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Original Research Article

Utility of Immunofluorescence in Immune-Mediated Vesiculobullous Lesions of Skin

Anil Dev Pant[1*], Sudip Parajuli[2]

[1]Consultant Pathologist, Department of Pathology, Grande International Hospital, Kathmandu, Nepal
[2]Associate Professor, Department of Dermatology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

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***Corresponding author:** Anil Dev Pant
Consultant Pathologist, Department of Pathology, Grande International Hospital, Kathmandu, Nepal

Abstract

Bullous lesions may be due to various causes, including bullous pemphigoid, pemphigus vulgaris, linear IgA disease, connective tissue disorders, and other rare vesico-bullous skin diseases. Though histopathology can help narrow down possible diagnoses, direct immunofluorescence is required for a definite diagnosis based on positivity patterns of different immunoglobulins. In this study, 47 cases of clinically suspected immune mediated vesiculo-bullous diseases (IMVBD) were confirmed with histopathology. Females were more commonly affected and the age range was from 10 to 93 years. Among these cases, 23 were diagnosed as pemphigus vulgaris, l6 as bullous pemphigoid, 3 each as pemphigus foliaceus and linear IgA disease. DIF, including testing for c1q, was instrumental for the diagnosis of the disease in order for the patient to get appropriate treatment.

Keywords: Skin Biopsy, Vesiculo-Bullous, Pemphigus Vulgaris, Bullous Pemphigoid, Direct Immunofluorescence, Immunoglobulins.

BACKGROUND

Autoimmune blistering diseases are associated with an autoimmune response directed to structural proteins mediating cell-cell and cell-matrix adhesion in the skin. These blistering diseases are classified based on the site of deposition of immunoglobulins and on the molecular target of auto antibodies [1]. The commonest immune mediated vesiculo-bullous diseases (IMVBD) include pemphigus vulgaris (PV) and bullous pemphigoid (BP).

Pemphigus vulgaris is a fatal disease which affects skin and mucosa. The term pemphigus was first used by Hippocrates to describe pemphigoid fever as pemphigoides pyertoi. De Sauvages proposed the term pemphigus in 1760 which was derived from pemphix which in Greek means pustule. In 1953, Lever described another entity which he termed as bullous pemphigoid and differentiated it from pemphigus. This is of great historical and clinical significance as bullous pemphigoid usually has a much better prognosis than pemphigus which may be fatal [2].

The recommended method for DIF (direct immunofluorescence) is a 4 mm punch biopsy; a shave biopsy can be done but should include reticular dermis. For bullous pemphigoid, perilesional skin should be sampled and this was further validated by Anstey *et al*., who found adequate results for both pre-treatment and post-treatment cases. Center of the blister or uninvolved sites should be avoided. Part of the bullous lesion may be sampled together with perilesional skin. Perilesional skin should be 3 mm to 10 mm away from the edge of the blister. Preferred sites include the trunk and flexural skin of forearms [3].

For diagnosis of bullous pemphigoid, DIF has been more sensitive than other testing methods like indirect immunofluorescence or enzyme-linked immunosorbent assay. Comparing their sensitivities, DIF was the most sensitive (90.8%), followed by IIF (76%) and finally ELISA for BP230 and BP180 (with variable rates ranging from 59% and 73% respectively) according to Eltson *et al*., [4].

MATERIALS AND METHODS

Retrospective analysis of all skin biopsies sent for assessment of vesico-bullous lesions over a period of

4 years was done (2018-2022). Samples were received in 10% formalin for routine histopathology, and normal saline for DIF studies. Sections were cut and stained with routine Hematoxylin and Eosin. For DIF, frozen sections were cut and stained with respective antibodies to IgG, IgA, IgM, c3 and c1q. Scoring for DIF positivity was done on a scale of 0 to 4+ with scores more than 2+ considered positive. Slides were analyzed in their respective microscopic configurations and diagnoses were made according to the site of positivity, i.e. basement membrane, intercellular, or both.

RESULTS

A total of 135 skin biopsies were received at the Department of Pathology and Laboratory Medicine, Grande International Hospital over the period of 2018 to 2022 for histopathology and immunofluorescence studies. Among these, 47 cases showed microscopic features of IMVBD, of which 28 were female and 19 were male. Age of these patients ranged from 10 years to 93 years of age.

The following table (Table 1) shows a comparison of different vesiculo-bullous disorders and their clinical site of presentation.

Table 1: Clinical site of vesiculo-bullous disorders

CLINICAL PRESENTATION	PV/ PF (26)	BP (16)	Others (5)
Bullae over trunk/back	21	12	2
Bullae over limbs	16	12	2
Bullae over face / scalp	3	3	0
Oral bulla / ulcer	11	1	2

Key: PV=pemphigus vulgaris, PF=pemphigus foliaceous, BP=bullous pemphigoid

The most common site for the pemphigus group included the trunk/back whereas in bullous pemphigoid, both trunk and limbs were common sites. In addition, oral lesions were far more common in the pemphigus group.

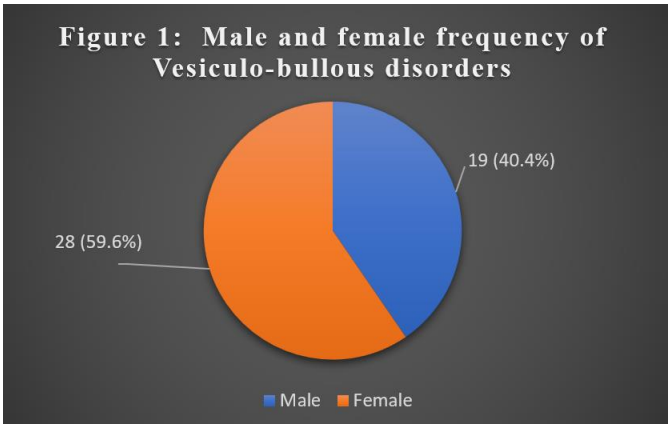

Figure 1: Frequency of vesiculo-bullous disorders according to sex

IMVBD were more common in females with a M:F ratio of 1:1.5.

Table 2: Age and sex distribution of vesiculo-bullous disorders

Age	Bullous Pemphigoid		Pemphigus Vulgaris / Foliaceous		Others		TOTAL
	M	F	M	F	M	F	
Up to 10	0	0	1	0	0	0	1
11-20	0	2	1	2	1	1	7
21-30	0	0	2	3	0	0	5
31-40	0	0	3	5	0	1	9
41-50	0	0	1	2	0	0	3
51-60	1	1	1	1	1	0	5
61-70	4	2	0	2	1	0	9
71-80	0	4	1	1	0	0	6
81-90	0	1	0	0	0	0	1
91-100	1	0	0	0	0	0	1
TOTAL	6	10	10	16	3	2	47

A wide age range was present in immune mediated IMVBD, with BP occurring most frequently in 61-80 years of age and pemphigus vulgaris/folliaceous occurring at a much younger age group of 21-40 years.

The following table (Table 3) shows positivity of different immunoglobulins in IMVBD.

Table 3: Positivity of different immunoglobulins according to different diseases

IMMUNOGLOBULIN	NUMBER OF POSITIVE CASES	PV/PF	BP	Linear IgA Disease	Others
IgG	43/47 (91.5%)	26/26 (100%)	14/16 (87.5%)	1 / 3 (33.3%)	2/2 (100%)
IgA	4/47 (8.5%)			3/3 (100%)	1 / 2 (50%)
IgM	1/47 (2.1%)				1 / 2 (50%)
C3	25/47 (53.2%)	9/26 (34.6%)	14/16 (87.5%)		2/2 (100%)
C1q	1/ 47 (2.1%)				1/2 (50%)

IgG was the most common immunoglobulin identified and showed 100% positivity in the pemphigus group and 87.5% of BP. C3 was the next most common immunoglobulin and it showed 87.5% positivity in BP and 34.6% positivity in the pemphigus group. As per definition, IgA was positive in all cases of linear IgA disease. A single case of bullous lupus (in the 'Others' group) showed a full house pattern with positivity of all five immunoglobulins along the dermo-epidermal junction. All other cases did not show any c1q positivity.

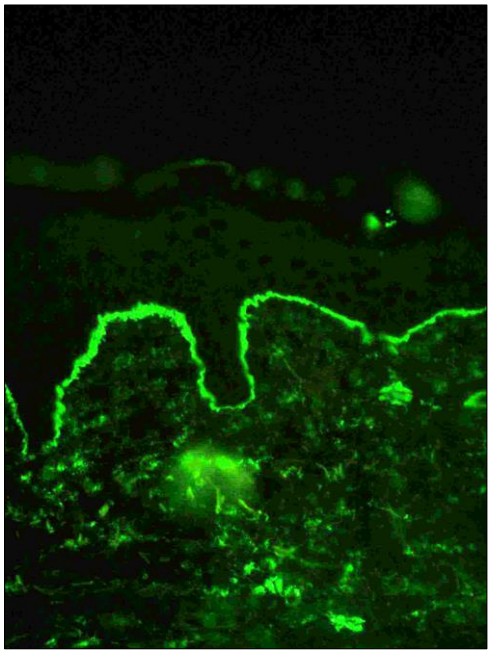

Figure 2: IgA positivity in Linear IgA disease. DIF findings in a case with Linear IgA disease showing linear staining of dermo-epidermal junction with IgA in the upper left region. Note the underlying dermis showing non-specific staining.

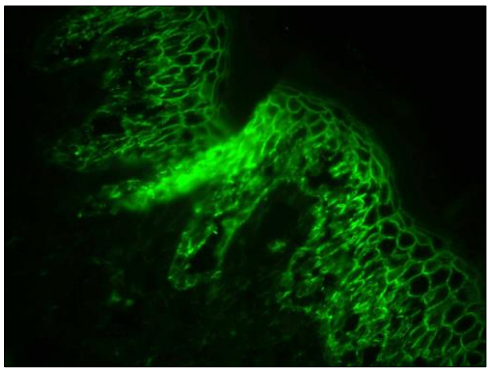

Figure 3: IgG in Pemphigus Vulgaris. DIF findings in a case of pemphigus vulgaris with typical fish-net pattern of IgG staining in between epithelial cells throughout the epidermis.

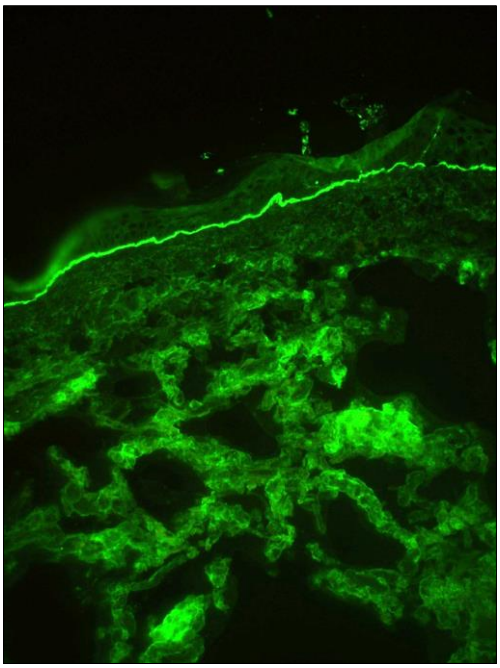

Figure 4: IgG in Bullous Pemphigoid. Case of bullous pemphigoid with linear staining of dermo-epidermal junction with IgG. C3 was also positive in the same region in this case.

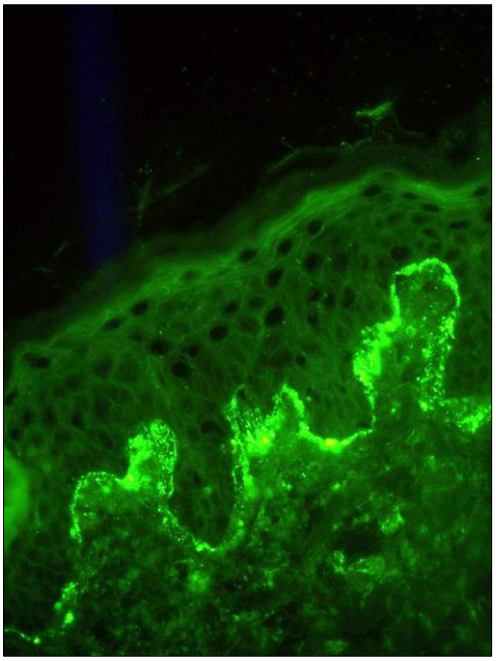

Figure 5: C1q in Bullous Lupus. Single case of bullous lupus showing positive band test for immunoglobulins IgG, IgA, IgM, and c3. The picture shows strong c1q positivity, which is usually not performed on skin biopsies.

Table 4: Frequency of final clinicopathological diagnoses of IMVBD

DIAGNOSIS	NUMBER OF CASES (%)
Pemphigus vulgaris	23 (48.9%)
Bullous pemphigoid	16 (34.1%)
Pemphigus foliaceus	3 (6.4%)
Linear IgA disease	3 (6.4%)
Bullous lupus	1 (2.1%)
Paraneoplastic pemphigus	1 (2.1%)
TOTAL	47 (100%)

The most common IMVID in this study was pemphigus vulgaris followed by bullous pemphigoid. A single case of paraneoplastic pemphigus was noted, which showed a mixed pattern of both BP and PV on DIF.

DISCUSSION

IMVBD can be associated with significant morbidity and mortality and it is important to recognize early clinical features for prompt and proper management [2-5]. Histopathology can help in diagnosis, but due to overlapping features, DIF is mandatory for accurate typing of these lesions [6].

In this study, the trunk and back were the most common sites for IMVBD followed by limbs. In some cases, oral lesions were also noted, more commonly in pemphigus vulgaris [7]. Some cases showed lesions all over the body and oral lesions as well. IMVBD involving the oral cavity may represent the oral manifestations of immune mediated dermatologic diseases. These lesions must be differentiated from other types of oral ulcerations as they may require specific treatment [8].

Females were more commonly affected in this study as compared to males accounting for 60% of cases. This was in accordance to a study by Ramalingam *et al*., who showed that IMVBD were more common in females which accounted for 54% of all cases [9].

Our patients were of a wide age range from 10 to 93 years. Though IMVBD are more commonly seen in middle aged to elderly patients, we should not exclude their possibility in children. BP occurred more commonly in the elderly age group while pemphigus group occurred more commonly in the middle-aged group. Similar findings were demonstrated in the aforementioned study [9].

IgG was the most common immunoglobulin in PV/PF and showed a typical fish-net pattern. This was similar to the findings by Deepti *et al*., in which 100% of cases showed fish-net pattern of immunoglobulin deposition [10]. Location of immune deposits was of utmost importance in order to help differentiate PV from PF. In PV, the fish-net pattern of IgG staining was mainly located in the lower half of the epidermis whereas it was more commonly in the upper half in PF. However, sometimes IgG staining may occur throughout the epidermis making distinction of pemphigus vulgaris vs foliaceous impossible on DIF.

Both linear C3 and IgG were the most common immunoglobulins in BP, present in the dermo-epidermal junction. This is in accordance with the study by Mejjer *et al*., in which 91.4% cases showed positivity for IgG and 73.6% cases were positive for c3.11 Similarly, Mysorekar *et al*., showed positivity for IgG and c3 as in our cases [12].

In IMVBD, Mejjer *et al*., demonstrated highest positivity of DIF in perilesional skin (90.4%) vs healthy skin (80.7%) and lesional skin (76.2%).11 All our cases were sampled from the perilesional skin, which was the ideal location as also shown by other studies [2-7].

Our study included a single case of paraneoplastic pemphigus who was a 56 year old man who presented with significant weight loss and oral lesions. DIF showed strong positivity of IgG and mild positivity for c3 in both patterns of BP and PV. After further investigation, the DIF findings and diagnosis were confirmed due to presence of underlying malignancy [14].

DIF staining for c1q was also included in the panel in our study. C1q has been shown to be an important marker of lupus cases in kidney biopsies. This has also been shown to be true in cases of leukocytoclastic vasculitis in the skin [15]. One of our cases also showed a full house pattern including c1q positivity which revealed a positive the lupus band test. We recommend the use of c1q in DIF testing of skin biopsies which may help confirm cases of lupus involving the skin, since c1q was negative in all the other cases in this study. Similar full house patterns, including c1q have also been seen in other studies of skin biopsies [16, 17].

CONCLUSION

IMVBD have been shown to occur in both sexes and may with a wide age range. Direct immunofluorescence is an invaluable tool for diagnosis of IMVBD and is required for targeted treatment according to their different types. Though PV and BP are the most common IMVBD, other rarer forms like lupus and paraneoplastic pemphigus should always be kept in the back of the mind. In addition, c1q testing also should be routinely done, to rule out any possibility of skin lesions due to lupus.

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