

TITLE: DIRECT IMMUNOFLUORESCENCE STUDY IN DISCOID LUPUS  
ERYTHEMATOSUS

Authors: Reema Bhushan, Shilpi Agarwal, Ram Chander, Kiran Agarwal

**ABSTRACT:** Diagnosis of a classical case of Discoid lupus erythematosus (DLE) is generally clinical. However, histopathological study of skin biopsy is helpful to confirm the diagnosis in early DLE lesions and in DLE variants. There is however paucity of large studies on its direct immunofluorescence (DIF) findings in India. The study was conducted in the Department of Pathology on 34 clinically suspected cases of Discoid lupus erythematosus who presented in the Department of Dermatology, Venereology & Leprosy at Smt. Sucheta Kriplani Hospital from 2012 to 2014. Histopathological diagnosis of DLE could be made in 30/34 (88.2%) cases while 4/34 (11.8%) cases showed non specific findings on histopathology. These four cases showed positive lupus band test on DIF examination, hence rendering the diagnosis of DLE. DIF was positive in 30/34 (85.3%) cases at the dermoepidermal junction (DEJ) in the form of lupus band test (LBT) in the lesional skin biopsy. The majority of cases showed deposit of multiple immunoreactants at DEJ (61.7% cases) with combined positivity for IgM plus IgG in 7/34 (20.6%) cases. Five cases (14.8%) cases in addition also showed DIF positivity in the dermal blood vessel (DBV) wall. Direct immunofluorescence is a useful diagnostic technique in the diagnosis of DLE. However, it should be used in conjunction with histopathology.

**KEY-WORDS:** Direct immunofluorescence, Discoid lupus erythematosus, Lupus band test

**INTRODUCTION:** Discoid lupus erythematosus (DLE) is a chronic dermatological disease that can lead to scarring, hair loss, and hyperpigmentation. Hence, early recognition and treatment is essential<sup>1</sup>. Although the diagnosis of classical DLE is generally clinical, histopathological study of skin biopsy is helpful to confirm the diagnosis in early DLE

lesions and in DLE variants. The histopathological findings in DLE are well described, and there is a paucity of large studies on its direct immunofluorescence (DIF) findings in India<sup>2</sup>. Lupus band test (LBT) in the lesional skin is a very sensitive and specific test for DLE. Approximately 90-95% of the patients with discoid lupus erythematosus have positive LBT in the involved skin.

Lupus band test is considered positive when one or more immunoreactants (IgG, IgM, C3, and IgA) are found at the DEJ<sup>3</sup>. The purpose of this study was to analyse the direct immunofluorescence features in the cases of DLE, cases in which the clinical presentation of DLE was not classical, such as early DLE lesions, DLE variants and to correlate DIF findings with histopathology.

**MATERIAL AND METHODS:** The study was conducted from 2012 to 2014 in the Department of Pathology on 34 clinically suspected cases of Discoid lupus erythematosus presenting in the Department of Dermatology, Venereology & Leprosy at Smt. Sucheta Kriplani Hospital. A single 4 mm punch biopsy of lesional skin was taken & it was bisected. One half was 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 standardised 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 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 immunoreactants, site of deposition, pattern and intensity of fluorescence. Final diagnosis of DLE was given after combining clinical findings, histopathology and DIF result. 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: The age of the patients ranged from 22-85 years with M:F =1:1.26. Final diagnosis of DLE (n=34/34) was given after combining histopathological and direct immunofluorescence features (100%). Histopathological diagnosis of DLE could be made in 30/34 (88.2%) cases while 4/34 (11.8%) cases showed non specific findings like focal basal cell vacuolization & perivascular inflammatory infiltrate. DIF examination in these four cases was positive in the form of lupus band test, hence suggesting the importance of using the direct immunofluorescence in conjunction with histopathology. DIF was positive in 29/34 (85.3%) cases at DEJ in the form of lupus band test. Multiple immunoreactants were seen in 21/34 (61.7%) cases. The cases showed a combined positivity for IgG plus IgM in 7/34 (20.6%) cases (Figure 1 and 2) followed by IgG plus fibrinogen in 5/34 (14.7%) (Figure3). However, the most common immunoreactant at DEJ was IgG (52.9 %). The most common pattern of deposit at DEJ was granular (24/29, 82.7%).

In addition, 5/34 (14.8%) cases showed DIF positivity in dermal blood vessels (DBV) most commonly with fibrinogen in 3/34 (8.9%) cases followed by IgG in 2/34 (5.9%) cases. On histopathology, these cases showed superficial and deep dermal perivascular chronic inflammatory infiltrate. These cases also showed positive lupus band test hence aiding in the diagnosis of DLE. All the immunoreactants in DIF study showed strong immunofluorescence intensity. In the present study, 25/34 (73.5%) cases showed good histo-immunological correlation with positive DIF and characteristic histopathology.

DISCUSSION: The age of patients in this study ranged from 22 years to 85 years (mean age 39.7 years) similar to Sandra et al<sup>4</sup> who reported age range of 22 - 48 years (mean age 36.25 years).

There was a slight female preponderance (M:F=0.78:1) which is in concordance with Kulthanan et al<sup>5</sup> (M:F = 0.75:1).

The definitive histopathological diagnosis of DLE was made in 30/34 (88.2%) cases which was consistent with observations of Minz et al<sup>6</sup> & Naqqash et al<sup>7</sup> who reported a diagnostic histopathology in 82% & 79.7% of DLE respectively. However Sandra et al<sup>4</sup> found diagnostic histopathology in 100% cases. Four cases showed non specific findings like focal basal cell vacuolization & perivascular inflammatory infiltrate. These cases showed positive LBT. Hence, DIF played a role in the diagnosis of these four cases in which histopathology with clinical findings were unable to make the definitive diagnosis.

In the present study, on DIF examination, 29/34 (85.3%) cases of DLE showed immunoreactant deposition in the form of lupus band test while 5/34 (14.7%) cases were negative. In these five cases, histopathological finding showed characteristic findings, hence rendering the diagnosis of DLE. Minz et al<sup>6</sup> & Isfer et al<sup>8</sup> and Bharti S et al<sup>2</sup> found positive lupus band test at DEJ in 59% , 69% and 68% of DLE patients respectively. Sandra et al<sup>4</sup> found that DIF was diagnostic in 100% cases. IgG was the most common immunoreactant seen at DEJ in 18/34 (52.9%) cases in this study, which is similar to the findings of Naqqash et al<sup>7</sup>, Kulthanan et al<sup>5</sup>, Al Swaid et al<sup>9</sup>, Bharti S et al<sup>2</sup> & Beutner et al<sup>10</sup>. However, Sandra et al<sup>4</sup> & Isfer et al<sup>8</sup> found IgM to be the predominant immunoreactant.

Deposit at the dermal blood vessels was an additional finding in the study. Five out of thirty four (14.7%) cases also showed DIF positivity in the DBV with fibrinogen in 3/34 (8.8%) cases and with IgG in 2/34(5.9%) cases. Kulthanan et al<sup>5</sup> reported DIF positivity of 15% in the DBV with C3 (9%), IgM(5%) and IgG (2%) while Isfer et al<sup>8</sup> reported 6.9% DIF positivity in the DBV with IgM (5%) and C3 (1.7%).

Immunoreactant deposits at blood vessel walls or sometimes a combination of deposits at the DEJ and immunofluorescence of vascular wall may occur in LE<sup>5</sup>. DIF positivity at the DEJ and at the DBV were strong in intensity in all the cases.

The quality of lupus band positivity can be affected by duration, distribution and type of lesions, sun exposure, prior steroid therapy, etc<sup>7</sup>. Some studies demonstrated that lesions of less than 3 months duration may not reveal immunoglobulin or complement deposition at DEJ<sup>11</sup>. This parameter could not be defined in our study as the duration of the lesions were not specified.

In the present study, the sensitivity of histopathology is not significantly greater than that of DIF (P value=1.000). However, histopathology gave positive or suggestive results in almost all the cases, in contrast to DIF that gave negative results in five patients. A combination of the two techniques yielded more reliable results (100%) than either technique alone (HPE=88.2%, DIF=85.3%). This signify the importance of using and incorporating the direct immunofluorescence findings of the lesional skin biopsy in this study. The yield of combined sensitivity of both techniques together was greater than either of the two techniques separately. This is similar to the study by Nieboer<sup>12</sup>. DIF, thus helps in providing additional diagnostic information in the histopathologically equivocal cases.

## CONCLUSION

Direct immunofluorescence is a useful diagnostic technique in the diagnosis of DLE. However, it should be used in conjunction with histopathology.

## REFERENCES:

1. Panjwani S. Early Diagnosis and Treatment of Discoid Lupus Erythematosus. *J Am Board Fam Med* 2009; 22:206 –213. PMID19264946
2. Bharti S, Dogra S, Saikia B, Walker R M, Chhabra S, Saikia U N. Immunofluorescence profile of discoid lupus erythematosus. *Indian J Pathol Microbiol* 2015; 58:479-82. PMID26549071
3. Mehta V, Sarda A, Balachandran C. Lupus band test. *Indian J Dermatol Venereol Leprol* 2010; 76:298-300. PMID20445312
4. Sandra A, Srinivas CR, Balachandran C, Shenoi SD. Direct immunofluorescence in lesional and uninvolved skin in DLE. *Ind J Dermatol Venereol Leprol* 1998; 64:150-152. PMID20921748
5. Kulthanan K, Roongphiboolsopit P, Chanjanakijskul S, Kullavanijaya P. Chronic Discoid Lupus Erythematosus in Thailand: Direct Immunofluorescence Study. *Int J Dermatol* 1996; 35:711-714. PMID8891819
6. Minz RW, Chhabra S, Singh S, Radotra BD, Kumar B. Direct immunofluorescence of skin biopsy: Perspective of an immunopathologist. *Ind J Dermatol Venereol Leprol* 2010; 76:150-157. PMID20228544
7. Naqqash S, Asad F, Pal SS. Direct immunofluorescence and Histopathology in chronic discoid lupus erythematosus. *Journal of Pakistan Association of Dermatologists* 2011; 21: 98-101.
8. Isfer RS, Sanches JA, Neto CF, Cernea SS, de Oliveira ZNP, Vilela MAC, Silva ML, Miyachi LM, Fukumori LMI, da Silva SR. Direct immunofluorescence in Lupus Erythematosus (LE). *Sao Paulo Med J* 1996; 114(2):1141-1147. PMID9077025

9. Al-Suwaid AR, Venkataram MN, Bhushnurmath SR. Cutaneous lupus erythematosus: comparison of direct immunofluorescence findings with histopathology. *Int J Dermatol* 1995; 34:480-482.PMID7591411
10. Beutner E, Jablonska S, Kumar V. Direct immunofluorescence in lupus erythematosus. In: Beutner E, Chorzelski TP, Beau SF, eds. *Immunopathology of Skin*. New York: Wiley Medical Publication. 1987; p499-523.
11. Blaszczyk M, Dahl MV. Usefulness of direct immunofluorescence in patients with lupus erythematosus. *Arch Dermatol* 1983; 119: 1010-7.PMID6360051
12. Nieboer C. The reliability of immunofluorescence and histopathology in the diagnosis of discoid lupus erythematosus and lichen planus. *Br J Dermatol* 1987; 116: 189-98.PMID3548795

Figures and legends:

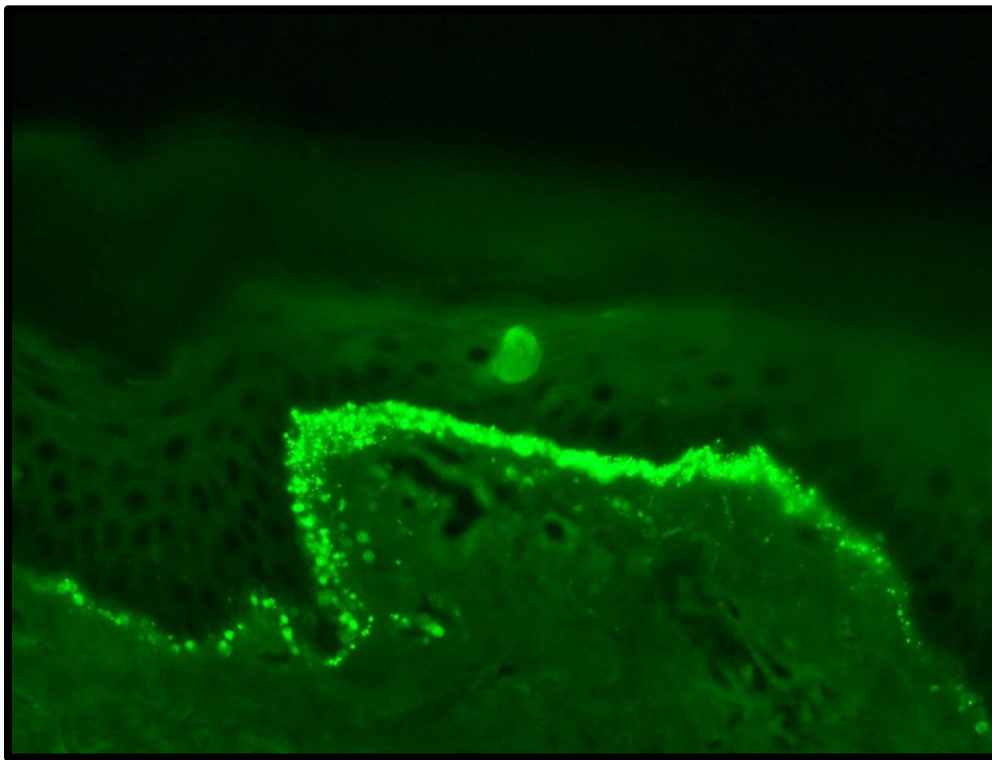


Figure 1: DIF of Discoid Lupus Erythematosus showing deposit of IgG in a linear granular pattern at basement membrane zone (Lupus band)

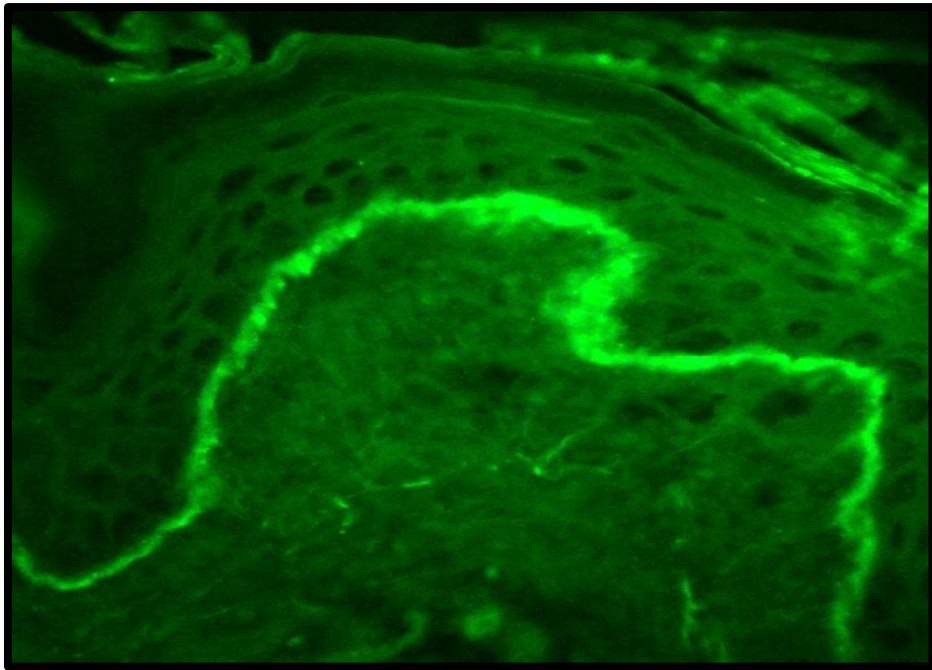


Figure 2: DIF of Discoid Lupus Erythematosus showing deposit of IgM in a linear granular pattern at basement membrane zone (Lupus band)

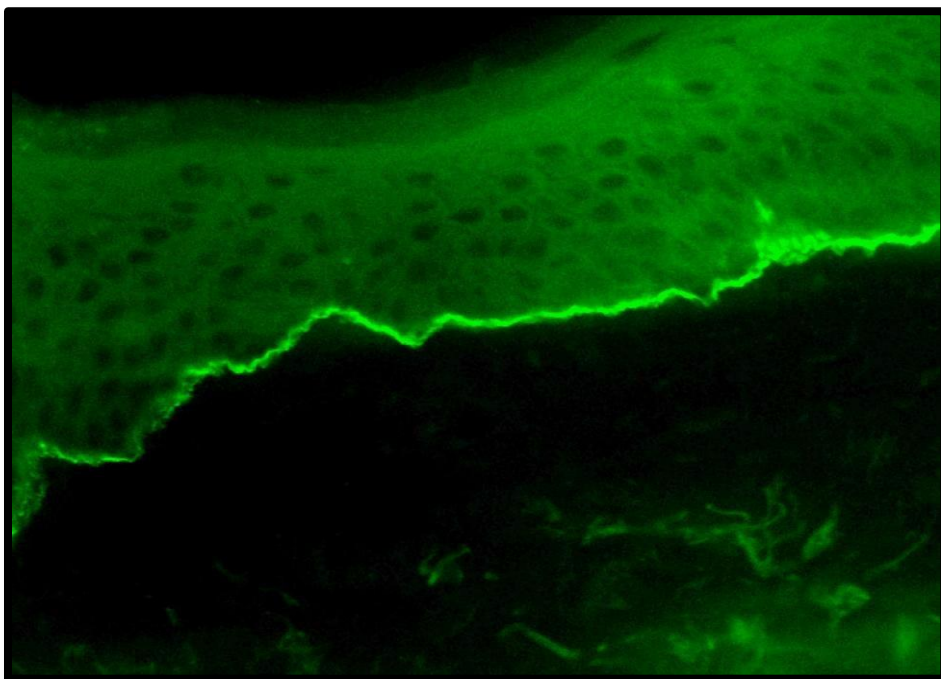


Figure 3: DIF of Discoid Lupus Erythematosus showing deposits of fibrinogen in a linear homogeneous pattern at basement membrane zone



TABLES :

1.Details Of Immunoreactant Deposits At The Dermoepidermal Junction In DLE

	Dermoepiermal Junction (DEJ)	Blood Vessels (BV)
IgG,IgM	7	0
IgG,FIB	5	0
IgM	4	0
IgG,C3,FIB	3	0
IgM,FIB	3	0
FIB	2	3
IgM,C3	2	0
IgG	2	2
IgG,IgM,C3,FIB	1	0
NEGATIVE	5	29

2.Site & Pattern of Immunoreactant Deposition in cases of DLE (n=29/34)

SITE – PATTERN	DLE	PERCENTAGE (%)
DEJ – Granular	21	72.4%
DEJ - Granular + DBV	5	17.3%
DEJ - Granular +DEJ - Homogenous	3	10.3%
Total	29	100%

### 3.Comparison of this study with other studies in DLE

DLE	Kulthanan et al(1996)	Sandra et al(1998)	Isfer et al (1996)	Naqqash et al(2011)	Bharti S et al(2015)	This study
AGE	15-68 yrs	22-48yrs	6-79 yrs	14-60 yrs	6-65yrs	22-85 yrs
M:F	0.75:1	0.23:1	0.4:1	0.2:1	0.66:1	0.78:1
HPE	100%	100%	100%	79.7%	60%	88.2%
DIF	90%	100%	69%	79.3%	68%	85.3%
Multiple immunoreactants at DEJ	62%	87.5%	66.6%	57.6%	34%	61.7%
MC @ DEJ	IgG(57%)	C3& IgM	IgM(52.3%)	IgG(74%)	IgG(41.3%)	IgG(52.9%)
DIF @DBV	15%	-	6.9%	-	12%	14.7%

