Original Article

USE OF DIRECT IMMUNOFLUORESCENT MICROSCOPY IN THE DIAGNOSIS OF VESICULOBULLOUS DISORDERS OF SKIN

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ABSTRACT

Objective: To determine the relative frequencies of various vesiculobullous disorders of skin in our patients and the morphological and direct immunofluorescent patterns of these disorders.

Methodology: This retrospective study was carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan on all the cases of vesiculobullous disorders of skin diagnosed in the department from January 2002 to June 2007. All the skin biopsies received during the study period were reviewed and cases of vesiculobullous disorders were selected for detailed study. Direct immunofluorescent (DIF) staining was done on these cases using fluorescein isothiocyanate conjugate (FITC) labelled antibodies for IgG, IgA, IgM, C3c and Fibrinogen. The data was analysed statistically using SPSS software.

Results: A total of 62 DIF proven cases of vesiculobullous disorders of skin were studied. Pemphigus vulgaris (PV) was found to be the most frequent disorder (32.25%) followed by bullous pemphigoid (BP) with a frequency of 27.42%. The relative frequencies of pemphigus foliaceus (PF), dermatitis herpetiformis (DH), childhood bullous pemphigoid (CBP) and chronic bullous dermatosis of childhood (CBDC) were 20.96%, 6.45%, 4.83% and 6.45% respectively. One case each of IgA pemphigus (IgAP) and herpes gestationis (HG) were seen. IgA pemphigus can only be diagnosed after having observed the immunofluorescence pattern of this disorder.

Conclusions: Definitive diagnosis of certain blistering lesions of skin requires the DIF microscopy. However, in special circumstances it needs to be reinforced with either salt split skin technique or immune electron microscopy.

KEY WORDS: Vesiculobullous Lesion, Direct Immunofluorescent Staining.

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INTRODUCTION

Vesiculobullous disorders of skin are a heterogeneous group of dermatological disorders that are included in the non infectious predominantly superficial inflammatory disorders of skin.[1] These disorders are characterized by the presence of a vesicle (< 0.5 cm in size) or bulla (>0.5 cm in size) that lie within or beneath the epidermis.[2] Some blistering lesions are potentially life threatening. A number of diagnostic procedures such as histopathologic

analysis, direct and indirect immunofluo rescence, Tzanck smear, etc. are frequently required for an accurate diagnosis to be established.[3] Many developments have revolutionized the field of autoimmune bullous disorders.[4] Detection of tissue-bound and circulating serum antibodies and characterization of their molecular specificity is mandatory for the diagnosis of autoimmune blistering diseases. For this purpose, various immunofluorescence methods as well as immunoassays, including immunoblotting, ELISA and immunoprecipitation have been developed.[5]

In many cases, bullous diseases can not be differentiated clinically and needs the help of histopathological examination and immunofluorescence findings.[1] To our knowledge no study in Pakistan has been conducted so far using DIF technique in the diagnosis of vesiculobullous disorders of skin. It was therefore planned to carry out a study on vesiculobullous disorders of skin

METHODOLOGY

This was a retrospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. The study was conducted on all the diagnosed cases of vesiculobullous disorders of skin over a period of 5 ½ years from January 2002 to June 2007. All skin biopsies were reviewed. Non neoplastic lesions were separated from neoplastic lesions and cases of vesiculobullous lesions were selected for detailed study.

H&E and other special stained (e.g. PAS, Trichrome etc.) slides were studied of these cases and morphological features were noted. Five microns thick sections were cut and stained for a panel of FITC labelled antibodies comprising of IgA, IgG, IgM, C3 and Fibrinogen by DIF staining method after the application of antigen retrieval solution (pronase) over them. The DIF stained slides were immediately studied under immunofluorescent microscope using scanner (4x), low power (10x) and high power (40x) objective lenses. Nature, pattern and location of various antibodies deposition were noted in every individual case and the definitive diagnosis was established. The data thus obtained was statistically analyzed and *p-value* was determined by using chi-square test.

RESULTS

A total of 1761 skin biopsies were received during the study period. Vesiculobullous disorders accounted for 79(4.49%) cases. This represented 9.82% cases amongst all non-neoplastic skin diseases. DIF staining could be performed on 62 cases subsequently. Intraepidermal blisters which accounted for 34/62 (54.83%) cases were found to be more frequent than subepidermal blisters which accounted for 28/62 (45.16%) of cases.

Pemphigus vulgaris was found to be the most frequent lesion and accounted for 32.25% (20/62) cases. This was followed by 27.42% (17/62) cases of BP and 20.96% (13/62) cases of PF. Dermatitis herpetiformis accounted for 6.45% (4/62) cases and 4.83% (3/62) cases each of CBP and CBDC, were diagnosed. IgAP and HG were the least frequent disorders observed and accounted for 1.61% (1/62) cases each (Table-I).

Maximum number of cases i.e. 12 out of a total of 62 (19.35%) were seen in patients older than 60 years, followed by 11/62 (17.74%) cases in the fourth decade of life. Cases of PV had an increased frequency in fourth and fifth decade of life with a mean age of 41.85 years. Even distribution of PF was seen in amongst all age groups with a mean age of 41.9 years. Bullous

Table-I: Distribution of 62 cases of vesiculobullous disorders on direct immunofluorescent staining

Type	*No of cases*	*%*	*95% Confidence Interval*
PV	20	32.25%	27.6-47.1
PF	13	20.96%	50.67-64.7
IgAP	01	01.61%	-
BP	17	27.42%	46.7-65.4
CBP	03	04.83%	5.0-6.3
DH	04	06.45%	9.4-41.4
CBDC	03	04.83%	5.7-6.7
HG	01	01.61%	-

Table-II: Age distribution of 62 cases of vesiculobullous disorders of skin

Type	*01-10 years*	*11-20 years*	*21-30 years*	*31-40 years*	*41-50 years*	*51-60 years*	*61-70 years*	*71-80 years*	*Mean Age*	*Standard Deviation*	*Total*
PV	-	02	-	09	06	02	-	01	41.85	11.12	20
PF	02	02	-	01	03	01	03	01	41.9	9.49	13
IgAP	-	-	-	-	01	-	-	-	50	-	01
BP	-	-	01	01	02	05	05	02	58.37	15.78	16*
CBP	03	-	-	-	-	-	-	-	5.6	0.57	03
DH	02	-	01	-	01	-	-	-	21.5	18.38	04
CBDC	03	-	-	-	-	-	-	-	6.5	0.35	03
HG	-	01	-	-	-	-	-	-	20	-	01
Total	10	05	02	11	13	08	08	04			

(AGE RANGES: PV= 18-80; PF= 06-73; IgAP = 50; BP = 28-80; CBP = 5-6; DH = 4-50; CBDC = 6-7; HG = 20)
* One case of BP showed negative DIF staining for IgG, IgA, IgM, C3c and fibrinogen.

pemphigoid was found to be more frequent in the sixth and seventh decade of life with a mean age of 58.37 years. The mean ages for the cases of CBDC and childhood BP were 6.5 years and 5.6 years respectively (Table-II).

Vesiculobullous disorders displayed a predilection for male sex in our study. All individual lesions were found to be more frequent in males than in females, except for cases of PF, which was found to be three times more frequent in female patients than male patients. Male to female ratio varied from lesion to lesion and was found to be as high as 2.4:1 in BP cases to as low as 1:1 in cases of DH (Table-III).

DISCUSSION

An attempt has been made through this retrospective study to determine the relative frequency, and morphological and DIF patterns of vesiculobullous disorders of skin. The exact incidence and prevalence of these disorders in our country is not known. Data reported from other countries has shown that these disorders are not very common.[1,6-9] In our study we found

Table-III: Sex distribution of 62 cases of vesiculobullous disorders of skin

Type	*Sex*								*Male to female ratio*	*Total*
	Male				*Female*					
	No	*%*	*Mean age*	*Median age*	*No*	*%*	*Mean age*	*Median age*		
PV	05	25	37.4	40	15	75	43.3	40	1:3	20
PF	08	61.5	51.5	52.5	05	38.5	26.6	14	1.6:1	13
IgAP	01	100	50	50	-	-	-	-		01
BP	12	70.58	56.41	60	05	29.41	63.4	65	2.4:1	17
CBP	03	100	5.66	06	-	-	-	-	-	03
DH	02	50	16	16	02	50	27	27	1:1	04
CBDC	02	66.66	6.25	6.25	01	33.33	7	7	2:1	03
HG	-	-	-	-	01	100	20	20	-	01
Total	33				29					

(Legend for Tables 1, 2 and 3: PV = pemphigus vulgaris, PF = pemphigus foliaceus, IgAP= IgA pemphigus, BP= bullous pemphigoid, CBP = childhood bullous pemphigoid, DH = dermatitis herpetiformis, CBDC = chronic bullous dermatosis of childhood, HG = herpes gestationis).

79 (8.23%) cases of vesiculobullous disorders out of a total of 959 non neoplastic skin lesions over a period of 5 ½ years. Direct immunofluorescent staining patterns could only be studied in 62 cases subsequently. The incidence of these bewildering disorders can not be commented upon the basis of present data.

The most frequent disorder in our study was PV. This finding is in accordance with other regional and international studies.[6] This also showed that PV is the most frequent disorder amongst pemphigus group, a finding that has been generally agreed upon. The relative frequency of PF in our patients was 20.96% (13/62 cases). This finding is also in accordance with the findings of Peiying et al.[6] Amongst the subepidermal blisters we found that BP had a lion's share accounting for 60.17% (17/28) cases of subepidermal blisters. This finding is in accordance with the finding of Mulyowa et al who found BP being the most common subepidermal blistering lesion.[7] BP has been described as the most common subepidermal autoimmune blistering skin disease seen in the elderly[10] (Table-I).

Mean age for PV was found to be 41.85 years with a male to female ratio of 3:1 in our patients. Mean age reported by Bickle et al[11] is also similar but Su and Chong described relatively later mean age for cases of PV. Our finding in this regard did not correlate with the mean age described by Su and Chong.[8] However, a large proportion of senior citizens have been included in the study of Su and Chong.[8] This may have resulted in an increased mean ages for all the vesiculobullous disorders included in their study.

In our study the mean age for cases of PF was found to be 41.9 years. The male to female ratio was 1.6:1. Our finding of mean age again is younger than the mean age described by Su and Chong.[8]

Mean age for BP was found to be 58.37 years with 2.4:1 male to female ratio in present study. The mean age described by Su and Chong was 70 years. The relatively younger onset of this disease in our study further augments the hypothesis given by Su and Chong regarding the age expectancy and life style of Hong Kong population.[8]

Childhood bullous pemphigoid is a variant of BP that primarily affects children. We had three such cases that were provisionally diagnosed as CBDC when only H&E stained slides were observed. After having seen the DIF patterns the diagnosis of these cases was reviewed. The ages of patients were five, six and six years. The histopathologic and immunofluorescent features are indistinguishable from adult BP.[12] In our series we found 6.45% (4/62) cases of DH with granular IgA deposition at the basement membrane zone. The age range for this disorder was 3-50 years with 1:1 male to female ratio. Although our finding in this regard coincides the finding of Su and Chong, however, cases of DH are seen in scattered age groups.[8]

Three cases of CBDC in our study belonged to first decade of life (6, 6½ and 7 years) with the mean age of 6.5 years. Our finding of mean

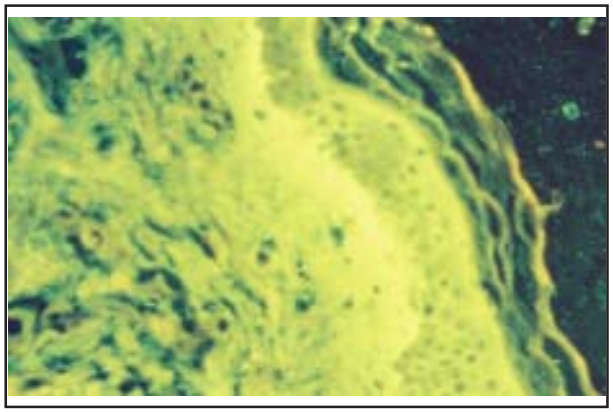

Fig-1: Linear deposition of IgG at the basement membrane zone in a case of BP (DIFx20)

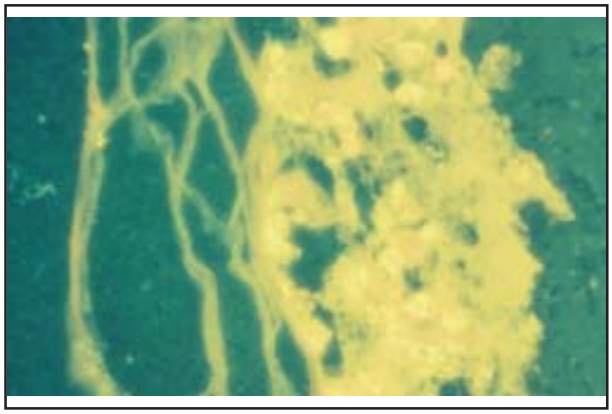

Fig-2: Lacelike deposition of IgG in intercellular spaces in a case of PV (DIF X 20)

age is in accordance to the findings of Peiying et al and Arti et al.[6,13]

IgA pemphigus is a pruritic pustular eruption that is characterized by squamous intercellular IgA deposits and intraepidermal neutrophils. It occurs primarily in middle aged and elderly individuals.[14] Clinical features are similar to those seen in PF or subcorneal pustular dermatosis.[15] IgAP is a heterogeneous group reflecting differences in autoantigen involved.[16]

In our series we had one case of IgAP diagnosed on DIF microscopy. Histopathological examination of this lesion revealed a subcorneal blister with few acantholytic cells, neutrophils and lymphocytes in the blister cavity. On DIF staining and subsequent microscopy the intercellular deposition of IgA was noted in a lacelike pattern. The definitive diagnosis of IgAP can only be established with immunofluorescent microscopy as there are a few diseases that look morphologically identical. The same pattern can be observed in Seddon-Wilkinson disease, PF, pustular psoriasis, bullous impetigo and PV.[2] There were, however, not enough cases of DH, CBDC, IgAP and HG, for any definite conclusion to be drawn.

CONCLUSION

Pemphigus vulgaris was found to be the most frequent autoimmune blistering lesion of skin in our patients and BP was the most frequent subepidermal autoimmune blistering lesions. Direct immunofluorescent staining and subsequent microscopy is a useful tool in establishing the exact diagnosis of vesiculobullous disorders of skin. However, in certain instances, especially in cases of subepidermal blistering lesions it needs to be reinforced with either immune electron microscopy or salt split skin technique and subsequent immunofluorescent microscopy in exactly localizing the site of immunodeposits.

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