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Pathology

A STUDY OF VESICULOBULLOUS LESIONS IN A HIMALAYAN REGION

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ABSTRACT Vesiculobullous (VB) lesions are a group of disorders with wide spectrum and varied pathogenesis which eventually result in common clinical presentation of vesicle, bullae, papules and pustules. Variety of lesions are included in this group of disorders. Broadly VB lesions can be Immunobullous or non-immunobullous and require clinical, Histopathological and Direct Immunoflourescence examination for diagnosis. This study is an attempt to determine the spectrum of vesiculobullous lesions along with their histopathological and Direct immunofluorescence findings in our institute located in a Himalayan region. A total of 56 patients were clinically suspected of having VB disorder out of which 45 patients were confirmed on histopathology and/or DIF examination. An concordance study of clinico-histopathological-DIF was done which revealed concordance to be 82.6%. We concluded that the combination of clinical, histopathological and DIF gives the best results while dealing with this group of enigmatic disorders.

KEYWORDS : Vesiculobullous, Spectrum, Histopathology, DIF, Correlation

INTRODUCTION

Vesiculobullous (VB) disorders are one of the most important primary morphological patterns of skin reaction to various external and internal pathological stimuli.[1] These lesions are caused by immunological (autoimmune) or non-immunological (inflammatory, infective, drug induced as well as genetic) mechanisms.[1 - 4] These vesiculobullous lesions are characterized by the presence of blisters (vesicles<0.5cm and bullae>0.5cm) which are fluid filled cavities formed within or beneath the epidermis.[1,5,6] Spongiosis, acantholysis, reticular degeneration, cytolysis and basement zone destruction either alone or in combination leads to the formation of these blisters.[7,8] Histopathology in combination with clinical features, and aided by DIF which is considered as gold standard is generally required for diagnosis of these enigmatic disorders.[9-16]

Histopathological evaluation of a blister involves systematic analysis of skin biopsy which includes blister separation plane, mechanism of blister formation and character of inflammatory cell infiltrate while DIF determines the site (intraepidermal, basement membrane zone etc.) and pattern (linear granular etc.) of immunoglobulin and complement deposits in the skin.[2,14,17-20]

This study was conducted in a tertiary care hospital in a Himalayan region to determine the clinicopathological spectrum and evaluate various (Clinical, Histopathological and DIF) findings of these lesions.

MATERIALS & METHODS

Study design:

Hospital based cross- sectional observational study carried out on all new patients, clinically diagnosed as having a Vesiculobullous disorder(all age groups) and whose biopsies were sent to the Department of Pathology in one year formed the study cohort. Patients with traumatic blisters, patients on therapy and Immunocompromised patients were not included in study.

Ethics:

Permission was taken from the ethical committee of the institute. Written consent was taken after reading out the patient information sheet and consent to the patient in the language they understood which included the information about the disease, procedures to be carried out rationale of the study and rights of the patient.

METHODS:

Two skin punch biopsies were taken from each patient clinically suspicious of having VB disorder. A lesional biopsy from the junction of newly formed uncomplicated lesion was taken put immediately in 10% neutral buffered formalin and transported to department of Pathology for further processing[21] and routine H&E staining.[22] Slides were examined under routine light microscope and a histopathological diagnosis was given.

Perilesional biopsy was taken within 2cm of the lesion and transported immediately to Department of Pathology in normal saline where it was embedded in OCT (optimum cutting temperature) medium and 4-5µm section were cut using cryostat(biogenex®).

Multiple slides were prepared with two sections on each slide and immustaining was done using IgG, IgM, IgA and C3(FITC, Bigenex®) by following the procedure described by Seema chabra et al[17]. And the slides were examined under fluorescent microscope in terms of type of antibody , pattern and intensity of deposition.

Statistical Analysis

Data was entered in Microsoft Excel and was further transported to Epi info version 7 software. Percentages and proportions with respect to gender, type of disease, site and for other variables of concern were calculated. Sensitivity and specificity with 95% confidence interval of histopathology with reference to direct immunofluorescence was calculated in autoimmune vesiculobullous disorders.

RESULT

During the study period a total of 70,325 patients attended the skin OPD in our Institute. Out of these 59(.09%) patients were clinically suspected of having vesiculobullous lesions.

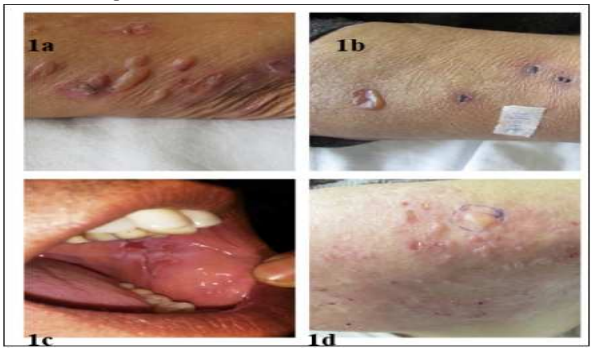
Biopsy was done in all (59) patients which comprised 9.8% of the total 615 skin biopsies received in the Department of Pathology in the same period.

Of these 59 patients, 45(76.27%) patients were confirmed (38 immunobullous and 7 non immunobullous) and subcategorized alone on histopathology and/or on DIF examination while 14(23.72%) biopsies did not show features of VB lesions (6 cases no definite diagnosis given, 8 cases other dermatological disease diagnosed).

Table 1 provides the details of the spectrum of the VB disorders. The most common lesion was Bullous Pemphigoid, followed by Pemphigus Vulgaris.

Table 1: Spectrum of Vesiculobullous lesion			
Diagnosis	Cases (n=45)	Percentage (%)	
Immunobullous disorders (total=38) (84.4%)	Bullous Pemphigoid (BP)	10	22.2
	Pemphigus Vulgaris (PV)	8	17.7
	Pemphigus Foliaceus (PF)	6	13.3
	IgA Pemphigus(IgA-P)	3	6.6
	Dermatitis Herpetiformis (DH)	6	13.3
	Erythema Multiforme (EM)	2	4.4
	Epidermolysis Bullosa Acquisita (EBA)	1	2.2
	Porphyria Cutanea Tarda	1	2.2
	Linear IgA disease	1	2.2
Non-immunobullous disorders (total=7) (15.6%)	Darier disease (DD)	3	6.6
	Hailey-Hailey disease (HHD)	2	4.4
	Bullous Fixed Drug Eruption (BFDE)	1	2.2
	Acute Spongiotic Dermatitis (ASD)	1	2.2

There was a male preponderance with M: F of 1.8 :1 and the lesions were most common in 31 to 50 year age group where 19(42%) cases were noted. Most 20(44.4%) patients presented within 2 weeks to 6 months of starting of the lesions and the most common presenting lesion was vesicles 45 (100%), followed by bullae 23(51.1%), Papule 18 (40%) and pustule 4 (8.9%) . Itching 19(42%) was another common complaint. The most common site of lesions was trunk 31(68.9%) followed by limbs 29 (64.4%) whereas mucosal involvement was seen in 4 belonged to Pemphigus Vulgaris. Figure 1a-1d shows few of the lesions in the patients.



1a: Bullous Pemphigoid: Multiple discrete, bullae and vesicles with evidence of erosion at places.

1b: Pemphigus: three well defined erythematous plaques and a single blister.

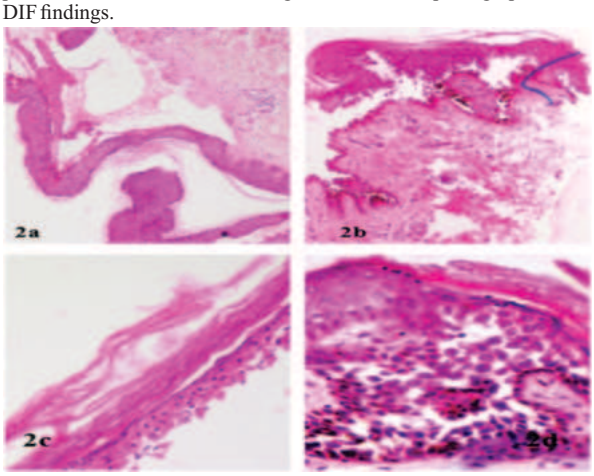
1c: Pemphigus: Oral lesion with ulceration.

1d: Dermatitis Herpetiformis: Papules, Plaques and blisters over the background of diffuse Erythema.

All biopsies of 45 cases were subjected to histopathological examination and a definite diagnosis was given in 39 (86.6%) cases the rest of cases were diagnosed on the basis of DIF findings. The biopsies were analysed for several parameters like plane of separation, predominant inflammatory cell infiltrate amongst others.

The most common site of separation was subepidermal in 19 (42%) cases followed by suprabasal in 11 (34.4%) cases. Acantholysis was seen in Pemphigus group of diseases (PV,PF,IgA Pemphigus) Darier disease, and HHD. All the cases of PF, HHD and Darier disease revealed acantholysis. Neutrophils were the most common inflammatory cell with 15(33.4%) of cases, while lymphocytes, eosinophils and a mixed inflammation was seen in 10 (22.2%) cases each. Hyperkeratosis of epidermis was the commonest additional histopathological finding seen in 9 (20%) cases followed by Acanthosis in 8(17.7%) cases. Dyskeratosis was seen exclusively in all cases of Darier disease. Figure 1a to 1d shows few of the histopathological findings in the cases.

DIF was performed on the perilesional biopsies in all vesiculobullous lesions. A Positive DIF was seen in 37/45 (82.2%) cases; all of which were Immunobullous lesions. However a single case of DH diagnosed on histopathology showed a negative DIF examination. The DIF was analyzed in terms of type of immunoreactant present, intensity of staining along with site and pattern of deposition. The details are provided in table 2 and table 3. Figure 3 shows few photographs of the DIF findings.



2a: Bullous Pemphigoid: Subepidermal plane of separation in Bullous Pemphigoid. (H&E, 100X)

2b: Pemphigus Vulgaris: Acantholytic cells in blister cavity and row of tombstone appearance of basal keratinocytes. (H&E, 100X)

2c: Pemphigus foliaceus: A subcorneal bullae with acantholytic cells. (H&E, 400X)

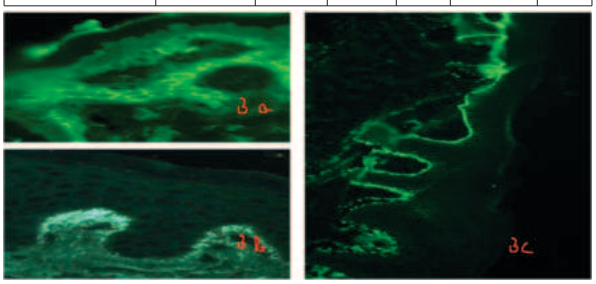
2d: Hailey-Hailey disease: subrabasal bullae with acantholytic cells in a dilapidated brick wall appearance. (H&E, 400X)

Table 2 : Type of immunoreactant deposited and intensity of deposition									
Diagnosis	Type of immunoreactant					Intensity			
	IgG	IgA	IgM	IgG/IgM +C3 (type of antibody)	negative	1+	2+	3+	4+
Pemphigus Vulgaris(n=8)	4 (50%)	0	0	4(50%) (IgG+ C3)	0	0	4(50 %)	4(50 %)	0
Pemphigus foliaceus (n=6)	6 (100 %)	0	0	0	0	0	1(16. 7%)	5(83 .3%)	0
IgA Pemphigus (n=3)	0	3 (10 0%)	0	0	0	0	2 (66. 7%)	1(33 .3%)	0
Bullous Pemphigoid (n=10)	2 (20%)	0	0	8(80%) (IgG+ C3)	0	0	5(50 %)	5(50 %)	0
Dermatitis herpetiformis (n=6)	0	5(8 3.3 %)	0	0	1(16. 7%)	0	3(60 %%)	2(40 %)	0
Erythema multiforme (n=2)	0	0		2 (100%) (IgG+ C3)	0	0	1(50 %)	1(50 %)	0
Linear IgA disease (n=1)	0	1 (10 0%)	0	0	0	0	0	1 (100 %)	0
Epidermolysis bullosa acquisita (n=1)	0	0	0	1(100%) (IgG+ C3)	0	0	0	0	0
Prophyria Cutanea Tarda (n=1)	0	0	0	1(100%) (IgM+ C3)	0	0	1(100 %)	0	0

Total(38)	12(31.6 %)	9(23.7 %)	0	16 (42.1%)	1(2.6 %)	0	17(46 %)	20(54 %)	0	

Table 3 : Site and pattern of deposition of immunoreactant

Diagnosis	Site of deposition	Pattern of deposition				
	ICS	BMZ	blood vessels and ducts	linear	granular	Lace like
Pemphigus Vulgaris(n=8)	8 (100%%	0	0	0	0	8
Pemphigus foliaceus (n=6)	6 (100%)	0	0	0	0	6
IgA Pemphigus (n=3)	3 (100%)	0	0	0	0	3
Bullous Pemphigoid (n=10)	0	10 (100%)	0	10 (100 %)	0	0
Dermatitis herpetiformis (n=5) (1 DIF negative)	0	5 (100%)	0	0	5 (100%)	0
Erythema multiforme (n=2)	0	2 (100%)	2(100 %)	0	2 (100%)	0
Linear IgA disease (n=1)	0	1 (100%)	0	1 (100 %)	0	0
Epidermolysis bullosa acquisita (n=1)	0	1 (100%)	0	1 (100 %)	0	0
Porphyria Cutanea Tarda (n=1)	0	0	1(100 %)	1 (100 %)	0	0
Total(n=37)	17(45.9%)	19(51.4 %)	3 (8.1%)	13 (35.1 %)	7(19%)	17 (45.9 %)



3a : Pemphigus Vulgaris: deposition of IgG in a lace like fashion in the intercellular spaces: **fishnet pattern**. (DIF, 100X) **3b: Dermatitis Herpetiformis:** deposition of in the tips of dermal papillae at basement membrane zone in a granular fashion. (DIF, 100X) **3c**: **Bullous Pemphigoid:** Linear deposits of IgG seen along the Basement membrane zone towards the roof of the bullae. (DIF, 100X)

Finally, a correlation was made between the clinical, Histo pathological and DIF findings. Of the total 46 cases clinically suspected to be Immunobullous lesions 33(71.7%) were proved on histopathology (Clinical-Histopathological concordance) while 37 (80.4%) were proved on DIF examination (Clical-Immuno flourescence concordance).Among the 37 cases diagnosed on DIF 33(89.2%) showed same results on histopathology (Histo pathological-Immunoflourescence concordance) and of the total 46 cases suspected to be VB lesions 38(82.6%) were proved by either histopathology or DIF(Clinical-histopathology-Immunoflourescence concordance).

On statistical analysis it was found that the positive predictive value of histopathological examination in diagnosing VB disorders, is 96.96%while negative predictive value is 61.54%.

The Sensitivity of histopathological examination is 86.49% while specificity is 88.89%. DIF being gold standard in diagnosis of Immunobullous lesions was found positive in 97.3% of cases and these findings in comparison to histopathology were statistically significant (p<.oo1) in the present study. (Table 4)

Table 4 Analysis of histopathology and immunofluorescence in immunobullous lesions

Disease	Immunofluorescence -Positive	Immunofluorescence-Negative	Total
Histopathology-Positive	32	1	33
Histopathology-Negative	5	8	13
Total	37	9	46

P<.001

DISCUSSION

The vesiculobullous disorders are a heterogeneous group of dermatosis [23]. Hippocrates first described these lesions as pemphigoides pyertoi[24], however in 1950's for the first time these lesions were subcategorized by Lever, when he differentiated BP from Pemphigus using histological criteria.[13]

The incidence of VB lesions reported by Kuldigi *et al*[23] was 0.128%, in our study it was .09% which is quite low but both these studies are hospital based studies and do not reflect the true incidence in general population ; the biopsies done for VB lesions generally vary from 4.16 % to 22.08 %. [2,4,24,25]

As far as the spectrum of VB lesions is concerned undoubtedly BP and PV are most common.[1,2,4,8,10,25-27] Though there is dispute amongst various studies regarding which is most common amongst them, Certain studies[2,18,24,28] found more cases of BP like Chananasayya *et al* [18] who had 46.2 % (n=91) cases of BP while others [4,8,23,25,26,28] had more cases of PV, Kuldigi *et al* [23] reported 62% (n=50) cases of PV. This variation can be attributed to sample size, geographical distribution and environmental factors . Environmental factors include exposure to pesticide, metal vapors and occupational exposure to UV which are the risk factors for Pemphigus.[8]

Most of the cases (42%) in present study occurred in 31-50 year age group as is also seen in studies by Patel *et al*[1], Mittal H *et al*[26] and Chopra D *et al*[28]. VB lesions are predominantly seen in males as is discerned in the present study and studies by Mohanty S *et al*[25], Murthy TK et al[2] and Kushtagi AS et al.[10] However Kudligi C et al[23],Patel PR et al[1] and Arundhati S et al[4] observed a female preponderance. In the present study number of cases of BP, DH and PF were more which are known to occur more in male patients. Hence, this could be the reason for a higher number of male patients in our study.

The time of presentation to seek medical advice varies from few days to several years and depends on awareness, severity of illness and accessibility of medical care.[3,8,28,29] Most of our patients presented within 2-6 months of illness and findings are similar to that observed by Chopra D *et al*[28] and Handa F et al[29]. Itching in autoimmune bullous lesions occurs due to inflammation caused by the autoimmune reaction. It is commonly seen in BP and the lesions of DH are extremely pruritic. Even PV and PF have an early pruritic phase. In the present study 42% patients complained of itching which is comparable to observations by Kudligi C et al[23].We had quite a large number of patients with papules as a presenting lesion, Mittal H *et al*[26] also had few cases of patients presenting with papules in VB lesions. Higher number of patients with papules in present study can be attributed to more number of cases of DH, Darier disease, HHD and IgA pemphigus in which papule is a common clinical complaint. Oral involvement in PV is found to vary from 25% to 84.7%[4,10,13,25,28],We observed 50% patients of PV with oral involvement.

Histopathology is considered gold standard for diagnosis in most of the dermatological disorders. VB lesions are one of the exceptions as DIF is considered the gold standard in their diagnosis; however histopathology is able to differentiate and diagnose most, if not all VB lesions. Non immunobullous VB lesions are primarily a histopathological diagnosis. Care should be taken during the punch biopsy procedure, since bullae roof is fragile and the sheer stress to the epidermis during biopsy may lead to separation of the epidermis /bullae roof . We had three (6.6%) such biopsies. Such biopsies were also encountered by Sharma G et al[13] but they did not include these cases in their study. We included these cases as eventually these patients turned out to be BP on DIF examination.

In our study the most common level of separation was subepidermal in 42% cases (7/8 Bp+6/6 DH+2/2 EM+1EBA+1 Linear IgA+ 1PCT+1BFDE). However literature is variable and range from 11.7% to 46.5%.[4,6,8,10,18,23,25,26,28] Our findings are in agreement with Chanabasaya V et al[18] who had 46.5% cases (all BP) with an subepidermal plane of separation. However higher than Arundhati S et al[4] who reported 11.7% of subepidermal bullae amongst all VB lesions. Ultimately the level of separation will depend upon the spectrum of diseases in the cohort and how many of the biopsies shows typical findings.

An inflammatory cell infiltrate is invariably present in the biopsies of VB lesions and is an important diagnostic clue towards the diagnosis. Certain lesions are typically associated with (eosinophils in BP,neutrophils in DH) one or the other inflammatory cell infiltrate which may be present inside the bullous cavity or the dermis.Neutrophils were the most commonly encountered inflammatory cell infiltrate in 33.3% of cases in the present study. The literature on this varies from from 12.2% to 72%.[2,10,23,25,26,28] Our observation was well within the range mentioned in literature and are closest to the findings of Kushtagi AS et al[10]. This too will mostly depend upon the spectrum of the diseases encountered.

DIF is a well established diagnostic technique in dermatopathology which detects wide variety of immunoreactants deposited in tissues. In the present study cohort a positive DIF was noted in 62.7% biopsies which is higher than observed by Kabir et al[9](50.4%) but less than Mittal H et al[26] (88.9%). The variation in positivity of DIF cases may be due to sample size, selection bias, technical issues and the spectrum of disease on which the DIF is performed. All but one case of DH showed positive DIF in our study (Table 2), the type and site of immunoreactant does of course vary depending on the type of disease present. The positivity of DIF in case of DH varies from 100% positive[24,26] to 100% negative[15,23,25] Special attention has to be observed in biopsy of DH as a lesional biopsy is always negative in DH due to degradation of immunoreactant by the inflammation. This can be attributed as the main cause of this variation.

The clinical-histopathology correlation in the present study was found to be 71.7% which is quite close to observation by Channabasaya et al[18] however Dhanabalan et al[24] Chopra D et al[28] and Mittal H et al[26] had higher clinico-histopathological correlation. The clinico-immuno flourescence correlation variees from 60.4% to 96.77% in literature, ours was 71.7%.[15,18,24,26,28] The Histopathology-immunoflourescence concordance was 89.2% in our study which is similar to many studies in literature.[15,18,24,26,28]

Clinical-Histopathological- Direct immunofluorescence concordance was 82.6% which is higher than both clinical-histopathological and clinical- immunofluorescence concordance. Hence all the three; clinical, histopathological and DIF findings are important in order to reach a final diagnosis in VB lesions.

CONCLUSION

Though clinical examination is the first step to make the diagnosis of VB lesions, histopathological examination and DIF are essential methods for definitive diagnosis and appropriate management of patients. DIF is more sensitive in comparison to histopathology but should always be used in conjunction with histopathology and clinical features since the combination of three yields the best results.

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