Original Article

MORPHOLOGIC AND DIRECT IMMUNOFLUORESCENT FEATURES OF INTRAEPIDERMAL BLISTERING DISORDERS OF SKIN

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Objective: To determine the relative frequencies of various intraepidermal autoimmune blistering disorders (IEABD) of skin, and to study the morphological and direct immunofluorescent features of features of these disorders.

Methods: This was a retrospective study conducted in the department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre and Sirsyed College of Medical Sciences Karachi over a period of 31/2 years. All skin biopsies were reviewed and cases of intraepidermal blistering lesions of skin were selected for direct immunofluorescent (DIF) staining for a panel comprising of IgG, IgA, IgM, C3c and Fibrinogen.

Results: Out of a total of 34 cases of intraepidermal skin blisters, there were 20 (58.82%) cases of pemphigus vulgaris (PV), 13 (38.23%) cases of pemphigus foliaceus (PF) and 01 (02.94%) case of IgA pemphigus (IgAP). Mean age for PV was 41.85 + 11.12, and that for PF was 41.9 + 9.49 years. Pemphigus vulgaris was found to be more frequent in females (15/20 cases), while PF was found to be slightly more frequent in males (8/13 cases). Morphological and direct immunofluorescent features are in accordance with those reported in local and international literature.

Conclusions: Pemphigus vulgaris is the most frequent of IEABD. DIF is essential in the diagnosis establishment.

Keywords: Intraepidermal blisters, pemphigus vulgaris, direct immunofluorescent staining.

Results

Intraepidermal autoimmune blistering disorders (IEABD) are a heterogeneous group of disorders that are included in the non-infectious superficial inflammatory dermatoses. Autoimmune bullous diseases of skin and mucosa are uncommon, disabling, and potentially lethal diseases.[1] These disorders are characterized by the presence of vesicle or bulla within the epidermis. In many of these diseases, clinical appearance of the patient will suggest diagnosis to dermatologists. However, many IEABD look clinically identical and the clinicians rely heavily on histopathology and, in particular, immunofluorescent findings to establish the diagnosis.[2,3] Some IEABD have serious sequelae, necessitating early treatment and intervention to prevent further morbidity and mortality.[4] The key morphologic features in the microscopic evaluation of IEABD are the level of plane of separation and the types of cellular changes seen particularly the presence or absence of acantholysis supplemented by their direct or indirect immunofluorescent pattern.[5]

Immunofluorescence staining has become an indispensible tool for the diagnosis of these bewildering disorders. Even in the situations in which the morphological findings seems characteristic of a bullous disease, direct immunofluorescent (DIF) staining can add to the certainty of diagnosis, sometimes modify it, and occasionally reveals a different diagnosis.[6]

Direct immunofluorescence staining is considered "gold standard" for the diagnosis of IEABD,[7] however it is not widely available in Pakistan.

Methods

This was a retrospective study of cases of IEABD diagnosed in the department of Pathology, Sirsyed college of medical sciences for girls and Sirsyed Hospital Karachi. All the skin biopsies received over a period of $3^{1/2}$ years (January 2013 to June 2016) were reviewed and cases of IEABD were selected for detailed study. The H&E stained glass slides were observed for each case, added with PAS, Trichrome, and other stains where necessary. Direct immunofluorescent (DIF) staining was done on formalin fixed, paraffin embedded tissues for all cases of IEABD using a panel of fluorescein

conjugate (FITC) labeled antibodies for IgG, IgA, IgM, C3 and Fibrinogen. The DIF stained slides were immediately observed in a fluorescent microscope using scanner (4x), low power (10x), and high power (40X) objective lenses.[8] Results were analyzed statistically using SPSS software. The criterion was established for the morphological and DIF features of IEABD, and the cases were accordingly typified .[9]

Results

Out of a total of 62 DIF proven cases of skin blisters, there were 34 cases of IEABD. Diagnosis was made after having observed the morphological and direct immunofluorescent features as shown in **table-1**.[9] Of these pemphigus vulgaris (PV) had the lion's share with 20/34 (58.82%), followed by 13/34 (38.23%) cases of pemphigus floiaceus (PF) and 1/34 (02.94%) case of IgA pemphigus **(table 2)**.

Table-1: Morphological and direct immunofluorescent features criteria for intraepidermal autoimmune blistering disorder

Diagnosis	Morphological features	Morphological features
Pehmphigus vulgaris	Intraepidermal blister	Intercellular deposition of IgG + C3, in the epidermis
	Suprabasal blister	
	Tombstone appearance of basal layer	
	Acantholyitc cells	
	Little inflammation	
Pemphigus floiaceus	Intraepidermal blister	Intercellular deposition of IgG + C3, in the epidermis
	Subcorneal blister	
	Eosinophilic spongiosis	
	Scanty acantholyitc cells	
	Little inflammation	
IgA pemphigus	Suprabasal or subcorneal blister	Intercellular deposition of IgA predominantly + C3, in the epidermis
	Few acantholytic cells	
	Little inflammation	

Table-2: Relative frequencies of various intraepidermal blistering lesions of skin in our patients.

Type	No. Of Cases	Percentage	95% Confidence Interval
Pemphigus Vulgaris	20	58.82%	27.6-47.1
Pemphigus foliaceus	13	38.23%	50.67-64.7
IgA pemphigus	01	02.94%	-

Table-3: Age distribution of 34 cases of intraepidermal blistering disordes of skin.

Age Groups (in Years)	Pemphigus Vulgaris	Pemphigus Foliaceus	IgA pemphigus
1-10	0	2	0
11-20	2	2	0
21-30	0	0	0
31-40	9	1	0
51-50	6	3	1

51-60	2	1	0
61-70	0	3	0
71-80	1	1	0
Mean Age	41.85	41.9	50
Standard deviation	11.12	9.49	-
Total	20	13	01

Table-4: Sex distribution of 34 cases of intraepidermal blistering disorders of skin.

		Sex								
		Male			Female				Male to female ratio	Total
Type	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	51.5	15	38.5	26.6	14	1.6:1	13
IgAP	01	100	50	50	-	-	-	-		01

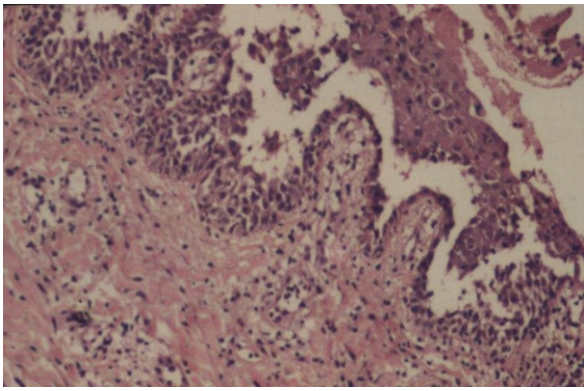

Fig-1: Photomicrograph showing suprabasal blister of pemphigus vulgaris (H&E x 200)

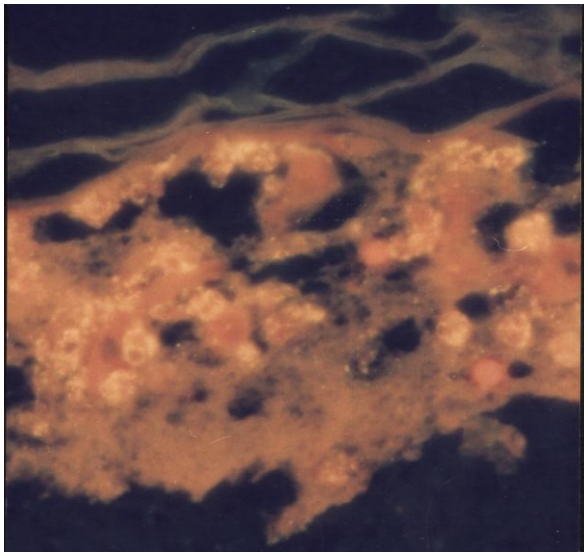

Fig-2: Lacelike deposition of IgG in intercellular spaces in pemphigus vulgaris (DIF x 200)

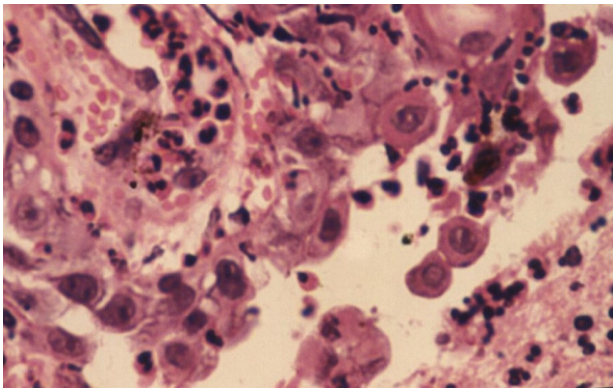

Fig-3: Blister cavity contains acantholytic cells in pemphigus foliaceus (H&E x 400)

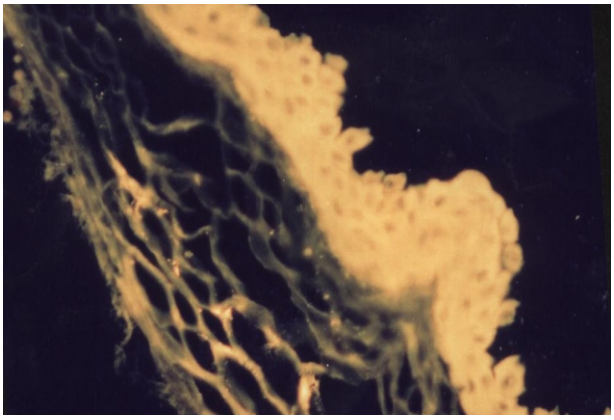

Fig-4: Intercellular deposition of IgG in a case of pemphigus foliaceus (DIF x 200)

Table 3 shows the age distribution of various IEABD in our study. It is quite obvious that majority of the cases were seen in the patients younger than 50 years of age with a peak incidence between 31 to 50 years. Only two cases of PF were seen in the first decade of life. The only case diagnosed as IgA pemphigus (IgAP) was by virtue of DIF only. Morphologically it resembled pemphigus foliaceus, with a subcorneal blister containing acantholytic cells. The patient was a 50 years old male. However there were not enough cases for IgAP to draw any conclusion.

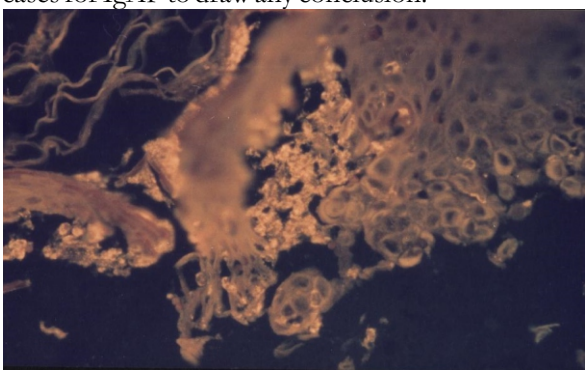

Fig-5: Intercellular deposition of IgA in a case of IgA pemphigus (DIF x 200)

Discussion

Although DIF is considered to be gold standard for the diagnosis of IEABD, it is not widely used in Pakistan.[8,9] An attempt has been made through this study to describe the morphological and DIF features of IEABD, as well as their relative frequencies in our patients. Our 20 cases of PV showed an intraepidermal blister. In 19 cases a suprabasal blister was seen with characteristic tomb stone appearance of the basal layer of epidermis. One case showed an intraspinous location of the blister. This relatively higher position of blister might have reflected growth of epidermis at its base. Acantholysis was seen in 19 (95%) cases. A mixed inflammatory cell infiltrate with predominant neutrophils was seen in 11 cases. Five cases showed mononuclear inflammatory infiltrate as well, while 02 cases revealed no evidence of inflammation. All (100%) cases revealed a lacelike deposition of FITC labeled IgG in the intercellular spaces. C3c was found in 06 (30%) cases. These findings are in accordance with the findings of other workers.[10,2]

Mean age for PF was a bit younger than the finding of Su and Chong who found mean age of 63 years in their study[11]. They described the difference related to increase life expectancy in Hong Kong population.

All cases of PF were characterized by the presence of a subcorneal blister with occasional or few acantholytic cells in all cases. An inflammatory infiltrate was found in 10 (76.92%) cases predominantly neutrophils. Lymphocytes and eosinophils were also seen in 08 cases. Plasma cells were the least frequent inflammatory cells and were present in 5 cases. On direct immunofluorescopy all lesions of PF demonstrated a lacelike deposition of IgG in the intercellular spaces within the epidermis. 05 (38.46%) cases demonstrated an additional deposition of C3c in a similar pattern. These findings are in accordance with the findings of Su and Chong, and Huilgol et al who found similar immuno fluorescent patterns.[11, 12] IgA pemphigus is a pruritic pustular eruption that is characterized by intracellular IgA deposits and intraepidermal neutrophils. It occurs primarily in middle aged and elderly individuals.[13] Clinical features are similar to those seen in pemphigus foliaceus or subcorneal pustular dermatosis.[14] IgA pemphigus is a heterogeneous group reflecting differences in the autoantigen involved [15]. Two patterns are observed in the histopathological examination: a subcorneal pustular dermatosis (SPD) type and an intraepidermal neutrophilic (IEN) dermatosis type.[16] Direct immunofluorescent testing reveals IgA deposition in the squamous intercellular substance throughout the epidermis with increased intensity in the upper layers. Complement and other immunoglobulins are usually not present.[14] In the subcorneal pustular dermatosis type IgA antibodies were shown to recognize desmoglein1 and 3.[15]In our series we had one case of IgAP diagnosed on direct immunofluorescent staining. Although histology suggested a provisional diagnosis of PF, however DIF showed intercellular deposition of IgA in a lacelike pattern.[14]

Definitive diagnosis of IgA pemphigus can only be made with immunofluorescent staining as there are a few diseases that look histopathologically identical. The same pattern can be observed in seddon-wilkinson disease, pemphigus foliaceus, pustular psoriasis, bullous impetigo and pemphigus vulgaris .[3]

Conclusion

Pemphigus vulgaris is the most frequent intraepidermal blistering disorder, followed byPemphigus floiaceus. Direct immunofluorescent staining is essential in establishing the diagnosis of these bewildering disorders.

References

1. Diercks GF, Pas HH, Jonkman MF. Immunofluorescence of Autoimmune Bullous Diseases. Surg Pathol Clin. 2017 Jun;10(2):505-512. doi: 10.1016/j.path.2017.01.011. Epub 2017 Mar 30.
2. Svecova D. Pemphigus vulgaris: a clinical study of 44 cases over a 20-year period. Int J Dermatol. 2015 Oct;54(10):1138-44. doi: 10.1111/ijd.12644.
3. Chu AC, Bhogal B. McLelland J. Imunohistochemistry in skin. In: Filipe MI, Lake BD eds. Histochemistry in Pathology. Churchil-Livingstone, 1990 (2nd edition): 159-174
4. Bickle KM, Roark TR, Hsu S. Bullous dermatoses: A review. Am Fam Physician 2002; 65: 1861-70
5. Wu H, Schapiro B, Harrist TJ. Noninfectious vesiculobullous and vesiculopustular diseases. In: Elder DE, Elenitsas R, Johnson BLJ, Murphy GF eds. Lever's histopathology of the skin, Philadelphia: Lippincott Williams and Wilkins, 2005: 243-291Seema and sangeeta 2002
6. Rani Z, Hussain I. Immunofluorescence in immunobullous diseases. JPAD 2003;13:76-88
7. Wojnarowska F, Eady RA, Berge SM. Bullous erution In:Champion RH, Burton JL, Burns DA, Breathnach SM, editors. Textbook of dermatology, 6th ed. Oxford (London): Blackwell Science;1998.p:1817-98
8. Rizvi SR, Sadiq S, Haque SU. Morphological and direct immunofluorescent features of subepidermal autoimmune bullous disease of skin in Karachi Pakistan. Med Forum Vol 22 Number 11. November 2011:38-41
9. Mehmood T, Haroon TS. Patterns of direct immunofluorescent in subepidermal autoimmune blistering diseases of skin in Lahore, Pakistan, JPAD 2003;13:67-71
10. Thorakkal S, Vengal IV, Maliyekkal SP. Pemphigus vulgaris in oral cavity a case report. Saudi Dental Journal 2006.Su and Ly 1996
11. RCW Su, LY Chong. Chronic bullous dermatoses in Hong Kong. HKMJ 1996; 366-72.
12. Huligol SC, Bhogal BS, Black MM. Immunofluorescence of the immunobullous disorders Part Two: The clinical disorders. Indian J Dermatol Venerol Leprol 1995;61:255-264
13. Saurat et al 1987 Saurat JH, Merot Y, Salomon D. Pemphigus-like IgA deposits and vesiculopustular dermatosisin a 10 year old girl. Dermatologica 1987; 20:89
14. Beutner AH, Prigenzi LS, Hale LS. Immunofluorescent studies of antibodies to intercellular are of epithelia in Brazilian Pemphigus Foliaceus. Proc Soc Exp Biol Med 1968;127:81
15. Harman KE, Holmes G, Bhogal BS. Inercellular IgA dermatosis (IgA pemphigus) - two cases illustrating the clinical heterogeneity of this disorder. Clin Exp Dermatol 1999; 24:464-466
16. Teraki Y, Amagai Z, Hashimoto T. Intercellular IgA dermatosis of childhood. Arch Dermatol 1991; 127: 221