A STUDY OF DIRECT IMMUNOFLUORESCENCE IN THE DIAGNOSIS OF IMMUNOBULLOUS DERMATOSES
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ABSTRACT: BACKGROUND: The immunobullous dermatoses are a group of blistering disorders characterised by pathogenic auto antibodies directed against the target antigens, which are components of the desmosomes or adhesion complex at the dermoepidermal junction. Direct immunofluorescence (DIF) is very much valuable in the diagnosis of these lesions. AIM: The aim of this study was to assess the diagnostic value of direct immunofluorescence in the commonly seen immunobullous dermatoses. The study also aimed at correlating the direct immunofluorescence patterns with clinical and histopathological findings. MATERIALS AND METHODS: A total of 30 patients of both sexes with suspected immunobullous disorders were selected among the patients attended over a period of one year from August, 1995 – July, 1996 in the Dermatology department of SVRR Govt. General Hospital, Tirupati, A.P. Clinical cutaneous examination along with a lesional and a perilesional skin biopsy specimens were collected from each of the 30 selected patients and were examined for histopathological study and Direct immunofluorescence study respectively at the Pathology department in the Sri Venkateswara Institute of Medical Sciences (SVIMS) Tirupati, A.P. Then they were analysed by correlating the clinical, histopathological and Direct immunofluorescence findings of immunobullous disorders of skin. RESULTS: Out of 30, 23 cases showed DIF patterns concordant with clinical and histopathological diagnosis. In the remaining 7 cases there was no correlation between the DIF, clinical and histopathological diagnosis, but the DIF proved to be highly valuable in confirming the diagnosis of the immunobullous dermatologic disorders. CONCLUSION: DIF is a very useful diagnostic tool in immunobullous skin disorders especially where there is overlap between clinical and histopathological diagnoses.

KEYWORDS: Autoimmune bullous dermatoses, Direct immunofluorescence, Pemphigus vulgaris, Bullous pemphigoid.

INTRODUCTION: Immunobullous dermatoses are associated with significant morbidity, considerable mortality and impaired quality of life.[1] Pemphigus vulgaris is the most common variety among the immunobullous dermatoses.

Direct immunofluorescence is a histochemical laboratory staining technique for detecting the presence and position of in vivo antibodies bound to tissue antigens.[2],[3] With direct immunofluorescence technique a rapid and accurate diagnosis can be made very early in the course of the immunobullous dermatoses.

MATERIALS AND METHODS: This study was conducted in the Department of Dermatology in SV Medical College & SVRR Govt. General Hospital, Tirupati, A.P and Department of Pathology in
SVIMS, Tirupati, A. P. for a period of one year from August, 1995 to July, 1996. Thirty patients with suspected autoimmune vesiculo bullos disorders of skin were included in the study. Patients with a known infective etiology of the bullous lesions were excluded. The clinical diagnosis of immunobullous dermatoses was made by detailed history, clinical findings and Tzanck test.[4]

After taking written informed consent of the patients, two biopsies were performed in each case under local anaesthesia, one from the fully developed vesiculobullous lesion (lesional biopsy) and the other from the perilesional area within 2 cm diameter of the lesion (perilesional biopsy). The lesional skin biopsy was sent in 10% formalin. Following standard processing, the sections were stained with H and E stain. Histopathological examination (HPE) findings were used as a further aid in the diagnosis.

The perilesional biopsy, placed in normal saline/phosphate buffered saline was immediately taken to the Pathology laboratory for DIF. The tissue was embedded in the cryostat embedding medium, frozen, and cut at 5μm thickness at−22°C. The cut sections were fixed with ether-alcohol mixture, and air dried for 10 min. Later the slides were rinsed in phosphate buffered saline for 30 min. The sections were incubated in respective fluorescein isothiocyanate conjugated antibody, i.e., IgG, IgA, IgM, and C3 respectively for 1hr. at 37°C. The sections were washed with phosphate buffer for 15 min. Then the sections were mounted with Glycerine jelly and preserved at 4°C. When observed under fluorescent microscope an apple green fluorescence was noticed in areas of antigen antibody reaction.[2],[3]

The DIF was reported based on the distribution and type of immune complexes. The type of Ig and the presence/ absence of Complement were noted. The deposition of immune complexes were noticed in the intercellular space (ICS) of epidermal region, basement membrane zone (BMZ) and in the walls of superficial dermal vessels (BMZ).[2] Immunobullous lesions were diagnosed based on the standard criteria.[4]

RESULTS: The patients age in this study ranged from 10 years (a boy diagnosed as Bullous pemphigoid) to 74 years (a male diagnosed as Bullous pemphigoid [BP]). Around 30% of patients were in the age group 41-50 years with a mean age of 40.73 years. A male to female ratio of 1.3:1.0 was observed. The distribution of immunobullous dermatoses in this study was as shown in the figure 1. In this study Pemphigus vulgaris is the commonest immunobullous dermatosis observed (43.33%). In the Pemphigus group female preponderance was observed with male to female ratio of 1.0:1.6. Vesicles and bullous lesions were the most common clinical finding followed by erosions and crusting. Flaccid vesicles and bullae were seen in 60% of cases and tense bullae were seen in 40% of cases. Itching was the most common symptom followed by pain and burning sensation of eroded skin. Oral lesions were seen in 15 patients (50% of cases). Tzanck smear was positive for acantholytic cells in 53.33% of cases. Subcorneal blister was seen in 16.66%, subepidermal in 40%, and suprabasal in 43.34% of the cases. Acantholysis (53.33% of cases) and inflammatory infiltrates were noted within the blisters. Histopathological features of various immunobullous dermatoses observed in this study are shown in the figures [Figure 2 (a)], [Figure 3(a)], [Figure 4(a)] and [Figure 5(a)].

On DIF examination, intercellular space (ICS) deposition of IgG was present in 5 out of 13 cases of pemphigus vulgaris resembling fishnet pattern as shown in the Figure 2(b). 8 cases also
showed C3 deposition along with IgG at the ICS. All the cases of pemphigus foliaceus showed ICS deposition of IgG in upper layers of epidermis as shown in figure 3(b). Majority (4 of 5 cases i.e. 80%) of Bullous pemphigoid showed linear IgG and C3 deposition in the basement membrane zone (BMZ) as shown in figure 4(b). One case showed IgM deposited in addition to IgG and C3 along the BMZ. One case of Lichen planus pemphigoides showed IgG and C3 deposit in granular pattern at BMZ as shown in figure 5(b). The DIF patterns observed in various immunobullous dermatoses in this study are depicted in the table 1.

All the 30 cases in this study showed positive DIF findings. Out of these 30 cases, IgG was present in 27 cases (90%), Ig M was present in 5 cases (16.66%) and C3 was present in 22 cases (73.33%) as was shown in table 2.

Out of total 30 cases of immunobullous dermatoses in this study 23 cases (76.66%) showed positive correlation between clinical, histopathological and direct immunofluorescence patterns. The remaining 7 cases (23.34%) did not show correlation between clinical, histopathological and DIF patterns

**Fig. 1: Distribution of immunobullous dermatoses in this study.**

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**Fig. 2a:** Pemphigus vulgaris showing suprabasal intraepidermal blister and row of tombstone appearance. (H and E, ×100). Blister contains eosinophils and few neutrophils.

**Fig. 2b:** Direct immunofluorescence of pemphigus vulgaris showing lacelike/fishnet pattern intercellular space deposition of IgG.
Fig. 3a: Pemphigus foliaceus: Subcorneal blister with dyskeratotic acantholytic granular cells. Secondary cleft formation is seen in the mid-level of epidermis (H and E, ×100).
Fig. 3b: Pemphigus foliaceus: Direct immunofluorescence shows subcorneal intercellular space deposition of IgG and C3.

Fig. 4a: Bullous pemphigoid (a) Subepidermal blister formation with an inflammatory infiltrate in the bullous cavity. (H and E, ×100).
Fig. 4b: Bullous pemphigoid: Direct immunofluorescence shows linear deposition of IgG in the basement membrane zone.

Fig. 5a: Histopathology of L. P. Pemphigoides shows sub epidermal bulla with lichenoid changes in adjacent skin.
Fig. 5b: DIF shows sub epidermal bulla with IgG and C3 deposit in granular pattern at BMZ.
**Table 1: Common patterns of immunoglobulin deposits seen in immunobullous dermatoses in this study**

| Name of the Immunobullous dermatosis | Site, type and pattern of immunoglobulin deposition |
|--------------------------------------|--------------------------------------------------|
| Pemphigus vulgaris                   | Intercellular deposit of IgG along with/without C3 in suprabasal intra epidermal, fish net pattern. |
| Pemphigus foliaceus                  | Intercellular deposit of IgG along with/without C3 in subcorneal intra epidermal, fish net pattern. |
| Bullous pemphigoid                   | Linear deposit of IgG along with/without Ig M and C3 along the BMZ, Shoreline fluorescence pattern. |
| Epidermolysis bullosa acquisita      | Granular pattern of deposit of IgM and C3 at BMZ. |
| Lichen planus pemphigoides           | Deposit of Ig G and C3 in a granular pattern at BMZ. |
| Bullous systemic lupus erythematosus  | Linear pattern of deposit of IgG, IgM and C3 at BMZ. |

BMZ: Basement membrane zone.
Ig G: Immunoglobulin G.
Ig M: Immunoglobulin M.

**DISCUSSION:** The immunobullous dermatoses result from an immune response in which autoantibodies react with antigens present in adhesion molecules of epidermis or Basement membrane zone. Considerable overlap in the clinical findings is present among the immunobullous dermatoses so that confirmation of the clinical diagnosis is difficult. For instance, Linear IgA dermatosis may mimic Bullous pemphigoid or Dermatitis herpetiformis. IgA pemphigus may mimic pemphigus foliaceus, pemphigus herpetiformis or subcorneal pustular dermatosis. Inflammatory epidermolysis bullosa acquisita is indistinguishable from Bullous pemphigoid.⁴ In
all these diseases clinical and histopathology findings are inconclusive, but the Direct immunofluorescence pattern is helpful to make the correct diagnosis.[5]

By histopathological examination of immunobullous dermatoses there is considerable overlap of findings which leads to difficulty in confirming the diagnosis, particularly in case of sub epidermal immunobullous dermatoses.[6]

Direct immunofluorescence is a key adjunct in the investigation and diagnosis of immunobullous dermatoses along with clinical and histopathological findings. DIF helps to detect antibodies and complement bound to tissue antigens within the biopsy specimen.[7] In the evaluation of immunobullous dermatoses with Direct immunofluorescence technique, the perilesional skin biopsy is mandatory because the immune deposits can be detected easily in comparison to the lesional skin biopsy where the immune deposits are degraded.[8] Based on site, type, number and pattern of deposition of immune complexes an accurate diagnosis of the immunobullous dermatoses can be made.[8]

The most frequent immunobullous disorder in this study was pemphigus vulgaris and the finding is in accordance with other regional and international studies.[9] Among the Pemphigus group of immunobullous dermatoses in this study majority of the patients (15 out of 18 cases, 83.3%) were in the age group of 30-59 years, similar observation was noted in other Indian studies.[10] Male to female ratio was 1:1.6, which is comparable to the findings of Shamim et al.[11] DIF findings in pemphigus vulgaris showed IgG positivity in ICS in fishnet pattern in 38.46%. Few cases showed both IgG and C3 deposition in ICS. This is comparable with other studies.[12] Depending on the level of blister formation, fluorescence can be observed in the lower epidermal layers in Pemphigus vulgaris and in upper epidermal layers or subcorneal layers in Pemphigus foliaceus.[8] This is because of the presence of target antigens desmoglein 1 in pemhigus foliaceus and desmoglein 3 in pemphigus vulgaris. DIF findings in case of pemphigus foliaceus shows that the IgG and C3 deposition occurs in intercellular space in subcorneal layers of the epidermis which differentiates it from pemphigus vulgaris.[13]

Bullous pemphigoid (BP) is commonly seen in the elderly people in their 5th to 7th decade of life with 65 years as the average age of onset.[14] BP occurred in 16.66% in this study. Nearly, 60% of the patients were in the age group of 50-80 years. Male to female ratio of BP patients in this study was 1:0.25. The reported sex ratios in BP have varied from 2.4:1 to 1:5.75 in different studies due to variation in the sample size.[15],[16] In this study, all the patients with BP showed linear IgG and C3 deposition in the BMZ. One case also had IgM deposited along the BMZ. IgG and C3 deposition at BMZ occurs in linear, continuous and fine pattern.[17]

By analysis of the results in this study, out of total 30 cases of immunobullous dermatoses 23 cases showed positive correlation between clinical, histopathological and DIF patterns. The Remaining 7 cases did not show correlation between Clinical, histopathological and direct immunofluorescence patterns, that means the clinical diagnosis was different from histopathological and direct immunofluorescence pattern. Even in 3 of these cases histopathology also did not give the conclusive diagnosis. But with the direct immunofluorescence conclusive diagnosis was done. Similar observation in which a child was clinically diagnosed as Chronic bullous dermatosis of childhood and was later diagnosed as Bullous pemphigoid based on direct immunofluorescence pattern was reported by Rajeev Sharma et al, 1996.[18] A case presented in
this study had overlapping clinical features of Lichen planus pemphigoides and Bullous lichen planus, but the histopathology and direct immunofluorescence pattern confirmed the diagnosis of Lichen planus pemphigoides. A similar observation was reported by Gawkrodger DJ et al, 1989. Direct immunofluorescence is a very reliable diagnostic test for pemphigus, which becomes positive at an early stage and remains positive for a long period after clinical remission.

CONCLUSION: This study gives the conclusion that the direct immunofluorescence is an essential tool for diagnosing autoimmune blistering diseases of skin with the clinical and the histopathological overlap.

With direct immunofluorescence rapid and accurate diagnosis can be made very early in the course of immunobullous dermatoses, so that it gives an opportunity to institute effective management of these disorders at an early stage of the disease, because these disorders are associated with considerable morbidity and mortality.

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