Original Article

Morphological and Immunofluorescent Patterns of Subepidermal Autoimmune Bullous Diseases of Skin in Karachi Pakistan

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ABSTRACT

Introduction: Subepidermal autoimmune blistering disorders (SEABD) are common in dermatological practice. Direct immunoflourescence (DIF) staining is considered gold standard for the diagnosis of these disorders. This study was conducted to determine the morphological and DIF patterns of these disorders.

Study Design: Retrospective Study.

Place and Duration of Study: This study was conducted at the department of Pathology, Basic Medical sciences Institute, Jinnah Postgraduate Medical centre, Karachi, from January 2002 to July 2007.

Materials and Methods: Morphological and DIF patterns were recorded and categorical diagnoses of these disorders were established.

Results: Bullous pemphigoid (BP) was found to the most frequent disorder with a relative frequency of 60.71% and mean age of 54.82 years. Relative frequencies of childhood bullous pemphigoid (Ch BP), dermatitis herpetiformis (DH), chronic bullous dermatosis of childhood (CBDC) and herpes gestationis (HG) were 10.71%, 14.28%, 10.71% and 3.57% respectively.

Conclusion: Direct immunofluorescent staining is a one step procedure that should be done in all cases of SEABD. Use of salt split technique and immune electron microscopy would further enhance the level of certainty in SEABD.

Key words: subepidermal blister, direct immunofluorescent staining, bullous pemphigoid

INTRODUCTION

Subepidermal autoimmune blistering disorders are a heterogeneous group of disorders in which the blister is formed along the dermoepidermal junction. This group of diseases includes conditions with different clinical presentations, morphological findings and pathogenesis. Many bullous diseases look clinically identical, and clinicians rely heavily on morphological and in particular immunofluorescent patterns of these disorders[1]. Subepidermal autoimmune blistering disorders include bullous pemphigoid (BP), cicatricial pemphigoid (CP), linear IgA dermatosis (LAD), chronic bullous dermatosis of childhood (CBDC), herpes gestationis (HG), epidermolysis bullosa acquisita (EBA) and bullous systemic lupus erythematosus (bullous SLE)[2]. Pathogenetically they are characterized by the presence of antibodies directed against the structural components of dermoepidermal junction or basement membrane zone[3].

Immunoflourescence staining has become an indispensable tool in the diagnosis of autoimmune bullous diseases. In many cases, bullous diseases can not be differentiated clinically and needs the help of histopathological examination and immunoflourescence findings[4]. Direct immunofluorescent staining in particular is important in the diagnosis of SEABD[3]. Even in situation in which the histopathological finding seem characteristic of a specific bullous disease, DIF testing can add to the certainty of diagnosis, sometimes modify it, and occasionally reveals a different diagnosis[2].

Direct immunofluorescent staining is not widely available in Pakistan it is considered the "gold standard" for the diagnosis of autoimmune bullous disorders[5]. This study was planned to establish the morphological and immunofluorescent patterns of these bewildering disorders in our population.

MATERIALS AND METHODS

This was a retrospective study conducted at the department of Pathology, Basic Medical sciences Institute, Jinnah Postgraduate Medical centre, Karachi, from January 2002 to July 2007. All skin biopsies were reviewed and cases of bullous diseases were selected for detailed study. Paraffin blocks of SEABD cases were retrieved and DIF staining was performed on these cases, after the application of pronase as antigen retrieving solution. Panel of antibodies comprised of fluorescein isothiocyanate conjugate (FITC) labeled IgG, IgA, IgM, C3 and Fibrinogen. Slides were studied immediately under immunofluorescent microscope using scanner (4x), low power (10x) and high power (40x) objective lenses. Type, pattern, and location of deposition of various antibodies were recorded on the designed proforma and photographs were taken[6]. The definitive diagnosis was established with the help of morphological and immunofluorescent patterns of various cases. Different SAEBD were diagnosed according to criteria described in table 1[3]. Statistical

analysis was done using SPSS software. The results thus obtained were analyzed and compared with the results obtained from other locally and internationally published studies.

RESULT

A total of 62 cases of bullous disorders of skin were studied during the study period. Of these 34 cases belonged to intraepidermal bullous disorders and 28 cases of SEABD were found. Of the 28 cases of SEABD, there were 19 (67.85%) males and 9 (32.14%) females. The age ranged from 5-73 years. Morphological and DIF criteria, that was used for the categorization of various SEABD is shown in Table 1.

Table No.1: Morphological and DIF criteria for the categorical diagnosis of SEBD[3]

Diagnosis	Morphological features	DIF patterns
Bullous Pemphigoid	• Subepidermal bulla • Mixed inflammatory cells in blister cavity predominantly eosinophil • Festooning of dermal papillae	• Linear deposition of IgG ± C3 at the dermoepidermal junction
Dermatitis herpetiformis	▪ Subepidermal bulla ▪ ± Microabscesses at the tip of dermal papillae	• Granular deposition of IgA at the basement membrane zone
Chronic bullous dermatosis of childhood	▪ Subepidermal bulla Neutrophilic ± eosinophilic infiltrate at dermoepiderm	• Thick linear deposition of IgA at the basement membrane zone.
Herpes gestationis	▪ Subepidermal bulla ▪ Necrosis of basal keratinocytes ▪ Papillary dermal edema ± eosinophilic infiltrate	• Linear deposition of C3 ± IgG, IgM etc at the basement membrane zone.

In the present study BP had the lions share with 17/28 (60.71%) of cases. Old males were predominantly affected with a mean age of 54.82 years and 2.4:1 male to female ratio. Child hood Bullous pemphigoid (Ch BP) accounted for 3/28 (10.71%) cases. Mean age for this disorder in the present study was found to be 5.6 years.

Table No. 2: Various SEABD in the study (n=28)

Diag-nosis	No	%	Male	Female	Male to female ratio	Mean age
BP	17	60.71	12	05	2.4:1	54.82
Ch BP	03	10.71	03	-	-	05.66
CBDC	03	10.71	02	01	2:1	06.33
DH	04	14.28	02	02	1:1	21.50
HG	01	03.57	-	01	-	20.00

Key to table 2: (BP= bullous pemphigoid, Ch BP= childhood bullous pemphigoid, CBDC= chronic bullous dermatosis of childhood, DH= dermatitis herpetiformis, HG = herpes gestationis)

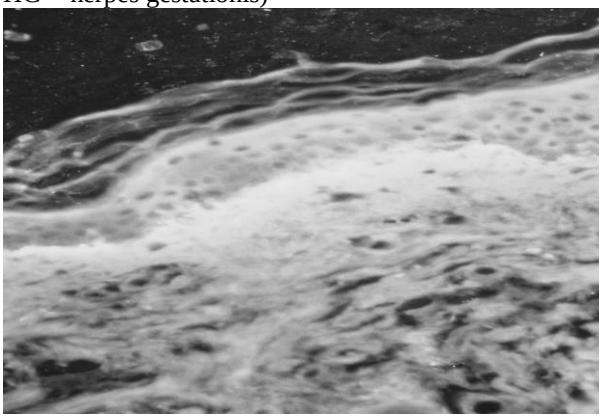

Figure No.1: Linear deposition of IgG at basement membrane zone in a case of bullous pemphigoid

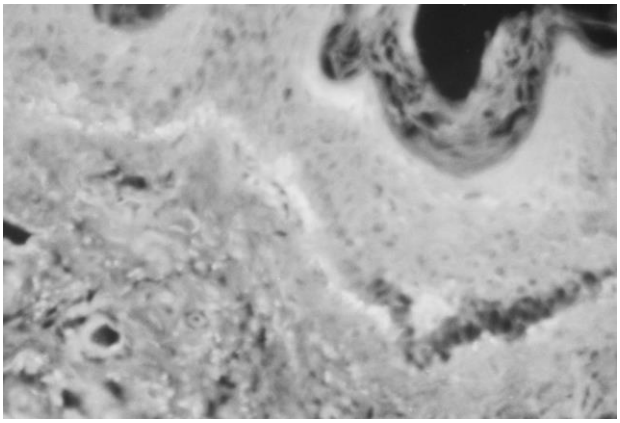

Figure 2: Granular deposition of IgA in a case of Dermatitis herpetiformis at dermoepidermal junction

All cases of BP (including Ch BP) showed subepidermal bullae with festooning of dermal papillae in 16/20 (80%) cases. Mixed inflammatory cell infiltrate predominantly eosinophil, neutrophil, lymphocytes and plasma cells was seen in all cases. Fibrin deposition was seen in 17/20 (85%) cases. Linear deposition of IgG was seen on DIF microscopy along with dermo-epidermal junction in 19/20 (95%) cases. Intensity of staining varied from + to +++. Linear deposition of C3 was also noted in 9/20 (45%) cases.

Diagnosis of DH was established in 4/28 (14.28%) cases. The age ranged from 3-50 years with a mean age of 21.50 years. Male to female ratio was 1:1. All cases of DH showed subepidermal bullae with formation of microabscesses at the tip of dermal papillae in 3/4 (75%) cases. Granular deposition of IgA was noted on DIF microscopy in all cases along basement membrane zone with additional deposition of C3 in 1/4 (25%) cases.

Mean age for 3/28 (10.71%) cases of CBDC was 6.33 years in our study with 2:1 male to female ratio. Subepidermal bulla with linear deposition of IgA along the basement membrane zone was noted in all the cases.

The only case that was diagnosed as HG showed a subepidermal bulla with concurrent history of pregnancy in a 20 years old lady. Presence of eosinophilic infiltrate and necrosis of basal keratinocytes was particularly noted. On DIF microscopy deposition of IgG, IgM and C3 was seen along the basement membrane zone in a linear pattern.

DISCUSSION

Although DIF is considered as the gold standard for the diagnosis of SEABD, it is not widely available in Pakistan[3]. An effort has been made through this study to describe the morphological and DIF patterns of these bewildering disorders.

Mean age for cases of BP in our study was slightly younger than that was described by Su and Ly in Hong Kong population. This relatively younger onset of BP in our study is supported by the hypothesis proposed by Su and Ly regarding the life style and life expectancy in Hong Kong population[7]. Although this finding is in accordance with the findings of Mehmood and Haroon, Mylowa et al and Adam BA[2,8,9]. Histopathological and DIF features are in accordance with those described by Fisler et al and Stern[10,11].

Mean age for the cases of DH was also younger in our study than described by Su and Ly[7]. This further augments the hypothesis proposed by Su and Ly[7]. Morphological and DIF features are compatible with the findings described by Williams et al and Browsi et al[12,13]. However DH is found in scattered age groups.

Morphological and DIF findings of CBDC in our series of patients were found to be identical as described by Navi et al and Kulthanan et al[16,15]. Mean age and male to female ratio were in accordance with those described by Peiying et al and Kulthanan et al[14,15].

Our morphological and DIF findings of HG are in accordance with the findings of Villegas et al[17]. However, there were not enough cases of DH, CBDC and HG, for any conclusion to be drawn.

CONCLUSION

DIF should be carried out in all the cases of SEABD in order to make the categorical diagnosis of these cases. Large population based studies using salt split technique and immune electron microscopy should be carried out to determine the nature of antibodies.

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