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*Original Research Article*

# Histopathological and Direct Immunofluorescence Spectrum of Vesiculobullous Skin Disorders

Dr Gautam Goyal[1*], Dr Hilda Fernandes[2*], Dr Nada C.K[1**]

[1]PG Resident, [2]Prof. and HOD
[*]Father Muller Medical College, Mangalore, [**]A J Institute of Medical Sciences, Mangalore.

Corresponding Author: Gautam Goyal

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## ABSTRACT

Vesiculobullous lesions comprises of a group of heterogeneous skin diseases, treatment of which greatly depend upon correct diagnosis. There is overlap in clinical and histopathological features of various autoimmune vesiculobullous lesions. Such overlap is more so with subepidermal lesions where the role of Immunofluorescence is critical. A diagnosis based solely on clinical and histological findings may not be accurate. Direct Immunofluorescence is extremely useful in distinguishing closely related groups. [1] This forms the basis of this study which aims to evaluate the role of Direct Immunofluorescence and Histopathology in diagnosis of vesiculobullous lesions. A total of 58 skin biopsies of suspected vesiculobullous lesions were studied over a period of 2 years. For Direct Immunofluorescence biopsies Optimally Diluted Fluorescein Isothiocyanate (FITC) labeled monospecific Immunoglobulins viz IgG, IgA, IgM and C3 was layered over the sections and incubated. Sections were then examined under fluorescent microscope and the type and pattern of Immunoreactant. For histopathological analysis formalin fixed skin biopsy of the vesiculobullous lesion was processed, stained with Hand E stain and then observed under light microscope Slight male preponderence was noted with 55.2% males as oppose to 44.8% females. Bullous pemphigoid constituted the most common vesiculobullous disorders with 27.9%% followed by pemphigus vulgaris 22.4%. Peak incidence was seen between 40-49 yrs for pemphigus and 70 to 80 in Bullous Pemphigoid. DIF was done for all cases.DIF showed positive findings. In 15 cases there was discordance between the clinical diagnosis and the final diagnosis offered considering both histopathological and DIF findings. 5 cases showed discordance between histopathological diagnosis and DIF findings. Out of these cases in 3 cases DIF was helpful and in another 2 cases final diagnosis was arrived by Histopathology.DIF was thus helpful in few overlapping cases. It should be used in conjugation with Histopathology and Clinical features to get the best diagnostic yield.

***Keywords:*** Direct Immunofluorescence, Vesiculobullous, Pemphigus

## INTRODUCTION

Immunobullous diseases constitute an important group of dermatological disorders caused by pathogenic autoantibodies directed against antigens in the intercellular substance or dermoepidermal junction. The treatment of these diseases is largely dependent on correct diagnosis. [2]

The diagnostic specificity of various clinical findings varies among bullous diseases. There is clinical overlap among various group of bullous diseases. Histological examination should be ideally performed on early vesicle to help reveal the site of formation and also the presence, intensity and composition of the inflammatory infiltrate. A differential

diagnosis is generated on the basis of combination of such findings. [3]

Immunofluorescence is a good technique that has greatly contributed to the diagnosis, treatment and understanding of the pathophysiology of vesiculobullous lesions of skin. It is used in both scientific research and clinical laboratories. The relative simplicity and accuracy of the technique has made imunofluorescence an unavoidable powerful technique in the diagnosis of bullous diseases. [2] With The availability of transport media like Michel's media, majority of dermatologists can have access to DIF. Therefore, this study was undertaken to evaluate the clinical features, histopathology and DIF findings of various vesiculobullous disorders of the skin for their role in various vesiculobullous skin lesion.

## MATERIALS AND METHODS

A descriptive hospital based study of clinical, histopathological and DIF features of vesiculobullous diseases was conducted on patients attending department of dermatology of father muller medical college Mangalore over a period of 2 years. In patients with vesiculobullous lesions, detailed history and clinical examination was done with particular reference to age, gender, morphology of lesions and site of involvement. The patients with clinical features suggestive of immunobullous disorders were included in the study as these disorders show varied clinical manifestations. Histopathology and DIF in these disorders help in the final diagnosis, exclusion of differential diagnosis and determining course of the disease and their response to treatment. Vesiculobullous lesions secondary to infections, eczemas and burns were excluded from the study as these disorders present with characteristic clinical features, and histopathology and DIF are not the main diagnostic methods.

In all the patients, punch biopsy from the lesional skin or oral mucosa preferably including intact vesicle was performed for histopathological study and another biopsy from perilesional normal looking skin or oral mucosa was taken for DIF. Of the two biopsies, one was sent in normal saline or Michel's medium for DIF and the other in 10% neutral buffered formalin for hematoxylin and eosin staining (H and E).

Histopathological diagnosis was based on level of blister separation, inflammatory infiltrate, altered keratinocytes such as acanthocytes and dyskeratotic cells and pattern of arrangement of keratinocytes e.g. row of tombstone, dilapidated brick wall appearance.

The DIF result was based on site (intercellular, along basement membrane zone or dermal papillae), type (IgG, IgM, IgA or C3), pattern (granular or linear) and intensity of deposition of immune reactants.

A final diagnosis was arrived for each case after considering clinical, histopathological and DIF findings.

## RESULTS

During the period of 24 months, 58 biopsy specimens of vesiculobullous lesions of skin were received which constituted 8% of all skin biopsies. Age ranged from 8 to 79 years Youngest patient being a 8 yr old boy who presented with Chronic Bullous Disease of Childhood (CBDC) while the oldest patient was 79 years old who presented with Bullous Pemphigoid (BP). Majority of patients presented between 30-49 years of age. Slight male preponderence was noted with 55.2% males as oppose to 44.8% females

Most Common pattern of involvement in Pemphigus vulgaris and Bullous pemphigoid was generalised involvement of whole body with blisters. The bullae in bullous pemphigoid were tense and did not rupture spontaneously. In limbs BP showed particularly involvement of the flexural aspect. Other cases like vasculitis were exclusively seen to involve lower limbs. suprabasal blister was seen in majority of cases of pemphigus vulgaris and subcorneal in the pemphigus foliaceous and

subcorneal pustular dermatoses patients. Subepidermal blisters were noted in most cases of Bullous pemphigoid, CBDC and LPP. However 2 cases each of PV and BP did not show any split in histopathology.

DIF was done for all cases. DIF pattern of deposition of immune reactants in different vesiculobullous disorders are shown in table 1.

Discordance between clinical and histopathological findings was noted in 15 cases (table 2). Of the Eight cases which were sent clinically as Bullous Pemphigoid, 6 turned out to be of non specific pathology and 2 were due to eczematous process on histopathology. Similarly a case of linear IgA turned out to be BP on histopathology.

**Table 1 - Patterns in IF study**

| FINAL | | | PATTERN | | | | |
|---|---|---|---|---|---|---|---|
| | | | Negative | Linear | Fishnet | Vessel wall | Total |
| | P.vulgaris & vegetans | Count | 0 | 0 | 13 | 0 | 13 |
| | | % | .0% | .0% | 100.0% | .0% | 100.0% |
| | P.follaceous | Count | 0 | 0 | 4 | 0 | 4 |
| | | % | .0% | .0% | 100.0% | .0% | 100.0% |
| | BP | Count | 0 | 16 | 0 | 0 | 16 |
| | | % | .0% | 100.0% | .0% | .0% | 100.0% |
| | HSF | Count | 0 | 0 | 0 | 1 | 1 |
| | | % | .0% | .0% | .0% | 100.0% | 100.0% |
| | vasculitis | Count | 1 | 0 | 0 | 4 | 5 |
| | | % | 20.0% | .0% | .0% | 80.0% | 100.0% |
| | LPP | Count | 0 | 1 | 0 | 0 | 1 |
| | | % | .0% | 100.0% | .0% | .0% | 100.0% |
| | CBDC | Count | 1 | 0 | 0 | 0 | 1 |
| | | % | 100.0% | .0% | .0% | .0% | 100.0% |
| | Linear IgA | Count | 1 | 0 | 0 | 0 | 2 |
| | | % | 50.0% | 50.0% | .0% | .0% | 100.0% |
| | DEE | Count | 1 | 0 | 0 | 0 | 1 |
| | | % | 100.0% | .0% | .0% | .0% | 100.0% |
| | Non Specific changes | Count | 2 | 0 | 0 | 0 | 2 |
| | | % | 100.0% | .0% | .0% | .0% | 100.0% |
| | Inflammatory prosess | Count | 2 | 0 | 0 | 0 | 2 |
| | | % | 100.0% | .0% | .0% | .0% | 100.0% |
| | BLP | Count | 1 | 0 | 0 | 0 | 1 |
| | | % | 100.0% | .0% | .0% | .0% | 100.0% |
| | dyshydrotic eczema | Count | 1 | 0 | 0 | 0 | 1 |
| | | % | 100.0% | .0% | .0% | .0% | 100.0% |
| | subcorneal pustular dermatosis | Count | 18 | 18 | 17 | 5 | 58 |
| | | % | 31.0% | 31.0% | 29.3% | 8.6% | 100.0% |
| Total | | Count | | | | | |
| | | % | | | | | |

**Table 2- Clinical Vs Histopathologic Daignosis**

| | Histopathology Diagnosis | | | | | |
|---|---|---|---|---|---|---|
| **Clinical Daig.** | **Scpd** | **Bp** | **Pv** | **Eczema** | **Non Specific** | **Infl.** |
| Pf (3) | | | 1 | | 1 | 1 |
| Bp (8) | | | | 2 | 6 | |
| Linear Iga (1) | | 1 | | | | |
| Iga Pemphigus (1) | 1 | | | | | |
| Dh (3) | | | | | 1 | 1 |

Five cases showed discordance between histopathological diagnosis and DIF findings. Two cases of Bullous pemphigoid showed only non specific changes on histopathology. Positivity in IF helped to arrive at the final diagnosis. One case of vasculitis had very suttle features on histopathology but IF was positive for C3 that helped to arrive at final diagnosis. One case of Linear IgA and DEB had clear clinical and histopathological features however IF was negative in both and could not give any appropriate diagnosis.

## DISCUSSION

Though, various primary cutaneous diseases present clinically with vesiculobullous lesions, their etiology, pathogenesis, severity and course differs. Therefore, accurate diagnosis of these diseases is essential for appropriate management to avoid or minimize associated morbidity and mortality. [3]

Clinically, all the patients with vesiculobullous diseases may not present with classical morphology and distribution of the lesions. [4] The number of patients presenting with clinical features like vesicles and bullae, involvement of mucous membranes, Nikolsky's sign and Bulla spread sign is different in various studies conducted in India. [5] The difference may be due to prevalence of the diseases, severity and stage of the disease at

presentation and status of the treatment. [6] Oral mucosa can be only site of involvement in the early stage of pemphigus vulgaris as noted in the present study. [7] In these clinical scenarios where clinical diagnosis is difficult, histopathology and DIF of biopsy specimen will help in arriving at final diagnosis. [8]

Vesiculobullous diseases show specific histopathological changes which are demonstrated only when early intact vesicle or bulla is included in the biopsy specimen. Histopathological features have been varied in many studies with PV showing showing suprabasal bulla 95% to 65%, [2] Row of tombstone in 88.5% [2] to 48.8%. [9] Many clinically daignosed vesivulobullous turned out to be non specific process in histopathology.

DIF positivity in PV has been recorded as 87.5% by A.K.M. Nurul et al [10] and 94.11% by S.P deepti et al. [11] As the DIF finding of PF is similar to PV, only histopathology helps in differentiating PV from PF.

Twenty four of clinical diagnoses were found discordant with final diagnosis. Similar findings were also noted by Nurul Kabir et al [10] where less than 50% of clinical diagnosis was in concordance with histopathology. This kind of clinico-histopathological discordance can be due to previous treatment taken by the patient who changes the morphology of lesions or due to presence of secondary changes. [2] Selection of biopsy site also can be critical for giving the appropriate diagnosis. This implies that clinicopathological correlation is more important than relying on clinical findings alone.

Five cases showed discordance in DIF and histopathology findings. Two cases clinically diagnosed as BP showed only non specific changes on Histopathology but IF was positive in both and helped to arrive at the diagnosis. A case of vasculitis was missed on HPE but was positive on IF. In a study by Srinath al it was noted that in two cases of BP and one of Linear IgA disease immunofluorescence was absolutely essential to come to a final diagnosis as histopathological finding were nonspecific.

One case of linear IgA and another one of DEB had characteristic clinical and histopathological findings. However IF was negative in both. In the study of Arundhati et al two cases with clinical and histopathological features of Pemphigus vulgaris DIF was negative. Selection of biopsy site, treatment status, and technical errors in procedure may result in false negativity of DIF. However in the absence of these factors, the negative DIF indicates prolonged remission of disease activity. [11]

## CONCLUSION

Histopathological examination and DIF are required for making a definitive diagnosis of vesiculobullous disorders. DIF is helpful to clarify the picture in cases of autoimmune bullous disorders where, clinically, no diagnosis has been made due to the atypical appearance and nonspecific characteristics of the lesions. IF requires special equipment and trained reporting person and may not be always affordable to the patient. In comparison to DIF, histopathology remains the cornerstone in differentiating PV from PF. Hence, clinical, histopathological and DIF features are considered together to arrive at final diagnosis as these methods may not be diagnostic individually in each and every case.

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