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Clinico-pathological spectrum of vesiculobullous diseases: A prospective cross-sectional study.

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Abstract

Background:
Vesiculobullous lesions are a heterogeneous group of disorders characterized by fluid-filled cutaneous lesions of varied etiology, including autoimmune, genetic, drug-induced, and infectious causes. Due to overlapping clinical presentations, diagnosis based on clinical features alone is challenging. Histopathology and direct immunofluorescence (DIF) are crucial adjuncts, enhancing accuracy, guiding therapy, and informing prognosis.

Objectives:
To evaluate the histopathological features of vesiculobullous skin disorders, correlate them with clinical findings, and assess the utility of DIF in achieving a definitive diagnosis. Additional objectives were to classify lesions by age, sex, and distribution.

Methods:
This prospective cross-sectional study was conducted in the Department of Pathology, D.Y. Patil School of Medicine, Navi Mumbai. A minimum of 50 clinically suspected cases were enrolled; 56 were analyzed. Clinical history, dermatological examination, and punch biopsies were performed. Routine histopathology was supplemented with DIF in selected cases. Data were analyzed using descriptive statistics.

Results:
The most commonly affected age group was 21–30 years (23.2%). A slight male predominance was noted (53.6%), with a male-to-female ratio of 1:0.87. The trunk was the most frequent site (41%). Clinically, pemphigus vulgaris was most common (23.2%), followed by bullous pemphigoid (12.5%) and Stevens–Johnson syndrome (12.5%). Histopathology revealed intraepidermal blisters as the most frequent pattern (35.7%). DIF showed granular IgG and C3 positivity in pemphigus vulgaris and linear IgG/C3 deposition along the dermoepidermal junction in bullous pemphigoid.

Conclusion:
Clinical features alone are insufficient for accurate categorization of vesiculobullous lesions. Histopathology remains the gold standard, while DIF provides valuable confirmation when findings are inconclusive. Integrating these approaches ensures precise diagnosis, timely treatment, and better prognostic guidance, particularly in autoimmune bullous diseases.

Recommendations:
Routine biopsies with clinicopathological correlation should be emphasized. DIF should be integrated whenever feasible, with improved accessibility and clinician–pathologist collaboration to optimize patient outcomes.

Keywords: *Vesiculobullous lesion, pemphigus, bullous pemphigoid, immunofluorescence, histopathology*
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Introduction

Vesiculobullous lesions comprise a diverse group of disorders in which the primary manifestation is a fluid-filled lesion of the skin, arising due to multiple etiological factors such as viral infections, autoimmune mechanisms, drug eruptions, genetic blistering disorders, and bullous changes secondary to physical, chemical, biological, or metabolic insults (1). Direct immunofluorescence (DIF) serves as an essential adjunct in establishing a definitive diagnosis and provides valuable guidance for both therapeutic decisions and prognostic evaluation, thereby justifying the need for the present study (2).

Vesicles are defined as circumscribed collections of free fluid located within or beneath the epidermis, measuring up to 0.5 cm in diameter; when the size exceeds 0.5 cm, the lesion is termed a bulla (3,4). Histopathologically, these lesions are categorized into subcorneal, intraepidermal, and subepidermal types, depending on the level of cleavage. Etiologically, they can be broadly classified into immunobullous, genetic, and inherited acantholytic disorders (5).

DIF is considered a highly reliable and sensitive diagnostic modality in pemphigus vulgaris, where it demonstrates a characteristic lace-like deposition of IgG along the squamous cell surfaces, often remaining positive even after clinical remission. Furthermore, DIF is valuable in other autoimmune blistering conditions such as pemphigus foliaceus, pemphigus erythematosus, pemphigus herpetiformis, and drug-induced pemphigus, with 80–90% of cases showing antibody deposition on keratinocyte cell surfaces (2).

Comprehensive evaluation of vesiculobullous lesions through clinicopathological correlation allows accurate categorization, while DIF testing provides further characterization of subgroups, thereby strengthening diagnostic certainty. In this context, the present study aims to correlate clinical and histopathological findings of vesiculobullous skin lesions with direct immunofluorescence wherever indicated.

MATERIALS AND METHODS

Study Design and Setting

This was a prospective cross-sectional, descriptive hospital-based study conducted in the Histopathology Section of the Department of Pathology, D.Y. Patil School of Medicine and Hospital, Navi Mumbai, Maharashtra. The institute is a tertiary care center catering to both urban and semi-urban populations, with the department receiving referral biopsies from dermatology and allied specialties. This setting ensured a representative case mix of vesiculobullous disorders. The study was conducted over 18 months, from December 2022 to June 2024.

Study Population and Sampling

A total of 56 patients clinically diagnosed with active vesiculobullous disorders of the skin, irrespective of age and sex, were included. Cases were selected using a simple random sampling technique.

Sample Size Determination

A minimum sample size was estimated using the formula for cross-sectional studies:

$n=Z2\times p\times(1-p)d2$

where Z = 1.96 (for 95% confidence interval), p = expected prevalence of vesiculobullous lesions among dermatological biopsies (20% based on prior Indian studies [6,12]), and d = allowable error (10%). This yielded a minimum required sample size of 50. To account for non-assessable or inadequate samples, 56 cases were ultimately included and analyzed.

Inclusion Criteria

Patients with clinically suspected vesiculobullous lesions of the skin.

Patients are willing to undergo a biopsy and give informed consent.

Exclusion Criteria

Patients with inadequate or autolyzed biopsy material.

Cases with incomplete clinical data.

Biopsy Procedure

Histopathological Examination (HPE)

A single lesional skin biopsy was obtained from each patient. The specimen was fixed in 10% neutral buffered formalin.

Routine processing and paraffin embedding were performed, and sections were stained with Hematoxylin and Eosin (H&E).

Slides were examined under light microscopy for the site of blister formation, epidermal and dermal changes, and inflammatory patterns.

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Direct Immunofluorescence (DIF)

Wherever possible, an additional perilesional skin biopsy (3–4 mm punch) was taken from clinically normal-appearing skin within 2 cm of the lesion.
The specimen was preserved in Michel's medium and stored at –70°C to –20°C until further processing.
Before cutting, the biopsy was washed thrice in phosphate-buffered saline (PBS) for 30 minutes each.
Tissue was embedded in a cryostat, and 3–5 µm-thick frozen sections were obtained.
Sections were brought to room temperature, rinsed thrice with PBS (10 minutes each), and stained with optimally diluted fluorescein isothiocyanate (FITC)-conjugated monoclonal antihuman antisera (IgG, IgA, IgM, and C3) for 45 minutes.
After washing thrice with PBS (10 minutes each), sections were mounted using PBS and buffered glycerine mountant.
Slides were examined under a fluorescence microscope for patterns of immune deposition.

Parameters Assessed

Clinical characteristics (age, sex, site, distribution of lesions).
Histopathological features (level of split, inflammatory infiltrate, epidermal/dermal changes).
DIF findings (type of immunoreactant and pattern of deposition).

Bias

To minimize selection bias, consecutive clinically suspected cases fulfilling the inclusion criteria were enrolled during the study period. Information bias was reduced by using a structured proforma for uniform recording of demographic, clinical, and histopathological details. Two independent pathologists reviewed the histopathological and DIF slides to limit observer bias, and discrepancies were resolved by consensus. Processing and staining protocols were standardized to reduce technical bias.

Data Management and Analysis

All clinical, histopathological, and immunofluorescence findings were recorded in a structured proforma. Data were entered into a master chart and analyzed using descriptive statistical methods to establish clinicopathological correlations.

Ethical Considerations

Ethical clearance for the study was obtained from the *Institutional Ethics Committee, D.Y. Patil School of Medicine and Hospital, Navi Mumbai, Maharashtra, India*. Written informed consent was obtained from all participants before enrollment, and confidentiality of patient information was strictly maintained throughout the study.

RESULTS

Patients Flow

During the study period, a total of 62 patients with clinically suspected vesiculobullous lesions were screened for eligibility. Of these, 4 patients declined consent for biopsy, and 2 had inadequate/autolyzed biopsy material. Thus, 56 patients were confirmed eligible and included in the final analysis. All 56 cases had complete demographic and clinical data, and their biopsy samples were successfully processed for histopathological evaluation. Direct immunofluorescence was performed in 12 cases where clinically indicated. No cases were excluded after enrollment, and all 56 patients were analyzed.

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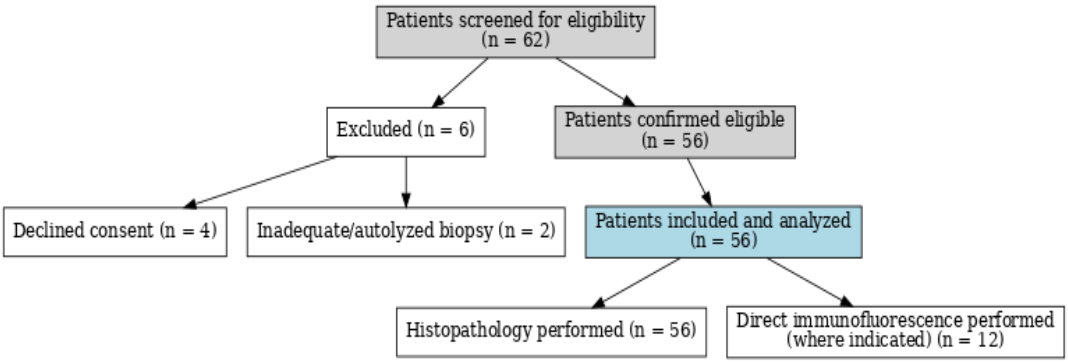


Figure 1. Participant Flow Diagram

Vesiculobullous lesions are a group of blistering disorders that present with many overlapping symptoms clinically and are difficult to diagnose based only on clinical findings. Hence, histopathology plays a significant role in the definitive diagnosis of vesiculobullous lesions. Correlation of the clinical findings along with the histopathological findings aids in a more confirmatory diagnosis.

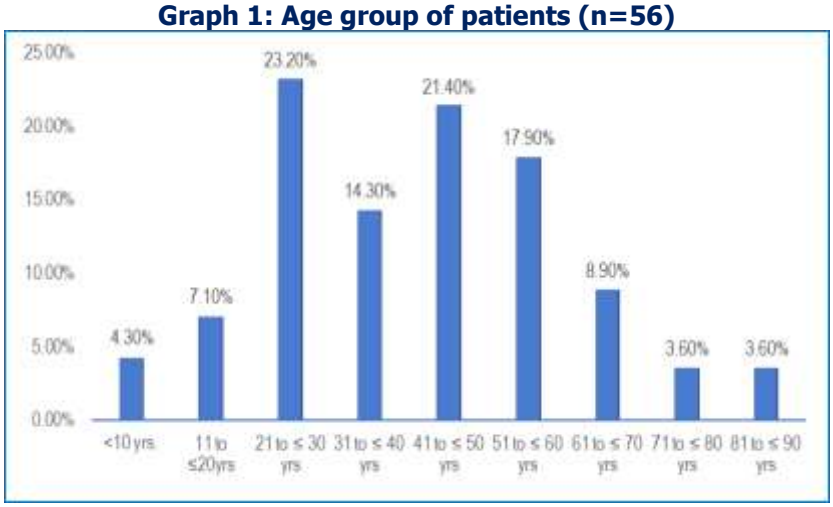
The present clinicopathological study of the spectrum of vesiculobullous lesions included retrospective and prospective evaluation of 56 skin biopsies in the Department of Pathology, in a tertiary care centre over a period of one and a half years.

DEMOGRAPHICS OF PATIENTS:

AGE GROUP:

The age group in this study varied from 10 to 90 years. The highest number of lesions was seen in the age group of 21 to 30 years (n=13, 23.2%), followed by 41 to 50 years (n=12, 21.4%). The least number of cases were noted in the older age group, particularly post the age of 70 years (n=2,3.6%).

Graph 1: Age group of patients (n=56)



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Graph 2: Spectrum of vesiculobullous lesions:

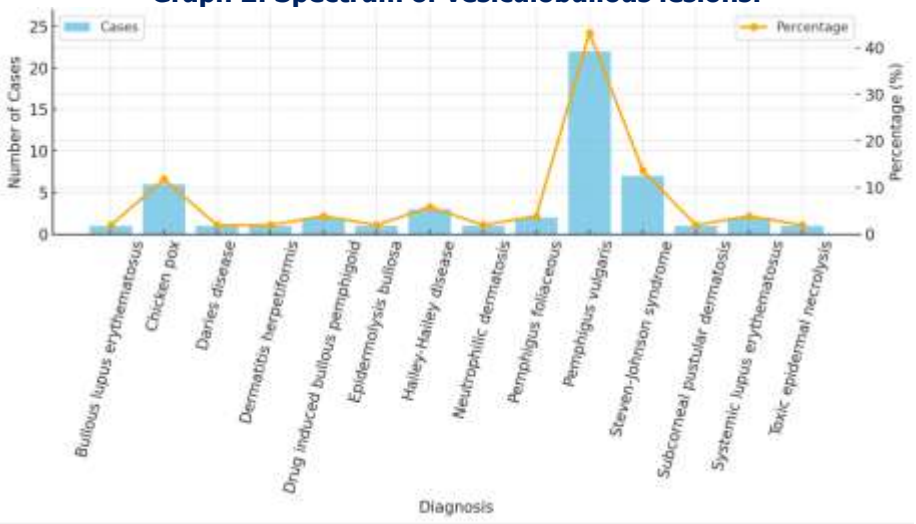

Table 1: Distribution of cases based on site of vesicle on histopathology (n=56)

		Intracorneal	Subcorneal	Intraepidermal	Suprabasal	Subepidermal
	N	*No.*	*No.*	*No.*	*No.*	*No.*
Bullous lupus erythematosus mononuclear type	1	0	0	0	0	1 (5.9%)
Bullous pemphigoid	9	0	0	0	1 (8.3%)	8 (47.1%)
Chicken pox	1	0	0	1 (5.0%)	0	0
Darier's disease	2	0	0	1 (5.0%)	1 (8.3%)	0
Dermatitis herpetiformis	2	0	1 (33.3%)	0	0	0
Drug-induced bullous pemphigoid	1	0	0	1 (5.0%)	0	0
Epidermolysis bullosa simplex	2	0	1 (33.3%)	1 (5.0%)	0	0
Hailey-Hailey disease	4	0	0	3 (15.0%)	1 (8.3%)	0
Herpes zoster	2	0	0	2 (100.0%)	0	0
Neutrophilic dermatosis	1	1	0	0	0	0
Pemphigoid nodularis	1	0	0	1 (5.0%)	0	0
Pemphigus Foliaceous	1	0	1 (33.3%)	0	0	0
Pemphigus vulgaris	20	1(50.0%)	0	8 (40.0%)	11 (78.6%)	0
Steven-Johnson syndrome	5	0	0	1 (5.0%)	0	4 (23.5%)
Subcorneal pustular dermatosis	1	0	0	1 (5.0%)	0	0
Systemic lupus erythematosus	2	0	0	0	0	2 (11.8%)
Toxic epidermal necrolysis	1	0	0	0	0	1 (5.9%)
Total	56	2	3	20	14	17

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BASED ON CONTENT OF BULLA:

As per our study, most cases contained red blood cells in the bullae (n=20, 35.7%), closely followed by acantholytic keratinocytes (n=19, 33.9%), only polymorphs/neutrophils (n=7), and a combination of eosinophils, lymphocytes, and neutrophils (n=6).

BASED ON EPIDERMAL AND DERMAL FINDINGS:

As per this study, the most common feature in the epidermis was acantholytic keratinocytes (n=9, 16%) and a row of tombstone (n=9, 16%), both commonly seen in cases of Pemphigus vulgaris.

Corps and grain type dyskeratosis was seen in both cases of Darier disease (n=2)

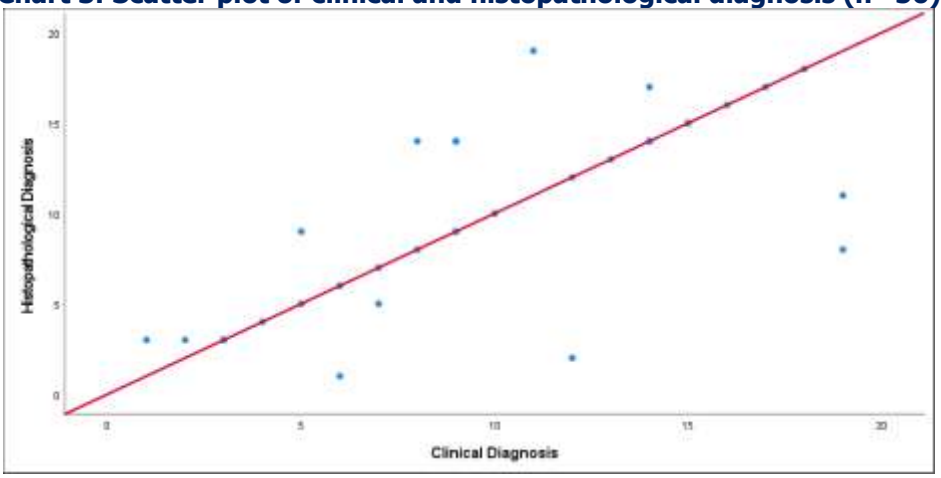
Basal layer vacuolization was seen in Bullous Pemphigoid (n=1) and Dermatitis herpetiformis (n=1)

Hyperkeratosis (n=6, 10.7%) was seen in Bullous Pemphigoid (n=3), Hailey-Hailey disease (n=1), Pemphigus Foliaceus (n=1), and Stevens-Johnson syndrome (n=1).

Spongiosis (n=2, 3.57%) was seen in one case of Neutrophilic dermatoses and one case of Pemphigus vulgaris.

In this study, maximum cases showed the presence of mixed inflammatory cell infiltrates (n=29) comprising polymorphs, eosinophils, and the rest showed the presence of chronic inflammatory cell infiltrates (n=24) comprising lymphocytes and plasma cells. Others included lesions that showed no significant findings in the dermis.

Chart 3: Scatter plot of clinical and histopathological diagnosis (n=56)



Pearson's correlation coefficient showed a positive relation between clinical and histopathology diagnosis, with r = 0.96.

DIRECT IMMUNOFLUORESCENCE FINDINGS:

Direct immunofluorescence was done on 12 samples in this study, only on those that were clinically indicated. In total, we received seven cases of pemphigus vulgaris, three cases of bullous pemphigoid, one case of neutrophilic dermatosis, and one case of systemic lupus erythematosus. Six cases were positive, four were inconclusive, and two cases were negative.

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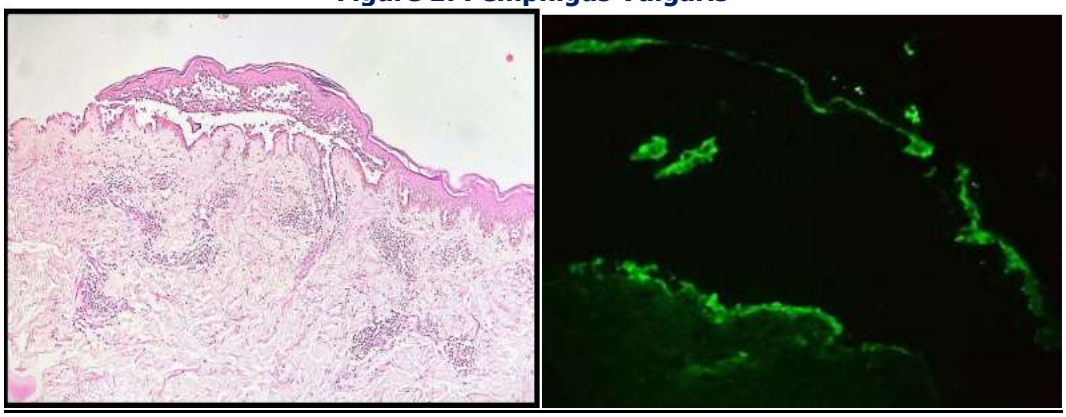
Table 2: Direct immunofluorescence findings:

Figure 1: Pemphigus Vulgaris



(H&E 10x) Suprabasal blister with row of tombstone appearance & IgG linear positivity on DIF

Figure 2: Bullous Pemphigoid

TYPE OF DISEASE	DIF POSITIVITY	SITE OF DEPOSITION	IgG	IgM	IgA	C3
Bullous pemphigoid	Positive	Basement membrane zone	Diffuse, granular +	-	-	-
Pemphigus vulgaris	Positive	Intercellular space, epidermis	Diffuse, granular +			Diffuse granular +
Systemic lupus erythematosus	Positive	Dermoepidermal junction	-	-	Linear, granular +	Linear, granular +
Bullous pemphigoid	Positive	Dermoepidermal junction	-	-	-	Granular, Diffuse +
Pemphigus vulgaris	Positive	Dermoepidermal junction	-	-	-	Granular, Diffuse +
Bullous pemphigoid	Positive	Dermoepidermal junction	Focal, linear ++	-	Focal, linear ++	Focal, linear ++
Neutrophilic dermatosis	Negative	-	-	-	-	-
Pemphigus vulgaris	Negative	-	-	-	-	-
Pemphigus vulgaris	Inconclusive	-	-	-	-	-
Pemphigus vulgaris	Inconclusive	-	-	-	-	-
Pemphigus vulgaris	Inconclusive	-	-	-	-	-
Pemphigus vulgaris	Inconclusive	-	-	-	-	-

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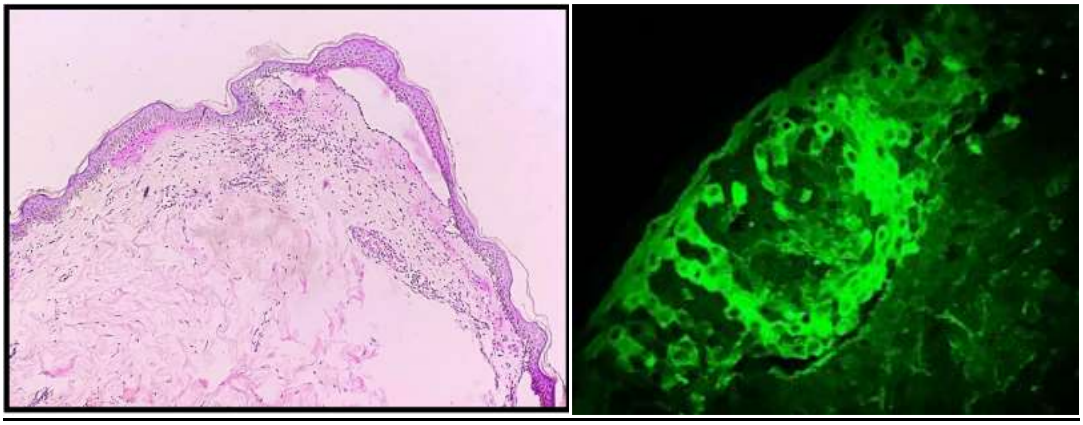
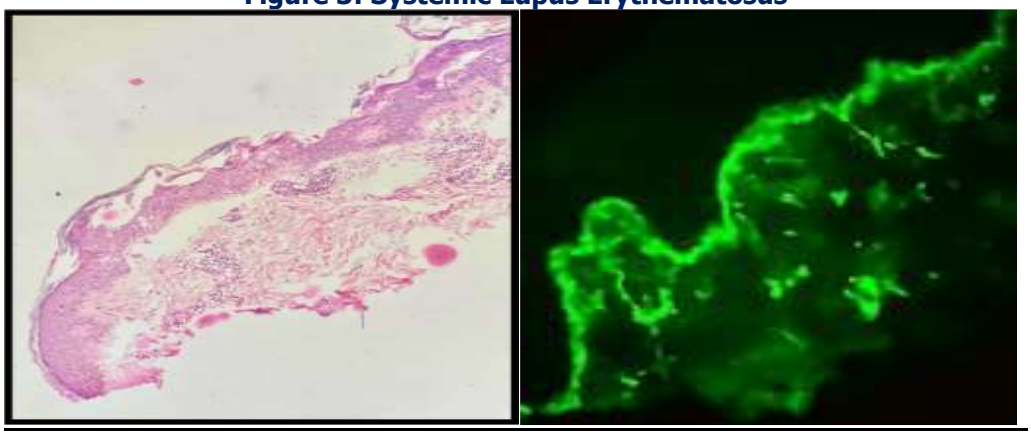


(H&E 10x) Subepidermal blister & C3 granular positive at DEJ

Figure 3: Systemic Lupus Erythematosus



(H&E 10x) Subepidermal blister & IgA granular positivity at DEJ

DISCUSSION

Vesiculobullous lesions are diagnostically challenging because of their overlapping clinical features. In this study of 56 cases, integrating histopathology with selective use of DIF provided a more robust diagnostic framework, emphasizing that no single modality is sufficient in isolation.

Age distribution

The clustering of cases in the 21–30-year group suggests that vesiculobullous diseases, particularly pemphigus, may disproportionately affect younger adults, which has important socioeconomic implications given the chronic nature of treatment [6,1]. This pattern indicates that early adulthood may represent a period of heightened vulnerability due to immunological or environmental triggers.

Gender distribution

The slight male predominance (53.6%) in this cohort, while modest, highlights demographic heterogeneity. Contrasting results across studies, some reporting female preponderance, reflect the influence of genetic, cultural, and referral factors [1,7–9]. This variation underscores the need for population-specific data rather than relying on universal assumptions.

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Clinical presentation

The predominance of vesicles as presenting lesions confirms their diagnostic significance in active disease [8,10,11]. However, the variation reported in different series indicates that lesion morphology alone cannot serve as a reliable diagnostic discriminator, reinforcing the indispensability of histopathological confirmation [12].

Spectrum of clinical diagnosis

Pemphigus vulgaris was the leading clinical entity, followed by bullous pemphigoid. This order mirrors most Indian and international reports [7,12–14,8]. The occasional reversal of this sequence, where bullous pemphigoid exceeds pemphigus vulgaris, suggests that regional case-mix, age demographics, and referral bias strongly influence disease patterns [1].

Histopathological patterns

The predominance of intraepidermal clefting in this study aligns with other series [10,15,13], reaffirming its value as a hallmark feature of pemphigus. Yet, the presence of subepidermal and suprabasal clefts in subsets of patients demonstrates that histological overlap is common, and a single biopsy may not capture the complete disease spectrum [12,8,11,1].

Pemphigus vulgaris

With over one-third of cases, pemphigus vulgaris continues to be the dominant autoimmune bullous disease, consistent with comparable series [12,8]. This reinforces its primacy in differential diagnosis whenever intraepidermal blistering is encountered.

Bullous pemphigoid

Bullous pemphigoid accounted for ~16% of cases, fitting within the range documented in prior cohorts [12,16,8]. Its frequency, particularly among older patients, highlights the importance of recognizing it as a key differential diagnosis in subepidermal blistering.

Clinicopathological correlation

The strong clinicopathological concordance (92.8%) and Pearson's correlation ($r = 0.96$) emphasize that combined assessment significantly reduces diagnostic ambiguity. These values are higher than or comparable to previously reported concordance rates (64.8–88.6%) [1,10,15,17,18]. Such high agreement demonstrates the reliability of integrating histopathology with clinical evaluation.

Direct immunofluorescence

Although DIF positivity was lower in this cohort compared with the 70–90% positivity reported elsewhere [8,10,18], this likely reflects the small DIF sample size, technical limitations in specimen handling, and biopsy timing. Nonetheless, DIF was crucial in distinguishing pemphigus from pemphigoid-spectrum disorders when histopathology alone was equivocal, underscoring its role as a confirmatory adjunct rather than a stand-alone tool.

Generalizability

The findings of this study can be generalized primarily to patients presenting with vesiculobullous lesions in tertiary care hospital settings. As the study was conducted in a single center with a limited sample size, regional variations, referral patterns, and case-mix differences may influence the distribution of lesions elsewhere. Nevertheless, the strong clinicopathological correlation and the supportive role of direct immunofluorescence highlight diagnostic principles that are applicable across similar clinical environments, thereby enhancing relevance for wider dermatopathology practice.

Conclusion

The skin, being the largest organ of the body, is vulnerable to a wide spectrum of disorders, among which vesiculobullous lesions form a distinct group presenting with vesicles, bullae, or pustules of varied etiology. Their overlapping clinical appearance often makes diagnosis challenging, emphasizing the indispensability of histopathological examination as the gold standard. However, no single modality is sufficient in all cases. A comprehensive approach that integrates clinical evaluation, histopathology, and direct immunofluorescence offers the highest diagnostic accuracy. Particularly in ambiguous cases, immunofluorescence serves as a crucial adjunct, enabling precise categorization and guiding management. Thus, multidisciplinary correlation ensures reliable diagnosis and optimal patient care.

Limitations

This study has certain limitations, including a relatively small sample size and restricted use of direct immunofluorescence, which limited the strength of

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conclusions. Overlapping histopathological features and biopsies taken at late stages posed diagnostic challenges. Delay in processing contributed to weak staining, while occasional false-negative DIF results and interobserver variability further affected accuracy. The absence of internal controls also restricted validation, thereby limiting the overall generalizability of the findings.

Recommendations

For improved diagnosis of vesiculobullous lesions, two biopsies should be obtained from the perilesional site within 24 hours of blister formation—one in formalin for histopathology and the other in Michel's medium for DIF, with normal saline as an alternative. Strict adherence to standard operating procedures for cutting and staining, faculty training for accurate DIF interpretation, and cost-effective strategies are essential. Additionally, robust internal and external quality control measures in both histopathology and DIF reporting will enhance reliability and consistency.

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ABBREVIATIONS

DIF- Direct immunofluorescence
IgG- Immunoglobulin G
IgA- Immunoglobulin A
PBS- Phosphate-buffered saline
FITC- Fluorescein isothiocyanate

Source of funding

The study has no funding

Conflict of interest

The Authors declare no conflict of interest.

Author contributions

NN- Sample collection, Review of literature, preparing the first draft of the manuscript, statistical analysis of data, preparing a master chart of data, and pictures of histopathological and DIF slides. **SS**- Concept and design of the study, reporting of histopathological and DIF slides, revision of the Review of literature, review of the first draft of the manuscript, review of statistical analysis of data, review of the master chart of data, review of pictures of histopathological and DIF slides. **PD**- Reporting of histopathological and DIF slides, revision of the Review of literature, review of the first draft of the manuscript, review of statistical analysis of data and interpretation, review of the master chart of data, review of pictures of histopathological and DIF slides.

Data Availability

Data is available on request.

Author Biography

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College, Hospital, and research center, Navi Mumbai, as an Assistant Professor in 2004 and, since then, has been teaching and guiding both MBBS and MD students in various projects and research activities. She has handled pivotal responsibilities including Histopathology, cytology, clinical Pathology, and other academic activities. She has published more than 70 research papers in both National and international journals. Currently, she is serving as Professor and Head of the Department of Pathology in the same institute.https://orcid.org/0000-0002-5213-6394

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