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A STUDY OF ROLE OF DIRECT IMMUNOFLUORESCENCE IN DIAGNOSIS OF CUTANEOUS VESICULOBULLOUS DISORDER AND ITS HISTOPATHOLOGICAL CORRELATION -A STUDY OF 30 CASES

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ABSTRACT

Background

Autoantibodies against antigens in intercellular substance or dermo-epidermal junction results in potentially debilitating cutaneous vesiculobullous disorder. As there is clinical overlap among this group, histopathological examination helps to generate differential diagnosis based on site of vesicle, presence, intensity and composition of inflammatory infiltrate. While direct immunofluorescence (DIF) is an expensive advance test which particularly contributes in diagnosis when histopathological findings are inconclusive.

Aim

To evaluate role of histopathological and direct immunofluorescence study in diagnosis of cutaneous vesiculobullous disorder.

Method

Present study is an analytical study of 30 clinically suspected cases of cutaneous vesiculobullous disorder carried out at Department of Pathology, S.S.G Hospital and Medical College, Baroda over a period of 1 year. Two biopsies were taken from each patient. One lesional skin biopsy for histopathological examination and one perilesional skin biopsy for direct immunofluorescence test. Findings of both were correlated and a final diagnosis for each patient was arrived.

Results

Out of 30 cases, pemphigus vulgaris was the most common with total of 13 cases (43.3%) followed by bullous pemphigoid 9 cases (30%), Dermatitis herpetiformis 3 cases (10%), pemphigus foliaceous 2 cases (6.7%), Linear IgA Dermatosis 2 cases (6.7%) and Cicatricial pemphigoid 1 case (3.3%). DIF test results of 29 cases concurred with histopathological findings. One case was negative for DIF test.

Conclusion

Histopathological examination and Direct Immunofluorescence both are required for making a definitive diagnosis of vesiculobullous lesions having overlapping and diverse presentation. DIF is particularly important when histopathological findings are non-diagnostic or non-confirmatory.

Keywords: Direct Immunofluorescence (DIF), Vesiculobullous Disorder.

INTRODUCTION

Autoimmune cutaneous vesiculobullous disorder is caused by autoantibody production against intercellular substances or antigens on dermo-epidermal junction resulting in varying clinical manifestations showing great overlap.[1,2] They result in significant mortality and morbidity, hence definitive diagnosis is required for treatment.[3,4]

Histopathological evaluation is a common and important examination ideally to be done on early lesions which helps to generate differentials based on plane of separation, presence of inflammatory infiltrate and its composition.[5] In past few years there is a great advance in diagnostic dermatology. Immunofluorescence though is an advanced test available in advanced laboratory but has gained a great popularity in recent years. As immune mediated bullous lesions elicit a disease specific immunopathogenic pattern, hence results of direct immunofluorescence contributes in diagnosis, treatment and prognosis by detecting early relapse.[6,7]

The present study was undertaken to evaluate role of both direct immunofluorescence and histopathology individually in diagnosis of cutaneous vesiculobullous disorder as well as their correlation.

MATERIALS AND METHOD

The present study is the time bound analytical study carried out at Department of Pathology, S.S.G Hospital and Medical College, Baroda, Gujarat, India for a period of 1 year in collaboration with Toprani Advance Laboratory, Vadodara for Direct Immunofluorescence test.

A total of 30 cases were studied. The patients with clinical suspicion of vesiculobullous skin lesions visiting outpatient department of Dermatology of S.S.G Hospital that require histopathological examination and direct immunofluorescence for definite diagnosis were included in the study. After counselling of patient a lesional skin biopsy was taken for histopathological examination which was received at histopathological section of S.S.G Hospital and was kept in 10% neutral buffered formalin for fixation. Another biopsy was taken from perilesional normal looking skin for direct immunofluorescence which was sent in Michel's medium at Toprani Advance Laboratory, Vadodara.

For histopathological analysis formalin fixed lesional skin biopsy was processed in tissue processor. Paraffin embedded block was made followed by 3-4 micron thick sections were cut with the semiautomated microtome. This slide was stained with Hematoxylin and Eosin stain and mounted with DPX with coverslip. The slide was examined under light microscope and findings were noted.

For Direct immunofluorescence after receiving biopsy in Michel's media at Toprani Lab, tissue was placed in cryostat and section of 4-6 micron thickness were taken on Poly-L-Lysin coated glass slides, fixed with ether alcohol mixture and air dried. The sections were than treated with Fluorescein Isothiocyanate (FITC) labelled and optimally diluted antisera (IgG, IgM, IgA and C3). The slides were incubated in wet chamber for 1 hr in dark room, washed with phosphate buffer and mounted with buffered glycerol. The slides were examined under fluorescent microscope at wavelength of 340-400 nm and the type and pattern of immunoreaction was noted.

The findings of both histopathological examination and direct immunofluorescence were noted and correlated. A final diagnosis was arrived for each case.

RESULTS

A total of 30 clinically suspected cases of vesiculobullous disorder were studied. Out of 30 cases, pemphigus vulgaris was the most common disorder diagnosed with total of 13 cases (43.3%) followed by bullous pemphigoid with 9 cases (30%). Rest cases were distributed as Dermatitis herpetiformis 3 cases (10%), pemphigus foliaceous 2 cases (6.7%), Linear IgA Dermatosis 2 cases (6.7%) and Cicatricial pemphigoid 1 case (3.3%). Among the study group age ranged from 5 years to 85 years. Youngest patient was of 5 years old diagnosed with Linear IgA Dermatosis (childhood type) while the

oldest patient was of 85 years diagnosed with Bullous Pemphigoid. Slight male predominance was seen with Male to Female ratio of 1.1:1.

Distribution of various histopathological features among various vesiculobullous disorder is shown in Table 1. Majority cases of pemphigus vulgaris showed suprabasal blister followed by intraepidermal blister. All cases of pemphigus foliaceous showed subcorneal blister. While all other cases of bullous pemphigoid, cicatricial pemphigoid, linear IgA dermatosis and Dermatitis herpetiformis showed subepidermal blisters. Predominant inflammatory infiltrate was neutrophils in pemphigus vulgaris cases and eosinophils in bullous pemphigoid cases. Majority of cases of pemphigus vulgaris showed Tombstone appearance. Dermal changes i.e dermal, perivascular and peri adnexal inflammation was seen in almost all cases.

Distribution of type of antibody deposition and its pattern seen on DIF among various vesiculobullous disorder is shown in Table 2. Of total 30 cases, 29 cases (96.6%) gave DIF results positive while only 1 case (3.3%) of bullous pemphigoid showed no deposit of antibody i.e DIF was negative. The most common antibody deposited in pemphigus vulgaris, pemphigus foliaceous, bullous pemphigoid and cicatricial pemphigoid was combination of IgG+C3. IgA deposition was seen in all cases of dermatitis herpetiformis and linear IgA dermatosis. All the cases of pemphigus vulgaris and pemphigus foliaceous showed antibody deposition in squamous intracellular space. 8 cases of bullous pemphigoid and all cases of cicatricial pemphigoid, linear IgA dermatosis and dermatitis herpetiformis showed antibody deposition in basement membrane zone.

Variable	**Category**	**PV (N=13)**	**BP (N=9)**	**PF (N=2)**	**DH (N=3)**	**LAD (N=2)**	**CP (N=1)**
Epidermal Changes	Tombstone appearance	9 (69.2%)	-	-	-	-	-
	Hyperkeratosis	2 (15.3%)	-	-	-	-	-
	Acanthosis	2 (15.3%)	-	1 (50%)	-	-	1 (100%)
	Acantholytic cells	11 (84.6%)	3 (100%)	2 (100%)	-	1 (50%)	1 (100%)
Dermal Changes	Dermal inflammation	12 (92.3%)	7 (77.7%)	2 (100%)	3 (100%)	2 (100%)	1 (100%)
	Peri adnexal inflammation	6 (46.1%)	4 (44.4%)	2 (100%)	1 (33.3%)	-	1 (100%)
	Perivascular inflammation	5 (38.4%)	6 (66.6%)	1 (50%)	2 (66.6%)	1 (50%)	1 (100%)
Level of blister	Intraepidermal	4 (30.7%)	-	-	-	-	-
	Suprabasal	9 (69.2%)	-	-	-	-	-
	Subcorneal	-	-	2 (100%)	-	-	-
	Subepidermal	-	9 (100%)	-	3 (100%)	2 (100%)	1 (100%)

Table 1: Distribution of various histopathological features among various vesiculobullous disorder

Abbreviations: PV- Pemphigus Vulgaris, BP- Bullous Pemphigoid, PF- Pemphigus Foliaceous, DH- Dermatitis Herpetiformis, LAD- Linear IgA Dermatosis, CP- Cicatricial Pemphigoid

Variable	**Category**	**PV (N=13)**	**BP (N=9)**	**PF (N=2)**	**DH (N=3)**	**LAD (N=2)**	**CP (N=1)**
Type of Antibody deposited	IgG+C3	8 (61%)	7 (78%)	1 (50%)	-	-	1 (100%)
	IgG	4 (31%)	-	1 (50%)	-	-	-
	IgA+C3	-	-	-	-	1 (50%)	-
	IgA	-	-	-	2 (67%)	-	-

	IgG+C3+IgA	1 (8%)	1 (11%)	-	1 (33%)	1 (50%)	-
	No deposit	-	1 (11%)	-	-	-	-
Pattern of Antibody Deposition	Dot like in Squamous intercellular space	3 (23%)	-	-	-	-	-
	Lace like in Squamous intercellular space	10 (77%)	-	2 (100%)	-	-	-
	Granular in Basement membrane zone	-	-	-	3 (100%)	-	-
	Linear in Basement membrane zone	-	8 (89%)	-	-	2 (100%)	1 (100%)

Table 2: Distribution of type of antibody deposition and its pattern seen on DIF among various vesiculobullous disorder

Abbreviations: PV- Pemphigus Vulgaris, BP- Bullous Pemphigoid, PF- Pemphigus Foliaceous, DH- Dermatitis Herpetiformis, LAD- Linear IgA Dermatosis, CP- Cicatricial Pemphigoid, Ig- Immunoglobulin, C3- Complement Factor Three, DIF- Direct Immunofluorescence

Pemphigus Vulgaris

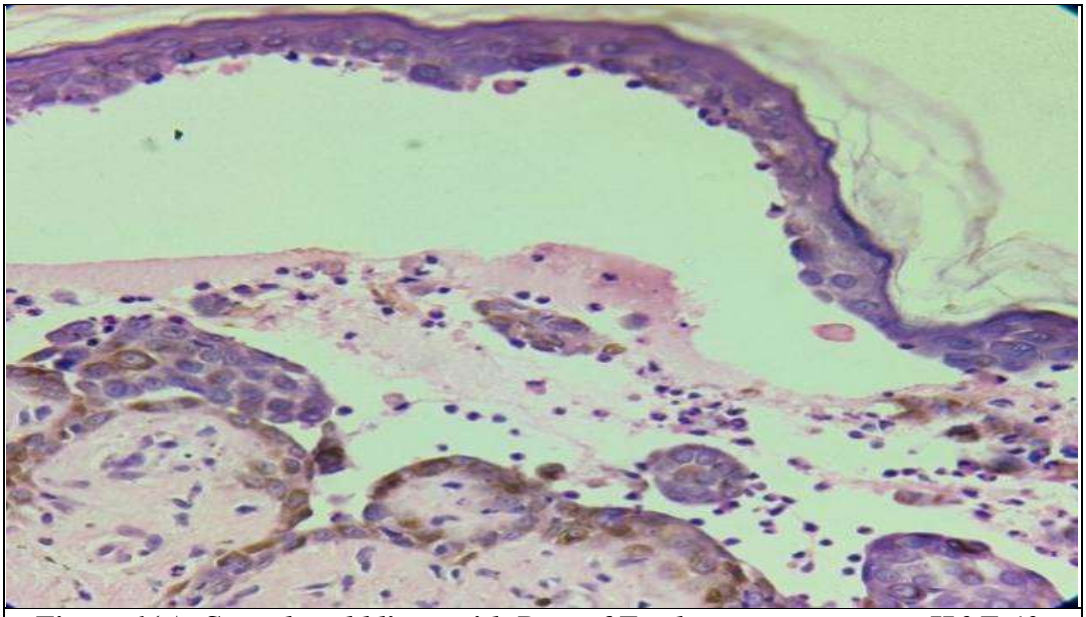

Figure 1(a): Suprabasal blister with Row of Tombstone appearance H&E 40x

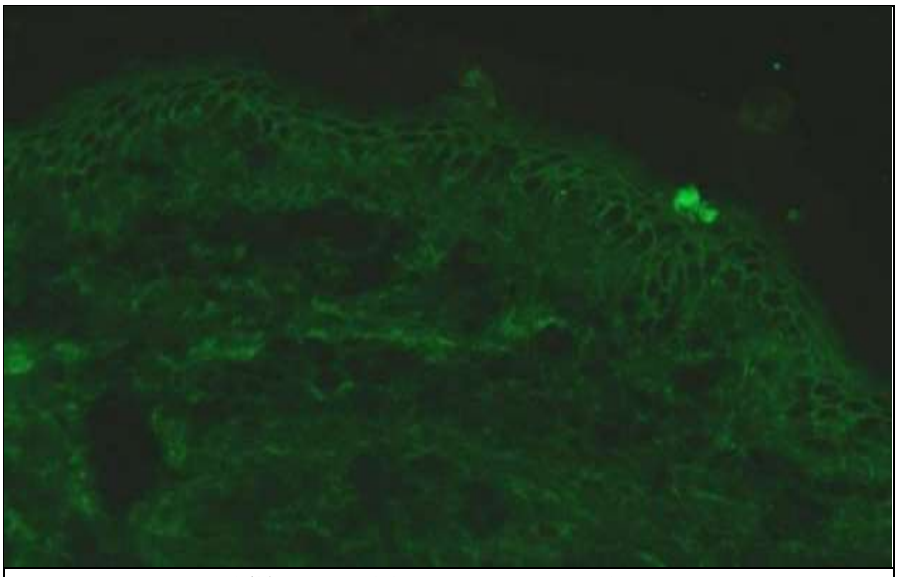

Figure 1(b): Lace like pattern of IgG on DIF 40x

Bullous Pemphigoid

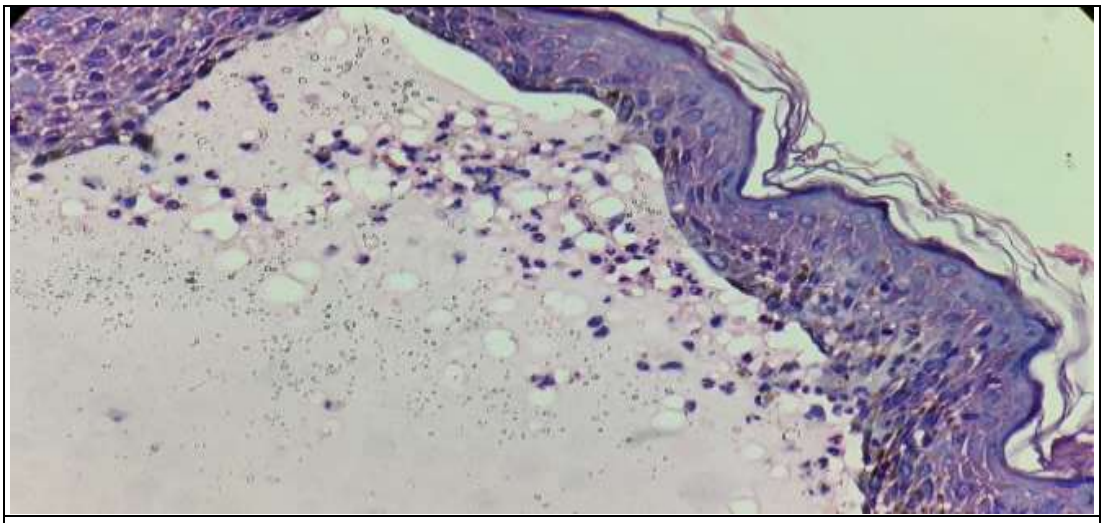

Figure 2(a): Subepidermal blister with neutrophils & eosinophils H&E 40x

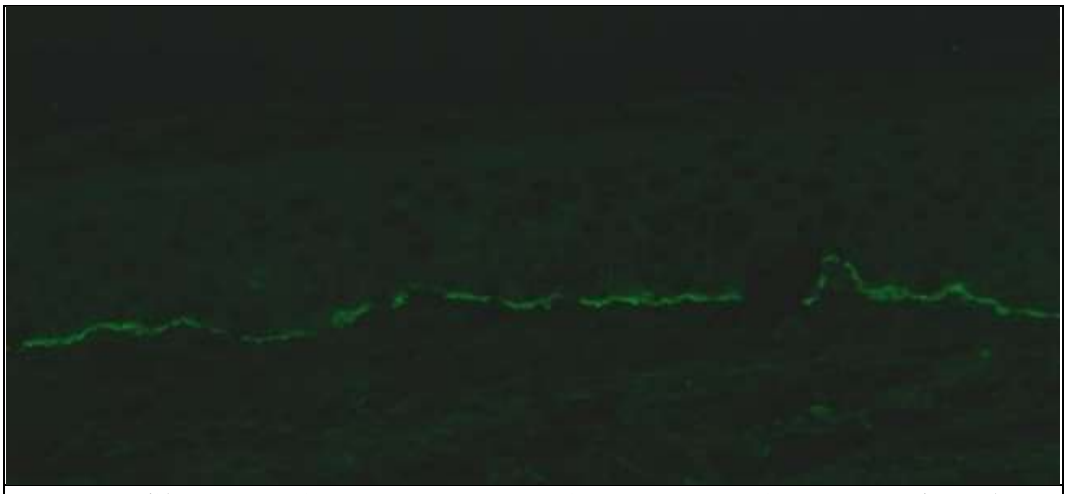

Figure 2(b): Linear deposition of IgG in basement membrane zone (BMZ) on DIF 40x

Cicatricial Pemphigoid

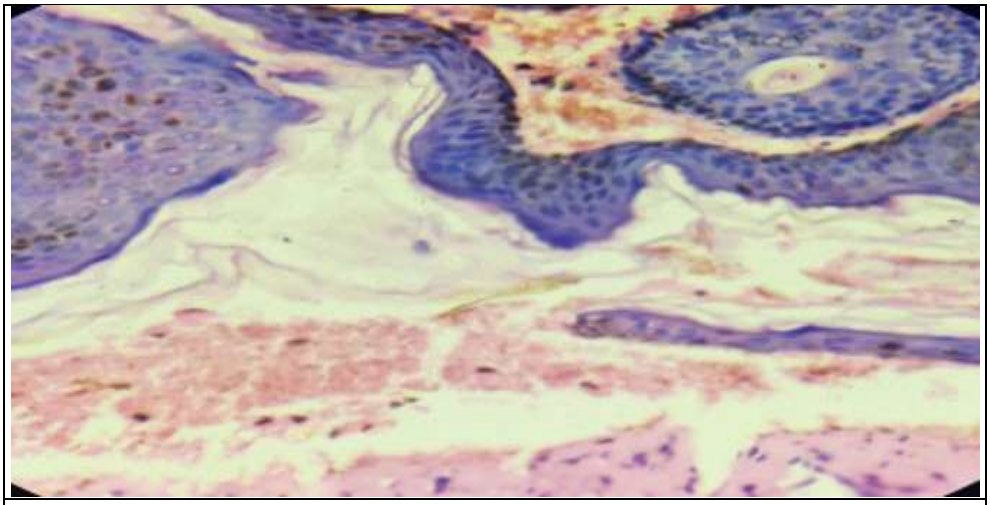

Figure 3(a): Subepidermal blister with dermal fibrosis H&E 40x

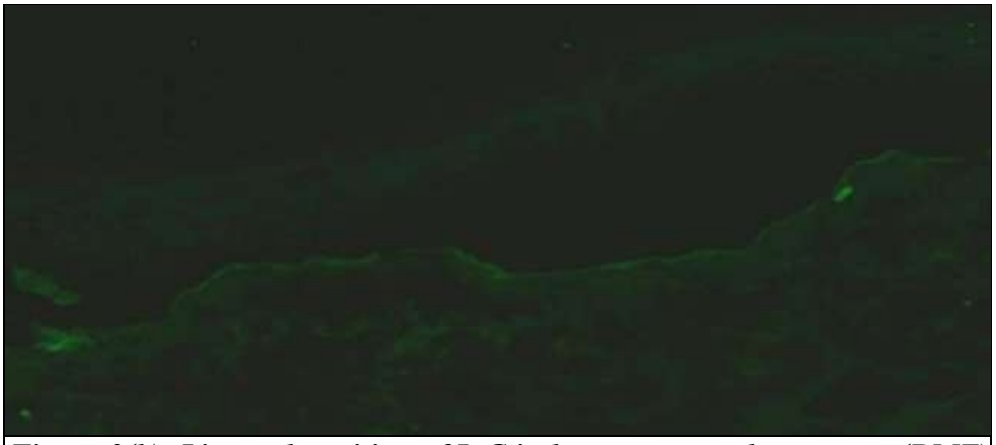

Figure 3(b): Linear deposition of IgG in basement membrane zone (BMZ) on DIF 40x

DISCUSSION

Vesiculobullous disorders are diagnostically challenging. They may show overlapping clinical and histopathological features. Accurate diagnosis is very essential for reducing overall morbidity and mortality by providing proper targeted treatment to patients with vesiculobullous lesions. Past few decades have shown a great advance in dermatopathology, thereby introducing new advance techniques like immunofluorescence which provides a great diagnostic help especially in cases where clinical and histopathological findings are overlapping or nondiagnostic or inconclusive. No particular test is 100% diagnostic, hence correlation of clinical, histopathological findings and direct immunofluorescence (DIF) proves to be more diagnostically helpful. The present study was conducted with the same aim.

In present study Pemphigus vulgaris was the most common diagnosis with 13 cases (43.3%) followed by bullous pemphigoid with 9 cases (30%). This findings were in concordance with Arundhati S. et al,[6] Shushruta Mohenty et al,[8] Ahmed et al,[9] Sarwat Fatma et al[10] and Kumar SS et al[11] studies. In our study 1 case of cicatricial pemphigoid was identified which was not seen in other studies. Overall patients in our study presented in majority in 51-70 years of age group which showed concordance with Anupama Raj Karattuthazhathu et al,[12] Kumar SS et al and Sarwat Fatma et al studies. In our study male to female ratio was 1.1:1 showing slight male predominance. This finding shows concordance with Shushruta Mohenty et al, Gautum Goyel et al[1] and Kumar SS et al studies. Among the pemphigus group, about 69.2% of pemphigus vulgaris cases showed suprabasal blisters which showed concordance with Sarwat Fatma et al. Predominantly inflammatory cells in the blister are neutrophils similar to Shushruta Mohenty et al. Histopathology was diagnostic in 12 cases out of 13 and direct immunofluorescence was positive in 100% cases. This is in concordance with Sarwat Fatma et al. 100% cases of pemphigus foliaceous showed subcorneal blisters. DIF was positive in 100% of cases. Overall the findings showed concordance with Arundhati S. et al and Shushruta Mohenty et al. Histopathology plays a very important role in diagnosis of pemphigus foliaceous and pemphigus vulgaris as both the entities can be differentiated only by the level of blisters.

Among the pemphigoid group 100% cases of bullous pemphigoid showed subepidermal blisters with eosinophils as the most common inflammatory infiltrate same as Shushruta Mohenty et al. DIF showed concordance with Histopathology in 88.8% cases. DIF finding was negative in one case. This can be justified as DIF is done on the separate biopsy, hence there can be error in selection of biopsy site or technical error. Patients treatment status also affects DIF results. Otherwise both DIF and histopathology are equally helpful in diagnosis of bullous pemphigoid. 100% cases of dermatitis herpetiformis were diagnostic on histopathological examination and DIF study which showed concordance with Sarwat Fatma et al. Out of two clinically suspected cases of linear IgA dermatosis (Childhood type) or chronic bullous disorder of childhood one case was non confirmatory on

histopathology but showed linear IgA deposition along the basement membrane on DIF. Hence DIF was helpful here.

Out of 30 cases, only 1 case was DIF negative. Hence in rest 29 cases histological diagnosis concurred with the direct immunofluorescence findings. Therefore 96.6% concordance was seen between histopathology and DIF diagnosis. DIF was helpful in diagnosis of a case of pemphigus vulgaris and linear IgA dermatosis (childhood type) where histopathological examination was non confirmatory.

CONCLUSION

Clinical examination is the initial step in making a diagnosis of vesiculobullous disorders. Histopathological examination and DIF are required for making a definitive diagnosis of vesiculobullous lesions having overlapping and diverse presentation. DIF being the most definitive diagnostic modality for vesiculobullous lesions can be negative at times. In addition to diagnosis, DIF also aids in monitoring of response to therapy predicting relapse. Hence DIF can be used as a supplement and not a substitute. In comparison to DIF, histopathology remains the cornerstone in differentiating pemphigus vulgaris from pemphigus foliaceous as DIF findings are similar in both cases. Hence no method is gold standard. 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. It becomes important to distinguish each of these entities and separate them for appropriate management.

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