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# HISTOPATHOLOGICAL STUDY OF VESICULOBULLOUS LESIONS OF SKIN WITH SPECIAL REFERENCE TO DIRECT IMMUNOFLUORESCENCE

Pathology

**Dr. P Sriram** Assistant Professor, Department Of Pathology, Government Villupuram Medical College, Villupuram, Tamil Nadu, India.

**Dr. M Premkumar** Assistant Professor, Department of Dermatology, Chettinad Medical science & research Institute, Kelambakkam, Tamil Nadu, India.

## ABSTRACT

Skin represents a window to the internal well-being of the disease. Vesiculobullous diseases represents a heterogenous group of dermatoses with protean manifestations. One of the main reason for continued identification of new bullous diseases is because the diagnosis is based on immunological & molecular basis in addition to clinical findings & immunomorphology. This study aims to correlate Direct Immunofluorescence with clinical and Histopathological findings and to analyze the utility of Immunofluorescence in the diagnosis of vesiculobullous lesions of skin.
A total of 50 cases were evaluated with vesiculobullous lesions with clinical history, signs, histopathological and immunofluorescence studies. In this study, Bullous Pemphigoid and Pemphigus vulgaris constituted 82 % of cases. Oral mucosal involvement was present in 89.5 % cases of Pemphigus Vulgaris & 22.7% cases of Bullous pemphigoid. Subepidermal blister/ bullae is commonest in the study, next being supra basal blister/ bullae. 71.4 % were positive for Direct IF in which 2/3 cases staining pattern was throughout the entire epidermis & in 1/3 cases staining pattern was restricted to lower 1/3 of epidermis. 50% of Dermatitis Herpetiformis showed positive staining by IgA in papillary dermis showing granular BM zone pattern. In Bullous Pemphigoid subjected for DIF, 70% cases were positive with linear basement membrane zone deposition of antibodies.

## KEYWORDS

Immunofluorescence, vesiculobullous lesions, basement membrane, Fluorochrome

## INTRODUCTION

Skin forms not only the protective covering but is a part of immune apparatus of the body. Skin is the single largest organ in the body. The pathophysiology of vesiculobullous diseases illustrates how impairment in epithelial adhesion molecules leads to disorders characterized by substantial morbidity & mortality. Blistering diseases can be acquired or inherited. Most of acquired disorders are autoimmune in nature and are characterized by autoantibodies that target junctional molecules promoting either cell to cell or cell to matrix adhesion in the skin. The pemphigus group of disease is associated with antibodies to desmosomal proteins.

Commonly used modalities for the diagnosis of immunobullous disorders are histopathology and Immunofluorescence [ Both Direct & Indirect ] . Clinical examination of skin bullous lesions provide dermatologist gross morphological findings upon which the differential diagnosis can be made. However histopathology is needed for definitive diagnosis. Bullous lesions are frequently a source of dismay to the Pathologists. Skin biopsy are easily intended with precision. Direct IF microscopy gives the best diagnostic yield in bullous lesions to give a clear reporting.

## MATERIALS AND METHODS

Patients with vesiculobullous lesions of skin, who visited the Department of Dermatology between Jan '17 and 'Jun 18 for whom both histopathology and direct IF had been requested were included in this study.

After evaluating the patient clinically, clinical signs i.e., Nikolsky 's sign and bullae spread sign were elicited. Two punch biopsies of at least 3 mm were taken from each patient. One specimen was fixed in 10% formalin for routine histopathology and the other was preserved in Michel 's medium for IF study. Sections of 3µm thickness were made and stained with H & E.

DIF technique was used to detect the presence of immunoreactants deposited in vivo in patients skin or mucosa. The immunoreactants include Ig, components of complement system and fibrinogen. This technique used fluorochrome dye tagged with antihuman Ig. They bind to specific immunoreactant already bound to epitopes within the epidermis. After washing to ensure removal of the excess dye, the tissue is viewed under UV light. The fluorochrome has the property to emit incident UV light as fluorescent radiation, thereby allowing visual localization of immunoreactant under the microscope.

The colour of fluorescent radiation depends on the dye used[1]. The most widely used fluorochrome is Fluorescein. It is best conjugated to protein in the form of fluorescein isothiocyanate which emits apple green fluorescence. The immunofluorescence pattern was studied and was reported as positive or negative for Direct IF.

## RESULTS

A total of 50 cases were evaluated with vesiculobullous lesion with history, clinical signs, histopathological and IF studies. In the entire study, majority of patients were male 58%. The Male : Female ratio was 4:3. This pattern was observed in all categories of diseases except for Pemphigus vulgaris where M:F ratio was 1:2 . In this study, Bullous Pemphigoid & Pemphigus vulgaris constituted 82% of cases.

**Table 1:- Distribution Of Various Types Of Vesiculobullous Lesions**

| Disorder | No Of Cases | Percentage |
|---|---|---|
| BULLOUS PEMPHIGOID | 22 | 44% |
| PEMPHIGUS VULGARIS | 19 | 38% |
| PEMPHIGUS FOLIACEOUS | 4 | 8% |
| DERMATITIS HERPETIFORMIS | 3 | 6% |
| EPIDERMOLYSIS BULLOSA | 1 | 2% |
| PEMPHIGUS VEGETANS | 1 | 2% |
| TOTAL | 50 | 100% |

Oral mucosal involvement in Vesiculobullous Lesions[2] was present in 89.5 % cases of Pemphigus Vulgaris and 22.7% cases of Bullous Pemphigoid. It was not seen in any of the cases of Pemphigus Foliaceous and Dermatitis Herpetiformis. Subepidermal blister/ bullae [3] is commonest in this study, next being suprabasal blister/ bullae. One case of Pemphigus vulgaris showed intraepidermal bullae.

Of the 22 cases of Bullous Pemphigoid, 18/22 cases were between the age s of 51 and 80. [Mean age 61 yrs]. Of these, 8/22 cases [36%] were between the ages of 51 and 60. Oldest patient in the study belonged to this group and was 80 yr old female. Of the 19 cases of Pemphigus Vulgaris, majority of cases 12/19 [63%] were between 41 and 60 yrs of age. The youngest patient in the entire study was a 6 days old female neonate who was a case of Epidermolysis Bullosa.

**Bullous Pemphigoid:**

8/22 cases [36.4%] of Bullous Pemphigoid shows positive bullae spread sign , with all other cases show negative for Nikolsky sign. 2/22 Cases [9%] showed subepidermal mixed inflammatory cell pattern in dermis and remaining 20/22 cases [90.9%] shows predominantly eosinophils. 2/22 Cases [9%] showed subepidermal mixed inflammatory cell pattern in dermis and remaining 20/22 cases [9o.9%] shows predominantly eosinophils. [Fig:1]

**Table 2: Bullous Pemphigoid - Clinical & HPE Features**

| S.NO | FEATURES | NO OF CASES | PERCENTAGE |
|---|---|---|---|
| 1. | NIKOLSKY SIGN + | 0 | 0 |
| 2. | BULLAE SPREAD SIGN + | 8 | 36.36% |

| | | | |
|---|---|---|---|
| 3. | ORAL INVOLVEMENT | 5 | 22.72% |
| 4. | SUBEPIDERMAL BLISTER | 22 | 100% |
| 5. | DERMAL INFILTRATE | 20 | 90.90% |
| 6. | DERMAL EDEMA | 5 | 22.72% |

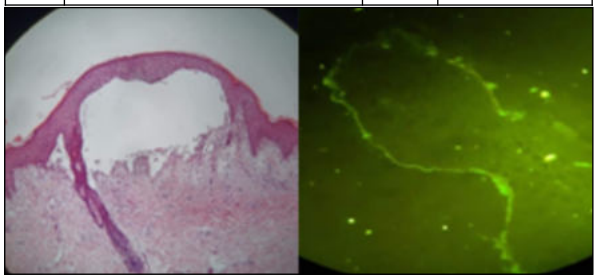

**Figure 1:** Subepidermal Bullae With Eosinophilic Infiltration In The DEMIS & Linear IgG, C3 Staining In DIF.

**Pemphigus Vulgaris:**
Nikolsky sign[4] was positive in 18 cases [94.7%].A majority of cases showed acantholysis and suprabasal cleavage with the classical tombstone appearance. [94.7 % and 100%] respectively [Fig:2]. Oral lesions was seen in 89.5% cases. Polymorphs was the predominant dermal inflammatory infiltrate in 52.6% cases. Negative DIF findings when the patient is seen in remission may be a good prognostic indicator[5,6].

**Table 3: Pemphigus Vulgaris - Clinical & HPE Features**

| S.NO | FEAUTRES | NO OF CASES | PERCENTAGE |
|---|---|---|---|
| 1. | NIKOLSKY SIGN | 18 | 94.7% |
| 2. | BULLAE SPREAD SIGN | 4 | 21.05% |
| 3. | ORAL LESIONS | 17 | 89.5% |
| 4. | SUPRABASAL BLISTER | 10 | 52.6% |
| 5. | TOMBSTONE APPEARANCE | 17 | 89.5% |
| 6. | SPONGIOSIS | 15 | 78.9% |
| 7. | ACANTHOLYSIS | 18 | 94.7% |
| 8. | DERMAL INFILTRATE – POLYMORPHS | 10 | 52.63% |
| 9. | LYMPHOCYTES | 4 | 21.05% |
| 10. | MIXED INFLAMMATORY INFILTRATE | 2 | 10.5% |

**Figure 2:** Pemphigus Vulgaris

**Pemphigus Foliaceous:** All 4 cases showed epidermal spongiosis with acantholysis. Sub-corneal bullae was seen in all 4 cases. The dermis showed perivascular lymphocytic infiltrate. No mucosal involvement was noticed[7].

**Pemphigus Vegetans:** Histopathology showed epidermis with a supra basal blister, acantholysis, marked acanthosis & papillomatosis [8]. Focal spongiosis [9] also seen. The blister cavity showed acantholytic cells and neutrophils.

**Dermatitis Herpetiformis:** 2 Cases presented with intensely pruritic bullae in the extensors of upper extremities. All 3 cases shows epidermis with subepidermal bullae and dermal papillary microabscesses containing neutrophils.

**Epidermolysis Bullosa:** A 6 day old female child with multiple large fluid filled blisters present all over the body. Few of them ruptured exposing erythematous base. HPE showed epidermis with a subepidermal bullae containing Neutrophils and plasma. The superficial dermis showed mild perivascular lymphocytic infiltrate.

**Direct Immunofluorescence Findings:** Of 7/19 cases of Pemphigus Vulgaris subjected for DIF, 5/7 cases [71.4%] were positive for DIF, with 3 cases [42.8%] showed squamous intercellular space] staining pattern alone. In 2/3 cases staining pattern was throughout the entire epidermis and in 1 case staining pattern was restricted to lower 1/3 of epidermis and 2 cases [28.6 %] showed squamous intercellular space staining pattern by both Ig G and $C_3$ throughout the entire epidermis. No case showed staining with Ig M and Fibrinogen. 2/7 case [28.6%] was negative for DIF.

In pemphigus foliaceous, 2/4 cases subjected for DIF, 50% showed squamous intercellular space pattern of staining throughout the epidermis by IgG and 50% showed negative reaction. No case showed positive staining for Ig M, $C_3$ and fibrinogen. Out of 10 cases of Bullous Pemphigoid subjected to DIF ,7/10 cases were positive with linear basement membrane zone deposition of antibodies and 3/10 [30%] cases came out as negative for DIF. These 3 cases showed subepidermal blister with characteristic eosinophils in the bullae as well as in the upper dermis.

In Dermatitis Herpetiformis, of 2/3 cases subjected for DIF,50% showed positive staining by Ig A in papillary dermis showing granular basement membrane zone pattern. As Epidermolysis Bullosa is a mechanobullous disorder, [results from genetic mutation in structural proteins in basement membrane] and not an immunobullous disorder, skin biopsy showed negative reaction for DIF.

**Table 4:- HPE & DIF Correlation**

| S.No | Disorder | No of cases | No of cases subjected for DIF | Cases with +ve DIF & diagnostic HPE | Cases with –ve DIF & diagnostic HPE |
|---|---|---|---|---|---|
| 1. | Bullous Pemphigoid | 22 | 10 | 7 [70%] | 3 [30%] |
| 2. | Pemphigus Vulgaris | 19 | 7 | 5 [71.4%] | 2 [28.6%] |
| 3. | Dermatitis herpetiformis | 3 | 2 | 1 [50%] | 1 [50%] |
| 4. | Pemphigus Vegetans | 1 | - | - | 1 [100%] |
| 5. | Pemphigus Foliaceous | 4 | 2 | 1 [50%] | 1 [50%] |
| 6. | Epidermolysis Bullosa | 1 | 1 | - | 1 [100%] |

## DISCUSSION

In the present study, clinical ,histopathological & DIF of various vesiculobullous diseases have been compared with various other studies. In present study, Bullous Pemphigoid was the most common vesiculobullous disorder constituting 44%[22 out of 50 cases ]followed by Pemphigus vulgaris [19 cases] 38%.Pemphigus Foliaceous being 8%[4cases] and Dermatitis Herpetiformis being 6%.This study showed similar results as that of Inchara YK etal[14].

Pemphigus vulgaris being the most common in all other studies i.e., Tsankov etal [11], Nanda A et al [12], and Nurul Kabir AKM et al [13], In present study, the most common age for Bullous Pemphigoid patient was above 60yrs similar to Bertram F et al[15] , Uzun S etal [16], Lagan SM et al [17], Jdy P et al [18] studies. DH also showed similarity with Betram et al [15] study.

In the present study, PV constituted 19 of the total number of cases of vesiculobullous disorder which is lower than that of Kanway AJ et al [21]., Vora et al [10]., Nafiesh et al [22], study.This shows that the disease has geographic changes. Mucosal involvement was seen in 89.5% in present study similar to other studies. Nikolsky sign was positive in 94.55% which was highest among all the other studies.

In Pemphigus Foliaceous, Nikolsky sign showed positivity in 75% cases which is lower than Arya et al study. Sub-corneal bulla and acantholysis showed 100% positivity similar to Vora et al.

In Dermatitis Herpetiformis, subepidermal bulla was present in all cases showing papillary microabscesses. DIF was positive in 1 of 2 cases [50%] showing granular deposits of Ig A in dermo-epidermal junction similar to Banu et al [24] study.

## CONCLUSIONS

The objective of the study was to observe the various histopathological

& IF findings in patients with vesiculobullous disorders and to correlate the clinical and pathological findings with DIF findings. Out of 50 cases of vesiculobullous disorders, Bullous Pemphigoid constituted the most common form with 44% followed by Pemphigus Vulgaris 38%. Nikolsky sign and Bullae spread sign were positive in majority of Pemphigus patients and negative in most of the patients with Bullous Pemphigoid. Oral mucosa was commonly involved in Pemphigus lesions except Pemphigus Foliaceous. Level of blister was supra-basal in most of PV cases and sub-corneal was seen in Pemphigus foliaceous.

Dermoepidermal junction separation was noted in Bullous pemphigoid, DH and epidermolysis bullosa. Acanthocytes was predominantly seen in PV, PF. PV also showed tombstone appearance and villi formation. Dyskeratosis was noted in PV. Papillary microabscess was noted in DH. Acantholytic cells were seen in blister cavity in PV along with inflammatory cells. Eosinophils were seen predominantly in BP.

DIF showed intercellular deposition of IgG and C3 in 5 out of 7 cases of which PV is a classical finding. Pemphigus foliaceous showed intercellular deposition of IgG in epidermis. Bullous Pemphigoid showed linear deposition of IgG and C3 in basement membrane zone. Granular deposition of IgA at dermoepidermal junction was noted in Dermatitis Herpetiformis.

IF studies have played a vital role in diagnosis and more importantly in understanding the pathophysiology of vesiculobullous lesions of skin. Although the technique has developed rapidly in western countries, its use as a diagnostic tool remain considerably low in our country. Two primary reasons that can be attributed to this are very high cost and technical expertise.

Although we found a high correlation between DIF and HPE in our study, role of DIF was highlighted especially in those histopathology was inconclusive or not compatible with clinical diagnosis. This is particularly true in subepidermal blistering disease where overlapping clinical and histological features a common diagnostic difficulty. DIF differentiates these disorders based on the type of autoantibody present and the pattern of staining of basement membrane zone.

Utility of DIF in the diagnosis of immune bullous lesion is unquestionable. The high correlation obtained in our study also indicates that Histopathology was accurate in most of the cases. DIF, HPE and clinical features forms a triad of diagnostic tool which complement each other. However if and when there is a need for a cost effective solution, DIF can be used selectively in lesions belonging to the grey zone which includes cases with clinical variation are those with inconclusive histopathological features or both.

Immunobullous lesions carry a grave prognosis and hence an accurate diagnosis with early therapeutic intervention is crucial in reducing the mortality of the patient. Clinical and histopathological features along with the judicious use of IF techniques together provide an accurate diagnosis.

## REFERENCES:


[1] Zahida R , Ijaz H.Immunofluorescence in immunobullous Diseases.Journal of Pakistan Association of Dermatologists. 2003;13:76-88.
[2] Harman K E Albert S ,Black M.M.Guidelines for the management of P.V. British Journal of Dermatology,2003.
[3] Yancey KB, Fairley JA. Introduction to the milestones in autoimmune bullous diseases, J Invest Dermatol.208 Oct ; 128:E15.
[4] Ratnarathorn.M, Newman J.Subcorneal pustular dermatosis(Sneddon- Wilkinson disease) occurring in association with nodal marginal zone Lymphoma: A case report.Dermatology Online Journa.2008 Aug;14(8):6
[5] David Elder 009),Hong Wu , Heather A , Brandling – Bennett, Terence J harrist, Non infectious vesiculobullous and vesiculopustular diseases.In Lever's Histopathology of Skin, David Elder (ed), 10th edition. Lippincott William & Wilkins.
[6] David Weedon (20020, The vesiculobullous reaction pattern. In: Skin Pathology , David Weedon (ed) 2nd edition .Churchill Livingstone ; Elsevier science Ltd.
[7] Kaul Holubar , M.D., Buffalo, ny; Herbert Honigsmann, M.D., Klaus Wolff,M.D. Cicatrical pemphigoid; Immunofluorescence investigations.Arch Dermatol 1973 : 108:50-52 .
[8] Ashish D, Paschal D, Ashok M, Raj KK . Pemphigus vegetans: an unusual presentation, Indian Dermatol Online J . 2012 Sep –Dec ; 3 (3 ): 193 -195
[9] Soni N, ChanderG,Vijay KG, Reddy BSN.Pmphigus vegetans of hallopeau.Indian journal of Dermatology , 2005; 50(3 ):166-167.
[10] Deval Vora,Vijay Popat, Viral Bhanvadia, Dimple A.hta, Bharat Bhetariya,Meet Kumar.A Study of 75 cases of Pemphigus in Saurashtra Region of dia. Ranian Journal of Dermatology 2010; 13 (2) 42 -46.
[11] Tsankov N, Vassileva S, Kamarashev J, Kazandjieva J, Kuzeva V.Epidemiology of pemphigusin Sofia,Bulgaria: A 16- year retrospective study 1980-1995). Int j dermatol 2000; 39:104 -108.
[12] Nanda A, Dvorak R , Al- Saeed K, Al-Sabah H, Alsaleh Q . A Spectrum of autoimmune bullous diseases in Kuwait.Int J Dermatol 2004 ; 43 (12) : 876 – 881.
[13] Nurul Kabir AKM , Kamal M Choudry A M. Clinicopathological correlation of blistering diseases of Skin ,Bangladesh. Med res counc bull 2008; 34 ; 48-53.
[14] Inchara YK Rajalakshmi T. – Direct immunofluorescence in cutaneous vesiculobullous lesions. Indian J Pathol Microbiol .2007 ; 50(4): 730-732.
[15] Bertram FBrocker E ,Zillikens D, Schmidt.Prospective analysis of the incidence of autoimmune bullous disorders in Lower Franconia.Germany Journal der deutschen Dermatologischen Gesellschaft.2009 May , 7(5) , 434-439.
[16] Soner U, MuratD, Ayse A ,Suhan G, CananU etal.Pemphigus in the mediterraneanregion of Turkey; A study of 148 cases. International Journal of Dermatology.2006; 45:523.
[17] LanganSm, Smeeth L, Hubbard H,Fleming KM, Smithand JW.Pemphigus: An epidemiological studyof patients treated in Finnish hospitals between 1978.Acta Derm Venerol 1982 ; 62 :491 -6.
[18] Pascal J, Sophie B, Agnes S ,Philippe B , Christophe etal .Incidence and Mortality of bullous Pemphigoid in France.Journal of Investigative Dermatology.2012 ; 132 : 1998-2004.
[19] CozzaniE, Parodi A,Rebora A , Delmonte S, Barile M, NigroA.Bullous pemohigoid in Liguria: a 2 year survey. Ur Acad Dermatol Venerol 2001 ; 15 (40 : 317-319.
[20] Deepthi PK, Santhosh Cs, Suguna B, Gopal M, Abhishek, .Direct immunofluorescence study in autoimmune bullous disorders of skin. IJBAR 2013 ; 04 (09): 611 -618.
[21] Kanwar Aj, De D. Pemphigus in India.Indian J Dermatol Venereol Leprol.2011; 77:439-49.
[22] Nafiesh E, Chams –davatchi C, Valikhani M, Daneshpazhooh M, Balighi Ket al. Pemphigus vulgaris in Iran. A clinical study of 140 cases. International Journal of Dermatology.2007 Nov, 46(11): 1166-1170.
[23] Arya SR, ValANAD Ag,Krishna K.A clinicopathological study of 70 cases of Pemphigus . Indian J dermatol Venerol Leprol 1999;65 94):168-171.
[24] Banu 1, Gulen GN , SelenS , Hulya E. Evaluation of Clinical and histopathologic/ direct Immunofluorescence Diagnosis in Autoimmune Vesiculobullous Dermatitis : Utility of Direct Immnluorescence.2012, 28(1): 11-16.