Analysis of the Utility of Direct Immunofluorescence in the Diagnosis of Common Immune Mediated Dermatological Conditions

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Abstract

Background: Accurate diagnosis of immune-mediated dermatological diseases requires an evaluation of clinical, histopathological, and immunofluorescence findings. Immunofluorescence detects antibodies bound to antigens in tissue and in circulating body fluids. It acts as a valuable adjunct to clinical and histopathological diagnosis in patients of autoimmune bullous diseases (AIBDs), connective tissue diseases (CTD), and vasculitis. Aim: This study aimed to analyze the utility of direct immunofluorescence (DIF) in the diagnosis of common immune-mediated dermatological diseases by its correlation with clinical features and histopathology. Methodology: This is an observational retrospective study. Medical records of 205 clinically suspected patients of AIBD, CTD, and vasculitis were retrieved for analysis where both histopathology and DIF had been carried out for patients treated at the dermatology department of a tertiary care hospital between November 2015 and November 2018. Data were analyzed using Statistical Package for the Social Sciences software version 20.0. Results: A total of 187 out of 205 patients were accurately diagnosed based on histopathology and DIF studies. Histopathology was positive in 177/187 (95.7%) patients and DIF was positive in 153 (81.8%) patients. Clinical, histopathological, and DIF discordance was seen in the remaining 18 out of 205 patients. The sensitivity of DIF in AIBD, lupus erythematosus (LE), and vasculitis was calculated to be 89%, 82.6%, and 60%, respectively. The overall sensitivity of DIF was 80.8%. The sensitivity of DIF was 100% in pemphigus foliaceous, acute cutaneous lupus erythematosus, and immunoglobulin A (IgA) vasculitis. Out of 118 clinically suspected AIBD patients, nine were diagnosed on the basis of DIF alone. Conclusion: DIF is an indispensable tool in the accurate diagnosis of autoimmune dermatological conditions, especially in cases where clinical and/or histopathological features are inconclusive. It is invaluable in confirming the diagnosis of IgA vasculitis.

Keywords: Autoimmune bullous diseases, connective tissue diseases, direct immunofluorescence, vasculitis

INTRODUCTION

Immune dysregulations of the skin as a part of systemic disease pathogenesis are reflected in the skin in patients of autoimmune bullous diseases (AIBDs) and many systemic conditions such as systemic lupus erythematosus (SLE), other autoimmune diseases, and systemic vasculitis. Thus, the skin can serve as a valuable marker of systemic diseases.\(^1\)

Histopathology is the gold standard for diagnosis in the majority of skin diseases. However, the same does not hold true for all lesions, especially immune-mediated conditions. Accurate diagnosis of these conditions requires an evaluation of clinical, histopathological, and immunofluorescence findings. Direct immunofluorescence (DIF) studies for tissue-bound antibodies play a vital role in the diagnosis of these conditions with the presence of immune complexes in the skin biopsy at various locations such as the intercellular spaces (ICS), dermo–epidermal junction (DEJ), and dermal blood vessels (BV). This aids in correctly classifying histopathologically similar diseases that differ in the treatment protocol. DIF also has an additional role in disease prognosis and in the prediction of relapse.\(^2,3\)

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The present study was undertaken to analyze the utility of DIF in the diagnosis of common immune-mediated dermatological diseases by its correlation with clinical features and histopathology and to evaluate the diagnostic potential of DIF.

**Methodology**

This study was done by analyzing the medical records of 205 clinically suspected patients of AIBD, CTD, and vasculitis where both histopathology and DIF studies had been carried out for patients treated at the dermatology department of a tertiary care hospital over a period of 3 years (November 2015 to November 2018). The clinical data providing demographic details of patients such as age and sex were collected from the files at the Department of Dermatology and the same were recorded along with the histopathological diagnosis, DIF study results, and the final diagnosis. Data were taken for only those patients in whom both histopathology and DIF studies were carried out.

The DIF results were recorded by taking into consideration the parameters such as nature of the immune deposits (immunoglobulin IgG, IgA, IgM, and C3); location of the immune deposits (intercellular spaces [ICS] in the epidermis, DEJ or the basement membrane zone [BMZ], and dermal blood vessels [BVs]); extent of the immune deposits (diffuse and focal); pattern of the immune complex deposits (linear and granular); and intensity of fluorescence (+ to ++++).[1,3]

Based on the DIF results, 153 patients with positive DIF were categorized into the following major headings:

1. **AIBDs**: Pemphigus vulgaris (PV), pemphigus foliaceous (PF), bullous pemphigoid (BP), pemphigoid gestationis (PG), and chronic bullous disease of childhood (CBDC)
2. **Vasculitis**: IgA vasculitis, immune complex-mediated vasculitis (ICV), and nonimmune complex-mediated vasculitis (NICV)
3. **LE**: Acute cutaneous LE (ACLE), subacute cutaneous LE (SCLE), and discoid LE (DLE).

The remaining 52 patients with negative DIF were clinically and histopathologically categorized into the above-mentioned headings or as miscellaneous conditions which were nonimmune mediated [Table 1].

**Statistical analysis**

The data were entered in MS Excel and were analyzed using the Statistical Package for the Social Sciences version 20.0, IBM, Armonk, New York, USA. Cohen’s Kappa statistics were used to test the concordance between the clinical, histopathological, and DIF diagnoses. Sensitivity was calculated for DIF considering histopathology as the gold standard. *P* < 0.05 was considered statistically significant.

**Results**

Out of the 205 records retrieved, there were 89 males and 116 females (M:F = 1:1.3). The age of the patients ranged from 5 to 97 years, with a mean age of 43.9 ± 17.3 years. Out of a total of 205 patients of suspected immune-mediated diseases, 153 had a positive diagnosis on DIF, 177 had a positive diagnosis on histopathology, while the remaining 18 were diagnosed to have nonimmune-mediated diseases [Tables 1 and 2]. The various diagnostic patterns seen on DIF are enumerated in Table 3.

AIBD was the largest group consisting of 118 (57.6%) patients of the total. PV was the most common disease in sixty (50.8%) patients. Out of 118 clinically suspected AIBD patients, nine were diagnosed on the basis of DIF alone. Histopathology was diagnostic in 92.4% of AIBD patients and DIF in 89.8% of patients [Table 2].

In the pemphigus group (*n* = 76), histopathology was positive in 70/76 (92.1%) patients and DIF in 69 (90.8%) patients. Histopathology was diagnostic in 56/60 (93.3%) patients of PV and DIF in 53 (88.3%) patients [Table 2]. In four clinically diagnosed patients of PV with inconclusive histopathological findings, DIF alone was diagnostic with characteristic fishnet pattern of immune deposits in the ICS in the epidermis [Figure 1]. Of the total of 16 clinically diagnosed patients of PF, 87.5% revealed histopathological diagnosis, while DIF was characteristic in 100% of patients. In two histopathological-negative patients of PF, a definite diagnosis was made on the basis of clinical features and DIF findings [Figure 2].

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**Table 1: Distribution of patients (n=205)**

| Final diagnosis | Number of patients (%) |
|-----------------|------------------------|
| PV              | 60 (29.2)              |
| PF              | 16 (7.8)               |
| PG              | 1 (0.5)                |
| BP              | 40 (19.5)              |
| CBDC            | 1 (0.5)                |
| ACLE            | 7 (3.4)                |
| SCLE            | 5 (2.4)                |
| DLE             | 12 (5.8)               |
| IgA vasculitis  | 5 (2.4)                |
| ICV             | 22 (10.7)              |
| NICV            | 18 (8.8)               |
| Psoriasis vulgaris | 1 (0.5)                |
| LP              | 1 (0.5)                |
| PLEVA           | 1 (0.5)                |
| EM              | 3 (1.5)                |
| Polymorphic eruption of pregnancy | 1 (0.5) |
| Allergic insect bite reaction | 4 (2) |
| Spongotic dermatitis | 3 (1.5) |
| Miscellaneous (nonspecific inflammation) | 4 (2) |
| **Total**       | **205 (100)**          |

PV: Pemphigus vulgaris, PF: Pemphigus foliaceous, PG: Pemphigoid gestationis, BP: Bullous pemphigoid, ICV: Immune complex vasculitis, NICV: Non-ICV, LP: Lichen planus, EM: Erythema multiforme, CBDC: Chronic bullous disease of childhood, ACLE: Acute cutaneous lupus erythematosus, SCLE: Subacute cutaneous lupus erythematosus, DLE: Discoid lupus erythematosus, IgA: Immunoglobulin A, PLEVA: Pityriasis lichenoides et varioliformis acuta
In the BP group \((n = 42)\), histopathology was diagnostic in 39 (92.9%) and DIF in 37 (88.1%) patients. Three patients of BP were diagnosed based on clinical and DIF findings with inconclusive histopathological findings. One patient clinically diagnosed as PV was subsequently labeled as BP on the basis of histopathology and DIF. In the single patient of PG and one patient of CBDC, histopathology and DIF were consistent with the clinical diagnosis.

Among the 24 LE patients, 23 (95.8%) and 20 (83.3%) patients were histopathologically and DIF consistent with clinical diagnosis, respectively. In all the seven (100%) patients ofACLE, diffuse granular deposits of immune complexes in BMZ were seen, whereas in SCLE, they were seen in 60% of patients [Figure 3]. In the 12 patients of DLE, DIF was consistent in 83.3% of patients. In one patient of DLE, DIF was clearly positive, although histology was not specific for

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**Table 2: Histopathological and direct immunofluorescence results in immune-mediated skin diseases (total=187, direct immunofluorescence=153/187)**

| Final diagnosis | Clinical | Histopathology | Immunofluorescence |
|-----------------|----------|----------------|--------------------|
|                 | Diagnostic (%) | Nondiagnostic | Diagnostic (%) | Negative |
| PV              | 60       | 56 (93.3) | 4 | 53 (88.3) | 7 |
| PF              | 16       | 14 (87.5) | 2 | 16 (100) | 0 |
| BP              | 40       | 37 (92.5) | 3 | 35 (87.5) | 5 |
| PG              | 1        | 1 (100)  | 0 | 1 (100)  | 0 |
| CBDC            | 1        | 1 (100)  | 0 | 1 (100)  | 0 |
|ACLE             | 7        | 7 (100)  | 0 | 7 (100)  | 0 |
| SCLE            | 5        | 5 (100)  | 0 | 3 (60)   | 2 |
| DLE             | 12       | 11 (91.7)| 1 | 10 (83.3)| 2 |
| IgA vasculitis  | 5        | 5 (100)  | 0 | 5 (100)  | 0 |
| Vasculitis (except IgA vasculitis) | 40 | 40 (100) | 0 | 22 (55)  | 18 |

**Table 3: Direct immunofluorescence pattern in skin disorders**

| Final diagnosis | Number of DIF-positive patients | Location of deposits | Nature of deposits | DIF pattern | Extent of deposits | Intensity of fluorescence |
|-----------------|---------------------------------|----------------------|-------------------|-------------|-------------------|--------------------------|
| PV              | 53                              | ICS=53               | IgG/C3=42         | Fishnet/lace like=53 | Diffuse throughout epidermis=53 | +++=4, +++=8, +++=13, +++=22 |
| PF              | 16                              | ICS=16               | IgG/C3=13         | Fishnet/lace like=16 | Diffuse throughout epidermis=16 | ++=1, +++=2, +++=13 |
| PG              | 1                               | BMZ=1                | C3=1              | Linear=1       | Diffuse=1          | ++++++1 |
| BP              | 35                              | BMZ=35               | IgG, IgA, C3=32   | Linear=35      | Diffuse=35         | +++++, +++++, +++++ |
| CBDC            | 1                               | BMZ=1                | IgA, IgG, C3=1    | Linear=1       | Diffuse=1          | IgA ++, IgG +, C3 + |
|ACLE             | 7                               | BMZ=7                | IgG/IgM/IgA/C3=7  | Granular=7     | Diffuse=7          | All ++, IgG ++, IgA +, C5 ++ |
| SCLE            | 3                               | BMZ=3                | IgG, IgM, IgA, C3=2 | Granular=3 | Diffuse=3          | All ++++, IgG ++, IgA +, C3 ++ |
| DLE             | 10                              | BMZ=10               | IgG, IgM, IgA, C3=06 | Granular=10 | Diffuse=10         | All ++, IgG +, IgA +, C3 + |
| IgA vasculitis  | 5                               | Walls of superficial dermal BV | IgA=5            | Granular=5     | Diffuse=5          | +++=2, +++=2 |
| Vasculitis      | 22                              | Walls of superficial dermal BV | IgG, IgM, C3=20   | Granular=22    | Diffuse=22         | All +++=20 |

PV: Pemphigus vulgaris, PF: Pemphigus foliaceous, PG: Pemphigoid gestationis, BP: Bullous pemphigoid, CBDC: Chronic bullous disease of childhood,ACLE: Acute cutaneous lupus erythematosus, SCLE: Subacute cutaneous lupus erythematosus, DLE: Discoid lupus erythematosus, IgA: Immunoglobulin A
it. In addition, two patients with a clinical impression of DLE, both histopathology and DIF were inconsistent with clinical diagnosis.

Among the 61 patients with clinical findings suggestive of vasculitis, only 45 (73.8%) patients were given a diagnosis of vasculitis based on the evidence of leukocytoclastic vasculitis on histopathology and DIF findings. Of these 45 patients, DIF was instrumental in the definitive diagnosis of IgA vasculitis in five patients, with granular diffuse deposit of IgA in superficial dermal BVs, and ICV in 22 (48.9%) patients, with the immune complex deposited in dermal BVs other than IgA, and NICV in the remaining 18 (40%) patients with negative DIF [Figure 4]. DIF was positive in 27/45 (60%) patients among vasculitis.

DIF was diagnostic in ten patients where the histopathology was inconsistent. Three of these patients showed the classical DIF pattern of BP with linear diffuse deposits of IgG and C3 at BMZ. Six out of these ten patients revealed diffuse fishnet-like pattern on DIF consistent with PV/PF, and one pattern with diffuse granular deposit of Ig at BMZ was classified as DLE.

The sensitivity of DIF in AIBD, LE, and vasculitis was calculated to be 89%, 82.6%, and 60%, respectively. The overall sensitivity of DIF was 80.8%. The sensitivity of DIF was 100% in PF, ACLE, and IgA vasculitis [Graph 1]. Kappa coefficient was calculated for estimating the concordance between clinical, histopathological, and DIF diagnoses. The value of Kappa coefficient for concordance between clinical diagnosis and DIF was calculated to be 0.662 (95% confidence interval [CI]: 0.595, 0.728), between histopathology and DIF was 0.742 (95% CI: 0.675, 0.808), and between clinical diagnosis and histopathology was 0.792 (95% CI: 0.731, 0.853). All the results were statistically significant with \( P < 0.001 \). The results showed good agreement in all the three groups [Table 4].

**DISCUSSION**

The diagnostic role of DIF in AIBD of the skin is well highlighted in various studies over previous years. This study reflects the mandatory role of DIF along with histopathology for proper diagnosis of bullous conditions of the skin. AIBD was the most common entity in 63.1% (118/187) of patients among the immune-mediated skin disorders and 57.6% of the total patients (\( n = 205 \)) studied. Among all patients, PV was the major group affecting 29.2% of patients. Similar findings were observed by Mysorekar et al. who had 20.5% cases of PV in their study.[19] Histopathology was diagnostic in 109/118 (92.4%) AIBD patients and DIF in 106 (89.8%) patients. Comparable results of DIF positivity in AIBD were seen in other studies by Kabir et al. (88.23%), Inchara and Rajalakshmi (73%), and Raj et al. (90.09%).[16-18] DIF was positive in 90.8% in the pemphigus group, which is comparable to the study by Inchara and Rajalakshmi (88%).[10] Mysorekar et al. had high DIF positivity with 78/80 (97.5%) in AIBD cases and 52/53 (98.1%) in pemphigus group.[19] Chanabasayya et al., however, had relatively less DIF positivity with 60.4%, and discordance was seen in 39.6% patients of AIBD.[19] All the 16 (100%) patients of PF had DIF positivity in our study, which is similar to the study by Mysorekar et al. where 8/8 patients had positive DIF.[19] In pemphigus group, 9.2% of patients were DIF negative in our study. As this was a retrospective study, the reason for DIF negativity could not be analyzed. DIF-negative PV had also been mentioned by Buch et al. in their study.[19] Out of the total ten patients diagnosed by DIF alone where histopathology was inconsistent, six patients could be classified to the pemphigus group based on the typical diffuse linear fishnet-face-like pattern of immunoprecipitants. This fact adds to the reliability of DIF being a diagnostic modality for pemphigus.
DIF positivity in BP group in our study was 35/40 (87.5%), which is again corroborating with other studies.\textsuperscript{[9-11]} We were able to confirm a single patient of PG and one patient of CBDC on DIF. In addition, 3/40 patients of BP were diagnosed based on DIF findings only.

Connective tissue diseases (CTDs) have cutaneous involvement as an important feature. Therefore, skin being accessible for biopsy easily plays an additive role in the diagnosis of CTD as well as in the classification of these diseases. DIF is valuable in confirming the diagnosis of clinically and histopathologically suspected patients of LE and in classifying LE based on morphology, site, and frequency of immune complex deposits.\textsuperscript{[6]} In the present study, 23/24 (95.8%) patients and 20 (83.3%) patients were histopathologically and DIF consistent with clinical diagnosis, respectively. All 7/7 (100%) clinically suspected patients of ACLE showed positive lupus band test (LBT), whereas in SCLE, it was seen in 3/5 (60%) patients. In the remaining 12 clinically suspected patients of DLE, DIF was consistent in 12 (83.3%) patients. This is consistent with a study by Mysorekar et al. with positive LBT in 90% of SLE patients, 60% of SCLE, and 90% of DLE patients.\textsuperscript{[3]} Low levels of positivity are generally seen due to lesions biopsied in treated patients or during the late stage of the disease.\textsuperscript{[1]} DIF was positive in all five patients and in 9/10 patients of LE in studies by Dhanabalan et al. and Kabir et al., respectively.\textsuperscript{[6,9]} In one patient of DLE, LBT was clearly positive, although histology was not specific for it. In addition, two patients with a clinical impression of DLE, both histology and DIF were inconsistent with clinical diagnosis. Few authors have found LBT positivity in other CTDs too, which was not specific to LE or as false-positive finding in other disorders.\textsuperscript{[1,14]}

DIF should be done to confirm all patients of IgA vasculitis and ICV. Out of the 61 clinically suspected patients of cutaneous small-vessel vasculitis or IgA vasculitis, histopathology was diagnostic in 45 (73.8%) patients. Among these, DIF positivity
was seen in 27/45 (60%) patients, with five patients diagnosed as IgA vasculitis and the remaining 22 patients as ICV with immune complex deposits other than IgA. Low DIF positivity was observed in 13/44 (29.5%) patients of vasculitis in studies by Mysorekar et al. and in 39% of patients by Khetan et al.\(^\text{[13,15]}\) Nandeesh and Tirumalae found 39% DIF positivity in cutaneous vasculitis, with 43/48 (90%) patients showing IgA vasculitis deposits.\(^\text{[16]}\) However, Poornimambaa et al. showed a much higher DIF positivity of 97%.\(^\text{[17]}\) According to Mutasim and Adams, lower limb lesions give false-positive DIF results and lesions more than a day old give false-negative result.\(^\text{[18]}\) In this study, among 45 patients with diagnostic histopathological findings of vasculitis, biopsy was done from lower limbs in 38 patients, with DIF positivity in 22 (57.9%) patients, and from upper limbs in seven patients, with 5 (71.4%) patients showing positive DIF. No immune deposits on DIF were seen in 18/45 patients, which can be attributed to the biopsy site being lower limbs in 16 patients.

DIF pattern in 153 patients was typical as shown in Table 3. In the present study, in 18 patients, both histopathology and DIF were discordant with clinical features, two patients were clinically suspected to be DLE initially, and the rest of the 16 patients were clinically diagnosed as vasculitis. These patients were given a final diagnosis based on the histopathology and clinical features as DIF was negative in these patients [Table 1].

In this study, the sensitivity of DIF in pemphigus group, BP, LE, and IgA vasculitis was 90%, 86.5%, 82.6%, and 100%, respectively. Mysorekar et al. reported slightly higher sensitivity, with 98.1% in pemphigus group (n=53), 96% in BP (n=27), 100% in LE (n=9), and 100% in IgA vasculitis (n=10).\(^\text{[3]}\) The overall sensitivity of DIF was 80.8% in the present study [Graph 1].

**Conclusion**

Over the past two decades, various studies have contributed to the role of DIF in diagnosis, treatment, and understanding of the pathophysiology of immune-mediated dermatological conditions. It is an indispensable supplement in the accurate diagnosis of autoimmune dermatological conditions, especially in patients where clinical and/or histopathological features are inconclusive, as brought out in the study. A negative DIF possibly rules out the immune basis in most of the dermatological diseases. It is valuable in confirming and classifying the diagnosis of small-vessel vasculitis as per immune deposits, particularly in IgA vasculitis. In situations with inconclusive or discordant clinical features or histopathology, DIF alone can be of diagnostic value in immune-mediated dermatological conditions, as highlighted in the present study. As clinical features, DIF, and histopathology may not be diagnostic individually in each case, we re-emphasize the combined analysis of all these methods to reach a final and the most accurate diagnosis.

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