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Original Article

Pathology Section

Immunobullous Disorder: A Diagnostic Dilemma Faced at a Tertiary Care Centre in Kanyakumari, Tamil Nadu, India

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ABSTRACT

Introduction: Immunobullous disorders are morphologically heterogenous and hence the differentiation between various subtypes is essential for proper treatment and prognosis. Prompt diagnosis needs clinicopathological concordance added with Immunofluorescence (IF) in particular Direct Immunofluorescence (DIF) study to avoid discrepancies.

Aim: To study the clinicopathological profile along with immunological features and to analyse the utility of IF in particular DIF of immunobullous disorders.

Materials and Methods: The present study was a single institution based cross-sectional observational hospital study done in the department of pathology in SreeMookambika Institute of Medical Sciences, Tamil Nadu over a period of one year (July 2019-August 2020) involving 70 outpatients in the department of dermatology with clinical evidence of bullous disorders. Two biopsies were taken from the patient, one from a newly formed bulla or vesicle for Haematoxylin and Eosin (H&E) stain and the other from a perilesional normal looking skin for DIF study. Statistical analysis was done by using SPSS software. Chi-square test was used for statistical analysis. A p-value of less than 0.05 was considered as statistically significant. However, the final diagnosis was arrived after considering clinical, bedside investigations, histopathology and DIF study.

Results: Out of seventy (70) patients included in this study, the most common age group of distribution was between 51-60 years in a frequency of 22 (31.4%). Out of 70 patients, 39 patients were diagnosed with intraepidermal bullous disorders and 31 patients were diagnosed with subepidermal bullous disorders. The most common disorder diagnosed was based on clinical findings was Pemphigus Vulgaris (PV) 31 (44.3%) followed by Bullous Pemphigoid (BP) 23 (32.9%). The common disorder diagnosed based on histopathology was PV 33 (47.1%). Clinical and histopathological concordance was conclusive in 31 cases of PV. The percentage of diagnosis of DIF was positive in 67 (95.71%). Patients which reached to inconclusive (DIF was not definite) diagnosis was in only three patients.

Conclusion: Since, in most of the cases there occurs an overlap in clinical and histopathological features, IF in particular, DIF plays a sensitive tool in confirming diagnosis as well as distinguishing immunobullous disorders from other disorders.

Keywords: Autoimmune, Direct immunoflorescence, Histopathology, Pemphigus

INTRODUCTION

Autoimmune bullous disorders are uncommon dermatological disorders, caused by autoantibodies. Their incidence ranging from 0.5-3.2 cases/1,00,000/year [1]. Due to their rare diversity and polymorphism, these disorders pose a diagnostic challenge, hence, it is essential to arrive at the diagnosis rapidly and plan the treatment accordingly [2].

Dermatopathology, as described by Sitaru C and Zillikens D, makes a keystone for modern dermatology as well as Immunofluorescence (IF) [3]. Lever WF has differentiated pemphigoid from pemphigus in his studies of histopathology [4]. Beutner E et al., made a combined effort in their studies on IF [5].

In addition, Direct Immunofluorescence (DIF) along with histopathology can be a supplement in the diagnosis of a variety of other conditions such as connective tissue disorders, vasculitis, lichen Planus, amyloidosis and psoriasis. DIF is more sensitive than Indirect Immunofluorescence (IIF) in patients on clinical remission and is valuable for detection of the immunological activity of the disease [5].

The present study was based on the clinical heterogeneity of immunobullous disorders, with a goal of understanding the demographic pattern and to predict the clinicopathological concordance of immunobullous disorders with respect to DIF in SreeMookambika Institute of Medical Sciences,Tamil Nadu.

MATERIALS AND METHODS

This single institution based cross-sectional observational study was conducted in the department of pathology in coordination with the department of dermatology, SreeMookambika institute of Medical Sciences, Tamil Nadu over a period of one year (July 2019-August 2020). A total of 70 cases (studied with people attending dermatology OPD having strong clinical evidence of immunobullous disorders).

Inclusion criteria:

1. Both male and female patients with clinical features of bulla, vesicle, erosions/suspected to be immunobullous disorder.
2. Patients who were willing to give consent for biopsy.

Exclusion criteria:

1. Both male and female patients who are already a known case of immunobullous disorder and are on treatment.
2. Pregnant and lactating women with clinical evidence of immunobullous disorder.
3. Patients not giving consent to participate in the study.

Approval from the institutional ethical committee- SMIMS/IHEC NO-58/2019 was obtained.

Study Procedure

The patients with clinical evidence of Bulla, vesicle, erosions were admitted in dermatology ward and a detailed case history of each patient was recorded. Bedside investigations such as Tzanck test for acantholytic cells, Nikolsky sign, Bullaspread sign were done. Clinical photographs were also taken.

A test dose of Inj. Lignocaine was given and patient was observed for any allergic reaction. If no allergic reaction was observed patient

was subjected to biopsy. The procedure of skin biopsy was explained and informed consent was taken from the patients.

Two separate biopsies were taken from the patient, one from a newly formed bulla or vesicle for H&E stain and the other sample from perilesional normal looking skin for DIF study. The sample was transported to a nearby IF centre in Michel's transport medium for DIF. DIF study was done using 5 primary antibodies derived from mice (anticytokeratin 5,14, antilaminin 332, anticollagen 7, 4). Anti-IgG antibody conjugated with Fluorescein Isothiocyanate (FITC) was used as a secondary antibody (Millipore). Control slides were examined separately. Slides were viewed under IF microscope. Results were interpreted and recorded in the proforma.

Based on 4 parameters- 1. Primary site of deposition of immunoreactant; 2. Type of immunoreactant; 3. Number of immunoreactant; 4. Site of deposition other than primary

STATISTICAL ANALYSIS

Results were evaluated and data was drawn in microsoft excel. Descriptive analysis of known data was done using SPSS software version 20.0. Chi-square test was done for checking statistical significance. A p-value <0.05 was considered statistically significant.

RESULTS

Among the total study population of 70 patients, the mean age group of distribution was 52.41 years (14-80 years) as mentioned in [Table/Fig-1].

Age group (years)	Frequency	Percentage (%)
<30	7	10.0
31-40	8	11.4
41-50	15	21.4
51-60	22	31.4
61-70	13	18.6
>70	5	7.1
Total	70	100

[Table/Fig-1]: Age wise distribution.

In this study, males 36 (51.42%) were more commonly affected than females 34 (48.75%). The mean duration of illness at the time of presentation was 1-3 months.

The most commonly presenting lesions was bulla 63(90%) followed by erosions 62 (88.6%) in order of frequency [Table/Fig-2].

Lesion	Present	Absent	Total
Bulla	63 (90%)	7 (10%)	70
Erosions	62 (88.6%)	8 (11.4%)	70

[Table/Fig-2]: Disorder distribution.

Trunk 67 (95.7%) was the most common site of presentation, followed by upper and lower limbs 64 (91.4%). Oral mucosal involvement was seen in the form of erosions in 31 (44.3%), followed by genital mucosal involvement in 12% of patients. Nail involvement was seen in 18 (25.7%) of patients. The common nail abnormality was subungual hyperkeratosis 6 (8.6%) followed by onycholysis 5 (7.1%).

Under laboratory parameters, the most common finding was anaemia 28 (40%) [Table/Fig-3].

Investigations	Frequency	Percentage (%)
Anaemia	28	40
Raised urea and creatinine	6	8.50
High ESR	2	2.8
Others*	34	48.57
Total	70	100

[Table/Fig-3]: Laboratory parameters.
*does not fit into above investigations

Tzanck smear was positive for acantholytic cells in 31 (44.3%) of patients. Indirect Nikolsky sign was positive in 35 (50%) of patients, Direct Nikolsky sign was positive in 34 (48.6%) of patients. Bulla spread sign was positive in 66 (94.27%) of patients [Table/Fig-4].

Indirect/direct	Frequency	Percentage (%)
Positive	35/34	50/48.6
Negative	35/36	50/51.4
Total	70	100

[Table/Fig-4]: Indirect/Direct Nikolsky sign.

Most common bullous disorder based on clinical diagnosis was PV followed by BP [Table/Fig-5].

Clinical diagnosis	Frequency	Percentage
Pemphigus Vulgaris (PV)	31	44.3
Bullous Pemphigoid (BP)	23	32.9
Bullous SLE	3	4.3
Pemphigus Foliaceus (PF)	2	2.9
Pemphigus Erythematosus	1	1.4
IgA pemphigus	1	1.4
Inconclusive	9	12.9
Total	70	100

[Table/Fig-5]: Clinical data.

The most common comorbid disease associated was type 2 diabetes mellitus 19 (27.10%) followed by systemic hypertension 14 (20.0%) cases [Table/Fig-6].

Comorbidities	Frequency	Percentage
Type 2 DM	19	27.1
Systemic hypertension	14	20.0
SLE	3	4.3
Latent syphilis	1	1.4
Dyslipidemia	1	1.4
Systemic HT/type 2 DM	13	18.6
Nil	18	25.7

[Table/Fig-6]: Comorbidities.

The most common disorder diagnosed by histopathology was PV. Clinical and histopathological concordance was evaluated [Table/Fig-7].

Clinical diagnosis	Histopathological inference						Chi-square p-value
	Bullous pemphigoid	Pemphigus vulgaris	Pemphigus foliaceus	Pemphigus erythematosus	Inconclusive	Total	
Bullous pemphigoid	23	0	0	0	0	23	0.0001
Pemphigus vulgaris	0	31	0	0	0	31	
Bullous SLE	0	0	0	0	3	3	
Pemphigus foliaceus	0	0	2	0	0	2	
Pemphigus erythematosus	0	0	0	1	0	1	
IgA pemphigus	0	0	0	0	1	1	
Inconclusive	4	2	1	0	2	9	
Total	27	33	3	1	6	70	

[Table/Fig-7]: Clinical and histopathological concordance.

C3 was found to be positive in about 34 (48.56%), IgG in about 52 (74.28%) and IgA in about 1 (1.42%) patient [Table/Fig-8].

The histopathological findings specific to diagnosis is mentioned in [Table/Fig-9].

Pattern	C3	IgG	IgA
Linear deposition along DEJ	26/37.14%	21/30%	1/1.42%
Fishnet pattern along Intercellular Space (ICS)	8/11.42%	31/44.28%	-
Negative	36/51.42%	18/25.71%	69/98.57%

[Table/Fig-8]: DIF findings.

HPE specific to diagnosis	Frequency
Pemphigus Vulgaris (PV)	33
Bullous Pemphigoid (BP)	27
Pemphigus Foliaceus (PF)	3
Pemphigus erythematosus	1
Inconclusive	6
Total	70

[Table/Fig-9]: Histopathological data.

The histopathological findings with regard to DIF has been concorded as follows [Table/Fig-10].

HPE and DIF	Frequency
Pemphigus Vulgaris (PV)	34
Bullous Pemphigoid (BP)	27
Bullous SLE	2
Pemphigus Foliaceus (PF)	3
Pemphigus erythematosus	1
Inconclusive	3
Total	70

[Table/Fig-10]: Histopathology and DIF concordance.

DIF is considered to be the gold standard in diagnosing immunobullous disorders. The concordance between histopathology and DIF findings were shown as in [Table/Fig-11].

Method	Conclusive diagnosis	Inconclusive diagnosis	Percentage of diagnosis (%)
Histopathology specific to diagnosis	64	6	91.4%
Direct Immunofluorescence (DIF)	67	3	95.71%

[Table/Fig-11]: The concordance between histopathology and DIF findings.

Among the total 70 cases, six cases were inconclusive with regard to histopathology specific to diagnosis and three cases showed negative DIF testing. DIF testing along with clinical and histopathological findings were reliable in arriving at a reliable diagnosis in 95.71% of patients (though none of the method gave 100% diagnosis).

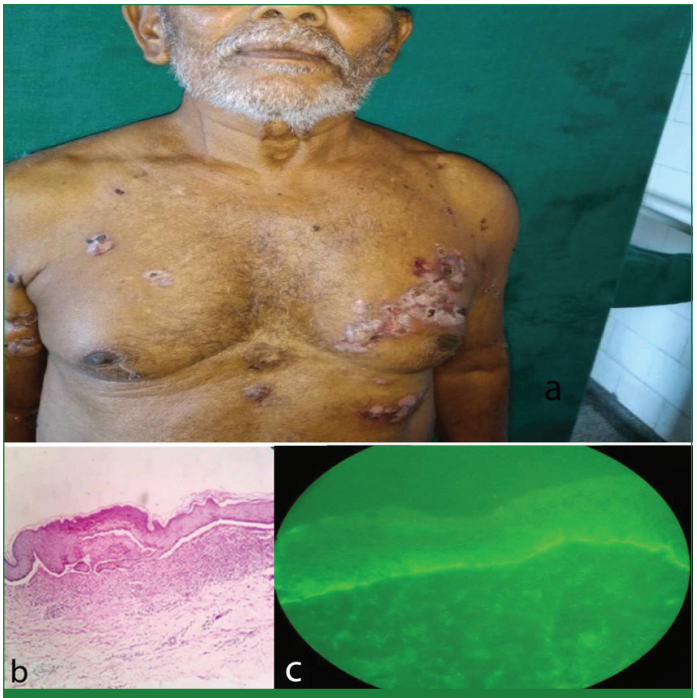
Below we have discussed few cases in which the histopath diagnosis was confirmed by DIF.

Case 1: A 52-year-old male patient clinically presented with extensive erosions presenting all over the trunk [Table/Fig-12a].

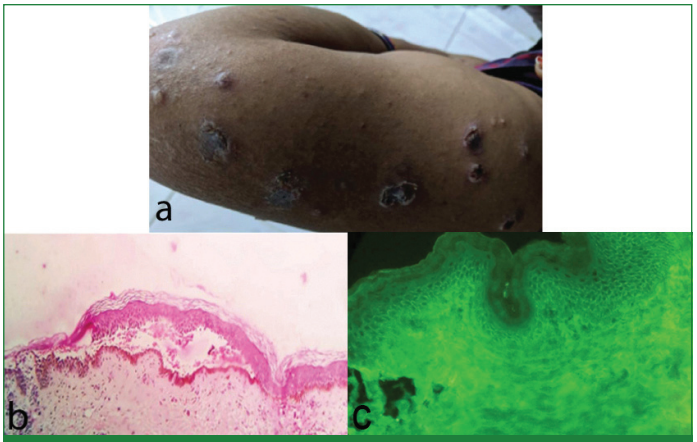
H&E of the same showed split formation in the subepidermis consisting of eosinophils and fibrin deposition which was confirmed by DIF which shows linear deposition of IgG over dermoepidermal junction suggestive of BP [Table/Fig-12b,c].

Case 2: A 32-year-old male patient clinically presented with numerous bulla and erosions all over the upper extremities [Table/Fig-13a]. Confirmation of the same was done by H&E section of skin showing supra bulla formation with a 'row of tomb stone appearance. DIF showing fishnet pattern of IgG deposition in the Intercellular Space (ICS) of epidermis suggestive of PV [Table/Fig-13 b,c].

Case-3: A 48-year-old female patient presenting with tense blisters with the formation of 'crown of jewels' appearance over

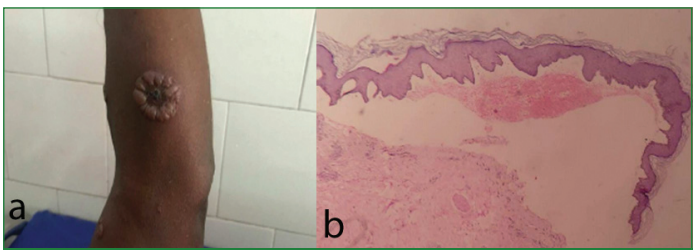


[Table/Fig-12]: a) Clinical presentation of extensive erosions over the trunk; b) H&E section (10x) showing split formation in the subepidermis consisting of Eosinophils and fibrin deposition; c) Direct Immunofluorescence (DIF) (10x) of the same shows linear deposition of IgG over dermoepidermal junction suggestive of BP.



[Table/Fig-13]: a) Flaccid bulla and erosions over upper extremities; b) H&E section showing supra bulla formation with 'Row of tomb stone' appearance. c) DIF showing fishnet pattern of IgG deposition in the ICS of epidermis suggestive of Pemphigus Vulgaris (PV).

the upper extremities [Table/Fig-14a]. H&E section showing subepidermal separation suggestive of subepidermal bullous diseases [Table/Fig-14b].

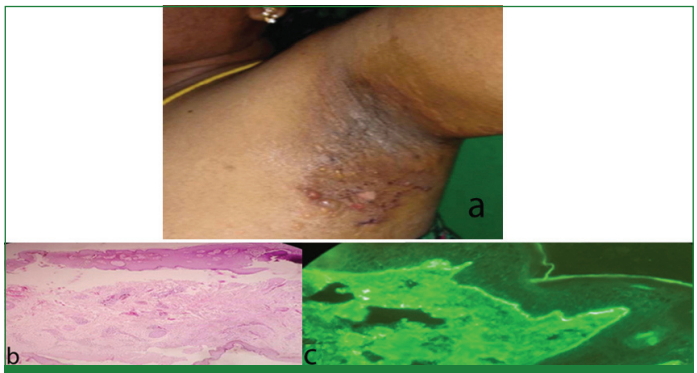


[Table/Fig-14a,b]: a) Tense blisters with the formation of 'crown of jewels' appearance over the upper extremities. b) H&E section showing subepidermal separation suggestive of subepidermal bullous diseases.

Direct immunofluorescence (IgA) was not done in this patient-treatment was not affordable to the patient. Based on gross appearance and histopathology, the diagnosis of Linear Iga Disease (LAD) was made and treated.

Case 4: A 48-year-old female presented with tense blisters over the left axilla [Table/Fig-15a]. H&E section showing subepidermal separation with inflammatory infiltrates in the upper dermis [Table/

Fig-15b] shows DIF testing showing linear IgG deposition in the dermoepidermal junction Suggestive of bullous systemic lupus erythematosus.



[Table/Fig-15]: a) Tense blisters presenting all over the left axilla; b, c) H&E section showing subepidermal separation with few inflammatory infiltrates in the upper dermis, DIF shows linear IgG deposition in the dermoepidermal junction suggestive of Systemic Lupus Erythematosus (SLE).

DISCUSSION

Immunobullous diseases are due to immunologically mediated immune responses against antigens of the skin. The classification is based on the level of split, which includes indraepidermal disorders like PV and Pemphigus Foliaceus (PF), subepidermal disorders like BP, Cicatricial Pemphigoid (CP), LAD, dermatitis herpetiformis, lichenplanus pemphigoides and bullous SLE [Table/Fig-16] [6].

	Autoimmune	Non autoimmune
Intra epidermal	Pemphigus	Epidermolysisbullosa simplex Hailey-Hailey disease
Sub epidermal	Bullous Pemphigoid (BP) Pemphigoid Dermatitis herpatiformis Linear IgA bullous acquisita Pemphigoid gestatoinis	Epidermolysisbullosa of the border Epidermolysisbullosadystropica

[Table/Fig-16]: The classification is as follows.

Which may be due to improper selection of biopsy site, previous treatment for some other conditions (which could have altered the immune status) and technical errors, patients detailed history not known/patient did not return back to treatment, remission of the disease may result in false negativity of DIF.

Overlapping of clinical, histopathological features of immunobullous diseases with other skin disorders like eczema, urticaria the accurate diagnosis depends purely on DIF which proves to be the gold standard method. Although DIF proves to be more sensitive than the serum testing, either may be positive while the other is negative and together they offer the most sensitivity. Precise diagnosis is key for prognosis, treatment decisions are importantly needed for response to therapy.

Clinically, all patients with bullous disorders may not present with classical morphology and distribution of disorders as observed by the studies conducted in India which may be due to differences in prevalence, staging, status of ongoing treatment and severity of the disease. Inspite of similar clinical presentations, they are remarkably histopathologically different from each other. Hence, proper diagnosis is essential to prevent the (fatality of the disease) which means that in the United States, the one year mortality rate for patients diagnosed with bullous disorders were reported to be 23% [7].

Light microscopy is the simple method to diagnose immunobullous disorders which should be correlated clinically. In order to arrive at a definitive diagnosis DIF is done in perilesional skin or mucosa as a substrate and IF done using patients serum [8]. In the present study, a detailed analysis of clinical, pathological, DIF features of bullous disorders were studied.

In males the most common age group of onset was 51.97 years. In females the most common age of onset was 52.88 years which was similar to the study conducted by Basu K et al., and Khannan CK et al., [9,10].

In the selected autoimmunobullous disorders most common gender group affected was males (51.42%). Among intraepidermal bullous disorders, the most common gender affected was females (56.4%), which was similar to the study conducted by Basu K et al., and Khannan CK et al., [9,10]. Among subepidermal bullous disorders, the most common gender affected was males (58.06%), in the study done by De D et al., and Tham SN et al., similar findings were observed [11,12]. The mean duration of illness at the mode of presentation was 1 to 3 months, which was similar to the study done by Jindal et al., [13]. The most common cutaneous disorder at the time of presentation was bulla with erosions, followed by erosions alone. In intraepidermal group of disorders, the most common cutaneous disorder was flaccid bulla with extensive erosions, which was similar to study done by Basu K et al., [9]. In subepidermal group of disorders, the most common cutaneous disorder was tense bulla followed by erosions, which is similar to the study done by De D et al., [11].

The most common comorbid illness observed was type 2 diabetes mellitus (27%) followed by systemic hypertension (20%), which is in contrast to the study done by De D et al., systemic hypertension (80%) followed by diabetes mellitus (35%) [11].

The disorders were most commonly distributed over the trunk (95.7%) followed by limbs (91.4%). This was similar to the study done by Khannan CK et al., in which trunk was involved in 52%, followed by limbs in 48% [10].

Mucosal involvement in the form of oral disorders was seen in 44.3% of patients. Among PV, oral disorders were seen in 82.3% (28/34) of patients. Genital erosions were seen in 12% of patients with pemphigus group of disorders. Oral disorders were seen with one patient with BP. De D et al., observed mucosal disorders in 40% of patients of BP [11].

Nail involvement was seen in 25.7% of patients. The most common finding was subungual hyperkeratosis followed by onycholysis. Gopal V et al., studies show, 72.5% of patients had, paronychia followed by onychorhexis [14].

Nikolsky sign was found to be positive in 79.4% of patients, which was similar to the study conducted by Basu K et al., who observed positivity in Nikolsky sign in 72% of patients [9]. Indirect Nikolsky sign was negative in 4 (6%) patients with pemphigus group of disorders. Direct Nikolsky sign was negative in 5 (7.14%) patients with pemphigus disorders.

Tzanck test for acantholytic cells was positive in 31(44.3%) patients. Among pemphigus group of disorders, Tzanck test was positive in 31(79.48%) patients. In the study done by Basu K et al., Tzanck test was positive in 88.24% of patients. Eight (11.42%) patients with Pemphigus group of disorders had negative Tzanck test [9].

Diagnosis was inconclusive in about 9 (12.9%) patients.

Diagnostic Dilemma: In about four cases, it was difficult to diagnose between PV and BP. These patients had both flaccid and few tense blisters, Tzanck test for acantholysis was inconclusive in all four cases. Nikolsky sign was positive in two cases and negative in two cases, IF was not done in those cases due to unwillingness of patient.

In about three cases, diagnosis of BP/LAD was made. In the first case, patient had classical string of pearls appearance in one region, but had tense blisters and few erosions similar to BP in other areas. In the 2nd and 3rd case, patient had prodromal symptoms such as itching and urticarial disorder prior to the onset of bulla and had a negative drug history and disorders mimicking both LAD and BP.

Differential diagnosis between PV and pemphigus was difficult. These patients had vegetative disorders over the scalp and chest region and also had flaccid bulla and erosions over other areas. One patient had no mucosal involvement; the other patient had few erosions over the buccal mucosa. Nikolsky sign and Tzanck test was positive in both cases.

Four patients with inconclusive clinical diagnosis were found to have features suggestive of BP. Two patients with inconclusive clinical diagnosis had suprabasal split with acantholytic cells, indicative of PV. One patient with inconclusive clinical differential diagnosis of PV/PF had subcorneal split on DIF which was suggestive of PF.

DIF was found overall positive in 67 (95.71%) patients. Three patients had a negative DIF testing, which could be due to various reasons such as previous treatment for some other conditions which could have altered the immune status of the patient or due to remission of the disease or due to laboratory errors (patients detailed history of the past was not known/patient did not return back to treatment).

Among the 34 patients, 29 (85.29%) patients with PV had DIF positivity. On DIF examination, ICS deposition of IgG was present in 31 out of 34 cases (91.17%) of PV resembling fishnet pattern. In the study conducted by Buch AC et al., fishnet pattern of IgG staining was observed in 87.93% of patients. C3 deposition in fishnet pattern was seen in 8 (23.5%) patients in our study. Buch AC et al., studies show that, fishnet pattern of C3 staining was observed in 12.06 % of patients [15].

Among 27 patients with BP, DIF was positive in 100% of patients. Linear deposition of C3 along the dermo-epidermal junction was present in 26 (96.29%) patients. Linear deposition of IgG along the dermo-epidermal junction was present in 21 (77.77%) patients. Linear deposition of IgA along the dermo-epidermal junction in one patient. In the study conducted by Buch A C et al., linear pattern of IgG and C3 staining was observed in 68% of patients. IF done on salt split skin provides a higher rate of positivity in contrast with the conventional DIF [16].

Among the three cases of PF, DIF was positive in two cases (66.67%). Fishnet pattern of IgG deposition in ICS was seen in both cases in the upper layers of epidermis. Fishnet pattern of C3 deposition in ICS was seen in one patient. In PF, DIF shows IgG and C3 deposition in upper layers of epidermis in ICS. This finding is helpful to differentiate PF from PV [17].

Vesicobullous disorders are extremely debilitating, can have serious sequelae and even fatal, necessating early treatment and invention to prevent morbidity and mortality [18]. In addition to diagnosis, DIF also aids in monitoring response to therapy and predicting relapse. It also plays an important prognostic tool as positive DIF findings in patients in remission predict early relapse of disease [19]. Clinical examination is the initial step, followed by histopathology and DIF. DIF is helpful in scenarios where clinical and or histopathological features are inconclusive [20]. Immunobullous disorders contribute a significant number of patients attending the dermatology OPD, which has varied presentation DIF study of perilesional skin combined with histopathology will help the pathologist to make accurate and prompt diagnosis [21].

Limitation(s)

The sample size obtained was lesser in number, hence further studies with larger sample size will be done in the future. Salt split skin technique was not performed due to unavailability in our institution, hence further categorisation of subepidermal bullous disorders was not possible.

CONCLUSION(S)

DIF proves to be the gold standard in diagnosing as well as distinguishing immune-mediated bullous disorders based on the tissue bound autoantibodies from other disorders. The diagnostic yield is enhanced by DIF in cases that pose a diagnostic dilemma both clinically and histologically. In addition to diagnosis, DIF also aids in monitoring response to therapy and predicting its relapse. Therefore, DIF can be used as an additional tool with histopathology and hence we recommend that IF study is essentially needed in all cases of immunobullous disorders to arrive at a definite diagnosis and to confirm with the clinical findings of the same (as these methods may not be diagnostic individually in each and every case.

REFERENCES

[1] Khan WA, Valand AG. Pattern of non infectious vesiculobullous and vesiculopustular skin diseases in a large tertiary care hospital. Bombay Hosp J. 2010;52(2):172-76.
[2] Mysorekar VV, Sumathy TK, Shyam Prasad AL. Role of direct immunofluorescence in dermatological disorders. Indian Dermatol Online J. 2015;6(3):172-80.
[3] Sitaru C, Zillikens D. Mechanisms of blister induction by autoantibodies. Exp Dermatol. 2005;14(12):861-75.
[4] Lever WF. Pemphigus. Medicine. 1953;32:1-123.
[5] Beutner EH, Chorzelski TP, Jablonska S. Immunofluorescence tests. Clinical significance of sera and skin in bullous diseases. Int J Dermatol. 1985;24(7):405-21.
[6] Yancey KB. The pathophysiology of autoimmune blistering diseases. J Clin Invest. 2005;115(4):825-28.
[7] Colbert RL, Allen DM, Eastwood D, Fairley JA. Mortality rate of bullous pemphigoid in a US medical center.J Invest Dermatol. 2004;122(5):1091-1095.
[8] Chhabra S, Minz RW, Saikia B. Immunoflorescence in dermatology. Indian J Dermatol Venereol Leprol. 2012;78(6):677-91.
[9] Basu K, Chatterjee M, De A, Sengupta M, Datta C, Mitra P. A clinicopathological and immunofluorescence study of intraepidermal immunobullous diseases. Indian J Dermatol. 2019;64(2):101-05.
[10] Khannan CK, Bhat R. A retrospective study of clinical, histopathological and direct immunofluorescence spectrum of immunobullous disorders. Int J Sci Res Pub. 2015;5(9):01-06.
[11] De D, Khullar G, Handa S, Saikia UN, Radotra BD, Saikia B, et al. Clinical, [10] demographic and immunopathological spectrum of subepidermal autoimmune bullousdiseases at a tertiary center: A 1-year audit. Indian J Dermatol Venereol Leprol. 2016;82(3):358.
[12] Tham SN, Thirumoorthy T, Rajan VS. Bullous pemphigoid: Clinicoepidemiological study of patients seen at a Singapore skin hospital. Australas J Dermatol. 1984;25(2):68-72.
[13] Jindal A, Shah R, Patel N, Patel K, Mehta R, Barot Jigna P: A Cross sectional study of clinical,histopathological and direct immunoflorescence diagnosis in autoimmune bullous diseases. Indian journal of dermatopathology and diagnostic dermatology. 2014;1(1):25-31.
[14] Gopal V, Shenoy MM, Bejai V, Nargis T. Nail changes in autoimmune blistering disorders: A case-control study. Indian J Dermatol Venerol Leprol. 2018;84(3):375-79.
[15] 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.
[16] Satyapal S, Amladi S, Jerajani HR. Evaluation of salt split technique of immunofluorescence in bullous pemphigoid. Indian J Dermatol Venereol Leprol. 2002;68(6):3303.
[17] Schmidt E, Zillikens D. Pemphigoid diseases. Lancet Lond Engl. 2013;381(9863):320-32.
[18] Pavani M, Harika P, Deshpande AK. Clinicopathological study of vesiculobullous lesions of the skin and the diagnostic utility of immunofluorescence. Int J Clin Diag Pathol. 2020;3(1):252-57.
[19] Sinha P, Sandhu S, Bhatia JK, Anand N, Yadav AK. Analysis of the utility of direct immunofluorescence in the diagnosis of common immune mediated dermatological conditions. J Mar Med Soc. 2020;22(1):44.

[20] Khursheed S, Shah H, Ijaz A, Mehmood M, Tanvir N. Histopathological spectrum and role of clinicopathological correlation in the diagnosis of vesiculobullous lesions. J Ayub Med Coll Abbottabad. 2022;34(3 Suppl 1):635-39.

[21] Badge PA, Sunny B, Mathew R. Diagnostic efficacy of clinical diagnosis, histopathological diagnosis as compared to direct immunofluorescence in autoimmune blistering diseases-a study from south Kerala. J Evolution Med Dent Sci. 2021;10(45):3895-99. Doi: 10.14260/jemds/2021/787.

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