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Original Research Article

Utility of Direct Immunofluorescence in diagnosis of non – infectious / immune mediated diseases involving skin – Tertiary center

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Abstract

Background: Skin biopsy in Auto immune bullous and other immune mediated disorders are valuable for diagnosis but and additional test which may lead to get a better understanding of the disease likewise DIF and Indirect immunofluorescence could help clinicians as well pathologist to access more. To understand the Patterns and intensity shown by different disorders this method is of valuable tool.

Materials and Methods: An Ambispective study was conducted in the Department of Pathology in our hospital during a 28 month period extending from January 2022 to April 2024. Material for this study included two samples of biopsy, one for histopathology and one for DIF of 80 patients who clinically presented with autoimmune bullous disease, connective tissue disease or vasculitis from Department of Dermatology of our hospital.

Results: In the present study total 80 cases were studied, which were suspected as immune mediated skin disorders out of which 40 (50%) cases belonged to pemphigus group, 21(26.2%) cases of bullous group, 10 (12.5%) cases belonged to vasculitis disorders, 3 (3.7%) cases of connective tissue disorders and 6 (7.5%) cases were taken as miscellaneous which were either suspected as pemphigus/bullous/vasculitis/CTD in the period between January 2022 and April 2024.

Conclusion: DIF is a useful supplement in the accurate diagnosis of autoimmune mediated skin disorders. A negative DIF result helps to rule out the immunological cause of the skin disorder. In cases with inconclusive clinical features and histopathology, DIF acts as a confirmatory as well as diagnostic in immune mediated disorders. A combined analysis of clinical features, histopathology and DIF is required for an accurate diagnosis. A combination of investigations by a direct immunofluorescence and histological examination remains the gold standard in the diagnosis of immune mediated disorders.

Keywords: Biopsy, Direct immunofluorescence, Indirect, BMZ, ICS.

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1. Introduction

Skin biopsy is an established diagnostic procedure which connects clinical diagnostic methodology with the invisible to the unaided eye microscopic field of skin pathology. Taking under consideration the potentials and limitations of optical microscopy and the indications of performing an invasive technique, dermatologists often rely on skin biopsy for enhancing their diagnostic abilities. Accurate diagnosis of immune-mediated dermatological diseases requires an evaluation of clinical, histopathological, and immunofluorescence findings. Immunofluorescence (IF) has been in use for the past five decades, both to investigate the pathophysiology of skin disorders and to help the dermatologists in the diagnosis of various cutaneous disorders, especially bullous diseases and connective tissue diseases. Immunofluorescence (IF) is a histochemical technique employed to detect antibodies bound to antigens in the tissue or in the circulating body fluids. IF is a simple, reliable and reproducible technique in immunopathology. There are two main types of IF techniques, namely direct IF (DIF) and indirect IF (IIF). IF technique involves viewing of antigen–antibody complexes under ultraviolet microscope using corresponding antibodies tagged to a Fluorochromes. Fluorochromes, currently in use, are fluorescein isothiocyanate (FITC) which produces apple-green color, and tetramethylrhodamine isothiocyanate (TRITC) with a red color of fluorescence. DIF involves the application of antibody-fluorophore conjugate molecules to samples of patient tissue obtained from biopsies. These antibody-

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fluorophore conjugates target abnormal depositions of proteins in the patient's tissue. When exposed to light, the fluorophore emits its own frequency of light, and then it is visualized by fluorescence or confocal microscopy and quantified by a flow cytometer. The particular staining pattern and type of abnormal protein deposition seen in the tissue sample help diagnose the disease.

The present study is undertaken to analyse the utility of DIF in the diagnosis of common immune-mediated/non-infectious dermatological diseases by its correlation with clinical features and histopathology and to evaluate the diagnostic potential of DIF.

2. Materials and Methods

This Ambispective study was conducted in the Department of Pathology in our hospital during a 28 month period extending from January 2022 to April 2024. Material for this study included two samples of biopsy, one for histopathology and one for DIF of 80 patients who clinically presented with autoimmune bullous disease, connective tissue disease or vasculitis from Department of Dermatology of our hospital.

2.1. Inclusion criteria

Skin punch biopsy of all patients male, female of any age group presenting with autoimmune bullous diseases, connective tissue diseases and vasculitis and were confirmed on DIF.

2.2. Exclusion criteria

1. Autolysed Biopsies.
2. Pregnant and lactating women with clinical evidence of immunobullous disorder.
3. Those biopsies of immunobullous disorders and vasculitis that were not confirmed on DIF.

Detailed history and clinical details of the patients with particular reference to age, gender, morphology of lesions, site of involvement was taken. Clinical diagnosis and pathological findings were noted. DIF diagnoses were compared with histopathological findings and concordance rate between both the findings was analysed.

Two biopsies for taken for all the patients. One of the two biopsies obtained was immediately put in 10% neutral buffered formalin in a labelled container to prevent autolysis for histopathological examination and was sent to Department of Surgical Pathology of our Institute. The other biopsy was put in a labelled container containing saline solution (0.95 NaCl) for DIF examination.

In this study the Histopathological sections were evaluated after staining by Haematoxylin and Eosin technique. Skin biopsy specimen were embedded in optimal cutting temperature (OCT) medium.

4 micron-thick sections were cut on a cryostat. A minimum of 10 sections were cut for each case.

Two sections were taken on each slide and the slides were dipped in cold acetone for 5 min. The slides were stained immediately.

For staining, sections were brought to room temperature, air dried, washed with phosphate-buffered saline (PBS) at pH 7.4 for 10 min, and layered with fluorescein isothio- cyanate (FITC)-conjugated rabbit antihuman immunoglobulin G(IgG), IgA, IgM, and C3 (dilution 1:20). These slides were incubated for 2 h in a moist chamber at room temperature. The sec- tions were then washed with PBS for 10 min, mounted in buffered glycerine, and viewed under a fluorescence microscope.

The DIF results were recorded by taking into consideration the nature of the immune deposits (IgG/IgA/IgM/C3); the location of the immune deposits (intercellular spaces in epidermis/DEJ or basement membrane zone-(BMZ)/sub epidermal blood vessels/colloid bodies, etc.); the ex- tent (focal/diffuse); the intensity of fluorescence (+ to ++++); and the pattern of immune complex deposits (granular/linear).

The definite diagnoses were based on a combination of the clinical, histopathological and immunofluorescence findings.

3. Result

In the present study total 80 cases were studied, which were suspected as immune mediated skin disorders out of which 40 (50%) cases belonged to pemphigus group, 21(26.2%) cases of bullous group, 10 (12.5%) cases belonged to vasculitis disorders, 3 (3.7%) cases of connective tissue disorders and 6 (7.5%) cases were taken as miscellaneous which were either suspected as pemphigus/bullous/vasculitis/CTD in the period between January 2022 and April 2024. Diseases are clubbed under following headings:

Pemphigus group of disorders include – Pemphigus Vulgaris (PV), Pemphigus Foliaceous (PF), IgA Pemphigus.

Bullous group of disorders include – Bullous Pemphigoid (BP), Dermatitis herpetiformis (DH), mucous membrane pemphigoid (MMP), Pemphigoid Gestationis (PG), Epidermolysis Bullosa Acquisita (EBA).

Connective tissue disorders include – SLE, Dermatomyositis (DM).

Vasculitis disorders include – IgA vasculitis (HSP).

4. Discussion

The **Table 1** shows the distribution of the study population based on their age groups.

Table 1: Distribution of study population on basis of age groups

Age Group	Frequency	Percent
11-20 Years	7	8.8
21-30 Years	10	12.5
31-40 Years	17	21.3
41-50 Years	12	15.0
51-60 Years	14	17.5
61-70 Years	13	16.3
71-80 Years	7	8.8
Total	80	100.0

The maximum number of cases 21.3% was for 31-40 years, followed by 17.5% for 51-60 years, 16.3% for 61-70 years and 15% for 41-50 years. The lower number of cases 12.5%, falls under the age groups 21-30 years and 8.8% was for age groups 11-20 years and 71-80 years respectively.

Table 2: Distribution of study population on basis of age groups

Sex	Frequency	Percent
F	44	55.0
M	36	45.0
Total	80	100.0

The **Table 2** shows the distribution of the study population based on their sex group.

The maximum number of cases 55% was for females and the lower number of cases 45% was for males respectively.

Table 3: Measure of agreement and prediction of pemphigus by clinical and DIF tools

Clinical Pemphigus		DIF Pemphigus		Total
		Present	Absent	
Present	Count	32	8	40
	%	100.0%	16.7%	50.0%
Absent	Count	0	40	40
	%	0.0%	83.3%	50.0%
Total	Count	32	48	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.800	0.000	Sig
Sensitivity		100.00%		
Specificity		83.33%		
PPV		80.00%		
NPV		100.00%		
Accuracy		90.00%		

In the **Table 3** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between clinical and DIF tool for pemphigus outcome.

The higher value of Kappa 0.80 shows that the number of cases of DIF tool shows similar outcomes with clinical procedure with 100% for present and 83.3% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of clinical procedure against the DIF tool as gold standard tool for pemphigus outcome.

The high value of sensitivity 100% and PPV 80% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 83.33% and NPV 100% shows the higher degree of prediction of absence of disease.

Also, the higher value of 86.05% accuracy shows that the clinical procedure can be used as an alternate tool to predict the presence of pemphigus outcome against the DIF tool.

Table 4: Measure of agreement and prediction of pemphigus by HPE and DIF tools

HPE Pemphigus		DIF Pemphigus		Total
		Present	Absent	
Present	Count	31	8	39
	%	96.9%	16.7%	48.8%
Absent	Count	1	40	41
	%	3.1%	83.3%	51.3%
Total	Count	32	48	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.774	0.000	Sig
Sensitivity		96.88%		
Specificity		83.33%		
PPV		79.49%		
NPV		97.56%		
Accuracy		88.75%		

In the above **Table 4** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between Histopathology and DIF Tool for Pemphigus outcome.

The higher value of Kappa 0.774 shows that the number of cases of DIF tool shows similar outcomes with Histopathology with 96.90% for present and 83.3% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of Clinical procedure against the DIF tool as gold standard tool for pemphigus outcome.

The High value of sensitivity 96.88% and PPV 79.49% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 83.33% and NPV 97.56% shows the higher degree of prediction of absence of disease.

Also, the higher value of 88.75% accuracy shows that Histopathology can be used as an alternate tool to predict the presence of Pemphigus outcome against the DIF tool.

Table 5: Measure of agreement and prediction of bullous by clinical and DIF tools

Clinical Bullous		**DIF Bullous**		**Total**
		Present	**Absent**	
Present	Count	15	9	24
	%	100.0%	13.8%	30.0%
Absent	Count	0	56	56
	%	0.0%	86.2%	70.0%
Total	Count	15	65	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.700	0.000	Sig
Sensitivity		100.00%		
Specificity		86.15%		
PPV		62.50%		
NPV		100.00%		
Accuracy		88.75%		

In the **Table 5** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between Clinical and DIF Tool for Bullous outcome.

The higher value of Kappa 0.70 shows that the number of cases of DIF tool shows similar outcomes with clinical procedure with 100% for present and 86.2% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of Clinical procedure against the DIF tool as gold standard tool for Bullous outcome.

The high value of sensitivity 100% and moderate PPV 62.50% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 86.15% and NPV 100% shows the higher degree of prediction of absence of disease.

Also, the higher value of 88.75% accuracy shows that the clinical procedure can be used as an alternate tool to predict the presence of Bullous outcome against the DIF tool.

Table 6: Measure of agreement and prediction of Bullous by HPE and DIF tools

HPE Bullous		**DIF bullous**		**Total**
		Present	**Absent**	
Present	Count	15	6	21
	%	100.0%	9.2%	26.3%
Absent	Count	0	59	59
	%	0.0%	90.8%	73.8%
Total	Count	15	65	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.787	0.000	Sig
Sensitivity		100.00%		
Specificity		90.77%		
PPV		71.43%		
NPV		100.00%		
Accuracy		92.50%		

In the **Table 6** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between Histopathology and DIF Tool for Bullous outcome.

The higher value of Kappa 0.787 shows that the number of cases of DIF tool shows similar outcomes with Histopathology with 100% for present and 90.8% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of clinical procedure against the DIF tool as gold standard tool for Bullous outcome.

The High value of sensitivity 100% and PPV 71.43% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 90.77% and NPV 100% shows the higher degree of prediction of absence of disease.

Also, the higher value of 92.5% accuracy shows that Histopathology can be used as an alternate tool to predict the presence of Pemphigus outcome against the DIF tool.

Table 7: Measure of agreement and prediction of vasculitis by clinical and DIF tools

Clinical Vasculitis		**DIF Vasculitis**		**Total**
		Present	**Absent**	
Present	Count	8	3	11
	%	100.0%	4.2%	13.8%
Absent	Count	0	69	69
	%	0.0%	95.8%	86.3%
Total	Count	8	72	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.821	0.000	Sig
Sensitivity		100.00%		
Specificity		95.83%		
PPV		72.73%		
NPV		100.00%		
Accuracy		96.25%		

In the **Table 7** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of

agreement between clinical and DIF tool for vasculitis outcome.

The higher value of Kappa 0.821 shows that the number of cases of DIF tool shows similar outcomes with clinical procedure with 100% for present and 95.8% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of clinical procedure against the DIF tool as gold standard tool for vasculitis outcome.

The high value of sensitivity 100% and moderate PPV 72.73% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 95.33% and NPV 100% shows the higher degree of prediction of absence of disease.

Also, the higher value of 9625% accuracy shows that the clinical procedure can be used as an alternate tool to predict the presence of vasculitis outcome against the DIF tool.

Table 8: Measure of agreement and prediction of vasculitis by HPE and DIF tools

HPE Vasculitis		**DIF vasculitis**		**Total**
		Present	**Absent**	
Present	Count	8	2	10
	%	100.0%	2.8%	12.5%
Absent	Count	0	70	70
	%	0.0%	97.2%	87.5%
Total	Count	8	72	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.875	0.000	Sig
Sensitivity		100.00%		
Specificity		97.22%		
PPV		80.00%		
NPV		100.00%		
Accuracy		97.50%		

In the **Table 8** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between Histopathology and DIF Tool for vasculitis outcome.

The higher value of Kappa 0.875 shows that the number of cases of DIF tool shows similar outcomes with Histopathology with 100% for present and 97.2% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of Clinical procedure against the DIF tool as gold standard tool for vasculitis outcome.

The high value of sensitivity 100% and PPV 80% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 97.22% and NPV 100% shows the higher degree of prediction of absence of disease.

Also, the higher value of 97.5% accuracy shows that Histopathology can be used as an alternate tool to predict the presence of vasculitis outcome against the DIF tool.

Table 9: Measure of agreement and prediction of CTD by clinical and DIF tools

Clinical CTD		**DIF CTD**		**Total**
		Present	**Absent**	
Present	Count	1	4	5
	%	100.0%	5.1%	6.3%
Absent	Count	0	75	75
	%	0.0%	94.9%	93.8%
Total	Count	1	79	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.319	0.000	Sig
Sensitivity		100.00%		
Specificity		94.94%		
PPV		20.00%		
NPV		100.00%		
Accuracy		95.00%		

In the **Table 9** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between clinical and DIF Tool for CTD outcome.

The moderate value of Kappa 0.319, but significant shows that the number of cases of DIF tool shows similar outcomes with clinical procedure with 100% for present and 94.9% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of clinical procedure against the DIF tool as gold standard tool for CTD outcome.

Table 10: Measure of agreement and prediction of pemphigus by HPE and DIF tools

HPE CTD		**DIF CTD**		**Total**
		Present	**Absent**	
Present	Count	1	2	3
	%	100.0%	2.5%	3.8%
Absent	Count	0	77	77
	%	0.0%	97.5%	96.3%
Total	Count	1	79	80
	%	100.0%	100.0%	100.0%
Kappa		Value	P Value	Result
		0.490	0.000	Sig
Sensitivity		96.88%		
Specificity		83.33%		
PPV		79.49%		
NPV		97.56%		
Accuracy		88.75%		

The high value of sensitivity 100% but low PPV 20% shows that the moderate degree of prediction of presence of disease by clinical procedure. Whereas higher value of

specificity 94.94% and NPV 100% shows the higher degree of prediction of absence of disease.

Although the higher value of 95% accuracy shows that the clinical procedure can be used as an alternate tool to predict the presence of CTD outcome against the DIF tool.

In the **Table 10** Cohen's Kappa is a statistical measure of agreement between two tools for categorical variables was applied which shows the significant and high degree of agreement between Histopathology and DIF Tool for CTD outcome.

The moderate value of Kappa 0.490 shows that the number of cases of DIF tool shows similar outcomes with Histopathology with 100% for present and 97.5% for absent cases.

In the measure of sensitivity and specificity, accuracy and related terms of clinical procedure against the DIF tool as gold standard tool for CTD outcome.

The high value of sensitivity 96.88% and PPV 79.49% shows that the higher degree of prediction of presence of disease by clinical procedure. Similarly higher value of specificity 83.33% and NPV 97.56% shows the higher degree of prediction of absence of disease.

Also, the higher value of 88.75% accuracy shows that Histopathology can be used as an alternate tool to predict the presence of CTD outcome against the DIF tool.

5. Discussion

The diagnosis of autoimmune dermatological conditions is based on the combined evaluation of clinical features, histopathology, and immunofluorescence studies. Accurate distinction between similar clinical entities is important for both treatment modalities and prognosis.[10,11,12] The present study reaffirms that DIF is valuable for the accurate diagnosis of autoimmune dermatological disorders of the skin. The location and pattern of deposition of immunoreactants helps in classifying various immune-mediated diseases. The negative DIF results helped to rule out the immune basis for the disease in certain patients. Total 80 patients with clinically suspected immune diseases were analysed. A total of 74 out of 80 patients were accurately diagnosed based on histopathology and DIF studies. Histopathology was positive in 73/74 (98.6%) patients and DIF was positive in 56/74 (75.6%) patients which is comparable to 73% of Inchara YK et al[1] in DIF. Sinha P et al[2] had a result of 81.8% on DIF and 95.7% on HPE, while Mittal H et al[3] had overall 92.6% on DIF. Mysorekar VV et al[4] had overall high sensitivity of 98% on DIF and conclusive HPE in 92.2%. We found that the most common age group was 31-40 years (21.3%). The male-to-female ratio was 1:1.2, which is similar to studies by Mittal H et al,[3] Sinha P et al,[2] Minz RW et al[5] and Mysorekar VV et al.[4]

In the present study, there was a very good concordance between the clinical, histological and DIF results.

The diagnostic role of DIF in Auto immune bullous disorders of the skin is well highlighted in various studies over previous years. This study reflects the mandatory role of DIF along with histopathology for proper diagnosis of bullous conditions of the skin. Auto immune bullous disorders was the most common entity in 82.4% (61/74) of patients among the immune-mediated skin disorders and 76.25% of the total patients (n= 80) present studied. HPE was positive in 60/61 (98.3%) and DIF was positive in 48/61 (78.6%). Comparable results of DIF positivity in AIBD were seen in other studies by Inchara YK et al[1] 106 (73%), Kumar SS et al[6] (80%), Minz RW et al[5] 1 (70%), Deepti S et al[7] (70%) Sinha P et al[5] (89.8%), Mittal H et al (88%), Dhanabalan RT et al[8] (89.74%) and Raj KA et al[9] (90.09%). Mysorekar VV et al[4] had comparatively high DIF positivity for Auto immune bullous disorder with 97.5%.

Pemphigus Vulgaris makes the majority of diseases in the whole study (40%) and 80% of the pemphigus group. 100% cases of PV were positive on HPE, 81.25% (26/32) on DIF. 100% cases showed granular deposition in Intercellualar spaces was seen with 76.9% having lace like and 23.1% having fishnet pattern. IgG deposition alone was seen in maximum cases (61.5%).

8.7% cases were of Pemphigus Foliaceous (n=7) in total cases studied and made 17.5% of pemphigus group. 6/7 (87.5%) cases of PF were positive on HPE and DIF. One case of PF was negative on HPE but was positive on DIF. 100% cases showed granular deposition in intercellular spaces. IgG and C3 deposition was seen in maximum cases (83.3%).

In our present study there is only one case of clinically suspected IgA pemphigus (1.25%) which was consistent on HPE but DIF gave negative results. In our study Bullous Pemphigoid is the 2nd most common disease making 15% of total (12/80) and 16.2% of immune mediated (12/74). DIF was positive in 83.3% (10/12) cases. 100% cases showed linear depositon of Igs in DEJ. Maximum (50%) cases had IgG deposition alone.[13]

In our study, Dermatitis Herpetiformis is 6.25% of total cases studied. 100% cases were consistent on HPE, while 60% showed DIF positivity. 100% cases showed linear deposition of IgA in papillary dermis.

1 case (1.25%) of Pemphigoid Gestationis was seen in our study which was consistent on HPE but negative on DIF. 1 case (1.25%) of Mucous Membrane Pemphigoid was consistent on both HPE and DIF. On DIF IgG deposition was seen in intercellular spaces with fishnet pattern.[14]

In our study there were 2 cases (2.5%) of clinically suspected Epidermolysis Bullousa Acquisita and both were consistent on HPE while 50% case was positive on DIF with granular deposition of IgM in Dermo-epidermal junction.

Sensitivity of DIF in relation to HPE in bullous group is 100% with 86.15% of specificity, PPV 62.5%, NPV 100%, kappa 0.70. Sensitivity of DIF in relation to HPE in bullous group is 100% with specificity of 90.77%, PPV 71.43%, NPV 100% and kappa of 0.78. Sensitivity of DIF in relation to HPE in CTD group is 100% with 94.9% of specificity, PPV 20%, NPV 100%, kappa 0.319. Sensitivity of DIF in relation to HPE in CTD group is 96.88% with specificity of 83.33%, PPV 79.49%, NPV 97.56% and kappa of 0.49.

In our study, among Vasculitis group only IgA vasculitis were present. No other types of vasculitis were present. After BP, IgA vasculitis is the 3rd most common disease studied (12.5%) in our study. All the cases were consistent with HPE while only 80% revealed positive results on DIF. 100% cases had IgA deposition in granular pattern in superficial dermal vessels (62.5%) and around perivascular region (37.5%).[15]

Sensitivity of DIF in relation to HPE in IgA vasculitis group is 100% with 95% of specificity, PPV 72.7%, NPV 100%, kappa 0.82. Sensitivity of DIF in relation to HPE in IgA Vasculitis group is 100% with specificity of 97.2%, PPV 80%, NPV 100% and kappa of 0.87.

6. Conclusion

DIF is a useful supplement in the accurate diagnosis of autoimmune mediated skin disorders. A negative DIF result helps to rule out the immunological cause of the skin disorder. In cases with inconclusive clinical features and histopathology, DIF acts as a confirmatory as well as diagnostic in immune mediated disorders. A combined analysis of clinical features, histopathology and DIF is required for an accurate diagnosis. A combination of investigations by a direct immunofluorescence and histological examination, remains the gold standard in the diagnosis of immune mediated disorders.

7. Source of Funding

None.

8. Conflict of Interest

None.

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