A Clinicopathological and Immunofluorescence Study of Intraepidermal Immunobullous Diseases

Keya Basu, Moumita Chatterjee, Abhishek De1, Moumita Sengupta, Chhanda Datta, Pradip Mitra

Abstract

Context: Autoimmune blistering diseases (AIBDs) are characterized by autoantibodies directed against antigens of skin. Direct immunofluorescence (DIF) study helps in confirming the diagnosis where histopathology alone is noncontributory. Aims: This study aimed to evaluate the clinicopathological and DIF features of intraepidermal AIBD and to assess their relative diagnostic significance. Materials and Methods: It was an institution-based observational study. A total of 34 patients were studied over a period of 1½ years in the Department of Pathology in collaboration with the Department of Dermatology of a tertiary care hospital. The clinical, pathological, and DIF features were evaluated and documented. Statistical Analysis: Data were analyzed by statistical tests using GraphPad InStat. Results: Pemphigus vulgaris (PV) was the predominant type with 18 (53%) cases followed by 15 (44%) cases of pemphigus foliaceus (PF) and a single case of pemphigus erythematosus (PE). The age of the patients ranged from 17 to 85 years. Overall, there was a female preponderance in the study group. The most common presenting feature was pruritus (58.82%). Tzanck smear showed the presence of acantholytic cells in thirty (88.24%) patients. Characteristic histopathological features were present in all the cases of PV and PF except one case of PF which was found to be a case of PE. DIF study showed intraepidermal deposition of intercellular immunoglobulin G (IgG) and C3 both in PV and PF. The case of PE showed epidermal “antinuclear antibody” staining with IgG. Conclusion: Immunofluorescence study may be used as an additional tool for confirmation of diagnosis where histopathology alone is inconclusive.

Key Words: Direct immunofluorescence, histopathology, immunobullous disorder, tzanck smear

Introduction

Intraepidermal immunobullous diseases are one type of autoimmune bullous diseases (AIBDs) characterized by pathogenic autoantibodies directed against target antigens, which are components of desmosome complex. Pemphigus vulgaris (PV) is the most common with a worldwide prevalence of 0.1–0.5/100,000 population[1] followed by pemphigus foliaceus (PF).

Tzanck smear is used as a minimally invasive test for the diagnosis of the pemphigus group of AIBDs and was first used by Arnault Tzanck in 1947.[2,3] Although the histopathology findings are characteristic, direct immunofluorescence (DIF) test leads to the confirmation due to its accuracy. DIF is a procedure for the detection of in vivo-bound antibody, complement components, and fibrinogen in the patient’s skin.[4] Patients with PF may show fluorescence solely or predominantly in the superficial layers of the epidermis. In addition, 30%–50% of PV patients may have immunoglobulin (Ig) M or IgA deposits and C3 deposition in the intercellular substance in up to 50% of PV patients. Pemphigus erythematosus (PE) patients (about 80%) also show granular deposits of C3 and IgG at the dermo-epidermal junction.[5] In addition, a positive DIF test may indicate an impending relapse in a patient in remission.[6]

Limited literature is available regarding the clinicopathological correlation of intraepidermal AIBD from eastern part of India. Hence, our study aimed to correlate the clinicopathological and immunofluorescence findings of intraepidermal AIBD.

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Materials and Methods
This study was conducted in the Department of Pathology in collaboration with the Department of Dermatology of a tertiary care hospital, over a period of 1½ years. Our study was approved by the Institutional Ethics Committee. We had included 34 patients who attended the Dermatology OPD and were suspected with intraepidermal AIBD. Patients who presented with bullae, vesicles, pustules, and crusts on the skin and mucous membrane were included in the study. Patients with blistering diseases due to infective etiology (e.g., varicella) or drug reaction (e.g., Stevens–Johnson syndrome) were excluded from the study.

After taking written informed consent from the patients, history and clinical findings were recorded followed by appropriate routine laboratory investigations. Tzanck smears were done after scraping the floor and roof of a fresh blister and were stained with Giemsa stain by conventional method. The stained slides were evaluated under a light microscope and considered as positive on the basis of the presence of acantholytic or Tzanck cell. A punch biopsy was taken from lesion in 10% buffered formaldehyde solution for routine histopathological examination (HPE). All the hematoxylin and eosin (H and E) stained slides were assessed under the following parameters: level of blister, overlying epidermis, blister cavity content, and dermal infiltrate.

Another punch biopsy was taken from the perilesional skin for DIF in Michel's transport medium. The slides were examined under fluorescence microscope and the following parameters were noted: nature, site, and pattern of immune deposit. Positive and negative controls were tested simultaneously.

All 34 cases were analysed. Data were analyzed by statistical tests using GraphPad InStat (GraphPad Software Inc., 11452 El Camino Real, #215, San Diego 92130, USA).

Results
Clinical findings
In our study, out of a total of 34 cases, PV was found to be the predominant type with 18 (53%) cases followed by 15 (44%) cases of PF and a single case of PE. The age of the patients ranged from 17 to 85 years, with a mean age of 46.24 years (±15.48). The mean age of PF was slightly higher than that of PV. Overall, there was a female preponderance in the study group. As far as the clinical presentation was concerned, acute onset of the disease was noted in 15 (44%) patients and the duration ranged from 0.5 to 4 months with a mean of 2.08 months (±1.3 months). The most common presenting feature was pruritus (58.82%) followed by pain (41.18%). Majority of the patients (88.24%) presented with flaccid vesicles and bullae [Figures 1-3].

Nikolsky sign was positive in 25 (73.53%) cases. Oral mucosal lesion was detected in 13 (72.22%) patients of PV and three (20%) patients of PF, resulting in significant statistical differences (P=0.019) [Figure 4].

Table 1 summarizes the clinical details of all cases.

Pathological findings
In this study group, Tzanck smear tests showed the presence of acantholytic cells in 30 (88.24%) patients which included 17 cases of PV and 13 cases of PF but was negative in one case of PE. This was statistically significant (P=0.0420). The diagnostic accuracy of Tzanck test was 88.24% in this study. On HPE, PV and PF showed suprabasal and subcorneal bullae, respectively. The only case of PE showed subcorneal blister. Acantholytic cells were present in a majority (79.41%) of cases (P=0.0495). Row of tombstone appearance of basal layer was present in 10 (55.56%) patients out of 18 PV patients (P=0.0056). Dermal infiltration, comprising mostly of neutrophils, was present in 32 (94.12%) patients of pemphigus. We found intercellular edema and eosinophilic spongiosis in patients without bullous lesion.

Table 2 summarizes the histopathological features of all cases.

DIF could not be done in two cases of PV and two cases of PF as they were lost to follow-up. DIF study showed intraepidermal deposition of intercellular IgG and C3 both in 16 patients of PV and 13 patients of PF. The only case of PE showed epidermal “antinuclear antibody” (ANA) staining with IgG.

In this study, the correlation between histopathological diagnosis and DIF diagnosis of the cases of PV and PF was statistically significant (P<0.0001). Histopathologic and immunofluorescence discordant was noted in the
presentation included scaly lesions over the malar area and nose in a butterfly distribution, simulating cutaneous lupus erythematosus.

Table 3 summarizes histopathological and DIF diagnosis correlation.

**Discussion**

Vesiculobullous diseases are a heterogeneous group of diseases, presenting with vesicle or bulla arising over the skin and/or mucous membrane. In spite of similar clinical presentations, they are remarkably histopathologically different from each other. Proper diagnosis is essential to prevent the fatal outcome if untreated. Very few studies in India have dealt with the clinicopathological features of intraepidermal bullous diseases. In the present study, a comprehensive analysis of clinical, pathological, and DIF features of intraepidermal bullous diseases was done.

Out of the 34 cases of pemphigus studied, PV was the predominant type with 18 cases (53%) followed by 15 cases (44%) of PF and one case (3%) of PE. This corroborates with an earlier study by Arya *et al.* where 61.42% of cases were PV.\(^7\) Singh\(^8\) and Chowdhury *et al.*\(^9\) also found PV as the major group. In the present study, 34 patients were included with age ranging from 17 to 85 years (mean 46.24 ± 15.48 years). This finding is similar to the study done by Singh *et al.*\(^10\) and Khaninan *et al.*\(^11\) A recent study of pemphigus by Chowdhury *et al.* showed the age at presentation to be from 16 to 76 years (mean 49.48 ± 16.51 years).\(^9\) There was a slight female predominance with male:female ratio of 1:1.12. It was similar to that of a study by Chowdhury *et al.*, where female: male ratio was 1.9:1.\(^9\) Zaraa *et al.*\(^12\) and Deepti *et al.*\(^13\) also documented similar findings.

In our study, 15 (44%) patients presented with acute onset of the disease, while 19 (56%) patients presented with insidious onset and the duration of symptoms in pemphigus group ranged from 0.5 to 4 months (2.08 ± 1.3 months). In a study of 109 cases of pemphigus, conducted by Shafi *et al.*, the duration of disease at presentation varied from 3 to 13 days.\(^14\) Pruritus was present in 20 (58.82%) cases, while 14 (41.18%) patients presented with pain. Similarly, in the study by Singh *et al.*, the most common symptom was itching.\(^10\) Of all the cases, 16 (47.06%) patients presented with oral mucosal lesions and seven (20.59%) patients had involvement of other mucosa. Similarly, the most common mucosal involvement was oral mucosa in a study done by Javidi *et al.*\(^15\) However, oral mucosa was involved in only 28% of cases in a study done by Singh.\(^8\) In our study, one patient (3%) had only oral mucosal involvement with no cutaneous lesion and was diagnosed as a case of PV. In the study by Chowdhury *et al.*, two (6.25%) cases had only mucosal involvement and were diagnosed as mucosal PV.\(^9\) In a study done...
Table 1: Clinical profile of the study population

| Parameters                        | PV (n=18) | PF (n=15) | PE (n=1) | Total (n=34) | P     |
|-----------------------------------|-----------|-----------|----------|--------------|-------|
| Sex ratio (male:female)           | 1:1.57    | 1.5:1     | 0:1      | 1:1.12       | 0.4975|
| Mean age at presentation (years)  | 44.94±12.34 | 48.8±18.8 | 31       | 46.24±15.48 | 0.77  |
| Duration (months)                 | 1.75±1.43 | 2.43±1.28 | 3        | 2.08±1.3     | 0.3672|
| Symptoms                          |           |           |          |              |       |
| Pain                              | 10        | 3         | 1        | 14           | 0.1248|
| Pruritus                          | 8         | 12        | 0        | 20           |       |
| Oral mucosal involvement          | 13        | 3         | 0        | 16           | 0.0197*|
| Other mucosal involvement         | 6         | 1         | 0        | 7            | 0.2809|
| Nikolsky sign                     | 13        | 11        | 1        | 25           | 0.9451|
| Blister type                      |           |           |          |              |       |
| Flaccid                           | 16        | 13        | 1        | 30           | 0.9813|
| Tense                             | 2         | 2         | 0        | 4            |       |
| Blister arrangement               |           |           |          |              |       |
| Discrete                          | 13        | 8         | 0        | 21           | 0.4072|
| Grouped                           | 5         | 7         | 1        | 13           |       |

*Statistically significant. PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, PE: Pemphigus erythematosus

Table 2: Histopathological profile of the study population

| Parameters                        | PV (n=18) | PF (n=15) | PE (n=1) | Total (n=34) | P     |
|-----------------------------------|-----------|-----------|----------|--------------|-------|
| Acantholytic cell                 | 17        | 10        | 0        | 27           | 0.0495*|
| Neutrophil                        | 15        | 15        | 1        | 31           | 0.4034|
| Eosinophil                        | 3         | 1         | 0        | 4            | 0.8193|
| Tombstone appearance              | 10        | 0         | 0        | 10           | 0.0056*|
| Dermal infiltration               | 17        | 14        | 1        | 32           | 0.9938|

*Statistically significant. Test of significance of difference was calculated using Chi-square test. PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, PE: Pemphigus erythematosus

by Kambil and Madavamurthy where all the cases of PF had no mucosal involvement[14] though, we found mucosal involvement in three cases of PF. Nikolsky sign was found to be positive in 13 (72.22%) cases out of 18 cases of PV in 11 (73.33%) cases out of 15 cases of PF, and the only one case of PE. This corroborates with the study done by Chowdhury et al., where Nikolsky sign was seen in 29 (90.62%) cases of PV and in all the cases of PF.[9] Thirty-one (91.18%) patients had neutrophils and only 4 (11.76%) patients had eosinophils within the blisters. This result corroborated with a previous study done by Chowdhury et al.[9] Row of tombstone appearance of basal layer was present in 55.56% of PV patients, while Chowdhury et al. documented it in 53.12% cases of PV.[9]

DIF study showed intraepidermal deposition of both intercellular IgG and C3 in all (100%) patients of PV and PF. The case of PE showed epidermal “ANA” staining with IgG. Similarly, in the study done by Mimouni et al., DIF test showed deposition of IgG and C3 in the intercellular spaces (10 out of 14 patients) and variable immunostaining at the basement membrane zone (6 out of 14 patients).[17] In contrast, in the study done by Chowdhury et al., 87.50% of the PV patients showed IgG and C3 antibody deposition and 75% cases of PF showed only IgG antibody deposition in the upper epidermis.[9] In the study done by Arundhathi et al., DIF was positive in 24 out of 36 cases of PV and three out of six cases of PF.[18]

The correlation between histopathological and DIF diagnoses of PV and PF was statistically significant in our study. Similarly, in a study performed by Inchara and Rajalakshmi, 73 of 100 cases showed DIF findings concordant with histopathological findings.[19] Histopathologic and immunofluorescent discordance was noted in the case of PE. Similarly, in the study done by Deepti et al., only one case showed histopathological and DIF discordance.[13] We found 100% sensitivity in PV but 92.86% sensitivity in PF. The sensitivity of DIF was 94.44% in the pemphigus group of diseases in a study by Buch et al.[20]

Among the study population, on HPE, 100% cases of PV showed suprabasal blister and 100% cases of PF showed subcorneal bulla. Acantholytic cells were present in the intraepidermal blisters in 27 (79.41%) cases comprising of 17 (94.44%) cases of PV and ten (66.67%) cases of PF. Arya et al.[9] and Kambil and Madavamurthy[10] observed similar findings. Thirty-one (91.18%) patients had neutrophils and only 4 (11.76%) patients had eosinophils within the blisters. This result corroborated with a previous study done by Chowdhury et al.[9] Row of tombstone appearance of basal layer was present in 55.56% of PV patients, while Chowdhury et al. documented it in 53.12% cases of PV.[9]
However, immunofluorescence is a relatively simple, less time-consuming, and highly reproducible technique. It has immense importance for confirmation of diagnosis and also for subtyping the pemphigus group of diseases. Though in our study, histopathology played a great role in the diagnosis, immunofluorescence study helped in confirming the diagnosis in cases where histopathology alone was inconclusive. Therefore, DIF is a very useful diagnostic tool and should be used as an additional tool with histopathology.

**Conclusion**

Immunofluorescence study helps in confirming the diagnosis where clinical presentation and histopathology are inconclusive. Our study proved that DIF can be used as an additional tool in all intraepidermal autoimmune blistering diseases (AIBDs).

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**Conflicts of interest**

There are no conflicts of interest.

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**Table 3: Histopathological and direct immunofluorescent diagnosis correlation**

|            | PV (n=16) | PF (n=13) | PE (n=1) |
|------------|-----------|-----------|----------|
| Sensitivity| 100       | 92.86     | Only one case which was diagnosed as PF by histopathology |
| Specificity| 100       | 100       |          |
| Positive predictive value | 100       | 100       |          |
| Negative predictive value | 100       | 94.12     |          |
| P          | <0.0001   | <0.0001   |          |

PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, PE: Pemphigus erythematosus