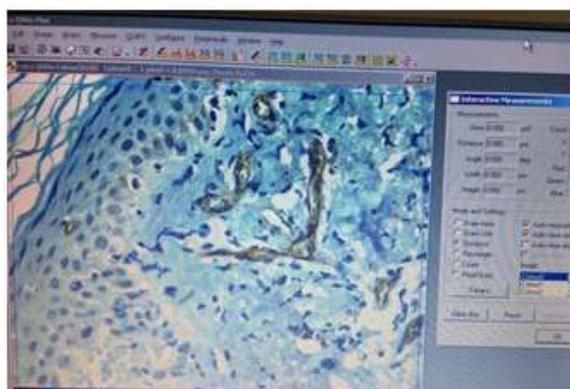


Fig-1: Morphometric Counting Of MVC

RESULTS

The study comprised of 65 skin biopsies of patients with inflammatory skin lesions.

The age of patients in this study ranged from 7 to 75 years. The Maximum cases, 20 (30.77%) were in 21-30 year of age. There were predominantly male patients 48 (73.85%) in the study group (Table 1).

Table -1: Age distribution

Age	No of cases	Percentage (%)
<20	10	15.38
21-30	20	30.77
31-40	9	13.85
41-50	13	20
>50	13	20
Total	65	100

Table-2: Association between age and disease in study group diagnosis

Diagnosis	Age					Total
	<20	21-30	31-40	41-50	>50	
Psoriasis	2	9	2	5	7	25
Lichen Planus	7	3	1	3	1	15
Bullous Lesions	0	0	4	1	3	8
Erythema Multiforme	0	4	2	1	0	7
Total	9	16	9	10	11	55

The maximum cases of psoriasis and EM were in the age group 21-30 years while LP was seen in at comparatively younger age group <20 years and bullous lesions were seen after 30 years of age.(TABLE 2)

The male to female ratio was 2.8:1. There was male predominance in entire study group as well in individual diseases (CHART 1).

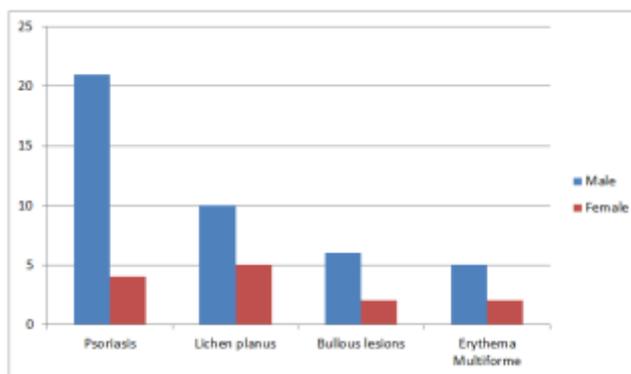


Chart-1: Association between gender and disease

Table-3: Association between MVD and diagnosis in study group

MVD/mm2	Psoriasis	Lichen planus	Bullous lesions	Erythema multiforme	Control	Total
40– 80	5 (20%)	6 (40%)	4 (50%)	5 (71.5%)	9(90%)	29
81-120	9 (36%)	5(33.3%)	3(37.5%)	2 (28.5%)	1(10)	20
121– 160	3(12%)	2 (13.3%)	1 (12.5%)	0	0	6
161 – 200	3(12%)	1 (6.6%)	0	0	0	4
>200	5 (20%)	1 (6.6%)	0	0	0	6
Total	25 (100%)	15(100%)	8 (100%)	7(100%)	10(100%)	65

Highest mean MVD was observed in psoriasis followed by lichen planus. When psoriasis was compared with control and EM, P values were 0.001 and 0.026 respectively, which were significant. However on comparison with LP and BL, P values were 0.21, 0.06, respectively. Comparison of LP with control,

BL and EM gave P values of 0.21, 0.88 and 0.68 respectively. When BL were compared with EM and control, the P values were 0.99 and 0.86 respectively. The P value of comparison between EM and the control was 0.98. All these showed statistical insignificance (TABLE 3).

Table-4: Association between MVC and diagnosis in study group

MVC μm	Psoriasis	Lichen planus	Bullous lesions	Erythema multiforme	Control	Total
<5	0	0	0	0	6 (60%)	6
5.1-10	10 (40%)	10(66.6%)	4 (50%)	3 (42.8%)	4 (40%)	31
>10	15 (60%)	5(33.3%)	4 (50%)	4 (57.1%)	0	28
Total	25(100%)	15 (100%)	8(100%)	7 (100%)	10(100%)	65

When psoriasis, LP, BL and EM were compared with control, the p value was <0.0001, which was statistically significant. When psoriasis was compared with LP, BL and EM, P values were 0.84,

0.82, and 0.99 respectively. When lichen planus was compared with BL and EM P values was 0.99 in both the cases. Comparison between BL and EM gave a P

value of 0.98. All these showed statistical insignificance. (TABLE 4)

We observed vascular elongation and tortuosity along with endothelial swelling in the form of nuclear hypertrophy, increased cytoplasm and

endothelial gaps in most of the patients with inflammatory skin disorders. (FIGURE 2,3,4)

Changes of vascular elongation and tortuosity were distinctly observed in psoriasis as compared to other lesions. (FIGURE 2)

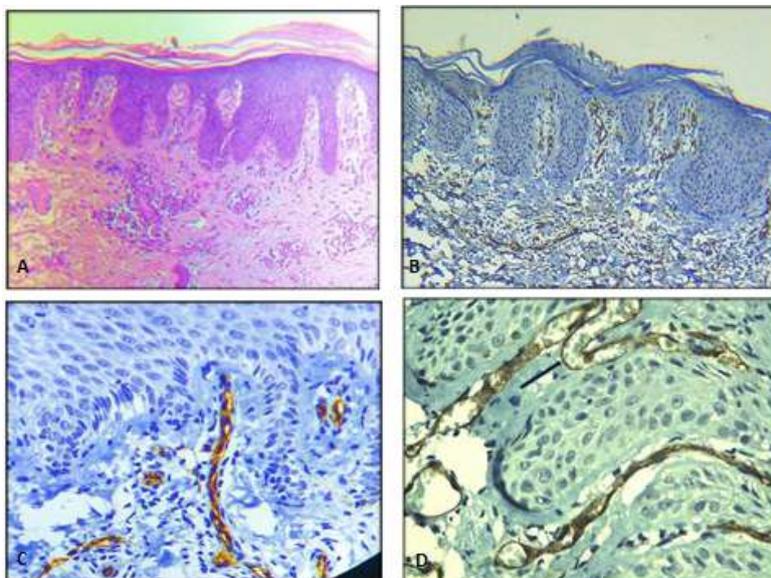


Fig-2A: Photomicrograph showing Psoriasis with parakeratosis, thinned suprapapillary layer, attenuated granular layer, elongation of rete ridges, and perivascular lymphocytic infiltrate in dermis. H&E (x 100)

Fig-2B: Photomicrograph shows psoriasis with increased papillary and reticular dermal capillaries, parakeratosis, suprapapillary thinning. IHC CD34 (x 100)

Fig-2C: Photomicrograph showing elongated blood vessel with endothelial gaps and nuclear hypertrophy in the papillary dermis of psoriatic skin. CD34 (x 400)

Fig-2D: Photomicrograph showing dilated and tortuous blood vessel in papillary dermis in case of psoriasis. IHC CD34 (x 400)

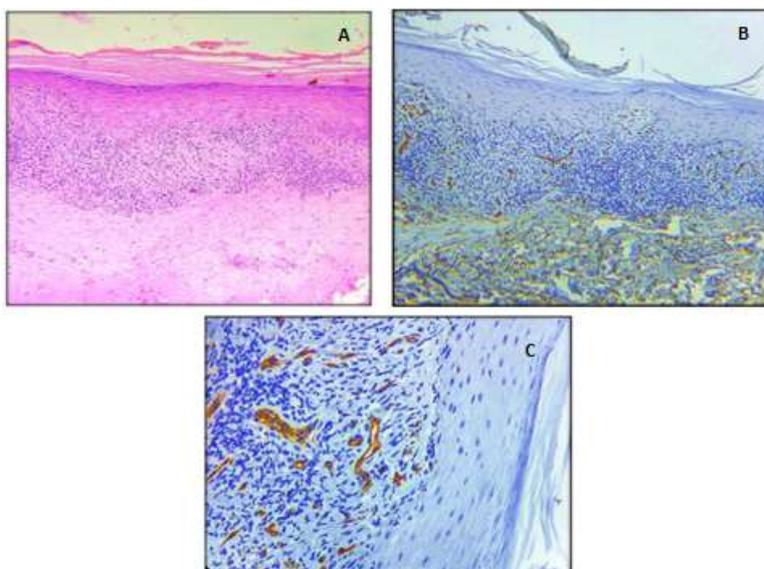


Fig-3A: Photomicrograph showing Lichen planus with orthokeratosis, wedge shaped hypergranulosis, band of inflammatory infiltrate at dermoepidermal junction comprising of lymphocytes, plasma cells. H&E (x100)

Fig-3B: Photomicrograph showing Lichen planus with dermal vasculature and inflammatory infiltrate band at dermoepidermal junction. IHC CD34 (x 100)

Fig-3C: Photomicrograph showing lichen planus with endothelial gaps and swelling in dermal vessels. IHC CD34 (x 400)

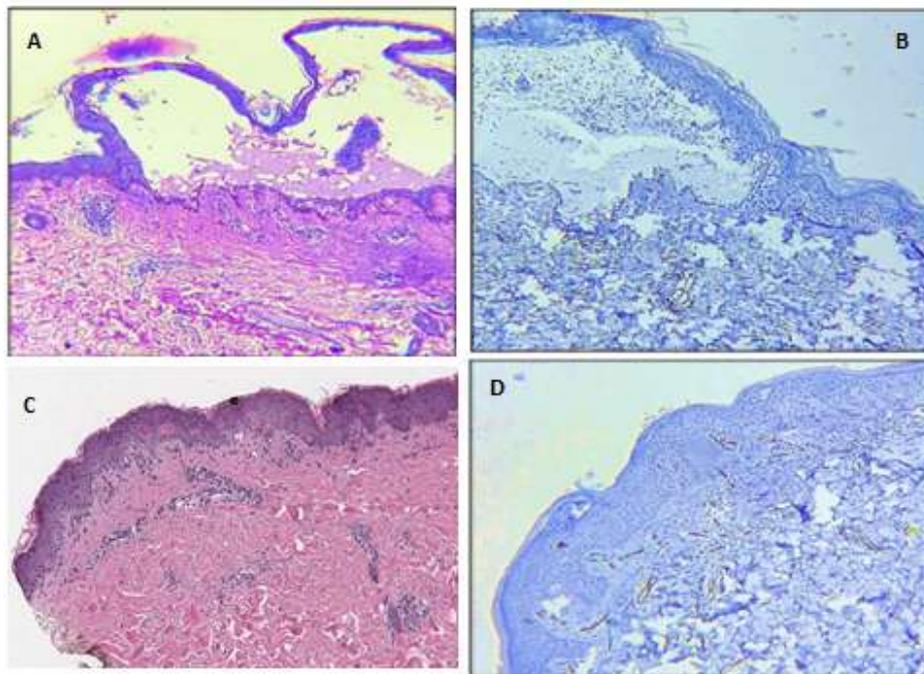


Fig-4A: Photomicrograph showing Pemphigus vulgaris with suprabasal blister containing acantholytic cells, dermal papillae lined by single layer of basal keratinocytes. HE (x 100)

Fig-4B: Photomicrograph showing Bullous lesion with suprabasal blister containing inflammatory infiltrate, and dermal vasculature. CD 34 (x100)

Fig-4C: Photomicrograph showing Erythema multiforme. HE (x 100)

Fig-4D: Photomicrograph showing Erythema multiforme with superficial perivascular, increased dermal vasculature. CD34 (x 100)

DISCUSSION

This was a retrospective and prospective study. The aim of this study was to analyze the dermal vascular alterations in various inflammatory skin diseases. Also to evaluate whether these alterations can help in the differential diagnosis of inflammatory skin diseases.

Total 65 skin biopsies were included in the study. Fifty five with clinical diagnosis of inflammatory skin diseases while 10 were control samples. The lesions included were psoriasis, lichen planus, bullous lesions and erythema multiforme.

All the biopsies were stained with routine H&E stain and CD34 IHC stain. Dermal microvasculature was analyzed in the form of microvascular density (MVD) and microvascular caliber (MVC) by using image analysis software.

Maximum number of patients 20 (30.77%) were in the age group of 21-30 years, followed by equal number of cases, 13 each in 41-50 and >50 year age group. There was a male predominance. (TABLE 1)

Psoriasis

There were 25 patients of psoriasis with male to female ratio of 2.8:1. (CHART NO 1) Male predominance has also been reported in few other

studies [8-10]. Our study revealed highest MVD in psoriasis. It ranged from 52 -230/mm² with a mean of 130.52 /mm². When we analyzed these cases, about 44% cases showed MVD more than 120 /mm² out of which 20% cases showed MVD> 200/mm². None of the control groups showed MVD more than 120/mm², which was statistically significant. (TABLE NO 3). In study done by Boruah and co-workers [11] the range of MVD was 87-329/mm² with mean of 185/mm² which was higher as compared to our study.

We observed highest MVC in psoriasis which ranged from 5.07- 14.81µm with mean of 10.33µm. In the study done by Hern S *et al.* [12], MVC ranged from 6-17µm and by Boruah and co-workers [11] 8.65-18.90 µm with mean of 11.82 µm [11,12]. We obtained statistically significant p value on comparing with controls. (TABLE NO 4)

Lichen planus

We found 15 cases of lichen planus, with male to female ratio of 2:1.(CHART NO 1) Maximum patients were young, with mean age of 27 years.(TABLE NO 1) Young age group was also observed by Parihar A *et al.*,¹³ however they observed female preponderance. They have concluded that, in India, lower age group is more affected when compared with western literature.

The range of MVD in LP cases was 47.82-213.04/mm² with mean of 99.12 /mm². The range of MVC 7.67-12.34µm with mean of 9.73. The MVD in LP was higher when compared with controls; however it was not statistically significant. The MVC in LP was significantly higher than in controls. (TABLE NO 4)

Vybohova D [14] *et al.* found 1.6 times enlargement in blood microcirculatory bed in lichen planus lesions as compared to healthy skin. Our results also showed, 1.6 times increase in MVD and 2 times increase in MVC [14] (TABLE NO 3,4).

Bullous Lesions

Total eight (12%) cases of BL were analyzed in this study, which included pemphigus vulgaris, pemphigus foliaceus and bullous pemphigoid. These were seen in comparatively older age group. The mean age of these patients was 48 years. There was a male predominance with M: F ratio of 3:1. The range of MVD in BL cases was 43.47-147.82 /mm² with mean of 80.97/mm². The range of MVC was 7.05-11.84µm with mean of 9.56. The MVC in BL was statistically significant when compared with control. Literature regarding MVD and MVC in bullous lesions was not available for comparison. This study has been done for the first time.

Erythema Multiforme

Seven cases of EM were analyzed in this study. The commonest age was 21-30 years, (TABLE NO 1) which was similar to psoriasis. There was male predominance with M: F ratio of 2.5:1. The mean age of these patients was 33.14 years. The MVD in EM cases ranged from 60.86-100 /mm² with mean of 72.35 /mm². It was higher than the control group but was not statistically significant. MVC in EM ranged from 8.6-11µm with mean of 10µm. The comparison of MVC between EM and control showed statistical significance. We haven't come across any study in the past on these parameters (MVD/MVC) in EM.

We observed vascular elongation and tortuosity along with endothelial swelling in the form of nuclear hypertrophy, increased cytoplasm and endothelial gaps in most of the patients with inflammatory skin disorders. (FIGURE 2). Changes of vascular elongation and tortuosity were distinctly observed in psoriasis as compared to other lesions. (FIGURE 2)

Barton *et al.* [15], indicated elevated endothelial volume and luminal volume in the lesional psoriatic skin compared to control subjects, in their study of 20 psoriatic patients and 10 healthy controls. The authors suggested that increase in these parameters was partly due to the increase in number of vascular profiles [15].

Some authors have reported that these vascular morphologic changes precede the visible epidermal hyperplasia [16]. Few ultrastructural studies have shown that dermal capillaries in psoriasis have a wider lumen, bridged fenestrations with edematous areas in endothelial cytoplasm [17].

Mordovtsev VN and his co-worker in 1989, studied skin microvasculature in psoriasis. He observed vascular dilatation, bridged fenestrations and gaps in endothelium, oedematous areas in the cytoplasm of endotheliocytes, myocytes and pericytes, basement membrane zone thickening and cell extravasation (signs of increased vascular permeability) in these biopsies[18].

As electron microscopy was not available at our set up, we were unable to quantitate the exact endothelial changes in various inflammatory skin diseases. So detail ultrastructural studies are necessary. Anti angiogenic drugs have been tried for the treatment of various inflammatory skin diseases showing good results and help to reduce the dose of steroids [19-23].

CONCLUSION

Inflammatory skin diseases were predominant in young males. The MVD was maximum in cases of psoriasis, followed by lichen planus, bullous lesions, and erythema multiforme. However it was least in the control group.

The high MVD value in psoriasis cases was statistically significant when compared with the controls. The MVC was maximum in psoriasis, followed by erythema multiforme, lichen planus, bullous lesions and was statistically significant when compared with control.

Dermal vessel elongation and tortuosity was significantly observed in psoriasis cases. There was endothelial swelling in the form of nuclear hypertrophy and increased cytoplasm, endothelial gaps in most of the patients with inflammatory skin disorders.

CD 34 stain was definitely helpful in studying the dermal vascular alterations which are otherwise difficult to appreciate on routine H&E.

Image analysis software is useful to determine MVD and MVC.

Thus we conclude that angiogenesis was increased in inflammatory skin diseases and it was highest in psoriasis. Therefore, therapeutic intervention at the level of vasoproliferation may prove to be useful for the treatment. Research on potent antiangiogenic drugs with least side effects can change the therapeutic outcome in these patients.

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