Direct Immunofluorescence Test of Skin Biopsy Samples – Results of 204 Cases

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Direct immunofluorescence (DIF) test of skin and renal biopsy specimens is being done on regular basis at the Pathology Department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka since July 2000. A total of 510 specimens were received for this test till 3rd March 2002, of which 204 (Male=76, Female = 128) were skin biopsy samples. Maximum number of patients came from 11-20 year age group. Clinically bullous diseases were suspected in 96 cases of which 39 correlated with histological and DIF diagnoses (pemphigus = 15, bullous pemphigoid = 12, dermatitis herpetiformis = 5, linear IgA dermatosis = 3, erythema multiforme = 3 and epidermolysis bullosa = 1). Remaining 57 cases were diagnosed as bullous disorders of other groups (n = 20) or as non-blistering dermatoses (n = 37). Five linear IgA dermatosis cases were detected of which two were not suspected clinically. Non-bullous disorders diagnosed by DIF included lichen planus (12), lupus erythematosus (9), vasculitis (6), scleroderma (3) and a small number of other disorders (DIF positive). DIF technique helped in segregating disorders, which do not show deposits of immunoglobulin or complement. Thus DIF technique combined with routine histology has proved a useful technique in distinguishing bullous diseases and confirming connective tissue and other skin disorder.

[Dinajpur Med Col J 2009 Jan; 2 (1):8-12]

Key words: Immunofluorescence, skin, biopsy

Introduction

Clinical examination of skin lesions provides to the dermatologist the gross morphological findings upon which a differential diagnosis can be made. However, histopathologic examination is sometimes needed for definitive diagnosis. Some skin diseases are immune mediated and immunopathologic patterns are disease specific and are of diagnostic importance. Other patterns are less specific and are of diagnostic value only when combined with clinical features and other laboratory findings.[1] Direct immunofluoresence (DIF) test of skin and renal biopsy specimen is being done on regular basis at the Department of pathology of BSMMU, Dhaka since July 2000. A total of 510 specimens were received for histological and immunofluoresence study till 3rd March, 2002. Out of 510 specimens 306 were kidney samples and 204 were skin. A retrospective study was carried out to evaluate the role of direct immunofluorescence (DIF) technique: in categorizing the bullous skin diseases correctly, in confirming immune mediated connective tissue and other skin disorders and in segregating non-immune skin disorders.

Methods

Skin biopsy samples were collected from the Dermatology Department of BSMMU and other hospitals and clinics of Dhaka City. The patients were either of bullous disorder or suspected cases of immune mediated skin disorders. Two samples were taken from each

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patient, lesional and perilesional. Lesional samples were collected in 10% formalin for routine higtopathological examination and perilesional skin samples in phosphate-buffered saline (PBS) or normal saline and immediately frozen for DIF examination. The DIF sample are properly oriented in optimum cutting temperature (OCT, ThermoShandon,USA) compound and frozen in the cryostat at –20ºC. Sections were prepared at 4-6 micron thickness and mounted on glass slides. The sections were air dried and washed in PBS, for 10 minute with three changes. After washing excess PBS was removed and one drop of appropriately diluted commercially prepared fluorescein-isothiocyanate (FITC) labelled rabbit anti-humam IgG, IgM, IgA, C3 and fibrin (Medic,Italy) was applied individually to separate biopsy sections, which were incubated at room temperature for 30 minutes in a moist chamber. Following three 15 minute rinses with PBS, the sections were coverslipped with glycerol-PBS solution and examined using an immunofluorescence microscope with epi-illumination (Hertel and Reuss, Germany with Exciter filter BP 485 and Barrier BP 520) and high pressure mercury lamps as the light source. The lesional skin processed as in routine procedure and stained with H&E stain and examined under light microscope. A final diagnosis was made combining the histological and immunofluorescence findings.

Results

Of 204 patients 76 were male and 128 were female and maximum number of patient came from 11-20 year age group (n = 64). Combining the histological and immunofluorescence findings the DIF positive cases (Table I) were categorized as three major groups: 1) blistering disorders such as: pemphigus group of lesions, bullous pemphigoid, dermatitis herpetiformis and others. 2) non-bullous immune mediated disorders: lichen planus, systemic lupus erythematosus/discoid lupus erythematosus, vasculitis etc. and 3) non-specific deposition cases of different disorders (prurigo nodularis, prurigo simplex, non-specific dermatitis and other small number of disorders) which showed positive immune reaction of different classes.

Non-immune disorders (DIF negative) were 101 cases (Table-II). These included prurigo nodulars, prurigo simplex, lichen simplex chronicus, non-specific dermatitis, Hailey-Hailey disease and other disorders (n = 30). Beside these two subcorneal pustular dermatosis were DIF negative. Five cases of lichen planus, five cases of seleroderma/ morphea and two cases of lymphocytic vasculitis also were DIF negative.

Table I: DIF positive cases

Diagnosis	Number
Bullous disease (N=58)	
Pemphigus	20
Pemphigoid	17
Dermatitis herpetiformis	07
Linear IgA Dermatosis	05
Erythema multiforme	04
Epidermolysis bullosa acquisita	04
Subcorneal pustular dermatosis	01
Non bullous (connective tissue and others) (N=21)	
Lichen planus	12
SLE/DLE	09
Vasculitis	06
Scleroderma	03
Lichen amyloidosis	01
Non specific deposits (Prurigo nodularis, prurigo simplex, non-specific dermatitis etc.)	14
Total	103

SLE = Systemic lupus erythematosus
DLE = Discoid lupus erythematosus

Table II: Direct immunofluorescence negative cases.

Diagnosis	Number
Prurigo nodularis	23
Prurigo simplex	12
Lichen simplex chronicus	12
Non-specific dermatitis	09
Lichen planus	05
Scleroderma/morphea	05
Lymphocytic vasculitis	02
Subcorneal pustular dermatosis	02
Hailey-Hailey disease	01
Others	30
Total	101

Discussion

A total of 204 skin samples were received from Department of Skin, BSMMU, BIRDEM Hospital, DMCH and some private clinics. The age range of patients was 5 years to 99 years and were female predominating (F:M =1.68:1)

Of immune mediated blistering diseases the most common DIF positive diagnosis was pemphigus group including eight patients of pemphigus foliaceus followed by bullous pemphigoid and dermatitis herpetiformis (DH). All pemphigus, including pemphigus foliaceus, samples showed deposition of IgG in the intercellular substance (ICS) in the full thickness of epidermis (Table III). Previous study also shows that full thickness ICS deposition is more common DIF pattern in Pemphigus foliaceus[2]. Of 17 cases of bullous pemphigoid the depositions were- C3, C3+IgG, C3+IgG+IgM and C3+IgA at the basement membrane zone. These are nearly similar to the result of study done by Provot *et al* (1979).[3] Of seven DH cases all showed

Table III Pattern of immune deposits in bullous diseases

Diagnosis	Deposits	Location	Pattern
Pemphigus (20)			
	IgG 100%	ICS of epidermis	Lace-like
Bullous pemphigoid (17)	C3 100% IgG 64.7% IgM 11.46% IgA 06.57%	BMZ of epidermis	Linear/granular
Dermatitis herpetiformis (7)	IgA 100% C3 28.57% IgM 14.28% C3+IgM 14.28%	Dermal papillae Dermal capillary wall	Granular
Linear IgA dermatosis (5)	IgA 100%	BMZ of epidermis	Linear
Erythema multiforme	C3 100% IgM 25%	Blood vessel wall BMZ of epidermis	Shaggy
Epidermolysis bullosa acquisita (4)	C3 75% IgG 50% IgM 25% Fibrin 25%	BMZ of epidermis	Linear/granular
Subcorneal pustular dermatosis	C3+ IgM	BMZ of epidermis	Granular

ICS = Intercellular substance, BMZ= Basement membrane zone.

Table IV: Clinicopathological correlation bullous diseases

Diagnosis	Clinically suspected		Clinically not suspected	Final Diagnosis Total
	Total	Confirmed by Routine & DIF		
Pemphigus	20	15	05	20
Bullous pemphigoid	13	12	05	17
Dermatitis herpetiformis	38	05	02	07
Linear IgA dermatosis	04	03	02	05
Erythema multiforme	06	03	01	04
Epidermolysis bullosa	04	01	-	01
Epidermolysis bullosa acquisita	-		04	04
Bullous drug eruption	06	-	-	-
Bullous SLE	01	-	-	-
Subcorneal pustular dermatosis	01	-	03	03
Vesiculobullous disease (unclassified)	02	-	-	-
Total	96	39	22	61

Table V: Diagnosis of immune mediated non-bullous skin disorders.

Disorders	Clinically		DIF positive	Deposits	Pattern of deposits	Final diagnosis
	Suspected	Not suspected				
Lichen planus	52	01	12	F.IgM, C3 IgG	Irregular, DEJ	17
SLE/DLE	29	0I	09	IgM,C3, IgG, IgA	Granular, BMZ	10
Vasculitis	06	03	06	C3,IgM, IgA	Dermal cap. Wall	08
Scleroderma	03	05	03	IgM,C3, IgG, IgA	Upper dermis or BMZ	08
Lichen amyloidosis	-	01	01	IgM,IgA C3	In clumps dermal papillae Cap. wall	01

SLE = Systemic lupus erythematosus, DLE = Discoid lupus erythematosus, F = Fibrin, DEJ = dermoepidermal junction, BMZ = Basement membrane zone, Cap.= Capillary.

granular deposits of IgA in dermal papillae, in addition C3 in one and IgM in one cases. One case showed C3 and IgM in dermal capillary wall. Of five linear IgA diagnosis (<15years= 3 and >18yeas=2 cases) two cases were not clinically suspected. One case (male, 15yrs) addition C3 in one and IgM in one cases. One case showed C3 and IgM in dermal capillary wall. Of five linear IgA diagnosis (<15years= 3 and >18yeas=2 cases) two cases were not clinically suspected. One case (male, 15yrs) was suspected as bullous erythema multiforme and one case (male, 24 years) was suspected as psoriasis. These showed linear deposits of IgA at the basement membrane zone. Four cases of epidermolysis bullosa acquisita were diagnosed showing C3 in three cases, IgG in two and IgM and fibrin in one case. But it is some time difficult to classifying epidermolysis bullosa acquisita or

differentiating it from pemphigoid, when immunofluorescence on salt-split skin or immunoelectron microscopic examination is essential.[4] Three subcorneal pustular dermatosis cases were diagnosed, one of which was DIF positive (C3 and IgM at the basement membrane zone) and two cases were immunonegative. Previous study showed IgG and C3 deposits at basement membrane zone.[5]

A total of 96 cases were suspected clinically as blistering diseases of which 39 patients correlated with histological and DIF diagnosis e.g. pemphigus = 15, bullous pemphigoid = 12, DH = 5, linear IgA = 3, erythema multiforme = 3 and epidermolysis bullosa = 1 (Table IV).

Twenty patients who were suspected as one type of blistering disease but diagnosed hispathologically and with DIF as another type of blistering diseases.

Of 37 remaining patients with suspected bullous diseases were diagnosed as non-bullous dermatoses. Clinically 33 suspected cases of dermatitis herpetiformis were diagnosed as prurigo nodularis, lichen simplex chronicus , prurigo simplex and other non-bullous diseases. These may due to associated itching and more or less bilateral distribution, common features of dermatitis herpetiformis and most of these diagnoses.

Immune mediated non-bullous (connective tissue disorders, vasculitis and other) skin disorders revealed variable DIF results (Table V) such as: of seven cases of systemic lupus erythematosus all showed depositions of immune reactants and two of three discoid lupus erythematosus patients showed deposits. Six cases of vasculitis, of which five were leukocytoclastic and one was lymphocytic, showed immune deposits in vessel wall. Two cases of lymphocytic vasculitis were DIF negative. Three cases of scleroderma showed immune deposits that may be seen in some cases.[2] One case of lichen amyloidosis showed immune deposits.

Non-immune disorders identified were prurigo nodularis, prurigo simplex, lichen simplex chronicus, Hailey-Hailey disease and small number of other disorders. However, some cases of these showed non-specific immune deposits.

Conclusion

Immunofluorescence technique is an essential method for the diagnosis and correctly classifying immune mediated skin diseases and segregating non-immune skin disorders.

References

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