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A Study of Direct Immunofluorescence in Autoimmune Vesiculobullous Lesions with Clinico - Pathologic Correlation

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ABSTRACT

Vesiculobullous diseases are a group of disorders in which primary lesion is a vesicle or a bullae on the skin or mucous membrane or both. Vesiculobullous lesions are mostly immune mediated. Direct immunofluorescence (DIF) is an immunological technique which is used to identify such autoantibodies that are bound to target antigens in the tissue.

Aims:

To identify the immunofluorescence pattern in autoimmune vesiculobullous lesions.

To correlate with the histopathologic findings and clinical diagnosis.

Settings and Design: Descriptive study

Methods and Material:

This is a descriptive study done over the period of 2 years. Skin biopsies sent for both Histopathologic examination and DIF with clinical diagnosis of autoimmune vesiculobullous lesions were included in the study. The histopathologic and DIF findings were studied and correlated.

Results:

A total of 63 cases were included. Out of 63 cases, 51 cases (80.9%) showed DIF positivity and 12 (19.1%) cases were negative on DIF. Among the positive cases, Pemphigus vulgaris was the most common

[n=26 (50.9%)] followed by bullous pemphigoid[n=20(39.2%)]and 2 cases of pemphigus foliaceus and 1 case each of pemphigus vegetans , pemphigoid gestationis and linear IgA bullous dermatosis. 51 out of 63 cases(80.9%) correlated with clinical findings. All cases (100 %) correlated with histopathologic findings.

Conclusion

Clinical examination is the initial step in making a diagnosis of AVBL. Histopathology remains the cornerstone in diagnosis and DIF studies aid as a complementary yet gold standard diagnostic test for autoimmune vesiculobullous lesions.

Keywords

Autoimmune vesiculobullous lesions, Direct immunofluorescence, immunofluorescence pattern, pemphigus, pemphigoid.

INTRODUCTION

The Vesiculobullous reaction pattern is characterized by the presence of vesicles or bullae at any level within the epidermis or at the dermoepidermal junction.[1] Vesiculobullous lesions are mostly immune mediated and are termed autoimmune vesiculobullous lesions (AVBL).[2]

AVBL are characterised by pathogenic autoantibodies directed at target antigens. The function of these antigens is either cell-cell adhesion within the epidermis or adhesion of stratified squamous epithelium to the dermis. There is clinical overlap among various groups of bullous diseases. [3] Histological examination should be ideally performed on early vesicle which reveals the site of formation and also the presence, intensity and composition of the inflammatory infiltrate. [4]

Immunofluorescence studies have greatly contributed to the diagnosis, treatment, and understanding of the pathophysiology of vesiculobullous lesions of skin.[4] Direct immunofluorescence (DIF) is an immunological technique which is used to identify such autoantibodies that are bound to target antigens in the tissue. [5]The relative simplicity and accuracy of the technique has made direct immunofluorescence an important technique in the diagnosis of bullous diseases. [6]

MATERIALS AND METHODS

The present descriptive study was conducted during the period of 2 years at a tertiary care hospital attached to medical college. All patients with clinical diagnosis of AVBL were included for the study. The detailed history including age, gender, occupation, personal and family history, presenting complaints, duration, general condition and findings on clinical examination were recorded from the medical case files. Skin biopsies, one from the vesicle (fixed in 10% buffered formalin) and another from the perilesional area (normal saline) sent to the Department of Histopathology were included.

Lesional biopies were subjected to routine processing and stained with haematoxylin and eosin(H&E).

Frozen sections 5 μm in thickness were cut from the perilesional biopsy with the freezing microtome and placed on slides. These were air dried for 10 min, then washed in phosphate-buffered saline (PBS) at a pH of 7.4 for 10 min and then were fan-dried once more and incubated with monospecific fluorescein isothiocyanate-labeled antisera for 30 min at 37°C. Antisera IgG, IgA, IgM, the C3 were employed. The sections were observed under fluorescence microscope.

The class of immunoglobulins and/or complement and their pattern and location were recorded.

Clinicohistopathological correlation was done for all cases.

RESULTS

A total of 63 cases were included in the present study. The Age range was 18 to 80 years with mean age being 38 years. There was a female preponderance with Male to female ratio of 0.8:1.

Pemphigus vulgaris was the most common lesion [n=26 cases(50.9%)].All these cases showed similar histopathological findings which included suprabasal bullae and intact row of basal cells (row of tombstone appearance) with few acantholytic cells within the bullae. Some cases also had neutrophils and/or eosinophils within the bullae.(fig 1a).All of these cases showed IgG and/or C3 lace like positivity intraepidermally on DIF. (fig 1b)

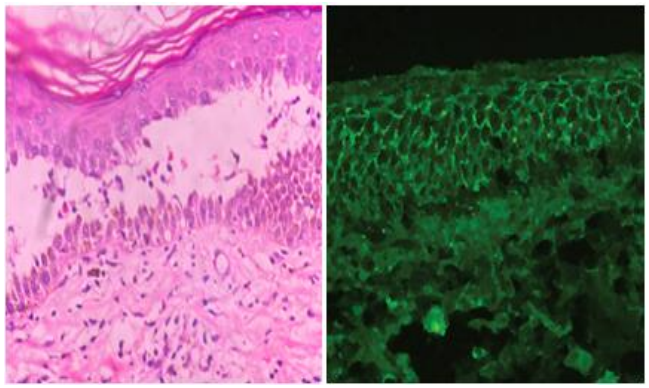

Fig 1 – (a) Suprabasal bullae with few acantholytic cells within and an intact row of basal cells (row of tombstone appearance).(H&E, 400x). (b) DIF showing lace like IgG positivity in the intraepidermal location.

Bullous pemphigoid was the second most common lesion. [n=20 cases(39.2%)] All these cases on histopathology showed subepidermal blister with variable number of eosinophils within the blister.(fig 2a).On DIF, the findings were IgG and/or C3 positivity with a linear homogenous deposition along the basement membrane.(fig 2b)

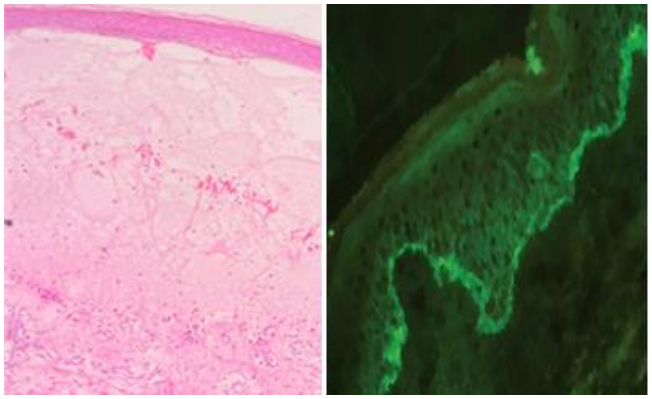

Fig 2 – (a) Subepidermal bullae with few eosinophils within. (H&E, 200x). (b)DIF showing linear IgG deposit along the basement membrane.

2 cases of were reported as Pemphigus foliaceus. The histopathologic finding in these cases was a subcorneal bullae containing occasional acantholytic cells.(fig 3a)On DIF, these cases showed IgG and C3 positivity in a lacelike pattern within the epidermis which was more pronounced in the superficial layers.(fig 3b)

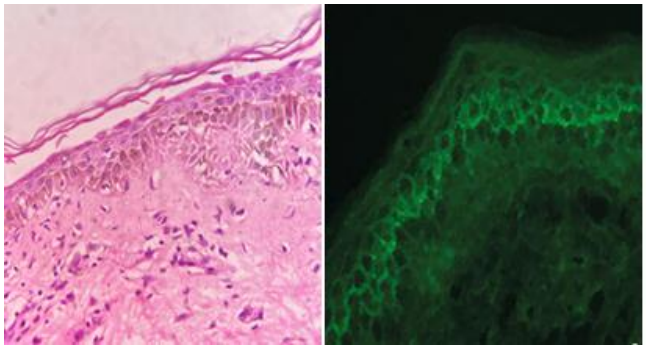

Fig 3- (a) Subcorneal bullae. (H&E, 400x). (b) DIF showing lace like IgG positivity in intraepidermal location but more pronounced in the superficial layers.

The present study also had 1 case each of pemphigus vegetans, pemphigoid gestationis and linear IgA bullous dermatosis.

Pemphigus vegetans had intraepidermal vesicle with occasional eosinophils with in. DIF findings were similar to those seen in Pemphigus vulgaris with intercellular IgG and C3 deposition.

Linear IgA bullous dermatosis showed subepidermal blister with prominent neutrophilic collection within.(fig 4a) DIF studies showed homogenous and linear IgA positivity along the basement membrane. (fig 4b)

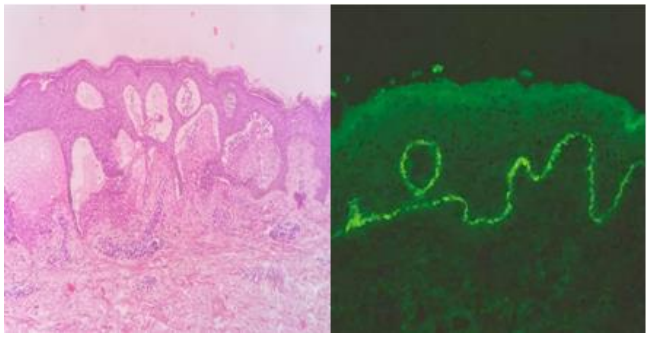

Fig 4- (a) subepidermal blister with prominent neutrophilic collection within. (b) DIF showing Linear IgA positivity along the basement membrane.

The histopathologic diagnosis of all the above cases[51/51 (100 %)] correlated with the corresponding DIF findings.

However 12/63 (19.1%) cases were negative on DIF studies and did not correlate with clinical diagnosis.

On histopathology 2/12 cases were diagnosed as lichen planus, 1 case as Polymorphic light eruption,1 case as reactive perforating collagenosis,1 case as acquired perforating dermatosis,1 case as eczema, 1 case as lymphangioma, 1 case as eosinophilic spongiotic reaction.

4/12 cases had nonspecific features on histopathology and were asked to correlate clinically.

Hence overall, 51/63 cases (80.9%) were considered as "correlating with clinical diagnosis".

Table 1- Distribution of cases which were positive on DIF and correlated with histopathologic findings (n=51)

Type	Frequency
Pemphigus vulagris	26(50.9%)
Bullous pemphigoid	20(39.2%)
Pemphigus foliaceus	2(3.9%)
Pemphigus vegetans	1(1.9%)
Pemphigoid gestationis	1(1.9%)
Linear IgA bullous dermatosis.	1(1.9%)
Total	51

DISCUSSION

Skin AVBL are alarming skin conditions which present variedly and cannot be differentiated clinically (fig 5, 6).

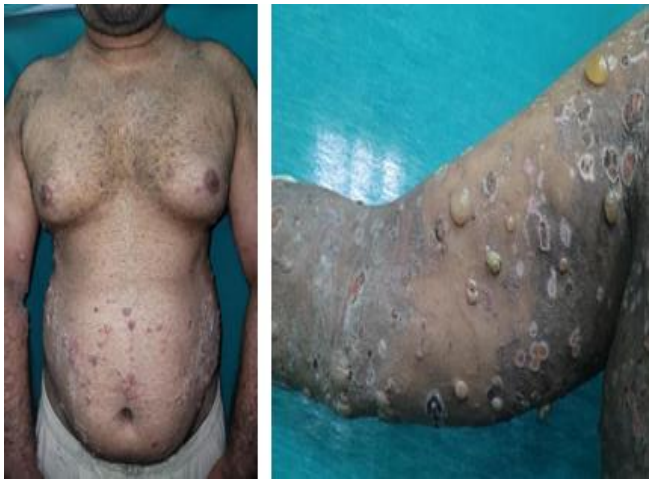

Fig 5(a & b)- 38 year old male with flaccid bullae all over the body. HPE diagnosis – Pemphigus vulgaris

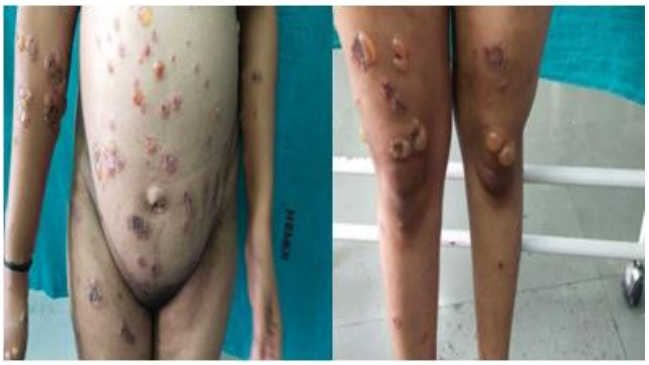

Fig 6(a & b)- 44 year old female tense bullae all over the body. HPE diagnosis- Bullous pemphigoid

Though some of the vesiculobullous lesions are characteristic in their appearance and distribution clinically, many a times definitive diagnosis cannot be made by physical examination alone. [7] Therefore dermatologists depend on histopathology for a definite diagnosis and classification. Basic histopathologic evaluation is helpful in knowing the level of cleavage in the bulla and the character of inflammatory infiltration.[8]

Diagnosis of AVBL is also challenging on histopathologic examination. The current gold standard of diagnostic testing for AVBL is DIF microscopy which demonstrates tissue-bound autoantibodies and/or C3 in the patient's skin or mucous membrane.[9]

Accurate early diagnosis is required for prompt treatment of these conditions to prevent substantial morbidity and mortality.

With this background, we thought that it would be important to study the gamut of AVBL with their clinical, histopathologic and DIF findings.

Hence, in the present study we analysed in depth the histological features and DIF patterns of AVBL and correlated with clinical diagnosis.

A total of 63 cases were included in the present study. The Age range was 18 to 80 years with mean age being 38 years. There was a female preponderance with Male to female ratio of 0.8:1. This finding was concordant with a study by Deepthi S P et al which also showed female preponderance with male to female ratio being 1:1.5. [11] Another study by Kumar SS et al had a slight male preponderance with male to female ratio of 1.08:1.[12]

Histopathologically, most common vesiculobullous disorder was pemphigus vulgaris which constituted 50.9% (26/51). Most Of the studies had similar finding with pemphigus vulgaris being the most common AVBL. Ahmed K et al found that pemphigus vulgaris constituted 24/59 (40.6%) cases of AVBL.[13] A study by Kumar SS et al had 32%(16/50) pemphigus vulgaris cases.[12] Another study by Deepthi et al had 17/50 (34%) of pemphigus vulgaris cases.[11]

On histopathological examination these cases showed suprabasal bullae and intact row of basal cells (row of tombstone appearance) with few acantholytic cells within the bullae. Some cases also had neutrophils and/or eosinophils within the bullae.(fig 1a).All of these cases showed IgG and/or C3 lace like positivity intraepidermally on DIF. (fig 1b)

The next common lesion was bullous pemphigoid constituted 20/51 (39.2%) cases in our study. This

finding was similar to other studies. In a study by Deepthi et al, there were 13/50(26%) bullous pemphigoid which was also the second common lesion.[11] A study by Ahmed K et al also had bullous pemphigoid as the second most common lesion with 10/59 (16.9%) cases. [13]All these cases on histopathology showed subepidermal blister with variable number of eosinophils within the blister.(fig 2a).On DIF, the findings were IgG and/or C3 positivity with a linear homogenous deposition along the basement membrane.(fig 2b).

Our study had 2 cases of pemphigus foliaceous which showed a subcorneal bullae containing occasional acantholytic cells on histopathology.(fig 3a).On DIF, these cases showed IgG and C3 positivity in a lacelike pattern within the epidermis similar to DIF findings of pemphigus vulgaris but positivity was more pronounced in the superficial layers.(fig 3b)

The present study had 1 case of pemphigus vegetans, which on DIF showed similar findings as that of pemphigus vulgaris with lace like positivity of IgG and/or C3 intraepidermally. So these two lesions are difficult to differentiate on DIF alone but they can be distinguished on histopathology based on the level of split. The level of split is intraepidermal in pemphigus vegetans whereas it is suprabasal in pemphigus vulgaris.

We also had one case of IgA bullous dermatosis which showed a subepidermal blister with prominent neutrophilic collection with in.(fig 4a) DIF studies showed homogenous and linear IgA positivity along the basement membrane. (fig 4b)

A close differential diagnosis on histopathology is dermatitis herpetiformis which has the same level of split i.e subepidermal, but there is accentuation of neutrophilic infiltrate in the dermal papillae. On DIF there will be IgA positivity, but the deposition is "granular" in contrast to "linear" deposition of IgA bullous dermatosis and this positivity is present mostly in the tips of dermal papillae.[3]

In the present study, histopathological diagnosis of all cases correlated with DIF findings (100%). A study by Ahmed K et al showed 93.2% correlation with histopathology.[13] Another study by Deepthi et al showed 70% correlation.[11]

The difference in DIF positivity occurs due to various reasons. It may be due to difference in time and site of biopsy, technical error or interpretation error. [10]

12/63(19.1%) cases were negative on DIF studies and did not correlate with clinical diagnosis.

On histopathology 2/12 cases were diagnosed as lichen planus. Lichen planus usually presents clinically as small, flat topped, shiny, polygonal, violaceous papules that may coalesce into plaques. Occasionally, small areas of artifactual separation between the epidermis and dermis, known as Max-Joseph spaces, are seen. In some instances, the seperaion occurs in vivo and subepidermal blisters form(vesicular lichen planus).[3] These vesicles develop as a result of extensive damage to the basal cells. In these cases Lichen planus can have a differential diagnosis of AVBL clinically. In these cases confirmation with histopathology and DIF is essential for proper management.

2/12 cases were diagnosed as Polymorphic light eruption(PMLE) on histopathology. Clinically PMLE presents as papules, plaques or vesicles. [1] When they present as vesicles, it can be difficult to distinguish with AVBL clinically. In these situations histopathology and DIF plays a major role in accurate diagnosis.

1/12 case was diagnosed as Reactive perforating collagenosis(RPC) and 1/12 case as acquired perforating dermatosis (APD). Both of these conditions are perforating disorders which present clinically as plaques, papules, nodules or occasionally as pustules. [3] A differential of AVBL clinically is made when these present as pustules hence histopathology and DIF is critical in such scenarios.

1/12 case was diagnosed as eczema and 1 case as eosinophilic spongiotic reaction, both of which can present as linear papules, plaques and vesicles. [3]Hence histopathology and DIF are required to confirm the diagnosis.

1/12 case was diagnosed as lymphangioma. "lymphangioma circumscriptum" a variant can present as papules with vesicle-like lesion containing clear fluid.[3] In this condition histopathology and DIF are confirmatory.

4/12 cases had nonspecific features on histopathology and were asked to correlate clinically.

51/63(80.9%) cases with clinical diagnosis of AVBL correlated with DIF findings in this study whereas this percentage were at 70%, 69.4%, and 84% in various other studies as compiled below.

Table 2 –Summarized comparison with other studies

Features	Present study	Kumar SS et al[7]	Ahmed k et al[8]	Deepthi SP et al[6]
No. of cases	63	50	59	50
M:F ratio	0.83:1	1.08:1	0.73:1	0.66:1
Pemphigus vulgaris %	53%	32%	40.6%	34%
% clinical and DIF correlation	80%	70%	69.4%	70%

CONCLUSION

Our study further proves that DIF is not only an ancillary technique in the diagnosis of autoimmune vesiculobullous disorders, but also a confirmatory test. Even though Clinical examination is the initial step in making a diagnosis of AVBL, it is difficult to ascertain treatment based only on clinical diagnosis due to the heterogeneity in the clinical manifestation. Since most of the autoimmune vesiculobullous disorders require prolonged and aggressive therapy, the diagnosis may not be confirmatory only with

histopathology, hence, DIF studies are a must for proper management.

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