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DIRECT IMMUNOFLUORESCENCE : REINFORCING ITS ROLE IN SKIN BIOPSY

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ABSTRACT

Context: Direct Immunofluorescence plays a vital role in the diagnosis of immune mediated dermatological disorders . The pattern of staining helps in the classification of bullous disorders.

Aims and Objectives: The aim of the present study was to analyze the corroboration between clinical , histopathological and DIF findings in arriving at the final diagnosis.

Material and Methods: Histopathology and DIF of 50 cases of clinically suspected immune-mediated diseases were studied and compared. Fluorescein Isothiocyanate –conjugated rabbit antihuman IgG, IgA, IgM, C3 and Fibrinogen was used.

Results: DIF was positive in 95.7% (44/46 cases)of immune mediated skin lesions , 4 being non immune lesions confirmed by histopathology. DIF was positive in 98.6% of Pemphigus group, 75% of Discoid lupus erythematosus and 100% each in Bullous Pemphigoid , vasculitis, congenital bullous dermatosis of childhood and lichen planus .

Conclusion: With appropriate sampling and good technique, DIF is a useful supplement for an accurate diagnosis of immune –mediated dermatological disorders .A combination of clinical features, histopathology and DIF solves the diagnostic dilemma in complicated cases.

KEY WORDS: immunofluorescence , bullous lesions, skin biopsy.

Introduction:
Immunoflourescence is a well established technique used for the detection of a variety of antigens in tissues and circulating antibodies. Direct Immunofluorescence (DIF) refers to the former wherein tissue bound autoantibodies are detected . The latter is the Indirect Immunofluorescence (IIF) [1] .The beginning of Direct IF dates back to 1942, when Albert Coons first used Fluorescein to label antipneumococcal antibodies in the lung tissue. Fluorescein Isothiocyanate (FITC) emitting green color is the most commonly used flurochrome followed by Rhodamine which emits red color [2]. DIF plays an important role in the diagnosis of immune-mediated dermatological lesions and in the classification of bullous disorders. A combination of clinical features, histopathology and DIF is most ideal for an accurate diagnosis. The aim of the present study was to analyze the corroboration between the three in arriving at the final diagnosis.

Material and Methods:
50 cases of clinically suspected immune-mediated diseases were included in this prospective study either to establish the diagnosis or to rule out a mimicker. The present study was done at the departments of Dermatology and Pathology at PSG Institute of Medical Sciences and Research ,Coimbatore from March 2009 to Feb 2010. For every patient whenever there was a clinical suspicion of Immunological disease two skin biopsy specimens were taken , one for Histopathological examination (HPE) and the other for DIF. The cases included in the study were Pemphigus group, Bullous Pemphigoid (BP), Dermatitis Herpitiformis (DH) , Vasculitis, Lupus erythematosus (LE), Lichen planus (LP), Congenital bullous dermatosis of childhood (CBDC) and Epidermolysis bullosa Acquisita (EBA) .
Histopathological (HP) diagnosis was made after examining the Hematoxylin & eosin stained sections. The site of biopsy for DIF was perilesional for bullous disorders and in others it was lesional. The skin biopsy for DIF was frozen in OCT medium ; 4 micron thick sections were cut in cryostat. A minimum of 5 slides were taken for each case .The sections were washed with PBS (Phosphate buffered saline) with pH 7.4 for 10 min before covering the sections with FITC –conjugated rabbit antihuman IgG, IgA, IgM, C3 and Fibrinogen (dil 1: 20) The slides were incubated in a moist chamber for one hour. The sections were again washed with PBS for 10 min, mounted in glycerine and viewed under a fluorescence microscope using blue filter (490 nm). Green fluorescence was observed. DIF report indicated the nature of the deposits (IgG, IgA, IgM, C3 and Fibrinogen) and location of deposits - intercellular spaces(ICS), basement membrane zone (BMZ) , around subepidermal blood vessels, colloid bodies in the subpidermal region .

Results :
Of the 50 cases studied, 38 were females and 12 males . The age of the patients ranged from 2yrs to 64yrs.Of the 50 cases, four were non –immune disorders as assessed by HPE though there was a clinical suspicion and all these cases did not reveal any immune deposits (DIF negative). The four cases included lymphocytic vasculitis (2 cases), Hailey-Hailey disease (1 case) and contact dermatitis (1 case). Of the 46 cases of immune mediated disorders, 44 (95.7%) showed DIF positivity .The two (4.3%) DIF negative cases included Pemphigus vulgaris & DLE one each. The clinical , histopathological , DIF & final diagnosis of the 46 DIF positive cases are shown in Table 1.

Table 1 :Shows clinical, histopathological, DIF and final diagnosis of the immune –mediated dermatological lesions :

Case no	Clinical diagnosis	Histopathological diagnosis	Direct IF diagnosis	Final diagnosis
1	PV/BP	PV	PV	PV
2	PV	PV	PV	PV
3	PV	PV	PV	PV
4	LP/DLE	DLE	DLE	DLE
5	PV/HHD	PV	PV	PV
6	PV/PF	PV	PV	PV
7	PV/PF	PF	PF	PF
8	BP/PV	NOT CONCLUSIVE	PV	PV
9	DLE/LP	LP	LP	LP
10	PV	PV	PV	PV
11	PV	PV	PV	PV
12	DLE	NOT CONCLUSIVE	DLE	DLE
13	BP/PV	BP	BP	BP
14	PV/HHD	PV	PV	PV
15	BP	PV	PV	PV
16	HSP	HSP	HSP	HSP
17	PV	PV	PV	PV
18	PV	PV	INCONCLUSIVE	PV
19	PV/PF	PF	PF	PF
20	PV	PV	PV	PV
21	PV/HHD/BP	PV	PV	PV
22	BP/EBA	NOT CONCLUSIVE	BP	BP

23	PV	PV	PV	PV
24	DLE	LP	LP	LP
25	PV	PV	PV	PV
26	BP	PV	PV	PV
27	DH/BP	BP	BP	BP
28	PV	PV	PV	PV
29	DLE	DLE	INCONCLUSIVE	DLE
30	BP/PV	PV	PV	PV
31	PV/PF	PV	PV	PV
32	DLE/PE	PE	PE	PE
33	PV	PV	PV	PV
34	PV	NOT CONCLUSIVE	PV	PV
35	DLE	DLE	DLE	DLE
36	PV	PV	PV	PV
37	PV/BP	NOT CONCLUSIVE	IG A DERMATOSIS	IG A DERMATOSIS
38	PV/BP	PV	PV	PV
39	EM/BP	BP	BP	BP
40	HHD/BP	PV	PV	PV
41	PV	PV	PV	PV
42	DLE/HSP	HSP	HSP	HSP
43	PV	PV	PV	PV
44	PV	PV	PV	PV
45	DLE/BP	BP	BP	BP
46	BP	BP	BP	BP

PV- pemphiguc vulgaris, PF Pemphigus foliaceous, PE pemphigus erythematosus, BP-Bullous Pemphigoid, HSP-Henoch-schonlein purpura, LP-lichen planus, DLE- discoid lupus erythematosus ,EM-erythema multiforme, HHD- Hailey Hailey disease .

The Pemphigus group showed intercellular pattern (Fishnet) of staining of the epidermis(30 of the 31 cases ,98.6%) .In Pemphigus vulgaris, ICS pattern was seen either throughout the thickness of epidermis or was confined to the lower third of the epidermis. The staining was seen with IgG,IgM nad C3 in all the 28 cases Fig 1A, while Fibrinogen was seen in 20 cases. IgA was not seen in any of our cases. One of the cases of pemphigus Vulgaris was DIF negative. The two cases of Pemphigus Foliaceous showed ICS pattern (IgG, C3) confined to the upper third of the epidermis(Fig 1B). The single case of Pemphigus erythematosus (Fig1C) showed both ICS and BMZ patterns of staining (IgG,C3,Fib).Histopathology was not conclusive in two cases of PV.

In the BP category, linear BMZ DIF positivity(IgG,C3,IgM,Fib) was seen in all the six cases(100%, Fig 1D). Histopathology was not diagnostic in one of these cases. Three of the four cases (75%) of DLE showed characteristic granular BMZ deposits of IgG and C3 - lupus band test (LBT) (Fig 2A). One of the cases failed to show any deposit. However histopathology and clinical findings (Anti-ds-DNAAb positive) were diagnostic.

Of the four cases of Vasculitis histopathology revealed leucocytoclasia in two , both of which showed granular perivascular IgA and C3 deposits(Fig 2B). The other two DIF negative cases were lymphocytic vasculitis by HPE.

The single case of congenital bullous dermatosis of childhood showed linear IgA BMZ deposit (Fig 7). The two cases of lichen planus included in the study showed civatte bodies in the subepidermal region (fig).

Discussion :

In the present study, a good correlation between clinical, histopathological and DIF findings was seen. DIF diagnosis correlated with histopathological diagnosis in 95.7% of the cases. These findings were concordant with those by Vijaya and others who studied 102 cases of Immune mediated dermatological lesions [3] .

The sensitivity of DIF in Pemphigus group was 96.8%(30of 31 cases) . This is in agreement with the observations of Inchara and others [4] . Extensive immune mediated tissue destruction is likely to have resulted in the single DIF negative PV. However clinical and HP finding favoured a diagnosis of PV .On the contrary, in one of the cases, DIF played an important role in the diagnosis as HP was inconclusive, the roof of the bulla being completely lost. DIF becomes positive early in the disease and complement deposition shows an increase in patients with relapse [5] . In all our cases in addition to IgG, C3 was also positive.

All cases of BP were DIF positive. A similar high positivity has been reported by Vijaya [3]. In addition in cell poor type of BP with bulla not included in the biopsy, DIF clinches the diagnosis along with clinical findings as was seen in one of our cases.

Among the Lupus group of disorders, a positive LBT has been demonstrated in 59% of DLE patients and 71% with SLE by Minz and others [6]. In the present study, a 75% positivity was noted. The single DIF negative case had characteristic histopathological features .

Both cases of Henoch-Schonlein purpura were DIF positive in our study(100%). This is comparable with 90% DIF positivity observed by Nandeesh and others who also analysed that the positivity rates are highest when biopsy is taken within 48hrs of onset of lesions [7].

DIF becomes very crucial in the diagnosis of CBDC., wherein clinical and histopathological findings can be only suggestive but not conclusive of the condition. A linear IgA is the hallmark of the condition [2].

The presence of colloid (civatte) bodies though not diagnostic of Lichen planus can be a useful adjunct.The final diagnosis however is made with histopathological findings since colloid bodies are found in other lesions as DLE and erythema multiforme . Both our cases showed colloid bodies. 60% of the cases of LP have shown civatte bodies in other studies [8].

Conclusion :

With appropriate sampling and good technique, DIF is a useful supplement for an accurate diagnosis of immune –mediated dermatological disorders. DIF confirms the diagnosis when clinical and histopathological findings are typical. On the other hand, when the latter are inconclusive then a diagnosis is made on the basis of DIF findings.

Images :

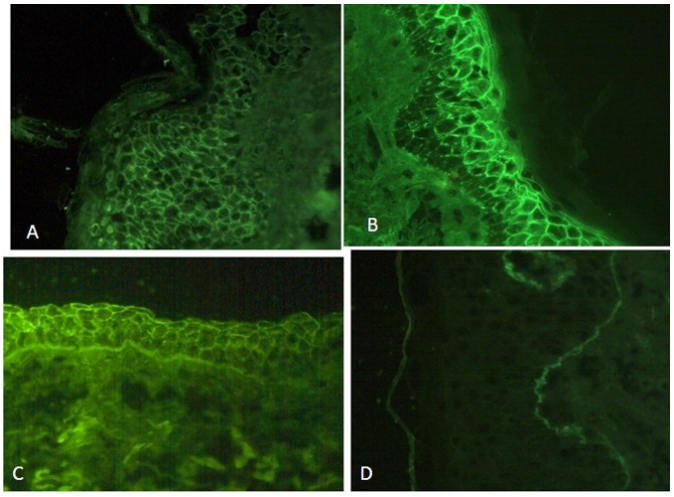

Fig1 A shows ICS pattern in pemphigus vulgaris, B pemphigus foliaceous confined to upper epidermid, C pemphigus erythematosus both ICS and BMZ patterns, D linear BMZ in Bullous Pemphigoid,400x

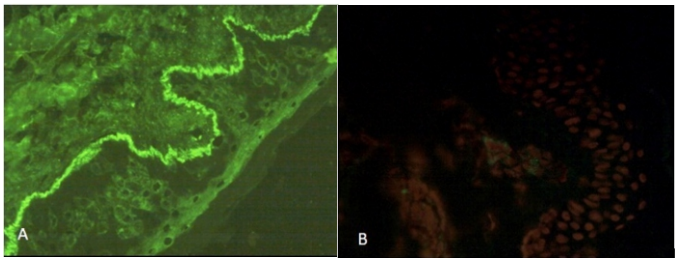

Fig2 A shows granular BMZ in DLE, B shows perivascular granular deposits in HSP, propidium iodide stains nucleus of epidermal cells red,400X

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