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Original Research Article

Vesiculo Bullous Lesions - Histomorphological Analysis vs. Immunofluorescence Study

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Abstract:

Objective: Our study aims to analyze the concordance between clinical, histopathological and direct histological examination in the diagnosis of the immunobullous diseases, detect specific pattern of direct immunofluorescence staining in different vesiculobullous disorders, and supplement the clinical and histopathological findings in the investigation of vesiculo-bullous disorders for confirmatory diagnosis of vesiculo bullous disorders.

Methods: This was a hospital-based study conducted among 50 patients who presented with bullous lesions of skin to the Department of Dermatology, Osmania General Hospital, from October 19 and October 21 over a period of two years, after obtaining clearance from Institutional Ethics Committee and written informed consent from the study participants.

Results: Level of blister was suprabasal in majority of cases (86.3 %) of *pemphigus vulgaris* and sub corneal in the pemphigus foliaceous. Acantholytic cells, lymphocytes, and few eosinophils and neutrophils were present in the blister cavity of *pemphigus vulgaris* and foliaceous. DIF showed intercellular deposition of IgG/IgG and C3 in 18 out of 22 cases of *pemphigus vulgaris* which is a classical finding, 4 out of 6 cases of pemphigus foliaceous also showed intercellular deposition of IgG in the epidermis similar to *pemphigus vulgaris*. 10 out of 16 cases of bullous pemphigoid showed linear deposition of IgG and C3 in the basement membrane zone. 2 cases each of BSLE and one case of CBDC were observed.

Conclusion: Considering the socio-economic status of the patients and unavailability of immunofluorescence technique in many centres, clinical diagnosis and histopathology are generally enough for arriving at the final diagnosis. DIF is only used as supplement and not a substitute. It is important to distinguish each of these separate entities for appropriate management, failure of which in few cases is associated with significant mortality and morbidity.

Keywords: Vesiculobullous Lesions, Histomorphological, Analysis, Immunofluorescence

Introduction

There has been extraordinary advancement beyond fifty years in our comprehension of the science of the skin as it identifies with the bullous diseases. One of the principle purposes behind preceded with distinguishing proof of new bullous diseases on the grounds that its determination depends on immunological and molecular basis in addition to clinical and histological findings. Stratified squamous epithelium of the human epidermis frames a persistent boundary against the outer climate. The pathophysiology of bullous diseases outlines how impairments epithelial attachment leads to disorders characterized by substantial morbidity/mortality. Blistering diseases which can be either inherited or acquired, most instances of the later are autoimmune that target junctional molecules promoting either cell-cell or cell matrix adhesion in skin. This later group together comprises the spectrum of immunobullous diseases proteins. The autoantibody in each type of pemphigus is directed against a desmosomal protein or a combination of desmosomal proteins. The subepidermal bullous diseases are associated with antibodies against components of the basement membrane zone (BMZ). Immunobullous diseases are commonly diagnosed by histopathology and immunofluorescence both direct and

indirect direct immunofluorescence help to detect antibodies and complement proteins from perilesional skin biopsy. In this study, we wanted to analyse the concordance between clinical, histopathological and direct histological examination in the diagnosis of the immunobullous diseases, detect specific pattern of direct immunofluorescence staining in different vesiculobullous disorders, and supplement the clinical and histopathological findings in the investigation of vesiculo-bullous disorders for confirmatory diagnosis of vesiculo bullous disorders.

Material and Methods

This was a hospital-based study conducted among 50 patients who presented with bullous lesions of skin to the Department of Dermatology, Osmania General Hospital, from October 19 and October 21 over a period of two years, after obtaining clearance from Institutional Ethics Committee and written informed consent from the study participants.

Inclusion Criteria

a) Type of immune deposit
b) Location of immune deposit
c) Extent of staining: focal or diffuse
d) Intensity of staining: + to ++++
e) Pattern of immune complex deposit: linear or granular

Statistical Methods

Data was entered in MS Excel and analysed using Statistical Package for Social Science (SPSS) software. Results were presented as tables and graphs.

Results

Table 1: Types of Vesiculobullous Diseases

Sl. No.	Disorder	No of Cases	Percentage
1	Pemphigus vulgaris	22	44
2	Bullous pemphigoid	16	32
3	Pemphigus foliaceous	6	12
4	Pemphigus vegetans	3	6
5	Chronic bullous dermatoses of childhood	2	4
6	Bullous SLE	1	2
	Total	50	

22/50 cases (44 %) studied were of pemphigus vulgaris, while 16/50 cases (32%) were of bullous pemphigoid. Majority of cases showed acantholysis and suprabasal cleavage with classical “tombstone" appearance (81.8 and 86.3 % respectively). Extension of acantholysis into follicular epithelium was seen in almost half of the cases studied (45.4 %) one case (18.1 %), showed non-specific histological findings. Other cases had a dense band-like lymphohistiocytic infiltrate within lamina propria consistent with oral lichen planus.

Table 2: Histopathological Change of Bullous Pemphigoid

Sl. no	Histopathological Changes	No of Cases	Percentage
1	Sub-epidermal blister	12	75
2	Superficial dermal oedema	8	50
3	Dermal inflammation		
	(i) cell rich	12	75
	(ii) cell poor	4	25
4	Predominant inflammatory cell		
	(i) eosinophils	12	75
	(ii) mononuclear	4	25
5	Spongiosis	2	12.5

12/16 cases (75 %) showed a sub-epidermal blister; two cases showed an intact epidermis with spongiosis. The superficial dermis showed oedema and an eosinophilic rich perivascular inflammatory infiltrate (consistent with early bullous pemphigoid). There were two cases with spongiosis (12.5 %) 4/16 cases (12.5 %) showed a cell-poor pattern and 12/16 cases (75 %) showed predominant inflammatory cell eosinophils.

Table 3: Histopathological Changes of Pemphigus Foliaceous

Sl. No.	Histopathological Changes	No of Cases	Percentage
1	Spongiosis	6	100
2	Sub corneal blister	4	67
3	Acantholytic/ Dyskeratotic/keratinocytes	4	67
4	Dermal inflammation	6	100

4 /6 cases (67 %) showed a sub-corneal blister with occasional acantholytic keratinocytes. Two cases showed epidermal spongiosis with absence of granular layer and stratum corneum with neutrophilic infiltrate. The dermis showed a perivascular lymphocytic collection. Separated fragment of lamellated keratin with neutrophils was seen. No evidence of acantholysis and hence diagnosis of pustular psoriasis was given, which was the clinical differential diagnosis

Pemphigus Vegetans

Histopathology showed epidermis along with supra-basal blister, acantholysis, papillomatosis and marked acanthosis. Focal spongiosis was also seen. Blister cavity showed acantholytic cells and neutrophils. Acantholysis also involved the follicular epithelium.

Chronic Bullous Dermatoses of Childhood

One case showed epidermis with spongiosis and a sub-epidermal bulla containing eosinophils neutrophils, and scattered lymphocytes superficial dermis showed oedema and inflammatory cell infiltrate. Other case showed only sub-epidermal cleft filled with plasma and neutrophils mild dermal perivascular inflammation composed predominantly of neutrophils was seen.

Bullous SLE

Only one case showed epidermis with sub-epidermal cleft containing neutrophils and plasma. Superficial dermis showed a mild perivascular lymphocytic infiltrate with vasculitis. Special stains (Alcian Blue) revealed increase in dermal mucin.

No	Disorder	No of Cases	Cases with positive DIF	Cases with Positive HPE and DIF	Cases with Positive DIF and discordant HPE	Cases with Negative DIF and Diaghpe
1	PV	22	20 (90.9)	18	2	2
2	BP	16	15 (93.7)	13	1	2
3	PF	6	6 (100)	5	1	0
4	CBDC	3	3 (100)	3	0	0
5	VEG	2	2 (100)	2	0	0
6	Bullous SLE	1	1 (100)		0	0

Correlation with DIF and HPE

VBD	No. of Cases	DIF Positive Cases (No and %)	Pattern	Strong Staining	Moderate Staining	Weak Staining	IgG	IgA	(+) Ig M	(+) Ig A	IgG+ C3
PV	22	20(90.9)	ICS	12	6	2	6		3	2	12
BP	16	15(93.7)	Linear BMZ	10	4	1			2	2	10
PF	6	6(100)	ICS	3	3	0			2		
VEG	3	3(100)	ICS	2	1	0					
CBDC	2	2(100)	Linear BMZ		2			1	1	1	
B SLE	1	1(100)	Granular BMZ		1						1

Types of Immunofluorescence Staining *Pemphigus Vulgaris*

8/ 22 cases (36.3 %) showed (Inter-cellular substance) ICS staining pattern restricted to the lower third of the epidermis while the rest of the 13 cases (63.6 %), which had shown a positive reaction, showed full thickness ICS pattern of staining. 9/22 cases (40.9 %) showed a weaker ICS staining pattern for C3 when compared to IgG. One case had staining with IgM in addition to IgG and C3 while another case showed positive reaction with both IgM and IgA along with IgG and fibrinogen. 1/22 cases were negative for DIF.

Bullous Pemphigoid

Of the 15 cases showing a positive reaction (93.7 %), 12 cases (75 %) showed linear basement membrane zone (BMZ) pattern of staining with IgG and C3 alone (with varying intensity between IgG and C3). Positive reaction with IgM was seen in two cases, while another two cases showed positivity with IgA 1/ 16 cases was negative for DIF.

Pemphigus Foliaceous

5/ 6 cases (83 %) showed ICS pattern of staining restricted to upper third of the epidermis while one case showed full thickness ICS staining pattern. IgM was positive in two cases in addition to IgG.

Pemphigus Vegetans

These cases showed moderate to strong full thickness ICS staining pattern with IgG and weak ICS staining pattern with C3.

Chronic Bullous Dermatoses of Childhood

One case showed weak positive linear BMZ pattern of staining with IgM and IgG in addition to moderately positive staining pattern with IgA. The other case showed moderately strong linear BMZ pattern of staining with IgA alone.

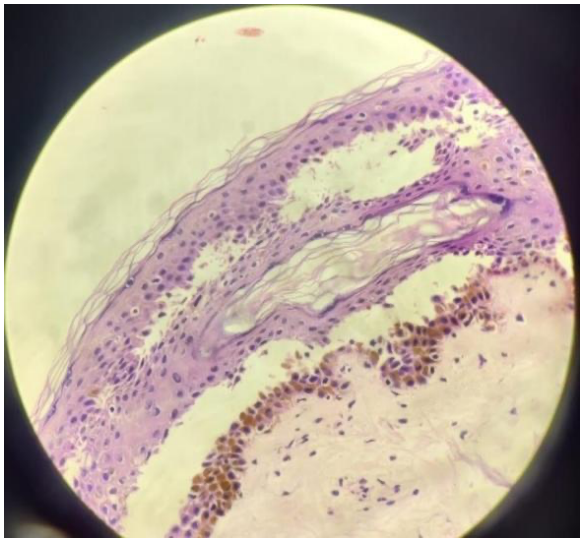

Figure 1: *Pemphigus vulgaris* X40 H&E

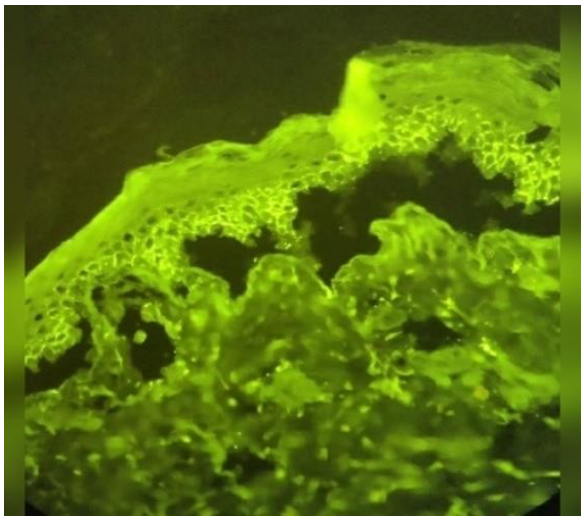

Figure 2: DIF -Intercellular Deposits OF Ig G

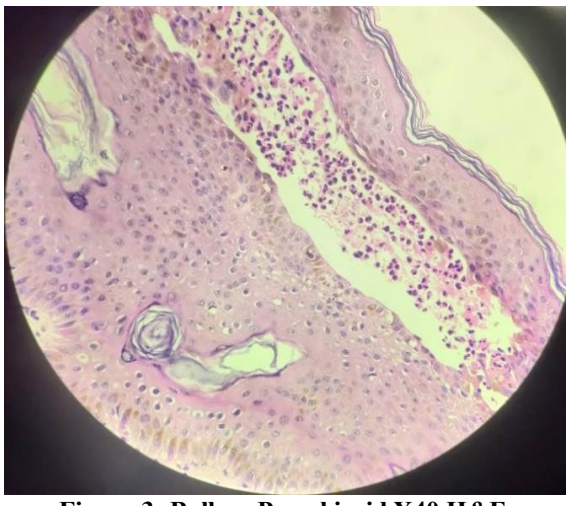

Figure 3: Bullous Pemphigoid X40 H&E

Discussion

Pemphigus vulgaris

20/22 cases (90.9 %) were positive for DIF showing an ICS pattern of staining within the epidermis. One of the patients with negative DIF was 26 year old female who had presented with multiple flaccid blisters all over the body with involvement of the oral mucosa for a period of three months. Histopathology showed features characteristic of *pemphigus vulgaris.*

Other patient was 30 year old male with blisters restricted to back and lower limbs and face with involvement of oral mucosa. Histopathology diagnosis was pemphigus vulgaris.

Of the 20 cases that were positive with DIF, two cases showed discordant findings with histopathology. One of these two cases that did not show a blister, showed an intact epidermis with a separated strip of degenerate epithelium.

The other case was from the oral mucosa that showed a band-like dense infiltrate of lymphohistiocytes in lamina propria with surface epithelium showing spongiosis with no evidence of blister formation. The findings were consistent with oral lichen planus. DIF showed focal weak ICS pattern of staining with IgG alone.

Bullous pemphigoid

15/ 16 cases (93.75 %) showed a positive linear BMZ pattern of staining on DIF. The case which showed negative reaction was of a 32 year old female with tense bullae on both hands. Histopathology showed features of *bullous pemphigoid.*

Pemphigus foliaceous

5/ 6 cases (83 %) showed ICS pattern of staining restricted to the upper 2/3rd of the epidermis on DIF. Another case showed full thickness ICS pattern of staining but had sub-corneal blister on histopathology with presence of acantholytic cells. One of the three cases with the characteristic DIF pattern, on biopsy showed epidermal collections of neutrophils with absent granular layer. The vesiculo bullous diseases are group of autoimmune diseases in which components of epidermis and basement membrane zone are the main focus of attack, resulting in formation of cutaneous and mucosal blisters. Early diagnosis and treatment of these disorders which are severe and potentially life threatening disorders are vital.

Several disorders are under the category of vesiculobullous diseases and are classified as intra-epidermal and sub-epidermal blistering disorders. Intra-epidermal blistering disorders, other than regulated by immunological mechanisms, include inherited disorders like Darier's and Hailey-Hailey disease. Secondary damage due to severe intercellular oedema can cause acantholysis, as seen in spongiotic dermatitis along with transient acantholytic dermatosis. Sub-epidermal blistering disorders include epidermolysis bullosa congenita which are mechanobullous disorders along with immunobullous lesions.

Pemphigus vulgaris

The predominant lesion of the fifty cases involved in the study was *pemphigus vulgaris* with 22 cases (44 %). This is in concordance to a few studies done earlier in India.[1] DIF was positive in the majority of cases (20/22 cases, 90.9 %), similar to the study conducted by Krasey et al. *Pemphigus vulgaris* is the most common vesiculobullous disorder similar to the studies conducted by Nanda A et al., Chams-Davatchi et al. Inchara YK et.al.

The mean age of the patients was 34 years which is consistent with that of Western literature and studies conducted in India.

Though previous studies show no sex predilection, our study showed a predominance of females with 12/22 cases (54.5 %) being women. Similar results were observed in a study conducted in Libya. Histopathology showed 19/22 cases (86.3 %) with a suprabasal blister and 10/22 cases (45.4 %) showed acantholysis extending into follicular epithelium

DIF showed strongly positive intercellular pattern of staining (ICS) in 12/19 cases. Most cases showed positive reactions with both IgG and C3. IgM and IgA were positive in few cases along with IgG. Isolated IgA and IgM can also be positive in *pemphigus vulgaris* as demonstrated by Judd and Lever which was not seen in our present study. One case showed non-specific findings on histopathology with DIF positive. It was from the perilesional skin of symptomatic patients clinically suspected to have pemphigus. In this case, biopsy had intact epidermis with mild dermal inflammation.

There was also a separate strip of degenerate epithelium lying detached from the skin in the case. Since the case had given a strong positive reaction with DIF, the patients were started on treatment for pemphigus vulgaris, but subsequent follow up was lost. It is possible that histopathology was inconclusive in these cases due to either epidermal regeneration The other patient had only oral erosions with no skin lesions. The oral mucosa of the other patient, who also had erosions over both legs, histopathological diagnosis was given of oral lichen planus. In this patient, DIF showed weak ICS pattern with IgG alone. Based on clinical and DIF findings, both patients were started on treatment for pemphigus vulgaris, but were subsequently lost on follow-up. Patients with only oral lesions can be seen in the initial phase of the disease showing only a dense band-like lympho-histiocytic infiltrate within the lamina propria, without classical histo-

logical features. Hence, in the absence of the characteristic histopathological features, which could be due to various causes, DIF becomes an important modality in the diagnosis of *pemphigus vulgaris* described by Maurer et al.

Table 5: Comparison of the Results of Our Study with Those of Other Studies

	STUDIES	PV	BP	PF	VEG	CBD	BSLE
1	Nanda A et al[2]	48 %	27 %				
2	Chams-Davatchi et al	91.9 %	2.7 %				
3	Inchara YK et.al	29 %	22 %	7 %		4 %	2 %
4	Present study	44 %	32 %				

Analysis of *Vesiculobullous Disorders*

Lesions	Soner Uzun et al[3]	Arti Nanda etal	SM Lagan et al.[4]	Present study
PV	18 - 70 years	25 - 48 years	21 - 102years	1 – 70
BP	49 - 89 years	49 - 83 years		21 – 80
PF	30 - 70 years	24 - 50 years	23 - 102 years	21 – 70

Age Comparison of VBD with Other Studies

S no	Features	No. of Cases	% of Total
1	Present study	16	32 %
2	Nanda A et al. Study	43	27 %
3	Chan PT et al.[5]	149	63.7 %
4	Bernard. P et al.[6]	94	73.4 %

Bullous Pemphigoid Cases Comparison with Other Studies

	Features	Present Study	Nishioka K et al. study[7]
	Subepidermal blister	75 %	100 %

HPE Change in *Bullous Pemphigoid*

	Studies	No. of Cases of BP	DIF Done	DIF Positive
1	Present study	16	15	93.75 %
2	Kippes W et al.[8]	115	115 (100 %)	115 (100 %)
3	Cozzani E et al.[9]	32	32 (100 %)	32 (100 %)
4	Marazza et al.[10]	140	134 (95.7 %)	128 (95.5 %)

DIF Positive of *Bullous Pemphigoid*

	Features	Fernandez J et al.	Arya SR et al.	Jamila Aboobaker et al.[11]	Present study
1	Number of cases	18 (18 %)	25 (35.7 %)	62 (55.3 %)	16 (32 %)
2	Sub corneal bulla	80 %	60 %		4 (67 %)
3	Acantholysis	100 %	96 %		4 (67 %)

Histopathology Changes in PF

		No. of Patients with PF	DIF Done	DIF Positive
1	Chams-Davatchi C et al. Study	89	34 (38.2 %)	30 (88 %)
2	Inchara et al.	7	7	7 (100 %)
3	KanwarAJ et al. Study	2	2	2 (100 %)
4	Present study	6	6	6 (100 %)

DIF Positive in PF

Age distribution of *bullous pemphigoid* (21 - 80) was greater than *pemphigus vulgaris* (170) suprabasal bulla was observed and was higher than Arya SR et al.[12] and less than Handaet al.[13] Acantholysis was seen less on comparison with both studies.

DIF was positive in 90.9 % patients which was similar to Chams Davatchi et al., Kumar S et al. KanwarAJ et al.[14] studies.

Bullous pemphigoid

The second most common lesion observed in our study group was bullous pemphigoid (16/50 cases, 32 %) with the mean age of presentation being 60 years. Only one patient aged between 20 - 30 years. Earlier studies and literature from western John's hospital, Bangalore, India showed same pattern with higher number of cases with pemphigus vulgaris. The affected patients were predominantly females (75 %). DIF was strongly positive in 15/16 (93.7 %) cases. All positive cases showed linear staining in the basement membrane zone (BMZ)

The deposits were of homogenous linear type. Complement component C3 was present in all 12 positive cases in combination with IgG. Studies by Jordan et al. and Chorzelski and Carmone have shown that C3 was present in almost every single case of bullous pemphigoid when compared to pemphigus vulgaris, where a minority of cases was invariably negative. IgA or IgM were present in

four cases along with IgG and C3. One case was negative for DIF.

Correlation between DIF and histopathology was high. 15/16 histopathological diagnosis matched with DIF with most cases showing a subepidermal bulla (12/16). Eosinophils (12/16) were the predominant inflammatory cells. Four cases showed a sparse dermal inflammatory infiltrate (cell–poor pattern). The case which was negative on DIF and histopathological diagnosis favouring bullous pemphigoid dermatitis herpatiformis was one of the differential diagnoses and had subepidermal bulla along with dermal inflammation composed of eosinophils and neutrophils. Other case was positive on DIF but histopathology did not give diagnosis as only dermis was included. patient was treated as bullous pemphigoid patient and follow up was lost.

In our study, bullous pemphigoid constituted about 32 % of the cases which is more than Nanda A et al. study and less than other studies due to lesser sample size. In our study, sub epidermal blister was seen in 75 % cases. In our study, DIF positive was 93.75 % which was almost similar to other studies.

Pemphigus foliaceous

Of the six cases of pemphigus foliaceous, four cases were between the ages of 51 and 70. One case was of 26 year old. The age group affected by foliaceous is usually quite variable. There was equal distribution of both sexes which was in concordance with earlier studies. All six cases showed ICS pattern of staining, of which five had staining restricted to the upper 2/3rd of the epidermis. The sixth case had full thickness ICS pattern of staining. 4 cases showed a sub-corneal blister on histopathology. One case did not match with histopathological diagnosis but was positive DIF on histopathology pustular psoriasis diagnosis patient was treated with common treatment regimen both for psoriasis and pemphigus foliaceous and no new lesions developed after treatment. Biopsies from patients with pemphigus foliaceous may occasionally show only collections of neutrophils in the sub corneal plane and hence may mimic sub corneal pustular lesions like pustular psoriasis so differential diagnosis becomes very important.

In our study, sub corneal bulla was similar to Arya SR et al. and acantholysis did not match any of other study. In our study, DIF was positive in 100 % cases which matched with majority of our comparison studies.

Pemphigus vegetans

We had three patients, three female patients, one patient presented with erosions from axilla and other patient presented with oral lesions and third patients also had lesions over axilla legs and oral mucosa both histopathology and DIF correlation was done. Histopathology and DIF gave same diagnosis as pemphigus vegetans. Histopathology showed supra-basal blister with epidermis exhibiting hyperkeratosis, papillomatosis marked acanthosis and acantholysis was seen extending into follicular epithelium. DIF showed moderately strong ICS staining pattern with IgG and C3 involving entire thickness of the epidermis.

Pemphigus vegetans is clinical variant of *pemphigus vulgaris* and carries better prognosis. In addition to the clinical findings, histopathology may show a few characteristic features such as marked acanthosis, pseudoepitheliomatous hyperplasia and multiple epidermal micro abscesses which distinguish it from *pemphigus vulgaris*

Chronic bullous dermatosis of childhood

There were two cases of chronic bullous dermatosis of childhood – A boy and a girl aged 12 and 8 years respectively both patients had vesicles and annular distribution, pruritis and post inflammatory pigmentation. Histopathology showed sub-epidermal bullae with neutrophil rich dermal inflammatory infiltrate. DIF showed moderately strong homogenous linear pattern of staining along the basement membrane zone with IgA.

One of the cases showed IgA staining and focal linear BMZ staining with IgG and IgM. The weak IgG along with IgA in one of the patients went towards childhood bullous pemphigoid as a possibility since they too can present with linear IgA deposits but the staining intensity of IgA is more and C3 was negative according to studies done by Beutner et al. are more suggestive towards chronic bullous dermatoses of childhood.

***Bullous* SLE**

This patient was 45-year-old female who presented with pruritic raised lesions over extensor aspect of both upper extremities. Some of the lesions developed into blisters. Photosensitivity was present (ANA) and was negative. Histopathology report showed subepidermal vesicle and a lymphocyte predominant perivascular inflammatory cell infiltrate with mild vasculitis in the dermis. Alcian blue with periodic acid Schiff (PAS) showed mild increase in dermal mucin. DIF suggested moderately strong granular BMZ pattern of staining with both IgG and C3 Granular BMZ staining can be seen in up to 40 % of cases when compared to linear deposits. Although a salt-split skin was negative with DIF, patient was on dapsone, and she responded well and did not develop any further lesions.

Conclusion

Vesiculobullous diseases are group of disorders in which primary lesion is either vesicle or a bulla, on the skin or mucous membrane or may be both. They form a bulla either in epidermis (suprabasal or sub corneal) or beneath the epidermis. *Pemphi-*

gus vulgaris is the most common subtype of vesiculobullous disorder in the present study followed by bullous pemphigoid. Histopathological examination showed features of each subtype of vesiculobullous disorder.

Clinical examination is the first step in making the diagnosis of vesiculobullous disorders. Histopathological examination and direct immunofluorescence are useful in definitive diagnosis of vesiculobullous disorders. Direct immunofluorescence is useful in confirmation of diagnosis. DIF features are almost same in all types of pemphigus group of disorders, and thus histopathology remains cornerstone in diagnosing. Considering the socio-economic status of the patients and unavailability of immunofluorescence technique in many centres, clinical diagnosis and histopathology are generally enough for arriving at the final diagnosis. DIF is only used as supplement and not a substitute. It is important to distinguish each of these separate entities for appropriate management, failure of which in few cases is associated with significant mortality and morbidity. However, a large sample sized study is essential to confirm these observations.

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