

# Study of direct immunofluorescence in various autoimmune vesiculobullous disorders: an observational study

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## Abstract

**Objectives** The aim of the study was to see the clinical, histological and immunological correlation of various immunobullous diseases and to evaluate the sensitivity of direct immunofluorescence (DIF) in specific diagnosis of various immunobullous diseases.

**Methods** 29 patients of immunobullous diseases attending skin OPD and IPD of a tertiary care hospital in Ujjain were included in the study and were thoroughly examined.

**Results** Out of 29, 26 cases showed DIF patterns concordant with histologic diagnosis. The sensitivity of DIF was 92.3% (12/13) in the pemphigus group, 75% (3/4) in bullous pemphigoid, 66.7% (2/3) in dermatitis herpetiformis, while it was 100% (3/3) each in linear IgA disease and chronic bullous dermatosis of childhood.

**Conclusion** DIF is essential for diagnosing autoimmune blistering diseases with the clinical and the histopathological overlap.

## Key words

Direct immunofluorescence, immunobullous diseases, histopathology, sensitivity, diagnosis.

## Introduction

Immunobullous disorders represent a heterogeneous group of dermatoses with protean manifestations. The most important techniques for the investigation of patients with the diseases are conventional histopathology and confirmative tests like direct and indirect immunofluorescence.<sup>1</sup> Immunofluorescence studies have greatly contributed to the diagnosis, treatment, and understanding of the pathophysiology of vesiculobullous lesions of skin.<sup>2</sup> It is also an important prognostic tool as positive direct immunofluorescence (DIF)

findings in patients in remission predict early relapse of disease.<sup>3</sup> The location and pattern of deposition of immunoreactants help in classifying various immune-mediated diseases. DIF in conjunction with histopathology, can be a useful supplement to clinical and histological examination in the diagnosis of a variety of vesicobullous diseases like pemphigus group, pemphigoid, linear IgA disease (LAD), chronic bullous dermatosis of childhood (CBDC), dermatitis herpetiformis (DH), epidermolysis bullosa acquisita (EBA), herpes gestations, bullous systemic lupus erythematosus (SLE) and other dermatological diseases which include discoid LE, SLE, subacute LE, scleroderma, vasculitides, lichen planus, erythema multiforme and psoriasis etc.<sup>4</sup> The detection of antibodies against the adhesion molecules by DIF technique in both groups of autoimmune

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blistering diseases (ABD) has almost 100% diagnostic accuracy.<sup>5</sup> By DIF, the presence of immune complexes in skin biopsy at various locations such as intraepidermal, the dermoepidermal junction (DEJ), dermal blood vessels, etc. helps to arrive to a specific diagnosis.<sup>6,7</sup>

**Methods**

The present study was carried out in a tertiary care hospital in Ujjain, M.P, India. A total of 29 cases clinically diagnosed as autoimmune vesicobullous disease patients attending the outpatient department or admitted in ward constituted the subject material for the present study. A detailed history of each case was taken. Cases were thoroughly examined and routine investigations were sent. Biopsies were sent for histopathological and DIF studies and on the basis of clinical, histopathology and DIF findings correlation between the three was seen.

Analysis was done by using SPSS (Statistical Package for the Social Sciences) software and necessary test of significance (McNemar’s test) was applied. Specific treatment was given to them and they were asked to come for follow-up.

**Results**

In the present study, the most common immunobullous disease was found to be pemphigus vulgaris 13 (44.8%). The age of

patients ranged from 5 years to 65 years and maximum number of patients of pemphigus, BP and DH were between the age group of 45-65 years, while majority of the cases of CBDC were seen under the age of 14 years. The majority of patients were males and the maximum cases 20 (77%) were found to have duration of onset less than six months, except 2 (67%) cases of DH with duration of onset between 7-12 months. Nikolsky sign was positive in 14 (48%) patients, Asboe Hansen sign was elicited in 13.7% and string of pearl appearances was observed in 20.6% of cases. Subcorneal cleft was observed in 10.3% cases, suprabasal cleft in 45% cases and subepidermal cleft was seen in 41% of cases.

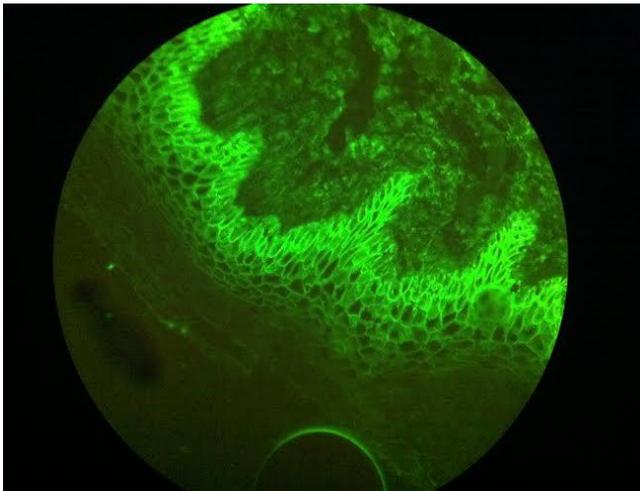
DIF was positive in 26 ( ) cases. IgG and C3 deposits were seen in maximum number of cases (11/16) of pemphigus patients intraepidermally and all patients of pemphigoid at basement membrane zone (BMZ). IgA deposits were seen in all the cases of LAD, CBDC and DH at DEJ (dermoepidermal junction). Out of 3, DIF negative cases, 1 was pemphigus vulgaris, 1 was of BP and 1 was of DH. Histopathological diagnosis of various immunobullous diseases along with corresponding DIF findings is depicted in **Table 1**. DIF was diagnostic in all the pemphigus variants including the pemphigus vulgaris (41%) and the pemphigus foliaceus (10%). DIF also helped to confirm the diagnosis of DH, LAD and CBDC. The statistical analysis of DIF is shown in **Table 2**.

**Table1** Histopathological diagnosis and DIF findings of immunobullous lesions

<i>Histopathological diagnosis</i>	<i>DIF positive cases</i>	<i>DIF negative cases</i>	<i>Total N (%)</i>
Pemphigus vulgaris	12	1	13 (44.8)
Pemphigus foliaceus	03	0	03 (10.3)
Bullous pemphigoid	03	1	04 (14)
Linear IgA disease	03	0	03 (10.3)
Chronic bullous dermatosis of childhood	03	0	03 (10.3)
Dermatitis herpetiformis	02	1	03 (10.3)
Total	26	3	29 (100)



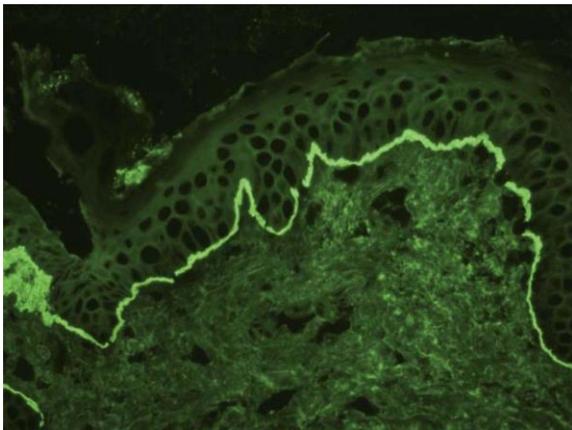
**Figure 1 & 2** a case of pemphigus vulgaris with flaccid bullae and oral lesions



**Figure 3** DIF finding in a case of pemphigus vulgaris, showing fishnet pattern intraepidermally



**Figure 4** a case of linear IgA disease, showing string of pearl appearance



**Figure 5** DIF finding in a case of linear IgA disease, showing IgA immunoglobulins at basement membrane zone

## Discussion

The autoimmune bullous lesions result from an immune response against adhesion molecules of epidermis or BMZ. The diagnostic specificity of clinical findings varies among bullous diseases. There is clinical overlap among various groups of bullous diseases for example: LAD may mimic BP or DH. IgA pemphigus may mimic pemphigus foliaceus, pemphigus herpetiformis or subcorneal pustular dermatosis. Inflammatory epidermolysis bullosa acquisita is

indistinguishable from BP.<sup>1</sup> Histopathology reveals the site of formation of bulla, the presence of infiltrate and its composition. The ideal site for DIF in immunobullous diseases is perilesional skin. The immune deposits are completely or partially degraded in inflamed or blistered skin and DIF may be negative. The differential diagnosis of a DIF test depends on, type and primary site of immune deposition, class of immunoglobulin and the number of immune deposits. With these parameters, a patterned approach can lead to an accurate diagnosis in the majority of specimens.<sup>1</sup>

In our study, the most common immunobullous disease was found to be pemphigus vulgaris (13/29, 45%), followed by 4 (14%) cases of BP, 3 (10.3%) cases of pemphigus foliaceus, 3 (10.3%) cases of LAD, 3 (10.3%) cases of CBDC and 3 (10.3%) cases of DH. These observations are comparable to the findings of the studies done by Buch *et al.*<sup>8</sup> (2014), Khannan *et al.*<sup>9</sup> (2015), Nageswaramma *et al.*<sup>10</sup> (2015), where pemphigus was the most common immunobullous disorder.

In the present study, pemphigus group was more common in females than males while BP and LAD were more common in males, which was similar to the studies done by Kambil and Madavamurthy<sup>11</sup> (2014) and Shamim *et al.*<sup>12</sup> (2008). Male preponderance was seen in DH in our study as compared to more number of females in the study done by West *et al.*<sup>13</sup>

All cases belonging to the pemphigus groups showed presence of flaccid blisters. Erosions, ulcers were most commonly seen in cases of pemphigus vulgaris. These findings are comparable with the findings of Arya *et al.*<sup>14</sup> and Kambil and Madavamurthy<sup>11</sup>, who have reported flaccid bullae in all cases belonging to pemphigus group. They found erosions in 27 (62.8%) cases out of the 43 cases of pemphigus

vulgaris. All cases of BP showed tense blisters. Erosions and crusting were seen less frequently. This can be explained by the fact that blisters in BP are subepidermal and are therefore, tense and are less likely to show erosions. The above mentioned observations are consistent with studies of Kambil and Madavamurthy.<sup>11</sup>

The present study showed IgG deposition in 100% cases of pemphigus group (intra-dermally) and BP (at BMZ). IgM deposition was seen in 31.2% cases of pemphigus group (intra-dermally), 66.7% cases of BP. While IgA deposits were seen at BMZ in all the cases of LAD, CBDC and DH. IgG and C3 deposits together were seen in 68.7% cases of pemphigus group (intra-dermally), 100% cases of BP (at BMZ). Our results are more or less similar to the study done by Deepti *et al.*<sup>15</sup> which showed IgG and C3 deposition together in 57.1% cases of pemphigus group and 46% cases of BP, while IgG + IgA deposition was seen in all cases of DH.<sup>15</sup> Similar results were seen in the studies done by Arundhati *et al.*<sup>16</sup>, Narsimha Rao Nethal *et al.*<sup>17</sup>, Buch *et al.*<sup>8</sup> and Isfer *et al.*<sup>19</sup> (1996).

The clinico-histological and histological-immunological concordance was tested using McNemar's test of significance. It was found that histopathology and DIF gave comparable results, that is, the difference in results by two methods was not statistically significant ( $P=0.5$ ). Comparison of clinical and histopathological results (clinico-histological concordance), showed p value of 0.5 which again indicated good concordance.

In our study, histopathology was taken as gold standard since the results were consistent. Histopathology was conclusive in all 29 cases. In the pemphigus group, out of 13 cases, results of DIF and histopathology correlated well in 12 cases, with sensitivity of 92.3%, while in case of

BP, out of 4 cases DIF and histopathology showed concurrent results in 3 cases with sensitivity of 75%. In case of LAD and CBDC, DIF sensitivity was found to be 100%, while it was 66.7% in cases of DH. The above results are comparable with the studies done by Mysorekar *et al.*<sup>17</sup> with sensitivity of 98.1% in each pemphigus vulgaris, as well as, pemphigus foliaceus and 96% in BP and Buch *et al.*<sup>8</sup> with sensitivity of 94.4% in case of pemphigus group and 84% in BP. The study done by Inchara and Rajalakshmi.<sup>3</sup> showed DIF sensitivity of 88% in pemphigus group and 82% in BP, while the study done by Lebe *et al.*<sup>19</sup> found clinical and histopathological/DIF concordance of only 5.2% in DH cases.

Hence, a diagnosis based solely on the clinical or histologic findings may not be accurate at times. DIF is extremely helpful in confirming a suspected diagnosis and in distinguishing among closely related cases of immunobullous lesions.<sup>6</sup>

### Conclusion

The results of the present study show that, although there are several variables of racial and climatic order, it was possible to reproduce positive results reported in studies done in other parts of the world. Our study concludes that the DIF is an essential tool for diagnosing autoimmune blistering disorders with the clinical and the histopathological overlap. Histopathology and DIF help in diagnosing various immunobullous disorders with similar picture.

With direct immunofluorescence rapid and accurate diagnosis can be made very early in the course of immunobullous dermatoses, so that it gives us an opportunity to institute effective management of these disorders at an early stage of the disease, because these disorders are

associated with considerable morbidity and mortality.

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