Section: Pathology

Original Research Article

HISTOPATHOLOGICAL AND IMMUNOLOGICAL STUDY OF VESICULOBULLOUS LESIONS

Mynampati V R Krishna Prasanthi[1], Itha Muni Bhavani[2], V. Kalyan Chakravarthy[3]

[1]Assistant Professor, Department of Pathology, Katuri Medical College & Hospital, Guntur, Andhra Pradesh, India.
[2]Assistant Professor, Department of Pathology, Guntur Medical College, Guntur, Andhra Pradesh, India.
[3]Professor, Department of Pathology, Dr PSIMS &RF, Gannavaram, Andhra Pradesh, India.

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Corresponding Author:
Dr. Mynampati V R Krishna Prasanthi,
Email: ramya.mynampati@gmail.com

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ABSTRACT

Background: Vesiculobullous disorders are a heterogeneous group of dermatoses with protean manifestations. The accurate diagnosis of bullous diseases of the skin requires clinical history, histopathological, DIF findings and serological tests. ANA IF study helps in diagnosis of autoimmune bullous disorders, which are reliable and rapid techniques and aids the early diagnosis and treatment of potentially life-threatening disorders. The aim and objective are to study the histopathological spectrum and immunofluorescence patterns of various vesiculobullous skin lesions. To correlate clinical and histopathological diagnosis of vesiculobullous skin lesions by incorporating the immunofluorescence and serologicals tests. **Materials and Methods:** Total of 60 cases of vesiculobullous disorders were studied over a span of 2 years. This is an observational study. Skin biopsies from patients with vesiculobullous skin lesions were taken at a tertiary care hospital. Punch biopsies were taken for histopathological diagnosis. H& E stain was applied. Perilesional skin taken in normal saline for DIF procedure and Patients blood samples were taken for serological tests. **Result:** In the present study pemphigus vulgaris is the most common vesiculobullous disorders with 34% followed by bullous pemphigoid 26%. Majority of patients are between 40-49 yrs of age, with female prepondarance. In most of the cases HPE along with DIF provided accurate diagnosis. Only 1 case showed HPE and DIF discordance. ANA IF positivity is seen in 65% of the cases which helps to know the autoimmune nature among these diseases. **Conclusion:** DIF is not a substitute but supplement to histopathological diagnosis. Thus clinical history, histoopathological correlation with DIF is required for definite diagnosis. ANA study can be considered as markers for evaluation and prognosis of the autoimmune vesiculobullous lesions of skin.

INTRODUCTION

The skin forms a protective covering and is also a part of the immune apparatus of the body.[1] Skin acts as a window to the inner well-being of disease. Many internal disorders may manifest themselves in the skin.[2]

Vesiculobullous disorders represent a heterogeneous group of dermatoses with protean manifestations. They dramatically impact the patient and their family and have severe economic consequences for the health services and the family. The diseases have been the point of intensive investigation in recent years.[2]

A wide variety of bullous diseases are present, among them serious sequelae may be seen in some bullous lesions, necessitating early treatment and intervention to prevent further morbidity and mortality.[3] Clinical examination of skin bullous lesion provides a dermatologist the gross morphological finding upon which differential diagnosis can be found. However, HPE is essential for a definite diagnosis.[4]

As there is a wide variety of bullous lesions, they are approached based on the site, size, and shape of the bulla and the changes in the bulla, epidermis, and dermis.[5] Blisters occur at different levels within the skin in the various disorders; histologic assessment is essential for accurate diagnosis and gives an insight into the pathogenic mechanisms.

Knowledge regarding the molecular structure of the intercellular and cell-to-matrix attachments that provide the skin with mechanical stability and helps to understand this disease.[6]

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For an accurate diagnosis of disease, a thorough histopathological examination is required. Initial identification starts with the lesion site followed by classification according to location as an intraepidermal and subepidermal group, and the changes within the bullous lesion are also seen.

Adjacent to the lesion, epidermal changes are also noted like villi, hyperkeratosis, parakeratosis, spongiosis and acanthosis, accentuation of normal dermal papillae, and also the site of disease and age of the patient is vital in diagnosis.[5]

Histopathology alone in bullous lesions does not give a satisfactory result. Bullous lesions are immune-mediated, and the immunopathogenesis patterns are disease- specific and are of diagnostic importance. As a part of disease pathogenesis, many of these bullous lesions show immune responses at several locations as a dermo- epidermal junction, dermal blood vessels, etc. Immune deposits typically used in DIF are IgG, IgA, IgM, and C3.[7]

In addition to the clinical findings and histopathology, Immunofluorescence techniques improve the diagnosis of immune-bullous disorders. These reliable and rapid techniques permit early diagnosis and treatment of potentially life-threatening disorders.[8]

By Direct Immunofluorescent Microscopy, presence of immunocomplex can be detected and helps to arrive at a diagnosis. DIF is considered as a diagnostic tool for the detection of mostly subepidermal autoimmune diseases. Over the past two decades, many advances have been made in understanding the clinical behavior and molecular nature of autoimmune diseases.[6]

Aim of the study

1. To study the histopathological spectrum of vesiculobullous skin lesions.
2. To study immunofluorescence patterns in various vesiculobullous skin lesions.
3. To correlate clinical and histopathological diagnosis of vesiculobullous skin lesions incorporating the immunofluorescence and serological tests.

MATERIALS AND METHODS

This cross-sectional study was done on a total of 60 consecutive cases of vesiculobullous skin lesions received in the Department of Pathology over a two-year period from November 2018 to October 2020. The patient clinical information such as age, gender., type of lesion, site, size, number, duration, previous medical history, family history was obtained from clinical records. For every case, two conventional skin punch biopsies were taken, one from a representative skin lesion for histopathology study and the second one from the perilesional skin for immunofluorescence study and also patient's serum for ANA-IF. The biopsy tissue for histopathology was sent in formalin and the biopsy tissue for DIF was sent in saline and transported to the pathology laboratory immediately. Transport medium (Michel's medium) was not required for any of the cases, as transit time for the samples was minimal.

Inclusion Criterion

All cases of vesiculobullous disorder with fresh, active lesion and not on treatment attending the Dermatology Department irrespective of age, sex and associated diseases.

Exclusion Criterion

1. Autolysis specimen
2. Autoimmune bullous disorders on steroids, immunosuppressive drugs.
3. Infectious diseases- Viral- herpes simplex, varicella, hand-foot-mouth disease, herpes zoster. Fungal- candidiasis. Bacterial- congenital syphilis, bullous impetigo, staphylococcal scalded skin syndrome.
4. Inflammatory conditions- Bullous mastocytosis, erythema toxicumneonatorum e.t.c
5. Metabolic –Acrodermatitis enteropathica, lead poisoning, Diabetic bullae, porphyria cutanea tarda.
6. Drug induced-Bullous drug reactions, bullous fixed drug reaction, erythema multiforme, Steven Johnsons syndrome/TEN.
7. Dermatitis- Allergic contact dermatitis, Irritant contact dermatitis.
8. Traumatic conditions- Chemical injury, thermal injury, friction blister.
9. Environmental-Phototoxic reactions, photoallergic reactions, insect bite reactions.

Method of collection: Histopathology study: The specimen is received in 10% formalin. After scrutinizing the patient details, the specimen of skin bullous lesion is fixed in fresh formalin for 24 hrs. After overnight fixation of specimen in formalin, dehydration of tissue done with graded alcohol, then it is cleared in chloroform, followed by paraffin embedding and section cutting in rotary microtome. Sections of 3-4 micrometer thickness will be made & stained with haematoxylin and eosin staining and eosin staining and studied under light microscope

Direct immunofluorescence study: Skin specimen was obtained by 3-5mm punch or surgical biopsy. Biopsy specimen was snap frozen, if delayed tissue was kept in cold saline and transported directly to histopathology department immediately. For the frozen sections, skin biopsy specimens were embedded in optimal cutting temperature (OCT) medium and 4-6 micron-thick sections were cut on a cryostat. A minimum of 8-10 sections were cut for each case. Two sections were taken on each slide and the slides were dipped in cold acetone for 5 min. The slides were either stained immediately or stored at –20°C before staining. For staining, sections were brought to room temperature, air dried, washed with phosphate-buffered saline (PBS) at pH 7.2 with three changes over a period of 30 min. Drain off the excess PBS and wipe around the section with tissue paper and layered with fluorescein isothiocyanate (FITC)-conjugated rabbit antihuman

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immunoglobulin IgG, IgA, IgM, and C3c (dilution 1:30). These slides were incubated for 2 h in a moist chamber at room temperature. The sections were then washed with PBS with three changes over a period of 30 minutes and the slides were mounted in buffered glycerol using glycerol and PBS mixed in equal volume, and viewed under a fluorescence microscope and observations were recorded. During entire process avoid direct light on the slides all the time.

ANA-IF Technique: To perform the ANA blood test, a blood sample is drawn from the patient and sent to the lab for testing. Serum from the blood specimen is added to microscope slides which have commercially prepared cells on the slide surface. If the patient's serum contains antinuclear antibodies, they bind to the cells (specifically the nuclei of the cells) on the slide. A second antibody, commercially tagged with a fluorescent dye, is added to the mix of patient's serum and commercially prepared cells on the slide. The second (fluorescent) antibody attaches to the serum antibodies and cells which have bound together. When the slide is viewed under an ultraviolet microscope, antinuclear antibodies appear as fluorescent cells.

- If fluorescent cells are observed, the ANA blood test result is positive.
- If fluorescent cells are not observed, the ANA blood test result is negative.

RESULTS

The present study was conducted over a period of 24 months from November 2018 to October 2020 in the department of Pathology, at tertiary care hospital.

Table 1: Distribution of Vesiculobullous Cases

Type	Frequency	Percentage
Pemphigus Vulgaris -PV	21	35%
Bullous Pemphigoid - BP	16	26.6%
Pemphigus Foliaceus -PF	12	20%
Hailey Hailey Disease - HHD	2	3.3%
Dariers Disease - DD	2	3.3%
Erythema Multiforme EM	2	3.3%
Dermatitis Herpetiformis - DH	3	5%
Chronic Bullous Disorder -CBD	1	1.6%
Pemphigus Erythematosus -PE	1	1.6%
Total	60	100%

In the present study pemphigus vulgaris constituted the most common vesiculobullous disorders constituting 35% [21 out of 60 cases] followed by Bullous pemphigoid 26.6% [16 out of 60 cases], Pemphigus foliaceus 20% (12 of 60 cases), Hailey-Hailey disease, Dariers disease, erythema multiforme constituted 3.3% [2 out of 60 cases]. Dermatitis herpetiformis cases accounts for 5% (3 out of 60 cases) and the Least common was chronic bullous disorder of childhood and pemphigus erythematosus which constituted 1.6% [1 out of 60 cases].

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 &>
PV	-	-	2(9.5%)	11(52.3%)	5(23.8%)	2(9.5%)	-	1(4.7%)	-
BP	-	-	-	2(12.5%)	4(25%)	2(12.5%)	2(12.5%)	6(37.5%)	-
PF	-	2(16.6%)	6(50%)	4(33.3%)	-	-	-	-	-
HHD	-	-	-	2(100%)	-	-	-	-	-
DD	-	-	-	1(50%)	1(50%)	-	-	-	-
EM	-	1(50%)	1(50%)	-	-	-	-	-	-
DH	-	1(25%)	2(75%)	-	-	-	-	-	-
CBD	1(100%)	-	-	-	-	-	-	-	-
PE	-	-	1(100%)	-	-	-	-	-	-

In present study Pemphigus vulgaris presented most commonly in age group of 30-39 years [52.3%] followed by 40-49 [23.8 %] years age group. Bullous pemphigoid presented commonly in the age group of 70-79 years [37.5%]. Pemphigus foliaceus, DH and PE were common at age group of 20-29 years [50% ,75%and 100% respectively].

Table 3: Sex Disrtibution of Vesiculobullous Disorder

FD	Male	Female
PV	5(23.8%)	16(76.19%)
BP	7(43.75%)	9(56.25%)
PF	3(25%)	9(75%)
Hailey Hailey disease	1(50%)	1(50%)
Dariers disease	1(50%)	1(50%)
DH	1(33.3%)	2(66.6%)
EM	1(50%)	1(50%)

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CBD	-	1(100%)
PE	-	1(100%)
Total cases	19	41

In Present study PV, BP, PF, DH, PE and CBD showed predominantly female predominance.

Table 4: Blisters In Vesiculobullous Disorder

FD	Present	Absent
PV	20 (95.2%)	1(4.7%)
BP	16(100%)	-
PF	10(83.3%)	2(16.6%)
HAILEY HAILEY DISEASE	1(50%)	1(50%)
DARIERS DISEASE	1(50%)	1(50%)
EM	1(50%)	1(50%)
DH	3(100%)	-
CBD	1(100%)	-
PE	1(100%)	-
Total	54(90%)	6(10%)

In this study it was noticed that 54 cases [90%] presented with blisters. PV showed blisters in 95.2% of cases, BP, DH showed blisters in 100% of cases.

Table 5: Level of blister

FD	No separation	Suprabasal	Subcorneal	D:E Junction	Intraepidermal
PV	1(4.76%)	19(90.47%)	0	0	1(4.76%)
BP	0	0	0	16(100%)	0
PF	2(16.7%)	0	10(83.3%)	0	0
HHD	1(50%)	1(50%)	0	0	0
DD	1(50%)	1(50%)	0	0	0
EM	1(50%)	0	0	1(50%)	0
DH	0	0	0	3(100%)	0
CBD	0	0	0	1(100%)	0
PE	0	0	0	1(100%)	0

In present study 90.47% of PV and 50 % of Hailey Hailey disease, Dariers disease, showed supra-basal separation. Dermo- epidermal junction separation was seen in 100% cases of BP, 50% of EM, 100% of DH, CBD and PE respectively. 4.76% cases of PV showed intraepidermal separation.83.3% cases of PF show sub-corneal separation. Remaining 4.76% of PV, 16.7% of PF, 50% of HHD,DDand EM did not show any separation.

Table 6: inflammatory cells in blister

FD	Absent	Neutrophil	Lymphocyte	Eosinophil	Macrophage	Mixed
PV	1(4.8%)	12(57%)	0	0	0	8(38%)
BP	1(6.2%)	0	0	13(81.2%)	0	2(12.5%)
PF	3(25%)	6(50%)	3(25%)	0	0	0
HHD	0	2(100%)	0	0	0	0
DD	0	2(100%)	0	0	0	0
EM	1(50%)	0	1(50%)	0	0	0
DH	0	3(100%)	0	0	0	0
CBD	0	1(100%)	0	0	0	0
PE	0	1(100%)	0	0	0	0

PV, PF, DH, HHD, DD, CBD and PE predominantly showed neutrophils. Eosinophils were seen in Bullous pemphigoid. Mixed inflammation was seen in PV and BP.

Table 7: direct immunofloresence results

DIF	Frequency	Percentage
Not done	10	17%
Positive	42	70%
Negative	08	13%

DIF was positive in 70% of cases. 17% was negative.

Table 8: antibody deposition

FD	IgG	IgM	IgA	C3	Negative	Not done	IgG+c3	IgG+IgA
PV	11(52.3%)	0	0	0	2(9.5%)	4(19%)	4(19%)	0
BP	0	0	0	8(50%)	0	3(18.7%)	5(31.3%)	0
PF	6(50%)	0	0	0	0	3(50%)	3(25%)	0
HHD	0	0	0	0	2(100%)	0	0	0
DD	0	0	0	0	2(100%)	0	0	0
EM	0	0	0	0	2(100%)	0	0	0

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DH	0	0	1(33.3%)	0	0	0	0	2(66.6%)
CBD	0	0	1(100%)	0	0	0	0	0
PE	1(100%)	0	0	0	0	0	0	0

IgG was predominantly positive in PV (52.3%), PF (50%). C3 was seen in BP (50%). Both IgG and C3 was positive in PV (19%), BP (31.3%), PF (25%). DIF was negative in EM, HHD and DD. IgA + IgG positive in 66.6 % of DH cases.

Table 9: Pattern of deposition of antibodies in positive cases

FD	Squamous intercellular spaces	Dermo-epidermal junction
PV	15(100%)	-
BP	-	13(100%)
PF	9(100%)	-
Hailey Hailey disease	-	-
Dariers Disease	-	-
EM	-	-
DH	-	3(100%)
CBD	-	1(100%)
PE	-	1(100%)

Table 10: discordance of HPE with DIF

Histopathology Diagnosis	DIF
PV	Negative

Only one case of pemphigus vulgaris showed discordance with DIF

Table 11: ANA IF table

Disease	Total no of cases	No of Cases test done	Test not done in	Pattern of ana-if
PV	21	15(71.4%)	06(28.57%)	Homogenous with few speckled
BP	16	10(62.5%)	06 (37.5%)	Homogenous
PF	12	07(58.3%)	05(41.6%)	Homogenous
HHD	2	2(100%)	0	Homogenous
DD	2	1(50%)	1(50%)	Homogenous
EM	2	0	2 (100%)	-
DH	3	2(66.6%)	1(33.33%)	Homogenous
CBD	1	1(100%)	0	Homogenous
PE	1	1(100%)	0	Homogenous
Total	60	39(65%)	21(35%)	

Homogenous with speckled pattern is the predominant pattern seen in the pemphigus vulgaris (71.4% of total case, 100% among the tests done), Homogenous pattern is most common pattern in remaining all the other vesiculobullous cases. Homogenous pattern is seen in bullous pemphigoid (62.5%), pemphigus foliaceous (58.3%), Hailey Hailey disease (100%), Dariers disease (50%), Dermatitis Herpetiformis (66.6%), Chronic bullous disorder of Childhood (100%)

Figure 1a: Clinicalpicturewith Crusted erosion.

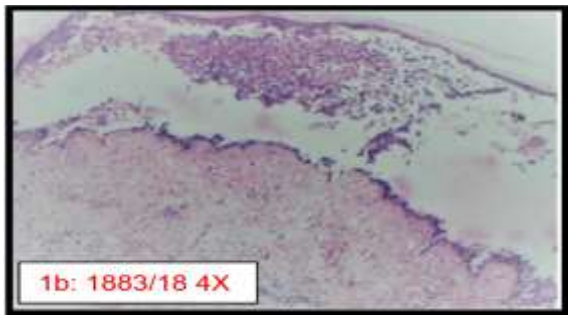

Figure1b:H&E4X, photomicrograph showing Tombstone appearance with acanthocytes in blister cavity

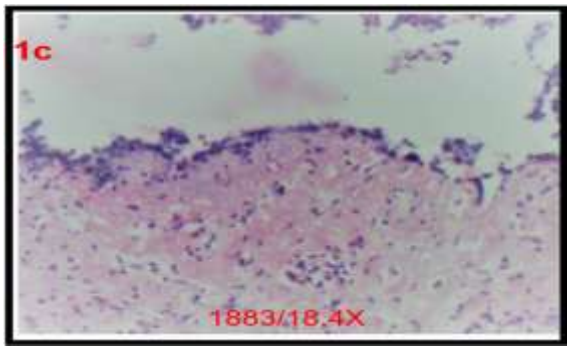

Figure 1c: H&E,4X photomicrograph showing Supra basal bulla.

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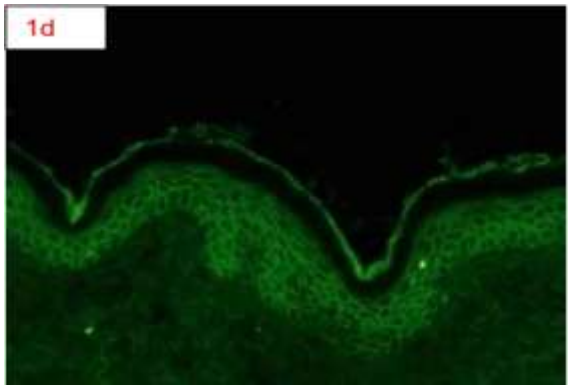

Figure 1d: DIF showing Intercellular deposition of IgG lace like/fish net appearance.

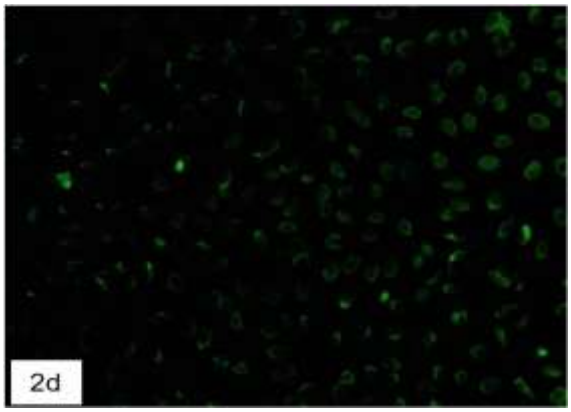

Figure 2d: ANA-IF showing homogenous positivity

Bullous Pemphigoid: [Figure 2a to 2d]

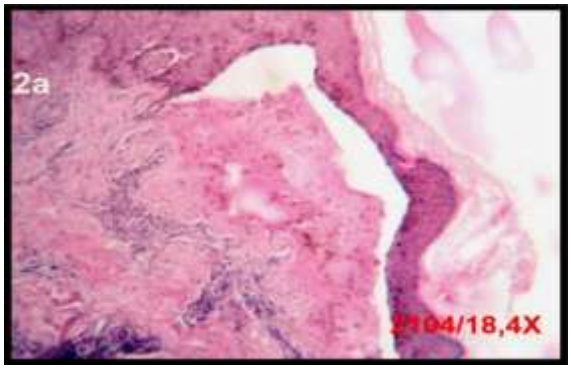

Figure 2a: H&E,4x photomicrograph showing Subepidermal blister

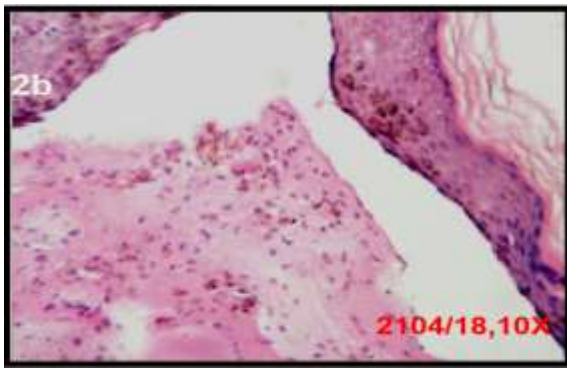

Figure 2b:H&E,10x photomicrograph showing sub-epidermal blister with Eosinophils.

Figure 2c: DIF showing focal linear deposits of IgG and junction

Pemphigus Foliaceus: [Figure 3a to 3c]

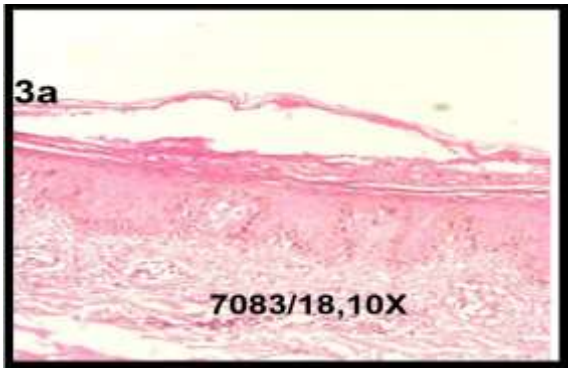

Figure 3a: H& E, 4x photomicrograph showing Sub corneal separation of bulla.

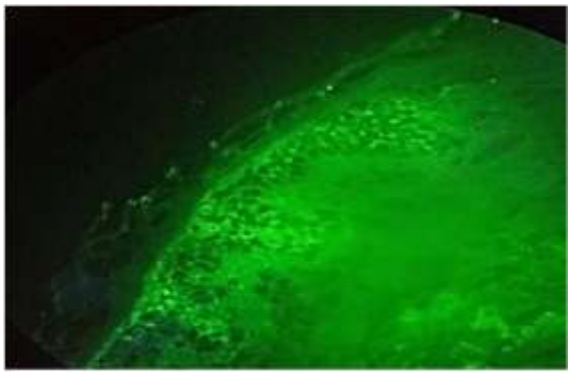

Figure 3b: DIF showing intercellular lace like deposition of IgG at D: E junction.

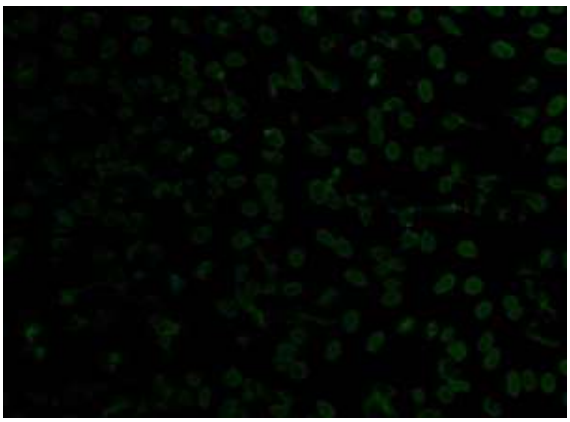

Figure 3c: ANA–IF showing homogenous pattern

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Chronic Bullous Dematosis of Childhood: [Figure 4a to 4c]

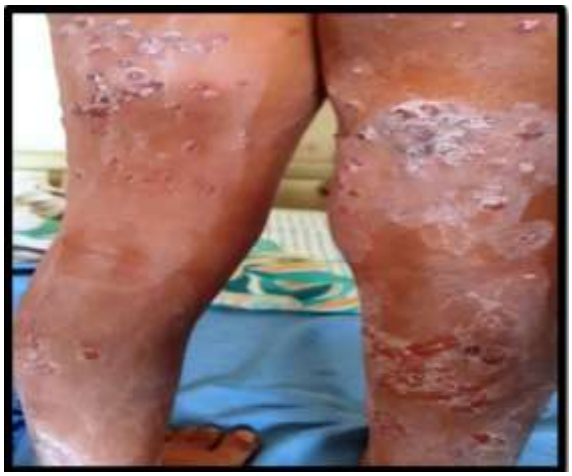

Figure 4a: Multiple vesicles and bullae are present on erythematous base in crops overneck, upperlimbs, chest, buttocks, lower limbs.

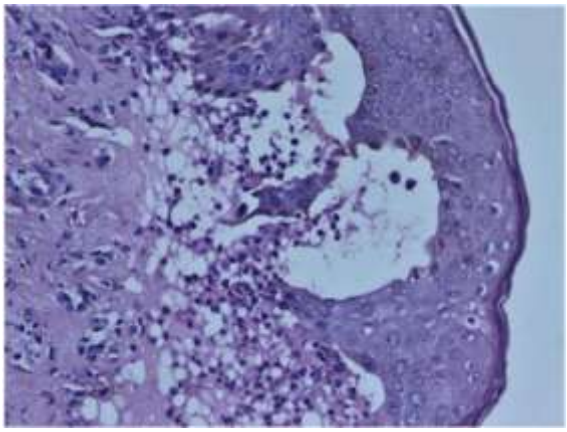

Figure 4b: H&E 20x photomicrograph showing Subepidermalblister with neutrophil infiltration.

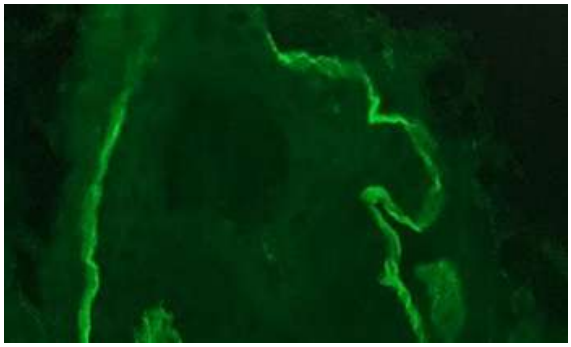

Figure 4c: DIF showing Ig Apositiveat dermo-epidermal junction

DISCUSSION

The immune-bullous diseases are a group of autoimmune diseases in which components of the epidermis and basement membrane zone are the focus of attack, resulting in the formation of cutaneous and mucosal blisters.

Early diagnosis and treatment of these severe and potentially life-threatening disorders are vital. Several disorders fall under the category of immune-bullous diseases and are broadly classified as intra-epidermal and sub-epidermal blistering disorders. Intra-epidermal blistering disorders, apart from those mediated by immunological mechanisms, include inherited disorders like Darier's and Hailey-Hailey disease. Secondary damage by severe intercellular edema can also cause acantholysis, as seen in spongiotic dermatitis and transient acantholytic dermatosis. In a similar fashion, sub-epidermal blistering disorders include mechano-bullous disorders like epidermolysis bullosa congenita, in addition to the immune-bullous lesions. Direct immune-fluorescence (DIF) has been widely used as a supplement to clinical examination and histopathology in the evaluation of vesiculobullous lesions of the skin. As a prognostic tool it is limited by the fact that DIF continues to remain positive many years after the patient has gone into clinical remission.

The present study was conducted across a period of 24 months from November 2018 to October 2020 in the department of Pathology, at Tertiary care hospital.

In the present study, clinical, histopathological, and direct immunofluorescence along with serological tests in the required cases of various vesiculobullous diseases have been discussed and compared with various other studies in detail below.

It is an observational study with a short study case; no statistical tests are applied for the analysis of data, and the results are expressed in numbers and percentages. As it is a hospital-based study and so the above number does not reflect the true incidence of vesiculobullous disorders in the community.

Pemphigus Vulgaris was the most prevalent vesiculobullous disorder constituting 35% (21 out of 60 cases) followed by bullous pemphigoid 26.6%(16 cases). PF being 20% (12 cases) and EM, HHD, DD, DH being 3.3% (2 cases each).

This study showed similar results as that of Patel et al., Deepti et al.study. The present study also included PE, Hailey Hailey disease, Dariers disease and chronic bullous disorder of childhood [Table 1].

PV being the most common is similar to Vora D et al,[9] Tsankov N et al,[10] Nanda A et al,[11] Nurul Kabir AKM et al,[12] Mysorekar et al studies.[13-15]

The present study showed various vesiculobullous disorders like DH, PE, Hailey Hailey disease, Dariers disease, and chronic bullous disorder of childhood, which are not seen in other studies.[16]

In the present study, bullous pemphigoid most common age was above 70 years similar to Bertram F et al,[17] Uzun s et al,[18] Lagan SM et al,[19] Joly P et al,[20] and Deepti et al studies.[14] PV ranged from 30-49 years similar to Lagan SM et al study.[19] DH did not show similarity with Bertram F et al,[17] study but similar to Deepti et al study.[14]

Pemphigus Vulgaris

The predominant lesion among the sixty cases involved in the study was pemphigus Vulgaris [Table 1] with 21 cases (35%). DIF was positive in

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the majority of cases (15 /17 cases test done [Table 8]. The most age group affected was 30-39 years [Table 2], which is consistent with Arya SR et al.[21] Study. Our study showed female predominance with 16/21cases (76.19%) shown in [Table 3].

Histopathology: Histopathology showed blister formation in 20/21 cases [Table 4] which is predominantly supra-basal in location 19/20 cases [Table 5]. The predominant inflammatory infiltrate of these blisters being neutrophils in majority of the cases (12 cases) i.e, 57% and mixed inflammatory cells are seen in 7 cases (38%). One case i.e, 4.8% in the current study showed no inflammatory infiltrate in the blister cavity. Other features like Acantholysis, row of tombstone appearance was seen and are well correlated with other studies.

Supra-basal bulla was seen in 19 cases (90.4%) close to the study of Deepti et al study.[14] Acantholysis was seen in 20 cases (95.2%), which is similar to that observed by Arya SR et al.[21] Vora D et al,[9] and Deepti et al study. Row of tombstone appearance seen in 16 (76.1%) of cases which is higher than the Arya SR et al,[21] and Vora D et al,[9] study and similar to Deepti et al study. Inflammatory cells were noted in 95.2%, which is again higher than that of all the four above mentioned study.

Direct immunofluorescence was done in 17 cases of pemphigus Vulgaris. 15 (88.23%) cases were positive, two cases were negative. As compared to Kaur JS et al,[24] study which showed 100 % positive DIF. Chams-Davatchi C et al,[23] study had 417 cases of pemphigus vulgaris out of 1111, among which 389 (93.28%) were positive. Deepti et al,[14] study also showed 94.11% of positive DIF. This shows that even the most definitive investigation may be negative in some cases. So, the diagnosis depends on clinical, histopathological, and immunofluorescence study.

Out of 17/21 cases of DIF performed, most of the cases showed positive reactions with IgG 11/17 cases (64.7%) and 4/17 cases showed both IgG and C3 (23.52%) shown in [Table 8].

In the current study ANA IF was done in 15/21 cases (71.4%) [Table 11] and in remaining cases ANA-IF was not performed. Positive ANA-IF cases show homogenous with speckled pattern indicating that most of cases have autoantibodies in them and are autoimmune in nature. Various studies show that the most of the vesiculobullous lesions of the skin show autoantibodies in them. 25,26,27.

Pemphigus foliaceus: Of the 12/60 cases (20%) were pemphigus foliaceous [Table 1] and six cases were between the ages of 20 and 29. Two cases were between 10 to 19 years [Table 2]. The age group affected by foliaceous is usually quite variable. There was female preponderance in 9 cases i.e. 75% of the cases and males constitute about 25% of the cases.

Histopathology: In the current study blister formation is seen in 10 cases out of 12 cases (i.e. 83.3%) and in about 16.6% (2 cases) no blister formation was seen. All the 10 cases show blister in sub-corneal location and the two cases showed no plane of separation.

In the present study the presence of Sub-corneal bulla was similar to Vora D et al study. Inflammatory cell was seen in 75% cases which did not show similarity with the above study. The Predominant inflammatory infiltrate was neutrophils (in 50% cases) followed by the lymphocytes (25% cases), remaining 25% of the cases did not show any inflammatory infiltrate in the blister cavity.

DIF showed a moderately strong ICS pattern of staining with IgG alone, which was restricted to the upper 2/3rd of the epidermis. Due to the discordant findings and the need for rapid therapeutic intervention, the patient was started empirically on both methotrexate and omnacortil (treated for both pustular psoriasis and pemphigus foliaceous). The lesions subsided. Advised further investigations. Biopsies from patients with pemphigus foliaceous may occasionally show only collections of neutrophils in the subcorneal plane and hence may mimic subcorneal pustular lesions like pustular psoriasis. The findings in DIF proved invaluable in this case as both clinical and histological findings had been misleading.

Direct immunofluorescence was done in 9 cases of pemphigus foliaceus, and the findings were suggestive of pemphigus foliaceus in all the cases (100%). Deepti et al,[14] Chams- Davatchi C et al,[23] study showed 88.2 %. DIF positive and Inchara YK et al,[13] showed 100 % DIF positive. DIF finding are similar to PV, but histopathology differentiates between PV and PF. So DIF is just supplement but not a substitute ANA IF done in 7/12 cases (58.3%) [Table 11] shows a homogenous pattern.

Saleh, Marwah Adly et al 25 study showed that ANA was recognized in 40% of Egyptian PV patients compared to 5.7% Tunisian PF patients, 13.7% Tunisian PV and 0%–2.5% Brazilian PF. Opposite to Tunisians in whom the pattern of the ANA was speckled in the majority of patients, the pattern was homogenous (50%) and speckled (50%) in the Egyptians. Homogenous pattern reflected antibodies to histones, double-stranded DNA, or chromatin. On the other hand, speckled pattern reflected antibodies to non-DNA nuclear antigens.

Bullous pemphigoid: The second most common lesion observed in our study group was bullous pemphigoid [Table 1] (16/60 cases, 26.6%) and the most common age group affected was between 70 - 79 years [Table 2]. The affected patients were predominantly females accounting for 56.25% [Table 3]. In our study it was second to pemphigus Vulgaris. Correlation between DIF and histopathology was high, with most cases showing blister at dermo-epidermal junction.

All the 16 cases (100%) showed blister at dermo-epidermal junction [Table 5]. out of 16 cases, 15 cases (93.7) showed Inflammatory cells in the bulla and dermal infiltrate (100%), similar to Leena JB et al study.[26] Predominant inflammatory cells were

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eosinophils similar to Leena JB et al.26and Nishioka K et al study.[27]

In the present study, DIF was done in 13 /16 cases. DIF was not done in 3/16 cases because of delay in sample collection and a few because the patient was not willing for a repeat biopsy. All 13 cases showed 100% positivity similar to Deepthi PK et al,[29] Cozzani E et al study,[28] Deepti et al.[14]

DIF [Table 8] was strongly positive in 13/16 (81.3%) cases. All positive cases showed linear staining in the Basement Membrane Zone (BMZ). The deposits were of homogenous linear type shown in [Table 11]. Out of 13 cases, complement component C3 was present in 5 positive cases, and in combination with IgG was seen in 8 cases shown in [Table 8].

ANA IF done in 10/16 cases (62.5%) [Table 11] shows a homogenous pattern. ANA IF was not done in 6 cases as the patients were not willing for the test. All the positive cases indicate that they have autoimmune etiology.

Dermatitis herpitiformis: Out of 60 cases, three cases presented with DH, which constituted 5%. [Table 1] Most of the Cases were in the age group of 20-29 and 10-19 years [Table 2] respectively. Both of them presented as pustules and with female predominance.

Subepidermal bulla was present in all cases with papillary micro-abscess. DIF was positive in one of the three cases showing granular deposit of IgA in dermo-epidermal junction similar to Banu L et al,[30] and Deepti et al study.[14] Two cases showed deposition of both IgG and IgA.

ANA IF done in 2 cases (66.6%) shows homogenous pattern indicating the autoimmune nature. ANA IF was not done in one case as patient was not cooperative.

Erythema multiforme: Current study includes 2 cases (3.3%) out of the total 60 cases. The present study had one patient in the pediatric age group i.e, 10year old [Table 2] same as Mateos M et al study.[31] Of the 2 patients, one is male and the other is female. [Table 3].

Histopathology: Only one patient presented with bulla [Table 4]. These is mild inflammatory infiltrate predominantly of lymphocytes along the dermo-epidermal junction. Necrosis of individual basal keratinocytes is seen. One patient had pigmentation similar to Mateos M et al study.[31]

DIF was negative in both the cases shown in [Table 8], Advised repeat biopsy and further investigations. Based on the clinical features and the histopathological features it was reported as EM.

ANA IF was not done as patients were not willing.

Chronic bullous dermatosis of childhood: There was one case of chronic bullous dermatosis of childhood – a girl aged 8 years [Table 1-3]. The patient had characteristic clinical features such as an annular pattern of distribution of the vesicles, pruritus, and post-inflammatory pigmentation throughout the body predominantly on the trunk and limbs.

Histopathology: Histopathology showed sub-epidermal bullae with a neutrophil rich dermal inflammatory infiltrate. Neutrophils are seen along the dermo-epidermal junction [Table 5,6].

DIF [Table 8] was done in that case and showed moderately strong homogenous linear pattern of staining along the basement membrane zone with IgA similar to Leonard et al study.[32]

ANA IF was done in 1 case (100%) shows a homogenous pattern. [Table 11] showing autoantibodies. Patient was put on the treatment and responded well to the therapy, indicating that DIF along with the histopathology could help patients for early diagnosis and prompt treatment.

Hailey hailey disease: Present study included 2 cases (3.3%) of HHD, Arundathi et al,[33] and Jindal et al,[34] showed 1.5 % and 1.6% of cases, which was slightly high in our study, Age ranged between 30 -39 years with equal sex distribution [Table 2,3].

Histopathology: one patient had the characteristic supra-basal separation [Table 5] and other case with no line of blister with acantholysis effecting the large portion of epidermis. Other adnexal involvement is absent. No eosinophils are noted in the blister. Dyskeratosis is frequent in both the cases. Histopathology differential diagnosis was HHD and PV, advised further investigation like DIF to distinguish from the PV.

DIF was done and is negative, this distinguishes them from pemphigus Vulgaris. Indicating the DIF role in the diagnosis.

ANA IF done in 2 cases (100%) shows a homogenous pattern shown in Table 15.

ANA IF was done in 1 case (100%) shows a homogenous pattern. [Table 11] showing autoantibodies. Patient was put on the treatment and responded well to the therapy, indicating that DIF along with the histopathology could help patients for early diagnosis and prompt treatment.

Dariers disease: The present study included 2 cases (4%) [Table 4], aged 39 and 40, with equal sex Distribution [Table 6,7].

Histopathology shows parakeratosis in columns, suprabasal clefts, Crops ronds are noted in the granular layer, and grains in the corneal layer. Acantholytic, dyskeratotic cells are also seen. No dermal edema or eosinophils noted [Table 10].

DIF was done in both the cases and found to be negative, ruling out the PV diagnosis. ANA IF done in 50% cases shows a homogenous pattern. [Table 15].

Pemphigus erythematosus: Present study showed one case of PE (2%) who was 28year old female Histopathology shows Subcorneal bulla and acantholysis identical to pemphigus foliaceus.

Immunofluorescence: DIF testing of perilesional skin granular deposition of IgG (i.e., a positive lupus band test) at the dermo-epidermal junction. Antinuclear antibodies are observed (100%) in this case, ANA IF shows homogenous pattern similar to the study by Maria Elena et al.[35]

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CONCLUSION

Vesiculobullous disorders represent a heterogeneous group of dermatoses with protean manifestations. Classified according to location as suprabasal, intraepidermal, subcorneal and subepidermal group.
Pemphigus Vulgaris constituted the most common subtype of vesiculobullous disorder in the present study, followed by bullous pemphigoid. Clinical examination of skin bullous lesion provide dermatologist gross morphological finding upon which differential diagnosis can be found out.
Immunofluorescence techniques are essential to supplement clinical findings and histopathology in the diagnosis of immune-bullous disorders.
In pemphigus Vulgaris and pemphigus foliaceus both show the same pattern in DIF. Hence definite diagnosis cannot be made without the help of histopathology. Thus, DIF is just a supplement, not a substitute.
Considering the economical constrain of DIF, clinical and histomorphological study of vesiculobullous diseases can be still used in confirming the diagnosis of diseases.
ANAs can also be considered markers of evolution and prognosis, which is why the possibility of correlation between IF patterns and certain autoantibodies conveys a major advantage in clinical evaluation. It will be important to develop IF methods in the future, to improve the accuracy of discrimination between healthy people who test ANA positive and patients with autoimmune disease.

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