

A Prospective Study of Clinical, Histopathological and Direct Immunofluorescence Spectrum of Cutaneous Vesiculobullous Lesions

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ABSTRACT

Background: Vesiculobullous lesions are heterogenous group of dermatoses characterized by autoantibodies directed against various autoantigens resulting in blister formation. Over the last two decades great advances have been made in understanding clinical behavior and molecular nature of autoimmune disease. Immunofluorescence has greatly contributed to diagnosis, treatment and understanding of pathophysiology of vesiculobullous lesions of skin.

Objectives: 1.To evaluate the histopathological patterns and immunofluorescence findings of various bullous disorders of skin for its diagnostic potential which would help the clinicians in appropriate treatment and 2. To record the sensitivity of immunofluorescence in vesiculobullous lesions.

Materials and Methods: The present study was carried out in the Department of Pathology at S.C.B.M.C.H, Cuttack, Odisha over a period of 24 months from September 2014 to August 2016 in collaboration with Department of Dermatology and K.I.M.S Bhubaneswar. A total of 40 cases of confirmed vesiculobullous lesions were analysed clinically and histopathologically and DIF were done in these cases to record the sensitivity.

Results: Pemphigus vulgaris was the most common bullous lesion constituting 16 cases (40%). Majority of PV cases and all cases of PF showed antibody deposition in squamous intercellular spaces giving a fish net appearance and all cases of BP, LIgAD and EBA showed antibody deposition in dermoepidermal junction, giving a linear pattern. DIF was done for 40 cases of which 30 cases showed positive results.

Conclusion: Immunofluorescence techniques supplements clinical and histopathological findings in diagnosis of vesiculobullous lesions whenever there is an overlap. DIF sensitivity of our study was 75%.

Keywords: Vesiculobullous Disorders, Pemphigus Vulgaris, DIF.

Abbreviations

PV- Pemphigus vulgaris, BP- Bullous pemphigoid, PF- Pemphigus foliaceus, LAD- Linear IgA disease, SCPD- Sub-corneal pustular dermatoses, EBA- Epidermolysis bullosa acquisita, HH- Hailey Hailey, DH- Dermatitis herpetiformis., CBDC- Chronic bullous dermatosis of childhood, BSLE- Bullous systemic lupus erythematosus. DIF- Direct immunofluorescence, BMZ- Basement membrane zone, DEJ- Dermoepidermal junction.

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INTRODUCTION

Autoimmune blistering disorders have a great impact on the patient and their family, some of which are extremely debilitating and fatal which if not diagnosed early can result in mortality and morbidity.^{1,2} Although clinical examination of bullous lesions helps clinicians to arrive at differential diagnosis by assessing the gross

morphology of the lesion but histopathology in conjunction with direct immunofluorescent microscopy is the gold standard. Bullous disorders are subdivided into intraepidermal and subepidermal.³ Histopathological assessment gives a clue to the site and level of blister within the skin, site shape and size of bulla

along with epidermal and dermal changes.⁴ It also throws light into the pathogenetic mechanism underlying the bullous disorders. Bullous lesions are immune mediated and they elicit immunopathogenetic pattern which is disease specific. The DIF result interpretation was based on site (i.e intercellular/along BMZ/or dermal papillae), type of immunoreactant deposited (i.e IgG, IgM, IgA, C3) pattern of deposition (granular/linear) and intensity of deposition of immunoreactants.⁵

MATERIALS AND METHODS

The present study was carried out in the Department of Pathology and Dermatology at Sri Ram Chandra Bhanja Medical College, Cuttack, Odisha over a period of 24 months from September 2014 to August 2016 in collaboration with Department of Pathology, K.I.M.S Bhubaneswar.

Methods of Collection of Data

All skin biopsies from patients with vesiculobullous lesions were included in our study. After taking due consent of the patient, they were referred to department of pathology, S.C.B Medical College. The detailed clinical history including duration, site, onset, progression, associated symptoms like itching, photosensitivity, pain was collected from the patients.

The family history, history of drug intake and past history was noted. The clinical diagnosis was recorded. Vesiculobullous lesions due to thermal, traumatic causes and drug induced vesiculobullous lesions were excluded.

Procedure

Cytological examinations were done from vesiculobullous lesion by Tzanck smears from a recent vesicle or bulla without any sign of secondary infections. Cytological smears were fixed in 95% ethanol and stained with haematoxylin and eosin stain. Unfixed smears were stained with Diff quick. Tzanck test was considered positive when the smear showed characteristic acantholytic cells, balloon cells and syncytial giant cells.

Two skin biopsies were obtained by 5 mm punch biopsy. The skin biopsies were performed by the clinicians as an aid to diagnosis and treatment. Of the two biopsies, perilesional skin biopsy was

sent in Michel's medium for Immunofluorescence study whereas lesional biopsy was sent in 10% neutral buffered formalin for routine histopathological studies. The tissue sent in Michel's media was frozen and cut using Leica CM 15105 model cryostat at 5 micrometer thickness at -22 deg c. Sections were taken on poly L Lysine coated glass slides ,fixed with ether -alcohol mixture and air dried for 10 mins. Later the slides were rinsed in phosphate buffer saline at PH 7.3 for 15 mins. Sections were treated with Fluorescein isothiocyanate labeled and optimally diluted antisera (1:40) i.e IgA, IgG, IgM and C3 Positive and negative controls were taken. The slides were incubated in wet chamber for 1hr in dark room, washed with phosphate buffered saline (PBS) and mounted with glycerine jelly and observed under green filter of fluorescence microscope at 494 nm wavelength.

RESULTS

A total of 960 skin biopsies were received in the Department of Pathology, out of which 40 cases were diagnosed as vesiculobullous lesions which constituted 4.16% of all skin biopsies. Tzanck smear was positive for acantholytic cells.-20 cases, 16 cases (Pemphigus group), 4 cases-(Viral) [Fig 1]

Table 1 shows distribution of demographic changes and associated symptoms. In our study, Pemphigus vulgaris constituted most common vesiculobullous disorders. Males outnumbered females in the present study with M:F ratio =2.07:1. Vesicle/bulla was the prominent primary lesion noted in the study. Crusting and erosions was the most common secondary lesion seen. Oral musosa involvement was seen in 87.5% (14/16) of Pemphigus vulgaris and 10% of Bullous pemphigoid cases.

Table 2 shows distribution of histopathological features among various vesiculobullous lesions. Tomb stone appearance was the most common epidermal change seen in majority of cases of Pemphigus vulgaris. Dermal infiltration was seen predominant in all the conditions. Predominant inflammatory cells in the blister cavity are neutrophils (68.75%) in Pemphigus vulgaris and eosinophils in Bullous pemphigoid (80%).

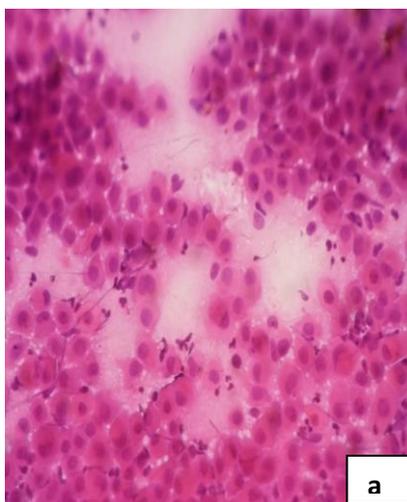


Fig 1(a): Photomicrograph of Tzanck Smear Showing Acantholytic Cells



Fig 1(b): Clinical photograph of 1/M showing dermatomal distribution of blisters.

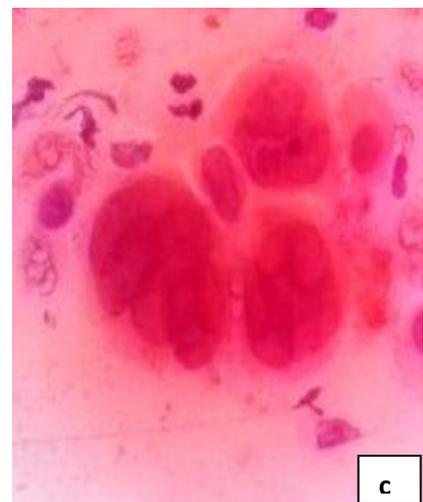


Fig 1(c): Tzanck smear of viral blister showing multinucleated giant cells.

Table 1: Distribution of Demographic Changes and Associated Symptoms

Variable	Category	PV (N=16)	BP (N=10)	PF (N=03)	LAD (N=02)	EBA (N=1)	SCPD (N=03)	HH (N=02)	DH (N=01)	CBDC (N=01)	BSLE (N=01)
Age (in years)	0-10	0	0	0	0	0	0	0	0	1 (100%)	0
	11-20	0	0	0	0	0	0	0	1 (100%)	0	0
	21-30	2 (12%)	0	0	0	0	0	0	0	0	0
	31-40	3 (18%)	0	0	0	0	1 (33%)	2 (100%)	0	0	0
	41-50	5 (31.2%)	1 (10%)	1 (33.3%)	1 (50%)	0	2 (67%)	0	0	0	1 (100%)
	51-60	5 (31.2%)	0	2 (66.7%)	1 (50%)	1 (100%)	0	0	0	0	0
	61-70	1 (6.25%)	4 (40%)	0	0	0	0	0	0	0	0
	>70	0	5 (50%)	0	0	0	0	0	0	0	0
Sex	Male	12	07	01	01	00	02	00	01	01	00
	Female	04	03	02	01	01	01	02	00	00	01
Associated symptoms	Blistering	12 (75%)	10 (100%)	03 (100%)	01 (50%)	01 (100%)	03 (100%)	01 (50%)	01 (100%)	01 (100%)	01 (100%)
	Burning	12 (75%)	03	02 (66.7%)	00	00	00	00	01 (50%)	00	01 (100%)
	Pain	03 (18.75%)	00	00	00	00	01 (50%)	00	00	01 (50%)	00
Primary lesion	Vesicle/ Bullae	14 (87.5%)	10 (100%)	02 (66.7%)	02 (66.7%)	01 (100%)	01 (33.4%)	01 (50%)	01 (100%)	01 (100%)	01 (100%)
	Pustule	00	00	00	00	00	02 (66.7%)	00	00	00	00
	Papule	00	00	01	00	00	00	00	01	00	00
Secondary lesions	Crusts	9 (56.2%)	3 (30%)	02 (66.7%)	01 (50%)	00	03 (100%)	01	01 (100%)	00	00
	Erosions	7 (43.7%)	07 (70%)	01 (33.3%)	01 (50%)	00	03 (100%)	01	00	00	00

Table 2: Distribution of Histopathological Features among Various Vesiculobullous Lesions

Variable	Category	PV (N=16)	BP (N=10)	PF (N=03)	LAD (N=02)	EBA (N=1)	SCPD (N=03)	HH (N=02)	DH (N=01)	CBDC (N=01)	BSLE (N=01)
Epidermal changes	Tomb stones appearance	13 (81.25%)	-	-	-	-	-	-	-	-	-
	Villi	5 (31.25%)	-	-	-	-	-	1	-	-	-
	Hyperkeratosis	2 (12.5%)	-	-	-	-	-	-	-	-	-
	Acanthosis	2 (12.5%)	-	-	-	-	1	-	-	-	-
Dermal changes	Acantholytic cells	13 (81.25%)	-	3 (100%)	-	-	2	1 (50%)	-	-	-
	Dermal edema	1	1	-	-	-	1 (33.4%)	-	1	-	-
	Dermal infiltration	13 (81.25%)	8 (80%)	3 (100%)	2 (100%)	1	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)
	Perivascular inflammation	06	03	02	-	-	1	-	1 (100%)	-	-
	Adnexal inflammation	-	-	-	-	-	-	-	1 (100%)	-	-
	Papillary microabcess	-	-	-	-	-	-	-	1	-	-
	Level of blister	Suprabasal	15 (93.75%)	1	-	-	-	-	2	-	-
Subcorneal		-	-	3 (100%)	-	1	3	-	-	-	-
DE Junction		-	9 (90%)	-	2	-	-	-	1	1	1 (100%)

Table 3: Distribution of Antibody Deposition and Its Pattern Using DIF Among Vesiculobullous Lesions

VARIABLE	Category	PV (n=16)	BP (n=10)	PF (n=3)	LAD (n=2)	EBA (n=1)	SCPD (n=3)	HH (n=2)	DH (n=1)	CBDC (n=1)	BSLE (n=1)
ANTIBODY DEPOSITED											
IgG		10 (62.5%)	2 (20%)	2 (67.3%)	0	1 (100%)	0	0	0	0	0
IgM		0	0	0	0	0	0	0	0	0	0
IgA		0	0	0	2 (100%)	0	0	0	0	0	0
C3		0	3 (30%)	0	0	0	0	0	0	0	0
IgG+C3		4 (25%)	5 (50%)	1 (33.3%)	0	0	0	0	0	0	0
Negative		12.5%	0	0	0	0	03	01	01	01	01
PATTERN OF ANTIBODY DEPOSITION											
Squamous intercellular Spaces.	Frequency	14 (87.5%)	03 (100%)
Dermoepidermal junction	Frequency	10 (100%)	02 (100%)	01 (100%)

Table 3 shows distribution of antibody deposition and its pattern using DIF among vesiculobullous lesions. IgG was the most common antibody deposited in majority of cases of PV, PF and 1 case of EBA. IgA was deposited in 2 cases of LAD. IgG+c3 was deposited in 1/4th cases of PV to 1/2 cases of BP. Out of all the positive cases more than 3/4th cases of PV and all cases of PF showed antibody deposition in squamous intercellular spaces giving a fish net appearance and all cases of BP, LIgAD and EBA showed antibody deposition in Dermoepidermal junction, giving a linear pattern. DIF was done for 40 cases of which 30 cases showed positive results. Sensitivity of DIF analysis in our study is 75%.

DISCUSSION

Autoimmune vesiculobullous disease, like other inflammatory dermatoses are morphologically heterogenous. They do not present with classical morphology and distribution of lesions which is most likely due to prevalence of the disease, severity of disease at presentation and status of treatment. Certain subepidermal disease also shows overlapping histopathological findings, while in others there is clinical overlap for e.g inflammatory EBA and Bullous pemphigoid, Linear IgA disease may mimic BP or DH. So an accurate diagnosis is required in these cases to minimize the morbidity and mortality. In this study Pemphigus vulgaris was the most common bullous lesion constituting 16 cases (40%). Other

types of bullous diseases found in our study were Pemphigus foliaceus and Sub corneal pustular dermatoses 03 cases each (7.5%), Bullous pemphigoid 10 cases (25%), Linear IgA Disease 2 cases and Hailey-Haley disease 2 cases (5%) each. Epidermolysis bullosa acquisita, Dermatitis herpetiformis, Chronic bullous Dermatitis of childhood and Bullous SLE 1 case each (2.5%). Similar frequency of distribution was found in literatures of Nanda A et al and Dr P.T.Chan, Sabir F et al and Leena JB et al. Bullous diseases like P.erythematosus, Pemphigoid gestationis were not documented in our study. Our study encountered a case of linear IgA dermatoses, Chronic bullous dermatoses of childhood, Bullous SLE and Hailey-Hailey which was not seen in other studies. [Table 4.]

In the present study Pemphigus vulgaris constituted 40% of total number of cases of vesiculobullous disorders, which is lower than that of Arya SR et al study.⁶ Handa F et al⁷ showed PV as high as 97.6%. This shows that disease has geographic changes. Pemphigus foliaceus was seen in 3 out of 40 cases that constituted 7.5%. When compared to Arya SR et al.⁶ study, the number of cases is very less probably because of less number of sample size. In our study, Bullous pemphigoid constituted 25% (10 cases) of the total cases of vesiculobullous disorders, which is lesser compared to studies done by Nanda et al 43 cases (27%)⁸, Chan P et al 149 cases (63.7%)⁹ and Bernard P et al 94 cases (73.4%).¹⁰ This might be because of small sample size.

Table 4: Comparative Analysis of Our Study with Other Literatures

Studies	Year	P. vulgaris	P. folia.	P.E	B.P	EBA	IgA Pemp.	SCPD	BSLE	LAD	HH	DH
Tsankov et al	2000	77.3%	-	-	-	-	-	-	-	-	-	-
Ljubojevic et al	2002	76%	2%	-	-	-	-	-	-	-	-	-
Nanda A et al	2004	48 %	-	-	27%	-	-	-	-	-	-	-
Chams-Davatchi et al	2005	91.9%	2.7 %	-	-	-	-	-	-	-	-	-
Inchara YK et al	2007	29 %	7%	1%	22 %	3 %	-	-	-	-	-	-
Daneshpazhooh M et al	2012	81.2%	4.4%	0.1%	11.6%	0.5%	0.2%	-	-	-	-	-
Leena JB et al	2012	45%	-	2.5%	40%	2.5%	2.5%	-	-	-	-	2.5%
Present study	2014	40%	7.5%	--	25%	2.5%	-	7.5%	2.5%	5%	5%	2.5%

The age range in Bullous pemphigoid (50-79 years) is higher than in Pemphigus vulgaris (20-69) years which are similar to Soner uzan et al¹¹ and Arti Nanda et al study. Bullous pemphigoid was common at 7th decade (50%) in the present study. Mean age at onset of Bullous pemphigoid was 65.5 years in our study similar to Nanda A et al (65.97 years) but it was higher (77 years) in Wong et al study.¹² The age group most commonly affected in Pemphigus foliaceus was between 51-60 years (50% of cases). Youngest age was 34 years where as other studies found vulnerable age between 20 and 60 years.

Hailey Hailey was found more commonly in age group of 2nd to 4th decade similar to study conducted by Susan M.Burge.¹³ CBDC was seen in 1st decade while the sole case of DH was seen mostly in second decade.

One unique feature noted in our study was predominance of males affected. Male:female ratio was 2.07:1 which is similar to Handa F et al⁷, Arya SR et al⁶ study, Javidi Z et al¹⁴ in contrast to

other studies like Vora et al study (2010);¹⁵ where there is female predominance.

Mucosal involvement was present in 87.5% of cases of Pemphigus vulgaris. Similar findings have been reported by other studies like Arya SR et al (72.1%)⁶ and Handa F et al (85.5%).⁷ Mucous membrane involvement was absent in the Pemphigus foliaceus which is consistent with studies done by Fernandez J et al study and Jamila Aboobaker study.¹⁶ It differs from findings of Arya SR et al study where mucous membrane was involved in 20% cases. This variation could be possibly due to decreased involvement of mucous membrane in Pemphigus foliaceus as compared to Pemphigus vulgaris. Only 1 patient of Bullous pemphigoid had oral lesions which is very small, in comparison to Kanwar AJ et al study of 70%¹⁷, Venning VA et al study of 50%¹⁸ and Bernard et al study of 24.63%.¹⁰ This might be because of small sample size. Mucosal involvement was not seen in any other case.

PEMPHIGUS VULGARIS



Fig 2 (a,b): Clinical photograph of 35/m, showing extensive crusting with oral lesion.

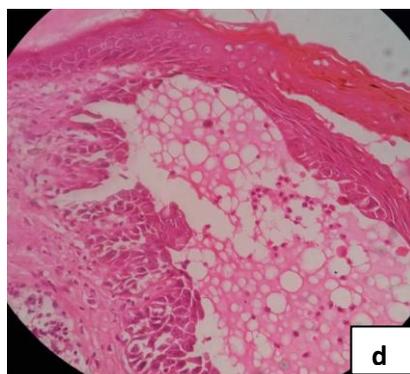
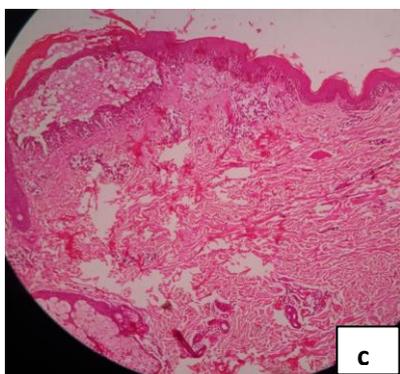


Fig 2 (c,d): Suprabasal cleft containing acantholytic cells with row of tomb stone appearance of basal keratinocytes.

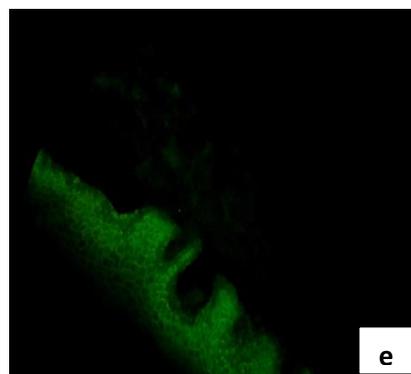


Fig 2 (e): DIF- Intercellular IgG

All the 16 patients had histopathological features of Pemphigus vulgaris which is comparable to other studies. Histopathology is confirmatory in most of the Pemphigus vulgaris cases. It showed suprabasal bulla in 15 (93.75%) cases and one case showed mid-epidermal vesicles. Fig 2 (c,d) Mid epidermal vesicles were seen in old bullae, due to regeneration of the cells from the floor of the bulla. Findings

were concordant with that of Arya SR et al (81.4%) cases. Acantholysis was seen in 13 cases out of 16 (81.25%), slightly lower than Arya SR et al (93%) and Handa et al (97%). Row of tomb stone appearance was seen in 13 cases (81.25%), but Arya SR et al had 41.8% cases and 55% in Vora et al. This higher percentage in our study may be due to small study group. Blister cavity showed inflammatory cells in 14 cases (87.5%) which is

similar to Handa et al (97%). Neutrophils were predominant in 11 cases (68.75%) and eosinophils in 3 cases (18.75%). On histopathology subcorneal bulla was seen in 100% cases of Pemphigus foliaceus which is compatible with study done by Fernandez J et al and more than that found in Arya SR et al. Fig 5 (c,d). Acantholysis was seen in 100% cases in the present study which is similar to Fernandez J et al and Arya SR et al. Inflammatory cells in the bulla cavity are present in 100% cases and neutrophils were predominant in 67% slightly comparable with Arya SR et al (83.3%). This shows most of the Pemphigus foliaceus have subcorneal blisters with acantholysis and inflammatory cells in the bulla cavity. Nine (9 cases) out of ten (10 cases) cases (90%) of Bullous pemphigoid showed subepidermal blister. One case showed suprabasal cleft which might be due to an older lesion being biopsied Fig 3 (c,d).

Eosinophil was predominant inflammatory infiltrate in 9 (90%) cases which coincides with the finding of Farmer et al.¹⁹ SCPD showed subcorneal blisters containing neutrophils findings which were consistent with study of Mittal RR, Singla A, and Gill SS.²⁰ [Fig 8 (c,d)]. Hailey Hailey showed a suprabasal layer containing acantholytic cells similar to study done by Susan M. Burge. [Fig 7(b,c)] Histopathological features of Dermatitis herpetiformis showed subepidermal blister with neutrophils in the blister cavity. There were neutrophilic papillary microabscesses within the dermal papillae with intense perivascular inflammatory infiltrate. Fig 9 (c,d) These features were concordant with other studies of Fraser NG et al (1973);²¹ and Kint A et al (1976).²² Bullous SLE showed a subepidermal blister containing fibrin, neutrophils and nuclear dust on histopathology which was similar to findings of Karen C Parviainen.²³ Fig 11(b,c)

BULLOUS PEMPHIGOID

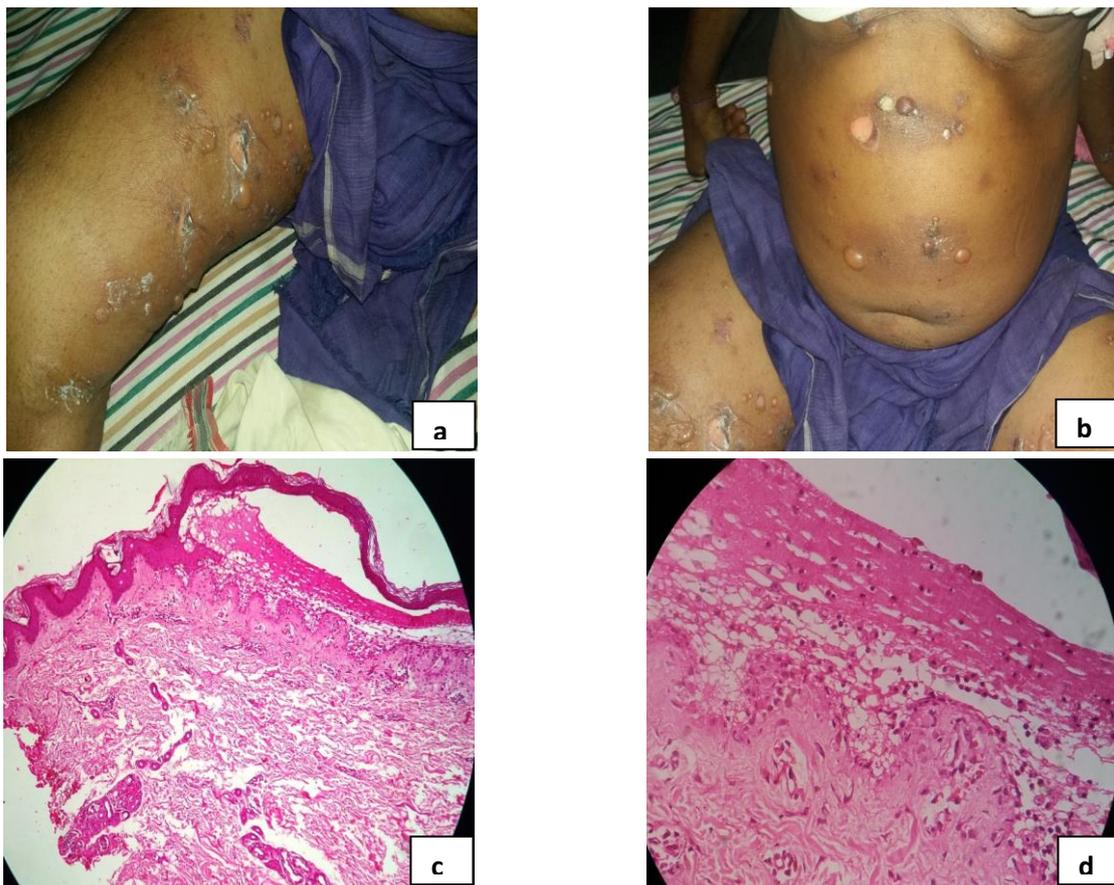


Fig 3 (c,d): Photomicrograph showing subepidermal bullae containing eosinophils

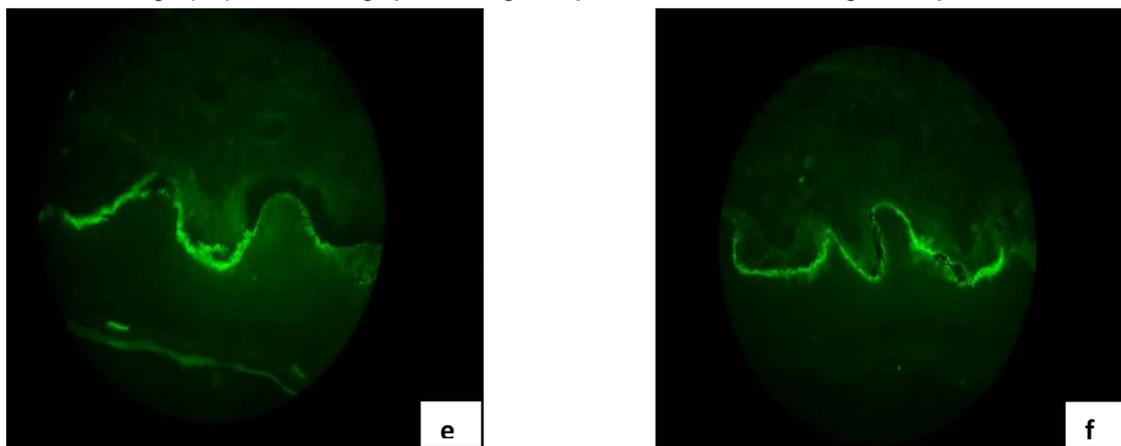


Fig 3 (e,f): DIF - linear IgG & C3 deposits at dermoepidermal junction-Bullous pemphigoid.

LINEAR IgA DERMATOSIS



Fig 4(a,b):Clinical photograph of 55/f, showing erosions covered with scales and crusts predominantly in limbs.

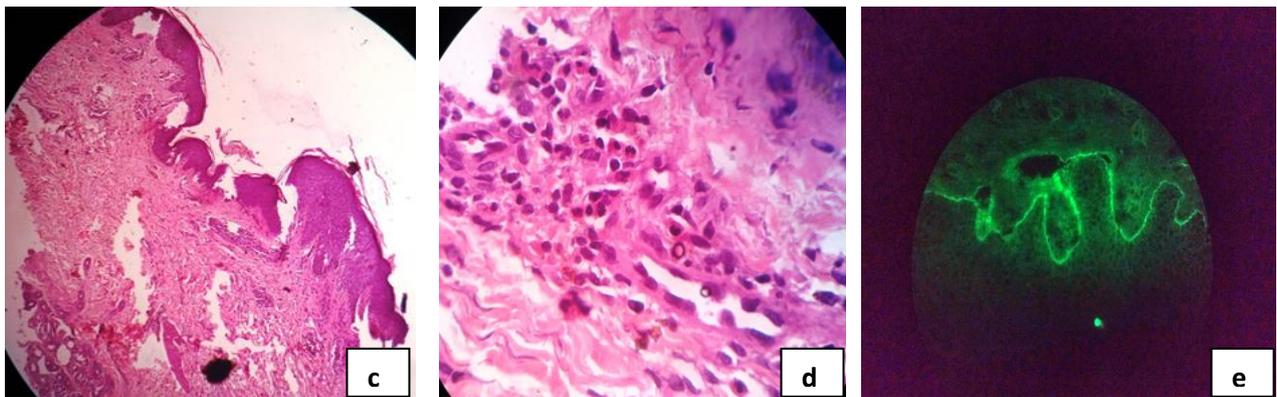


Fig 4(c,d):Photomicrograph showing subepidermal clefting containing neutrophils

Fig 4(e): DIF- IgA deposits at dermoepidermal junction.

PEMPHIGUS FOLIACEOUS (PF)



Fig 5 (a,b): Clinical photograph of 25/m ,with superficial erosions and extensive scaling and crusting

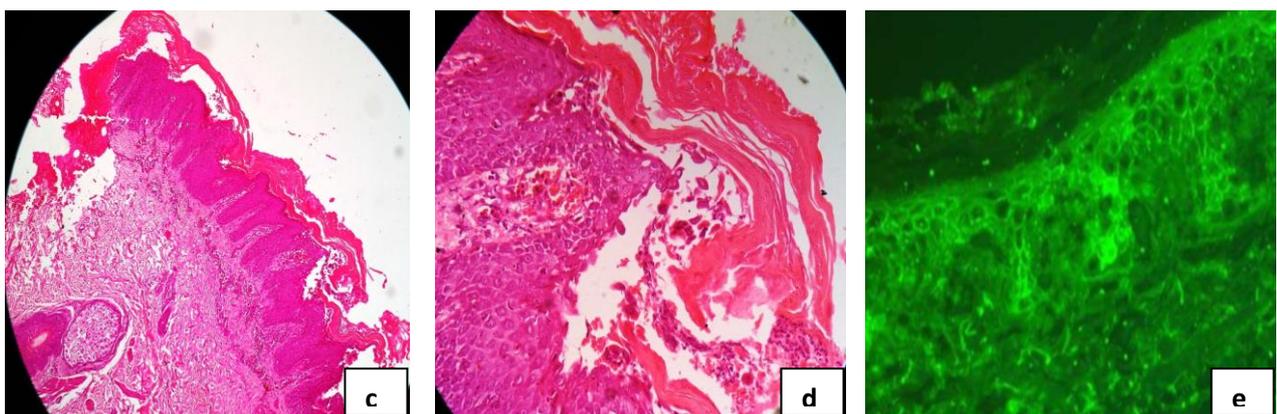


Fig 5(c,d):Photomicrograph showing sub corneal clefting containing neutrophils

Fig 5 (e):DIF - IgG deposition in squamous intercellular spaces

EPIDERMOLYSIS BULLOSA ACQUISITA (EBA)



Fig 6 (a,b): Clinical picture of :58/f, tense bullae, multiple eroded areas healing with atrophy and milia, at places showing contractures.

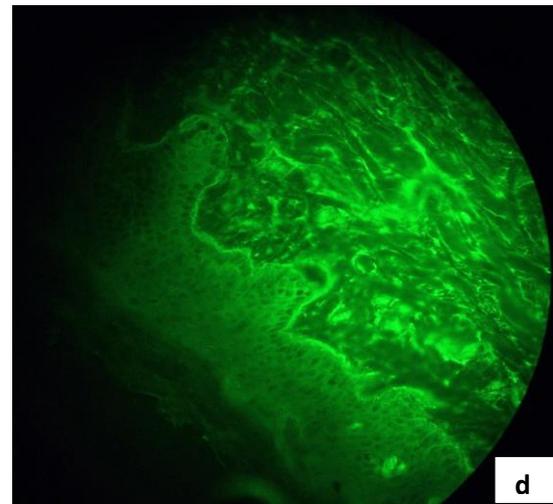


Fig 6 (c): Inconclusive histopathological finding

Fig 6(d):DIF -IgG deposits at dermoepidermal junction.

HAILEY-HAILEY DISEASE(HH)

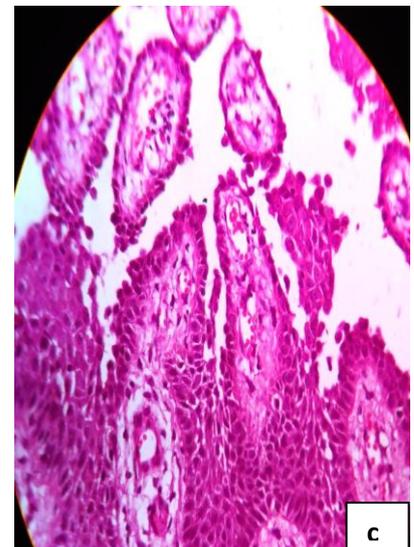


Fig 7 (a): 40/M, Plaques with erosions and areas of macerations, involving axilla, groins, flexures.

Fig 7 (b,c): 400x; Photomicrograph showing dilapidated brick wall appearance

SUB-CORNEAL PUSTULAR DERMATOSIS (SCPD)

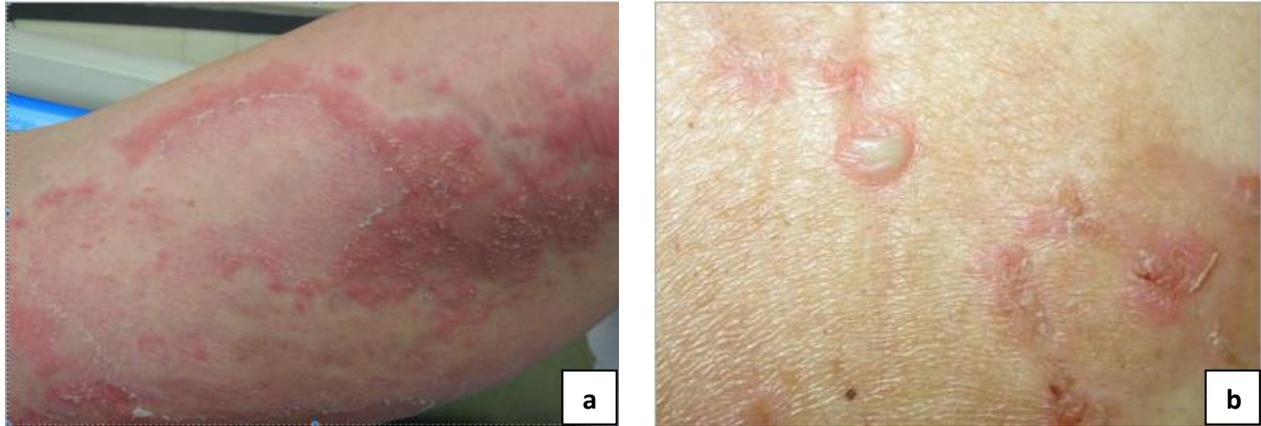


Fig 8 (a,b): Clinical photograph of 45/m, presenting with superficial vesicles with hypopyon and centre clearing up with activity at periphery.

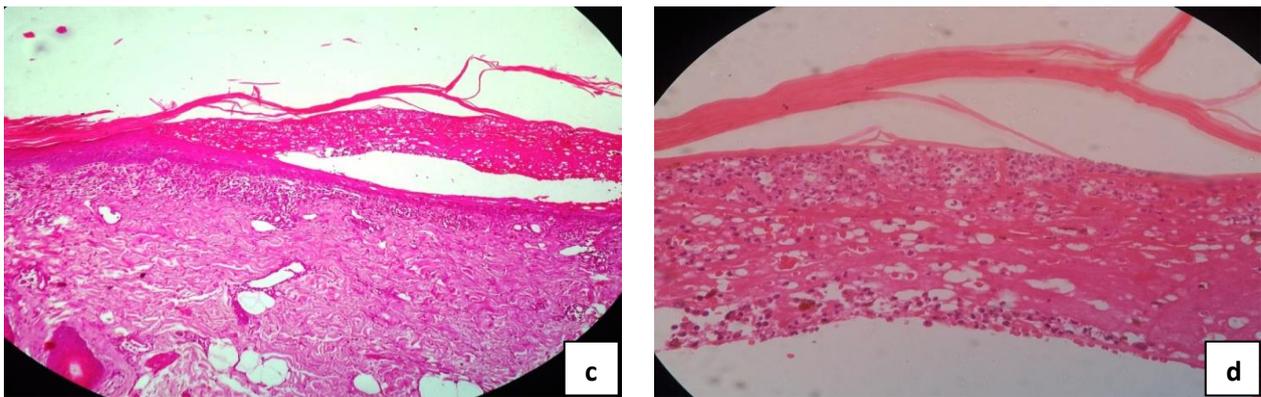


Fig 8 (c,d): Photomicrograph showing subcorneal pustule containing neutrophils predominantly

DERMATITIS HERPETIFORMIS(DH)

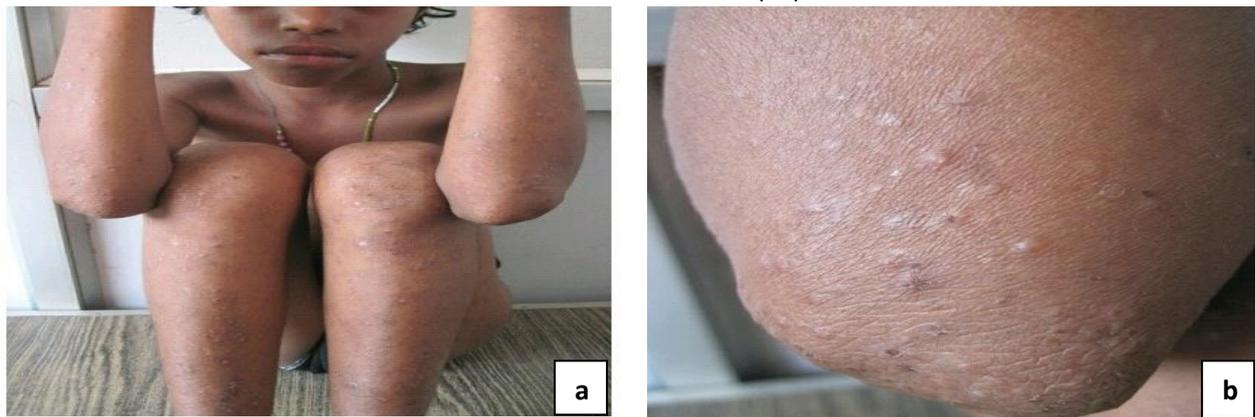


Fig 9 (a,b): Clinical photograph of 10/f, presenting with symmetrical grouped papules around elbow and knee joint

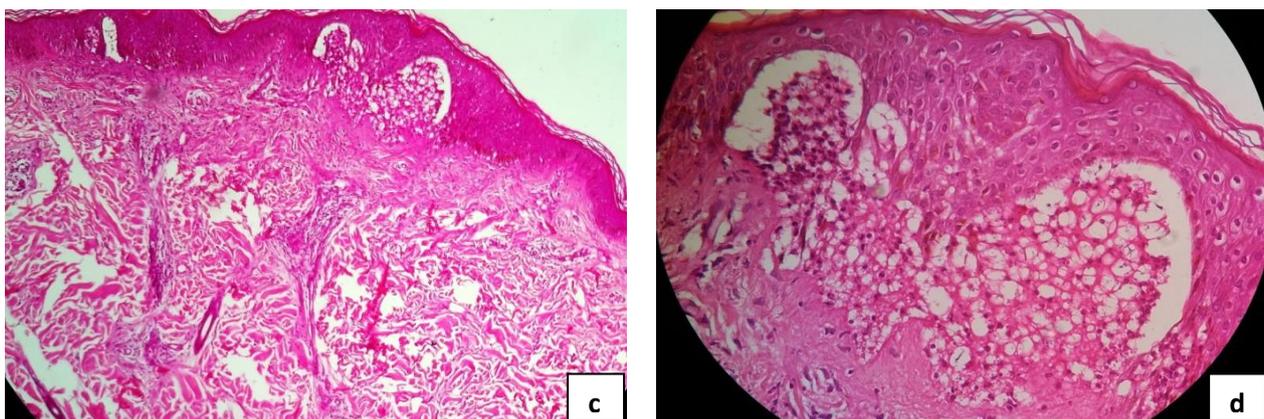


Fig 9 (c,d): Photomicrograph showing subepidermal cleft with dermal papillary neutrophilic microabscess

CHRONIC BULLOUS DERMATOSIS OF CHILDHOOD(CBDC)



Fig 10 (a,b): 8 yr old with tense bullae on inflammatory base giving a “cluster of jewels appearance”

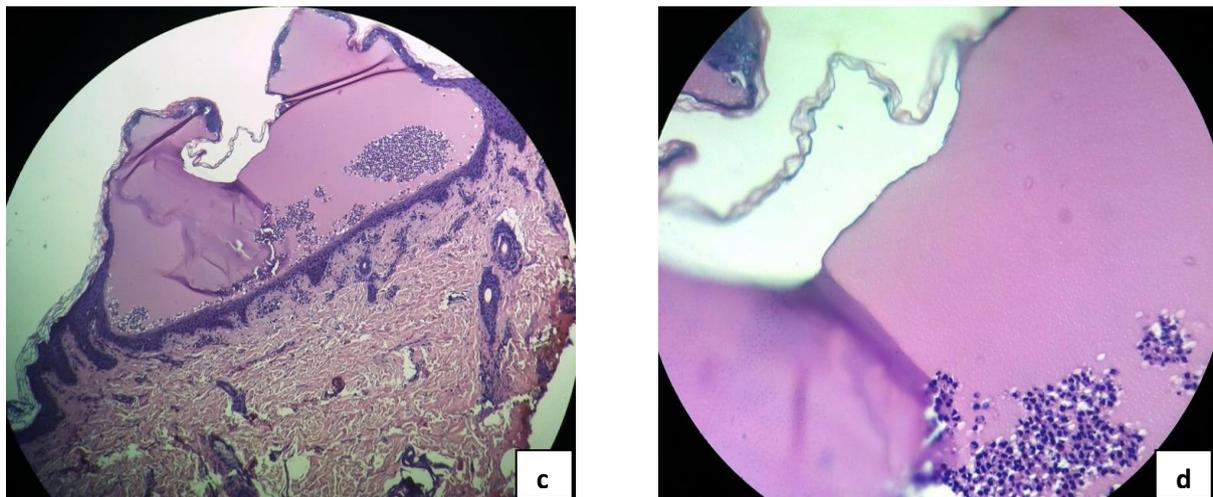


Fig 10 (c,d): Photomicrograph showing subcorneal cleft containing polymorphs-Bullous impetigo..

BULLOUS SYSTEMIC LUPUS ERYTHEMATOSUS(BSLE)

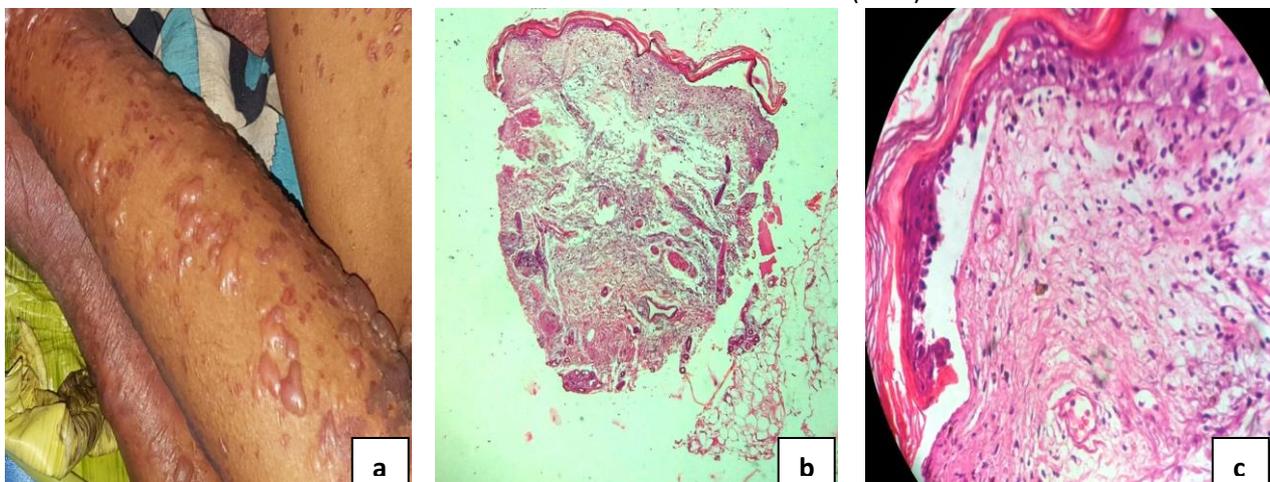


Fig 11 (a): Clinical photograph of 48/f multiple tense bullae over the limbs, h/o fever, joint pains, serum ana positive

Fig 11 (b,c): Photomicrograph showing Subepidermal cleft with neutrophilic infiltrate and acantholytic cells.

Direct immunofluorescence was done in all 16 cases of Pemphigus vulgaris. 14 cases (87.5%) were positive, 2 were negative. Kumar S et al and Kanwar AJ et al study showed DIF (100%) positive. On DIF intercellular space deposition of IgG was

seen in almost all cases of Pemphigus vulgaris. Fig 2 (e) Chams-Davatchi C et al²⁴ had studied 417 cases of Pemphigus vulgaris out of 1111, among which 389 (93.28%) were positive. This shows even the most definitive investigation may be negative. So

diagnosis solely depends on histopathology. Direct immunofluorescence was done in all cases of Pemphigus foliaceus [Fig 5 (e)] and the findings were suggestive of Pemphigus foliaceus in all the 3 cases (100%) which is similar to Inchara et al²⁵ and Kanwar AJ et al. All cases of PF showed intercellular spaces deposition of IgG. The DIF finding is similar to PV. So histopathology differentiates between PV and PF. A unique feature observed in this study was DIF which was done in 10 cases of Bullous pemphigoid of which 10 were positive (100%), similar to other studies like Kippes W et al²⁶ and Cozzani E et al²⁷ studies which showed 100% positivity in DIF. On DIF we get linear deposition of IgG and C3 deposits along the dermoepidermal junction. Both the patients of LAD showed linear deposition of IgA along basement membrane zone. DIF finding in EBA shows IgG deposition at dermoepidermal junction. DIF was negative in all the three cases of SCPD consistent with study of Mittal RR, Singla A, and Gill SS. DIF was negative in both the cases of Hailey-Hailey consistent with the finding of Susan M. Burge.¹³ DIF was negative in Dermatitis herpetiformis. All the 4 histopathologically diagnosed cases of DH in Inchara YK et al were also DIF negative. DIF was negative in single case of Bullous SLE similar to findings of Karen C Parviainen. DIF sensitivity of our study was 75% which is similar to that of S.P. Deepti et al (77.7%), Charishma Kunhi et al (78%). Our study encountered a case of Linear IgA Dermatosis, Chronic bullous dermatosis of childhood, Bullous SLE and Hailey-Hailey which was not seen in other studies. Clinicohistopathological discordance was seen in 1 case which was clinically diagnosed as Chronic bullous dermatosis of childhood but on histopathology was diagnosed as Bullous impetigo Fig 10 (a,b,c,d). Histopathological findings in case of Epidermolysis bullosa acquisita was inconclusive in the present study, but DIF in this case was helpful for diagnosis. DIF patterns of all the Pemphigus disorders shows similar findings causing difficulty in diagnosis. In those cases histopathology play a valuable role, where Pemphigus vulgaris shows a suprabasal blister and Pemphigus foliaceus shows a subcorneal blister. DIF was negative in 10 cases, two (2) cases of Pemphigus vulgaris, three (3) cases of SCPD, two (2) Cases of HH, one (1) case of CBDC, one (1) case of DH, one (1) case of BSLE. Selection of biopsy site, treatment status, and technical errors may result in false negativity of DIF which explains the cause of DIF results being negative for 2 cases of PV. Thus DIF being the most definitive diagnostic modality for vesiculobullous lesions can be negative at times. It plays an important role where clinical and histopathological results are inconclusive.

CONCLUSION

Considering the socioeconomic situations of the patient's and unavailability of immunofluorescence technique easily, clinical diagnosis and histopathology though help in arriving at the diagnosis, DIF can be used as a supplement and not a substitute. It becomes important to distinguish each of these entities and separate them for appropriate management, failure of which in some cases is associated with significant morbidity and mortality. However a large sample sized study is required to further confirm these observations.

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