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Direct Immunofluorescence in Cutaneous Vesiculobullous Lesions: A Cross-sectional Study in a Tertiary Care Center

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Abstract

Introduction: Cutaneous noninfectious autoimmune vesiculobullous diseases are a wide variety of lesion. They can show clinical and histopathological overlap, posing diagnostic challenges. Clinically, vesiculobullous lesions might not present with typical morphology and distribution in all cases. The specific histomorphological changes are observed only in an early and intact bulla. Immunofluorescence (IF) is a histochemical staining technique which detects antibodies in the tissue and body fluids. IF an adjuvant to histopathology is considered as gold standard in the diagnosis of vesiculobullous diseases. **Methodology**: A two-year retrospective cross-sectional descriptive study was conducted in the department of pathology, in a tertiary care center from January 2018 to December 2019. Paraffin embedded hematoxylin and eosin slides were retrieved from the archives and analyzed. Biopsies received in Michel's medium were utilized for immunofluorescence study. **Results**: A total of 67 cases with Vesiculobullous lesions were analysed. The most common lesion diagnosed was Bullous Pemphigoid (BP) (29 cases -46.7%), followed by Pemphigus Vulgaris (PV) (19 cases- 30.6%). The highest number of cases were noted in the 7th decade, with a slight female preponderance. Sixteen out of 19 cases of pemphigus vulgaris and 28 out of 29 cases of bullous pemphigoid showed positive histomorphological correlation with IF. The PPV for pemphigus vulgaris was 100% and sensitivity was 84.2%, and in bullous pemphigoid, both the PPV and sensitivity was 96.5%. Higher concordance was noted between Histopathology and IF diagnosis in cases of BP in comparison to PV. **Conclusion**: Vesiculobullous lesions are heterogeneous in nature and can have clinical and histopathological overlap, posing diagnostic challenges. IF is considered gold standard and is necessary for the diagnosis in every case. A combined clinical, histopathological and IF examination is the best approach to accurate diagnosis.

Keywords: Bullous; Pemphigus; Vesiculobullous; Direct Immunofluorescence

1 Introduction

Cutaneous noninfectious autoimmune vesiculobullous diseases are a wide variety of Lesions.[1] They can show clinical and histopathological overlap, posing diagnostic challenges. Clinically, vesiculobullous lesions might not present with typical morphology and distribution in all cases[2].

The specific histomorphological changes are observed only in an early and intact bulla. Immunofluorescence (IF) is a histochemical staining technique which detects antibodies in the tissue and body fluids[1]. IF an adjuvant to histopathology is considered as gold standard in the diagnosis of vesiculobullous diseases[3]. The incidence of vesiculobullous lesions varies from 0.5-3.2 cases/100,000 population/year[4]. They are categorized into inherited or autoimmune subepidermal blistering diseases and acantholytic diseases[5]. While the exact etiology of these disorders is not known, an HLA association is with DRB10402 and DQ0503 in pemphigus vulgaris (PV).[6] Clinically they are one of the most common reaction patterns of skin reaction. Itching and burning sensation of are common symptoms, vesicles, bullae and erosion and crusting are the common signs observed[2]. Vesicles are lesions less than 0.5 cm in diameter and bullae are greater than 0.5 cm[6].

In histomorphology, there are various planes of blister formation including subcorneal, suprabasal, subepidermal, interspinous and dermal depending on the antigen involved[3]. The pathogenic mechanism involved is the binding of antibodies to the antigen, altering the desmosomal function and resulting in spongiosis, acantholysis, basement membrane zone destruction and blister formation. The nature of inflammatory infiltrate is different in different vesiculobullous lesions making it diagnostically significant, but ageing of lesion, superadded infections and treatment can alter the inflammatory response.[3]

Immunofluorescence (IF) was first used in the 1940s by Coons to demonstrate the microorganisms in infected tissue. Later in 1964 by Beutner and Jordan, to demonstrate antibodies in pemphigus patients[5]. Since then, it has been extensively used autoimmune blistering disorders of the skin. Direct Immunofluorescence (DIF) is more commonly used and involves identification of tissue bound auto-antibodies. Indirect Immunofluorescence (IIF) on the other hand demonstrates circulation antibodies in a patient's sera. Diagnosis in DIF is made based on the specific patterns of immune complex deposition and nature of antibody (IgG, IgA, IgM, and C3c). Vesiculobullous diseases can be extremely debilitating with serious sequelae, and even fatal, necessitating their early and proper diagnosis.[7]

1.1 Objective of the study

1. To study the clinicopathological features of non-infectious vesiculobullous lesions.
2. To correlate the clinical and histopathological features of the skin lesions and to confirm with Direct immunofluorescence (DIF) findings.

2 Materials and Methods

A two-year descriptive retrospective time bound study was conducted in the department of pathology, in a tertiary care center from January 2018 to December 2019. The institutional scientific and ethical committee clearance was obtained. All the biopsy specimens from patients with clinically diagnosed vesiculobullous lesions with adequately filled requisition forms were included in the study.

All the skin biopsies that were fixed in 10% Buffered Formalin and tissues were routinely processed, slides stained with hematoxylin and eosin were retrieved and studied. Biopsy received in Michel's medium used for immunofluorescence

study. Vesiculobullous lesions due to infectious etiology or other non- immune etiology such as Inherited disorders, metabolic disorders, arthropod bite reaction, trauma and dermal acute inflammatory process were excluded from the study.

Histopathological diagnosis was made based on the level of separation of the lesion, inflammatory infiltrate, features of the epidermal keratinocytes such as presence of acantholysis. Immunofluorescence diagnosis was made by taking into consideration the type of antibody (IgG, IgM, IgA, or C3), site (intercellular, basement membrane) and pattern of deposition (linear, granular, or fishnet).

3 Results

Total of 67 cases with Vesiculobullous lesions were analyzed during the study period. In our study the age ranged from 13 to 91 years with a mean age of 54.2 years and the largest number of cases were noted in the 7th decade followed by 5th decade (Figure 1), with a slight female preponderance with Male:Female ratio being 1:1.3.

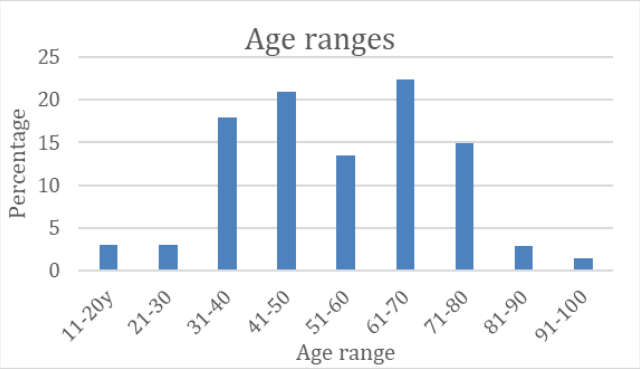

Fig 1. Distribution of cases according to age

Patients presented with itching, fluid filled lesions and erosion. The signs observed were tense and flaccid bullae, vesicle, crusted plaque, papule, pustule, and erosion. Only oral mucocutaneous lesions were noted in 4 cases.

Cases of BP mostly presented with tense bullae (69%), followed by flaccid bullae (15%) or vesicles (11%) while cases of PV showed vesicles (31.2%), flaccid bullae (25%), 18% presented only with oral lesions, other lesions seen were erosion and crusted plaques. Oral mucocutaneous lesions were present in most (89%) patients of PV and some 20% of patients of BP and 1 patient presented with subcorneal pustular dermatosis.

The distribution of the lesions according to the site of involvement is shown in Table 1. Almost all cases of BP, 92% showed a subepidermal bulla, and the remaining 8% there was absence of bullae. Most cases 87.5% cases of PV showed suprabasal bulla and 12% showed intraepithelial bullae. Cases of pemphigus foliaceus (PF) show a subcorneal bulla and rarely an intraepithelial bulla. Cases of linear IGA (IGA) mostly showed subepidermal bullae.

Table 1. Table showing distribution of lesion on clinical examination

Distribution of lesions	Frequency (n=67)	Percentage (%)
Trunk	32	48.4
Upper Limb	17	25.7
Generalised	6	9.09
Lower limb	5	7.5
Oral lesions	4	6.06
SCALP	1	1.5
FACE	1	1.5
Total	**66**	**100%**

Eosinophilic inflammatory infiltrate was seen in 89% of cases of BP, while neutrophils were the most common (48%) inflammatory infiltrate in PV, cases with Linear IgA and PF also showed predominantly a neutrophilic inflammatory infiltrate (Table 2).

Other significant histopathological findings include acantholytic cells in 25% of cases in PV and row of tombstone appearance in 36% cases of PV.

The DIF pattern of deposition of immune antibodies is shown in (Table 3). Almost all cases, 96.5% of BP and 89% that is majority of PV cases showed characteristic IF patterns.

Discordance between clinical and histopathological diagnosis were seen in 14 cases. Discordance between histopathology and IF diagnosis were seen in 13 cases (Table 4).

4 Discussion

Clinically, vesiculobullous lesions might not present with typical morphology and distribution in all cases[2]. Variations in presentation are seen depending on duration, severity, presence of superadded infection and previous treatment. The specific histomorphological changes are observed only in an early and intact bulla. These lesions are immune mediated with immune-complex deposition at various locations including dermoepidermal junction, intraepidermal, dermal blood vessels etc. They can be detected by Direct immunofluorescence (DIF) test for tissue-bound autoantibodies.

The most common age group of patients in the study was 7th decade and mean age 54.2 this was similar to other studies. Study by Karattuthazhathu et al. showed the most common age group being 46-60 years[3] Pavani D et al. showed the common age group as 61-70 years and mean age 47.7 years[7]. Handa F et al. on the other hand showed prevalence of pemphigus in younger age group[8]. There was a slight female preponderance seen in our study which was in concordance to studies done by Karattuthazhathu et al.[3], Pavani D et al.[7],

Table 2. Table showing type of inflammatory infiltrate in various lesions

Histopatho- logical diagnosis	Eosinophils	Neutrophils	Lymphocytes	No infiltrate	Total
BP	25 (89%)	0	2 (7%)	1 (3%)	28 (100%)
PV	0	8 (50%)	1 (6%)	7 (43%)	16 (100%)
DH	100%	0	0	0	1 (100%)
HH	0	100%	0	0	1 (100%)
IgA	0	100%	0	0	3 (100%)

Table 3. Showing the antibody deposition in immunofluorescence

IF positivity	Ig G	Ig A	Ig M	C 3	Total
PV	17 (89.5%)	3 (15.8%)	2 (10.5%)	15 (79%)	19 (100%)
BP	28 (96.5%)	3 (10.3%)	1 (3.4%)	28 (96.5%)	29 (100%)
PF	4 (100%)	0	0	3 (75%)	4 (100%)
IgA	0	2 (100%)	0	1 (50%)	2 (100%)
PE	2 (100%)	0	1 (50%)	1 (50%)	2 (100%)

Table 4. Table showing discordance between clinical, histopathological and immunofluorescence findings

Disease	Clinical Impression	Histopathological Diagnosis	DIF Diagnosis
PV	20 (30.3%)	16 (27.5.8%)	19 (32.7%)
BP	33 (50%)	28 (48.2%)	29 (50%)
PF	5 (7.5%)	6 (10.3%)	4 (6.89%)
IgA	2 (3.03%)	3 (5.17%)	2 (3.4%)
DH	1 (1.5%)	1 (1.72%)	0
HH	2 (3.03%)	2 (3.4%)	0
PE	2 (3.03%)	0	2 (3.4%)
SPD	0	1 (1.72%)	0
BLP	1 (1.5%)	1 (1.72%)	1 (1.72%)
P VEGETENCE	1 (1.5%)	1 (1.72%)	1 (1.72%)
Total	66 (100%)	58* (100%)	58* (100%)

* 7 Cases had no histopathological evidence of the vesiculo- bullous disease and IF was negative.
Bullous pemphigoid (BP), *Pemphigus vulgaris* (PV), *Pemphigus foliaceus* (PF) linear IgA disease (IGA) and *Pemphigus erythematosus* (PE), haileyhailey (HH) disease, dermatitis herpetiformis (DH), superficial pustular dermatosis (SPD), bullous lichen planus (BLP), *Pemphigus vegetans* (PV).

Deepti SP et al.[9] and Arundhati S et al.[10].

Majority of the bullous pemphigoid cases presented with tense bullae (Figure 2).

Fig 2. Bullous pemphigoid clinical picture

Majority of *Pemphigus vulgaris* cases presented with flaccid bullae and oral lesions (Figure 3).

Trunk and upper limbs are the most common site involved. This was similar to the findings in study by Bhat R M et al.[2]. On the other hand, Deepti SP et al.[9] and Kumar A et al.[11] showed generalized involvement of the body. Oral mucocutaneous lesions were present in most (89%) patients of PV and some 20% of patients of BP. Many studies have shown the association between oral lesions and *Pemphigus*

Fig 3. *Pemphigus vulgaris* clinical image

vulgaris[3,7,7,10,11].

Our study showed BP as the most common lesion identified, this was similar to finding by Pavani et al.[7] and Thejasvi et al.[12]. But most other studies showed PV as the most common lesion[3,7,10,11].

On histomorphology most cases of BP showed subepidermal bullae with eosinophilic inflammatory infiltrate (Figure 4).

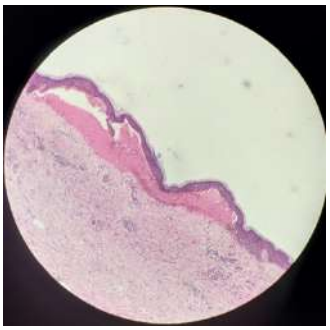

Fig 4. Bullous pemphigoid 10x (H & E)

PV showed a suprabasal bulla with a mixed inflammatory infiltrate (Figure 5).

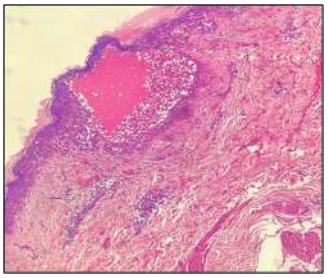

Fig 5. *Pemphigus vulgaris* with a suprabasal bulla 10x (H & E)

IgA disease showed a subepithelial plane of separation (Figure 6), and HH disease showed suprabasal bulla and acantholysis giving a 'dilapidated brick wall' appearance.

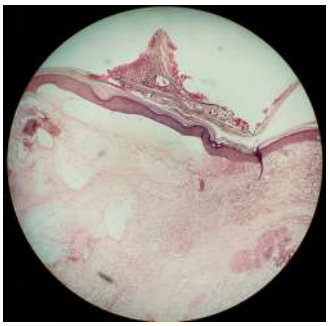

Fig 6. Linear IgA disease, 10 x, H & E

In immunofluorescence, most cases of BP showed linear IgG, C3 deposition along the basement membrane zone (BMZ) (Figure 7).

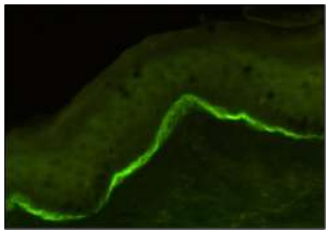

Fig 7. DIF showing linear deposition of IgG along the basement membrane (10x)

PV, PF, P vegetans, showed an intercellular IgG, C3 deposition giving a fishnet appearance (Figures 8 and 9).

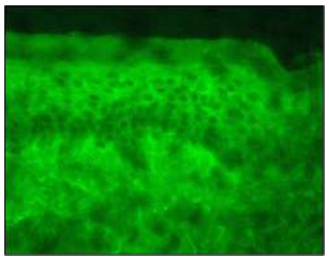

Fig 8. DIF with inter cellular deposition of IgG in a fishnet pattern (10x)

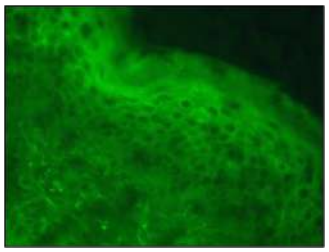

Fig 9. DIF with inter cellular deposition of C3 in a fishnet pattern (10x)

Cases of PF appear similar to PV with sparing of basal layers of the epidermis. IGA disease showed linear IgA deposition along the BMZ (Figure 10).

Our study showed 16 out of 19 cases of pemphigus and 28 out of 29 cases of bullous pemphigoid showed positive histopathology correlation with IF. The positive predictive value (PPV) and sensitivity of histopathology was calculated. The PPV for pemphigus vulgaris was 100% and sensitivity was 84.2%. and in bullous pemphigoid, both the PPV and sensitivity was 96.5%.

In the current study a higher concordance between Histopathology and IF diagnosis in cases of BP group

Table 7. Distribution of lesions seen in various studies

Lesions	Pavani et al. [4]	Deepti et al. [5]	Kumar et al. [8]	Thejasvi et al. [11]	Present study
BP	38%	26%	34%	21.6%	46.7%
PV	23%	34%	31%	18.9%	30.6%
PF	2.3%	8%	-	8.1%	6.4%
IGA	4.7%	-	-	2.7%	3.2%
DH	-	4%	-	8.1%	1.6%
HH	-	-	-	1.3%	2.6%
PE	-	-	-	1.3%	3.25%
SPD	-	8%	2.8%	2.7%	1.6%
BLP	-	-	-	-	1.6%
P VEGETANS	-	-	2.8%	2.7%	1.6%

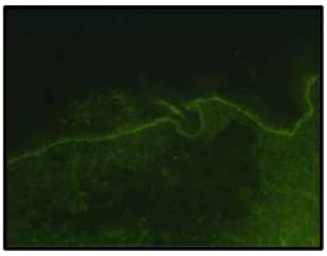

Fig 10. DIF with linear deposition of IgA along the basement membrane (100x)

compared to PV group was observed compared to previous studies (Tables 5, 6 and 7).

Table 5. Analysis of histopathology and Immunofluorescence in Pemphigus group

Disease	Immunofluorescence positive	Immunofluorescence negative	Total
HP positive	16	0	16
HP negative	3	1	4
TOTAL	19	1	20

Positive predictive value, 16/16=100%, sensitivity 16/19=84.2%

Table 6. Analysis of histopathology and Immunofluorescence in Bullous pemphigoid

Disease	Immunofluorescence positive	Immunofluorescence negative	Total
HP positive	28	1	29
HP negative	1	4	5
TOTAL	29	5	35

Positive predictive value, 28/29=96.5%, sensitivity 28/29=96.5%

Karattuthazhathu et al. reported PPV and sensitivity of 93.7% and 90.09% respectively in pemphigus vulgaris and 93.3% PPV and 77.7% sensitivity in Bullous pemphigoid[3]. Sensitivity of histopathology reported by a study by Fatma et al. are 95.83% for the pemphigus group and 92.85% for the pemphigoid group[6]. Bhat R et al. showed 39 out of 50 cases (78%) showed a positive correlation between clinical, histopathological and IF findings. In remaining cases the clinical diagnosis was discordant from the histopathological and IF diagnosis[2].

While histopathological features are extremely important and is as good as DIF in making a diagnosis (Tables 5 and 6), DIF is highly sensitive and can additionally help in differentiating nonimmune causes from immune causes making it necessary for definitive diagnosis.

5 Conclusion

Vesiculobullous lesions are a rare autoimmune disease. For cases with challenging clinical and histopathology diagnosis, Immunofluorescence studies should be considered to arrive at a final conclusive diagnosis. Histopathology provides valuable information and IF is an adjuvant that supplements the histopathology diagnosis. With IF, nonimmune causes of bullous lesions can accurately be distinguished from immune causes. A combined clinical, histopathological and IF examination is the best approach to accurate diagnosis.

References

1) Raman S, Sirka CS. A two year retrospective study of direct immunofluorescence in autoimmune vesiculobullous lesions with clinical correlation. *National Journal of Laboratory Medicine*. 2019;8(4):PO17–PO21. Available from: https://njlm.net/articles/PDF/2368/42247_CE[Ra1]_F(SHU)_PF1(AG_SHU)_PFA(SHU)_PB(AG_SHU)_PN(SHU).pdf.
2) Chanabasayya V, Jyothi J, Jacintha M, Sukumar D. A retrospective study of the clinical, histopathological, and direct immunofluorescence spectrum of immunobullous disorders. *Egyptian Journal of Dermatology and Venereology*. 2017;37(2):62–68. Available from: https://doi.org/10.4103/ejdv.ejdv_3_17.
3) Karattuthazhathu AR, Vilasiniamma L, Poothiode U. A Study of Vesiculobullous Lesions of Skin. *National Journal of Laboratory Medicine*. 2018;7(1):PO01–PO06. Available from: https://www.njlm.net/articles/PDF/2266/29611_CE[VSU]_F(AK)_PF1(VSU_GG)_PFA(GG)_PF2(VSU_GG).pdf.
4) Mysorekar VV, Sumathy TK, Prasad ALS. Role of direct immunofluorescence in dermatological disorders. *Indian Dermatology Online Journal*. 2015;6(3):172–180. Available from: https://doi.org/10.4103/2229-5178.156386.
5) Shetty VM, Subramaniam K, Rao R. Utility of immunofluorescence in dermatology. *Indian Dermatology Online Journal*. 2017;8(1):1–8. Available from: https://doi.org/10.4103/2229-5178.198774.
6) Fatma S, Narla S, Jacob S, Geetha N. Correlation of Histology of Vesiculobullous Disorders With Immunofluorescence: A Study at A Tertiary Care center. *National Journal of Laboratory Medicine*. 2018;7(4):PO28–PO33. Available from: https://www.njlm.net/articles/PDF/2319/36312_CE[Ra1]_F(SHU)_PF1(A_SHU)_PFA(SHU)_PB(A_SHU)_PN(SHU).pdf.
7) Pavani M, Harika P, Deshpande AK. Clinicopathological study of vesiculobullous lesions of the skin and the diagnostic utility of immunofluorescence. *International Journal of Clinical and Diagnostic Pathology*. 2020;3(1, Part D):252–257. Available from: https://doi.org/10.33545/pathol.2020.v3.i1d.183.
8) Handa F, Agarwal RR, Kumar R. A clinical study of 85 cases of Pemphigus. *Indian Journal of Dermatology, Venereology and Leprology*. 1973;39:106–111. Available from: https://ijdvl.com/a-clinical-study-of-85-case-of-pemphigus/.
9) Deepti SP, Sulakshana MS, Manjunatha YA, Jayaprakash HT. A histomorphological study of bullous lesions of skin with special reference to immunofluorescence. *International Journal of Current Research and Academic Review*. 2015;3(3):29–51. Available from: http://www.ijcrar.com/vol-3-3/S.%20P.Deepti,%20et%20al.pdf.
10) Arundhathi S, Raghunatha S, Mahadeva KC. A Cross-sectional study of clinical, histopathological and direct immunofluorescence spectrum of vesiculobullous disorders. *Journal of Clinical and Diagnostic Research*. 2013;7(12):2788–2792. Available from: https://doi.org/10.7860/JCDR/2013/7019.3760.
11) Kumar A, Shreya AS, Baig MA. Study of vesiculobullous lesions of the skin clinical and histopathology correlation. *Journal of Diagnostic Pathology and Oncology*. 2018;3(3):214–218. Available from: https://doi.org/10.18231/2581-3706.2018.0044.
12) Murthy TK, Shivarudrappa AS, Biligi DS, Shubhashree MN. Histopathological Study of Vesiculobullous lesions of skin. *International Journal of Biological & Medical Research*. 2015;6(2):4966–4972. Available from: https://www.biomedscidirect.com/1888/histopathological-study-of-vesiculobullous-lesions-of-skin.