Clinicopathological Spectrum of Vesiculobullous Skin Lesions with Special Reference to Direct Immunofluorescence Microscopy

Kuldeep Singh, Reeta Dhar, Shilpi Sahu, Hemangi R Jerajani

ABSTRACT

Introduction: Accurate diagnosis of vesiculobullous lesions of skin requires evaluation of clinical, histopathological, and immunofluorescence findings.

Materials and methods: A clinicopathological study of vesiculobullous lesions of skin was carried out over a period of 2 years in a tertiary hospital, Navi Mumbai, India. Forty-one cases of vesiculobullous disease were reported. Patients were evaluated based on their clinical findings, histopathological, and direct immunofluorescence (DIF) features to arrive at a definitive diagnosis in case of cutaneous vesiculobullous diseases. Biopsies comprised lesional as well as perilesional skin.

Results: Majority of the patients (34.15%) were in the age group of 40 to 49 years. Male to female ratio was 1:1.4. All the cases presented with blister. Pemphigus vulgaris (PV) was the most common vesiculobullous disease accounting for 39.02% followed by bullous pemphigoid (BP) in 29.27%. Pemphigus foliaceus (PF) and Hailey-Hailey disease (HDD) constituted 7.32% each. 4.88% were of spongiotic dermatitis, while one case each of pemphigus erythematosus (PE), subcorneal pustular dermatoses (SCPD), epidermolysis bullosa acquatica (EBA), lichen planus pemphigoid (LPP), and bullous lichen planus (BLP) accounting for 2.44%. Histopathological features were conclusive in 85.37% cases. Direct immunofluorescence was contributory in 14.63% cases, where histopathology was inconclusive.

Conclusion: Histopathology was important for differentiating PV from PF. A good clinicopathological correlation was seen. Thus, correlation of clinical, histopathological, and DIF required for definitive diagnosis of vesiculobullous lesions of the skin.

Keywords: Clinical pathology, Direct immunofluorescence microscopy, Epidermolysis bullosa, Vesiculobullous lesions.


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Conflict of interest: None

INTRODUCTION

Vesiculobullous disorders represent a heterogeneous group of dermatoses with protean manifestations. The accurate diagnosis of bullous diseases of the skin requires evaluation of clinical, histologic, and immunofluorescence findings. Pathologic evaluation of blisters involves systematic analysis, which includes the blister separation plane and the character of inflammatory infiltrate. Direct immunofluorescence (DIF) is performed on perilesional skin for patients with bullous diseases and lesional skin for patients with connective tissue diseases and vasculitis. The clinicopathological spectrum of various vesiculobullous skin lesion by their clinical presentation, age, sex, physical and local examination of lesion, provisional diagnosis by clinician, histopathology by routine hematoxylin and eosin (H&E) stains, and DIF microscopy using perilesional skin in selected cases have been studied.

AIMS AND OBJECTIVES

- To study the incidence of various vesiculobullous lesions of skin.
- To study the various vesiculobullous skin lesions in relation to morphology, site, different age group, and sex.
- To correlate clinical and histopathological diagnosis of skin lesions and to confirm with DIF wherever possible.
- Use of immunofluorescence where histopathological features are not diagnostic.

MATERIALS AND METHODS

Study of vesiculobullous diseases with clinical, histopathological, and DIF examination wherever applicable was conducted from October 2013 to October 2015. During this period, 41 biopsy specimens of vesiculobullous lesions of skin were received. In all the cases, punch biopsy from the lesional skin, including intact vesicles, was taken for histopathological study. Another biopsy from perilesional normal looking skin was taken for DIF. Of the two biopsies, one was sent in 10% buffered formalin for H&E staining and another was sent in Michel’s medium for DIF.
OBSERVATION AND RESULTS
From October 2013 to October 2015, 1358 skin biopsies were received for histopathological examination. Forty-one cases were vesiculobullous lesions, representing 3.02%. Approximately 30,000 patients attended Dermatology Clinic, out of which 41 cases presented clinically as immunobullous diseases, constituting 0.14%.

Distribution of Cases of Vesiculobullous Diseases
Pemphigus vulgaris (PV) was the most common vesiculobullous disease constituting 39.02% (16 out of total 41 cases), followed by bullous pemphigoid (BP) 29.27% (12 cases), pemphigus foliaceus (PF) 7.32% (3 cases), Hailey-Hailey disease (HDD) 7.32% (3 cases), spongiform dermatitis (SD) 4.88% (2 cases), one case each of pemphigus erythematosus (PE), bullous lichen planus (BLP), epidermolysis bullosa acquisita (EBA), subcorneal pustular dermatoses (SCPD), and lichen planus pemphigoid (LPP) were encountered accounting to 2.44% each.

Age Group and Gender Distribution
Out of 41 cases, 17 were males (41.46%) and 24 females (58.54%). Ratio was 1:1.4. Youngest was 4-year-old boy. A 84 years male patient was the oldest. Majority of the patients of vesiculobullous lesions were in the age range of 40 to 49 years (34.15%) followed by 20 to 29 (17.07%) and 60 to 69 (14.63%) respectively. In the age range of 40 to 49 years, 11 were female and 3 male patients. Maximum male patients were in the age group of 20 to 29 and 60 to 69 years, comprising 4 cases (23.53% of males) in each group. Maximum age group for females was 40 to 49 years, comprising 11 cases (45.83% of females) (Table 1).

Gender Distribution of Vesiculobullous Disorder
Pemphigus vulgaris showed female predominance. Out of 16 cases of PV, 62.5% cases (10 out of 16) were females and 37.5% cases (6 out of 16) were males. All the cases of PF and HHD were females (100%). Bullous pemphigoid showed male predominance. 66.67% cases (8 out of 12) were males and four were females (33.33%). Patients of SCPD, BLP were males and EBA, LPP were females.

Consistency of Blisters in Vesiculobullous Diseases
81.25% cases of PV (13 out of 16) presented with flaccid blisters, 100% cases of PF, HHD, and BLP presented with flaccid blisters. All cases of BP presented with tense bullae. Blisters were tense in SD, SCPD, EBA, and LPP.

Site Distribution of Vesiculobullous Diseases
Trunk was the most common site of the blister. Blisters were present on trunk in 93.75% cases (15 out of 16) of PV. In BP trunk, involvement was seen in 91.67% cases (11 out of 12). Axilla and groin was involved in all cases of HHD. Trunk was involved in all the three cases of PF. In 81.25% cases (13 out of 16) of PV, oral mucosa was involved, while one case of each BP, BLP, and PF showed the involvement of oral mucosa (Table 2).

Direct Immunofluorescence Results
Out of 41 cases, DIF was done in 38 cases, and DIF was positive in 93.75% cases of PV and 100% cases of BP. In these disorders, it helped in the confirmation of disease. In diseases like SCPD, EBA, SD, LP pemphigoid and BLP, DIF was contributory in arriving at the final diagnosis. But DIF was not done in the cases of HHD.

Site of Antibody Deposition in DIF
The site of antibody deposition in majority of the pemphigus group was squamous intercellular spaces in epidermis and at dermoepidermal junction in case of BP lesions (Table 3).

Direct Immunofluorescence Microscopy: Type of Antibody
IgG was the most common antibody observed in 93.75% cases (15 out of 16) of PV. IgG along with IgA, C3 and IgM showed male predominance. 66.67% cases (8 out of 12) were males and four were females (33.33%). Patients of SCPD, BLP were males and EBA, LPP were females.

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Age groups in years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–9</td>
<td>10–19</td>
</tr>
<tr>
<td>PV</td>
<td>–</td>
<td>1 (6.25%)</td>
</tr>
<tr>
<td>PF</td>
<td>–</td>
<td>1 (33.33%)</td>
</tr>
<tr>
<td>PE</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BP</td>
<td>1 (8.33%)</td>
<td>–</td>
</tr>
<tr>
<td>HHD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SCPD</td>
<td>–</td>
<td>–</td>
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<tr>
<td>EBA</td>
<td>–</td>
<td>–</td>
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<tr>
<td>LPP</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BLP</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
was seen in 5, 3, 1 case of PV respectively. IgG alone was detected in 6 cases (37.5%). C3 antibody was observed in 100% cases of BP. C3 was positive in 6 cases (50%), C3 + IgG in 3 cases (25%), and C3 + IgG + IgA in 3 cases (25%) (Table 4).

### Table 4: Type of antibodies in vesiculobullous disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>C3</th>
<th>IgG + IgA</th>
<th>IgG + C3</th>
<th>IgG + C3 + IgA</th>
<th>IgG + IgM</th>
<th>Negative</th>
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<tr>
<td>PV</td>
<td>6 (37.5%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5 (31.25%)</td>
<td>3 (18.75%)</td>
<td>–</td>
<td>1 (6.25%)</td>
<td>1 (6.25%)</td>
</tr>
<tr>
<td>PF</td>
<td>1 (33.33%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2 (66.67%)</td>
<td>–</td>
</tr>
<tr>
<td>PE</td>
<td>1 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BP</td>
<td>–</td>
<td>–</td>
<td>6 (50%)</td>
<td>–</td>
<td>3 (25%)</td>
<td>3 (25%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HHD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2 (100%)</td>
<td>–</td>
</tr>
<tr>
<td>SD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1 (100%)</td>
<td>–</td>
</tr>
<tr>
<td>SCPD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EBA</td>
<td>1 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LPP</td>
<td>–</td>
<td>–</td>
<td>1 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>BLP</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1 (100%)</td>
<td>–</td>
</tr>
</tbody>
</table>

### Accordance and Discordance in Histopathological and Final Diagnosis

Final diagnosis was made after DIF. Histopathology was conclusive in majority of cases (85.37%). However, it was inconclusive in 6 cases (14.63%), either due to damage to blister during biopsy procedure or during tissue processing or requirement of DIF microscopy to differentiate it from their close differential diagnosis. Direct immunofluorescence helped in such cases to arrive at final diagnosis. Among such cases, one case was PV. One was of PE. In both these cases, blister was eroded, so histopathology was noncontributory. In each case of EBA, SCPD, LPP, and BLP, histopathology was noncontributory.

### Accordance and Discordance between Clinical and Final Diagnosis

Accordance between clinical and final diagnosis was seen in 70.73% (29 out of 41 cases). Discordance between clinical and final diagnosis was seen in 29.27% (12 out of 41 cases) (Table 5).

### Table 5: Discordance between clinical and final diagnosis

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>PV</th>
<th>PF</th>
<th>BP</th>
<th>SCPD</th>
<th>SD</th>
<th>EBA</th>
<th>LPP</th>
<th>BLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive seborrhoeic dermatitis</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Linear IgA bullous disease</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bullous pemphigoid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IgA pemphigus (3 cases)</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bullous lichen planus (2 cases)</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Pemphigus erythematosus (2 cases)</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bullous herpes zoster</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Lichen planus pemphigoid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
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</tbody>
</table>
DISCUSSION

Pemphigus vulgaris

Pemphigus vulgaris was the most common vesiculobullous disease, constituting 39.02% (16 out of 41 cases). It was reported as common disease in the studies of Leena et al\(^3\) (45%), Arundhati et al\(^7\) (38.2%), and Deepti et al\(^4\) (34%). Fifty percent (8 out of 16) cases were in the age group of 40 to 49 years. This is akin to Indian literature where majority of patients are younger than Western patients. Youngest patient of PV was 19 years and oldest was 59. Similarly, in the study of Arya et al\(^6\) and Leena et al\(^3\), most of the patients were between 21 and 60 years.

Most of the cases presented with large flaccid bullae (Fig. 1). Involvement of oral mucosa was seen in 81.25% (13 out of 16 cases). This was similar to the Arundhati et al (84.6%) and Deepti et al (88.2%) and slightly higher in number than seen by Leena et al (66%) and Arya et al (72.1%). Most common site was trunk (93.75%) followed by upper extremities (81.25%) and lower extremities (75%). Scalp and face involvement was seen in 43.75 and 50% cases respectively. In 7 cases (43.75%) lesions were generalized. Similar site predilection was seen by Leena et al, Deepti et al, Arya et al, and Arundhati et al.

In 68.75% cases mixed inflammatory infiltrate comprising of neutrophils, lymphocytes, and eosinophils seen in the blister cavity. In 18.75% cases, neutrophils were chief inflammatory cell and in 6.25% cases eosinophils were predominantly seen (Fig. 2). In our study, inflammatory cells were absent in blister cavity in 6.25% (1 out of 16 cases) possibly due to less severity of disease and due to the effect of steroids. Similar findings were seen by Leena et al, where mixed inflammatory infiltrate was seen in 94.4%, and in the study of Arya, neutrophils were seen in 20.9% cases. While in the study of Deepti et al neutrophils were predominant (58.8%) and mixed infiltrates were seen in 35.2%, and eosinophils were seen in 25.6% cases in the study of Arya et al. In all the 16 cases of PV, dermal infiltrates were seen. Most commonly dermal interstitial infiltrate was seen (93.75%), which was comparable to the study of Leena et al (100%) and Deepti et al (88.2%).

Direct immunofluorescence was done in all the 16 cases of PV. In 93.75% cases, DIF was positive and in 6.25% DIF was negative. Most common site of deposition was squamous intercellular junction in the epidermis which was seen in 93.75% cases. Direct immunofluorescence positivity was 97.67, 92.3, 94.7, and 94.12% in the studies of Narsimha Rao et al, Arundhati et al, Jindal et al, and Deepti et al respectively, which was similar to our study. Pattern of deposition was fishnet or Lace-like (Fig. 3). IgG was seen in all the positive cases. In addition to IgG, IgA was seen in 31.25% cases, C3 in 18.75% cases, and IgM in 6.25% cases. Similarly study of Deepti too showed IgG in all positive cases. IgG at squamous intercellular spaces was seen in 93.02% in the study of Narsimha Rao et al.

Fig. 1: Pemphigus vulgaris – large flaccid bullae on trunk

Fig. 2: Pemphigus vulgaris—suprabasal separation plane (H&E: 100×)

Fig. 3: Pemphigus vulgaris – positive DIF (IgG) at squamous intercellular spaces in a lace-like pattern
In 12.5% cases, DIF deposition was seen at dermo-epidermal junction with squamous intercellular junction in the epidermis. Deposition at both the site was also observed in 13.95% cases by Narsimha Rao et al. Both patients had severe disease and were not responding to therapy. There were also multiple relapses in the past. This dual deposition may be due to coexistence of BP antigens or due to some other antibody to basement membrane antigens. Direct immunofluorescence was negative in one case and disease was less severe with few vesicles. Negative DIF could be due to ongoing steroid therapy, a good prognostic sign for the patient.

**Pemphigus foliaceus**

Out of 41 cases, 3 cases (7.32%) were diagnosed as PF, comparable to the study of Deepti et al (8%) and Jindal et al where 3 cases of PF were reported. All the 3 patients were females. Female preponderance was seen by Shafi et al and Deepti et al. Oral lesion was present in 1 case (33.33%), which was similar to the study of Deepti et al. (25%) and Arya et al, where oral mucosa was involved in 4 out of 25 cases (16%). Involvement of oral mucosa was less in comparison to the study of Shafi et al (47.69%). Nikolsky’s sign was positive in 100% cases. Bulla spread sign was negative in all the cases. Nikolsky’s sign positivity was 50, 75, and 94.7% in the study of Arundhati et al, Deepti et al, and Arya et al respectively.

The H&E stained sections showed subcorneal bulla in all the cases of PF. Bulla cavity contained mild neutrophilic infiltrate and dyskeratotic keratinocytes (Fig. 4). Deepti et al and Arundhati et al also noted subcorneal separation in 100% cases. Predominance of neutrophils in the bulla cavity was also noted by Arya et al. Acantholytic cells were seen in one case (33.33%). This was in discordance with the studies of Deepti et al, Arya et al and Arundhati et al, who reported 100, 93, and 75% acantholytic cells in their study respectively. Our study showed dyskeratotic cells in 100% cases, similar to the study of Deepti et al; it was seen in only 8% cases by Arya et al.

Direct immunofluorescence was done in all the 3 cases of PF and was positive in 1 case (33.33%). Intercellular IgG deposition was seen in upper layer of epidermis (Fig. 5). Positive IgG in epidermal squamous intercellular spaces was also reported by Jindal et al and Arundhati et al. Direct immunofluorescence was negative in 2 cases (66.67%). Negative DIF in PF was also reported by Arundhati et al (25%). Both the patients were referred cases and had already started with treatment. One patient showed remission within 1 week and other did not come for follow-up. Thus negative DIF was a good prognostic indicator.

**Pemphigus erythematous**

Out of 41 cases, 1 case (2.44%) of PE was diagnosed. The incidence was comparable to the studies of Leena et al (2.5%) and Arundhati et al (2.9%). The patient was a 76-year-old female and had vesicles, erosion, and scaly lesions over face and trunk. Similar site predilection and scaly lesions were seen by Leena et al in their study. Vesicles were situated on erythematous base. The present study showed absence of oral lesions similar to the study of Leena et al. Histopathology was inconspicuous as the blister was eroded either during the biopsy procedure or tissue processing. Blister was also eroded in the study of Leena et al. Direct immunofluorescence showed the deposition of IgG at both squamous intercellular spaces and at dermoepidermal junction. The findings were similar to the study of Arundhati et al and Jindal et al; they also observed 100% positivity of IgG in DIF at both squamous intercellular spaces and at dermoepidermal junction. Thus,
the clinical findings of scaly lesions on face and characteristic positive DIF finding helped in arriving at final diagnosis in spite of damage to the blister in paraffin sections (Fig. 6).

Bullous pemphigoid

Bullous pemphigoid was the second most common disease comprising 12 cases (29.27%). The youngest patient was 4-year-old boy and oldest was 84 years male.

Tense blisters were seen in all the 12 cases (100%) of BP. Blisters were situated on erythematous base in 10 cases (83.33%). Similarly an erythematous base was seen in 9 cases (69.9%) in the study of Deepti et al. Tense vesicles on erythematous base were seen in 90.9% cases in the study of Leena et al. Tense vesicles on erythematous base were seen in 90.9% cases in the study of Deepti et al. Involvement of oral mucosa was seen in only 1 case (8.33%) in the present study. The study by Deepti et al and Leena et al also showed similar findings. Arundhati et al reported involvement of oral mucosa in 18.2% cases (Fig. 7).

The H&E stained sections show a subepidermal bulla in all the cases (100%). In 7 cases (58.33%) eosinophils were predominant inflammatory cells in bulla cavity and in 5 cases (41.67%) there were mixed inflammatory infiltrate comprising eosinophils, lymphocytes, and neutrophils (Fig. 8). Comparison of histopathological features of different studies is shown in Table 6.

Direct immunofluorescence was positive in all the 12 cases and showed linear deposition at dermoeipidermal junction. C3 was positive in all the cases (100%) (Fig. 9). Similarly 100% positivity in DIF at dermoeipidermal junction was reported by Jindal et al, Mahmood et al, and Deepti et al. C3 was the most common antibody in all the studies. We also observed C3, IgG, and IgA positive cases were more severe and of longer duration than rest of the cases.

Hailey-Hailey Disease

Three cases (7.32%) were diagnosed as HHD. Arundhati et al7 reported 1 case (1.5%) and Jindal et al8 reported 1 case (1.6%) of HHD in their study of 68 cases and 60 cases of vesiculobullous disease respectively. Incidence of HHD was slightly higher in the present study than in other studies. Two patients were from the same family. Yordanova et al10 and Sangoram et al11 also reported positive family history.

All the patients had characteristic involvement of the intertriginous areas like axilla, groin, and submammary area. No other part of the body or oral mucosa was affected. Similar site of predilection was also seen by Yordanova et al and Sangoram et al. Vesicles and papules were situated on erythematous bases in all the cases (100%). Yordanova et al and Sangoram et al also observed the erythematous base of vesicles and papules.

Table 6: Comparison of histopathological changes in BP

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Histopathological changes</th>
<th>Present study (%)</th>
<th>Deepti et al (%)</th>
<th>Arundhati et al (%)</th>
<th>Leena et al (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of split</td>
<td>Subepidermal</td>
<td>100</td>
<td>92.3</td>
<td>72.3</td>
<td>100</td>
</tr>
<tr>
<td>Bulla content</td>
<td>Eosinophils predominance</td>
<td>58.33</td>
<td>76.4</td>
<td>90.91</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Mixed inflammation</td>
<td>41.67</td>
<td>15.3</td>
<td>9.09</td>
<td>100</td>
</tr>
<tr>
<td>Epidermal changes</td>
<td>Spongiosis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>18</td>
</tr>
<tr>
<td>Dermal infiltrate</td>
<td>Interstitial inflammatory infiltrate</td>
<td>83.33</td>
<td>84.6</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
The H&E stained sections revealed the suprabasal blister, acantholytic cells, villi, dilapidated brick wall in all the 3 cases (100%). Dilapidated brick wall formed due to partial detachment of keratinocytes in the epidermis. Arundhati et al7 also reported villi and acantholytic cells in 100% of the cases. Characteristic “dilapidated brick wall” was also observed by Yordanova et al and Sangoram et al. Direct immunofluorescence was not done in cases of HHD in our study, as DIF is reported negative in all the cases in literature, and diagnosis was based on high clinical suspicion and also the site of involvement being axilla and groin. Histopathological findings of villi, “dilapidated brick wall” like structure of epidermis and absence of inflammatory infiltrate were quite characteristic of HHD (Fig. 10).

**Spongiotic dermatitis**

Two cases (4.88%) were diagnosed as spongiotic dermatitis. Deepti et al4 reported 4 cases (8%) of spongiotic dermatitis in their study of 50 cases of vesiculobullous lesions, which was slightly higher than our study. Blisters were tense and seen on erythematous base in both cases (100%). Trunk was the most common site (100% cases). Similar site of predilection was also noted by Deepti et al. The H&E stained sections showed intraepidermal blister along with spongiosis and lymphocytic infiltrate within the blisters. Dermis also showed the interstitial infiltrate (100% cases). Acantholytic cells were also seen (Fig. 11). This was similar to the findings of Deepti et al, who observed intraepidermal blister and spongiosis in 100% of the cases. However, neutrophils were predominant in their study, suggesting the acute spongiotic dermatitis and in our study lymphocytic predominance suggested the chronic spongiotic dermatitis. Direct immunofluorescence was done to confirm the diagnosis of spongiotic dermatitis. Direct immunofluorescence was negative in both the cases (Fig. 12). Thus negative DIF finding confirms the diagnosis of spongiotic dermatitis and ruled out PE which was given as clinical diagnosis in one case.
Epidermolysis Bullosa Acquisita

Out of 41 cases, 1 case (2.44%) was diagnosed as EBA. The incidence was similar to Leena et al.\(^2\) (2.5%) and Narsimha Rao et al.\(^5\) (3.03%) in their study of 40 and 66 vesiculobullous lesions respectively. The patient was a 48-year-old female and tense vesicle and bulla were seen on bilateral lower extremities following trauma in this study. Previous history of trauma was also present in the case of Leena et al. Some papules were also seen. Base of the blister was erythematous. Bulla spread sign and Nikolsky’s sign both were negative.

Histopathological examination of blister revealed subepidermal bullae with plenty of lymphocytes within blister cavity. Mild acanthosis was also noted. Histopathological diagnosis of BP was made. Leena et al also reported lymphocytes in the blister cavity in case of EBA (Fig. 13). Direct immunofluorescence revealed IgG deposition at dermoepidermal junction. C3 was negative. This was similar to the findings of Narsimha Rao et al.\(^5\); they also reported 100% positivity of IgG in EBA. Previous history of trauma, subepidermal blister with plenty of lymphocytes, and presence of IgG at dermoepidermal junction favors diagnosis of EBA over BP (Fig. 14).

Subcorneal Pustular Dermatoses

One case (2.44%) was diagnosed as SCPD. Similar incidence was seen in the study of Arundhati et al.\(^7\) (2.9%). The patient was a 20-year-old male. He had tense vesicles, pustules, and few crusted lesions over face, upper extremities, lower extremities, and trunk. Oral mucosa was not involved. Vesicles were situated on erythematous base and associated with itching and burning.

The H&E stained sections showed a subcorneal blister with dense infiltrate of neutrophils. Mild acanthosis and few acantholytic cells were seen. Papillary dermis also showed the interstitial and perivascular inflammatory infiltrate. Similar clinical presentation and histopathological findings were seen by Arundhati et al.\(^7\), Velez et al.\(^12\) Direct immunofluorescence was negative in this case. Negative DIF was also reported by Arundhati et al. Blisters in the SCPD are indistinguishable from the blister of PF and IgA pemphigus. Direct immunofluorescence proved to be the only tool to differentiate these disorders.

Lichen Planus Pemphigoid

Out of 41 cases, 1 case (2.44%) was LPP. The patient was a 40-year-old female similar to the findings of Harting et al.\(^13\) Patients had lesions of lichen planus over trunk and lower extremities. Later tense vesicles developed on lower extremities over the lesions of lichen planus and also on normal appearing skin. Oral mucosal involvement was absent. Nikolsky’s sign and Bulla spread sign was negative. Blisters were situated on erythematous base and associated with itching. Similar clinical presentation and site of distribution was seen by Harting and Hsu\(^13\) and Farshchian and Rahmatpour.\(^14\)

The H&E stained sections showed a subepidermal bulla with mixed inflammatory infiltrate of neutrophils and lymphocytes. Eosinophils were not seen. Epidermis was showing mild acanthosis. No lichenoid change of the epidermis and dermis seen. Similar
histopathological findings were seen by Gawakrodger et al. Direct immunofluorescence showed a linear deposition of C3 at dermoepidermal junction. Positive C3 was reported by Harting and Hsu and Farshchian and Rahmatpour.

In this case, more blisters were seen on the lichenoid lesion and few on the normal skin, thus the clinical diagnosis of BLP was made. Histopathological examination was inconspicuous. It could not differentiate between BLP and LPP. Positive DIF finding was essential for the diagnosis of LPP.

**Bullous Lichen Planus**

One case (2.44%) was diagnosed as BLP. The patient was a 67-year-old male and had lesions of lichen planus. Later vesicles developed on normal skin as well as on the lesions of lichen planus. Thus the clinical diagnosis of LPP was made. Vesicles were flaccid and present on the neck, upper extremity, and lower extremity. Oral lesions were present and blisters were seen on nonerythematous skin. Nikolsky’s sign and Bulla spread sign were negative. Itching was present. Similar clinical presentation was also noted by Verma et al and normal skin was also involved in their study.

The H&E stained sections revealed a subepidermal blister and the cavity contained mixed inflammatory infiltrate comprising dense lymphocytic infiltrate along with eosinophils and neutrophils. Dense lymphocytic dermal infiltrate was seen in papillary dermis; however, other changes of lichen planus were not seen. Predominance of lymphocyte was seen by Gawkrodger et al and Verma et al. Direct immunofluorescence was negative. This led to the diagnosis of BLP. Negative DIF was reported by Verma et al and Gawkrodger et al. Histopathology could not reliably distinguish between BLP and LPP, hence DIF was essential for the confirmation of the diagnosis.

**CONCLUSION**

Clinical examination is the first step in diagnosis of vesiculobullous diseases. Histopathological examination and DIF are required for making definitive diagnosis. Direct immunofluorescence is helpful where histopathological features are inconclusive. However, histopathology remains the gold standard in differentiating PV from PF. Direct immunofluorescence from perilesional skin biopsies is helpful in arriving at definitive diagnosis, particularly in cases where there is loss of epidermis of lesional skin for histopathology during biopsy procedure or tissue processing. Hence, clinical histopathological and DIF features are considered together to arrive at final diagnosis, as these methods may not be diagnostic individually in each and every case.

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