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HISTOPATHOLOGICAL AND IMMUNOFLUORESCENCE ANALYSIS OF VESICULOBULLOUS LESIONS: A DIAGNOSTIC APPROACH

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Abstract
Introduction: Vesiculobullous skin lesions encompass a range of disorders with diverse etiologies, necessitating accurate diagnosis for effective management. Direct immunofluorescence (DIF) and histopathological analysis are essential for differentiating between these disorders.

Methodology: A retrospective analysis of 150 cases of vesiculobullous lesions was conducted at Saveetha Hospital, Chennai. Clinical data, histopathological findings, and DIF results were analyzed to categorize lesions based on cleavage level and immunological markers.

Results: Patients were mostly over 50 (42.5%), while women made up the majority (56.2%). The most frequent diagnoses were bullous pemphigoid (32.8%), pemphigus vulgaris (26.1%), and dermatitis herpetiformis (16.7%). C3c was the most often found protein (55.2%) in the 58 cases where DIF was done.

Conclusion: Bullous pemphigoid and pemphigus vulgaris were the predominant vesiculobullous disorders. Histopathology and DIF were essential for accurate diagnosis, though incomplete DIF testing was a limitation. Future studies with larger sample sizes and comprehensive immunofluorescence analysis are recommended.

Keywords: Vesiculobullous lesions, pemphigus vulgaris, bullous pemphigoid, histopathology, DIF

INTRODUCTION:

Vesiculobullous skin lesions encompass a diverse group of dermatological disorders with varied clinical and pathological presentations. These lesions are divided into two groups according to their size: vesicles have a diameter of 0.5cm or less, whereas lesions larger than 0.5cm are called bullae. Numerous factors, such as viral, inflammatory, drug-induced, hereditary, as well as autoimmune disorders, might result in them. Accurate diagnosis is crucial for efficient care and to reduce related morbidity as well as mortality because of their varied etiologies.

By determining important characteristics such the process of bulla development, the degree of cleavage, the kind of inflammatory infiltrates (if present), and the state of the surrounding epidermis and dermis, histopathological investigation is essential in the diagnosis of vesiculobullous illnesses. Nevertheless, as the lesion progresses, variations in these characteristics can obscure the diagnosis and mimic unrelated conditions, complicating accurate assessment.

A skin punch biopsy remains the cornerstone of dermatological diagnosis, particularly when supplemented with confirmatory tests like direct immunofluorescence (DIF). DIF not only aids in diagnosis but also serves as a valuable prognostic tool by detecting subclinical lesions and predicting disease relapse.(2,3)

With a focus on the diagnostic difficulties and the contribution of immunofluorescence and histology to enhancing diagnostic precision, our study aimed to examine the clinicopathological spectrum of vesiculobullous lesions.

METHODOLOGY:

A retrospective analysis was conducted on 150 cases of vesiculobullous lesions at the "Department of Histopathology, Saveetha Hospital and Medical College, Chennai, Tamil Nadu". Clinical information such as patient demographics (age and sex), lesion characteristics (kind, place, size, number), and length of disease were gathered from referral reports.

All patients gave their written informed consent before the skin punch biopsy was performed. Three biopsy samples in all, two from the peri-lesional region and one from the lesion, were taken. Two peri-lesional biopsies were fixed in formalin and processed according to conventional histopathological procedures, while one was preserved in cryomatrix for direct immunofluorescence (DIF) investigation. All samples were sectioned vertically after being bisected perpendicular to the bulla plane and individually inserted in cassettes after formalin fixation. A skilled histopathologist then assessed the slides that had been stained with "hematoxylin and eosin (H&E)".

The final histological diagnosis was determined by classifying the lesions as subcorneal, intraepidermal, suprabasal, and subepidermal bullae according to the degree of cleavage. To rule out fungal infections, periodic acid-Schiff (PAS) staining was also carried out.

RESULTS:

Our study revealed that the most of the patients (42.5%) were above 50yrs. of age, falling into the geriatric category. Female patients comprised 56.2% of the total cases. The most common lesion distribution was generalized (72.8%), followed by the lower limbs (10.5%) and trunk (8.1%).

The most common diagnosis was bullous pemphigoid (32.8%), followed by paraneoplastic pemphigus (0.9%), dermatitis herpetiformis (16.7%), Darier's disease (13.5%), pemphigus vulgaris (26.1%), pemphigus foliaceus (5.2%), epidermolysis bullosa (1.8%), linear immunoglobulin A (IgA) dermatosis (1.5%), and drug-induced reactions (1.5%).

Complement C3c protein had been found to be the most frequently deposited protein (55.2%) in 58 cases that underwent direct immunofluorescence (DIF) investigation (Table 1).

Table1 : Clinicopathological Characteristics of Patients with Bullous Disorders (n=100)

Characteristic	**Value (n=100)**
Age (years), mean±SD	50.2±18.9
Age group	
<18 years, n (%)	9 (9)
18-35 years, n (%)	20 (20)
36-50years, n (%)	35 (35)
>50years, n (%)	36 (36)
Gender	
Female, n (%)	52 (52)
Male, n (%)	48 (48)
Site	
Generalized, n (%)	75 (75)

Characteristic	**Value (n=100)**
Face/neck, n (%)	1 (1)
Lower limbs, n (%)	9 (9)
Abdomen, n (%)	2 (2)
Trunk, n (%)	5 (5)
Upper limbs, n (%)	7 (7)
Back, n (%)	1 (1)
Level of bulla	
Subcorneal, n (%)	5 (5)
Intraepidermal, n (%)	3 (3)
Suprabasal, n (%)	28 (28)
Subepidermal, n (%)	64 (64)
Direct Immunofluorescence	
Not performed, n (%)	50 (50)
Performed, n (%)	50 (50)
IgA (n=50)	
Negative, n (%)	45 (90)
Positive, n (%)	5 (10)
IgG (n=50)	
Positive, n (%)	22 (44)
Negative, n (%)	28 (56)
IgM (n=50)	
Negative, n (%)	43 (86)
Positive, n (%)	7 (14)
C3c (n=50)	
Positive, n (%)	30 (60)
Negative, n (%)	20 (40)
C1q (n=50)	

Characteristic	Value (n=100)
Positive, n (%)	6 (12)
Negative, n (%)	44 (88)
Diagnosis	
Pemphigus vulgaris, n (%)	30 (30)
Dermatitis herpetiformis, n (%)	15 (15)
Epidermolysis bullosa, n (%)	4 (4)
Drug reaction, n (%)	2 (2)
Pemphigus foliaceus, n (%)	5 (5)
Bullous pemphigoid, n (%)	33 (33)
Darier's disease, n (%)	12 (12)
Erythema multiforme, n (%)	3 (3)
Linear IgA dermatosis, n (%)	4 (4)
Paraneoplastic pemphigus, n (%)	2 (2)

Table 1, summarizes the clinicopathological characteristics of 100 patients with blistering diseases. The mean age is **50.2±18.9 years**, with a nearly equal gender distribution (**48% male, 52% female**). The majority of cases (**75%**) present with generalized lesions, while **64%** exhibit subepidermal bullae. Direct immunofluorescence was performed in **50%** of cases, with **IgG (44%)** and **C3c (60%)** showing the highest positivity. Pemphigus vulgaris (**30%**) and bullous pemphigoid (**33%**) are the most common diagnoses, followed by "dermatitis herpetiformis (**15%**)" and Darier's disease (**12%**). The findings highlight the immunopathological diversity of blistering disorders.

Table 2: Association of Direct Immunofluorescence Studies with the Final Diagnosis (n=100)

Direct Immunofluorescence	Pemphigus Vulgaris	Paraneoplastic Pemphigus	Epidermolysis Bullosa	Drug Reaction	Erythema Multiforme	Bullous Pemphigoid	Dermatitis Herpetiformis	Linear IgA Dermatosis	Pemphigus Foliaceus	Darier's Disease	p-value
IgA Positive, n (%)	5 (20)	0 (0)	0 (0)	0 (0)	0 (0)	2 (7.1)	0 (0)	3 (42.9)	0 (0)	2 (9.1)	0.298
IgA Negative, n (%)	20 (80)	1 (100)	2 (100)	1 (100)	2 (100)	26 (92.9)	10 (100)	4 (57.1)	7 (100)	20 (90.9)	
IgG Positive, n (%)	18 (72)	1 (100)	0 (0)	1 (100)	0 (0)	15 (53.6)	3 (30)	1 (14.3)	2 (28.6)	5 (22.7)	0.351

Direct Immunofluorescence	Pemphigus Vulgaris	Paraneoplastic Pemphigus	Epidermolysis Bullosa	Drug Reaction	Erythema Multiforme	Bullous Pemphigoid	Dermatitis Herpetiformis	Linear IgA Dermatosis	Pemphigus Foliaceus	Darier's Disease	p-value
IgG Negative, n (%)	7 (28)	0 (0)	2 (100)	0 (0)	2 (100)	13 (46.4)	7 (70)	6 (85.7)	5 (71.4)	17 (77.3)	
IgM Positive, n (%)	4 (16)	0 (0)	0 (0)	0 (0)	0 (0)	3 (10.7)	1 (10)	2 (28.6)	1 (14.3)	4 (18.2)	0.403
IgM Negative, n (%)	21 (84)	1 (100)	2 (100)	1 (100)	2 (100)	25 (89.3)	9 (90)	5 (71.4)	6 (85.7)	18 (81.8)	
C3c Positive, n (%)	20 (80)	1 (100)	1 (50)	1 (100)	1 (50)	18 (64.3)	5 (50)	2 (28.6)	3 (42.9)	9 (40.9)	0.569
C3c Negative, n (%)	5 (20)	0 (0)	1 (50)	0 (0)	1 (50)	10 (35.7)	5 (50)	5 (71.4)	4 (57.1)	13 (59.1)	
C1q Positive, n (%)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)	4 (14.3)	2 (20)	0 (0)	1 (14.3)	5 (22.7)	0.627
C1q Negative, n (%)	23 (92)	1 (100)	2 (100)	1 (100)	2 (100)	24 (85.7)	8 (80)	7 (100)	6 (85.7)	17 (77.3)	

Fisher's exact test was applied.

The table2 presents the association between direct immunofluorescence (DIF) findings and various blistering diseases. Pemphigus vulgaris and bullous pemphigoid show higher IgG and C3c positivity, reflecting their autoimmune nature, while linear IgA dermatosis is characterized by IgA positivity. Dermatitis herpetiformis has lower IgG and IgA positivity, aligning with its gluten-sensitive pathogenesis. C1q positivity is rare and not a significant distinguishing marker. However, the antibody patterns provide valuable diagnostic insights for clinical correlation.

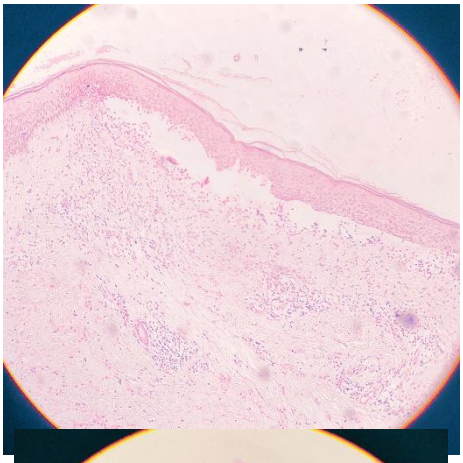

Figure 1: Bullous Pemphigoid: The direct immunofluorescence (DIF) pattern of bullous pemphigoid usually shows a uniform, linear deposition of C3 and IgG along the basement membrane zone. (10)

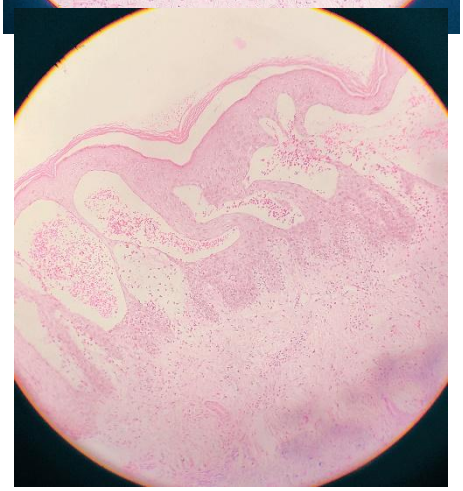

Figure 2:Pemphigous Vulgaris: Just above the base layer of the epidermis, histology usually shows keratinocytes (acantholytic cells) that have been rounded up and separated. (11)

DISCUSSION

The most common cause of vesiculobullous skin lesions in our investigation was “bullous pemphigoid”, which was followed by pemphigus vulgaris. According to Pavani et al., who examined 42 patients, pemphigus vulgaris was the most prevalent vesiculobullous condition, followed by “bullous pemphigoid” (38.1%). Our findings are consistent with their findings. Direct immunofluorescence (DIF) is an essential supplemental technique, and skin punch biopsy is a dependable diagnostic technique, according to their findings [4].

On the other hand, pemphigus vulgaris was the most frequently identified histological diagnosis in a study that examined 68 cases of vesiculobullous lesions. 38.2% of patients had typical histological characteristics, 17.7% had nonspecific alterations, and 34.9% had a negative DIF. These results imply that since no single method is always conclusive, a combination of clinical, histological, and DIF evaluations is required for an appropriate diagnosis [5].

Our study also observed that DIF played a crucial role in diagnosing vesiculobullous disorders. However, it was not performed in all cases, which limited the ability to confirm diagnoses definitively. In cases where DIF was performed, pemphigus vulgaris cases showed intercellular IgG deposition in the epidermis, but “bullous pemphigoid” cases showed significant linear IgG as well as C3 deposition along the basement membrane zone. These findings further highlight the utility of DIF in differentiating autoimmune blistering diseases.

“Pemphigus vulgaris” was the most frequently diagnosed condition, followed by “bullous pemphigoid” and linear IgA dermatosis, according to a different investigation that included indirect immunofluorescence and Tzanck smears in addition to DIF in 34 instances [6]. Similarly, Basu et al. highlighted the importance of DIF as a vital supplementary tool in a study of 34 cases with intraepidermal vesiculobullous lesions, where pemphigus vulgaris was the most commonly identified entity [7].

The distribution of vesiculobullous skin lesions varies geographically and ethnically. Our findings align with reports indicating a higher incidence of pemphigus vulgaris in “Indian”, “Southeast” “European”, and “Middle Eastern” populations [8]. The most prevalent autoimmune condition in our analysis was bullous pemphigoid,

which is known to mostly affect the elderly and is typified by autoantibodies that target hemidesmosomal antigens. The reported incidence is 7.63 per 100,000 person-years [9].

Despite the significance of our findings, the study is limited by its single-center design and the fact that less than half of the instances involved DIF. There is a need for more extensive multicentric research with thorough immunofluorescence analysis to improve our understanding of vesiculobullous skin lesions in our population.

CONCLUSION

The most prevalent vesiculobullous illness in our study was "bullous pemphigoid", which was followed by pemphigus vulgaris. Histopathology and DIF played crucial roles in diagnosis, though incomplete DIF testing limited accuracy. Geographic and ethnic variations influence disease distribution, highlighting the need for region-specific strategies. Despite study limitations, our findings emphasize the importance of a comprehensive diagnostic approach. Future multicentric studies with standardized immunofluorescence and molecular techniques are needed to enhance diagnostic accuracy and patient care.

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