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IP Indian Journal of Clinical and Experimental Dermatology

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Original Research Article

A clinicopathological and immunofluorescence study of immunobullous disorders

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Abstract

Aim and Objective: The aim of the study was to evaluate the clinicodemographic profile, histopathology and DIF findings of patients with immunobullous disorders to facilitate diagnostic accuracy and optimal treatment.

Background: Immunobullous disorders are blistering disorders in which autoantibodies are directed against target antigens present in the epidermis and dermo-epidermal junction. With a myriad of presentations, they still remain an enigma. Histopathology and direct immunofluorescence (DIF) play a major role in diagnosing such conditions.

Materials and Methods: A retrospective study was conducted in a tertiary hospital in North Karnataka over a period of 24 months. Data was collected from the hospital medical records department. All clinically diagnosed cases of immunobullous disorders who were subjected to histopathology and direct immunofluorescence were included in the study.

Results: In the present study, among the total 63 cases of immunobullous disorders, the predominant type was pemphigus vulgaris (63.49%), followed by bullous pemphigoid (26.98%), pemphigus foliaceous (6.34%), and one case each of pemphigus vegetans and chronic bullous disorder of childhood. In 85.71% of the cases, histopathology showed suprabasal, subcorneal or sub-epidermal blister. Histopathology findings were consistent with clinical diagnosis in 73.01% cases. Cases with inconclusive biopsy findings were diagnosed using DIF. DIF was diagnostic in majority (90.47%) of patients.

Conclusion: Histopathology with clinical correlation plays a major role in differentiating various subtypes of immunobullous disorders and DIF can be used as a confirmatory tool in inconclusive cases.

Keywords: Immunobullous disorders, Histopathology, Direct immunofluorescence

Received: 08-01-2025; **Accepted:** 24-03-2025; **Available Online**: 26-05-2025

1. Introduction

Immunobullous disorders are characterized by a reaction pattern of vesicles or bullae on the skin or mucous membrane.[1] They are classified as intraepidermal blistering disorders such as pemphigus vulgaris (PV), pemphigus foliaceous (PF), pemphigus erythematosus and subepidermal blistering disorders such as bullous pemphigoid (BP), linear IgA disease, dermatitis herpetiformis, lichen planus pemphigoides, epidermolysis bullosa acquisita and bullous systemic lupus erythematosus.[2] Histopathology is a valuable tool in differentiating the various subtypes. Direct immunofluorescence (DIF) acts as an adjunct when histopathology alone is non-contributory. This study aims to evaluate the clinical profile, histopathology and DIF findings of patients with immunobullous disorders for their diagnostic potential.

2. Materials and Methods

This retrospective study was conducted in the Department of Dermatology of a tertiary hospital in North Karnataka. The study period was for two years from August 2020 to August 2022. Ethical clearance was obtained from the institutional ethical committee. A total of 71 case records of patients who had presented to the outpatient department with immunobullous disorders were audited from the medical

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http://doi.org/: 10.18231/j.ijced.2025.033

records section of our institute. Out of these, 63 patients who had consented to undergo skin biopsy for histopathology and direct immunofluorescence were included in the study. Patients with blistering diseases due to infective, thermal or drug reactions and metabolic disorders were excluded from the study. Detailed history regarding age, gender, presenting complaints, associated symptoms, distribution and morphology of blisters, presence or absence of mucosal involvement and severity of the disease were recorded. Tzanck smear wherever done was observed for the presence of acantholytic cells and nature of inflammatory cells. Histopathological findings such as the level of split and nature of inflammatory cells were noted along with the pattern of IgG, C3, IgA and IgM deposition on DIF. Descriptive data was presented as percentages and frequencies. Data was collected, compiled and analyzed using Microsoft Excel. The results were compared to previous similar studies.

3. Results

A total of 63 cases of immunobullous disorders who presented to the department of dermatology were evaluated in detail. A male preponderance was noted in the study with 35 males (55.55%) as compared to 28 females (44.44%). Maximum number of patients in the study were in the age group of 51-60 years with 16 cases (25.39%), followed by age group of 61-70 years with 11 cases (17.46%). The mean age of the study population was 50.72 years, age ranging from 14 to 86 years. **Figure 1** shows age and gender distribution of the patients. Majority of the cases were that of pemphigus vulgaris with 40 cases (63.49%), followed by bullous pemphigoid with 17 cases (26.98%) and others [**Figure 2**]. It was noted that pemphigus vulgaris was more common in slightly younger patients with mean age of 46.77 years as compared to bullous pemphigoid with mean age of 68.58 years [**Table 1**].

The duration of symptoms ranged from three months to one year in 48 cases (76.19%). All the cases presented with a history of vesicles and bullae on the skin. Active vesicles or bullae were noted in 39 cases (61.9%) at the time of physical examination [**Figure 3**], and the remaining 24 cases (38.09%) presented with secondary lesions such as erosions, crust, plaque or hyperpigmented macules. Pruritus was an associated symptom in 53 cases (84.12%). Trunk was the most common site of involvement in 51 cases (80.95%) followed by extremities in 48 cases (76.19%), mucous membranes in 40 cases (63.49%) and scalp in 13 cases (32.5%). Oral cavity was involved in 31 cases of pemphigus vulgaris (77.5%) and in six cases of bullous pemphigoid (15%). Both flaccid and tense bullae were noted in the patients. Tense bullae were common in patients with bullous pemphigoid. Nikolsky sign was positive in 30 cases of PV (75%) and 2 cases of PF (50%). The mean pemphigus area and activity scoring (PAAS) for cases of pemphigus vulgaris was 3.09. A perifollicular pattern of repigmentation suggestive of bullous pemphigoid was observed in a few cases [**Figure 4**]. Tzanck smear was done in 34 cases (53.96%), out of which 16 cases (47.05%) showed acantholytic cells and the remaining 18 cases (52.94%) showed only inflammatory cells. All cases of bullous pemphigoid who were subjected to Tzanck smear showed eosinophils.

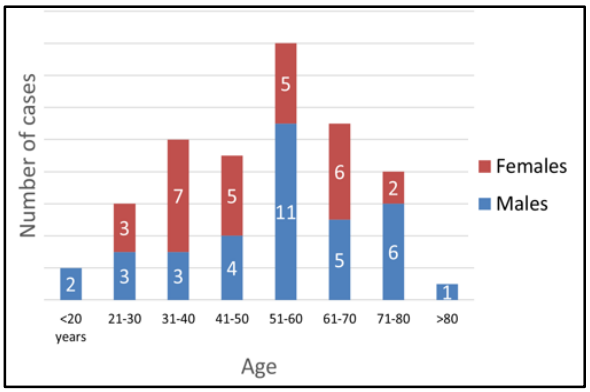

Figure 1: Age and sex distribution of cases

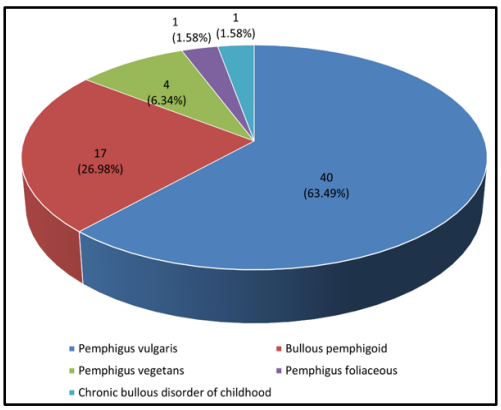

Figure 2: Frequency distribution of various immunobullous disorders

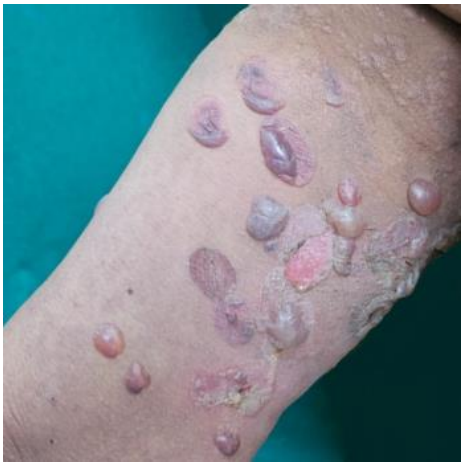

Figure 3: Multiple tense bullae on the thigh of a patient with bullous pemphigoid

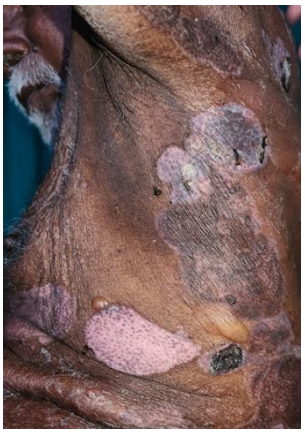

Figure 4: Perifollicular pattern of repigmentation noted in a patient with bullous pemphigoid

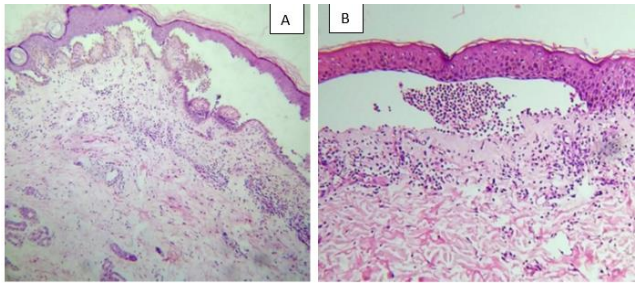

Figure 5: Suprabasal blister with tombstone appearance in pemphigus vulgaris and Subepidermal blister in bullous pemphigoid

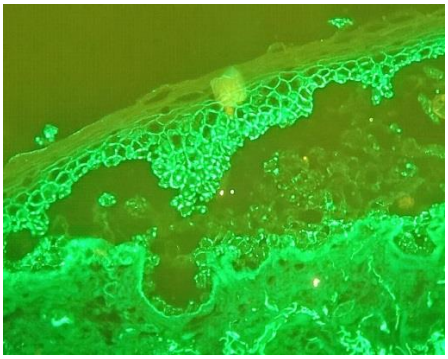

Figure 6: Fishnet or chickenwire pattern of deposition of immunoreactants in pemphigus vulgaris

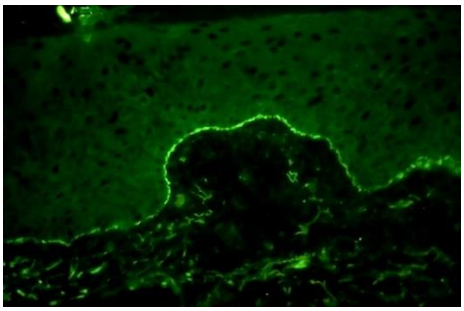

Figure 7: Linear deposition of immunoreactants along dermo-epidermal junction in bullous pemphigoid

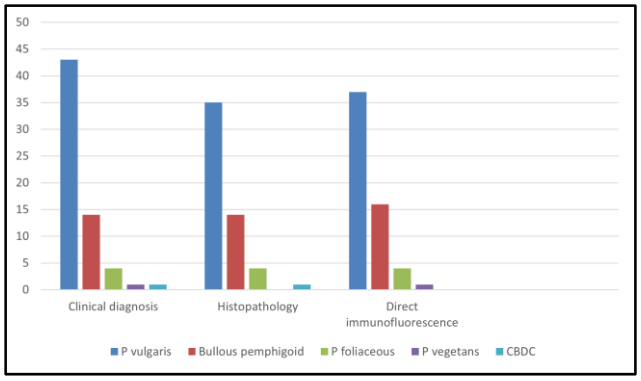

Figure 8: Concordance between clinical, histopathological and direct immunofluorescence based diagnosis

Histopathological evaluation showed suprabasal blister and tombstone appearance in 33 cases (52.38%), subepidermal blister in 15 cases (23.8%) and subcorneal blister in 4 cases (6.34%). Inflammatory cell infiltrates were a common content of the blister in 28 cases (45%). Acantholytic cells were seen in only 12 cases (19.04%). Scattered dermal, perivascular, and adnexal inflammatory infiltrates were seen in almost all cases regardless of the level of blister formation. Lymphocytic infiltrate was found in 61 cases (96.82%), followed by eosinophils in 20 cases (31.74%) and neutrophils in 10 cases (15.87%) [**Table 2**]. Figure 5 shows microscopic pictures of some of the cases seen in the present study. Histopathology findings were consistent with clinical diagnosis in 46 cases (73.01%). However, 9 cases (14.28%) showed inconclusive findings.

Direct immunofluorescence showed 3+ positivity of IgG and C3 along epidermal intercellular spaces in a fishnet or chicken-wire pattern in 40 cases (63.49%) and 3+ linear granular deposition along the dermo-epidermal junction in 15 cases (23.80%) [**Figure 6**, **Figure 7**]. In 3 cases (4.76%), DIF showed deposition in both fishnet as well as linear pattern. DIF was diagnostic in 57 cases (90.47%) in the present study. In 13 cases (20.63%), the diagnosis differed between histopathology and DIF; in such cases the diagnosis given by DIF was considered final. However, DIF showed no deposits in 6 cases (9.52 %), including 3 cases of pemphigus vulgaris and one each of bullous pemphigoid and Chronic bullous disease of childhood. There was one case of clinically diagnosed pemphigus vegetans which showed inconclusive findings on histopathology where DIF showed IgG deposition in fishnet pattern, along with C3 and IgM at dermo-epidermal junction. In two cases of pemphigus vulgaris and one case of bullous pemphigoid, IgA deposition was also noted along with the characteristic IgG, C3 deposits [**Table 3**]. **Figure 8** shows correlation of clinical, histopathological and direct immunofluorescence findings in present study.

Table 1: Age wise distribution of immunobullous disorders

Disease	Number of patients in each age group								Total
	10-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	71-80 years	81-90 years	
Pemphigus vulgaris	-	6 (15%)	8 (20%)	7(17.5%)	13(32.5%)	4(10%)	2(5%)	-	40
Bullous pemphigoid	-	-	-	-	3(17.64%)	7(41.17%)	6(35.29%)	1(5.88%)	17
Pemphigus foliaceous	1(25%)	-	2(50%)	1(25%)	-	-	-	-	4
Pemphigus vegetans	-	-	-	1(100%)	-	-	-	-	1
Chronic bullous disorder of childhood	1(100%)	-	-	-	-	-	-	-	1

Table 2: Distribution of histopathological features among various vesiculobullous lesions

Variable	Category	Pemphigus vulgaris	Bullous pemphigoid	Pemphigus foliaceous	Pemphigus vegetans	Chronic bullous disorder of childhood
Level of split	Suprabasal	34 (85%)	1 (5.88%)	-	-	-
	Subepidermal	-	14 (82.35%)	-	-	1 (100%)
	Subcorneal	-	-	4 (100%)	-	-
Epidermal changes	Row of tombstone	34 (85%)	-	-	-	-
	Acantholytic cell	12 (30%)	-	-	-	-
Dermal infiltrate	Lymphocytes	38 (95%)	17 (100%)	4 (100%)	1(100%)	1(100%)
	Eosinophils	3 (7.5%)	17 (100%)	-	-	-
	Neutrophils	7 (17.5%)	1 (5.88%)	1 (25%)	-	1 (100%)

Table 3: Distribution of antibody deposition and its pattern using DIF among vesiculobullous lesions

Variable	Category	Pemphigus vulgaris	Bullous pemphigoid	Pemphigus foliaceous	Pemphigus vegetans	Chronic bullous disorder of childhood
Antibody deposited	IgG + C3	36 (90%)	16 (94.11%)	4 (100%)	1 (100%)	-
	IgA	2 (5%)	1 (5.88%)	-	-	-
	IgM	-	-	-	1 (100%)	-
	Negative	3 (7.5%)	1 (5.88%)	1 (25%)	-	1 (100%)
Pattern of deposition	Fishnet	36 (90%)	1 (5.88%)	4 (100%)	1 (100%)	-
	Linear along dermo-epidermal junction	-	15 (88.23%)	-	-	-

Table 4: Analysis of types of immunobullous disorders

Disease	Present study	Gupta et al.[1] (2022)	Patel et al.[23] (2022)	Daniel et al.[4] (2020)	Mittal et al.[7] (2017)	Chanabasayya et al.[11] (2017)
Pemphigus vulgaris	63.49%	32.35%	30.3%	38	48.18%	18.68%
Bullous pemphigoid	26.98%	17.2%	13.63%	31	27.27%	46.15%
Pemphigus foliaceous	6.34%	4.3%	24.24%	7	3.63%	10.98%
Pemphigus vegetans	1.58%	-	-	-	-	-
Chronic bullous disorder of childhood	1.58%	-	-	-	-	-

4. Discussion

Immunobullous disorders are a heterogenous group of disorders characterized by antibodies against target antigens on the epidermis and dermo-epidermal junction.[3] They present with a reaction pattern consisting of vesicles and bullae. Based on the level of split, they can be classified as intraepidermal and subepidermal blistering disorders. A good clinical acumen helps us identify these disorders. However, many of them mimic each other clinically, therefore it is essential to have adjunct methods for their diagnosis. Biopsy is considered to be the gold standard for diagnosis of immunobullous disorders.[4] Due to their high sensitivity it describes the plane of separation and also the mechanism of blister formation based on the nature of inflammatory infiltrate.[5] DIF is a one-step procedure that involves application of fluorescent antibodies to a frozen section of the perilesional skin. Direct immunofluorescence studies have now become an invaluable supplement to clinical and histological examination in a variety of dermatological diseases including immunobullous disorders.[6]

In the present study, the maximum number of patients were in the age group of 51-60 years (25.39%), followed by 61-70 years (17.46%). The mean age of patients was 50.72 years, consistent with the studies of Daniel et al. (51.88 ± 32.16 years) and Chowdhury et al.(49.48 ± 16.51 years),[4,7] but not consistent with a study done by Kabir et al. where mean age was 35.1 years.[8] Pemphigus vulgaris was most common in the age group of 51-60 years. This is in accordance with the observations of Chanabasayya et al. and Kudligi et al.[9,10] However, studies by Rokde et al. observed maximum number of PV cases between 40-49 years.[11] Bullous pemphigoid was common in older individuals with maximum number being in the age group of 61-70 years, comparable to studies done by Kudligi et al. and Kushtagi et al.[10,12] This observation conforms to literature where BP is found more commonly in an older age group compared to PV.[13] Males were affected more than females in a male to female ratio of 1.25:1, similar to findings of studies by Mohanty et al. and Madhavi et al.[14,15] This is in contrast to literature and some studies by Khursheed et al. and Pavani et al. showing female preponderance.[5,16] This discrepancy could be due to greater number of males presenting to our institute.

The most predominant type of immunobullous disorder observed in the present study was pemphigus vulgaris followed by bullous pemphigoid. This pattern of distribution was similar to various other studies including those by Deepti et al., Selvaraj et al. and Gupta et al.[1,17,18] However, a study done by Wong et al. in Singapore observed bullous pemphigoid as the most common group.[19] This could be due to the regional variations in prevalence of various immunobullous disorders. Oral cavity was involved in 77.5% cases of pemphigus vulgaris and 15% cases of bullous pemphigoid, compared to 64.2% involvement in PV and 10% involvement in BP according to Mittal et al.[20] Another noteworthy finding was the oral cavity involvement in 50% cases of pemphigus foliaceous in the present study, since according to literature, oral cavity is rarely seen to be involved in PF.[21] Thus presence of oral lesions cannot rule out a diagnosis of pemphigus foliaceous. Nikolsky sign was positive in 75% cases of PV and 50% cases of PF. This rate is comparable to the study by Arundhati et al. with Nikolsky positivity in 84.6% cases of PV and in 50% cases of PF.[22]

Histopathological evaluation showed suprabasal cleft and tombstone appearance in 33 out of 43 clinically diagnosed cases of PV (76.74%). Additionally, two cases which were diagnosed clinically as BP showed suprabasal cleft. Acantholytic cells were seen only in 12 cases out of these. Biopsy findings were inconclusive in 6 cases of PV. Out of 14 cases that were clinically diagnosed as BP, 10 showed subepidermal blister (71.42%). Four cases which were diagnosed clinically as PV were later diagnosed histologically as BP. One case of BP showed non-specific features on histopathology. In the present study, Histopathology findings were consistent with clinical diagnosis in 73.01% cases compared to 89.24% concordance in a study by Gupta et al. and 96% concordance in another study by Kudligi et al.[10] This discrepancy could have arisen due to the heterogeneous clinical presentations of immunobullous disorders.

Direct immunofluorescence showed fishnet pattern of IgG and C3 deposits in 35 cases (81.39%) and negative findings in three cases out of 43 clinically diagnosed cases of PV. In addition, one clinically diagnosed case of BP showed a fishnet pattern. Out of the 14 clinically diagnosed cases of BP, 12 cases showed linear IgG and C3 deposits along the

dermo-epidermal junction (85.71%) and one case showed negative findings. Additionally, four cases that were clinically diagnosed as PV showed linear deposits on DIF. DIF was diagnostic in 90.47% cases and DIF findings were consistent with clinical features in 85.71% cases. Concordance between histopathological and DIF findings was 76.19% against a value of 89% observed by Kudligi et al.[10] This finding highlights the need for direct immunofluorescence in accurate diagnosis of immunobullous disorders, since histopathology alone can give dubious results in some cases.[23]

Limitations of the study include a low sample size, inability to perform salt splitting and non-availability of indirect immunofluorescence to further confirm the subtyping.

5. Conclusion

The study shows that it is ideal to perform skin biopsy in all cases of immunobullous disorders for its diagnosis. Although histopathology is considered gold standard for the diagnosis, it may not be diagnostic individually in every case, which further curtails optimal treatment and a good prognosis. Hence it is recommended to perform a direct immunofluorescence test which plays a crucial role in arriving at a final diagnosis in cases where histopathology gives inconsistent results.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Cite this article: Krishna S, Sreedharan AV, Naveen KN, Agnihotri A, Prabhu SR. A clinicopathological and immunofluorescence study of immunobullous disorders. *IP Indian J Clin Exp Dermatol*. 2025;11(2):202-207.