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Original Research Paper

Pathology

DIRECT IMMUNOFLUORESCENCE PROFILE OF LICHEN PLANUS - A FRESH LOOK ON OLD DISEASE. A STUDY OF 15 CASES.

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ABSTRACT

Introduction: Lichen planus (LP) is a chronic inflammatory mucocutaneous diasease that is often diagnosed on the basis of clinical &/or histopathologic findings. However, it has some characteristic direct immunofluorescence (DIF) findings that can help in diagnosing confusing cases.

Aim: We analyzed DIF findings in skin biopsy in LP compared to histopathology.

Materials & methods: A skin biopsy of lesion was taken from 15 cases of LP (13 classic LP & 2 LP pigmentosus) & subjected to histopathology & DIF examination.

Results: The sensitivity of histopathology and DIF was found to be 100% & 93.3% respectively for the diagnosis of LP & LPP.

KEYWORDS : Direct immunofluorescence, Lichen planus, skin biopsy

Introduction

Lichen planus (LP) is a chronic inflammatory mucocutaneous diasease that commonly affects middle age patients with slight female predominance. Pruritic, Purple, Polygonal, Planar, Papules, and Plaques are the classical "6 P's" of LP that often render a diagnosis on clinical examination alone. [1] The widely described characteristic histologic features are sufficient to confirm the diagnosis in most cases. [2] However due to clinical heterogeneity of the lesions, cutaneous LP can sometimes be virtually indistinguishable from other lichenoid lesions, lupus erythematosus, drug reactions etc.; both clinically and histopathologically. Chronicity, erosive nature and recurrence of LP lesions can have a detrimental effect on patients' quality of life, if left untreated. [1] Therefore an early and correct diagnosis is essential. The direct immunofluorescence (DIF) examination of skin biopsy is a useful adjunct for confirmation of diagnosis in such confounding cases. [1,3] [Table 1]

Table 1: The pattern of DIF findings in potential differential diagnoses of lichen planus.[1]

Differential Diagnosis	DIF Findings
Lichen planus	Globular deposits of immunoreactants at cytoid bodies particularly in clusters + shaggy fibrin deposition at DEJ
Discoid lupus erythematosus	Linear continuous band of granular/ homogenous IgG, IgM, IgA and C3 at DEJ in various combinations (LBT) in lesional skin + dermal blood vessel deposits
Systemic lupus erythematosus	Linear continuous band of granular/ homogenous IgG, IgM, IgA and C3 at DEJ in various combinations (LBT) in lesional AND nonlesional non sun-exposed skin + dermal blood vessel deposits
Bullous pemphigoid	Linear C3, IgG at DEJ
Porphyria	Less intense immunoreactivity at DEJ + strong intensity in dermal blood vessels (exactly opposite of LE), complement is rarely found
Erythema multiforme	Negative
Hailey-Hailey disease	Negative
Chronic ulcerative stomatitis	Speckled or granular perinuclear IgG in the lower third and basal layer of epithelium
Aphthous ulcers	Negative
Lichen nitidus	Negative

Since there is a paucity of Indian literature describing the immunofluorescence profile of lichen planus, we conducted this study to characterize DIF findings in LP & its variants and their correlation with histopathology.

Materials and methods

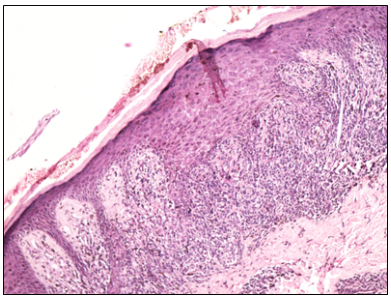
Skin biopsies received from Dermatology outpatient clinic over a 2-year period from freshly diagnosed patients of LP (n = 15) were enrolled in the study. The study was conducted in Department of Pathology after obtaining clearance from the institutional ethical committee and informed written consent from patients. Patients with no active skin lesions, history of steroids/ immunosuppressive therapy in last 4 weeks were excluded. A 4 mm punch biopsy of skin lesion was taken. One half was sent for histopathological examination in 10% neutral buffered formalin and other half in Michel's medium for DIF examination. Five µm thin frozen tissue sections were taken for DIF. Slides were stained with fluorescein isothiocyanate (FITC) conjugated antibodies against IgG, IgM, IgA, C3 and fibrinogen in optimal dilutions by a standardized method and incubated in dark moist chamber at 37°C for 1 hour. The slides were washed multiple times in Phosphate buffer saline (PBS) and mounted in glycerol-PBS mixture and viewed under the immunofluorescence microscope fitted with an UV-light source. Each slide was assessed for deposition of immunoreactants, their type, site, pattern and intensity of fluorescence. Final diagnosis of LP was made after correlating clinical, histopathology and DIF findings.

Results

Fifteen clinically suspected cases comprising of classic Lichen Planus (LP, 13/15) and Lichen Planus Pigmentosus (LPP, 2/15) were studied. The age distribution ranged from 5-58 years with slight male preponderance (M:F = 1.14:1). A definitive histopathological diagnosis could be made in all (15/15, 100%) cases, diagnosed as LP in 13/15 (86.7%) cases and LPP in 2/15 (13.3%) cases. On DIF, 14/15

(93.3%) cases showed positive findings while one case of LP (6.7%) was negative. This case was confirmed on the basis of characteristic histopathologic findings. Therefore the sensitivity of histopathology and DIF was found to be 100% & 93.3% respectively for the diagnosis of LP & LPP. [Figure 1]

Figure 1: A case of Lichen planus demonstrating hyperkeratosis, hypergranulosis, irregular acanthosis, saw toothing of the rete ridges and a dense band-like chronic inflammatory infiltrate at dermo-epidermal junction. [H&E, 400X]



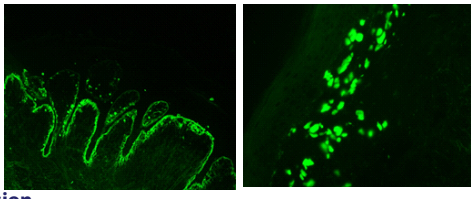
The most common site of immunoreactant deposition in DIF positive (14/15) cases was a combination of basement membrane zone (BMZ) & cytoid bodies (CB) seen in 7/12 (58.3%) including 5 cases of LP & 2 cases of LPP, followed by isolated BMZ positivity seen in 6/12 (50%) cases of LP. [Table 2]

Table 2: Distribution of immunoreactants in Lichen planus according to site, pattern & type of immune deposits

Diagnosis (n)	Site & pattern of immune deposits	
	BMZ (Shaggy deposits)	Cytoid Body (globular deposits)
LP (13)	Fibrinogen (2)	C3 (2)
	Fibrinogen (1)	IgM (1)
	Fibrinogen (1)	C3+IgM+A (1)
	Fibrinogen (1)	IgG (1)
	Fibrinogen (5)	Negative (5)
	IgM+C3 (1)	Negative (1)
	Negative (1)	IgA (1)
	Negative (1)	Negative (1)
LPP (2)	Fibrinogen (1)	IgM+C3 (1)
	IgM+Fibrinogen (1)	IgM (1)
	Negative (0)	Negative (0)

Fibrinogen (shaggy deposits) was the most common immunoreactant seen at the basement membrane zone (BMZ) in 12/15 (80%) cases while C3 was the most common immunoreactant seen at cytoid bodies (CB) seen in 4/15 (26.7%) cases. [Figure 2]

Figure 2: a) Shaggy deposits of fibrinogen at BMZ. b) Globular deposits of IgM in numerous cytoid bodies. [400X]



Discussion

The cases of LP and LPP ranged from 5-58 years with mean age of 30 yrs. Bhushan R et al, Parihar A at al, Kulthanan et al and Dhar et al reported an age range of 5-68 years, 5-76 years, 6-76 years and 29-45 years respectively among the LP patients. [4-7] Notably 20% (3/15) cases in our study were less than 18 years of age which is similar to the findings of Parihar A et al who reported 28% (41/145) cases of LP in the pediatric age group. [5] Nangia A et al [8] also reported a peak incidence of LP between 11-20 years of age. This is in contrast to Western literature where LP is considered to be rare in children.

In the present study, among LP, there was slight male preponderance (53.3%) with M:F ratio of 1.14:1 in concordance with Bhushan R et al and Singh OP et al with M:F ratio of 1.06:1 and 3:2 respectively. [4,9] Kulthanan et al reported an equal gender distribution while Dhar et al reported marked female predominance (M:F = 1:6.5). [6,7] Among LPP, both cases were women.

On DIF, 14/15 (93.3%) cases showed positive findings suggestive of LP. Bhushan R et al, Kulthanan K et al, Kabir AK et al & Minz RW et al reported DIF positivity of 87.9%, 75%, 70.5% & 57% respectively in LP. [4,6,10,11] Nangia A et al reported an overall DIF positivity of 80% in various types of LP (Lichen Planus Hypertrophicus, Lichen Planus Actinicus & Lichen planopilaris) & 100% positivity in classic LP. The BMZ was the most common site of immunoreactant deposition seen in 13/14 (92.8%) DIF positive cases while CB deposition was seen in 8/14 cases (57.1%). Nangia A et al also found BMZ deposits in majority (80%) of cases followed by blood vessel deposits (16%) & cytoid bodies (8%). [8] However none of our cases showed blood vessel positivity.

A combination of BMZ & CB was seen in 8/14 (57.1%) cases followed by deposition at BMZ alone in 6/14 (42.8%) cases. These findings are concordant with the observations of Nangia A et al who reported that a combination of BMZ & CB (38%) deposits was the predominant pattern in LP. [8] Chularojanamontri L et al also found that the most common pattern in LP was immunoreactant deposition at CB with BMZ (62%), however presence of numerous strong positive cytoid bodies alone favours LP over LE. [12] We also found isolated CB positivity with strong IgA in single case (7.1%) diagnosed as LP.

Fibrinogen was the most common deposit at BMZ seen in 12/15 (80%) cases. Nangia A et al, Bhushan R et al and Kulthanan K et al also reported fibrinogen to be the most common deposit at BMZ found in 52%, 72.7% and 100% cases respectively. [4,6,8] While Minz RW et al reported irregular deposits of fibrinogen/ IgM/ C3/ IgG at DEJ in 70.5% LP cases. [11] Camisa C et al suggested that fibrinogen at BMZ in the absence of fluorescence by other immunoreactants is sufficiently unique to be used as a diagnostic criterion for LP. [13] Similarly we also found 5 cases of LP showing shaggy fibrinogen deposits at BMZ without any other immunoreactants. Kulthanan K et al also emphasized that fibrin was often seen as shaggy / broad linear band at DEJ while immunoglobulins were usually granular. [6] Contrastingly, Arora SK et al reported DIF positivity in 55% cases & BMZ positivity in only 7% cases. However, fibrinogen was not included in their study that might be responsible for low DIF positivity. [14]

The cytoid bodies were most frequently positive with IgM & C3 in 4/14 (28.6%) cases each. Kulthanan K et al found that the most common deposit at cytoid bodies was IgM (93%) followed by C3 (47%). [6] While Bhushan R et al found C3 to be predominantat immunoreactant at cytoid bodies (38.4%) followed by IgM (30.7%) cases. [4]

Histopathology was 100% sensitive for both LP and LPP while DIF was 92.3% (12/13) and 100% (2/2) sensitive respectively. Bhushan R et al and Kulthanan et al also reported higher sensitivity of histopathology (100%) for diagnosis of LP versus DIF (87.9% & 75% respectively). [4,6] In contrast, Minz RW et al reported DIF positivity of 57% in LP in comparison to 43% by histopathology. [11] [Table 3]

Table 3: Comparison of results of DIF and histopathology in Lichen planus and its variants

LP and its variants	Nangia A et al (2000)	Kulthanan et al (2007)	Arora SK et al (2014)	Bhushan R et al (2017)	This study
Mean age	(peak incidence in 11-20 years)	44.7 years	46.6 years	32.4 years	30 years
M:F	1:1.7	1:1	1:1.1	1.06:1	1.14:1
HPE	100%	100%	89%	100%	100%

DIF	80%	75%	55%	87.9%	93.3%
DIF positivity at BMZ	72%	53%	7%	75.6%	86.7%
DIF positivity at CB	8%	60%	47%	54.5%	53.3%
Most common immunoreactant at BMZ	Fibrinogen (72%)	Fibrinogen (100%)	IgG (67%)	Fibrinogen (96%)	Fibrinogen (80%)
Most common immunoreactant at CB	C3 (8%)	IgM (93%)	IgM (72%)	C3 (36.3%)	IgM & C3 (28.6%)
Histo-DIF correlation	80%	75%	55%	87.9%,	93.3%

In this study, 14/15 (93.3%) cases showed good histo-immunological correlation (p value < 0.01). Bhushan R et al, Nangia A et al, Kulthanan K et al & Kabir AK et al reported histo-immunological correlation in 87.9%, 80%, 75% & 70.5% cases respectively. [4,6,8,10]

Conclusion

Both histopathology & direct immunofluorescence examination of skin biopsy are useful tools for confirming the diagnosis of Lichen Planus & its variants. Direct immunofluorescence examination is not required in every case however the characteristic findings might help in cases with overlapping clinico-histopathological features. It should also be kept in mind that since deposits at basement membrane zone or cytoid bodies on DIF can also be seen in diseases like Discoid Lupus Erythematosus, Erythema Multiforme, scleroderma, morphea etc., DIF should always be used in conjunction with histopathology.

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