Section: Pathology

Research

HISTMORPHOLOGICAL PROFILE AND DIRECT IMMUNOFLORESENCE IN SUBEPIDERMAL BULLOUS LESION OF SKIN

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Received : 24/08/2022
Received in revised form : 05/09/2022
Accepted : 17/09/2022

Keywords:
Vesiculobullous disorders, Subepidermal blisters, Bullous pemphigoid

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DOI: 10.47009/jamp.2022.4.5.51

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2022; 4 (5); 249-252

Abstract

Background: Subepidermal Vesiculobullous disorder are subset of immunobullous disease less encountered in India.It requires histomorphological diagnosis along with DIF and clinical findings. This study is undertaken to evaluate the utility of DIF in histopathological diagnosis of Subepidermal bullous lesions of skin. **Materials and Methods:** A total of 21 cases of Subepidermal vesiculobullous disorders were studied over a span of 24 months. Total of 21 skin biopsies from patients with vesiculobullous skin lesions were sent to the Department of Pathology, B R Ambedkar medical college Bangalore. Punch biopsies were taken for histopathological diagnosis. H& E stain was applied. Perilesional skin was taken in normal saline for DIF procedure. **Result:** In the present study Bullous Pemphgoid constituted the most common Subepidermal vesiculobullous disorders with 62% followed by Erthyema Multiforme26%. Majority of patients presented between 60-80 yrs of age with female preponderance. All most all cases presented with blister.D: E junction separation was seen in all the cases. DIF showed positive findings in 71.4% cases. **Conclusion:** In Subepidermal Blisters are not uncommon in India but the prevalence is relatively low Thus a clincopathological correlation with DIF is required for better characterize the pattern of Subepidermal Bullous diseases.

INTRODUCTION

Autoimmune and inherited bullous disorder are rare skin diseases that may have a profound negative impact on quality of life.[1] Vesiculobullous disorders represent a heterogenous group of dermatoses with protean manifestations. The diseases have been the subject of intensive investigation in recent years.[2]

There is wide variety of bullous disease, some of which can be extremely debilitating and even fatal, some bullous lesion may have serious sequele, necessating early treatment and intervention to prevent further morbidity and mortality.[3] Clinical examination of skin bullous lesion provides dermatologist gross morphological finding upon which differential diagnosis can be found out. However HPE is needed for definite diagnosis.[4] There is wide variety of bullous lesion. So it is presented based on site, shape and size of the bulla and also changes in the bulla, epidermis and dermis.[5]

This bullous lesion show immune perturbation as a part of disease pathogenesis at various location such as dermo-epidermal junction, dermal blood vessels etc. Nature of immune deposits usually used in DIF is IgG, IgA, IgM and C3.[6,7]

Immunofluorescence techniques are essential to supplement clinical findings and histopathology in the diagnosis of the immunobullous disorders. These rapid and reliable techniques permit early diagnosis and treatment of potentially life-threatening disorders.[6]

Subepidermal bullous diseases are disorders in which a blister forms along the dermo-epidermal junction and on immunofluorescence by the deposition of immunoglobulins and/or complement at the basement membrane zone, with the exception of dermatitis herpetiformis, where the deposits are in the dermal papillae.[8,9] This group of disorders

ISSN (O): 2687-5365; ISSN (P): 2753-6556

encompasses bullous pemphigoid(BP), epidermolysis bullosa acquisita, cicatricial pemphigoid, pemphigoid gestationis, linear IgA dermatosis, Erythema Mutliforme(EM)dermatitis herpetiformis(DH)and bullous systemic lupus erythematosus(BSLE), Bullous Drug Eruption(BDE).[9] By Direct Fluorescent Microscopy presence of immunocomplex can be detected and will help to arrive at diagnosis. DIF is considered diagnostic tool in detection of mostly subepidermal autoimmune diseases.[8]

MATERIALS AND METHODS

Source of Data

Study was conducted in pathology department of Dr B R Ambedkar medical college & hospital in collaboration with Department of dermatology

Collection of Data

Minimum of 21 cases of bullous lesion of skin was collected from 2 years study. These patients had clinical history of bullous lesions. Biopsy was fixed in 10% formalin and PBS. Histological slides will be prepared and studied using H&E stain and immunofluorescence

Direct Immunofluorescence Technique

Skin specimen was obtained by 3-5mm punch or surgical biopsy

Biopsy specimen was snap frozen, if delayed was kept in cold saline

Frozen 4-6micron section were cut on cryostat and placed on glass slide before being air dried for 15 min

After rinsing in phosphate buffer saline (PBS) ph 7.2 for 15 minutes in 3 cycle.

Slides are overlaid in moist chamber with FITC conjugates with following specificities anti IgG, IgA, and C3. Each reagent on separate Slide for 1 hour.

After rinsing in PBS again for 15 minutes in 3 cycle

Slides are mounted on buffered glycerine and examined in fluorescence microscopy.

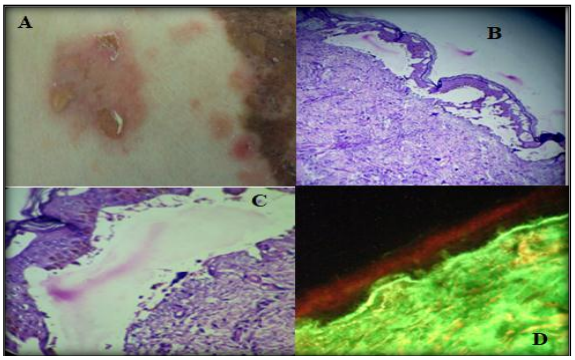

Figure 1: Bullous Pemphigoid: a.Small vesicles noted, b. Seperation at D:E junction, c. Cell poor blister cavity, d. Linear deposition of C3 at D-E junction.

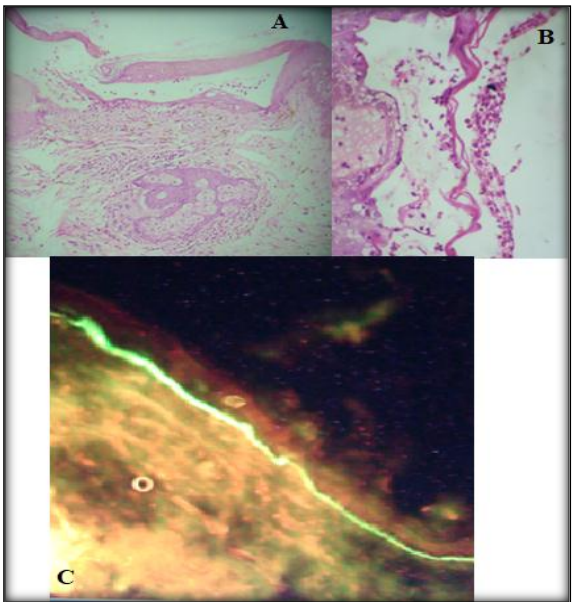

Figure 2: Bullous SLE, a. Blister at D:E junction with adnexal Inflammation, b. Inflammatory cellspredominatly Neutrophilis noted, c. Linear deposition of IgG at D-E junction.

RESULTS

The present study was conducted over a period of 24 months in the department of Pathology, at Dr B R Ambedkar Medical College, Bangalore.

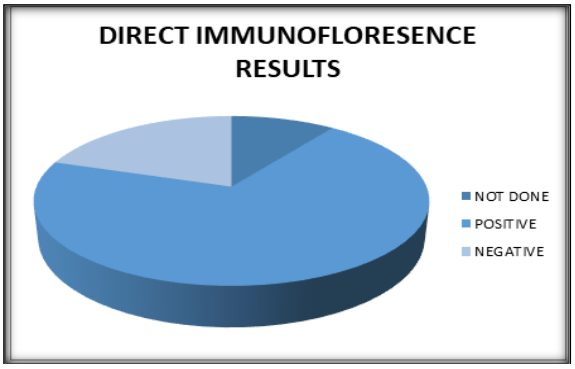

Figure 3: Direct immunofloresence results

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 1: Distribution of Cases.

Type	Frequency	Percentage
Bullous Pemphigoid(BP)	13	62%
Erythema multiforme (EM)	3	14%
Dermatitis Herptiformis(DH)	2	9.5%
Bullous drug eruption(BDE)	2	9.5%
BullousSLE(BSLE)	1	4.7%

In the present study Bullous Pemphigoids constituted the most common Subepidermal vesiculobullous disorders constituting 62% followed by Erythema Multiforme 14%.

Table 2: Age distribution of vesiculobullous disorders

FD	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80 &>
BP	-	-	-	1(7.6%)	2(15.3%)	-	4(31%)	59(38%)	1(7.6%)
EM	1(33.3%)	1(33.3%)	-	1(33.3%)	-	-	-	-	-
DH	-	1(50%)	1(50%)	-	-	-	-	-	-
BDE	-	-	-	1(50%)	1(50%)	-	-	-	-
BSLE	-	-	1(100%)	-	-	-	-	-	-

In present study the most common age group of presentation was between age group of 60-80 years with slight female prepondarnce.

Table 3: Blisters in Vesiculobullous Disorder

FD	Present	Absent
BP	13(100%)	-
EM	1(33.3%)	2(66.6%)
DH	2(100%)	-
BDE	2(100%)	-
BSLE	1(100%)	-

In this study BP showed blisters in 100% of cases, EM less commonly presented with blisters.

Table 4: Dermal Changes.

FD	Dermal edema	Papillary microabseces	Melanin incontinence	Dermal inflitration	Perivarscular infiltration	Adenxal infiltration
BP	1(7.6%)	0	0	11(84.6%)	6(46.15%)	1(7.6%)
EM	1(33.3%)	0	0	1(33.3%)	1(33.35)	0
DH	1(50%)	2(100%)	0	2(100%)	2(100%)	0
BDE	0	0	0	2(100%)	2(100%)	0
BSLE	0	0	0	1(100%)	1(100%)	0

BP showed 84.6% of dermal infiltration and 47% of perivascular infiltration. Dermal edema was noted in Bullous Pemphigoid [7.6%], Erythema Multiforme [33.3%], and Dermatitis Herpitformis [50%].

Table 5: Inflammatory Cells in Blister

FD	Absent	Neutrophil	Lymphocyte	Eosinophil	Macrophage	Mixed
BP	1(7.6%)	0	0	10(76.4%)	0	2(15.3%)
EM	1(33.3%)	0	1(33.3%)	1(33.3%)	0	0
DH	0	2(100%)	0	0	0	0
BDE	0	0	0	2(100%)	0	0
BSLE	0	1(100%)	0	0	0	0

DH and BSLE predominantly showed neutrophils. Eosinophils was seen in Bullous pemphigoid and BDE. Mixed inflammation was seen in BP.

DIF was positive in 71.4% of cases. 23.8% was negative.1 case it was not done

IgG was predominantly positive in DH (50%). C3 was seen in BP (46.15%). Both IgG and C3 was positive in PV, BP (46.15%). DIF was negative in EM, BDE.

DISCUSSION

In present study bullous pemphigoid constituted 62% with mean age of the patient in the range of 40-79 years. Male to female ratio (M: F) ratio being 1:1.1 which is similar to Lagan SM et al,[10] study.

All patient of bullous pemphigoid presented with bulla.

11 cases out of 13 (76.4%) showed sub epidermal blister. One case had suprabasal cleft. This might be due to an older lesion being biopsied. Inflammatory cells were noted in bulla (92.3%) and dermal infiltrate (84.7%) similar to Leena JB et al study Predominant.[11]

In present study DIF was done in 12 cases. DIF was not done in 1 case because of delay in sample collection. All 12 cases showed 100% positivity similar to Deepthi PK et al.[12]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

2 cases presented with Dermatitis herpitiformis which constituted 8%. Cases were in the age group of 10-19 and20-29 years respectively. Both of them presented as pustules. In one bulla was also noted.
Sub epidermal bulla was present in both cases with both showing papillary micro abscess. DIF was positive in 2 of the cases showing granular deposit of IgA in dermoepidermal junction similar to Banu L et al study.[13]
One female patient aged 24 years presented Bullous SLE with bulla and pigmentation with face involvement. Positive Nikolsky sign and erythematous base was noted. This study had similar findings to that of Chan LS - 1999 et al study of a 15 year old female.[14]
HPE: Showing sub epidermal blister with neutrophil infiltration in blister cavity and dermis.
DIF showed linear deposition of IgG and C3 along dermo- epidermal junction.
Present study had 3 Erythema multiforme patients in paediatric age group same as Mateos M et al study.[15] 2 out of 3 patients were females. Only1 patient presented with bulla. One patient had pigmentation similar to Mateos M et al study.[15]
HPE: 2 cases showed subepidermal blister with inflammatory cell predominantly lymphocytes and eosinophils. 1 case did not show any separation. DIF was negative in all 3 cases.
2 cases with Bullous Drug Eruption where noted in present study. Both cases were male patient similar to cheng –Han L et al study.[16] Histopathology showed bulla in dermoepidermal junction, perivascular infiltration was predominant. Blister showed predominantly eosinophilic infiltration similar to Chen- Han L et al study. DIF in both cases showed negativity.[16]

CONCLUSION

Subepidermal bullous lesions of skin are most devastating diseases they target basement membrane by autoantibodies. Bullous Pempigoid constituted the most common Subepidermal bullous disease of skin. Immunofluorescence techniques are essential to supplement clinical findings and histopathology in the diagnosis of the Immunobullous disorders.[17]

REFERENCES

1. Padniewski JJ, Shaver RL, Schultz B, Pearson DR. Patient Quality of Life Improvement in Bullous Disease: A Review of Primary Literature and Considerations for the Clinician. Clin Cosmet Investig Dermatol. 2022;15:27-42. doi: 10.2147/CCID.S324331.
2. Hofmann SC, Juratli HA, Eming R. Bullous autoimmune dermatoses. J Dtsch Dermatol Ges. 2018;16(11):1339-1358. doi: 10.1111/ddg.13688.
3. Tani M, Shimi R. SLE with vesiculobullous lesion: immunoelecton microscopic study. Arch Dermatol.1984;120(11): 149-501
4. Mysorekar VV, Sumathy TK, Shyam Prasad AL. Role of direct immunofluorescence in dermatological disorders. Indian Dermatol Online J. 2015;6(3):172-80. doi: 10.4103/2229-5178.156386.
5. Gane NF. A guide to bullous lesions of the skin. J Clin Pathol. 1973;26(3):235-7. doi: 10.1136/jcp.26.3.235.
6. Huilgol SC, Bhogal BS, Black MM. Immunofluorescence of the immunobullous disorders part one : Methodology. Indian J Dermatol Venereol Leprol. 1995;61(4):187-95.
7. Arbesman J, Grover R, Helm TN, Beutner EH. Can direct immunofluorescence testing still be accurate if performed on biopsy specimens after brief inadvertent immersion in formalin? J Am Acad Dermatol. 2011;65(1):106-11. doi: 10.1016/j.jaad.2010.06.019.
8. Tariq M, Tahir SH. Patterns of autoimmune bullous diseases of skin in Lahore, Pakistan. J Pak Assoc Dermatol. 2003;13:67-69
9. Inchara YK, Rajalakshmi T. Direct immunofluorescence in cutaneous vesiculobullous lesions. Indian J Pathol Microbiol. 2007;50(4):730-2.
10. Langan SM, Smeeth L, Hubbard R, Fleming KM, Smith CJ, West J. Bullous pemphigoid and pemphigus vulgaris--incidence and mortality in the UK: population based cohort study. BMJ. 2008;337(7662):a180. doi: 10.1136/bmj.a180.
11. Leena JB, Chandrashekar M, Vijaya B, Sunila R, Manjunath GV. A clinicopathological Study of immunobullous lesions of the skin a 2 years study. Adv Lab Med Int. 2012; 2(2): 49 - 60.
12. Deepthi PK, Santosh CS, Suguna B, Gopal M, Abhishek. Direct immunofluorescence study in autoimmune bullous disordersof skin. IJBAR. 2013;04(09):611-618.
13. Lebe B, Gül Nıflıoğlu G, Seyrek S, Ellıdokuz H. Evaluation of clinical and histopathologic/direct immunofluorescence diagnosis in autoimmune vesiculobullous dermatitis: utility of direct immunofluorescence. Turk Patoloji Derg. 2012;28(1):11-6. doi: 10.5146/tjpath.2012.01091.
14. Tristano AG. A patient with multiple blisters in the skin and mucous membranes. BMJ Case Rep. 2010;2010:bcr06.2009.1962. doi: 10.1136/bcr.06.2009.1962.
15. Martín Mateos MA, Roldán Ros A, Muñoz-López F. Erythema multiforme: a review of twenty cases. Allergol Immunopathol (Madr). 1998;26(6):283-7.
16. Anderson HJ, Lee JB. A Review of Fixed Drug Eruption with a Special Focus on Generalized Bullous Fixed Drug Eruption. Medicina (Kaunas). 2021;57(9):925. doi: 10.3390/medicina57090925.
17. Amber KT, Murrell DF, Schmidt E, Joly P, Borradori L. Autoimmune Subepidermal Bullous Diseases of the Skin and Mucosae: Clinical Features, Diagnosis, and Management. Clin Rev Allergy Immunol. 2018;54(1):26-51. doi: 10.1007/s12016-017-8633-4.

ISSN (O): 2687-5365; ISSN (P): 2753-6556