# Concordance of clinical, histopathologic and direct Immunofluorescence findings in patients with intraepidermal immunobullous disorders

Erum Ashraf, Faryal Shareef

## Abstract

**Objective:** To determine the concordance among clinical, histopathological and immunofluorescence as diagnostic methods for intraepidermal immunobullous disorders.

**Method:** The prospective cross-sectional study was conducted at the Institute of Skin Diseases, Karachi, from December 2020 to December 2022, and comprised adult patients of either gender presenting with complaints of bullae, vesicles, pustules and crusts on the skin or mucous membrane. Diagnostic findings of each patient as obtained by clinical assessment, microscopy and direct immunofluorescence were compared. Data was analysed using SPSS 19.

**Results:** Of the 81 patients, 41(50.6%) were males and 40(49.4%) were females. The overall median age was 35 years (interquartile range: 23 years), with 66(75%) patients aged 19-55 years. The predominant body site involved was the trunk 49(60.5%), followed by mucosa 26(32.1%). Clinical diagnosis detected 80(98.7%) cases, compared to 76(93.8%) by microscopy and 81(100%) by direct immunofluorescence.

**Conclusion:** Direct immunofluorescence was found to be the gold standard for a confirmatory diagnosis of intraepidermal immunobullous disorders, especially when clinical and histopathology findings were inconclusive.

**Keywords:** Intraepidermal immunobullous disorders, Pemphigus vulgaris, Histopathology, Direct immunofluorescence.

## Introduction

Bullae are a common skin condition that can be a result of bacterial or viral infections, trauma, genetic disorders and autoantibodies. Intraepidermal autoimmune bullous disorders are autoimmune disorders resulting from the formation of autoantibodies directed against the structural proteins of the epidermis or the dermal-epidermal junction.[1-3] These disorders are characterised by blister formation at mucosal as well as cutaneous surfaces, while the severity of the diseases may widely vary.[3,4] There are various types of immunobullous disorders that are specifically characterised on the basis of the location of the bullae in the skin and the specific antigens targeted by the antibodies. These can be largely categorised into intraepidermal and subepidermal bullous disorders.[2,5] The most common intraepidermal immunobullous disorder is pemphigus vulgaris, with a worldwide prevalence of 0.1-0.5 per 100,000 population. The other intraepidermal immunobllous disorders are pemphigus foliaceus (PF), paraneoplastic pemphigus, intercellular immunoglobulin A (IgA) pemphigus, and pemphigus erythematous (PE).[5-8] The disease can be life-threatening in 5-10% of cases. An accurate diagnosis of disease requires a correlation of clinical findings and microscopy with findings on direct immunofluorescence (DIF).[9,10] Hence, DIF is crucial to the diagnosis of such disorders. Nevertheless, in resource-limited settings, like Pakistan, due to economic or cost-related reasons, many patients never opt for DIF, and dermatologists are dependent on clinical diagnosis and histopathology for diagnosis and treatment. A recent study found 100% concordance among all the three modes for diagnosing autoimmune bullous disorders.[11] However, there is lack of sufficient local evidence regarding the spectrum of clinical presentation and frequency of various diagnostic and clinical features of intraepidermal immunobullous disorders that may vary from population to population. The current study was planned to fill the gap by determining the concordance among clinical, histopathological and DIF as diagnostic methods for intraepidermal immunobullous disorders.

## Patients and Methods

The prospective cross-sectional study was conducted at the Institute of Skin Diseases (ISD), Karachi, between December 2020 and December 2022. After approval from the institutional ethics review committee, adult patients attending the Dermatology out-patient department (OPD) and inpatients presenting with complaints of bullae, vesicles, pustules and crusts on the skin or mucous membrane, and diagnosed with intraepidermal immunobullous disorders were included using convenience sampling technique. Patients presenting with blisters due to infectious diseases, such as measles and rubella, any drug reaction or allergies, and hereditary

Department of Dermatology, Institute of Skin Diseases, Karachi, Pakistan.

**Correspondence:** Erum Ashraf. e-mail: drerum_ashraf@yahoo.com

ORCID ID. 0000-0001-5302-3044

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disorders were excluded.

After taking informed consent from all the subjects, data was collected by trained data-collectors regarding the disease, related complications, and laboratory investigations. Clinical examination and diagnosis was done directly by a qualified consultant dermatologist. Skin smears were taken after scraping the base of a fresh blister, and were stained with Giemsa stain using the standard method. The stained slides were evaluated under a light microscope, and were considered positive for the disease on the basis of the presence of acantholytic or Tzanck cells. For skin biopsy 2 samples were taken, one from the lesional skin for routine histopathological examination and another from the perilesional skin for DIF. The sample for DIF was immediately stored in Michel's medium to be sent to the laboratory for examination of immunoreactants under a fluorescence microscope. Diagnosis obtained by clinical assessment, microscopy, and DIF were compared to determine possible concordance among these diagnostic methods. Data was analysed using SPSS 19. Descriptive statistics were calculated for basic demographic characteristics. Data was expressed as frequencies and percentages. Chi-square test was used to assess the differences in the clinical spectrum of immunobullous disorders based on differences in gender and age groups. $P<0.05$ was considered statistically significant.

## Results

Of the 81 patients, 41(50.6%) were males and 40(49.4%) were females. The overall median age was 35 years (interquartile range [IQR]: 23 years), with 66(75%) patients aged 19-55 years. The predominant body site involved was the trunk 49(60.5%), followed by mucosa 26(32.1%). Among the participants, 19(23.5%) presented with disease duration <1 month, 39(48.1%) 1-3 months, 19(23.5%) 4-6 months, and 4(4.9%) >6 months.

Among non-specific signs and symptoms, oral ulcer, Nikolsky sign and weight-loss were the most frequently observed, with a frequency of 65(80.2%), 41(50.6%) and 30(37%), respectively. Among the blisters, 38(46.9%) were discrete and 25(30.9%) were mixed. However, the characterization of blister was not possible or applicable in 18(22.2%) lesions (Table 1).

Clinical diagnosis detected 80(98.7%) cases, compared to 76(93.8%) by microscopy and 81(100%) by direct immunofluorescence (Table 2). Among the participants, 61(75.3%) were identified as positive for intercellular IgG, while 3(3.7%) were found positive for IgA, and 1(1.2%) case was diagnosed as intercellular and subepidermal IgG-positive paraneoplastic pemphigus. Also, 1(1.2%) case clinically presenting as bullous SLE and non-representative

on histopathology was diagnosed positive for complement proteins C3 and C1q deposited along the dermoepidermal junction which is the interface between epidermis and dermis.

**Table-1:** Demographic and clinical characteristics of patients with intraepidermal immunobullous disorders (n=81).

| Variable | n (%) |
|---|---|
| **Median Age** 35 years (IQR =24 years) | |
| **Age** (years) | |
| ≤18 | 8 (9.9 |
| 19-35 | 35 (43.2 |
| 36-55 | 28 (34.6 |
| ≥56 | 10 (12.3 |
| **Gender** | |
| Male | 40 (49.4 |
| Female | 41 (50.6 |
| **Disease duration** (months) | |
| <1 | 19 (23.5 |
| 1-3 | 39 (48.1 |
| 4-6 | 19 (23.5 |
| >6 | 04 (4.9 |
| **Predominant body area involved** | |
| Mucosa | 26 (32.1 |
| Head and Neck | 6 (7.4 |
| Trunk | 49 (60.5 |
| **Predominant skin lesions** | |
| Blisters | 34 (41.9 |
| Erosions | 37 (45.6 |
| Scaling | 1 (1.2 |
| Scaling and erosions | 09 (11.1 |
| **Other signs and symptoms ** ** | |
| Itching | 14 (17.3 |
| Alopecia | 14 (17.3 |
| Joint Pian | 15 (18.5 |
| Oral ulcer | 65 (80.2 |
| Photosensitivity | 8 (9.8 |
| Nikolsky sign | 41 (50.6 |
| Weight loss | 30 (37.0 |
| **Blister Characteristic** | |
| Discrete | 38 (46.9 |
| Mixed | 25 (30.9 |
| Not applicable | 18 (22.2 |
| **Type of split present on Histopathology** | |
| 1. Subcorneal | 11 (13.6 |
| 2. Suprabasal | 51 (63 |
| 3. Subepidermal | 4 (4.9 |
| 4. Absent | 15 (18.5 |
| **Type of infiltrate** | |
| Mixed | 11 (136. |
| Lymphocytic | 35 (43.2 |
| Lymphoplasma | 1 (1.2 |
| Neutrophilic | 6 (7.4 |
| Non-specific | 28 (34.6 |
| **Serum ANA** | |
| Positive | 3 (3.7 |
| Negative | 78 (96.3 |
| **Photo-sensitivity** | |
| Yes | 07 (8.6 |
| No | 74 (91.4 |
| **History of Stress** | |
| Yes | 53 (65.4 |
| No | 28 (34.6 |

**multiple responses possible; ANA: Antinuclear antibody.

**Table-2:** Concordance between findings of clinical examination, histopathology and DIF in patients with intraepidermal immunobullous disorders (n=81).

| Disease type | Findings n (%) | | |
| --- | --- | --- | --- |
| | Clinical Diagnosis | Histopathology | DIF * |
| Pemphigus Vulgaris | 55 (67.9) | 55 (67.9) | 46(56.7) |
| Pemphigus Folaceous | 10(12.3) | 07(8.6) | 05(6.1) |
| **Pemphigus** | | | |
| Paraneoplastic | 04(4.9) | 01(1.2) | 01(1.2) |
| Senear-Usher Syndrome | 02(2.5) | 0 | 02(2.5) |
| Bullous SLE | 04(4.9) | 0 | 03(3.7) |
| SLE | 01(1.2) | 0 | 01(1.2) |
| Linear IgA | 0 | 03(3.7) | 03(3.7) |
| Eczema | 0 | 06(7.4) | 0 |
| Drug Reaction | 04(4.9) | 05 | 04(4.9) |
| Inconclusive | 0 | 02(2.5) | 0 |
| Non representative | 0 | 02 (2.5) | 01(1.2) |
| Negative | 0 | 0 | 15(18.5) |

*Direct Immunofluorescence, Ig: Immunoglobulin, SLE: Systemic lupus erythematosus.
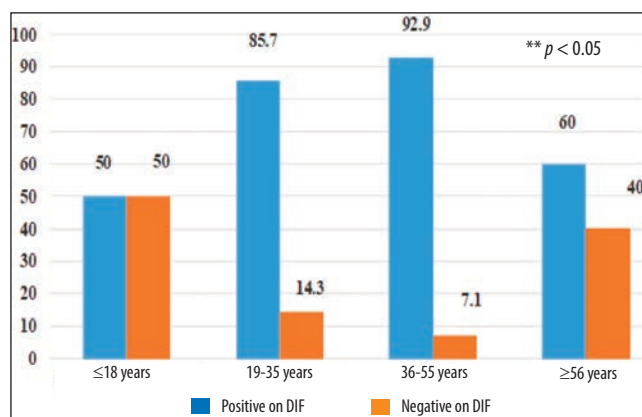


**Figure:** Distribution of diagnostic outcome on direct immunoflorecense (DIF) on the basis of age group (n=81).

There were significant differences in cases confirmed on DIF among different age groups ($p$=0.01) (Figure).

Among the cases diagnosed on DIF, gender stratification was not significant ($p$>0.05).

## Discussion

The current study mainly focussed on intraepidermal immunobullous disorders among patients presenting at one of the biggest public-sector dermatology hospitals in the country. The median age of disease presentation was 35 years (IQR: 24 years). The age range of the sample was 12-72 years. The average age at presentation was slightly lower compared to a previous study,[11] which can be explained by the slight difference in the targeted age group. Also, the selective inclusion of intraepidermal immunobullous cases in the contrast study was in contrast to the previous study which included dermatology patients with all kinds of immunobullous disorders.

On clinical diagnosis, the most common type of intraepidermal immunobullous disorder was pemphigus vulgaris affecting 67.9% patients, while only 56.7% of those cases were confirmed on DIF. Similarly, of the 12.3% patients clinically diagnosed with pemphigus folaceous, only 6.1% were confirmed on DIF. The clinical diagnosis of two patients with Senear-Usher Syndrome was also confirmed on DIF which showed positive intercellular IgG along with a subcorneal split consistent with pemphigus folaceous. The diagnosis was supported by a positive antinuclear antibody (ANA). In the current study, majority of the cases showed significant concordance across clinical findings, histopathology and DIF. These observations were similar to earlier findings.[12] Slight discordance was observed in a few cases. For example, in 3 cases that were clinically suspected as pemphigus vulgaris were diagnosed as linear IgA disease based on histopathology and DIF as the cases lacked characteristic clinical features of the disease. Frequent observations of slight to moderate discordance among clinical diagnosis, histopathology and DIF are well supported by most studies conducted in similar populations.[13-17] Whenever there is a lack of correlation or discordance with clinical and histopathological parameters, DIF is considered an effective tool to diagnose intraepidermal immunobullous disorders.[7,18-20] However, in cases where DIF is not available, diagnosis should be made on clinical assessment and histopathology.

The most common non-specific lesion was oral ulcer, but Nikolsky sign was positive among half of the patients. The predominantly affected region was mainly the trunk, followed by mucosa, head and neck. Moreover, stress was reported by the majority of the patients with intraepidermal immunobullous disorders.

The current study faced serious challenges in the collection of data which coincided with the active phase of coronavirus diseases-2019 (COVID-19) pandemic, which resulted in a temporary shutdown of dermatology OPDs. The current findings lack generalisability because of certain limitations. It did not calculate the sample size, and, instead, recruited all the available patients. Also, the findings relate to a single centre.

Multi-centre studies with larger sample sizes are required to identify any possible concordance of clinical diagnosis, histopathology and DIF with autoimmune diseases.

## Conclusion

DIF was found to be the gold standard for a confirmatory diagnosis of intraepidermal immunobullous disorders, especially when clinical and histopathology findings were inconclusive.

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**Author Contribution:**

EA: Study design, data collection and writing.

FS: Data collection and writing.