

A cross-sectional study of clinical, histopathological and direct immunofluorescence diagnosis in autoimmune bullous diseases

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INTRODUCTION

Dermatopathology, pioneered by Unna, affords a keystone not only for modern dermatology but also for the use of immunofluorescence in studies of skin immunopathology ¹. Histopathologic studies of Walter Lever differentiated what

Background: Immunobullous diseases are morphologically heterogeneous and the differentiation between various subtypes is essential for proper treatment and prognosis. The aim of our study was to analyze and correlate clinical, histopathological, and immunofluorescence findings in autoimmune bullous diseases.

Method: A cross-sectional study was conducted over a period of two years (2010-2012) after receiving the ethics committee approval. Sixty patients who met the inclusion criteria of immunobullous disease were included in the study. Skin biopsy for histopathological and direct immunofluorescence (DIF) examination was taken. DIF was also done in a few of the cases using the salt-split technique. The final diagnosis was based on clinical, histopathological, and DIF findings.

Result: Sixty cases with a clinical diagnosis of autoimmune bullous diseases were evaluated. In 95% of the cases, the histopathological diagnosis was consistent with the clinical diagnosis and in 75% of the cases, the DIF diagnosis was consistent with the clinical diagnosis. A positive relationship was seen between clinical and DIF findings with $r = 0.67$ and between histopathologic and DIF findings with $r = 0.76$. DIF positivity was seen in 100% of the cases of bullous pemphigoid (BP), 100% of the cases of pemphigus foliaceus, and 94.7% of the cases of pemphigus vulgaris which was statistically significant with $P < 0.05$.

Conclusion: Our study provides evidence-based guidance for the diagnosis and classification of various immunobullous disorders. DIF test should be done in conjunction with histopathology to make a definite diagnosis and minimize both false-positive and false-negative results.

Keywords: autoimmune bullous disease, diagnosis, direct immunofluorescence, histopathology, pemphigus, pemphigoid

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we now call pemphigus and pemphigoid ². Immunofluorescence studies by Ernst Beutner et al revealed the autoimmune etiology of pemphigus and pemphigoid ¹. Now, other immunologic methods also contribute to our understanding ^{3,4}. Autoimmune blistering diseases are associated with an autoimmune response directed to structural

proteins mediating cell-cell and cell-matrix adhesion in the skin⁵. Autoimmune blistering diseases are classified based on the ultrastructural site of deposition of immunoreactants and on the molecular target of auto antibodies⁶.

Diseases of the pemphigus group are associated with autoantibodies to epidermal components mediating cell-cell adhesion and are characterized by acantholytic blisters within the epidermis⁷. Tissue-bound and circulating autoantibodies to the dermal-epidermal junction are characteristic immunopathological features of subepidermal autoimmune bullous diseases^{5,8}.

Direct immunofluorescence (DIF) is more sensitive and also more frequently positive than indirect immunofluorescence (IIF) in patients in clinical remission and is more valuable for detection of immunological activity of the disease⁹.

PATIENTS AND METHODS

A cross-sectional study was conducted in the Department of Dermatology, Smt.S.C.L Hospital, Smt. N.H.L Municipal Medical College, from June 2010 to November 2012 after receiving the approval of our Institutional Ethics Committee Board. The patients were selected from the inpatient and outpatient wards of the Department of Dermatology and informed consent was obtained from the recruited patients. Patients with a clinical diagnosis of autoimmune bullous disorders who were willing to participate in the study were included irrespective of age and gender. Patients who were not willing to be part of the study or undergo the required investigations were excluded.

Clinical data was recorded in form of:

- Demographic information
- Detailed clinical history
- General and dermatological examination
- Clinical photographs
- Routine investigations
- Tzanck smear
- Skin biopsy for histopathological evaluation
- Direct immunofluorescence staining of the perilesional skin specimen

A total 60 cases that were clinically diagnosed with autoimmune dermatological disorders were included in the study. Skin biopsy sample (punch biopsy/ excision biopsy) was the standard procedure for obtaining samples.

The biopsy specimen was sent in 10% formalin for histopathology and in the Michel medium for DIF. In the laboratory, specimens were processed and stained, and findings were recorded. DIF using the salt-split method was performed in few cases on the specimen that were previously investigated by routine DIF. The specimens were incubated in 10ml 1mol/L NaCl at 40°C for 48-72 hours. The epidermis was separated from the dermis easily and DIF staining was carried out. Facilities for IIF were not available at our centre and it was not performed. While reporting DIF findings of the skin biopsy, fluorescent staining was described under the following headings:

1. Type of immunoreactant: IgG, IgA, IgM, C3, and fibrin.
2. Location of immune deposits: intercellular spaces (ICS) in the epidermis/basement membrane zone (BMZ) /blood vessels/hair shaft/cytoid bodies.
3. Pattern of immune complex deposits: granular or linear

The description of all these staining characteristics leads to an immunopathological diagnosis. Digital photography was done to document the results. In this way, both histopathological and DIF examinations were carried out in all included cases and the findings were recorded. The definite diagnosis of these patients was based on clinical, histopathological, and immunofluorescence findings.

Statistical Analysis

Analysis was done with Epi info version 7 and Microsoft Excel. The chi-square test was used wherever appropriate. Pearson's correlation coefficient was calculated. The above-mentioned tests were used wherever applicable according to the sample size and type of data.

RESULTS

The study group comprised 60 cases of autoimmune bullous disorders of the skin including 34 females (56.6%) and 26 males (43.3%). The age range of the cases was between 5 months and 88 years with a mean age of 39.7 years. The clinical diagnoses of the patients were pemphigus vulgaris (PV) in 19 cases (31.6%), dermatitis herpetiformis in 11 cases (18.3%), bullous pemphigoid (BP) in

10 cases (16.6%), and pemphigus foliaceus in 3 cases (5%). Two cases (3.3%) of pemphigus erythematosus, subcorneal pustular dermatosis, bullous SLE, linear IgA dermatosis, and Darier's disease were seen (Table 1).

Overall, in 57 cases (95%), histopathology findings were consistent with the clinical diagnosis and in 45 cases (75%), DIF findings were consistent with the clinical and histopathological diagnosis. Figure 1 shows the correlation between clinical and histopathological diagnosis. On applying Pearson's

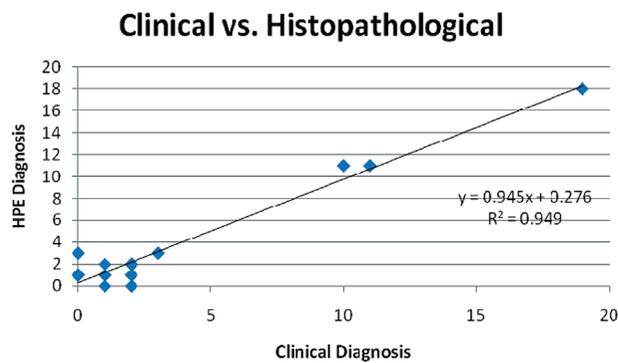


Figure 1. Correlation between clinical and histopathological diagnosis

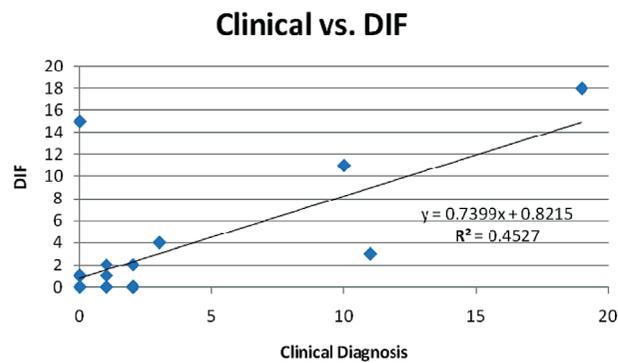


Figure 2. Correlation between clinical and DIF diagnosis

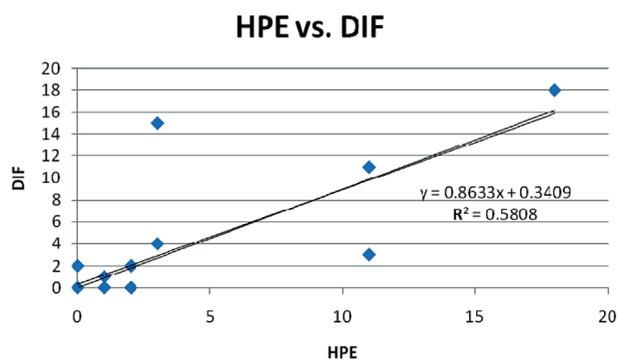


Figure 3. Correlation between histopathological and DIF findings.

correlation coefficient, a positive relation was seen between clinical and histopathological diagnosis with $r = 0.97$. Figure 2 shows the correlation between clinical and DIF diagnosis. A positive correlation was observed in between clinical and DIF diagnosis with $r = 0.67$. Figure 3 shows the correlation between histopathological and DIF diagnosis. A positive correlation was found in between histopathological and DIF diagnosis with $r = 0.76$.

DIF findings were noted as follows:

1. Pemphigus vulgaris: Out of a total 19 cases, 94.7% showed DIF positivity ($n = 18$). IgG was the most common immunoreactant found to be deposited in 83.3% of the cases, followed by C3 in 16.7% (Figure 4).
2. Pemphigus foliaceus: 100% of the cases showed DIF positivity ($n = 4$). IgG was found in all cases, i.e.100%, followed by C3 in 25% of the cases.
3. Pemphigus erythematosus: IgG and IgM deposition at ICS and BMZ was found in 100% of the cases ($n = 2$).
4. Bullous pemphigoid: 100% ($n = 10$) of the cases had DIF positivity. One case that was clinically diagnosed with LAD showed histopathological and DIF findings consistent with a diagnosis of BP. Therefore, a total of 11 patients had DIF findings suggestive of BP. IgG and C3 were the predominant immunoreactants, found in 90.9% ($n = 10$) of the cases. IgG alone was present in only one case, i.e. 9% (Figure 5).
5. Dermatitis herpetiformis: Out of a total 11 cases who were clinically diagnosed with DH, only 27.27% ($n = 3$) of the cases had a positive DIF

Table 1. Types of blistering disorders on clinical examination

Clinical types of disease	No.	Percentage (%)
Pemphigus Vulgaris	19	31.6
Pemphigus Foliaceus	3	5
Pemphigus Vegetans	1	1.6
Pemphigus Erythematosus	2	3.3
Bullous Pemphigoid	10	16.6
Lichen Planus Pemphigoid	1	1.6
Pemphigoid Gestationis	1	1.6
Subcorneal Pustular Dermatitis	2	3.3
Linear IgA Dermatitis	2	3.3
Bullous SLE	2	3.3
Dermatitis Herpetiformis	11	18.3
Darier's Disease	2	3.3
Hailey-Hailey Disease	1	1.6
Erythema multiforme	2	3.3
Chronic Bullous Disease of Childhood	1	1.6

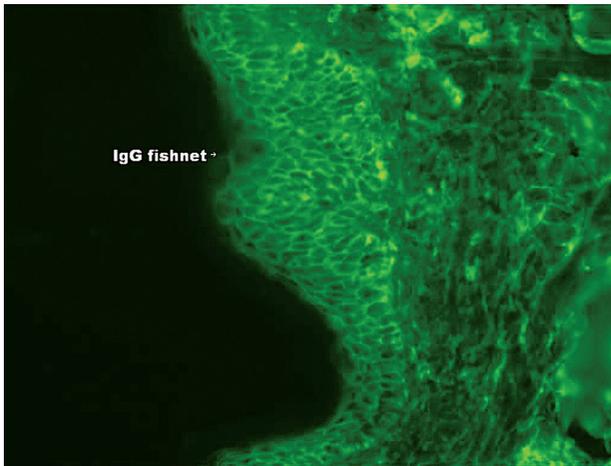


Figure 4. Reticular IgG deposition in epiderm in pemphigus vulgaris (DIF $\times 100$)

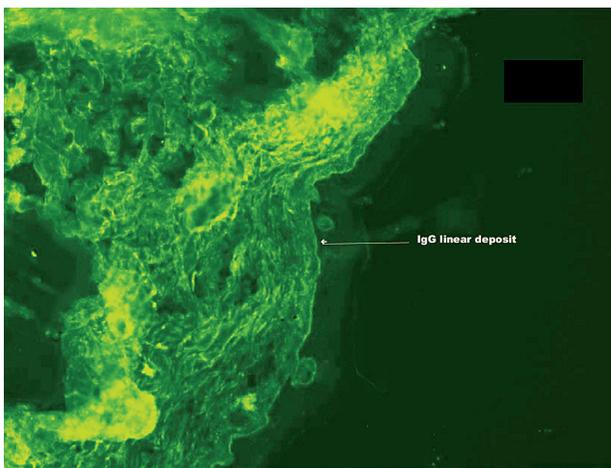


Figure 5. Linear IgG deposition in BMZ in bollous pemphigoid (DIF $\times 100$)

test. IgA was the main immunoreactant deposited in 67% of the cases.

Among the subepidermal group of disorders, bullous pemphigoid can mimic epidermolysis bullousa aquisita (EBA) in clinical presentation, histopathological findings, and DIF findings. In the present study, in 4 patients with clinical and histopathological differential diagnoses of BP and EBA, the direct salt split immunofluorescence test was performed. In three cases of BP, the site of deposition was the roof of the blister and in one case of EBA, the site of deposition was the floor of the blister cavity.

DISCUSSION

The diagnosis of autoimmune bullous diseases is based on the evaluation of clinical findings,

histopathology, direct immunofluorescence (DIF), and indirect immunofluorescence ¹⁰.

The differentiation between these entities is important for both treatment modalities and prognosis ¹¹. Our study validated that DIF was requisite for accurate diagnosis of vesicobullous disorders of the skin. Thus, improved detection and affirmation of clinical diagnosis of diseases like DH, linear IgA dermatosis (LAD), chronic bullous disease of childhood (CBDC), and bullous SLE is attainable with DIF only. In the present study, DIF was able to confirm 75% of clinically diagnosed cases. In a study by Minz et al, DIF was able to detect 70% of clinically diagnosed vesicobullous lesions of the skin ¹².

Out of 19 cases that were clinically suspected as PV, DIF was found to be consistent with the clinical diagnosis in 18 cases. In all the 3 clinically suspected cases of pemphigus foliaceus, light microscopy and DIF diagnosis was consistent with the clinical diagnosis. One case that was clinically suspected as PV, after histopathology and DIF examination, features turned out to be of pemphigus foliaceus.

A total of 10 cases of clinically suspected BP were analyzed by DIF of whom 100% were consistent and one additional case was picked up on DIF and light microscopy when there was no clinical finding in favor of BP. Clinically, this case was labeled as LAD. Through DIF microscopy, linear IgG and/or C3 deposition in BMZ was observed in all cases. However, DIF findings confirmed the diagnosis in 3 of 11 patients with clinical diagnosis of DH. This result is in agreement with the sensitivity of DIF in the diagnosis of DH reported in the literature ¹³. In a study by Banu Lebe et al, histopathology and DIF findings were in accordance with the clinical diagnosis in only 3 out of 58 cases. The authors suggested that the possible reason for this discordance may be due to the rapid evolution and disappearance of pruritic papulovesicular eruptions in DH. Therefore, it leads to inclusion of DH in the list of clinical differential diagnoses in many pruritic papulovesicular eruptions ¹⁴. We encountered one case with clinical differential diagnosis of DH and EBA on light microscopy while the DIF examination diagnosis was in favor of BP and EBA. On DIF- salt split examination, immunoreactant deposition was seen in the floor of the blister cavity, favoring a diagnosis of epidermolysis bullosa aquisita.

DIF microscopy analyzes the tissue for the presence of autoantibodies, complements, and fibrin. For accurate results, the site of biopsy, transport media, and sending the specimen to the laboratory without delay is important. In conclusion, only histopathological findings are not sufficient to confirm the diagnosis of autoimmune bullous diseases and DIF findings should be correlated with light microscopy.

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