Original Research Paper

Volume-8 | Issue-8 | August-2018 | PRINT ISSN No 2249-555X

Pathology

DIRECT IMMUNOFLUORESCENCE DECODING THE DIAGNOSTIC DILEMMAS IN DISCOID LUPUS ERYTHEMATOSUS - A STUDY OF 19 CASES.

Dr Deeksha Singh* MD, Senior Resident Pathology, Lady Hardinge Medical College, New Delhi *Correspondig Author

Dr Shilpi Agarwal MD, Director Professor Pathology, Lady Hardinge Medical College, New Delhi

Dr Ram Chander MD, Director Professor & Head, Department of Dermatology and STD, Lady Hardinge Medical College, New Delhi

Dr Kiran Agarwal MD, Director Professor Pathology, Lady Hardinge Medical College, New Delhi

Dr Preksha Singh MD, Junior Resident, Department of Dermatology and STD, Lady Hardinge Medical College, New Delhi

ABSTRACT **Background:** Discoid lupus erythematosus (DLE) is an autoimmune connective tissue disorder affecting skin. The diagnosis is usually clinical with serologic evidence. However skin biopsy is often needed in early or atypical lesions of DLE. A positive Lupus band test is diagnostic of DLE.

Aim: We analyzed direct immunofluorescence findings in skin biopsy in DLE compared to histopathology.

Materials & methods: A skin biopsy of lesion was taken from 19 cases of DLE & subjected to histopathogy & DIF examination.

Results: The sensitivity of histopathology & DIF for diagnosing DLE was found to be 84.2% each, however combined use of both techniques could diagnose 100% cases. The DIF was particularly helpful in diagnosing 3 histopathologically equivocal cases.

KEYWORDS : Discoid lupus erythematosus, direct immunofluorescence, lupus band test

Introduction

Cutaneous lupus erythematosus (LE) commonly presents as discoid lupus erythematosus (DLE). The classical lesion is a plaque with central depigmentation, surrounding hyperpigmentation with adherent scales, telangiectasia and scarring alopecia. [1] However in some early lesions and atypical cases of DLE, the clinical features may be confounding. In such cases, histopathology and direct immunofluorescence (DIF) examination of lesional skin biopsy are important for early diagnosis. [1]

The continuous linear deposition of immunoglobulins (Ig) and/or complement (C3) at the dermo-epidermal junction (DEJ) of skin biopsy from patients with LE, is known as Lupus Band Test (LBT). [2] However, mere presence of deposits at DEJ does not signify a positive LBT and needs to be differentiated from wide variety of lesions showing DEJ deposits. **[Table 1]** Therefore LBT should be correlated clinically.

Table 1: The potential pitfalls in diagnosis of Lupus Band Test on DIF showing depositions of immunoreactants at DEJ. [2,12]

Diseases	DIF Findings
Discoid lupus erythematosus (DLE)	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 (SLE)	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
Lichen planus	Shaggy fibrin deposition at DEJ + Globular deposits of immunoreactants at civette bodies
Bullous pemphigoid	Linear C3, IgG at DEJ
Epidermolysis bullosa acquisita	Linear IgG and C3 at DEJ
Linear IgA bullous dermatosis	Linear IgA at DEJ, less common IgG, IgM, and C3
Dermatitis herpetiformis	Granular IgA at DEJ with concentration at the papillary tips
Para-neoplastic pemphigus	Intercellular IgG and C3 with or without DEJ involvement
Porphyria	Less intense immunoreactivity at DEJ + strong intensity in dermal blood vessels (exactly reverse of what is seen in LE), complement is rarely found
Actinic keratosis, Polymorphic light eruption and Rosacea	Weak, focal & interrupted deposits at DEJ
Autofluorescence	Artefactual fibrillar pseudobands of dermal collagen at low power can mimic positive LBT
Healthy young adults	An interrupted weak deposition at DEJ in normal sun-exposed skin of head and neck in 20% cases

The lesional skin demonstrating positive LBT is very sensitive (90-95%) and highly specific for SLE/DLE. It is also useful in differentiating LE from other antinuclear-antibody positive interface dermatitis like rheumatoid arthritis, scleroderma, dermatomyositis etc. [2] Positive LBT in sun protected normal skin in patients of DLE, also has prognostic value in predicting the severity of the disease and correlates positively with risk of developing nephritis. [3] Only few studies have described DIF findings in DLE cases among Indian population.

Materials and methods

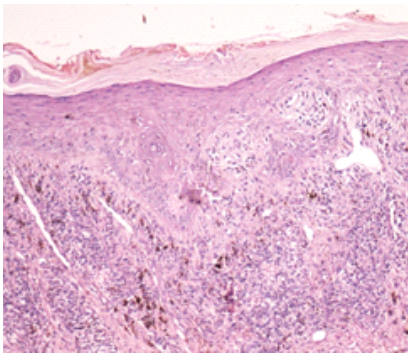
Skin biopsies received from Dermatology outpatient clinic over a 2-year period with a clinical diagnosis of DLE (n = 19) were included in the study. The study was undertaken in Department of Pathology after obtaining clearance from the institutional ethics committee and informed written consent from patients. Patients with no active skin lesions or history of steroids / immunosuppressive therapy within last 4 weeks were excluded. A 4 mm punch biopsy of lesion was taken. Additional normal non sun-exposed skin biopsy was also received in 2 cases suspected of having SLE. One bisected half was sent for histopathological examination in 10% neutral buffered formalin and other half in Michel's medium for DIF examination. Five µm thick frozen tissue sections were taken for DIF. Slides were stained with fluorescein isothiocyanate (FITC) conjugated antibodies (Dako) directed against IgG, IgM, IgA, C3 and fibrinogen by a standardized method and incubated in moist chamber in dark at 37°C for 1 hour. The slides were washed in Phosphate buffer saline (PBS) multiple times to remove unbound/non-specifically bound antibodies to reduce background fluorescence. Slides were mounted in glycerol-PBS mixture and viewed under the fluorescence microscope fitted with a UV-light source. Each slide was assessed for presence or absence of

deposits, site of deposition, pattern as well as intensity (1+ to 4+) of fluorescence.

Results

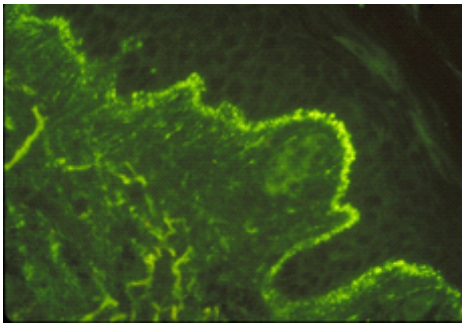
The age of the patients ranged from 1-85 years (mean age = 37.5 years) with slight female preponderance (M:F = 0.6:1). Final diagnosis of DLE (n=19/19) was made after amalgamation of histopathological and direct immunofluorescence features (100%). Histopathologic diagnosis could be made in 16/19 (84.2%) cases. **[Figure 1]** While 3/19 (15.8%) cases showed non-specific findings suggestive of interface dermatitis like hyperkeratosis, focal basal cell vacuolization, pigment incontinence & perivascular / periadnexal lymhocytic inflammation however features were not pathognomic of DLE. Direct immunofluorescence examination in these cases revealed positive lupus band test, hence highlighting the diagnostic importance of DIF.

Figure 1: Histopathological picture of a case of DLE [H&E, 400X].



On DIF examination of lesional skin, 16/19 (84.2%) cases showed positive lupus band test, while 3/19 (15.8%) cases were negative. These 3 DIF negative cases showed typical features of DLE on histopathology. Also, DIF on normal non sun-exposed skin biopsy was negative in 2 cases of suspected SLE. Majority of the cases (14/19, 73.7%) had deposition of multiple immunoreactants at DEJ. **[Figure 2]**

Figure 2: Granular deposits of IgM in a linear pattern (Lupus Band) at DEJ in a case of DLE [400X].



The most common combined deposition was of IgM & fibrinogen seen in 4/19 (21.5%) cases. The most common immunoreactant was IgM (11/19, 57.9%) followed by IgG (10/19, 52.6%) cases. **[Table 2]** However none of the cases showed IgA deposits. The most common pattern of deposition at DEJ was granular (12/16, 75%). In addition to positive LBT, 3/19 (15.8%) cases showed immunoreactivity in dermal blood vessels (DBV) with presence of fibrinogen in all 3 cases along with IgG and C3 in one case (5.2%) each. **[Table 3]**

Table 2: Distribution of immunoreactant(s) in DLE

Immunoreactants	Number of cases showing deposition at DEJ	Number of cases showing deposition at DBV
IgM+Fibrinogen	4	0
IgG+IgM+C3+Fibrinogen	2	0
IgG+Fibrinogen	2	1
IgG+IgM+Fibrinogen	1	0
IgG+C3+Fibrinogen	1	0
IgM+C3	1	0
IgG+C3	1	0
IgM	1	0
IgG	1	0
C3+Fibrinogen	0	1
Fibrinogen	0	1
Negative	3	16
Total	19	19

Table 3: Site and pattern of immunoreactant deposition in DIF positive cases of DLE (n = 16/19)

Site/Pattern	No. of DLE cases (n)	Percentage (%)
DEJ/Granular	9	56.2
DEJ/Homogenous	4	25
DEJ/Granular + DBV	3	18.7
Total DIF positive cases	16	100

In the present study, 13/19 (68.4%) cases showed good histo-immunofluorescence concordance with positive LBT on DIF and characteristic histopathology. All the immunoreactants in DIF study showed strong fluorescence intensity.

Discussion

Since DLE can lead to considerable disfigurement, permanent hair loss and may even progress to systemic LE in few cases, timely diagnosis & intervention is important. [1] This study was undertaken to assess the role of DIF vis-à-vis histopathology in the diagnosis of DLE.

The patients in this study had a wide age range from infancy to elderly however the mean age was 37.5 years similar to Isfer et al who reported an age range of 6-79 years with majority of patients in their thirties. [4] Sandra et al also reported the mean age of 36.25 years however the age range was narrow (22 - 48 years). [5] This study shows that DLE is slightly preponderant in women that is in concordance with findings of various other studies. [Table 4] On the contrary, Sampaio et al [6] noted a male predominance [M:F=2:1] but their study included pediatric DLE.

A definitive histopathologic diagnosis of DLE was made in 84.2% cases consistent with the observations of Minz RW et al & Naqqash S et al who reported clinico-histopathologic correlation in 82% & 79.3% cases respectively. [7, 8] However, 15.8% cases showed non-specific histopathologic findings similar to the observations of Sampaio MCS et al & Naqqash S et al. [6,8] These cases were confirmed as DLE on DIF that emphasizes the fact that a characteristic positive LBT may clinch the diagnosis in appropriate clinical setting even if histopathology is inconclusive.

On DIF, 16/19 (84.2%) cases showed immunoreactant positivity. Three (15.8%) cases that were negative on DIF showed characteristic histopathological features of DLE. Hence establishing the diagnosis. In the past, various authors have found DIF positivity of 45.4% to 69% in DLE. [1,4,6-8] However Sandra A et al reported DIF to be diagnostic in 100% cases. This could be due to longer duration and cephalic location of lesions (which tends to be sun-exposed) biopsied in their study. [5] Therefore site and duration of lesion should be kept in mind while doing the biopsy. Mysorekar VV et al also reported 100% sensitivity of DIF in 9 cases of LE in their study out of which only 2 were DLE.

Majority of the cases 14/16 (73.7%) showed multiple immunoreactants at DEJ, similar to previous literature. However none of our cases showed a full-house LBT positivity with all the immunoreactants. IgM was the most common immunoreactant seen in 11/19 (57.9%) cases similar to the findings of Sandra A et al, Sampaio MCA et al, and Isfer RS et al. [4-6] However Kulthanan et al, Bharti S et al, Naqqash S et al & Bhushan R et al found IgG to be the predominant immunoreactant. [1,8-10] We also found IgG positivity in half of the cases. [Table 4]

Table 4: Comparison of this study with other studies in DLE [1, 4, 5, 6, 8, 9, 10, 13]

DLE	Kulthanan et al (1996)	Isfer et al (1996)	Sandra et al (1998)	Sampaio et al (2008)	Naqqash et al (2011)	Bharti S et al (2015)	Mysorekar VV et al (2015)	Bhushan R et al (2017)	Present study

Age	15-68 years	6-79 years	22-48 years	0.5-16 years	14-60 years	6-65 years	-	22-85 years	1-85 years
M:F	0.75:1	0.4:1	0.23:1	2:1	0.2:1	0.66:1	-	0.78:1	0.6:1
HPE	100%	100%	100%	96.8%	79.7%	60%	66.6%	88.2%	84.25
DIF	90%	69%	100%	56.6%	79.3%	68%	100%	85.3%	84.2%
Multiple immuno-reactants at DEJ	62%	66.6%	87.5%	-	57.6%	34%	100%	61.7%	73.7%
Most common immuno-reactant at DEJ	IgG (57%)	IgM (52.3%)	C3 & IgM	IgM (94%)	IgG (74%)	IgG (41.3%)	IgG/ C3/IgA (100%)	IgG (52.9%)	IgM (57.9%)
Deposits at DBV	15%	6.9%	-	-	-	12%	-	14.7%	15.7%

Three (15.8%) cases showed immunoreactivity in dermal vessel walls with fibrinogen (15.8%), C3 and IgG (5.26% each) besides a positive LBT. Bhushan R et al also reported similar findings in 14.7% cases showing deposition in the dermal blood vessels with fibrinogen (8.8%) and IgG (5.9%). [10] Kulthanan et al also found deposition in the dermal blood vessels in 15% cases with C3 (9%), IgM (5%) and IgG (2%). [9]

Though LBT is very sensitive, a negative result does not exclude DLE. Many variables like duration, distribution and type of lesions, sun exposure etc. can affect LBT. Prior topical steroid therapy is common in patients presenting to tertiary care hospital like ours that can affect LBT. [8] It has also been suggested that lesions of less than 3 months duration may not show a positive LBT. [11] However these variables could not be analyzed in this study due to unreliable history and non-compliance of patients.

The sensitivity of histopathology & DIF in diagnosing DLE was found to be 84.2% each, however a combination of both techniques could diagnose 100% cases. Although histopathology gave diagnostic or suggestive findings in almost all the cases, as compared to DIF that gave negative results in 3 cases. The DIF was helpful in diagnosing 3 histopathologically equivocal cases. Naqqash S et al & Bhushan R et al reported that histopathology was diagnostic in 79.7% & 88.2% cases respectively as compared to DIF in 68.5% & 85.3% respectively and a combination of histopathology & DIF gave better diagnostic yield (85% & 100% respectively). [8,10] A histo-immunological concordance was seen in 68.4% cases that is similar to the findings of Isfer RS et al who reported a histo-immunological correlation of 69%. [4]

Conclusion

The present study reinforces that direct immunofluorescence examination (positive Lupus Band Test) of lesional skin biopsy is a useful indicator of DLE particularly in histopathologically equivocal cases. However as any other test, it is influenced by various factors and has its limitations. Therefore, it should always be used in conjunction with histopathology for more reliable results than by either technique alone.

Acknowledgement - We would like to thank Mr. Rakesh, Medical Lab Technician (Department of Pathology) for the technical support in the laboratory investigation of these cases.

Conflict of interests

None.

Funding

None.

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