

## Direct Immunofluorescence in Lichen Planus and Lichen Planus like Lesions

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### Abstract

**Background:** The direct immunofluorescence examination (DIF) in lichen planus (LP) and lichen planus like lesions can show deposition of multiple immunoreactants at dermoepidermal junction (DEJ) and cytooid bodies (CB).

**Patients and Methods:** Fifty one cases of lichen planus and lichen planus like lesions were included in the study on the basis of clinical and histological criteria at Department of Pathology, Lady Hardinge Medical College from November 2012 to March 2014. The results of direct immunofluorescence was analysed in these cases.

**Results:** Among lichen planus (LP), DIF positivity at dermoepidermal junction (DEJ) was seen in 25/33 (75.6%) cases followed by deposit at cytooid bodies in 18/33 (54.5%) cases. The immunoreactivity at DEJ was most commonly seen with fibrinogen in 24/33 (72.7%) cases. DIF positivity at cytooid bodies was most commonly seen with C3 in 12/33 (36.3%) cases. Among lichen planus pigmentosus (LPP), DIF positivity was seen in 9/13 (69.2%) cases. The deposit at DEJ was noted in 2/13 (15.3%) cases while deposit at cytooid bodies (CB) were seen in 8/13 (61.5%) cases. Most common immunoreactant at cytooid bodies was C3 in 5/13 (38.4%) cases. The sensitivity of histopathology and direct immunofluorescence for the diagnosis of lichen planus including lichen planus-like lesions was found to be 100% & 74.5% (LP-87.9% and LPP-69.2%) respectively.

**Conclusions:** The study showed that DIF is helpful in diagnosis of LP and LP like lesions. In LP, the shaggy fibrinogen deposit alone at DEJ & C3 alone or in combination of other immunoreactants at CB was found to be most characteristic

**Key words:** Direct immunofluorescence, Lichen planus, skin, pathology,

### Introduction

Lichen planus (LP) is an idiopathic subacute or chronic inflammatory disease of the skin, mucous membranes and nails [1]. Cutaneous lichen planus is characterized by polygonal flat-

topped, violaceous papules and plaques, which in some cases can be intensely itchy. The lesions may result in longstanding residual hyperpigmentation, especially in dark skinned patients [2]. According to one study LP represents 0.38% of all dermatology outpatients in India [3]. LP frequently occurs between the ages of 30 and 60 years without sexual or racial predilection [4].

The diagnosis of LP is based on the clinical character of the lesions and histological examination [5]. However, direct

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immunofluorescence (DIF) studies may be

**TABLE 1: Sensitivity of Histopathology and DIF for diagnosis of LP & LP like lesions**

Diagnosis (n)	HPE	DIF	Site
LP(33)	100%	87.9%	DEJ(75.6%) followed by deposit at CB(54.5%)
LPP(13)	100%	69.2%	CB(61.5%) followed by deposit at DEJ(15.4%)
LPH(2)	100%	NEG	-
L Planopilaris (1)	100%	NEG	-
L Nitidus(1)	100%	NEG	-
L Striatus(1)	100%	NEG	-

HPE- Histopathology; DIF – Direct immune fluorescence.

helpful in disease differentiation for cases with ambiguous features of other diseases, e.g. lupus erythematosus (LE) [6]. This study was undertaken to analyze the role of direct immunofluorescence in the diagnosis of lichen planus and lichen planus like lesions.

### Material and Methods

Fifty one cases of lichen planus and lichen planus like lesions were included in the study on the basis of clinical and histological criteria at Department of Pathology, Lady Hardinge Medical College from November 2012 to March 2014. A single 4mm punch biopsy of skin lesions was taken and it was bisected with one half sent for histopathological examination in 10% neutral formalin and other half in Michel's medium (pH = 7.25) for direct immunofluorescence examination. Five µm thick tissue sections were cut for immunofluorescence study by a standardized method.

Slides of each case was stained with fluorescein isothiocyanate (FITC) conjugated antibodies directed against IgG, IgM, IgA, C3 and fibrinogen and incubated in dark at 37°C for 1 hour. After several washings in phosphate buffer saline (PBS), the slides were mounted in glycerine PBS

mixture and viewed under the

**Table 2: Type of Immunoreactant According to the Site of Deposition in LP (N=33)**

Immunoreactant	DEJ	CB
FIB	24	0
C3	0	4
IgM	0	3
IgM+C3	1	3
C3+IgG	0	2
IgG,C3,IgM	0	1
IgG	0	1
C3,IgM,IgA,IgG	0	1
FIB,IgG,IgA	0	1
IgA,IgM,C3	0	1
IgA	0	1
Negative	8	15

immunofluorescence microscope fitted with an UV light source, under ideal excitation and barrier filter combination. Each slide was assessed for presence or absence of immunoreactants, type of immunoreactant, site of deposition (basement membrane zone, cytotoid body), pattern (linear, granular, homogenous, shaggy etc.) and intensity of fluorescence. The approval of Ethics Committee of Lady Hardinge Medical College was obtained prior to the study. Informed consent from the participants was also obtained.

### Results

Fifty one cases comprised of lichen planus (LP, 33/51), lichen planus pigmentosus (LPP, 13/51), lichen planus hypertrophicus (LPH, 2/51), lichen planopilaris (LPPil, 1/51), lichen nitidus (L nitidus, 1/51) and lichen striatus (L striatus, 1/51) (Table 1). The age distribution ranged from 5-69 years with slight female preponderance (50.99%). The cases of LP ranged from 5-68 years, cases of LPP ranged from 9-69 years. The mean age of LPH was 11.5 yrs. There was one case each of PPil, L nitidus and L striatus aged 30 years, 26 years and 12 years respectively.

**Table 3: Type of immunoreactant according to the site of deposition in LPP (N=13)**

Immunoreactant	DEJ	CB
C3	0	3
FIB	2	0
FIB,IgM,C3	0	1
IgM	0	1
IgM,C3	0	1
IgA	0	1
IgG, IgM, FIB	0	1
Negative	11	5

The sensitivity of histopathology and direct immunofluorescence for the diagnosis of lichen planus including lichen planus-like lesions was found to be 100% & 74.5% (LP-87.9%; p value=0.114 and LPP-69.2%; p value=0.096) respectively. Among LP, DIF positivity with the deposit at DEJ was the commonest (25/33, 75.6%) followed by deposit at cytoid bodies (18/33, 54.5%). The immunoreactivity at DEJ was most commonly seen with fibrinogen alone (24/33, 72.7%) (Table 2).

Among LPP, DIF positivity at cytoid bodies was seen in 8/13 (61.5%) cases, most commonly seen with C3 in 12/33 (36.3%) cases followed by IgM 9/33 (27.2%). The deposit at DEJ was noted in 2/13 (15.3%) cases, seen with fibrinogen (Table 3).

**Table 4: Comparison of this study with other studies in LP**

LP	Kulthanan <i>et al.</i> , (2007)	This study
Mean age	44.7 yrs	32.4yrs
M:F ratio	1:1	1.06:1
HPE positivity	100%	100%
DIF positivity	75%	87.9%
DIF positivity at DEJ	53%	75.6%
MC immunoreactant at DEJ (out of total DIF positive cases)	Fib (100%)	Fib(96%)
DIF positivity at CB	60%	54.5%
MC immunoreactant at CB	IgM(93%)> C3(47%)	C3(36.3%)> IgM(27.2%)
Histo-immunological	75%	87.9%

## Discussion

The cases of LP ranged from 5-68 years (mean age 32.42 yrs) in this study. Kulthanan *et al.*, [4] and Dhar *et al.*, [7] reported age range of 6-76 yrs and 29-45 years respectively among the LP patients. Cases of LPP ranged from 9-69 years (mean age 36.15 years) while Kanwar *et al.*, [8] reported age range between 13-62 years in his study on LPP. The mean age of LPH was 11.5 yrs. There was one case each of Lichen planopilaris, L nitidus and L striatus aged 30 years, 26 years and 12 years respectively. Chierigato C *et al.*, [9], Park *et al.*, [10] and Mu *et al.*, [11] reported mean age of patients of Lichen planopilaris, Lichen nitidus and Lichen striatus as 52 years, 45 yrs and 4.6 years respectively.

Among LP, there was slight male predominance (51.5%) with M:F ratio of 1.06:1 in concordance with Singh *et al.*, [12] (M: F ratio = 3:2). Kulthanan *et al.*, [4] reported an equal incidence in males & females while Dhar *et al.*, [7] reported M: F ratio of 1:6.5. Among LPP, there was female predominance (61.5%) with M: F ratio being 1:1.6, similar to the study of Kanwar *et al.*, [8] (M:F= 1:1.2). Among LPH, both cases were male. L PPil and L striatus have one female patient each while one case of L nitidus was a male.

### Direct Immunofluorescence Findings

Direct immunofluorescence positivity was seen in 29/33 (87.9%) cases of LP. Kulthanan *et al.*, [4] (Table 4) and Kabir *et al.*, [13] reported DIF positivity in 75% and 70.5% of LP respectively.

**Table 5: Comparison of this study with other studies in LPP**

LPP	Kanwar et al	This study
AGE	13-62 yrs	9-69 yrs
M:F	1:1.2	1:1.6
DIF+	14.2%	69.3%
@CB	-	61.5% mc with C3(5/13)
@DEJ	7.1%	15.3%

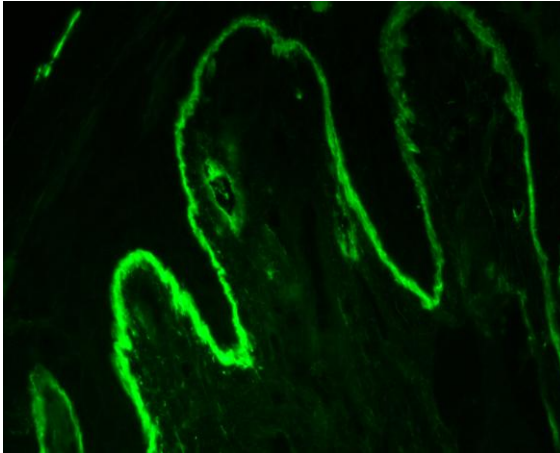


Figure 1: DIF of Lichen Planus showing shaggy deposits of fibrinogen at DEJ.

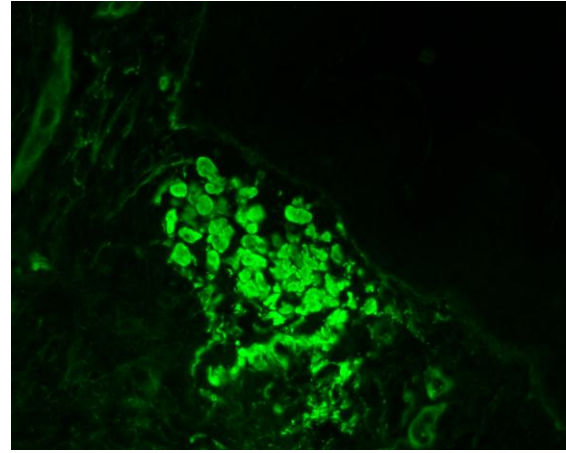


Figure 2: DIF of Lichen Planus showing C3 deposition at cytotoid bodies in upper dermis.

Nine out of thirteen (69.3%) cases of LPP showed DIF positivity. Kanwar *et al.*, [8] reported DIF positivity in 14.2% cases of LPP (Table 5).

Among LP, DIF positivity with the deposit at DEJ (Figure 1) was the commonest seen in 25/33 (75.6%) followed by deposit at cytotoid bodies in 18/33 (54.5%) cases. The immunoreactivity at DEJ was most commonly seen with fibrinogen alone in 24/33 (72.7%) cases. Kulthanan *et al.*, [4] found that either alone or in combination, the most common deposit at DEJ was fibrinogen (100%) followed by C3 (47%). Kabir *et al.*, [13] reported irregular deposits of fibrinogen/ IgM/ C3/ IgG at DEJ in 70.5% LP cases.

Among LPP, DIF positivity was seen in 9/13 (69.2%) cases. The deposit at DEJ was noted in 2/13 (15.3%) cases, seen with fibrinogen. Kanwar *et al.*, [8] reported DIF positivity with IgM and C3 at DEJ in 1/14 (7.1%) cases each. Among LP, DIF positivity at cytotoid bodies, either alone or in combination, was most commonly seen with C3 in 12/33 (36.3%) cases followed by IgM 9/33 (27.2%). Kulthanan *et al.*, [4] reported the cytotoid bodies staining most commonly with IgM (93%) followed by C3 (47%) (Table 4).

Among the 13 cases of LPP, deposit at cytotoid bodies was seen in 8/13 (61.5%) cases. Most

common immunoreactant at cytotoid bodies was C3 (Figure 2) in 5/13 (38.4%) cases followed by IgM in 4/13 (30.7%) cases. However, Kanwar *et al.*, [8] did not find positivity of any immunoreactant at cytotoid bodies in his study on LPP. Among LP, a combination of DEJ (shaggy deposit) with deposit at CB was the commonest seen in 14/29 (48.3%) cases followed by DEJ (shaggy) alone seen in 10/29 (34.5%) cases. Chularojanamontri *et al.*, [14] also found that the most common pattern in LP (62%) was immunoreactant deposition at DEJ and CB (62.1%) followed by CB alone (29.3%).

In cases of LPP, the most common site of immunoreactions deposition was at CB alone, seen in 7/9 (77.8%) cases, while DEJ (shaggy) alone and a combination of DEJ (shaggy) and CB was seen in one case (11.1%) each. In our study, the immunoreactions deposition at DEJ and /or CB with fibrinogen, IgM, IgG & C3 was of strong intensity. Only IgA deposition at cytotoid bodies was moderate in intensity. Nangia *et al.*, [15] observed that the intensity of DIF positivity was maximal in lesions of less than 3 months duration and which are associated with vasodilatation and dermal edema. The older lesions are DIF negative due to breakdown of fibrinogen by phagocytosis. Among LP, 29/33 (87.9 %) cases showed good histo-immunological correlation while among LPP, 9/13 (69.2%) cases showed good histo-

immunological correlation. Kulthanan *et al.*, [4] & Kabir *et al* [13] found histo-immunological correlation in 75% & 70.5% cases respectively among LP.

Histopathology was 100% sensitive for both LP and LPP while DIF was 87.9% (29/33) and 69.2% (9/13) sensitive respectively. Kulthanan *et al* [4] also found that sensitivity of HPE for LP was higher (100%) as compared to DIF (75%). Although CBs can be found in whole lot of conditions, yet they are an important diagnostic finding in conditions like LP and DLE. Presence of CBs alone or in combination with other immunoreactions deposits at DEJ can help in differentiating the diseases presenting sometimes with close overlapping features [16]. Lichenoid eruptions represent a heterogeneous group of conditions that resemble idiopathic LP in terms of their clinical appearance and demonstrate a lichenoid tissue reaction. Direct immunofluorescence can be similar at times in these conditions. Thus, considering all three modalities i.e. clinical features, histopathology and direct immunofluorescence, together can aid to reach a correct diagnosis [17].

### Conclusion

The study showed that DIF is helpful in diagnosis of LP and LP like lesions. In LP, the shaggy fibrinogen deposit alone at DEJ & C3 alone or in combination of other immunoreactants at CB was found to be most characteristic while in LPP, deposit of C3 alone or in combination with other immunoreactants at CB is characteristic. However, DIF must always be used in conjunction with histopathology.

### Authors' Contribution

**RB:** carried out the experiments and interpreted the results, carried out the literature search and prepared the draft manuscript, designed the study and performed the analysis, conceived the

study, participated in design and edited the final manuscript.

**SA:** interpreted the results, prepared the draft manuscript, designed the study and performed the analysis, conceived the study, participated in design and edited the final manuscript.

**RC:** conceived the study, participated in design and edited the final manuscript

**KA:** participated in design and edited the final manuscript

All authors read and approved the final manuscript for publication.

### Conflict of Interests

The authors declare that there are no conflicts of interests

### Ethical Considerations

The study was approved by Institute Ethics committee.

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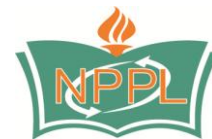
None

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