

Research Article

Direct immunofluorescence study in autoimmune bullous disorders of skin

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Abstract

Background and objectives: This study was taken up to know the utility of application of Direct Immunofluorescence on skin biopsies with particular reference to Autoimmune Bullous disorders.

The objectives of the present study was to detect specific patterns of DIF in different Autoimmune bullous disorders, to supplement clinical and histopathological features, and hence for confirmatory diagnosis of these disorders.

Methods: DIF using fluorescent labelled antibodies – IgG, IgM, IgA and C3 - was carried out in 60 clinically suspected cases of Autoimmune bullous disorders referred to Department of Pathology, KIMS Hospital and Research Center, Bangalore, over a period of 18 months from January 2011 to July 2012.

Results: Out of 60 cases analysed, 39 cases were given a final diagnosis as Autoimmune bullous disorder based on clinical, histopathological and DIF findings. DIF positivity was observed in 34 cases. DIF findings correlated with clinical and histopathological findings in 37 cases (94.8%).

Interpretation and Conclusion: In this study we found out that DIF serves as a simple, highly sensitive, cost-effective and hence gold standard test for Autoimmune bullous disorders. The technique is essential to supplement clinical findings and histopathology in the diagnosis of these Immunobullous disorders.

Keywords: Autoimmune bullous disorders; Direct Immunofluorescence

1. Introduction

Immunofluorescence is a histochemical laboratory staining technique used for demonstrating the presence of antibodies bound to antigens in tissue or circulating body fluids¹.

Coons *et al*² first described the technique of fluorescent antibody studies in 1942, while Burnham *et al*³ were the first to report a fluorescent band in the dermoepidermal junction in Lupus Erythematosus. The method used by Coons is known as the 'direct technique', where the antibody was conjugated directly with the fluorochrome.

The technique is essential to supplement clinical findings and histopathology in the diagnosis of Immunobullous disorders. It permits early diagnosis, treatment and subsequent monitoring of disease activity in patients with these life-threatening diseases¹. The direct method is the most widely used by most dermatologists for diagnosis of bullous disorders³.

It is important to pursue classification as accurately as possible so that useful clinical trends may become apparent, eg. differences in response to therapy⁴.

Considering this, we apply the technique of Direct Immunofluorescence to supplement clinical and histopathological findings in the investigation of Immunobullous disorders.

2. Methodology

2.1 Source of data: Clinically suspected cases of Immunobullous disorders presenting to department of dermatology and referred to the Department of Pathology, KIMS

2.2 Sampling method: Purposive sampling

2.3 Inclusion Criteria: Patients for this study were selected on the basis of clinical suspicion of auto immune bullous disorders, irrespective of age, gender, duration of disease and treatment received.

2.4 Exclusion Criteria: (1) Biopsy specimen dried up or fixed in formalin. (2) Inadequate biopsy specimen – no epidermal lining. (3) Clinical details / histopathological findings are not available.

2.5 Method of collection of Data:

A detailed history was taken and clinical examination done of patients and those who were clinically diagnosed to have immuno bullous disorder were selected and biopsy done from the fresh vesicle for histopathology and from the adjacent skin for DIF.

Biopsy specimen was snap frozen immediately. In case of delay between biopsy and snap freezing, it was refrigerated. Four frozen sections of 4microns taken on a cryostat, placed on a glass slide and fixed in cold acetone. Slides were then overlaid in a moist chamber and incubated for 2hours with fluorescein conjugated antibodies with following specificities: Anti IgG, Anti IgM, Anti IgA and Anti C3. Slides were then washed gently in phosphate buffer saline, mounted in buffer glycerin mixture and examined under fluorescence microscope.

The Immunofluorescence pattern was studied and reported, considering the following parameters:

- (1) Nature of immune deposits – IgG, IgA, IgM, C3
- (2) Site of immune deposits – Dermoepidermal junction / Intercellular spaces in epidermis
- (3) Semi quantitative grading of strength of fluorescence - + to ++++
- (4) Pattern of immune complex deposits – Granular / linear

The immunoreactants include antibodies, Complement components and fibrinogen. Immunoreactants are deposited in 2 main patterns:

1. In the epidermal intercellular space
2. Along the basement membrane zone

2.6 Statistics

The findings were tabulated and basic statistics of calculating percentages was used from Master chart windows excel 2000.

3. Results

A prospective study with 60 cases of clinically suspected Autoimmune Bullous diseases was undertaken over a period of 18 months. .

Out of 60 cases analyzed, 39 cases (65%) were diagnosed as AIBDs based on clinical, histopathological and DIF findings.

Percentage distribution of various AIBDs were as seen in Table 1, where **PV** cases were 15 (38%), **BP** cases were 8(20%), **PF** cases were 4(10%), **DH** cases were 3(8%) and so on.

Table 1: Total no. of clinically suspected bullous disorders

Diagnosis	Number of patients (n=60)	%
Pemphigus vulgaris	15	25
Bullous pemphigoid	8	13.3
Pemphigus foliaceus	4	6.7
Dermatitis herpetiformis	3	5.0
PLEVA	2	3.3
No opinion possible	2	3.3
<i>Spongiotic blistering disease</i>	3	5.0
<i>Prurigo simplex</i>	2	3.3
<i>Prurigo nodularis</i>	1	1.7
Bullous Erythema Multiforme	2	3.3
Scabies	1	1.7
Hailey Hailey disease	1	1.7
Pustular psoriasis	1	1.7
Transient acantholytic dermatosis	1	1.7
Linear IgA disease	1	1.7
Macular amyloidosis	1	1.7
Epidermolysis Bullosa Acquisita	1	1.7
Bullous Lupus Erythematosus	2	3.3
Dystrophic Epidermolysis Bullosa	3	5.0
Follicular eczema	1	1.7
Polymorphic light eruption	2	3.3
Bullous impetigo	1	1.7
Vesicular insect bite reaction	2	3.3

Most of the patients in the study were in middle age group like 36% in 41-50 yrs and 23.5% between 31-40 yrs.

The total no. of **PV** cases in the age groups of 21-30,31-40,41-50,51-60, 71-80 years are 2(13.3%), 5(33.3%), 6(40%), 1(6.6%), 1(6.6%) respectively, being most common in age group of 41-50 years. The total no. of **BP** cases in the age groups of 31-40,41-50,51-60,61-70 years are 1(12.5%),3(37.5%), 2(25%) and 2(25%) respectively, with 50% of cases in the age group 51-70 years. The total no. of **PF** cases in the age groups of 1-10,41-50,51-60 years are 1(25%), 2(50%) and 1(25%).

There was nearly equal incidence of AIBDs in males and females. 20 males (51.2%) and 19 females (48.8%) were diagnosed as AIBDs and gender distribution in individual AIBDs. Generalised skin lesions were observed in 13 cases (33.3%). The lesions were localized to head and neck, trunk and/or extremities in 26 cases (66.7%). Mucosal involvement was noted in 14 cases, all of which were Pemphigus vulgaris. One of these patients had nasal involvement, while the rest had oral mucosa involved.

Bulla was noted on histopathological examination in 31 out of 39 cases. Papillary micro abscess was seen in 4 cases, which includes 3 cases of DH and 1 case of LAD. (Table 2) Lichenoid infiltrate was seen in 4 cases, including 2 cases each Bullous LE and Bullous EMF. One of the cases showed focal acantholysis with spongiosis and dyskeratosis, which was diagnosed as TAD. One case showed dilapidated brick wall due to acantholysis with subepidermal clefting, typical of HH disease. Out of 31 cases showing bulla on histopathology, most common site of bulla was suprabasal, observed in 15 cases, corresponding to 15 cases of PV, followed by subepidermal bulla in 11 cases and subcorneal bulla in 5 cases.

Table 2: Histopathological findings

	No. of patients	%
Bulla	31	79.5
Papillary micro abscess	4	10.3
Lichenoid infiltrate	4	10.3
Dyskeratosis	1	2.6
Dilapidated brick wall	1	2.6

Out of 60 cases, DIF was positive in 34 cases (56.7%). DIF was negative in 26 cases (43.3%) Among 39 AIBD cases, DIF was positive in 34 cases (87%). The 5 AIBD patients (13%) with negative DIF test includes 3 DIF-negative AIBDs – SCPD, TAD, HH- and 2 false-negative cases – PV and BP.(Table 3 & Table 4)

Table 3 : DIF Positivity

DIF	Number of patients	%
Negative	26	43.3
• AIBDs	5	
• Others	21	
Positive	34	56.7
Total	60	100.0

Table 4: DIF positivity in autoimmune bullous disorders

DIF	Number of AIBD patients	%
Negative	5	
• Pemphigus vulgaris	1	
• Bullous pemphigoid	1	
• SCPD	1	
• TAD	1	
• Hailey Hailey	1	
Positive	34	87%
Total	39	100.0

The most common site of ICS immune deposits was observed in 18 cases (53%), followed by DEJ deposits in 13 cases (38.2%) and 3 cases (8.8%) showing deposits in the papillary dermis. 18 cases of ICS deposits included 14 cases of PV and 4 cases of PF.

The most common deposits was IgG and C3 (71%), only IgG in 21% and 8% only C3 deposits.

4. Discussion

The present study is a prospective study over a period of 18 months. Total of 60 clinically suspected cases of Autoimmune Bullous disorders, were included in the study.

Autoimmune Bullous disorders are a heterogenous group of diseases characterized by antibodies to structural components of the skin and mucous membranes⁵.

4.1 Age incidence: Most Autoimmune bullous disorders occur during the ages of 40 to 60. Our study found a peak in the age group (41-50) years. 72% of the cases are between 31 – 60 years in accordance with the Lieferman *et al* study⁶.

Peak age incidence in Pemphigus vulgaris is between 4th to 6th decade as per the study by Vodegel⁷. In our study also peak incidence was between 31 -50 years.

Bullous pemphigoid is found in older age group, mainly in the elderly, between 50-80 years as reported by Korman N J *et al*⁸. In our study also most cases were between 41–70 years.

4.2 Gender distribution: Autoimmune disorders did not show sex predilection in the study by Korman N J⁸ and Scott J E⁹. In our study also, overall sex distribution in AIBDs was equal, though 3:1 male predominance was noted in Bullous pemphigoid, in Dermatitis herpetiformis 2:12 in accordance with Korman N J study who noted 2:1 male predominance in BP and 1:1 ratio in DH.

4.3 Clinical presentation: Pemphigus vulgaris presents with flaccid blisters, most rupture leaving behind denuded areas¹⁰. A study by Rados J reported 100% cases of PV presenting with mucosal involvement⁵. In our study all except one, who was on remission had mucosal lesions (93%). One of these patients had nasal involvement alone which is a rare observation according to Weedon¹¹, while the rest had oral lesions. Pemphigus foliaceus cases showed similar lesions without oral lesions which correlates with Rados J study⁵.

Figure 1: Pemphigus vulgaris - Clinical pictures Flaccid bullae, ruptured leaving behind denuded areas, with oral mucosa.

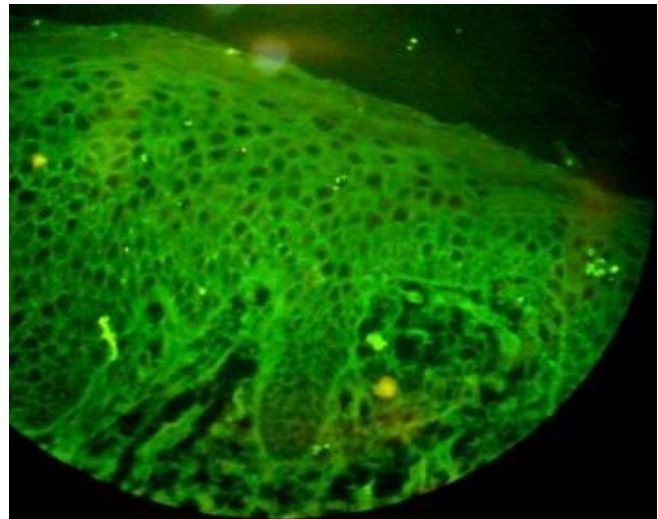
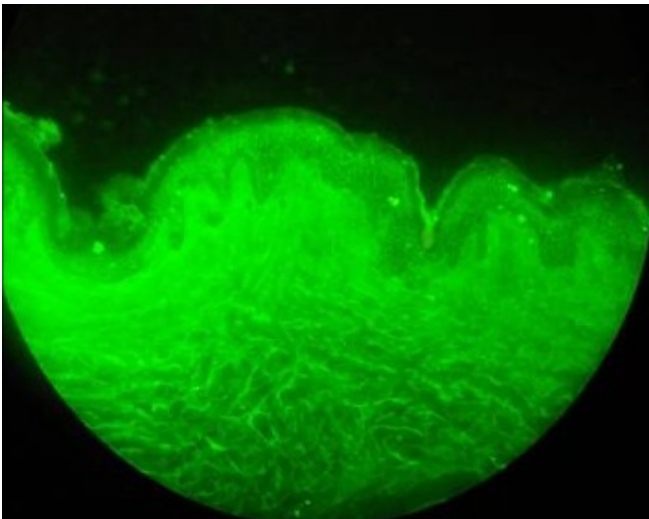


Fig 2: DIF in pemhigus vulgaris

Lace-like ICS deposits giving a fish-net appearance 2a. (X100), 2 b. (X400)

Figure 2 a

Figure 2 b



All cases of Bullous pemphigoid showed tense blisters, and all 3 cases of Dermatitis herpetiformis had vesicles with crusted lesions and none had any symptoms of gluten-sensitive enteropathy. This is in accordance with the reports by Nousari H C¹² and Hall R P¹³ studies that though all cases have histologic evidence of GSE, only 10% cases of DH are asymptomatic.

Figure 3: Bullous pemphigoid - Clinical pictures showing Tense blisters.



Bullous pemphigoid

Figure 4: Bullous pemphigoid - DIF.
Linear immune deposits along DEJ (X100).

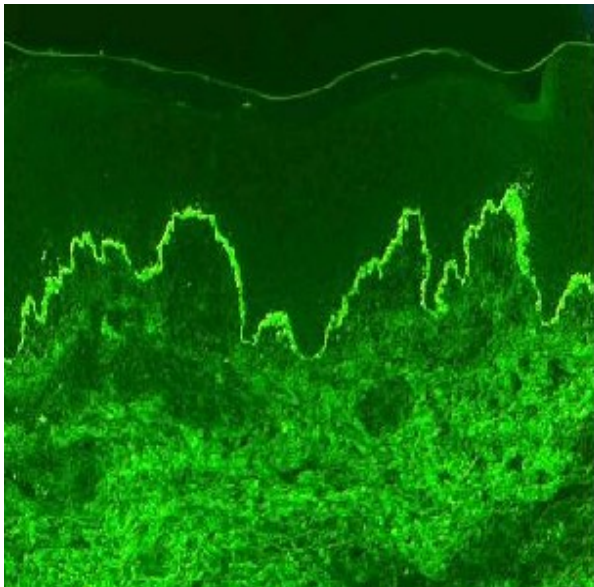
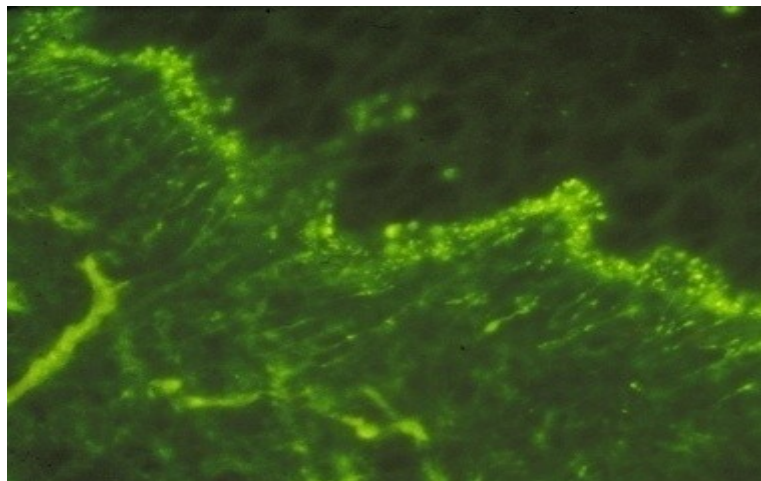


Figure 5: Dermatitis herpetiformis –
Clinical picture showing Small vesicles and crusted lesions.



Figure 6: Dermatitis herpetiformis DIF. Granular immune deposits along DEJ and papillary dermis (X400).



4.4 Histopathology: Out of 39 cases, bulla was observed in 31 cases. Subcorneal bulla was noted in 5 cases, which include all 4 cases of PF and 1 case of SCPD. Suprabasal bulla was seen in 15 cases, corresponding to all 15 cases of PV. Subepidermal bulla was seen in 11 cases which comprises of 8 cases of BP and one case each DH, EBA and Bullous LE. Only micropapillary abscess without bulla was found in 2 cases of DH and 1 case of LAD, which is a more important diagnostic finding as reported by Rados J study⁵.

4.5 DIF positivity: Of 60 cases for which DIF was performed, 34 were positive, all diagnosed as Autoimmune bullous disorders. 26 cases were DIF negative, out of which 5 were still diagnosed as AIBDs. These include 1 case each DIF-negative AIBDs - SCPD, TAD and HH; 2 DIF false negative cases – PV and BP. This is in accordance with the studies by Jablonska S¹⁴ and Beutner *et al*¹⁵ which report SCPD, TAD and HH as AIBDs which are DIF-negative. DIF positivity in PV, PF and DH observed in our study correlated with the literature. However DIF positivity was slightly lower for BP in comparison with the literature .

DIF false negative cases:

1. 49 years male, a case of PV, who was on remission. This is as per the studies by Huligol S C *et al*¹⁶ and Sethi K J¹⁷ which state that intensity of DIF in PV reduces and becomes negative with years of treatment.
2. 41 years male, a case of BP, a newly diagnosed case. The reason for false negativity could be one of the technical problems like delay in collection and inadequate preservation leading to loss of immunoreactants or biopsy taken from inflamed or blistered skin which are explained in the study by Kirtschig G¹⁸.

4.6 DIF patterns: DEJ deposits was found in 13cases (38.2%) – 7cases of BP with linear IgG &/or C3 deposits, 2cases of BEMF with granular IgG, IgM deposits, 2cases of BLE with granular IgG &/or C3 deposits, 1case of LAD with linear IgA deposits and 1case of EBA with linear IgG deposits. Pattern of immune deposits in our study correlated with the studies by Mutasim D F¹⁹ and Morrison L H²⁰. In our study, significant correlation between clinical, histopathological and DIF findings was established in 94.8% cases. This is in accordance with Harrist T J *et al* study²¹.

4.7 Immune deposits in Pemphigus vulgaris: Both IgG and C3 ICS deposits was found in 71% cases, only C3 in 21% cases and IgG alone in 8% cases, which correlated with study by Bhogal B *et al*²² which also showed both IgG and C3 positivity in 80% cases. Studies by Sethi K J¹⁷ and Bhogal *et al*²² state that both IgG and C3 positivity is found in all active cases. The intensity of IgG and then C3 decreases with treatment and disappears when the patient is in remission. Persistent C3 positivity is strong indication of disease relapse with cessation of treatment. C3 positivity is more often found in untreated patients according to Vassileva *et al*²³ study, the same observation also noted in our study.

5. Conclusion

- Autoimmune Bullous disorders are life-threatening diseases demanding early accurate diagnosis with clinical, histopathological and DIF findings.
- In our study most frequent cases were Pemphigus vulgaris (38.5%), followed by Bullous pemphigoid (20.5%).
- Most affect middle age adults with no sex predilection.
- While pemphigus vulgaris was found in middle aged, Bullous pemphigoid was found in older age group, in the elderly.
- DIF positivity in Autoimmune Bullous disorders was found in 87% cases.
- Linear intercellular space immune deposits (fish-net pattern) was the most frequent pattern encountered (53%), followed by dermo-epidermal junction deposits (linear and granular pattern) (38.2%).
- DIF positivity was 93.3% for Pemphigus vulgaris and 100% for Dermatitis herpetiformis, which is in accordance with other studies.
- DIF positivity for Bullous pemphigoid was 87.5%, slightly lower in comparison to other studies, probably due to fewer cases encountered in our study and false negativity.
- Clinical and histopathological features correlated with DIF findings in 37 of 39 cases – 94.8% correlation.

The present study reaffirms that apart from new sophisticated tests (immunoblotting, immunoprecipitation, immunoelectron microscopy), the diagnosis of Autoimmune bullous disorders still relies on DIF findings in most laboratories. It is cost effective. Thus DIF has been the gold standard.

It is important to integrate clinical findings, histopathology and DIF in reaching an accurate diagnosis of Autoimmune bullous disorders.

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