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DIAGNOSTIC UTILITY OF DIRECT IMMUNOFLUORESCENCE IN VARIOUS DERMATOLOGICAL CONDITIONS WITH CLINICAL AND HISTOPATHOLOGICAL CORRELATION

Dermatology

Dr Jaydip Tank MD, Assistant Professor, Department of Dermatology, Venereology & Leprosy, GMERS medical college, Civil Hospital, Junagadh, Gujarat

Dr Radha Dhudshia* MD, Registrar, Department of Dermatology, Venereology & Leprosy, GMERS medical college, Civil Hospital, Junagadh, Gujarat *Corresponding Author

Dr Kirti Parmar MD, Associate Professor, Department of Dermatology, Venereology & Leprosy, BJ medical college, Civil Hospital, Ahmedabad

Dr Bela Shah MD, Professor and Head, Department of Dermatology, Venereology & Leprosy, BJ medical college, Civil Hospital, Ahmedabad

ABSTRACT

DIF studies are done for the detection of tissue bound antibodies in immune-mediated skin diseases. This study aimed to assess the role of DIF in diagnosing the dermatological diseases by correlating the clinical, histological and DIF diagnosis. DIF testing was done on the biopsy samples taken from 150 suspected cases of autoimmune diseases, connective tissue diseases and vasculitis along with the histopathological examination. The overall positivity of DIF in immune-mediated skin diseases was 81.3% in this study. The sensitivity of DIF was 94.1% in the pemphigus group and 88.5% in pemphigoid group. A positive lupus band test was noted in 17/23 cases of lupus erythematosus. DIF was positive in 10/15 cases of dermatitis herpetiformis. All the suspected cases of vasculitis were confirmed by DIF studies. False-negative results were seen.DIF is very helpful for the definitive diagnosis in autoimmune blistering diseases with clinico-histological dilemmas.

KEYWORDS

immunoflourescence, autoimmune disease

INTRODUCTION

Direct immunofluorescence (DIF) studies are done on skin biopsies to look for the presence of immunoglobulin, complement, and fibrin deposited in tissue.[1] It has become the standard procedure for accurately diagnosing the autoimmune vesiculobullous diseases as there may be a great degree of clinical and histological overlap.[2] The immunofluorescence studies are also an important part of the laboratory evaluation of other immune-mediated skin diseases which include connective tissue disorders and vasculitides. Even in conditions such as lichen planus DIF help understand the immunological pathogenesis.[3]

Limited literature is available about immunoflourescence studies. DIF tests are mostly done in tertiary care hospitals since this technique has many difficulties requiring a thoroughly trained team and advanced laboratories proficient in the performance and interpretation of these tests. This study was undertaken to evaluate the DIF patterns in the immune-mediated dermatoses. This study aimed to assess the diagnostic utility of DIF in various dermatological conditions with respect to the clinical and histological diagnosis.

MATERIALS AND METHODS

This cross-sectional hospital-based study was conducted on 150 patients attending the department of dermatology for 2 years, after taking ethics committee approval from the institution. Patients having a strong clinical suspicion of immune-mediated diseases, vasculitides and other dermatoses were included in the study. Patient with bullous lesions secondary to infections, medication and burns (chemical and thermal) were excluded from the study.

After taking written informed consent, Tzanck smear was prepared from the patients with bullous lesions for the identification of acantholytic and inflammatory cells. Two samples of 3-5 mm sized punch biopsies were taken from every patient. Biopsy for histopathological examination was performed from the skin lesion, fixed in 10% formalin and subjected to hematoxylin and eosin staining. In cases of suspected autoimmune bullous diseases, the biopsy for DIF was taken from the perilesional skin, while in other conditions; it was taken from the sites including half normal and half the lesion. The tissue for DIF was put in saline and transported to the pathology laboratory immediately. Fluorescein isothiocyanate (FITC) labelled monospecific immunoglobulins (IgG, IgA, IgM, C3q) were layered onto the frozen sections and finally viewed under a fluorescent microscope. The DIF results were reported based on the nature and location of the immune deposits; the extent and the intensity of fluorescence; as well as the pattern (granular/linear) of immune deposits.[4]The final diagnoses were based on the combination of clinical, histopathological and immunofluorescence findings.

Data were analyzed in Microsoft Excel. To test for the correlation between clinical diagnosis, histopathology and DIF diagnosis, Kappa statistics were used.

OBSERVATION AND RESULTS

Amongst the 150 patients enrolled, 82(54.67%) were females and 68 (45.33 %) were male. The age of the patients varied from 3 to 76 years. Majority of the patients were in 31-40 years of age group (n= 46, 30.67%). In this study maximum number of patients were in the vesiculobullous group (n= 111) followed by connective tissue disease (n=23).

TABLE 1 enumerates the clinical, histopathological and DIF results in 150 immune-mediated skin diseases. DIF was able to diagnose the same condition in 86% of the clinically suspected immune-mediated diseases. Histopathology was conclusive in 79% of the cases. Thus, in the remaining 7% cases with non-specific histopathological findings, DIF helped us to confirm the diagnosis

TABLE 1: CLINICAL , HISTOPATHOLOGICAL AND DIF RESULTS IN IMMUNE-MEDIATED SKIN DISEASES

Final diagnosis	Clinical Diagnosis		Histopathology		Direct Immunofluorescence	
	Definitive	Provisional	Diagnostic	Nondiagnostic	Positive	Negative
Vesiculobullous disorders						
PV (n=48)	42	6 PV/BP	44	4	46	2
PF(n=16)	14	2PF/PV	15	1	14	2
PE (n=1)	0	1PE/PF	1	0	1	0
PNP(n=1)	0	1PNP/PV	1	0	1	0
PH(n=1)	0	1 PH/DH	1	0	1	0
P.veg.(n=1)	0	1 PVEG/PV	1	0	1	0

SCPD(n=2)	0	1SCPD/PF 1SCPD/DH	2	0	0	2
BP(n=20)	13	2BP/EM 5BP/PV	17	3	18	2
PG(n=1)	1		1	0	1	0
EBA(n=2)	0	2 EBA/BP	2	0	2	0
CBDC(n=2)	1	1CBDC/PV	1	1	1	1
LIBD(n=1)	0	1 LIBD/PV	1	0	1	0
DH(n=15)	13	2 DH/LIBD	6	9	10	5
Connective tissue disease						
SLE(n=5)	5		3	2	5	0
Bullous LE(n=2)	2		1	1	1	1
SCLE(n=3)	2	1	2	1	2	1
DLE(n=6)	6		4	2	5	1
MCTD(n=2)	2		0	2	2	0
SS(n=3)	3		2	1	1	2
DM(n=2)	2		1	1	1	1
Vasculitis						
HSP(n=4)	4		3	1	4	0
ICV(n=1)	0	1	1	0	1	0
Miscellaneous						
LP(n=7)	7		5	2	3	4
Psoriasis vulgaris(n=1)	1		1	0	0	1
EM(n=2)	2		2	0	0	2
PG(n=1)	1		1	0	0	1

Table 2 gives a detailed description of the DIF patterns in positive cases.

Table 2: DIF patterns in positive 122 skin biopsy

Intra epidermal blistering Disorders	Location of deposits	Nature of deposits	Extent	Pattern	Intensity of fluorescence
Pemphigus Vulgaris (DIF positive=46) (n=48)	ICS-46(100%)	IgG,C3-36(78.26%) IgG-10(21.74%)	Diffuse, in lower epidermis – 46(100%)	Granular, lace like– 43 (100%)	++7(15.22%), +++15 (32.61%), ++++24 (52.17%)
Pemphigus foliaceus (DIF positive=14) (n=16)	ICS–14(100%)	IgG, C3–9(64.29%) IgG–5(35.71%)	Diffuse, throughout epidermis, specially upper part– 14 (100%)	Granular, lace like – 14(100%)	+++6 (42.85%), ++++8 (57.15%)
Pemphigus erythematosus (DIF positive=1) (n=1)	ICS and BMZ–1(100%)	IgG, C3 at ICS, IgG, IgM at BMZ–1 (100%)	Diffuse, throughout epidermis – 1 (100%)	Granular, lace like at ICS, granular at BMZ – 1 (100%)	++++1 (100%)
Paraneoplastic pemphigus (DIF positive =1) (n=1)	ICS and BMZ-1(100%)	IgG and C3 at ICS,IgG at BMZ-1(100%)	Diffuse, throughout epidermis – 1 (100%)	Granular, lace like at ICS, granular at BMZ – 1 (100%)	+++1(100%)
Pemphigus Herpetiformis (DIF positive=1) (n=1)	ICS-1(100%)	IgG (100%)	Diffuse, throughout epidermis – 1 (100%)	Granular, lace like– 1(100%)	+++1(100%)
Pemphigus vegetans (DIF positive =1) (n=1)	ICS-1(100%)	IgG and C3(100%)	Diffuse, throughout epidermis – 1 (100%)	Granular, lace like –1(100%)	++1(100%)
Subepidermal blistering disorders					
Bullous Pemphigoid (DIF positive =18) (n=20)	BMZ-18(100%)	IgG, C3–13(72.22%) C3–5(27.78%)	Diffuse, throughout the BMZ – 18(100%)	Linear – 18 (100%)	++2(11.11%), +++6 (33.33%), ++++10 (55.55%)
Pemphigoid gestationis (DIF positive =1) (n=1)	BMZ-1(100%)	C3-1(100)	Diffuse, throughout the BMZ – 1 (100%)	Linear-1(100%)	+++3(100%)
Dermatitis Herpatiformis (DIF positive=10) (n=15)	BMZ+papillary dermis-10(100%)	IgA-10(100%)	At tips of dermal papillae-10(100%)	Granular-10(100%)	++3(30%) +++4(40%) ++++3(30%)
Childhood linear IgA bullous dermatosis (DIF positive=1) (n=2)	BMZ–1(100%)	IgA, IgG, C3–1 (100%)	Diffuse, throughout the BMZ – 1 (100%)	Linear – 1 (100%)	IgA ++++, IgG +, C3+ 1 (100%)
Linear IgA bullous dermatosis (DIF positive=1) (n=1)	BMZ–1(100%)	IgA, IgG,-1(100%)	Diffuse, throughout the BMZ – 1 (100%)	Linear – 1 (100	IgA ++++, IgG + 1 (100%)
Epidermolysis bullosa acquisita (DIF positive=2) (n=2)	BMZ-2(100%)	IgG,C3-2(100%)	At floor (dermal side)after salt split technique-2(100%)	Linear-2(100)	IgG +++ C3++2(100%)
Connective tissue diseases					
Systemic Lupus Erythematosus (DIF positive=5) (n=5)	BMZ-5(100%) CB-3(60%)	IgG,IgM,IgA,C3-3(60%) IgG,IgM,-2(40%)	Diffuse, throughout the BMZ-5(100%)/CB/SBV	Granular-5(100)	IgG-+++5(100%) IgM++5(100%) IgA++3(60%) C3-++3(60%)

Bullous LE (DIF positive=1) (n=2)	BMZ – 1(100%)	IgG, IgM, IgA, C3–1 (100%)	Diffuse, throughout the BMZ – 1 (100%)	Linear –1(100)	All-++++ 1(100%)
Subacute Cutaneous Lupus Erythematosus (DIF positive=2) (n=3)	BMZ – 2(100%)	IgG, IgM, IgA, C3 –1 (50%) IgG, IgA, C3 – 1 (50%)	Diffuse, throughout the BMZ – 2(100%)	Granular – 2(100)	All ++++2(75%) IgG ++++, IgA ++, C3++1 (50%)
Discoid Lupus Erythematosus (DIF positive=5) (n=6)	BMZ – 5(100%)	IgG, IgM, IgA, C3 – 3 (60%) IgG, IgA, C3 – 2 (40%)	Diffuse, throughout the BMZ – 5(100%)	Granular – 5 (100)	All ++++3(60%) IgG ++++, IgA ++, C3++2 (40%)
Mixed Connective Tissue disease (DIF positive=2) (n=2)	In vivo ANA (ENS) and/or BMZ-2(100%)	IgG-2(100%)	Diffuse -2(100%)	Granular-2(100%)	IgG ++1(100%)
Systemic Sclerosis (DIF positive=1) (n=3)	BMZ-1(100%)	IgM-1(100%)	Diffuse, throughout the BMZ-1(100%)	Granular-1(100%)	IgM ++1(100%)
Dermato-myosits (DIF positive=1) (n=2)	BMZ-1(100%)	IgG,IgM,C3 -1(100%)	Diffuse, throughout the BMZ-1(100%)	Granular-1(100%)	IgG,IgM,C3-++1(100%)
Vasculitis					
Henoch–Schönlein Purpura (IgA vasculitis) (DIF positive=4) (n=4)	Walls of superficial dermal vessels	IgA – 4 (100%)	Diffuse – 4(100%)	Granular – 4(100%)	++2 (50%) +++2(50%)
Immune complex vasculitis (DIF positive=1) (n=1)	Walls of superficial dermal vessels	IgG, IgM, C3 – 1 (100%)	Diffuse – 1(100%)	Granular – 1 (100%)	All +++1 (100)
Others					
Lichen planus (DIF positive=3) (n=7)	-Upper dermis -BMZ	IgM,, C3 – 3 (100%) Fibrin-2(66.67%)	Focal, in clusters – 3 (100%)	Homogenus colloid bodies – 3 (100%) Linear shaggy fibrin-2(66.67%)	+++3 (100%)

TABLE 3 shows the sensitivity of Clinical diagnosis, Histopathology and DIF in immune-mediated skin diseases DIF is having sensitivity comparable with that of histopathology in pemphigus and pemphigoid group. Both are superior to the clinical diagnosis. Considering the connective tissue disease, the clinical findings were more sensitive to detect the disease compared with both the DIF and histology.

Disease group	Clinical diagnosis		Histopathology		DIF	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
PEMPHIGUS	82.4	100	92.6	100	94.1	100
PEMPHIGOID	57.7	100	84.6	100	88.5	100
CTD	91.3	100	56.5	100	74	100
VASCULITIS	80	100	80	100	100	100

Chi-square test was used to test the difference between paired proportions. Comparison between clinical diagnosis and DIF showed p-value 0.135 and combined sensitivity of 70%. The histopathology and DIF results showed p-value 0.896 and combined sensitivity of 61.3%. Both the results are comparable. There was a moderate agreement observed between DIF with clinical diagnosis and histology with a kappa value of 0.57 and 0.48 respectively. P value was less than 0.05 in both cases.

In the Pemphigus group, Tzanck smear showed acantholytic cells in 40/48 patients of pemphigus vulgaris(PV) and 14/16 patients of pemphigus foliaceus(PF).On histology, a total of 44 cases were diagnosed as PV showing suprabasal intraepidermal blister with acantholytic cells, 15 cases as PF showing subcorneal intraepidermal blister. In cases with inconclusive histological findings, further DIF studies showed intercellular space (ICS) deposition of immune-reactants. In cases of para-neoplastic pemphigus, pemphigus erythematosus, pemphigus vegetans, the histopathological and DIF results correlated well. Of all the pemphigus patients who had positive DIF results, 75% had ICS staining with both IgG and C3 and the remaining had staining with IgG only (Figure 1). Only two cases each of pemphigus vulgaris and pemphigus foliaceus with characteristic clinical and histological features showed a negative DIF result.

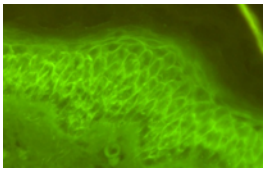

Figure 1- ICS deposition in PV

Tzanck smear from all 20 patients of Bullous pemphigoid (BP) showed the presence of inflammatory cells. The skin biopsy studied based on seven histological criteria were highly suggestive in 85% of cases(n=17).[5] Out of 18 patients who had positive DIF results linear deposition of both IgG and C3 at basement membrane zone(BMZ) was seen in 72% cases **(Figure 2)**.

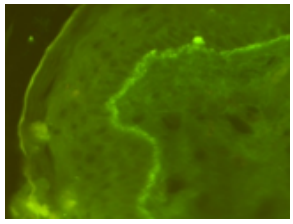

Figure 2- BMZ deposition in BP

The lone case each of Pemphigoid gestationis and Linear IgA bullous disease (LAD) both adult and children form was confirmed with DIF. The patients of Epidermolysis bullosa acquisita (EBA) showed subepidermal split with heavy neutrophilic infiltrate. DIF of direct salt-split skin biopsy showed linear deposits of IgG and C3 at the dermal aspect of the blister in both the patient.

DIF from 10/15 patients of Dermatitis herpetiformis (DH) showed granular IgA deposition at tips of dermal papillae**.** One suspected case clinically presenting as DH revealed IgG deposition in the ICS leading to the diagnosis of Pemphigus herpetiformis. Majority of the cases were diagnosed based on the clinical assessment.

A positive lupus band test (LBT) was noted in 13/16 cases of lupus

erythematosus**.** Of all the DIF positive cases, 62% stained with all the immunoreactants. DIF from lesional sun-exposed site demonstrated full house LBT positivity at DEJ with cytoid bodies while DIF from non-lesional sun-exposed skin showed IgG and IgM deposition at DEJ. Biopsy from 2 patients of SCLE demonstrated basal layer degeneration with lymphocytic infiltrate around vessels, appendages, and in subepidermal location with mild epidermal atrophy. All the cases of DLE were diagnosed clinically. DIF gave confirmatory results in 5 biopsies. In MCTD, DIF demonstrated IgG deposition in epidermal cell nuclei making a speckled pattern with no deposition at BMZ. Only single case each of SS and dermatomyositis had immune deposits on DIF studies.

DIF was positive in all 5 cases of vasculitis, of which 4 proved to be Henoch–Schönlein purpura (HSP) showing IgA deposition at walls of superficial dermal vessels. One case of leukocytoclastic vasculitis showed deposits of IgG, IgM and C3 in the dermal vessel walls, and was thus proved to be immune-complex mediated.

Only 3 cases of Lichen planus (LP) showed IgM and C3 stained colloid bodies in the papillary dermis with shaggy fibrin deposition. DIF study revealed no fluorescence in the cases of Erythema multiforme, Psoriasis vulgaris and Pyoderma gangrenosum while histopathological findings in all cases were consistent with their respective clinical diagnosis.

DISCUSSION

Autoimmune bullous diseases often presents with overlapping clinical and histological features. A diagnosis based solely on clinical or histologic findings may not be accurate. DIF is extremely helpful in confirming a suspected diagnosis and in distinguishing among closely related diseases.[6] In this study, 87/94 patients having vesiculobullous skin lesions gave diagnostic results on DIF. The results from the previous studies are summarized in the **Table 4**

Sensitivity Of DIF (%)	Present Study	Inchara YK et al[7]	MINZ et al[8]	Mysorekar et al[9]	Buch AC et al[10]
Vesiculobullous Disorders	87.38	73	70	97.5	89
Pemphigus Group	94.12	88	81.8	98.1	94.44
Bullous Pemphigoid	90	82	53.8	96	84

The sensitivity and specificity of DIF observed in all the study confirmed its utility in the pemphigus group. False-negative DIF result occurred in approximately 6% specimens, two cases each of PV and PF. The presence of inflammation in tissue, damaged biopsy specimen or technical error might be the cause. In the study by Minz et al[8] from 22 cases clinically suspected as PV, there were four cases where histopathology demonstrated the lesions but DIF failed to show the same due to technical faults or treatment-induced changes. Similarly, sampling errors contributed to false negative results in the study by inchara and rajalakshmi.[7] This finding emphasizes on the importance of obtaining an intact perilesional biopsy for accurate DIF assessment.

A negative DIF helped to confirm two cases of Sub-corneal pustular dermatoses (SCPD) which is comparable with the study by Arundhiti et al.[11] Nevertheless, repeat studies are recommended to detect a subgroup referred to as SCPD type IgA pemphigus.[12]

In the present study, 90% BP cases were DIF positive. Both histopathology and DIF results were significantly better than the clinical assessment. Thus, the combination of DIF with histology improves the sensitivity of detection of BP. False-negative results were seen in 10% cases that can be attributed to sampling or technical error. Minz et al suggested false negativity in some cases is also attributed to the longer stay of skin biopsies in the transport medium. This observation makes the use of fresh tissue the preferred substrate for DIF studies.

We were able to confirm the rare case of pemphigoid gestationis (PG) by the characteristic immunoflourescence pattern. DIF is the key assay to differentiate PG from pruritic urticarial papules and plaques of pregnancy.[13] With the DIF studies, we were able to diagnose the lone case of Linear IgA dermatoses and differentiate it from clinically similar conditions such as BP, DH.

The DIF studies using salt-split skin biopsy also confirmed the two cases of EBA. Intense IgG deposition is almost consistently present with the intensity of C3 deposition less than that of IgG.

Dermatitis herpetiformis is an exquisitely pruritic blistering disorder that is often associated with a gluten sensitive enteropathy. DIF of uninvolved skin collected from the perilesional site is the gold standard for the diagnosis of DH.[14] In the present study, histopathologic picture was unspecific in 60% cases. DIF helped to confirm 10/15 cases of DH. In the other suspected patients with negative DIF results, the site of biopsy should be reconsidered. In such cases, one must rely on the clinical findings and serological tests for the accurate diagnosis. Moreover, in patients on a gluten-free diet IgA deposits can disappear from the skin in period of times. Therefore, in such patients, a normal gluten-containing diet should be administered and the biopsy taken after at least 1 month.[15]

A positive lupus band test was demonstrated in 81 % of the clinically suspected cases of LE. DIF helped to confirm six cases with inconclusive histological findings. However, DIF showed negative results in three cases which were picked up only on histopathology. Minz et al. showed 71.43% (10/14) cases having positive LBT on DIF. The prevalence of immunoreactant deposition in LE depends on several factors including the clinical morphology of the lesions, biopsy site, past treatment, and disease activity. Therefore, one must always interpret DIF results in conjunction with clinical features and histopathology.[16] LBT was also seen in cases of MCTD, systemic sclerosis and dermatomyositis. In present study both the patients of MCTD demonstrated 100% positivity in DIF although histological features of both the patients were inconclusive. The clinical features of dermatomyositis and systemic sclerosis are usually characteristic. DIF is of little value in the diagnosis of these cases. Cases where clinico-histological findings are inconclusive, differentiation may be made by the use of serologic antibody testing as well as muscle enzyme chemistry findings.[17]

All the studies available in the literature had DIF sensitivity of 90% to 100% in diagnosis of vasculitis.[18] HSP is a common form of LCV described in children who have systemic involvement in addition to cutaneous lesions.[19] Diagnosis is usually clinical. Histopathological features can vary from typical leucocytoclastic vasculitis with fibrinoid necrosis to less specific perivascular lymphocytic infiltrate. DIF is very useful for the confirmation of vasculitis; the yield of positive result is much higher when biopsy sample is taken within 48 hours of clinical presentation. However, the number of vasculitis cases is very small in this study in comparison to the study by Minz et al

The presence of cytoid bodies in the upper dermis was noted in three patients of LP in this study. IgM was the most common immunoreactant present in the cytoid bodies along with the shaggy fibrin deposition in two cases. This is in accordance to the criteria suggested by Kulthanan et al in his study.[20] However, cytoid bodies are not characteristic of LP and may be seen in other conditions such as LE and vasculitis. The final diagnosis should be based on clinical and histological findings.

CONCLUSION

DIF is extremely helpful in confirming the immune mediated diseases and in distinguishing among the closely related diseases. False negative results do occur. So, the results should always be interpreted in conjunction with histopathology and clinical features. The combination of three usually yields the best results. Besides, DIF also provides a platform on which other advanced tests such as ELISA and immunoblotting can be done, if the facilities are available.

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