The utility of immunofluorescence in diagnosing dermatological lesions and its correlation with clinical and histopathological diagnosis in a tertiary health care setup

Rajeswari Thivy Dhanabalan, Sindhuja Ramalingam¹, Shifa Seyed Ibrahim², Bhuvaneswari M. Ganesan³, Lavanya Krishnagiri Balan⁴, Pavithra Thandavarayan⁴, Sakti Sankari Shanmuganathan⁴

Department of Pathology, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kanchipuram, ¹Department of Pathology, Tirunelveli Medical College, Tirunelveli, ²Department of Pathology, Madurai Medical College, Madurai, ³Department of Pathology, Coimbatore Medical College, ⁴Department of Pathology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India

A B S T R A C T

Context: Immunofluorescence (IF) is an immunological technique which is used for identifying antibodies against antigens that are bound to tissues or those circulating in body fluids. This study is an attempt to evaluate the dermatological lesions such as noninfectious vesiculobullous lesions, connective tissue disorders, vasculitis, and lichen planus using histopathology techniques and direct IF (DIF) studies. Furthermore, an attempt was made to evaluate the diagnostic utility of the IF technique in our hospital. Aims: (1) To determine the utility of IF in the diagnosis of skin lesions. (2) To correlate IF with histopathological findings. Settings and Design: A cross-sectional, observational study. Subjects and Methods: The present study was conducted in the Pathology Department, Coimbatore Medical College, Coimbatore, from August 2012 to August 2013. Fifty cases received during the period were included in our study. Two skin biopsies for each case - one in formalin for routine histopathological examination and other in phosphate buffer solution for IF studies - were received. Statistical Analysis Used: Fisher's exact value, mean, and coefficient of mean were calculated. Results: The lesions were diagnosed by both light microscopy and IF study. Out of fifty cases, 42 cases were positive with the IF technique and the overall sensitivity of IF was 84% in our study. When histopathological and IF findings were correlated, statistically significant two-tailed \( P = 0.0130 \) was obtained. Both light microscopic findings and IF findings complemented each other in our study. IF proved to be a necessary supplementary technique to both clinical and histopathological examinations, especially in cases of diagnostic dilemmas. Conclusions: IF is a rapid diagnostic procedure. IF was useful in early diagnosis, treatment, and monitoring of various disorders in our study. Positive and negative DIF had prognostic significance in certain cases. Clinicopathological correlation along with DIF studies proved to be a powerful tool in definite diagnosis of the skin lesions.

Key words: Autoimmune, connective tissue disorders, direct immunofluorescence, lichen planus, vasculitis, vesiculobullous

INTRODUCTION

Skin is the largest organ of the body. As it is the only external organ, skin is examined first. It is a window
to one’s well-being. Skin is also an easily accessible site for biopsy. Not only well-defined de novo skin lesions but also many systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and vasculitis can also be easily diagnosed by skin biopsy.

Although histopathology is the gold standard diagnosis for most dermatological lesions, all lesions cannot be diagnosed by histopathology alone. Ancillary techniques such as Tzanck smear, immunofluorescence (IF), serology, and recent advancements such as immunoelectron microscopy and immunoblotting have refined the diagnosis. This study was an attempt to evaluate the dermatological lesions such as vesiculobullous lesions, connective tissue disorders, vasculitis, and lichen planus (LP) using both histopathology and direct IF (DIF) studies and to assess the diagnostic potential of the IF studies.

SUBJECTS AND METHODS

The present study was conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore, from August 2012 to August 2013. All newly diagnosed cases of immunobullous disorders, connective diseases, vasculitis, and LP irrespective of age, sex, and associated disorders were included in the study. Patients treated with steroids in the past 3 weeks and specimens devoid of clinical details and histopathological specimens that were not fixed with formalin were excluded from our study.

Fifty cases with skin lesions were included in our study. The clinical details were collected from the patients. Two punch biopsies of early skin lesions were collected for each case. In case of autoimmune blistering disease (AIBD), perilesional biopsies were taken. In case of connective tissue disorders, LP and vasculitis, lesional biopsies were taken. One was sent in formalin for routine histopathological examination. These specimens were processed routinely and sections were cut and stained routinely using hematoxylin and eosin. The other was sent in phosphate buffer for IF studies. Four-micron-sized sections were cut using cryostat and tissues were transferred to six slides. One slide was used for rapid hematoxylin and eosin staining. The remaining slides were stained with fluorescein isothiocyanate-labeled immunoglobulin G (IgG), IgA, IgM, C3, and fibrinogen (in 1:25 dilution) for an hour and then viewed under a fluorescent microscope.

The site (whether dermoepidermal junction [DEJ] or the intercellular spaces [ICSs] of epidermis), nature (IgG, IgA, IgM, C3, fibrinogen), and pattern (linear or granular) of staining were noted.

RESULTS

Our study was an observational one for a period of 1 year from August 2012 to August 2013. During the study period, 8576 specimens were received, of which 264 were skin biopsies. Our study group comprised fifty patients with clinically suspected and newly diagnosed noninfectious vesiculobullous lesions, connective tissue disorders and vasculitis.

In our study group of 50, 26 were men and 24 were women with a sex ratio of 1.08:1 [Table 1]. Most cases were in the age group 41–50 years in both the sexes [Table 1].

Vesiculobullous disorders constituted 0.45% of the total specimens and 14% of all skin specimens [Table 2].

| Table 1: Age- and sex-wise distribution of cases |
|-----------------------------------------------|
| Age group | Sex, n (%) | Total (%) |
|-----------|------------|-----------|
|           | Male       | Female    |          |
| 0-10      | 0          | 0         | 0        |
| 11-20     | 1 (2)      | 4 (8)     | 5 (10)   |
| 21-30     | 1 (2)      | 2 (4)     | 3 (6)    |
| 31-40     | 2 (4)      | 4 (8)     | 6 (12)   |
| 41-50     | 13 (26)    | 8 (16)    | 21 (42)  |
| 51-60     | 4 (8)      | 3 (6)     | 7 (14)   |
| 61-70     | 5 (10)     | 3 (6)     | 8 (16)   |
| >70       | 0          | 0         | 0        |
| Total     | 26 (52)    | 24 (48)   | 50 (100) |

| Table 2: Spectrum of distribution of diseases |
|---------------------------------------------|
| Final diagnosis | Cases | Percentage |
|-----------------|-------|------------|
| PV              | 8     | 16         |
| PF              | 5     | 10         |
| IgAP            | 2     | 4          |
| PNP             | 1     | 2          |
| SCPD            | 1     | 2          |
| BP              | 18    | 36         |
| DH              | 2     | 4          |
| LIBD            | 2     | 4          |
| SLE             | 2     | 4          |
| DLE             | 3     | 6          |
| LCV             | 3     | 6          |
| PSS             | 1     | 2          |
| EED             | 1     | 2          |
| LP              | 1     | 2          |
| Total           | 50    | 100        |

PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus
Among the vesiculobullous disorders, bullous pemphigoid (BP) was most frequently diagnosed in our study (18 cases) [Figures 1 and 2]. Histopathological examination confirmed the diagnosis of pemphigus in only 14 cases [Figure 3]. A case each of pemphigus vulgaris (PV) and pemphigus foliaceus (PF) did not show acantholytic cells.\textsuperscript{[3]} Out of the 39 AIBD cases, DIF positivity was seen in 34 cases [Table 3]. PV, PF, and IgA pemphigus showed lace-like pattern in the ICS of the epidermis [Figure 4 and Table 4]. The sensitivity of DIF in diagnosing AIBD was calculated as 89.74\% in our study.

Among the connective tissue disorders, three cases of discoid lupus erythematosus (DLE) [Figure 5], two of SLE [Figure 6], and one case of progressive systemic sclerosis (PSS) were diagnosed. However, DIF positivity was seen in five cases and one case was DIF negative (PSS) [Table 3]. The sensitivity of DIF in diagnosing connective tissue disorder was 83.33\% in our study.

In the vasculitis category, leukocytoclastic vasculitis (LCV) was diagnosed in three cases and erythema elevatum diutinum in one case. DIF positivity was seen in only two of four histopathologically confirmed cases [Figures 7, 8 and Table 3]. The sensitivity of DIF in diagnosing vasculitis was 50\% in our study.

One case of LP was included in our study. DIF was positive and the sensitivity was 100\% [Table 3].

The discordant results observed between clinical, histopathological, and IF findings are tabulated in Table 5. In all the discordant cases, IF findings were taken to arrive at the final diagnosis in case of positive IF results.
Table 3: Direct immunofluorescence staining in individual cases

| Final diagnosis | IgG | IgA | IgM | C3 | Fibrinogen | IgG, C3 | All | Negative |
|-----------------|-----|-----|-----|----|------------|---------|-----|----------|
| PV              | 4   |     |     | 3  | 0          | 1       |     |          |
| PF              | 2   |     |     | 2  | 1          |         |     |          |
| IgAP            |     |     | 2   |    |            |         |     |          |
| PNP             |     |     |     |    |            | 1       |     |          |
| SCPD            |     |     |     |    |            |         |     |          |
| BP              | 1   |     | 4   | 11 | 2          |         |     |          |
| DH              |     |     | 2   |    |            |         |     |          |
| LIBD            |     |     |     | 2  |            |         |     |          |
| SLE             |     | 1   |     |    | 1          | 2       |     |          |
| DLE             |     |     |     |    |            | 1       |     |          |
| PSS             |     |     |     |    | 1          | 1       |     |          |
| LCV             |     | 1   |     |    |            | 1       |     |          |
| EED             |     |     |     |    | 1          | 1       |     |          |
| LP              |     |     |     |    | 1          | 1       |     |          |

DIF: Direct immunofluorescence, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus, ICS: Intercellular space, Ig: Immunoglobulin

Table 4: Pattern of direct immunofluorescence staining

| Final diagnosis | Pattern of DIF |
|-----------------|---------------|
|                 | ICS | DEJ | Dermal vessels | Negative |
|                 | Linear | Granular | Shaggy |         |
| PV              | 7     |     |               | 1        |
| PF              | 5     |     |               |          |
| IgAP            | 2     |     |               |          |
| PNP             |       | 1   |               |          |
| SCPD            |       |     |               |          |
| BP              | 16    | 0   |               | 2        |
| DH              | 2     |     |               |          |
| LIBD            | 2     | 2   |               | 1        |
| SLE             | 2     |     |               |          |
| DLE             | 2     |     |               |          |
| PSS             |       |     |               |          |
| LCV             | 2     |     |               | 1        |
| EED             |       | 1   |               |          |
| LP              |       | 1   |               |          |

DIF: Direct immunofluorescence, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus, ICS: Intercellular space, DEJ: Dermoepidermal junction

Figure 5: Granular immunoglobulin M deposits along the dermoepidermal junction - Discoid lupus erythematosus (black arrow, immunofluorescence)

Figure 6: Granular positivity of C3 along the dermoepidermal junction - Systemic lupus erythematosus (black arrow, immunofluorescence)
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Statistics
When histopathological and IF findings were correlated, the two-tailed $P = 0.0130$ which was statistically significant [Table 6]. The mean value calculated was 0.50 and the 95% confidence interval of this difference ranged from 0.12 to 0.88. Hence complementary nature of histopathology and IF was proved.

DISCUSSION

Autoimmune vesiculobullous disorders
Vesiculobullous disorders include a wide range of dermatoses, which are characterized by protean manifestations.[1] These disorders have varying etiology, prognostic factors, but united by the formation of blisters either within or beneath the epidermis. These lesions have a striking impact not only on the affected individuals but also for their family members. Histopathological evaluation of vesiculobullous lesions involves the systematic analysis, including the plane of separation of blisters and also the nature of inflammatory infiltrates. These have many differentials which can be sorted out using IF.

Pemphigus is a fatal disease affecting skin and mucosa. Pemphigus was first used by Hippocrates to describe pemphigoid fever as pemphigoides pyeortoi. In 1760, De-Sauvages coined the term pemphigus from *pemphix* meaning pustule in Greek.[4] Lever in 1953 described BP and differentiated it from pemphigus.[5-7] This is of great historical significance as BP has a much better prognosis than pemphigus which is commonly fatal.

The most common age group in our study was fourth to fifth decades. This finding was in accordance with Kabir et al., Kambi et al., and Rizvi and Sadiq studies.[8-10] Male:female ratio [1.08:1] was approximately equal in this study, which was similar to Kabir et al. and Kambi et al. studies.[8,9] However, Anuradha et al. and Rizvi and Sadiq’s studies showed a male predominance.[10,11]

From Table 1, BP (46.5%) constituted the majority followed by PV (20.5%) in the present study. This was in accordance with Kabir et al. and Monsef et al. studies.[8,12] Most of the other studies had PV as the predominant disorder. This discordance could be due to variation in the geographical distribution of vesiculobullous lesions. In BP, subepidermal bulla was seen in 77% patients. Other BP cases revealed only numerous eosinophils.

| Clinical diagnosis | HPE diagnosis | If diagnosis | Final diagnosis |
|-------------------|--------------|--------------|----------------|
| BP                | LIBD/DH      | LIBD         | LIBD           |
| PV                | PF           | PF           | PF             |
| DH                | BP           | BP           | BP             |
| SCPD              | IgAP/PF/SCPD | IgAP         | IgAP           |
| DH                | PF           | PF           | PF             |
| IgAP              | IgAP/PF/SCPD | IgAP         | IgAP           |
| SCPD              | IgAP/SCPD    | SCPD         | SCPD           |
| LIBD              | BP/LIBD      | LIBD         | LIBD           |
| LIBD              | BP           | BP           | BP             |
| Vasculitis        | LCV          | Negative     | LCV            |
| EED               | EED          | Negative     | EED            |
| BP                | BP           | Negative     | BP             |
| BP                | Negative     | BP            |
| PSS               | Negative     | PSS           |
| PNP               | Negative     | PNP           |
| PV                | Negative     | PV            |

PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus

Figure 7: Destruction of the blood vessels - Leukocytoclastic vasculitis (H and E, x40)

Figure 8: Fibrinogen positivity in the vessels - Leukocytoclastic vasculitis (immunofluorescence)
in the papillary dermis. This could be due to biopsy of early lesions as prodromal stage shows nonspecific changes such as edematous dermis with abundant eosinophilic infiltration.\textsuperscript{[13]}

The most common intraepidermal disorder in our study was PV. This finding was similar to Kabir et al. studies.\textsuperscript{[8]} Suprabasal bulla was seen in 100\% of cases and this was in accordance with Kambi et al. and Leena et al.’s studies.\textsuperscript{[9,14]} In our study, the incidence of PF was 12.82\%, which was similar as that of Kambi et al. study.\textsuperscript{[9]} All pemphigus lesions show staining in the ICS of epidermis in a fish net-like pattern; hence, they should be interpreted along with histological findings. IgA pemphigus and linear IgA bullous dermatoses can be diagnosed confidently only by immunofluorescent studies. Two cases of dermatitis herpetiformis were seen in this study. Shave biopsy is preferred in dermatitis herpetiformis as IgA is only focally positive in this case. Due to this, DIF should be done at least twice in shave biopsy specimens before awarding a negative DIF diagnosis in DH according to Sontheimer et al.'s study.\textsuperscript{[15]}

In the present study, lesions such as subcorneal pustular dermatoses (SCPDs) and paraneoplastic pemphigus (PNP) which was not seen in other studies were seen. The five cases were DIF negative in our study which includes two cases of BP, a case of PNP, a case of PV, and a case of SCPD. Grolleau-Rochiccioli et al. in their study had mentioned three BP cases that showed negative DIF results.\textsuperscript{[16]} This could be due to subthreshold immunoreactants. DIF negativity or delayed detection with IF technique had been observed in PNP.\textsuperscript{[17]} Mysorekar et al. and Buch et al. in their study had mentioned about DIF negative PV cases.\textsuperscript{[18,19]} Buch et al. in their study had mentioned that IF negative PV meant disease remission.\textsuperscript{[19]} DIF is normally negative in SCPD as it is a nonimmune bullous lesion associated with IgA gammopathy.\textsuperscript{[20]} Our DIF findings in vesiculobullous lesions were in accordance with the Wallach D's study.\textsuperscript{[20]}

**Connective tissue disorders**

Connective tissue disorders have multisystemic involvement. They have a remarkable diversity, but at the same time, they are clinically similar in many aspects.\textsuperscript{[20]} Cutaneous involvement is an important feature in these diseases. Therefore, skin biopsies play an important role in the classification of these diseases.\textsuperscript{[20]} DIF is helpful in confirming the diagnosis of LE when suspected histologically, clinically, or both. It also helps in classifying the various types of LE as the morphology, site and frequency of immunoreactants deposition vary with various subtypes of LE.

DIF positivity in SLE usually varies with biopsy site, morphology of lesions, treatment profile, and disease activity. In our study, all LE cases were IF positive. Anuradha et al. studied four LE cases, of which two belonged to SLE and both showed DIF negativity.\textsuperscript{[11]} In Kabir et al. study, seven cases of SLE were included and all were DIF positive which coincides with our study.\textsuperscript{[21]} According to Mutasim and Adams, DIF was more commonly seen in the sun unexposed, oldest, and untreated DLE lesion. Moreover, positivity of combined IgM and IgG deposit was in favor of DLE.\textsuperscript{[22]} In Kabir et al. study, of the 3 DLE cases, two showed DIF positivity.\textsuperscript{[21]} Sandra et al. study which included 16 LE cases (13 females and 3 males), they had concluded that if DIF was positive in nonlesional skin, there was a greater chance of developing SLE. Hence, those patients required close follow-up.\textsuperscript{[23]} One case of PSS was included in our study. Histopathological examination revealed findings consistent with scleroderma. However, DIF studies were negative. According to Mutasim and Adams, DIF was negative or nonspecific in PSS cases. PSS patients having positive DIF finding seem to have more often overlapping features of SLE, vasculitis, or dermatomyositis.\textsuperscript{[22]} Winkelmann et al. had reported ten PSS cases that were DIF negative.\textsuperscript{[4]} Kulthanan et al. have mentioned about the inconsistent IF results in PSS cases.\textsuperscript{[3]} Hence, in PSS, clinical examination and histopathology play a more important role than DIF. From this, we conclude that a combination of histopathology, serology and immunology with the background of clinical examination is essential for definite diagnosis of connective tissue disorders.

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**Table 6: Clinical, histopathological and direct immunofluorescence correlation**

| Diagnostic mode | PV | PF | IgAP | PNP | SCPD | BP | DH | LIBD | SLE | DLE | PSS | LCV | EED | LP | Total |
|-----------------|----|----|------|-----|------|----|-----|------|-----|-----|-----|-----|-----|-----|-----|-------|
| Clinical        | 9  | 3  | 1    | 1   | 2    | 17 | 3   | 3    | 2   | 3   | 1   | 3   | 1   | 1   | 50    |
| HP              | 8  | 5  | 3    | 1   | 2    | 14 | 2   | 2    | 2   | 3   | 1   | 3   | 1   | 1   | 48    |
| DIF            | 7  | 4  | 2    | -   | 1    | 16 | 2   | 2    | 2   | 3   | 1   | 3   | -   | 1   | 42    |
| Total case diagnosed | 8  | 5  | 2    | 1   | 1    | 18 | 2   | 2    | 2   | 3   | 1   | 3   | 1   | 1   | 50    |

DIF: Direct immunofluorescence, HP: Histopathological, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatoses, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus.
Vasculitis

Vasculitis refers to necrosis and inflammation of arteries, veins, or both. For confirmation of LCV and Henoch–Schönlein purpura, IF is very useful. In our study, four cases of vasculitis were noted. In Khetan et al.'s study of 61 cases, female predominance was noted unlike our study. In Nandeesh and Tirumalae's study of 198 patients also, a female predominance was noted unlike our study. In vasculitis, the immunoreactants are commonly deposited in the postcapillary venules in the papillary dermis. The pattern could be either fibrillary or granular. It could also be deposited either in the intravascular or extravascular space. In this study, staining was seen in intravascular space of vessels. Henoch–Schönlein purpura was common in other studies, but it was not seen in our study. In Gupta et al. study, of fifty cases, LCV was the predominant lesion akin to our study, histopathology gave a conclusive diagnosis in 42 cases (84%) and DIF was positive in 74% cases. However, similar to this study, in Nandeesh and Tirumalae's study, LCV was the predominant lesion and it had DIF positivity of 54%. However, the overall DIF positivity was 62% in their study. Like our study, LCV was the predominant lesion in Khetan et al.'s study (66%). But DIF positivity was seen in 39% and this was lower than our study. Leelavathi et al. (85 cases) revealed DIF positivity in 13% cases. DIF provides only supporting evidence in vasculitis. A definite diagnosis of vasculitis should be given after considering clinical, histopathological, laboratory investigations and IF studies. According to Mutasim and Adams, the sensitivity of DIF in vasculitis is limited by both duration and site. Lower limb lesions give false positive DIF results and lesions aged more than a day tend to give false negative test.

Lichen planus

In Kabir et al. study, 7 of 12 LP cases were DIF positive showing irregular immunoreactant deposit along the DEJ, constituting 58% positivity. In Anuradha et al. study, all six LP cases showed fibrinogen positivity along the DEJ (100%). The pattern was linear, granular, or shaggy. Sandra et al. showed fibrinogen positivity in all their 18 LP cases. According to Mutasim and Adams, DIF was essential only when histopathological and clinical findings were inconclusive and was not necessary in LP as most cases were diagnosed by clinical examination alone.

CONCLUSIONS

IF plays an ancillary role in the definite diagnosis of vesiculobullous disorders, connective tissue disorders and vasculitis and should be interpreted along with clinical features, laboratory workup and histopathology findings.
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