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Pathology

ROLE OF DIRECT IMMUNOFLUORESCENCE IN AUTOIMMUNE BULLOUS DISORDERS: A REPLACEMENT OR REINFORCEMENT FOR HISTOPATHOLOGY ?

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ABSTRACT Autoimmune bullous diseases (AIBD) constitute a wide spectrum of dermatological disorders. The diagnosis is often made by histopathology in correlation with clinical examination. In a resource poor country like India, the direct immunofluorescence (DIF) is still not widely available because of high cost of establishing the immunofluorescence laboratory, requirement of technical skill & expertise in interpretation. However, DIF is a valuable tool for diagnosing & classifying various AIBD. In this study, we analysed & correlated histopatologic & DIF findings in 29 cases of AIBD. The DIF was found to be highly useful particularly in cases with non-specific clinico-histopathologic features with a sensitivity of 86.2% vis-à-vis histopathology with a sensitivity of 82.7%. However, like any test it has got its limitaions that may lead to a false negative DIF & hence lower its sensitivity. Therefore it should not replace histopathology rather it should be used in conjunction with histopathology.

KEYWORDS : direct immunofluorescence, vesicobullous disorders, autoimmune bullous disorders

INTRODUCTION

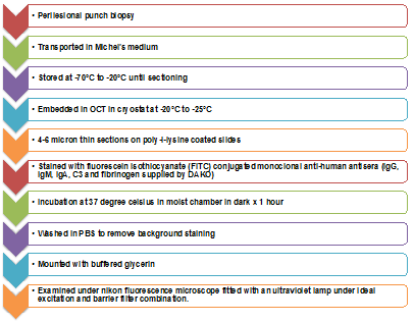
Immunological reactions account for most of the bullous diseases. Autoimmune Bullous Disorders (AIBD) are divided into intra-epidermal group and sub-epidermal group. Intraepidermal group consists of various subtypes of Pemphigus such as Pemphigus vulgaris (PV), Pemphigus foliaceous (PF) and their variants. Subepidermal group mainly consists of Bullous Pemphigoid (BP), Pemphigoid Gestationis or Herpes Gestationis (PG/HG), Dermatitis Herpetiformis (DH), Linear IgA Disease (LAD) and Chronic Bullous Disease of Childhood (CBDC). Clinical examination & histopathology have traditionally been used for the diagnosis of AIBD. However, the patterns obtained on Direct Immunofluorescence (DIF) are often diagnostic and considered gold standard. [1]

MATERIALS AND METHODS

A cross-sectional study was undertaken in a tertiary care hospital in Delhi. Aims were to study & correlate the histopathology and DIF results of clinically suspected AIBD cases. Informed written consent was obtained from all the study participants / parents / guardians. Patients with no active skin lesions, only mucosa involvement or history of steroids / immunosuppressive topical therapy in last 1 month and/or oral therapy in last 3 months were excluded. Two punch biopsies from lesional and peri-lesional skin for histopathology and DIF respectively; were taken from 29 cases of AIBD presenting with active vesicobullous lesions on skin in the department of Dermatology, Venereology & Leprosy over a period of 2 years. Histopathologically the lesions were categorized on the basis of (a) level of bulla (intraepidermal - subcorneal / suprabasal, subepidermal), (b) type of inflammatory infiltrate within the bulla & (c) presence or absence of acantholytic cells in the bulla.

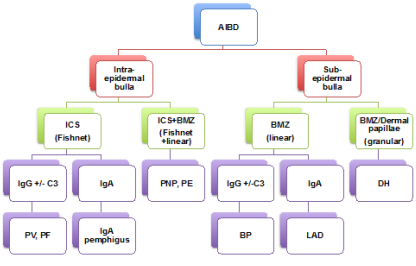
For DIF, peri-lesional skin biopsy frozen sections were stained with fluorescein isothiocyanate (FITC) conjugated monoclonal anti-human antisera (IgG, IgA, IgM, C3 and fibrinogen, supplied by Dako) and one control section with Phosphate Buffer Saline (PBS) by a standardized protocol. [Fig. 1]

Figure 1: Schematic representation of procedure for DIF examination



On DIF, all cases were examined for (a) site of deposition of immunoreactants (epidermis –intercellular surface staining (ICS) / basement membrane zone (BMZ) / dermis), (b) pattern of immunofluorescence (fish-net or lace-like, linear / granular or both), (c) semi-quantitative grading of fluorescence intensity (4+strong / 3+moderate / 2+weak / 1+faint / negative). An algorithmic approach was used to arrive at an accurate diagnosis in majority of cases.26 [Fig. 2]

Figure 2: Algorithmic representation of DIF features in various AIBD



RESULTS

Twenty nine patients were enrolled in the study. The age ranged from 1.5 to 79 years with mean age of 38.29 years. There was a slight female preponderance with M:F ratio of 0.6:1.

A histopathologic diagnosis was made in 24/29 (82.7%) cases of AIBD

while 5/29 (17.3%) cases showed non-specific findings (superficial dermal & peri-adnexal mixed inflammatory infiltrate without any blister formation) and could not be diagnosed on histopathology alone. The most commonly diagnosed cases on histopathology were of Pemphigus group - 9 (31%) followed by BP - 7 (24.1%), DH - 4 (13.5%), HG/PG - 3 (10.3%) and single case (3.4%) of CBDC. As DIF can't differentiate between various subtypes of Pemphigus, the data of cases of PV, PF and PE were clubbed together for analysis. Among 9 cases of Pemphigus group, there were 4 (44.4%) cases of PV & PF each and 1 (11.2%) case of pemphigus erythematosus (PE).

Twenty-five out of 29 (86.2%) cases of AIBD showed immunoreactant positivity on DIF while 4 (13.8%) cases were negative. The most common cases diagnosed on DIF were of Pemphigus group & BP - 9/29 (31%) cases each followed by HG/PG & DH, 3/29 (10.3%) cases each and single (3.4%) case of CBDC. [Table 1]

Table 1: Comparison of positive results of DIF and histopathology

Final Diagnosis (n=29)	Diagnosed on Histopathology (n=24)	Diagnosed on DIF (n=25)
Pemphigus group (11)	81.8% (9/11)	81.8% (9/11)
➢ PF (6)	66.6% (4/6)	83.3% (5/6)
➢ PV (4)	100% (4/4)	75% (3/4)
➢ PE (1)	100% (1/1)	100% (1/1)
Subepidermal group (18)	83.3% (15/18)	88.8% (16/18)
BP (9)	77.7% (7/9)	100% (9/9)
DH (5)	80% (4/5)	60% (3/5)
HG or PG (3)	100% (3/3)	100% (3/3)
CBDC (1)	100% (1/1)	100% (1/1)

In pemphigus group, presence of fish-net pattern of IgG deposition at intercellular spaces of epidermis (ICS) was consistently demonstrated in all 9 cases showing immunoreactivity. A single case of PE also showed linear deposits at BMZ in addition to fish-net pattern. [Figure 3 & 4]

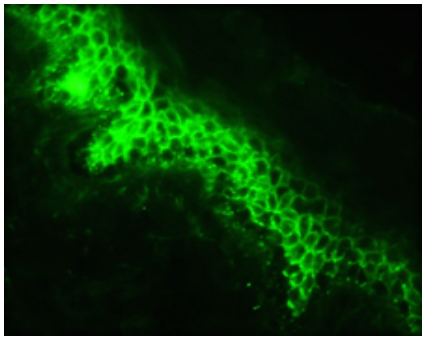


Figure 3: DIF of a case of PV showing full thickness fish-net pattern of IgG at ICS with strong intensity predominantly in lower part of the epidermis.

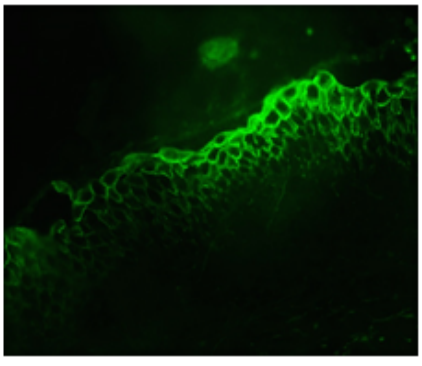


Figure 4: DIF of a case of PF showing lace-like pattern of IgG at ICS with immunofluorescence mainly in upper part of the epidermis.

All 9 cases (100%) of BP showed linear deposits at BMZ. IgG alone was seen in 4 (44.4%) cases whereas C3 alone was present in 2 (22.2%) cases. In rest 3 (33.3%) cases, combination of both was detected. [Figure 5]

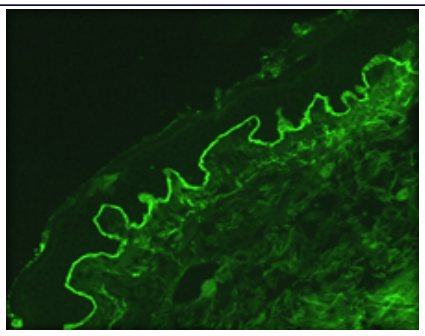


Figure 5: DIF of a case of BP showing strong C3 deposits in linear homogenous pattern at BMZ.

In 3 cases of HG or PG, linear deposits of IgG alone were seen in 2 (66.6%) cases & of C3 alone in 1 (33.3%) case at BMZ.

IgA was seen as granular deposits at the tips of dermal papillae in 3/5 (60%) cases of DH & in a linear pattern at BMZ in a single case of CBDC. [Figure 6]

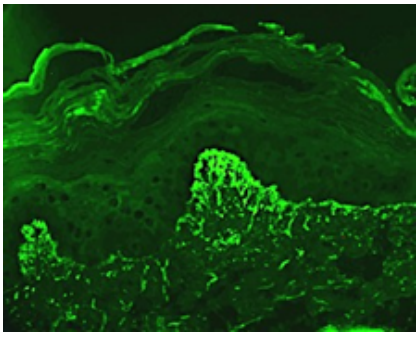


Figure 6: DIF of a case of DH showing strong IgA deposits in granular pattern at tips of dermal papillae.

Histopathology revealed non-specific findings in 5/29 (17.2%) cases which were picked up by DIF as 2 cases of PF, 2 cases of BP & 1 case of DH. However, 2 cases of DH, 1 PV & 1 PF diagnosed on histopathology, were found to be negative on DIF. Overall, 20/29 (68.9%) cases showed histo-immunological concordance.

Table 2: Distribution of immunoreactants in AIBD (Total 29 cases)

Diagnosis	Number of cases	Deposits (no. of cases)	Site	Pattern	Negative
PF	6	IgG (2), IgG+C3 (3)	ICS	Fish-net / lace-like	1
PV	4	IgG (1), IgG+C3 (1), IgG+C3+Fib (1)	ICS	Fish-net / lace-like	1
PE	1	IgG with IgM+C3	ICS with BMZ	Fish-net / lace-like with Linear deposits	0
BP	9	IgG (4), IgG+C3 (3), C3 (2),	BMZ	Linear	0
DH	5	IgA (3)	Dermal papillae	Granular	2
HG/PG	3	IgG (2), C3 (1)	BMZ	Linear	0
CBDC	1	IgA (1)	BMZ	Linear	0

DISCUSSION

Immunofluorescence has become an essential technique for making the diagnosis, subtyping and correctly classifying immune-mediated bullous diseases. Some immunopathologic patterns are disease-specific and are of diagnostic value like fish-net pattern in pemphigus. Other patterns like linear deposits at BMZ; are less specific and are of diagnostic value only when correlated with clinical features and histopathologic findings.[2]

Majority of the cases in this study; diagnosed after histopathology & DIF were of Pemphigus group (37.9%) followed by BP (31%), DH (17.2%), HG/PG (10.3%) and CBDC (3.4%). Inchara YK et al[3], Kabir AK et al[1,4], Kulthanan K et al[5] & Kanwar AJ et al[6] also found similar pattern of distribution of immunobullous diseases.

The histopathological diagnosis was made in 24/29 cases (82.7%) while 5/29 cases (17.3%) showed non-specific findings which is in concordance with the findings of Inchara YK et al[3], Kabir AK et al[1] and Lebe B et al[12].

In our study, 25/29 (86.2%) cases showed positivity on DIF whereas 4/29 (13.8%) cases were negative which is consistent with the observations of Inchara YK et al[3], Kabir AK et al[1] and Kulthanan K et al[5].

In all DIF positive cases of Pemphigus group including 5 PF, 3 PV & 1 PE cases, IgG was the most common immunoreactant which is in concordance with findings of Kanwar AJ et al[18] and Kelly MB et al[9]. All the cases of pemphigus group showed fish-net pattern of deposition at ICS which is consistent with the findings of Kanwar AJ et al[18], Kabir AK et al[1], Kabir AK et al[4] and Lebe B et al[12]. Single case of PE showed a combination of IgG in fish-net pattern at ICS with IgM and C3 in linear/granular pattern at BMZ. These findings are consistent with the earlier reports.[5]

All (100%) cases of BP, HG/PG and CBDC showed linear deposition of immunoreactants at BMZ which were concordant with the findings of Mahmood T et al[10], Kabir AK et al[1], De A et al[11], Monia K et al[15], Kabir AN et al[4], Kulthanan K et al[5] and Lebe B et al[12].

In cases of BP, IgG was the most common immunoreactant (77.8%) followed by C3 (55.5%) in concordance with the findings of Mahmood T et al[10] & De A et al[11]. In contrast, Kabir AK et al[1] & Satyapal S et al[19] found C3 to be the predominant deposit in 90% or more cases.

Among 3 cases of HG/PG, IgG was the most frequent immunoreactant (66.6%) followed by C3 (33.3%) which was similar to the findings of Mahmood T et al[10].

Out of 5 cases of DH, 3 (60%) cases showed granular IgA deposition at the tips of dermal papillae similar to the findings of Mahmood T et al[10] & Kabir AK et al[4].

Single case of CBDC (100%) showed linear IgA deposition at BMZ in concordance with the findings of Mahmood T et al[10], Kabir AK et al[1] and Monia K et al[15].

In the present study, 20/29 cases (68.9%) cases of AIBD showed histo-immunological concordance. Sharma G et al[20] reported good histo-immunological correlation in 25/31 (80.06%) cases. All histopathologically diagnosed cases of BP (7), HG/PG (3), CBDC (1) & PE (1), showed concordance with DIF findings which is similar to the observations of Satyapal S et al[19], Mahmood T et al[10] & Lebe B et al[12]. Five cases with non-specific findings on histopathology were picked up by DIF as 2 cases of PF (no epidermis in lesional skin biopsy), 2 BP (fragmented biopsy for histopathology) and 1 case of DH which is similar to the observations of Inchara YK et al[3], Kulthanan K et al[5,7] & Minz RW et al[2]. Whereas 2 cases of DH (DIF may be negative in few cases as reported earlier also), 1 case of PF (history of topical homoeopathic treatment) & 1 PV (biopsy of bullous skin was taken for DIF) each showed negative DIF findings and were diagnosed on histopathology, which is consistent with the observations of Mahmood T et al[10] & Inchara YK et al[3].

Overall 81.8% cases of Pemphigus group were diagnosed on histopathology, 66.6% cases of PF and 100% cases of both PV & PE, which is similar to the observations of Inchara YK et al[3] & Kulthanan K et al[7]. The overall positivity of DIF in Pemphigus group was 81.8% similar to the observations of Inchara YK et al[3]. One case of HG/PG & CBDC each, 80% of DH & 77.7% of BP were diagnosed on histopathology which is similar to the observations of Inchara YK et al[3], Kulthanan K et al[5] & Lebe B et al[12]. The positivity of DIF was 100% in BP in concordance with Mahmood T et al[10], De A et al[11] & Kulthanan K et al[5], 60% in DH, similar to the observations of Inchara et al[3], 100% in HG/PG in concordance with Mahmood T et al[10] & 100% in CBDC in concordance with Mahmood T et al[10] & Monia A et al[15].

Table 3: Comparative findings of the previous literature and this study [2-4,10,12,20-25]

S. No	Study (year)	No. of cases	Frequency of cases	Age	Sex	Most common type of immunoreactant	Site of deposits
1	**Mahmood T et al (2003)**	50 (26 patients with subepidermal blisters)	BP (14)	1-80 years	M>F (1.2:1)	IgG (14)	BMZ
			LAD (5)			IgA (5)	BMZ
			DH (4)			IgG (4)	BMZ
			PG (2)			IgG+C3 (2)	BMZ
			Bullous LE (1)			IgG+A+M (1)	BMZ
2	**Inchara YK et al (2007)**	100	PV (29)	-	-	IgG (26)	ICS, lace like (26)
			PF (22)			IgG+C3 (17)	Linear BMZ (17)
			NS (15)			IgG+C3 (2)	ICS, lace-like + C3 in dermal vessels (2)
3	**Kabir AN et al (2009)**	204 (various bullous & non-bullous diseases)	DH (38)	11-20 years	F>M (1.68:1)	IgA (5)	Granular in dermal papillae (5)
			PV (20)			IgG (15)	ICS, lace like (15)
			BP (13)			C3 (12)	Linear BMZ (12)
4	**Minz RW et al (2010)**	267 (various bullous & non-bullous diseases)	PV (22)	-	F>M (1.2:1)	IgG	ICS, lace like
			BP (13)			IgG+C3+ IgM	Linear BMZ
5	**Lebe et al (2012)**	197	BP (66)	5th- 6th decade	F>M (1.01:1)	IgG+C3 (25)	Linear BMZ Granular
			DH (58)			IgA+C3 (3)	BMZ & papillary dermis
			PV (51)			IgG (30)	ICS, lace like
6	**Arundhati S et al (2013)**	68	PV (36)	4th-5th decade	F>M (1.27:1)	IgG (24)	ICS, lace-like (24)
			BP (8)			IgG+C3 (8)	Linear BMZ (8)
			PF (6)			IgG (3)	ICS, lace-like (3)
7	**Buch AC (2014)**	100	PV (58)	16-87 years	-	IgG (51)	ICS (51)
			PF (12)			IgG (12)	ICS (12)
			PE (1)			IgG+M (1)	ICS+BMZ (1)
			P vegetans (1)			IgG (1)	ICS (1)
			BP (25)			IgG+C3 (18)	BMZ (21)
			DH (2)			IgA (2)	BMZ (2)
			LAD (1)			IgA (1)	BMZ (1)
8	**Arbache ST (2014)**	421 (intra-epidermal=277 & sub-epidermal =144)	PV (142)	-	-	IgG (130)	ICS (130)
			PF (117)			IgG (110)	ICS (110)
			PNP (3)			IgG (2)	ICS +/- BMZ (3)
			IgA P (8)			IgA (8)	ICS (8)
			BP (90)			C3 (82)	BMZ (88)
			EBA (19)			C3 (17)	BMZ (19)
			LAD (15)			IgA (15)	upper dermal papillae (11)
			DH (13)			IgA (12)	BMZ (12)

9	**Mysorekar VV et al (2015)**	215 (immune-mediated bullous & non-bullous diseases)	PV (44)	2 to 94 years	F>M (1.2:1)	IgG (43)	ICS (43)
			PF (8)			IgG (5)	ICS (8)
			PE (1)			IgG+C3+M (1)	ICS+BMZ (1)
			BP (25)			IgG+C3 (20)	BMZ (24)
			PG (1)			C3 (1)	BMZ (1)
			CBDC (1)			IgA (1)	BMZ (1)
			Bullous LE (3)			IgG+M+A+C3 (3)	BMZ (3)
10	**Sharma G et al (2016)**	31	PV (18)	4^{th}-5^{th} decade	F>M (2:1)	IgG (18)	ICS, lace-like (18)
			PF (11)			IgG (7)	ICS, lace-like (7)
			BP (4)			IgG+C3 (4)	Linear BMZ (4)
11	**Dhanabalan RT et al (2016)**	50	PF (8)	2^{nd}-7^{th} decade	M>F (1.08:1)	IgG (7)	ICS (7)
			PV (5)			IgG (4)	ICS (5)
			IgA P (2)			IgA (2)	ICS (2)
			PNP (1)			Negative (1)	None (1)
			BP (18)			BP (12)	BMZ linear (16)
			DH (2)			IgA (2)	BMZ granular (2)
			LAD (2)			IgA (2)	BMZ linear (2)
12	**Chanabasayya V et al (2017)**	91	PV (18)	2-85 years	M>F (1.2:1)	IgG (13)	ICS (13)
			PF (5)			IgG (5)	ICS (5)
			P vegetans (1)			IgG+C3 (1)	ICS (1)
			PE (1)			Negative	None
			BP (34)			C3 (27)	BMZ (31)
			DH (3)			IgA (1), C3 (1)	BMZ (2)
13	**Present study**	29	PF (6)	1.5 to 79 years	M<F (0.6:1)	IgG (5)	ICS
			PV (4)			IgG (3)	ICS
			PE (1)			IgG with IgM+C3 (1)	ICS with BMZ
			BP (9)			IgG (7)	BMZ
			DH (5)			IgA (3)	Dermal papillae
			HG/PG (3)			IgG (2)	BMZ
			CBDC (1)			IgA (1)	BMZ

CONCLUSION

Direct immunofluorescence is a useful tool in the diagnosis of autoimmune bullous disorders and corroborates with histopathology considerably. However DIF may yield false-negative results due to sampling errors like biopsy from incorrect site (lesional skin), formalin fixed bi-opsy, lack of epidermis in biopsy, partially treated cases and other technical errors. Therefore a negative DIF result does not completely rule out a diagnosis of AIBD.

The fish-net pattern on DIF is diagnostic of Pemphigus group however histopathological ex-amination is necessary to differentiate between its subtypes (PV vs PF). In the subepidermal group, DIF examination plays a crucial role in the diagnosis of various subtypes as the presence of subepidermal bulla on histopathology is seen in all subtypes. IgA exclusively in the dermal papillary tips is characteristic of DH. However, it should be noted that linear deposits at BMZ may be seen in non-bullous diseases like Discoid lupus erythematosus as well. Therefore, clinico-histopathological correlation is essential for making a definite diagnosis. Hence we conclude that DIF is a sensitive method for the diagnosis of autoimmune bullous diseases however it is complementary to histopathology and does not replace it.

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