ISSN : (Online): 1898-7249, ISSN (Print): 1898-7249
Abbreviation: J Derm Cse Rep
DOI:10.61705/jdcr.19.3.2026.55.64

Journal of Dermatological Case Reports

Original Research

Autoimmune vesiculobullous disorders- a study of clinicohistopathological correlation

Dr. Rakesh Kumar[1], Dr Sanjay Pancholi[2], Dr Abhishek Malviya[3], Dr Shivank Shrivastava[4], Dr. Varun Kumar[5], Dr. Trashita Rajani[6], Dr. Prachi Garg[7], Dr. Aanchal Malhotra[8], Dr. Shilpa Rajpoot[9]

[1,8,9]Postgraduate Resident, [2]Professor& Head of Department, [3]Professor, [4,5,6]Associate Professor, [7]Senior Resident, Department of Dermatology, Venereology and Leprosy, Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh, India.

Corresponding Author

Dr. Rakesh Kumar

Postgraduate Resident, Department of Dermatology, Venereology and Leprosy, Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh, India

Email:
Dr.rakesh.chauhanmd@gmail.com

Keywords:
Autoimmune vesiculobullous disorders; Pemphigus vulgaris; Histopathology; Tzanck smear; Clinicohistopathological correlation

Received: 07-06-2026
Accepted: 10-07-2026
Published: 13-07-2026

Abstract:

Background: Autoimmune vesiculobullous disorders (AVBDs) are a heterogeneous group of immune-mediated blistering diseases characterized by considerable clinical overlap, making accurate diagnosis challenging. Clinicohistopathological correlation plays a crucial role in establishing the diagnosis, particularly in settings where direct immunofluorescence is not routinely available.

Methods: This hospital-based cross-sectional observational study was conducted in the Department of Dermatology, Venereology and Leprosy, Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh. Ninety-two patients with clinically suspected, untreated autoimmune vesiculobullous disorders presenting with fresh lesions were enrolled. All patients underwent detailed clinical evaluation, Tzanck smear examination, and skin biopsy for histopathological assessment. Clinical diagnoses were compared with histopathological findings to determine clinicohistopathological concordance.

Results: The majority of patients were females (73.91%) and belonged to the 41–50-year age group (27.17%). Oral mucosal involvement (55.43%), vesicular lesions (76.09%), and positive Nikolsky's sign (79.34%) were the predominant clinical findings. Pemphigus vulgaris was the most common clinical (64.13%) and histopathological (60.87%) diagnosis, followed by pemphigus foliaceus and bullous pemphigoid. Suprabasal cleft formation (60.87%) and acantholysis (65.22%) were the principal histopathological findings. Tzanck smear was positive in 86.96% of cases, with acantholytic cells identified in the majority. Overall clinicohistopathological concordance was 93.48%, with excellent agreement between clinical and histopathological diagnoses (Cohen's $\kappa = 0.823$; $p < 0.001$).

Conclusion: Pemphigus vulgaris was the predominant autoimmune vesiculobullous disorder in this cohort. A combined approach incorporating clinical examination, Tzanck smear, and routine histopathological evaluation demonstrated excellent diagnostic accuracy and remains a practical, reliable, and cost-effective strategy for diagnosing autoimmune vesiculobullous disorders, particularly in resource-limited settings.

Introduction

Autoimmune vesiculobullous disorders (AVBDs) comprise a heterogeneous group of chronic mucocutaneous diseases characterized by the formation of vesicles and bullae resulting from autoantibody-mediated damage to adhesion molecules within the epidermis or at the dermoepidermal junction. Vesicles are fluid-filled lesions measuring less than 5 mm, whereas bullae exceed 5 mm in diameter. Although relatively uncommon, these disorders are associated with significant morbidity due to chronicity, recurrent

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blistering, mucosal involvement, secondary infections, and impaired quality of life (1).

The pathogenesis of AVBDs involves the production of pathogenic autoantibodies directed against structural proteins responsible for maintaining epidermal integrity. In pemphigus, antibodies against desmosomal proteins, particularly desmogleins, cause loss of keratinocyte adhesion (acantholysis), resulting in intraepidermal blister formation. In contrast, bullous pemphigoid and other subepidermal blistering disorders are characterized by autoantibodies targeting components of the basement membrane zone, leading to separation beneath the epidermis and the formation of tense bullae (2,3). The clinical spectrum includes pemphigus vulgaris, pemphigus foliaceus, bullous pemphigoid, dermatitis herpetiformis, linear IgA disease, and pemphigoid gestationis, each demonstrating characteristic yet overlapping clinical features (4,5).

Although clinical examination provides valuable diagnostic clues, considerable overlap exists among these disorders. Clinical signs such as Nikolsky's sign, bulla spread sign, mucosal involvement, and the morphology of blisters may suggest a diagnosis but are not always definitive (6). Histopathological examination remains the cornerstone for determining the level of blister formation and associated inflammatory changes. Features such as suprabasal acantholysis, subcornealclefting, subepidermal separation, and the nature of inflammatory infiltrates play an essential role in differentiating individual entities (7,8). While direct immunofluorescence is considered the diagnostic gold standard, its availability is often limited in resource-constrained settings, making clinicohistopathological correlation particularly important for establishing an accurate diagnosis (9).

Several studies have demonstrated varying degrees of agreement between clinical and histopathological diagnoses of autoimmune vesiculobullous disorders, emphasizing the importance of integrating both modalities for optimal diagnostic accuracy (10,11). The present study was therefore undertaken to evaluate the clinical profile of autoimmune vesiculobullous disorders and to assess the correlation between clinical diagnosis and histopathological findings in patients presenting to a tertiary care centre, thereby providing institution-specific evidence that may improve diagnostic precision and patient management.

Material and methods

This hospital-based, cross-sectional observational study was conducted in the Department of Dermatology, Venereology and Leprosy, Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh, India, over a period of 18 months (June 2024 to November 2025). The study aimed to evaluate the clinicohistopathological correlation in patients with autoimmune vesiculobullous disorders. Ethical approval was obtained from the Institutional Ethics Committee before commencement of the study, and written informed consent was obtained from all participants or their legal guardians. The study was conducted in accordance with the Declaration of Helsinki.

Patients of any age and either sex presenting with clinically suspected autoimmune vesiculobullous disorders with fresh untreated vesiculobullous lesions were included. Patients receiving systemic corticosteroids or immunosuppressive therapy and those with infectious, inflammatory, genetic, metabolic, traumatic, drug-induced, or other non-autoimmune vesiculobullous disorders were excluded from the study.

Sample size and sampling: The calculated minimum sample size was 92, based on the formula $n = Z^2p(1-p)/E^2$, assuming a prevalence of 40%, a 95% confidence level, and an allowable error of 10%. Eligible patients were enrolled using a convenience sampling technique until the required sample size was achieved.

Methodology

A detailed history was obtained from each participant, including age, sex, duration of illness, site of onset, progression of lesions, associated symptoms, drug history, family history, and associated systemic diseases. Comprehensive dermatological examination documented the morphology and distribution of lesions, mucosal involvement, Nikolsky's sign, and bulla spread sign. Clinical diagnosis was established before laboratory investigations.

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Tzanck smear and histopathological examination: Fresh intact vesicles or bullae were selected for Tzanck smear preparation. After deroofing the blister, material from the lesion base was collected, stained with May-Grünwald-Giemsa stain, and examined microscopically for acantholytic cells.

A representative skin biopsy including an intact blister and adjacent normal skin was obtained under local anesthesia using 2% lignocaine. Specimens were fixed in 10% buffered formalin, processed routinely, embedded in paraffin, sectioned at 3–5 µm, and stained with hematoxylin and eosin. Histopathological evaluation included determination of the level of blister formation, presence of acantholysis, epidermal alterations, and dermal inflammatory infiltrate. Final histopathological diagnosis was recorded by the pathology department.

Clinicohistopathological correlation: The provisional clinical diagnosis was compared with the final histopathological diagnosis for each participant. Cases were categorized as concordant when both diagnoses belonged to the same disease entity and discordant when they differed. The degree of clinicohistopathological correlation constituted the primary study outcome.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using Stata version 17.0 (StataCorp LLC, College Station, TX, USA). Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. The association between clinical and histopathological diagnoses was evaluated using the Chi-square test. A p-value of <0.05 was considered statistically significant.

Results

A total of **92 patients** with clinically suspected autoimmune vesiculobullous disorders were included in the study. The demographic characteristics of the study population are presented in **Table 1**. The highest proportion of patients belonged to the **41–50 years** age group (27.17%), followed by **31–40 years** (22.83%) and **21–30 years** (19.57%). Females constituted **73.91%** of the study population, while males accounted for **26.09%**. Most patients presented within **three months** of disease onset, with 42.39% reporting symptoms for less than one month and an equal proportion presenting within one to three months. **[Table 1, Figure 1]**

Table 1. Demographic Characteristics of Study Participants (N = 92)

Variable	Category	Frequency	Percentage (%)
Age Group (Years)	0–10	2	2.17
	11–20	2	2.17
	21–30	18	19.57
	31–40	21	22.83
	41–50	25	27.17
	51–60	13	14.13
	61–70	11	11.96
Sex	Male	24	26.09
	Female	68	73.91
Duration of Lesions	<1 month	39	42.39
	1–3 months	39	42.39
	4–6 months	4	4.35
	>6 months	10	10.87

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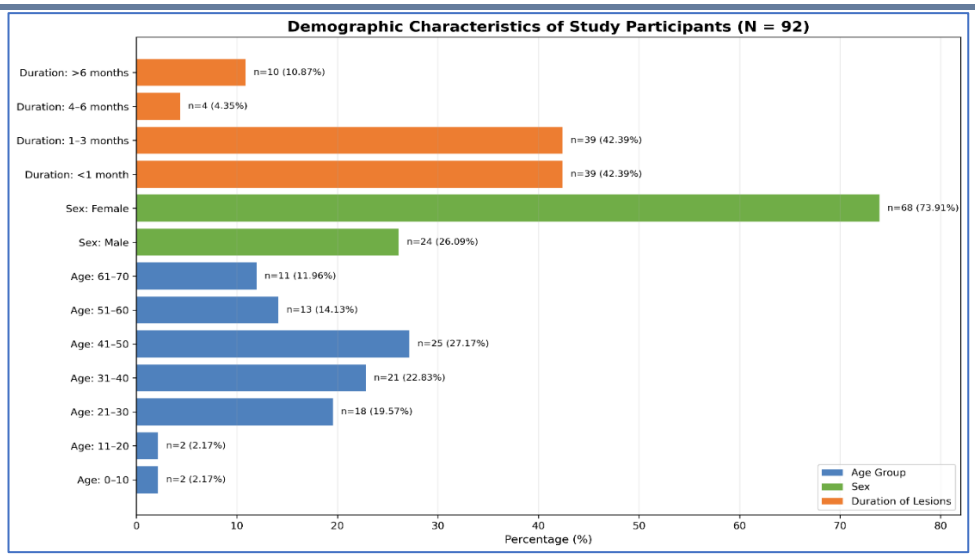

Figure 1: Demographic Characteristics of Study Participants

The most common site of onset was the **oral mucosa (55.43%)**, followed by the extremities (19.57%), scalp and face (14.13%), and trunk (10.87%). Vesicles were the predominant morphological lesion (76.09%), followed by raw mucosal erosions (52.17%) and crusting (36.96%). Mucosal involvement was observed in **55.43%** of patients, with oral mucosa being the most frequently affected site (62.75% of those with mucosal involvement). The commonest presenting complaint was **itching (70.70%)**, followed by pain (53.30%) and burning sensation (34.80%). Nikolsky's sign was positive in **79.34%** of patients, while bulla spread sign and Asboe-Hansen sign were present in 63.04% and 60.87%, respectively. **[Table 2, Figure 2]**

Table 2. Clinical Characteristics of Autoimmune Vesiculobullous Disorders

Variable	Category	Frequency	Percentage (%)
Site of Onset	Mucosa	51	55.43
	Scalp & face	13	14.13
	Trunk	10	10.87
	Extremities	18	19.57
Morphology*	Vesicles	70	76.09
	Bullae	13	14.13
	Pustules	22	23.91
	Crusting	34	36.96
	Oozing	18	19.57
	Raw mucosal lesions	48	52.17
Mucosal involvement	Present	51	55.43
	Absent	41	44.57
Type of mucosal involvement†	Oral	32	62.75
	Genital	16	31.37
	Conjunctival	2	3.92
	Nasal	1	1.96
Presenting complaints*	Itching	65	70.70
	Pain	49	53.30
	Burning sensation	32	34.80
Clinical signs*	Nikolsky's sign	73	79.34
	Bulla spread sign	58	63.04
	Asboe-Hansen sign	*56*	*60.87*

**Multiple responses possible.*

†Percentages calculated among patients with mucosal involvement (n=51).

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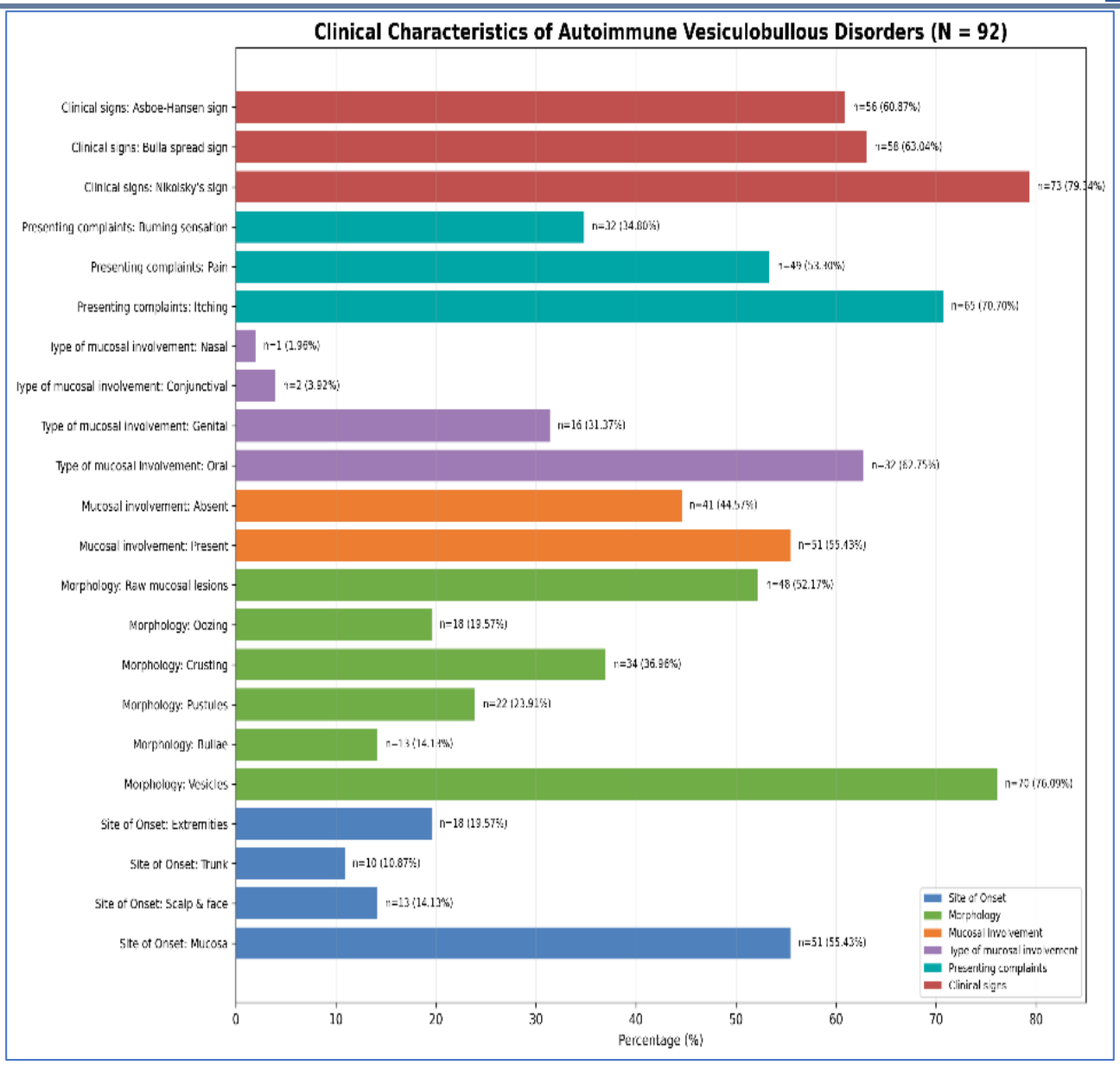

Figure 2: Clinical Characteristics of Autoimmune Vesiculobullous Disorders

Histopathological examination demonstrated that **pemphigus vulgaris** was the most common diagnosis (60.87%), followed by **pemphigus foliaceus** (26.09%) and **bullous pemphigoid** (13.04%). Suprabasalcleft formation was the predominant histological finding (60.87%). Acantholysis was the most frequent epidermal change (65.22%), whereas eosinophilic inflammatory infiltrate was the commonest dermal finding (16.30%). Tzanck smear was positive in **86.96%** of cases, with acantholytic cells identified in the majority of positive smears. **[Table 3]**

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Table 3. Histopathological and Cytological Findings

Variable	Category	Frequency	Percentage (%)
Histopathological diagnosis	Pemphigus vulgaris	56	60.87
	Pemphigus foliaceus	24	26.09
	Bullous pemphigoid	12	13.04
Level of cleft formation	Suprabasal	56	60.87
	Subcorneal	24	26.09
	Subepidermal	12	13.04
Epidermal changes*	Acantholysis	60	65.22
	Row of tombstone appearance	32	34.78
	Villi formation	26	28.26
	Hyperkeratosis	15	16.30
	Acanthosis	11	11.96
	Dyskeratosis	4	4.35
Dermal changes*	Eosinophilic infiltrate	15	16.30
	Papillary oedema	3	3.26
	Neutrophilic infiltrate	1	1.09
	Papillary microabscesses	0	0.00
Tzanck smear	Positive	80	86.96
	Negative	12	13.04
Tzanck smear findings*	Acantholytic cells	80	86.96
	Neutrophils	50	54.35
	Eosinophils	24	26.09

**Multiple findings were observed in the same patient.*

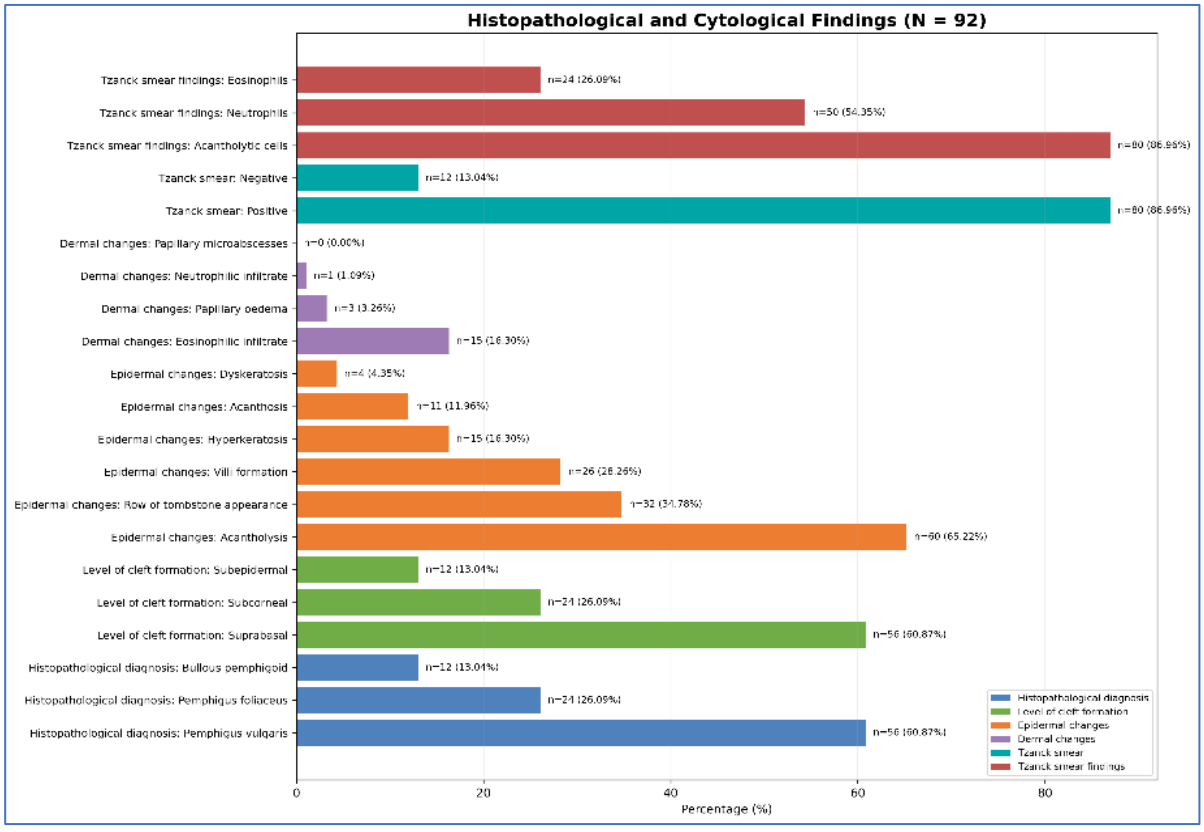

Figure 3: Histopathological and Cytological Findings

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Comparison of clinical and histopathological diagnoses revealed excellent diagnostic agreement. Clinically, **pemphigus vulgaris** was diagnosed in **64.13%** of patients and histopathologically confirmed in **60.87%**. Bullous pemphigoid demonstrated complete agreement between clinical and histopathological diagnoses. Overall clinicohistopathological agreement was **93.48%**, with a **Cohen's kappa coefficient of 0.823**, indicating excellent agreement beyond chance (**p < 0.001**). **[Table 4]**

Table 4. Comparison of Clinical and Histopathological Diagnosis

Diagnosis	Clinical Diagnosis n (%)	Histopathological Diagnosis n (%)
Pemphigus vulgaris	59 (64.13)	56 (60.87)
Pemphigus foliaceus	21 (22.83)	24 (26.09)
Bullous pemphigoid	12 (13.04)	12 (13.04)
Dermatitis herpetiformis	0	0
Linear IgA disease	0	0
Pemphigoid gestationis	0	0

Clinicohistopathological concordance was observed in **86 (93.48%)** patients, while discordance was noted in only **6 (6.52%)** patients. The observed agreement (93.48%) was substantially higher than the expected agreement (54.90%), confirming a high degree of concordance between clinical and histopathological diagnosis in autoimmune vesiculobullous disorders. **[Table 5]**

Table 5. Diagnostic Agreement Between Clinical and Histopathological Diagnosis

Parameter	Value
Overall agreement	93.48%
Expected agreement	54.90%
Cohen's kappa (κ)	0.823
Standard error	0.0672
p-value	<0.001
Concordant cases	86 (93.48%)
Discordant cases	6 (6.52%)

Overall, **pemphigus vulgaris** was the predominant autoimmune vesiculobullous disorder in the study population, followed by **pemphigus foliaceus** and **bullous pemphigoid**. Histopathological examination demonstrated predominantly suprabasal cleft formation with acantholysis as the characteristic epidermal change. Tzanck smear showed a high diagnostic yield, being positive in 86.96% of patients. The study demonstrated **excellent clinicohistopathological correlation (κ = 0.823, p < 0.001)**, supporting the diagnostic value of integrating careful clinical assessment with routine histopathological examination in the evaluation of autoimmune vesiculobullous disorders.

Discussion

Autoimmune vesiculobullous disorders (AVBDs) are chronic autoimmune blistering diseases that require early diagnosis because delayed treatment is associated with significant morbidity. Although direct immunofluorescence is considered the diagnostic gold standard, clinical evaluation combined with routine histopathological examination remains the cornerstone of diagnosis in many resource-limited settings. In the present study, excellent clinicohistopathological concordance was observed (93.48%; κ=0.823, p<0.001), demonstrating that careful clinical assessment supported by histopathology provides a reliable diagnostic approach.

Most patients in the present study belonged to the fourth and fifth decades of life, with the highest

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proportion in the 41–50-year age group (27.17%). Similar observations were reported by **Mittal et al.**, who found that autoimmune vesiculobullous disorders predominantly affected middle-aged adults (44). **Nageen et al.** also reported that these disorders commonly occurred during middle age, supporting the age distribution observed in the present study (12).

Females constituted 73.91% of the study population, indicating a marked female predominance. This finding agrees with **Mittal et al.**, who likewise reported higher disease frequency among females (13). The increased susceptibility of females has been attributed to hormonal and immunological factors. However, **Nageen et al.** observed an almost equal sex distribution, suggesting that demographic patterns may vary across different populations and study settings (12). Overall, the demographic findings of the present study are largely consistent with previous Indian studies.

The present study identified **pemphigus vulgaris** as the most common autoimmune vesiculobullous disorder, accounting for **60.87%** of histopathologically confirmed cases, followed by **pemphigus foliaceus (26.09%)** and **bullous pemphigoid (13.04%)**. Similar findings have been reported by **Mittal et al.**, who observed pemphigus vulgaris in 48.2% of cases, followed by bullous pemphigoid (27.3%), pemphigus foliaceus (3.6%), and dermatitis herpetiformis (8.3%) (13). Although pemphigus vulgaris was more frequent in the present study, the overall disease pattern was comparable. **Nageen et al.** also reported pemphigus vulgaris as the predominant autoimmune blistering disorder, supporting the present findings (12).

No cases of **dermatitis herpetiformis, linear IgA disease, or pemphigoid gestationis** were identified. While **Mittal et al.** reported dermatitis herpetiformis in 8.3% of patients (13), these disorders are generally uncommon. Previous studies have shown that **linear IgA disease** is rare and often requires direct immunofluorescence for confirmation (14,15), whereas **pemphigoid gestationis** is an uncommon pregnancy-associated disorder with very few reported cases (16,17). Their absence in the present study is therefore likely related to their low prevalence and the limited sample size rather than a true epidemiological difference.

The clinical presentation in the present study was typical of autoimmune vesiculobullous disorders. **Mucosal involvement** was observed in **55.43%** of patients, predominantly affecting the oral cavity, consistent with the high prevalence of pemphigus vulgaris. **Vesicles (76.09%)** were the most common lesion, while itching (70.7%), pain (53.3%), and burning sensation (34.8%) were the predominant presenting complaints. Similar clinical patterns have been reported by **Nageen et al.**and**Mittal et al.**, who emphasized that considerable overlap exists among autoimmune blistering disorders, making histopathological confirmation essential (12,13).

The present study demonstrated excellent **clinicohistopathological concordance (93.48%)**, with a **Cohen's kappa coefficient of 0.823 (p<0.001)**, indicating excellent agreement between clinical and histopathological diagnoses. Clinically, pemphigus vulgaris was diagnosed in 59 (64.13%) patients, whereas histopathology confirmed 56 (60.87%) cases, while bullous pemphigoid showed complete agreement between both modalities. These findings indicate that although clinical examination provides a reliable provisional diagnosis, histopathological evaluation remains indispensable for accurately differentiating morphologically overlapping autoimmune vesiculobullous disorders.

The **clinicohistopathological concordance** observed in the present study (**93.48%; κ=0.823, p<0.001**) was higher than that reported in previous studies. **Nageen et al.** reported an overall concordance of **80.7%** (12), while another study and a systematic review reported agreement rates of **72.6%** and **72.8%**, respectively (17,18,19). A large biopsy-based study documented concordance ranging from **55.36% to 61.01%** (20). The higher concordance in the present study may be attributed to the inclusion of fresh untreated lesions, standardized clinical evaluation, and careful biopsy site selection, all of which improved diagnostic accuracy.

Clinical examination also demonstrated good diagnostic utility. **Nikolsky's sign** was positive in **79.34%** of patients, while **bulla spread sign** and **Asboe-Hansen sign** were observed in **63.04%** and **60.80%**, respectively. These findings reflect the predominance of pemphigus vulgaris and are consistent with previous studies that have

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highlighted the diagnostic importance of these clinical signs (12,18). However, because significant overlap exists among autoimmune blistering disorders, histopathological confirmation remains essential for establishing the final diagnosis.

Overall, the present study demonstrates that a combined approach using **clinical examination, Tzanck smear, and routine histopathology** provides excellent diagnostic accuracy for autoimmune vesiculobullous disorders. This approach is particularly valuable in resource-limited settings where direct immunofluorescence is not routinely available and facilitates early diagnosis and appropriate patient management.

Conclusion

The present study demonstrates that pemphigus vulgaris is the predominant autoimmune vesiculobullous disorder in the study population, with characteristic clinical features including oral mucosal involvement, vesicular lesions, and positive Nikolsky's sign. Histopathologically, suprabasal cleft formation and acantholysis were the most consistent findings, while Tzanck smear proved to be a rapid and valuable adjunctive diagnostic tool. Most importantly, the study established excellent clinicohistopathological concordance, confirming that meticulous clinical examination closely correlates with histopathological diagnosis. These findings emphasize that an integrated approach combining detailed clinical assessment, Tzanck smear, and routine histopathological examination enables accurate diagnosis and early therapeutic intervention. In settings where direct immunofluorescence is not readily available, this practical clinicohistopathological approach remains an effective, reliable, and cost-efficient strategy for the diagnosis and management of autoimmune vesiculobullous disorders.

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