Original Article

Evaluation and Correlation of Clinical, Histopathological and Direct Immunofluorescence Findings in Vesicobullous Disorders of Skin: A Cross Sectional Study with Review of Literature

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ABSTRACT

Background: To assess and correlate the clinical, histopathological and DIF features and compare the sensitivity of DIF with that of histopathology in autoimmune bullous disorders of skin.

Methods: A cross-sectional descriptive hospital based study was conducted on 45 patients who had active vesicobullous lesions. After a detailed cutaneous examination, two punch biopsies were taken, one from lesional skin for histopathological study and another from perilesional skin for DIF. Biopsies from 31 patients were deemed fit to be included in the study.

Result: Based on clinical, histopathological and DIF findings the most common final diagnosis was Pemphigus Vulgaris (PV), 18/31 cases. On histopathology, characteristic histopathological features were seen in 15/18 cases of PV, 6/11 cases of Pemphigus Foliaceous (PF), 3/4 cases of Bullous Pemphigoid (BP) and a single case of Dermatitis Herpitiformis (DH) while 4/31 cases showed non specific findings (NS). DIF was positive in 30/31 cases (96.77%) except in a single case of DH. Good clinico–histo-immunological correlation was seen in 21/31 cases (67.7%). In 25/31 cases (80.06%) good histo-immunological correlation ($p < 0.05$; significant) was observed while 6/31 cases (19.3%) showed discordance between histological and DIF findings. The senstivity of the histopathology in the pemphigus group (PV + PF + Paraneoplastic Pemphigus), BP and DH was 88%, 75% and 100% respectively while on DIF it was 100% for the pemphigus group and BP. Single case of DH was negative on DIF.

Conclusion: As compared to histopathology, DIF has better sensitivity and it is an indispensible tool especially in vesicobullous skin lesions that are difficult to diagnose on the basis of clinical and histopathological features.

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Introduction

Autoimmune bullous disorders are a heterogeneous group of diseases in which components of the epidermis and basement membrane zone (BMZ) are the focus of attack. [1] Their accurate diagnosis requires detailed clinical examination, histopathological evaluation followed by direct immunofluorescence study (DIF). [2] DIF plays an essential role in the diagnosis, classification and treatment of various immunobullous disorders as it shows distinct immunofluorescence patterns. [3] Patients in clinical remission with positive DIF findings show early relapse of disease which also emphasizes the role of DIF in prognosticating and monitoring of disease activity. [1,4,5] Thus, DIF is considered gold standard for the diagnosis of autoimmune bullous disorders, specially in patients with clinical and/or histopathological dilemma. [4,6,7,8]

However, in developing countries like India, DIF is done only in the few centers due to its high cost, requirement of technical skill and difficulty in maintaining the facility. Therefore, this study was undertaken to assess and correlate the clinical, histopathological and DIF features of various vesicobullous diseases of the skin and compare the sensitivity of DIF with that of histopathology.

Materials and Methods

A cross-sectional descriptive hospital based study was conducted on 45 patients attending the Departments of Dermatology and Pathology, Lady Hardinge Medical College and associated Hospitals, New Delhi over a period of 2 years who were clinically diagnosed with active vesicobullous diseases, irrespective of age and sex. Patients with no active lesions and on systemic steroids/ immunosuppressive therapy for the last three months were excluded from the study.

In all the patients, two punch biopsies from skin were taken. Biopsy for histopathological examination was taken from the lesional skin, put in 10% neutral formalin solution and stained with haematoxyline and eosin.

On histopathological examination the lesions were categorized based on:

- Site of blister separation plane (Subcorneal/granular, Intraepidermal/spinous, Suprabasal, Subepidermal)
- Nature of inflammatory infiltrate (Neutrophil, Eosinophil, Lymphocytes & Mast cells) within the bulla cavity
- Presence/Absence of acantholytic cells within the bulla cavity

Another biopsy for DIF was taken from the perilesional skin (i.e. 3-4 mm punch biopsy from clinically normal appearing skin within 2 cm from the lesion except from hands, feet, neck, groin and mucous membrane), put in Michel's medium and kept at -70 °C to -20°C until cut. Before cutting, the biopsy for DIF was washed thrice in phosphate–buffered saline (PBS) at pH 7.2 for 15 minutes each and was embedded in OCT in cryostat and 4-6 micron sections were obtained on poly–L-Lysine slides at -20°C to -25°C (minimum of six sections per biopsy). For DIF staining, sections were brought at room temperature after rinsing thrice with PBS for 10 min each and five frozen sections of each biopsy were overlaid with 40-50 µl each of optimally diluted fluorescein isothiocyanate (FITC) conjugated monoclonal anti human antisera (IgG, IgA, IgM, C3 and fibrinogen, supplied by Diagnostic biosystems) and sixth section with PBS (Control) for at least 1 hour. After washing the sections again thrice in PBS they were mounted with Buffered glycerin mountant and finally examined under NIKON fluorescence microscope fitted with an ultraviolet lamp source, under ideal citation and barrier filter combination.

- On DIF examination the following parameters were evaluated:
- Site of deposition of immunoreactants (Epidermis/ BMZ/Dermis)
- Pattern of Immunofluorescence (Linear/Granular/ both) Intensity of fluorescence (graded as"+++": Strongly positive, "++": Moderately positive, "+": Weakly positive and "-": Negative)

Result

Among the 45 vesicobullous skin biopsies received, 14 biopsies were excluded from the study. Amongst these 14 biopsies, 8 biopsies without epidermis were inadequate for opinion, 4 biopsy samples were dried up & in 2 biopsies, patients were later found to be on steroids. Finally in the 31 cases studied, the most common age group was 4th – 5thdecade (29.03%) with M:F ratio of 0.82:1.

Single definitive clinical diagnosis given in 24/31 cases (77.4%) were: PV(13/31), PF(6/31), BP(3/31), PNP(1/31) & DH(1/31) which were consistent with the final diagnosis after histopathology and DIF findings. In 7/31 (22.5%) cases, differential clinical diagnosis were considered .

Definitive histopathological diagnoses were made in 27/31 cases (87.1%) while, 4/31 (12.9%) cases showed non-specific findings on histopathology. DIF positivity was seen in 30/31 cases (96.77%). Based on clinical, histopathological and DIF findings the most common final diagnosis was PV 18/31cases (58.06%). (Table 1)

In 18 clinically diagnosed cases of PV, females were affected twice more commonly than males. The most common

site of involvement was face in 15/18 cases (83.3%) with 12/18 cases (66.6%) showing oral involvement. The most common cutaneous lesion was "Flaccid normal"-15/18 cases (83.3%). On histopathology, 16 cases showed histopathological features of PV with presence of suprabasal bulla with characteristic tomb stone appearance and acantholytic cells which on DIF examination showed strong positivity for IgG+++ at Intercellular substance (ICS) with *lace like* Network Continuous pattern (NC) in the lower part of the epidermis with 4 cases showing additional weak C3 +. Two cases which showed non-specific findings on histopathology i.e. (superficial, dermal, perivascular and perineural mixed inflammatory infiltrate) on DIF examination, showed strong positivity for IgG+++ at Intercellular substance (ICS) with *lace like* Network Continuous pattern and hence diagnosed as PV. (Table 1&2 ; Figure1)

In 11 clinically diagnosed cases of PF, slight male preponderance was seen. The abdomen and back were involved in 100% of the cases however no oral invovement was seen. The most common cutaneous lesion was "Flaccid erythematous" (6/7 cases: 85.71%). On histopathology, only 6 cases showed characteristic histopathological features of PF i.e. subcorneal bullae with presence of acantholytic cells which on further, DIF examination showed ICS IgG +++ predominantly in the upper part of the epidermis.Three cases were diagnosed as PV both on histopathology as well as on DIF study. Two cases which showed non–specific findings on histopathology ,on DIF examination showed IgG +++ ICS *lace like* Network Continuous pattern in upper and lower epidermis and hence diagnosed as PF and PV respectively. (Table 1&2 ; Figure 2)

Among 4 clinically suspected cases of BP, males were more commonly affected than females. All the cases showed involvement of upper extremities with oral lesions seen in ¼ cases (25%). The most common cutaneous lesion was "Tense Erythematous" (3/4 cases: 75%). On histopathology, only 3 showed definitive histopathogical features of BP characterized by presence of subepidemal bulla with presence of acantolytic cells and eosinophils which on DIF examination showed continous homogenous linear (CHL) IgG +++ & C3 ++ at basement membrane zone (BMZ). Single case showed non-specific findings on histopathology which had IgG+++ & C3 +++ on DIF examination , hence diagnosed as BP. (Table 1&2 ; Figure 3a&b)

Single clinically suspected female patient of paraneoplastic pemphigus (PNP), a follow up case of chronic lymphocytic leukemia, in addition to oral lesions showed involvement of upper and lower extremity and was diagnosed as erythema multiforme (EM) on histopathology. On further DIF examination IgG +++ & C3 ++ deposition was seen at both ICS and BMZ with network continuous and continuous homogenous pattern and was confirmed as EM like variant of PNP. (Table 1&2 ; Figure 4a&b)

Another single female patient, clinically suspected to be having dermatitis herpetiformis with involvement of upper & lower extremities and scalp was concordant with the histopathological findings but DIF examination was negative. (Table 1,2)

In 21/31 cases (67.7%) good clinico–immuno-histopathological correlation was seen while ten cases i.e. 10/31 (32.25%) showed clinico-histo-immunological discordance. (Table 3) .In 25/31 cases (80.06%) good histo-immunological correlation (p <0.05significant) was observed while six out of 31 cases (6/31) i.e. 19.3% showed discordance between histological and DIF findings

Discussion

DIF is a robust tool for a proper diagnostic labeling of all vesicobullous lesions of the skin. The comparative findings of the previous studies on immunobullous lesions by various authors as compared to our present study are tabulated as follows: (Table 4,5)

Single case of dermatitis herpitiformis was negative on DIF and these findings were concordant with Zone JJ et al [11] & Lourdes Sousa et al [12] who emphasized the normal appearing skin adjacent to the active lesion as the preferred biopsy site in DH as immunoreactants degrades in the lesional site due to inflammation giving rise to negative DIF.

In 21/31 cases (67.7%) good clinico–immuno-histopathological correlation was seen while ten cases i.e. 10/31 (32.25%) showed clinico-histo-immunological discordance. (Table 3) .In 25/31 cases (80.06%) good histo-immunological correlation (p <0.05significant) was observed while six out of 31 cases (6/31) i.e. 19.3% showed discordance between histological and DIF findings

Clinico-histo-Immunoconcordance of present study was consistent with Walker Ranjana et al.

The senstivity of histopathology in the pemphigus group (PV+PF+PNP), BP and DH was 88%,75% and 100% respectively while senstivity on the DIF was 100% in pemphigus group and BP. Single case of DH was negative on DIF. (TABLE 6)

Table 1: Distribution of cases according to clinical diagnosis, gender, histopathological diagnosis, DIF findings and final diagnosis

Bullous Disorders	Clinical diagnosis		Male (%)	Female (%)	Histopathology Diagnosis	DIF Examinnation			Final Diagnosis (Frequency of cases &%)
	Definitive	Differential				Positive cases	Immuno reactant	Site/ Pattern	
Pemphigus vulgaris (PV)	13	5	6 (33.3)	12 (66.6)	16-PV 2-NS	18	IgG (14) IgG+ C3 (4)	ICS/ lace like NC	18 (58.06)
Pemphigus foliaceous (PF)	6	5	9 (81.8)	2 (18.18)	6- PF 3-PV 2-NS	7	IgG (7)	ICS/ lace like NC	7 (22.58)
Bullous pemphigoid (BP)	3	1	3 (75)	1 (25)	3 – BP 1-NS	4	IgG+ C3 (4)	BMZ/ CHL	4 (12.9)
Paraneoplastic pemphigus (PNP)	1	0	0	1 (100)	Erythema multiforme	1	IgG+ C3	ICS/NC + BMZ/ CHL	1 (3.32)
Dermatitis herpetiformis (DH)	1	0	0	1 (100)	DH	Negative		–	1(3.23)

[PV- Pemphigus vulgaris, PF- Pemphigus foliaceous, BP- Bullous pemphigoid, PNP- Paraneoplastic pemphigus, DH- Dermatitis herpetiformis, NS- Non-specific findings, ICS- Intercellular substance, NC- Network continuous, BMZ -Basement membrane zone, CHL- Continous homogenous linear, DIF- Direct immunofluorescence findings]

TABLE 2: Distribution of cases in the study group according to intensity of immunoreactant

	PV n=18	PF n=7	BP n=4	PNP n=1	DH n=1
IgG+++ & C3+	4	0	0	0	0
IgG+++ & C3++	0	0	3	1	0
IgG+++ & C3+++	0	0	1	0	0
IgG+++	14	7	0	0	0

[PV- Pemphigus vulgaris, PF- Pemphigus foliaceous, BP- Bullous pemphigoid, PNP- Paraneoplastic pemphigus, DH- Dermatitis herpetiformis]

TABLE 3: Discordant clinical and/or histo-immunological findings (10/31 cases)

Clinical diagnosis	Histopathological diagnosis	DIF diagnosis
5 PV/ PF/ PE	4 PV 1 Inconclusive	5 PV
1 PF/CBD	Inconclusive	1 PF
1 BP/ EBA/ EM	Inconclusive	1 BP
1 PV	Inconclusive	1 PV
1 DH	1 DH	Negative
1 PNP	1 EM	1 PNP
TOTAL	**10 CASES**	

[PV- Pemphigus vulgaris, PF- Pemphigus foliaceous, BP- Bullous pemphigoid, PNP- Paraneoplastic pemphigus, DH- Dermatitis herpetiformis, EM- Erythema Multiforme, PE- Pemphigus erythematosus, CBD- Chronic bullous disease of childhood, EBA- Epidermolysis bullosa acquisita]

Table 4: Comparative findings of the previous studies as compared to our present study

Study (year)	No. of cases	Maximum frequency of cases	Age	Sex	Most common type of immunoreactant	Site of deposition
Present study	31	PV (18) PF (11) BP (4)	4th-5th	F>M (2:1)	IgG (18) IgG (7) IgG+C3 (4)	ICS, lace like ICS, lacelike Linear BMZ
S. Arundaati et al, [8] (2013)	68	PV (36) BP (8) PF (6)	4th-5th	F>M (1.27:1)	IgG (24) IgG+C3 (8) IgG (3)	ICS, lace like Linear BMZ ICS, lace like
Lebe et al, [9] (2012)	197	BP (66) DH (58) PV (51)	5th- 6th	F>M (1.01:1)	IgG+C3 (25) IgA+C3 (3) IgG (30)	Linear BMZ Granular, BMZ & papillary dermis ICS, lace like

Study (year)	No. of cases	Maximum frequency of cases	Age	Sex	Most common type of immunoreactant	Site of deposition
Ranjana Walker Minz et al, [7] (2010)	267	Non –bullous immune complex vasculitis (45) Bullous PV (22) BP (13) Lichen Planus (7)	–	F>M (1.2:1)	IgA IgG IgG+C3 IgM	Rings in vessel wall ICS, lace like Linear BMZ In Cytoid bodies
Kabir AN et al, [10] (2009)	204	DH (38) PV (20) BP (13)	11-20 yrs	F>M (1.68:1)	IgA (5) IgG (15) C3 (12)	Granular in dermal papillae ICS, lace like Linear BMZ
Inchara et al, [6] (2007)	100	PV (29) BP (22) NS (15)	–	–	IgG (26) IgG+C3 (17) IgG +C3 (2)	ICS, lace like Linear BMZ ICS, lace like +C3 in dermal vessels

[PV- Pemphigus vulgaris, PF- Pemphigus foliaceous, BP- Bullous pemphigoid, PNP- Paraneoplastic pemphigus, DH- Dermatitis herpetiformis , NS- Non specific findings, ICS- Intercellular substance, BMZ -Basement membrane zone]

TABLE 5: Comparative findings regarding clinico- histo- immunological concordance as well as disconcordance of our study as compared to previous studies .

FINDINGS	PRESENT STUDY	Walker Ranjana et al [7] (2010)	KabirAN et al [10] (2009)	Inchara YK et al [6] (2007)	Lebe benu et al [9] (2012)
Clinico-histo-Immunoconcordance	67.7%	70%	40%	73%	33.5%
Histo-Immunoconcordance	80.06%	–	–	–	–
Clinico-immunoconcordance	–	77%	–	–	–
Clinico-histo-immunodisconcordance	32.25%	–	20.8%	9%	62.4%
Histo-immunodisconcordance	19.3%	7%	–	–	–

TABLE 6: Comparison of sensitivity of DIF with that of Histopathology

Final diagnosis	Sensitivity on histopathology	Sensitivity on DIF
Pemphigus group (PV+PF+PNP)	88.0%	100%
BP	75%	100%

[PV- Pemphigus vulgaris, PF- Pemphigus foliaceous, BP- Bullous pemphigoid, PNP- Paraneoplastic pemphigus]

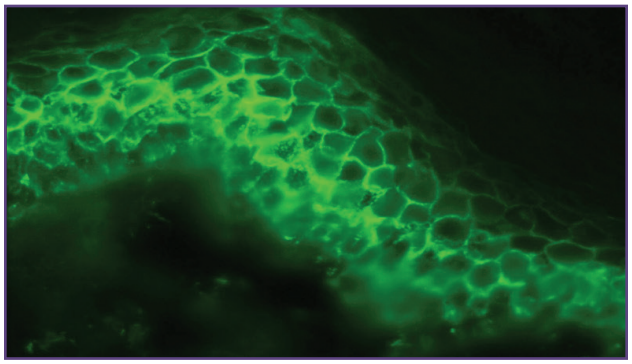

Fig. 1: DIF of PV shows full thickness lace like ICS IgG with Network Continuous pattern with strong intensity predominantly in lower part of the epidermis.

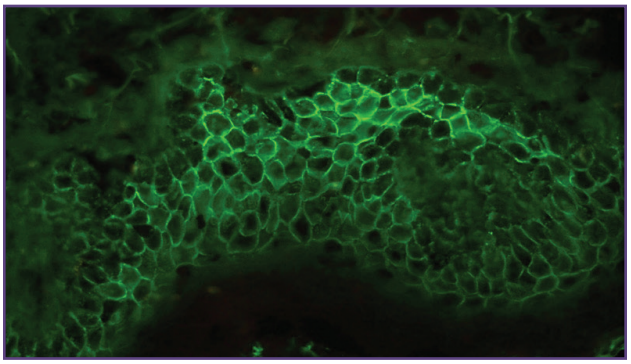

Fig. 2: DIF of PF shows full thickness lace like ICS IgG with Network Continuous pattern mainly in upper part of the epidermis

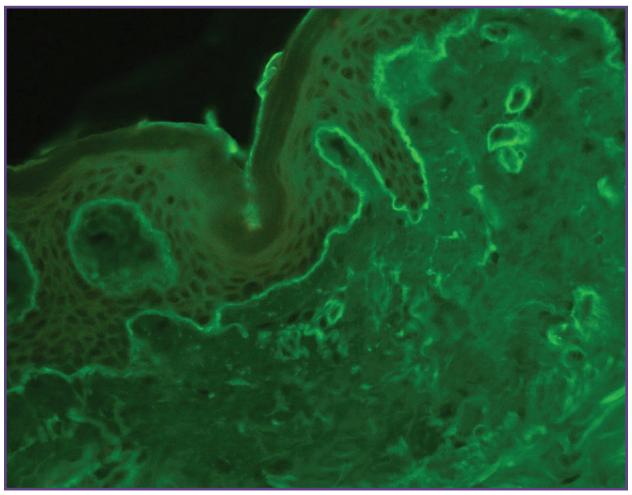

Fig. 3: a) DIF of BP shows strong linear homogenous IgG deposition at BMZ.

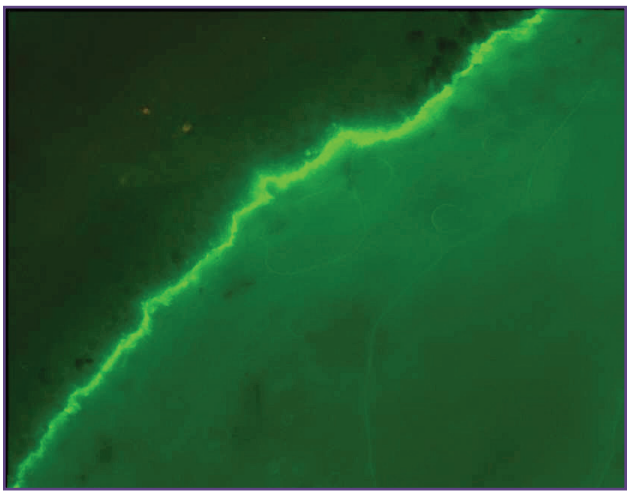

Fig. 3: b) Strong linear homogenous deposition of C3 at BMZ

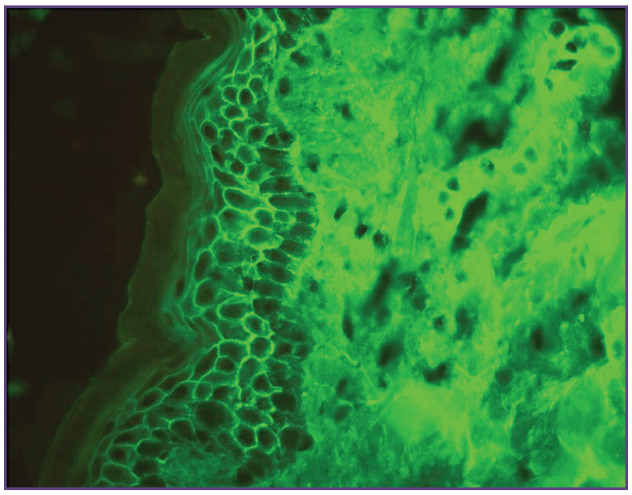

Fig. 4: a) DIF of PNP shows full thickness IgG deposition at ICS and BMZ.

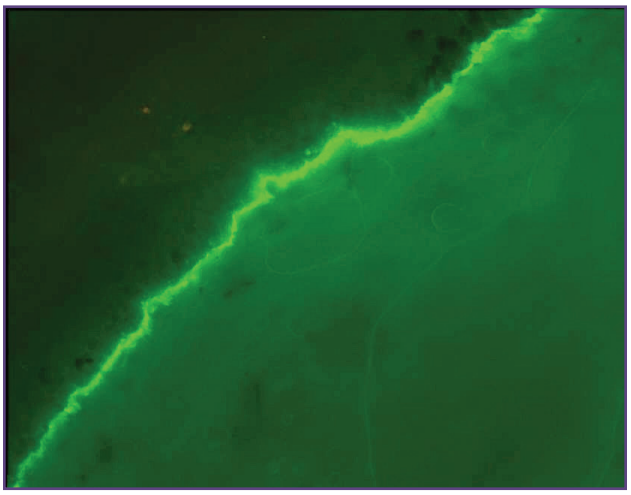

Fig. 4: b) Strong linear homogenous BMZ deposition of C3

Conclusion

Diagnosis of vesicobullous lesions of skin is enhanced by DIF in those cases that pose a diagnostic dilemma both clinically and histopathologically. DIF has better sensitivity as compared to histopathology, but it should always be used in conjuction with histopathology and clinical features as the combination of three yields the best results. Thus, histopathology and DIF examination are complementary and does not supplement one another.

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Competing Interests

None Declared

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