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HISTOPATHOLOGICAL AND IMMUNOFLUORESCENT STUDIES IN CERTAIN SKIN DISORDERS

Rekha S[1], Vineet Kumar[2], Vanita Kumar[3], R D Mehta[4]
[1,2] Senior Resident, [3] Professor [4] Senior Professor
[1,2,4] Department of Dermatology, Venereology and Leprosy, S.P. Medical College and A.G. of Hospitals Bikaner, Rajasthan
[3] Department of Pathology, S.P. Medical College and A.G. of Hospitals Bikaner, Rajasthan

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Address for Correspondence: Vineet Kumar
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Abstract
Background: This study was an attempt to evaluate the role of direct immunofluorescent technique to demonstrate the immunoglobulins in certain skin disorders, which are likely to have immunological mechanism in their pathogenesis.
Methods: This study was carried out in department of Pathology, Sardar Patel Medical College & Associated group of Hospitals, Bikaner. This study was hospital based study on skin biopsy specimen recieved in the department of Pathology during the study period.
Results: On immunofluorescent studies of these skin biopsies, 12 out of 16 cases of pemphigus were positive for fluorescence was intracellular area in epidermis. In lichen planus 5 out of 7 cases were positive for fluorescence and commonest site was dermo-epideremal junction. In dermatis-herpatiformis 2 out of 3cases were positive for fluorescence and commonest site was dermo-epideremal junction. IgG was commonest type of immunoglobulin's demonstrate in 12 out of 16 cases of pemphigus followed by IgM (5 cases), IgA(1cases). In lichen planus IgM was commonest type of immunoglobulin's demonstrate in 5 out of 7 cases of pemphigus followed by IgG (3 cases), IgA(1cases). In dermatitis herpatiformis IgA was commonest type of immunoglobulin's demonstrate in 2 out of 3 cases of pemphigus followed by IgM (1 cases).
Conclusion: We conclude that the demonstration of immunoglobulins in skin biopsies by direct fluorescent technique is a quite useful adjunct in diagnostic confirmation of pemphigus, lichen planus and dermatitis herpetiformis.
Keywords: Direct immunofluorescence, Pemphigus, Histopathology.

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.[1] 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.[2]

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 role of direct immunofluorescent technique to demonstrate the immunoglobulins in certain skin disorders, which are likely to have immunological mechanism in their pathogenesis.

Material and methods

This study was carried out in department of Pathology, Sardar Patel Medical College & Associated group of Hospitals, Bikaner. This study was hospital based study on skin biopsy specimen recieved in the department of Pathology during the study period.
Cases were divided in to four groups
Group-I. Pemphigus – 16 cases
Group-II. Lichen planus- 7 cases
Group-III. Dermatitis herpetiformis- 3 cases
Group-IV. Controls- 10 cases (Skin biopsies taken patients other than dermatological disease)
Exclusion Criteria: 1) Specimen without clinical detail 2) Autolysed specimen.

Clinical data was obtained from hospital record and requisition submitted along with tissue specimen received in the department. Tissue bits were routinely processed. A section was made from paraffin blocks and was stained with H&E stain. Special stains were done whenever necessary. Specimen obtained from eligible study population was examined microscopically.

Data Analysis

To collect required information from eligible patients a pre-structured pre-tested proforma was used. For data analysis Microsoft excel and statistical software SPSS was used and data was analyzed with the help of frequencies, figures, proportions, measures of central tendency and appropriate statistical test.

Results

Table 1: Age wise distribution

Age group (Yrs)	Control	Pemphigus	Lichen planus	Dermatitis herpetiformis
<10	1(10.00%)			
11-20		1(6.25%)	1(14.28%)	1(33.33%)
21-30	2(20.00%)	7(43.75%)	2(28.57%)	2(66.67%)
31-40	3(30.00%)	3(18.75%)	3(42.87%)	
41-50	1(10.00%)	3(18.75%)	1(14.28%)	
51-60	2(20.00%)	2(12.50%)		
61-70	1(10.00%)			
Total	10(100.00%)	16(100.00%)	7(100.00%)	3(100.00%)

The age range was 5 to 65 years in controls, 15 to 60 years in pemphigus, 19 to 45 years in lichen planus and 18 to 30 years in dermatitis herpatiformis.

Table 2: Sex wise distribution

Sex	Control	Pemphigus	Lichen planus	Dermatitis herpetiformis
Male	5(50.00%)	7(43.75%)	3(42.87%)	2(66.67%)
Female	5(50.00%)	9(56.25%)	4(57.13%)	2(66.67%)
Total	10(100.00%)	16(100.00%)	7(100.00%)	3(100.00%)

Lichen planus & pemphigus were more in female.

Table 3: distribution of histological features in pemphigus

Histological features		Number	Percentage
Bullae		16	100.00
Acantholysis		16	100.00
Inflammatory reaction		11	68.75
Inflammatory cells	Polymorphs	8	50.00
	Lymphocytes	7	43.75
	Macrophages	5	31.25
	Eosinophil	2	12.50

Table 4: distribution of histological features in lichen planus

Histological features		Number	Percentage
Basement membrane haziness		7	100.00
Acantholysis		7	100.00
Inflammatory reaction		7	100.00
Inflammatory cells	Lymphocytes	7	100.00
	Macrophages	7	100.00
Hyperkeratosis		5	71.43
Parakeratosis		2	57.14

Table 5: distribution of histological features in dermatitis herpetiformis

Histological features		Number	Percentage
Microabscess		3	100.00
Bullae		1	33.33
Inflammatory reaction		3	100.00
Inflammatory cells	Polymorphs	3	100.00
	Lymphocytes	3	100.00
	Macrophages	3	100.00
	Eosinophil	3	100.00

Table 6: Site of fluorescence in different disease

Site of fluorescence	Pemphigus	Lichen planus	Dermatitis herpetiformis
Intra cellular in epidermis	12(75.00%)	-	-
Dermo-epidermal junction	2(12.50%)	5(71.43%)	2(66.67%)

On immunofluorescent studies of these skin biopsies, 12 out of 16 cases of pemphigus were positive for fluorescence was intracellular area in epidermis. In lichen planus 5 out of 7 cases were positive for fluorescence and commonest site was dermo-epideremal junction. In dermatis-herpatiformis 2 out of 3cases were positive for fluorescence and commonest site was dermo-epideremal junction.

Table 7: Distribution of different immunoglobulins in different disease

Immunoglobulin	Pemphigus	Lichen planus	Dermatitis herpetiformis
Polyvalent	12(75.00%)	5(71.43%)	2(66.67%)
IgG	12(75.00%)	3(42.86%)	1(33.33%)
IgM	5(31.25%)	5(71.43%)	-
IgA	1(6.25%)	1(14.29%)	2(66.67%)
IgG,IgM,IgA,P	1(6.25%)	1(14.29%)	-
IgG,IgM,P	4(25.00%)	2(28.57%)	-
IgG ,P	7(43.75%)	-	-
IgM,P	-	2(28.57%)	-
IgG,IgA,P	-	-	1(33.33%)
IgA,P	-	-	1(33.33%)
IgA,IgM,P	-	-	-

IgG was commonest type of immunoglobulin's demonstrate in 12 out of 16 cases of pemphigus followed by IgM (5 cases), IgA(1cases). In lichen planus IgM was commonest type of immunoglobulin's demonstrate in 5 out of 7 cases of pemphigus followed by IgG (3 cases), IgA(1cases). In dermatitis herpatiformis IgA was commonest type of immunoglobulin's demonstrate in 2 out of 3 cases of pemphigus followed by IgM (1 cases).

Discussion

Direct Immunofluorescence is a single-step procedure that demonstrates the antibodies bound *in vivo* to antigens in the skin or mucosae.[3] A 3–4 mm punch biopsy is optimum for DIF study; to get a maximum yield, it is important to take biopsy from an appropriate site. An ideal site of biopsy in all autoimmune blistering diseases (AIBDs) is the perilesional skin; DIF microscopy may be negative if the biopsy is taken from lesional skin as the *in vivo*-bound autoantibodies are consumed by the inflammation. In cases of vasculitis, a freshly erupted purpuric spot in the most proximal part of the limb is preferred as IgA deposits may undergo degradation in older lesions. Lesional biopsy is also preferred in cases of discoid lupus erythematosus (DLE), amyloidosis, and lichen planus (LP). In systemic lupus erythematosus (SLE) and other connective tissue diseases, two or three biopsies are taken (lesional/sun exposed and nonlesional/sun protected skin). In porphyria cutanea tarda (PCT), biopsy should be taken preferably from the lesional skin; a second

biopsy from the perilesional, normal skin may be considered, especially if the patient has an intact blister.

It is important to avoid contamination of biopsy samples with formalin which render the skin specimen unsuitable for DIF study. Common scenario where formalin contamination of biopsy sample occurs is when two biopsies are planned for routine histopathology and DIF. In a situation like this, the first biopsy is taken for histopathology and the same forceps are used to pick up the second biopsy (for DIF) specimen leading to formalin contamination. Therefore, we advise, when two biopsies are planned, the first biopsy should always be taken for DIF.

In the present study, there was a very good concordance between the histological and DIF results. The overall results of DIF in immune-mediated skin disorders were very good. DIF was helpful in making an accurate diagnosis when the histology were not typical, and for confirmation of the diagnosis in all cases where the clinical features and histology were typical. In many situations, a negative DIF result was also important since it helped to exclude an immune basis for the disease, even though it could not provide a precise diagnosis. There were no false-positive DIF results.

In our study demonstration of immunoglobulins especially IgG and complement in the intercellular space by DIF is a very reliable diagnostic test for pemphigus, it becomes positive early at the onset and remains positive for a long period after clinical remission.

The role of complement in the pathogenesis of pemphigus is controversial, since antibody alone can induce acantholysis in vitro. [3,4] Although intercellular complement is reported to be mostly present in the involved skin,[5] it may also be demonstrated in the uninvolved skin. Its presence is thus a marker of the disease activity, the skin becomes negative for complement during remission.[6]

Conclusion

We conclude that the demonstration of immunoglobulins in skin biopsies by direct fluorescent technique is a quite useful adjunct in diagnostic confirmation of pemphigus, lichen planus and dermatitis herpetiformis.

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