INTRODUCTION

Vesicobullous disorders represent a heterogenous group of dermatoses with protean manifestations. They have a remarkable impact on the patients and their families with severe economic consequences. Five principle mechanisms that can result in blister formation are genetic derangement, physical, immunological, and inflammatory damage and the drug reactions. Of these immunological reactions accounts for most of the vesiculobullous dermatological diseases.¹

Many of these blistering diseases mimic each other clinically and on histopathology, therefore immunofluorescence methods are used as adjunct to routine histological examination for diagnosing vesiculobullous lesions of skin.²

We undertook this study to evaluate histopathologic and immunofluorescence staining patterns and establish clinicopathologic correlation in patients presenting with vesiculobullous lesions to our large tertiary care teaching hospital in north India.
METHODS

This was a prospective study conducted between July 2013 and June 2016. The study was cleared by the ethics committee of our institute and informed consent was obtained from all patients. Consecutive patients with vesiculobullous lesions were enrolled for the study and were subjected to a detailed history and a thorough clinical examination. A clinical diagnosis was made based on the characteristic clinical features. In case of overlap of clinical features, differential diagnoses were kept instead of a single diagnosis.

Biopsy for histopathologic examination was taken from lesional skin or oral mucosa, and stained with hematoxylin and eosin (H&E) in all cases. Histopathologic findings were recorded and analysed as per proforma. Biopsy for Direct immunofluorescence (DIF) was taken from perilesional skin or oral mucosa, snap frozen, sections cut, and stained with polyclonal fluorescence isothiocyanate (FITC) conjugated antisera specific for IgG, IgA, IgM and C3 and fibrinogen. The pattern and distribution of immune complex deposits was analysed under fluorescence microscope qualitatively, while the intensity was determined semi quantitatively. Correlation of light microscopy findings and immunofluorescent staining was done and data was analyzed.

RESULTS

A total of 110 patients of vesiculobullous lesions were included in this study. Approximately one-third of the patients were of the age group of 41-50 years followed by age group of 31-40 years (17.3%) and 51-60 years (14.5%) respectively. The mean age of the study population was 47.1 years. Out of 110 cases, 51 cases were males and 59 cases were females with a male:female ratio of 1:1.15.

Pemphigus vulgaris constituted the most common vesiculobullous disorder making 48.2% of the total cases followed by Bullous Pemphigoid (27.3%) and Dermatitis herpetiformis (8.3%). Pemphigus foliaceus and varicella constituted 2.7% of the total patients. In the present study, PV, PF and DH affected middle aged with mean age of 42 years, 46.3 ears and 41.4 years respectively. BP and DLE were common in older individuals with mean of 60.2 years and 70 years respectively. Female preponderance was seen in PV, BP and SJS whereas DH, PF, varicella, paraneoplastic pemphigus (PP), subcorneal pustular dermatosis (SCPD) and chronic bullous disease of childhood (CBDC) showed male preponderance (Table 1).

Vesicles/bullae were the primary presenting lesions in 100% patients of BP, PF, varicella, bullous SLE, SCPD, CBDC and HHD, 94.3% patients of PV and 88.8% of DH. Plaque/papules were seen in 100% patients of SJS, 50% cases of PE, 22.2% of DH and 11.3% of PV. Erosions and ulcers were presenting lesions in 100% patients of PP and CBDC, 25% of PF and 21% patients of PV.

Out of 110 cases, 8 cases (7.2%) showed no bulla clinically. Fragile bullae were seen in 62 cases (56.3%) out of which majority of the patients were of pemphigus group (87.0%). Tense bullae were present in 39 cases with majority of cases belonging to BP (58.9%) and DH (20.5%).

Nikolsky sign was noted in pemphigus group, being positive in 49.1% cases of PV, 100% cases of PF and 33.3% patients of varicella. Bulla spread sign was seen in 30.2% cases of PV and 75% cases of PF.

Upper and lower extremities and back were commonly involved in patients of PV (77.9% and 24.5%), BP (73.3% and 26.6%), DH (77.8% and 33.3%), PF (75% and 25%), SJS (100% and 66.7%) and SCPD (100% and 0%). Scalp was involved in PV (13.2%), BP (16.6%), DH (22.2%) and SJS (66.7%). Face involvement was seen only in PV (13.2%) and BP (6.6%) whereas chest involvement was present in PV (5.6%), DH (11.1%) and SJS (66.7%). Lesions all over the body were seen in PV (20.7%), BP (10%), PF (25%), PE (50%) and CBDC (100%). No cutaneous involvement occurred in PP, bullous SLE, DLE and HHD.

Table 1: Age and gender distribution of the cases.

| Diagnostic spectrum (n=110) | Mean age in years | Male (n=51) | Female (n=59) | M:F |
|----------------------------|------------------|------------|--------------|-----|
| PV (n=53)                  | 42.04            | 20         | 33           | 1:1.6|
| BP (n=30)                  | 60.17            | 14         | 16           | 1:1.1|
| DH (n=9)                   | 41.44            | 7          | 2            | 3:1  |
| PF (n=4)                   | 46.25            | 3          | 1            | 3:1  |
| SJS (n=3)                  | 44.67            | 1          | 2            | 1:2  |
| Varicella (n=3)            | 28.67            | 2          | 1            | 2:1  |
| PE (n=2)                   | 57.00            | 1          | 1            | 1:1  |
| PP (n=1)                   | 47.00            | 1          | 0            | 1:0  |
| SCPD (n=1)                 | 32.00            | 1          | 0            | 1:0  |
| Bullous SLE (n=1)          | 19.00            | 0          | 1            | 0:1  |
| CBDC (n=1)                 | 5.00             | 1          | 0            | 1:0  |
| DLE (n=1)                  | 70.00            | 0          | 1            | 0:1  |
| HHD (n=1)                  | 85.00            | 0          | 1            | 0:1  |
Table 2: Histopathological features of various vesiculobullous disorders.

| Histopathological change | PV (n=53) | BP (n=30) | DH (n=9) | PF (n=4) | SJS (n=5) | Varicella (n=3) | PE (n=2) | PP (n=1) | SCPD (n=1) | Bullous SLE (n=1) | CBD C (n=1) | DLE (n=1) | HHD (n=1) |
|--------------------------|-----------|-----------|----------|----------|-----------|----------------|---------|---------|-----------|-----------------|-------------|-----------|----------|
| Plane of separation      |           |           |          |          |           |                |         |         |           |                 |             |           |          |
| Subcorneal               | 0         | 0         | 0        | 2; 50%   | 0         | 0              | 2; 100% | 0       | 1; 100%   | 0               | 0           | 0         | 0        |
| Intraepidermal           | 18; 34.0% | 0         | 0        | 2; 50%   | 0         | 0              | 0       | 0       | 0         | 1; 100%         | 0           | 0         | 0        |
| Suprabasal               | 30; 56.6% | 0         | 0        | 0        | 0         | 0              | 1; 100% | 0       | 0         | 0               | 0           | 0         | 0        |
| DE junction              | 0         | 30; 100%  | 8; 88.9% | 0        | 0         | 0              | 0       | 0       | 1; 100%   | 0               | 0           | 0         | 0        |
| No bulla                 | 5; 9.4%   | 1; 11.1%  | 0        | 3; 100%  | 0         | 0              | 0       | 0       | 1; 100%   | 1; 100%         | 1; 100%     | 0         | 0        |
| Bulla content            |           |           |          |          |           |                |         |         |           |                 |             |           |          |
| Acantholytic cells       | 45; 84.9% | 0         | 0        | 4; 100%  | 0         | 1              | 33.3%  | 1; 100% | 1; 100%   | 1; 100%         | 0           | 0         | 1; 100% |
| Neutrophils              | 46; 86.7% | 1; 3.3%   | 8; 88.9% | 4; 100%  | 0         | 0              | 0       | 0       | 0         | 1; 100%         | 0           | 0         | 0        |
| Eosinophils              | 0         | 9; 30%    | 0        | 0        | 0         | 0              | 0       | 0       | 0         | 0               | 0           | 0         | 0        |
| Mixed                    | 7; 13.2%  | 19; 63.3% | 1; 11.1% | 0        | 1; 100%   | 0              | 0       | 0       | 1; 100%   | 0               | 0           | 0         | 0        |
| Adjacent epidermis       |           |           |          |          |           |                |         |         |           |                 |             |           |          |
| Acanthosis               | 13; 24.5% | 9; 30.0%  | 1; 11.1% | 0        | 1; 33.3%  | 0              | 0       | 0       | 1; 100%   | 0               | 0           | 0         | 0        |
| Spongiosis               | 9; 17.0%  | 6; 20.0%  | 1; 11.1% | 1; 25%   | 1; 33.3%  | 0              | 0       | 0       | 1; 100%   | 0               | 0           | 0         | 1; 100% |
| Inflammatory infiltrate  |           |           |          |          |           |                |         |         |           |                 |             |           |          |
| Eosinophils              | 0         | 9; 30%    | 0        | 0        | 0         | 0              | 0       | 0       | 0         | 0               | 0           | 0         | 0        |
| Lymphocytes              | 0         | 1; 3.3%   | 0        | 0        | 0         | 2; 66.7%       | 3; 100% | 2; 100% | 1; 100%   | 1; 100%         | 0           | 1         | 0        |
| Neutrophils              | 46; 86.8% | 1; 3.3%   | 8; 88.8% | 4; 100%  | 0         | 0              | 0       | 0       | 0         | 1; 100%         | 0           | 0         | 0        |
| Mixed                    | 7; 13.2%  | 19; 63.4% | 1; 11.1% | 0        | 1; 33.3%  | 0              | 0       | 0       | 1; 100%   | 0               | 0           | 0         | 0        |
| Others                   |           |           |          |          |           |                |         |         |           |                 |             |           |          |
| Hyperkeratosis           | 30; 56.6% | 17; 56.7% | 7; 77.8% | 2; 50%   | 2; 66.7%  | 0              | 1; 50%  | 0       | 1; 100%   | 1; 100%         | 1; 100%     | 1         | 1        |
| Parakeratosis            | 6; 11.3%  | 4; 13.3%  | 1; 11.1% | 0        | 1; 33.3%  | 0              | 0       | 0       | 0         | 0               | 0           | 0         | 0        |
| Characteristic histopathologic feature | | | | | | | | | | | | | |
| Row of tombstone appearance - 24; 45.3% | 0 | | | | | | | | | | | | |
| Papillary microabscess - 8; 88.9% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Villi formation          | 5; 9.4%   | 0         | 0        | 0        | 0         | 0              | 0       | 0       | 0         | 0               | 0           | 0         | 0        |
| Tzanck smear             | Positive  | 21; 39.6% | 0        | 0        | 2; 50%    | 0              | 0       | 0       | 0         | 0               | 0           | 0         | 0        |
Oral mucosa was involved in 100% patients of PP and bullous SLE, 64.2% patients of PV, 50% patients of PE and 10% patients of BP. Patients with DH, PF, varicella, SCPD, CBDC, Discoid lupus erythematosus (DLE) and Hailey-Hailey disease (HHD) showed no oral mucosal involvement.

Table 3: Findings of direct immunofluorescence in various vesiculobullous disorders.

| DIF findings                  | PV (n=37) | BP (n=27) | DH (n=6) | PF (n=3) | SJS (n=1) | Varicella (n=2) | PE (n=2) | PP (n=1) | CBDC (n=1) | Bullous SLE (n=1) |
|-------------------------------|-----------|-----------|----------|-----------|------------|-----------------|----------|----------|------------|------------------|
| Pattern of immune complex deposits | Granular  | 0         | 0        | 5         | 0          | 0               | 0        | 0        | 0          | 0                |
|                               | (n=5)     |           |          |           |            |                 |          |          |            |                  |
|                               | Lace-like  | 35        | 0        | 0         | 3          | 0               | 2        | 1        | 0          | 0                |
|                               | (n=41)    |           |          |           |            |                 |          |          |            |                  |
|                               | Linear    | 0         | 25       | 0         | 0          | 0               | 0        | 0        | 1          | 0                |
|                               | (n=26)    |           |          |           |            |                 |          |          |            |                  |
|                               | No antibody deposition | 2       | 2        | 1         | 0          | 1               | 2        | 0        | 0          | 0                |
|                               | (n=9)     |           |          |           |            |                 |          |          |            |                  |
| Site of antibody deposition   | Intercellular space | 35     | 0        | 0         | 3          | 0               | 0        | 2        | 1          | 0                |
|                               | (n=41)    |           |          |           |            |                 |          |          |            |                  |
|                               | Dermo-epidermal junction | 0     | 25       | 0         | 0          | 0               | 0        | 0        | 1          | 0                |
|                               | (n=26)    |           |          |           |            |                 |          |          |            |                  |
|                               | Dermal papillae | 0     | 0        | 5         | 0          | 0               | 0        | 0        | 0          | 0                |
|                               | (n=5)     |           |          |           |            |                 |          |          |            |                  |
| Type of antibody deposited    | IgG       | 33; 94.2% | 9; 36%   | 0         | 3; 100%    | 0               | 0        | 1; 50%   | 1; 100%    | 0                |
|                               |           |           |          |           |            |                 |          |          |            |                  |
|                               | C3        | 21; 60%   | 23; 92%  | 1; 20%    | 2; 66.7%   | 0               | 0        | 2; 100%  | 0          | 0                |
|                               |           |           |          |           |            |                 |          |          |            |                  |
|                               | IgA       | 1; 2.8%   | 1; 4%    | 5; 100%   | 0          | 0               | 0        | 0        | 1; 100%    | 0                |
|                               |           |           |          |           |            |                 |          |          |            |                  |
|                               | IgE       | 0         | 0        | 0          | 0          | 0               | 0        | 0        | 0          | 0                |
|                               |           |           |          |           |            |                 |          |          |            |                  |
|                               | IgM       | 1; 2.8%   | 0        | 0          | 0          | 0               | 0        | 0        | 0          | 0                |

Table 4: Clinico-histopathological-direct immunofluorescence correlation in various vesiculobullous disorders.
Table 5: Age distribution pattern of bullous lesions (in years).

| Disorder | Bertram et al<sup>12</sup> | Uzun et al<sup>13</sup> | Deepti et al<sup>1</sup> | Present study |
|----------|-----------------------------|------------------------|-------------------------|---------------|
| PV       | 70 -80                      | 49 - 89                | 20-49                   | 12 - 60       |
| BP       | 60 -70                      | 18-70                  | 60 - 80                 | 19 -88        |
| PF       | -                           | 30 -70                 | 20 - 40                 | 30 -65        |
| DH       | 40 -50                      | -                      | 10 -29                  | 28 -62        |

Table 6: Comparison of plane of separation in various vesiculobullous disorders.

| Plane of separation | Arundhati et al<sup>4</sup> | Deepti et al<sup>3</sup> | Present study |
|---------------------|-----------------------------|-------------------------|---------------|
| Subcorneal          | 100% PF, SCPD and 50% PE    | 100% PF                 | 50% PF, 100% PE and SCPD |
| Intraepidermal      | 9.1% BP                     | 100% spongiotic dermatitis | 34% PV, 50% PF and 100% varicella and HHD |
| Suprabasal          | 96.2% PV, 9.1% BP, 100% HHD | 88.2% PV                | 56.6% PV, 100% PP |
| D.E.J.              | 72.3% BP, 50% EM, 100% bullous SLE | 92.3% BP, 66.6% EM, 100% DH and bullous SLE | 100% BP and bullous SLE, 88.9% DH |
| No bulla            | 3.8% PV, 50% PE, 9.1% BP, 50% EM, 5.8% PV, 7.6% BP, 33.3% EM | 9.4% PV, 11.1% DH and 100% SJS |

Table 7: Comparison of histopathologic features in various studies.

| Microscopic features | Arundhati et al<sup>6</sup> | Deepti et al<sup>6</sup> | Present study |
|----------------------|-----------------------------|-------------------------|---------------|
| Hyperkeratosis       | 7.7% PV, 50% EM and bullous SLE | 29.4% PV, 100% bullous SLE, 25% EM | 56.6% PV and BP, 77.8% DH, 50% PF and PE, 100% SCPD, bullous SLE, CBDC, DLE and HHD |
| Parakeratosis        | -                           | -                       | 11.3% PV and DH, 13.3% BP and 33.3% SJS |
| Spongiosis           | -                           | -                       | 17% PV, 20% BP, 11.1% DH, 100% SCPD and HHD |
| Acanthosis           | 11.5% PV, 50% SCPD          | 94% PV, 100% PF         | 24.5% PV, 30% BP, 11.1% DH, 100% SCPD |
| Acantholytic cells   | 76.9% PV, 75% PF, 100% PE and HHD, 50% SCPD | 94.5% PV, 100% PF, 50% SCPD | 84.9% PV, 100% PF, PE, PP, SCPD and HHD, 33.3% varicella |
| Row of tombstone appearance | 88.5% PV   | 70.5% PV                | 45.3% PV |
| Villi formation      | 38.5% PV, 25% PF, 100% HHD  | 17.6% PV                | 9.4% PV |
| Papillary microabscess | -                     | -                       | 88.9% DH |

Tzanck smear was conducted in 42.7% of cases. Tzanck smear for epidermal acantholytic cells was positive in 65.6% of the cases of PV and 66.7% cases of PF. This test was negative in 34.4% of the patients of PV, 33.3% cases of PF and 100% cases of PP, BP, DH, HHD and SJS.

Histopathologically, subcorneal bulla was seen in 100% cases of PE and SCPD whereas only 50% of PF cases showed subcorneal bulla. Intraepidermal bulla was seen in 34% cases of PV, 50% cases of PF and 100% cases of HHD and varicella. Suprabasal bulla was seen in 56.6% cases of PV and 100% cases of PP. Subepidermal bulla was seen in 100% cases of BP and bullous SLE whereas...
it was seen in only 88.95% cases of DH. Eleven cases showed no bulla on microscopy.

The details of histopathological findings are summarized in Table 2.

Out of 110 cases, DIF was conducted in 81 (73.6%) cases and 72 cases showed antibody deposition. Out of 37 cases of PV where DIF was performed, 34 patients were clinically suspected, characteristic histopathology was observed in 32 patients and 35 cases were found to be positive on DIF. Sensitivity on histopathology was 86.4% and on DIF 94.5%. In cases of BP, there were 24 clinically suspected cases, 27 cases which were diagnosed on histopathology and 25 cases that were proved on DIF. Hence sensitivity on DIF was less than sensitivity on histopathology in cases of BP. There was 100% agreement between histopathological and DIF findings in PF, PP, SJS and varicella. In cases of DH, there were 6 clinically suspected cases, 5 cases which were diagnosed on histopathology which were also proved on DIF. There was one clinically suspected case of SJS and DLE each that were proved on histopathology but on DIF showed no deposits. CBDC was suspected in one patient clinically which was confirmed only on DIF. Details of DIF are shown in Table 3; and its correlation with clinical and histopathological findings is shown in Table 4.

**DISCUSSION**

Blistering diseases are alarming skin conditions where blister formation occurs in various ways and cannot be differentiated clinically. Autoimmune blistering diseases are a group of bullous disorders characterized by pathogenic antibodies directed at target antigens in the epidermis or dermoepidermal junction. For confirmation of diagnosis, along with routine histopathological examination, immunofluorescence studies are essential.

**Age and sex distribution**

In the present study, majority of the patients presented in the age group of 41-50 years (30.9%). This was in accordance with the studies conducted by Deepti et al and Arundhathi et al in which the majority of patients belonged to age group of 40-49 years of age. In the current study, the youngest patient was 5 years old and the oldest being 88 years. In a study conducted by Khannan et al the youngest patient was 4 years old whereas the oldest patient was 80 years old. The mean age of study population was 47.1 years in the current study. This is in accordance with a study by Kabir et al who found the mean age of 35.1±19.4 years whereas study by Buch et al described mean age of 57 years.

Females were affected slightly more than the males. This finding was similar to the previous study by Deepti et al, Arundhathi et al and Khannan et al whereas studies by Kabir et al, Mahmood et al and David et al showed male predominance.

In the present study, BP was common in older individuals with mean age of 60.2 years which is similar to that reported by Selvaraj et al. The mean age of presentation in patients of PV in present study was 42 years. This was in concordance with study conducted by Shamim et al which also showed a mean age of 42.7 years. Age distribution pattern of various vesiculobullous lesions in different studies is shown under in Table 5.

In the present study, PV, BP and SJS showed a female preponderance whereas DH, PF, varicella, PP, SCPD and CBDC showed a male preponderance. Study conducted by Deepti et al showed PV, BP and PF as female preponderant. Chandrashekar et al described PV as male predominant and equal incidence of males and females in BP.

**Spectrum of vesiculobullous disorders**

In the present study PV was the most common vesiculobullous disorders constituting 48.2% followed by BP constituting 27.3% of the study population. This is in agreement with the study by Collier et al, Inchara et al, Arya et al, Deepi et al, Khannan et al and Buch et al all of which showed pemphigus vulgaris to be the commonest entity among all vesiculobullous disorders followed by bullous pemphigoid.

**Clinical correlation**

In the present study, oral mucosal involvement was seen in 64.2% cases of PV, 10% cases of BP, 100% cases of bullous SLE and no oral involvement in patients of PF and DH. This showed result similar to study conducted by Arundhathi et al in which oral mucosal involvement was present in 84.6% (22/26) cases in PV and 18.2% cases in BP. Deepi et al showed oral mucosal involvement in 88.2% of the cases of PV and 100% cases of DH and bullous SLE.

In the present study patients of PV showed only mucosal involvement in 22.6% of patients, only skin involvement in 41.5% patients and both skin and mucosal involvement in 35.8% of patients. Various studies showed varied results. Study by Javidi et al showed mucosal involvement in 14% and skin involvement in 21.7% and involvement of both the skin and mucosa in 64.3% of the patients. 50% patients of PE showed only skin involvement whereas other 50% showed involvement of both skin and mucosa. Study conducted by Chandrashekar et al showed majority of patients having both oral and skin involvement in 66% of cases. This observation was also described by Fernandez et al.

In the current study Nikolsky sign was positive in 49.1% patients of PV and 100% patients of PF. This positivity
rate was low in case of PV and was comparable in cases of PF when compared to studies conducted by Deepti et al, Vora et al and Arya et al.3,19,16

Present study showed 65.5% and 66.7% positivity of Tzanck smear in PV and PF respectively which was concordant with the study conducted by Sabir et al which showed 75% positivity of Tzanck smear in pemphigus.20 Other studies which showed 100% positivity of the smear were conducted by Selvaraj et al and Chandrashekar et al.10,14

**Histopathological findings**

On comparison of plane of separation of various vesiculobullous disorders, variable results have been reported in different studies. Studies conducted by Khannan et al and Selvaraj et al described 100% cases of PV showing suprabasal bulla in contrast to the current study in which only 56.6% cases of PV showed suprabasal plane of separation.3,10 All the cases of PF showed subcorneal bulla as shown by Srinath et al and Khannan et al whereas in current study only 50% cases of PF showed subcorneal plane of separation.3,21 Rest 50% cases of PF showed intraepidermal plane of separation which might be due to secondary clefts leading to detachment of the epidermis in its mid-level and epithelial migration and regeneration may result in an intraepidermal location in older blisters.2 Different planes of separation by different studies are shown in Table 6.

On comparison of histopathological features on microscopy concordant results were found with studies conducted by Arundhathi et al and Deepti et al shown in Table 7.3,4

In the current study neutrophils were the predominant inflammatory cells in cases of PV, PF and DH. Eosinophils were the predominant inflammatory cells in BP whereas mixed inflammatory infiltrate was seen in cases of BP and bullous SLE. This was in concordance with the study conducted by Deepti et al, Arundhathi et al and Khannan et al which showed similar findings.3,5

**Pemphigus**

The present study showed PV as the major type of pemphigus followed by PF, PE and PP constituted 3.33% and 1.67% of the proportion respectively. This was in concordance with studies conducted by Deepti et al, Khannan et al and Chandrashekar et al which also showed PV as the commonest type in pemphigus group.3,5,14

**Pemphigus vulgaris**

Female preponderance of pemphigus vulgaris similar to our study was shown by various studies done by Nafiseh et al, Vora et al and Deepti et al which is contrary to Kanwar et al who found no gender predilection.3,19,22,23 Mucosal involvement was seen in 58.4% cases which is comparable to Kanwar et al.23 Rate of Nikolsky sign positivity was much lower than the other studies by Vora et al and Deepti et al.3,19

Suprabasal bulla was present in 56.6% of our cases which is similar to the findings of Vora et al and contrary to Arya et al and Deepti et al.3,16,19 Acantholytic cells were seen in 84.9% cases which is lower than that reported by Vora et al, Arya et al and Deepti et al.3,16,19

**Bullous pemphigoid**

In present study BP constituted 30% of the study population with M: F ratio of 1:1.1 which is similar to studies by Lagan et al, Budimir et al and Deepti et al.1,24,25 Subepidermal blister was seen in 100% cases in present study which was similar to studies by Nishioka et al and Leena et al.26,27 Inflammatory cells were noted in bulla (100%) and dermal infiltrate (100%) similar to study by Leena et al.27 Predominant inflammatory cells were eosinophils similar to that of Nishioka et al and Deepi et al.26

**Dermatitis herpetiformis**

Nine cases presented with DH which constituted 27.3% which is much higher than reported by others (Deepti et al - 4% and Banu et al – 5.6%).28 Subepidermal bullae and papillary microabscess were present in 88.9% cases where as Deepti et al and Banu et al reported 100% cases showing subepidermal bulla and papillary microabscess.3,28

**Pemphigus foliaceous**

Age group affected by Pemphigus foliaceous was mainly between 30-65 years in this study with M: F ratio 3:1 which was similar to study by Vora et al and opposite to that of Deepi et al.3,19 Mucosal membrane involvement was noted in 25% cases which is similar to study by Deepi et al and Arya et al.3,16 Nikolsky’s sign showed positivity in 75% cases which was similar to study to Deepi et al and lower than Arya et al.3,16 Subcorneal bulla were present in 50% of the cases which was similar to Arya et al, lower than Vora et al and Deepi et al.3,19 Acantholytic cells showed 100% positivity which is similar to study by Vora et al and Deepi et al.3,19

**Comparison of DIF positivity in various vesiculobullous lesions**

Out of 110 cases, DIF was performed in 81 cases in our study. In this present study, 72 cases showed positivity on direct immunofluorescence and 9 cases showed no antibody deposition. This is comparable with other studies by Kabir et al (88.23%), Inchara et al (73%), and Minz et al (70%).6,15,25 The positivity rate of DIF in cases of pemphigus vulgaris was comparable to studies by Deepti et al and Chams-Duvatchi et al and was higher.
when compared to the studies conducted by Kabir et al, Inchara et al, and Minz et al. The DIF had a sensitivity of 92.6% in cases of BP in our study. The findings of our study are supported by study conducted by Deepthi et al which had lower sensitivity. The studies of Kabir et al and Mahmood et al had 100% sensitivity of DIF in their studies.

The sensitivity of DIF and histopathology in cases of DH was observed to be 83.3% in our study. The previous studies have yielded variable results. Hertz KC et al and Minz et al in their studies on five and three cases of DH respectively found a 100% sensitivity of DIF. On the other hand, Inchara et al studied 4 cases of DH, none of which showed positivity on DIF, sensitivity of DIF being 0%. Only one case of SJS was found in this study which was negative for any antibody on DIF. Similar findings were also observed in the study by Minz et al which also showed DIF negative cases. However in the study conducted by Kabir et al there was only one case of EM which revealed deposits of C3 in dermal blood vessels on DIF. A critical review of literature indicates that DIF studies serve primarily to rule out bullous pemphigoid, dermatitis herpetiformis and pemphigus in the differential diagnosis.

**Patterns and site of antibodies deposition on DIF in vesiculobullous lesions**

In the present study, lace like squamous intercellular pattern was noted in all types of pemphigus. This was in concordance with study conducted by Deepti et al, Chandrashekar et al and Khannan et al. Current study described linear deposition of antibodies at dermo-epidermal juncton in BP and CBDC. This was in concordance with study conducted by Deepti et al, Khannan et al and Kabir et al. Present study showed granular deposition of antibodies at dermal papillae in cases of DH. Buch et al and Deepti et al both included 2 cases and Banu et al which included 3 cases of DH, all showed similar findings.

**Type of antibodies deposition on DIF in vesiculobullous lesions**

In current study, majority of cases of PV showed IgG (94.2%) and C3 (60%) deposition with 3.8% of cases showing IgM and IgA deposition each. This was in concordance to study conducted by Khannan et al which showed 100% cases of PV having IgG deposition and 84% having C3 deposition. IgG showed higher percentage in our study when compared to studies conducted by Deepti et al and Arundhati et al which showed 52.5% and 57.7% of IgG respectively.

Cases of BP in present study showed C3, IgG and IgA deposition in 92%, 36% and 4% of the cases respectively. The previous studies have yielded variable results. According to study by Kabir et al, 50% cases showed deposition of C3, 40% cases showed C3 and IgG, and 10% showed C3, IgG and IgM along the BMZ. Study by Buch et al described majority (18/25) of BP showing linear IgG and C3 deposition in the basement membrane zone (BMZ). In our study 100% cases of DH showed IgA deposition and 20% of the cases showed additional C3 deposition. This was in concordance to study by Buch et al which showed 100% of cases having granular IgA deposition at dermal papillae.

Present study showed 100% cases of PF having IgG deposition and 66.7% showing additional C3 deposition also. Study by Deepti et al showed 75% of cases having only IgG deposition and 25% having both IgG and C3 deposition. Khannan et al showed 100% of cases of PF showing IgG intercellular deposition. Kabir et al study showed intercellular deposition of IgG 100% cases along with intercellular depositions of C3 in 33.33% cases and focal BMZ deposition of C3 and IgM in one case (16.66%).

Our study described only one case of PP which showed IgG deposition whereas study by Kabir et al revealed deposition of IgA and C3 along the BMZ along with deposition of IgG both in epidermis and BMZ.

**Clinical, histopathological and DIF correlation**

In present study DIF was performed in 81 cases. A clinico pathological and DIF correlation was made in these cases. Out of these 81 cases, 91.3% of the clinically suspected cases of vesiculobullous lesions, 90.12% were proved on histopathology and 92.6% on DIF showing that immunofluorescence is a confirmatory test in addition to histopathology. Minz et al in their study proved that 77% of their DIF diagnosis correlated with the clinical diagnosis whereas histopathology correlated with 70% of the clinical diagnosis. However, in the study conducted by Kabir et al less than 50% cases showed concordance of histopathology with DIF diagnosis.

According to a study conducted by Buch et al, DIF is a very reliable diagnostic test for pemphigus, which becomes positive at an early stage and remains positive for a long period after clinical remission. In the absence of the characteristic DIF pattern, the combination of clinical, histologic, and immunologic data is needed to support the definite diagnosis of different immunobullous disorders.

Similarly in single suspected case of PE, histopathology did not prove beneficial due to similarity in findings of PE and PF on microscopy. DIF is extremely helpful in
distinguishing among closely related cases of immunobullous lesions. Study by Kabir et al concluded that although clinical findings and histological examination were sufficient for the diagnosis of most cases, direct immunofluorescence study is essential in many cases.

**Funding:** No funding sources

**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the institutional ethics committee

**REFERENCES**

1. Collier PM, Wojnarowska F. Blistering diseases. Med Int. 1997;11:51-3.
2. Wu H, Brandling-Bennett HA, Harrist TJ. Noninfectious vesicobullous and vesicopustular diseases. In: Elder DE, editor. Lever’s Histopathology of Skin. 10th ed. New Delhi: Wolters Kluwer (India); 2009: 235-278.
3. Deepti S, Sulakshana MS, Manjunatha YA, Jayaprakash HT. A histomorphological study of bullous lesions of skin with special reference to immunofluorescence. Int J Curr Res Aca Rev. 2015;3(3):29-51.
4. Arundhati S, Raghunatha S, Mahadeva KC. A Cross-sectional study of clinical, histopathological and direct immunofluorescence spectrum of vesiculobullous disorders. J Clin Diagn Res. 2013;7(12):2788-92.
5. Khanna CK, Bhat R. A retrospective study of clinical, histopathological and direct immunofluorescence spectrum of immunobullous disorders. Int J Sci Res Pub. 2015;5(9):1-6.
6. Kabir AKMN, Kamal M, Choudhury AM. Clinicopathological correlation of blistering diseases of skin. Bangladesh Med Res Counc Bull. 2008;34:48-53.
7. Buch AC, Kumar H, Panicker NK, Misal S, Sharma YK, Gore CR. A cross sectional study of direct immunofluorescence in the diagnosis of immunobullous dermatoses. Indian J Dermatol. 2014;59(4):364-68.
8. Mahmood T, Haroon TS. Patterns of direct immunofluorescence in subepidermal autoimmune bullous diseases of skin in Lahore, Pakistan. J Pak Assoc Derma. 2003;13:67-71.
9. David M, Weissman-Katznelson V, Ben-Chetrit A, Hazaz B, Ingber A, Sandbank M. The usefulness of immunofluorescent tests in pemphigus patients in clinical remission. Br J Dermatol. 1989;120:391-5.
10. Selvaraj U, Ramamoorthy M. Clinicopathological study of autoimmune vesiculobullous disorders: a case series from a resource poor rural tertiary care center in southern Tamil Nadu. Int J Sci Study. 2016;4(4):27-30.
11. Shamim T, Varghese VI, Shameena PM, Sudha S. Pemphigus vulgaris in oral cavity: Clinical analysis of 71 cases. Med Oral Patol Oral Cir Bucal. 2008;13(10):622-6.
12. Bertram F, Brocker E, Zillikens D, Schmidt E. Prospective analysis of the incidence of autoimmune bullous disorders in Lower Franconia. Germany Journal der Deutschen Dermatologischen Gesellschaft. 2009;7(5):434-9.
13. Urun S, Durdu M, Akman A, Gunasti S, Uslular C, Memisoglu HR, et al. Pemphigus in the Mediterranean region of Turkey: a study of 148 cases. Int J Dermatol. 2006;45:523-8.
14. Chandrashekar M, Leena JB, Vijay B, Sunila R, Manjunath GV. A Clinicopathological study of immunobullous lesions of the skin a two years study. Adv Lab Med Int. 2012;2(2):19-26.
15. Inchara YK, Rajalakshmi T. Direct immunofluorescence in cutaneous vesicobullous lesions. Ind J Pathol Microbiol. 2007;50:730-2.
16. Arya SR, Valand AG, Krishna K. A clinicopathological study of 70 cases of pemphigus. Indian J Dermatol Venereol Leprol. 1999;65(4):168-71.
17. Javidi Z, Meibodi NT, Nahidi Y. Epidemiology of pemphigus in northeast Iran: A 10-year retrospective study. Indian J Dermatol. 2007;52:188-91.
18. Fernandez JC, Dharni JB, Desai SC. A study of 100 cases of pemphigus-clinical features. Indian J Dermatol Venereol Leprol. 1970;36:1-9.
19. Vora D, Popat V, Bhanvadia V, Mehta DA, Bhetariya B, Kumar M. A study of 75 cases of pemphigus in Saurashtra region of India. Iranian J Dermatol. 2010;13(2):42-6.
20. Sabir F, Aziz M, Afroz N, Amin SS. Clinical and cyto-histopathological evaluation of skin lesions with special reference to bullous lesions. Indian J Pathol Microbiol. 2010;53(1):41-6.
21. Srinath M, Kambli, Madavamurthy P. Immunobullous disorders clinical, histopathological and immunofluorescence study of 36 cases. Mullers J Med Res. 2014;5:134-8.
22. Nafiseh E, Chams-Davatchi C, Valikhani M, Daneshpazhooh M, Baligi K, et al. Pemphigus vulgaris in Iran: a clinical study of 140 cases. Int J Dermatol. 2007;46(11):1166-70.
23. Kanwar AJ, Singh M, El-Mangoush IM, Bharija SC, Belhaj MS. Clinical pattern of bullous disorders in Eastern Libya. Indian J Dermatol Venereol Leprol. 1987;53:337-9.
24. Langan SM, Smith CIP, West J. Bullous pemphigoid and pemphigus vulgaris incidence and mortality in the UK: population based cohort study. BMJ. 2008;337:180.
25. Budimir J, Liborija L M, Situm M, Bulat V, Persic S, Tomljancovic-Veselski M. Oral lesions in patient with pemphigus vulgaris and Bullous pemphigoid. Acta Clin Croat. 2008;47:13-8.
26. Nishioka K, Hashimoto K, Katayama I, Sarashi C, Kubo T, Sano S. Eosinophilic spongiosis in bullous pemphigoid. Arch Dermatol. 1984;120:1166-8.
27. Leena JB, Chandrashekar M, Vijay B, Sunila R, Manjunath GV. A clinico-pathological study of 22 cases of pemphigus. J Clin Diagn Res. 2010;4:2446-51.
28. Banu I, Gulen GN, Selen S, Hulya E. Evaluation of clinical and histopathologic/direct immuno-fluorescence. 2012;28(1):11-6.
29. Minz RW, Chhabra S, Singh S, Radotra BD, Kumar B. Direct immunofluorescence of skin biopsy: perspective of an immunopathologist. Ind J Dermatol Venerol Leprol. 2010;76:150-8.
30. Chams-Davatchi C, Valikhani M, Daneshpazhooh M, Esmaili N, Balighi K, Hallaji Z, et al. Pemphigus: analysis of 1209 cases. Int J Dermatol. 2005;44:470-6.
31. Deepthi PK, Santosh CS, Suguna B, Gopal M, Abhishek. Direct immunofluorescence study in autoimmune bullous disorders of skin. IJBAR. 2013;4(9):611-8.
32. Hertz KC, Gazze LA, Katz SI. Immunofluorescent localization of basement membrane in lesions of dermatitis herpetiformis. J Invest Dermatol. 1976;67:683-7.
33. Beutner EH, Chorzelski TP, Jablonska S. Immunofluorescence Tests: Clinical significance of sera and skin in bullous diseases. Int J Dermatol. 1985;24:405-21.