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Clinicopathological study of vesiculobullous lesions of the skin and the diagnostic utility of immunofluorescence

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Abstract
Introduction: Vesicobullous disorders represent a heterogenous group of dermatoses with protean manifestations in which the primary lesion is a vesicle or a bulla on the skin or mucous membrane or both. These lesions can be extremely debilitating, may have serious sequelae, and even fatal, necessating early treatment and intervention to prevent further morbidity and mortality. Many of these blistering diseases mimic each other clinically, Histopathology together with immunofluorescence are used for diagnosing vesiculobullous lesions of skin.
Materials and Methods: The present study is done at out institute for a period of 3 years from 2016 to 2019 All the vesiculo bullous lesions which we received during this period have been included in our study. We received a total of 42 cases during our study period. Light microscopic examination followed by DIF was done for definitive diagnosis. The pattern and distribution of immune complex deposits was analysed under fluorescence microscope and correlated with histological findings
Results: Bullous pemphigoid was the common vesiculobullous disease (38.09%) followed by pemphigus vulgaris. dif showed linear type of immune deposits in epidermal basement membrane zone in bullous pemphigoid and in pemphigus vulgaris intercellular lace like deposition of immune complexes was seen.
Conclusion: Vesiculobullous disorders represent a heterogeneous group of dermatoses Punch biopsy of the skin is a simple, inexpensive, safe OPD procedure, causing minimal discomfort lesions and DIF studies are an adjunct to the histomorphology.
DIF which is the gold standard and sensitive test in making a definitive diagnosis aids in distinguishing non immune lesions from immune mediated lesions which pose a diagnostic dilemma both clinically and histologically An integrated approach of clinical findings in conjunct with histomorphology and DIF assist in accurate diagnosis and proper patient management.

Keywords: Vesiculo bullous lesions, bullous pemphigoid, pemphigus vulgaris, direct immunofluorescence

Introduction
Vesicobullous disorders represent a heterogenous group of dermatoses with protean manifestations in which primary lesion is a vesicle or a bulla on the skin or mucous membrane or both. Among the various dermatological conditions, vesiculobullous lesions form one of the most frequent clinical problems [1] and are one of the most important primary morphological patterns of skin reaction to various external and internal pathologic stimuli. Wide variety of pathologic processes can lead to development of vesiculobullous eruptions over body [2]. They may occur in various conditions inflammatory, infective, autoimmune, drug induced as well as genetic.(3) These lesions have dramatic impact on the patients, their families and have severe economic consequences for the family and health services [4].
Vesicles and bullae are fluid filled cavities formed within or beneath the epidermis. Vesicles are less than 0.5cm in diameter and bullae are blisters greater than 0.5 cm in diameter. A blister is a fluid-filled cavity formed within or beneath the epidermis [5]. Vesiculobullous diseases have been the focus of intensive investigation in recent years as some of these can be extremely debilitating with serious sequelae, and even fatal, necessating early intervention and treatment to prevent further morbidity and mortality [4, 6].
Clinical examination of skin bullous lesion provides the gross morphological findings upon which differential diagnosis can be made.

Though some of the veisculobullous lesions are characteristic in their appearance and distribution, still many of the subepidermal blistering disorders may strikingly mimic each other clinically, and many a times a definitive diagnosis cannot be made by physical examination alone [1, 3]. Mortality and morbidity in various vesiculobullous lesions differ greatly, therefore accurate diagnosis is important.

Histologic assessment is essential for accurate diagnosis and provides insight into the pathogenic mechanisms [4]. Punch biopsy is the most commonly employed technique and is simple, inexpensive, safe OPD procedure without any major complications, causing minimal discomfort to the patient and no scarring [7].

As the majority of vesiculobullous skin disorders are immune mediated the molecular nature of autoimmune diseases, the role of cell to cell interactions and desmosomes have been identified over the last two decades.The main pathogenic mechanism involved is the antigen antibody immune complex deposition which may directly interfere with desmosomal function [8].

Most of these lesions have characteristic light microscopic and immunofluorescence patterns which are of great help in reaching an accurate diagnosis. The blister separation plane, type of inflammatory infiltrate and mechanism of blistering are specific for each disease [9].

Though histopathological study is sufficient in most of the cases, it is often accompanied by immunofluorescence antibody tests. Direct immunofluorescence (DIF) antibody testing is a gold standard for confirmation of the diagnosis as many lesions have overlapping microscopic features [1]. Immune mediated patterns are disease specific and immune complex depositions at various location such as dermo-epidermal junction, within the epidermis, dermal blood vessels etc are of diagnostic importance [10]. Nature of immune deposits usually used in DIF is IgG, IgA, IgM and C3c [10]. These rapid and reliable immunofluroscent techniques permit early diagnosis and treatment of potentially life-threatening disorders [11].

Aims and Objectives

1. To study various vesiculobullous lesions in relation to site, age and gender encountered in our region.
2. To correlate the clinical and histopathological features of the skin lesions and to confirm with Direct immunofluorescence (DIF) findings.
3. To evaluate the diagnostic utility and sensitivity of DIF studies.

Materials and methods

The present study was done at our institute for a period of 3 years from 2016 to 2019 All the vesiculobullous lesions which we received during this period have been included in our study. The skin biopsies were taken by the dermatologist from the site of lesion including the intact vesicle and from perilesional skin, under strict aseptic conditions. The specimens were sent in 10% buffered formalin for routine histological analysis and in normal saline or Michel's medium for DIF. Skin biopsy slides were analysed initially by light microscopy, followed by DIF with IgG, IgA, IgM, C3c antibodies. The pattern and distribution of immune complex deposits was analysed under fluorescence microscope and correlated withlight microscopy.

Results

We received a total of 42 cases during our study period. Females out numbered males in our study. Out of the 42 patients, 23 cases were females (54%) and 19 cases were males (45%). In our study, the maximum number of patients were in the age group of 61-70 years (26%) followed by 20-30 years (16%). Mean age was 47.71 years. Youngest case was 1 year old and oldest case was 87 years. The commonest site of disease involvement was limbs followed by trunk.

Table 1: Sites of involvement

Site	Number of lesions
Extremities	19
Trunk	10
Back	08
Face	05
Oral cavity	02
All over the body	02

Majority of the patients presented with blisters associated with itching. Other presenting features were erythematous crusted plaques, ulceration with raw painful areas and burning sensation. The blisters were of variable sizes from tiny fluid filled vesicles to very large bullae. The number of lesions varied from single lesion to multiple lesions.

Lesions clinically diagnosed as Bullous pemphigoid presented as multiple, tense bullae of varying sizes, and pemphigus vulgaris, in which oral and skin involvement occured showed flaccid bulla of varying sizes. The duration of the presenting complaints in the present study ranged from 3 days to 2 years. In all the cases of pemphigus vulgaris Nikolsky's sign was positive and oral/ mucosal involvement was also observed in cases of pemphigus vulgaris.

Approach to the light microscopic assessment was based on the following findings. 1. Blister separation plane 2. Mechanism of blistering 3. Type of inflammatory infiltrate. On the basis of the plane of separation, the vesiculobullous lesions have been broadly divided into two categories, intraepidermal, and subepidermal. The intraepidermal lesions are further divided into suprabasal and subcorneal. In the present study majority of the cases showed plane of separation at the subepidermal region ie at the dermoepidermal junction (56.7%) followed by suprabasal (23.8%) and subcorneal (19.04%) planes.

Table 2: Plane of separation of the lesions

S. No	Plane of Seperation	No: of Cases	Percentage
1.	Intra Epidermal- Suprabasal	10	23.8%
2.	Intra Epidermal -Subcorneal	08	19.04%
3.	Subepidermal	24	57.1%

In our present study epidermal basement membrane destruction leading to subepidermal bullae formation was the most common mechanism involved (56%) followed by acantholysis (25%) leading to intraepidermal bullae. Other mechanisms like spongiosis and keratinocytes degeneration were also observed. The most common inflammatory infiltrate in the bullous cavity was neutrophilic infiltrate followed by mixed inflammatory infiltrate, eosinophilic, and lymphocytic infiltrate.

Table 3: Type of Inflammatory infiltrate observed

S. No	Type of infiltrate	Number of cases	Percentage
1.	Neutrophils	20	47.6%
2.	Mixed	14	33.3%
3.	Eosinophils	04	9.5%
4.	Lymphocytes	04	9.5%

As many of the histological features may be overlapping in various lesions, a provisional light microscopic diagnosis was made, and then DIF testing is done for the final histopathological diagnosis. DIF testing was done with IgA, IgG, IgM, and C3C flurochrome conjugated antibodies in all the 42 cases and 37 cases showed antibody deposition. The DIF findings are based on the type, site, pattern and intensity of antigen antibody complex deposition.

Table 4: Pattern of immunofluorescence and histopathological diagnosis in the lesions.

Histopathological diagnosis	No: of cases	No: of cases showing DIF positivity	IgG	IGA	C3c	IgG+ C3C	Negative	Location	Pattern
Bullous pemphigoid	16	16	2	0	1	13	0	EBMZ	Linear
Pemphigus vulgaris	10	10	6	0	0	4	0	ISR	Lace like
Subcorneal pustular dermatosis	4	Nil	0	0	0	0	4	Nil	Nil
Epidermolysis bullous aquista	3	2	1	0	0	1	1	EBMZ	Linear
BP,EBA	3	1	0	0	1	1	1	EBMZ	Linear
Pemphigus foliaceous	1	1	0	0	0	1	0	ISR	Intercellular
IgA linear Dermatosis	2	2	0	2	0	0	0	EBMZ	Linear
Drug induced bullous disorder	2	Nil	0	0	0	0	2	nil	Nil
Dishydrotic Eczema	1	Nil	nil	0	0	0	1	Nil	nil

*EBMZ-Epidermal Basement Membrane Zone, ISR-Intercellular Squamous Region

All the cases of BP showed linear deposits predominantly of IgG and C3c at the EBMZ and the cases of Pemphigus vulgaris showed lace like deposits of IgG and IgG + C3c at the ISR. Pemphigus foliaceous showed IgG and C3c deposition in the ISR. All the non immune causes for vesiculobullous lesions were confirmed after negative DIF. Details provided in the table. Ambiguity in the diagnosis remained in two cases where provisional diagnosis was made as BP or EBA as the DIF was inconclusive in these two cases. Final impression was given as a differential diagnosis and was advised to correlate with clinical details.

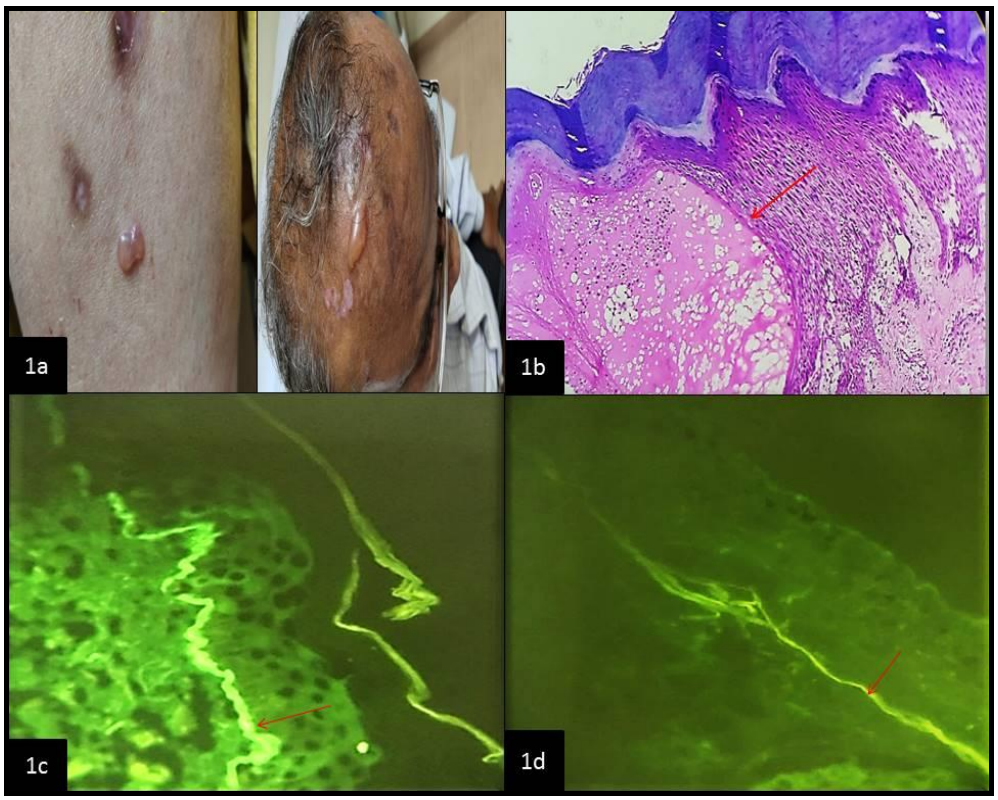

Fig 1: 1a: Clinical presentation of bullous pemphigoid. 1b: Subepidermal blister. H&E staining. Low power view 1c, 1d: Linear IgG and C3c deposit at the EBMZ. 1b: Subepidermal blister.

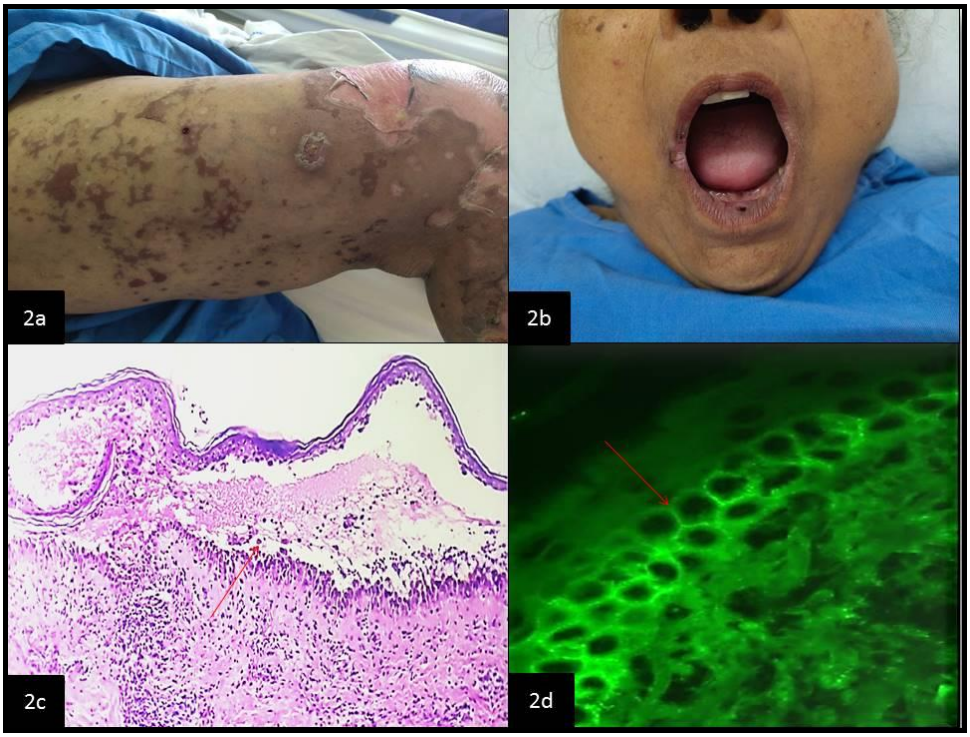

Fig 2: 2a: cutaneous and oral lesions of Pemphigus vulgaris 2b, 2c. Suprabasal blister H&E staining. Low power view 2d. Lace like deposition of IgG in the ISR

Discussion

Skin is the single largest organ of the body. It represents a window to the internal well-being. Various diseases along with its manifestations can commonly involve the skin and mucous membranes out of which vesiculobullous lesions form a predominant group [12].

Although vesicles and bullae occur in several nonspecific conditions there exists a group of disorders in which blisters are the primary and most distinctive feature of presentation.These bullous diseases, in some instances are fatal if untreated [13]. Blisters occur at different levels within the skin in the various disorders and histologic assessment is essential for accurate diagnosis providing insight into the pathogenic mechanisms.

Skin not only has protective function but also has immune functions. The main function of our immune system is to protect an individual from foreign or non-self-antigens without reacting with self-antigens. Sometimes this is distorted and leads to auto immune disorders [13].

Vesiculobullous lesions are mostly immune mediated, and the immunopathogenesis is specific for each disease, which is of diagnostic importance. The immune complex depositions occur at various locations as intraepidermal, dermo-epidermal junction, dermal blood vessels etc. Nature of immune deposits usually used in DIF is IgG, IgA, IgM and C3c. [4] Light microscopy alone does not yield much results in these lesions.

Knowledge of the molecular structure of the intercellular and cell-to-matrix attachments that provide the skin with mechanical stability is helpful in understanding these diseases [5].

Desmosomes are the intercellular adhering junctions which attach the neighbouring cells to each other, and are seen in tissues such as the stratified squamous epithelia of the skin, mucous membranes and myocardium [5]. Significant insights into the regulation of desmosomal adhesion demonstrated the role of autoantibodies in patients suffering from the autoimmune blistering skin diseases [16]. Autoantibodies against desmoglein cause the intraepidermal blistering seen in pemphigus, while autoantibodies against hemidesmosomes are the reason for the subepidermal blistering seen in pemphigoid [5]. Thorough clinical examination aided by light microscopy and immunofluorescence helps in definitive diagnosis and proper patient management.

In the present study females out numbered males. Out of the 42 patients, 23 were females (54%) and 19 were males (45%). Our study is in concordance with the studies by Kumar A, *et al*. [1] Deepthi *et al*,[4] Anupama [17] Arundhathi *et al*,[18] Heena mittal [19] and many other studies.The maximum number of patients were in the age group of 61-70 years (26%) followed by 20-30 years (16%). Mean age was 47.71 years. The mean age of presentation in various studies was comparable with this present study [1, 2, 4, 17, 18] Youngest case was 1 year old and oldest was 87 years. Extremities were commonly involved (45%) followed by trunk (23.8%) and back (19%). Similar findings were observed by Anupama [17] and Heena mittal. [19] Lesions were seen all over the body in studies by Deepti [4] Kumar SS [6]. Oral cavity involvement was seen in all the cases of pemphigus vulgaris and 2 cases showed only oral lesions without skin involvement. Many studies done show oral lesions associated predominantly with pemphigus vulgaris [1, 2, 4, 6, 17, 18].

Bullous pemphigoid presented clinically as multiple, tense bullae of varying sizes commonly in adults, had the highest incidence in both males and females. Pemphigus vulgaris presented as oral and skin lesions showing flaccid bullae of varying sizes. The most common lesion encountered in our study was Bullous pemphigoid (38%) followed by Pemphigus vulgaris (23%). We have encountered other lesions also like subcorneal pustular dermatosis, IgA linear

dermatosis etc. Our findings are similar to the findings from studiies by Thejasvi *et al* where Bullous pemphigoid was the predominant lesion [20]. Majority of the other studies showed pemphigus vulgaris as the predominant lesion [1, 4, 6, 17, 18]. This can be attributed to the genetic, environmental and geographical variations.

The Nikolsky's sign is a clinical sign which manifests as dislodgement of intact superficial epidermis by a shearing force, indicating a plane of cleavage in the epidermis. The defect may be due to epidermal antibodies as in Pemphigus or staphylococcal toxin as in Staphylococcal scalded skin syndrome. It is characteristically associated with Pemphigus vulgaris [21]. Nikolsky's sign which is a significant indicator of altered structural epidermal integrity and active acantholysis enables the dermatologist to clinically distinguish between intraepidermal and subepidermal blistering diseases [21]. In our study Nikolsky sign was present in all the cases of Pemphigus vulgaris and negative in Bullous pemphigoid. This is in correlation with study by Deepthi *et al.* and Kumar SS [4, 6].

In the present study the plane of separation was subepidermal in majority of the cases (57.1%) followed by suprabasal (23.8%) and subcorneal planes (19.04%). Our study is similar to the study by Thejasvi *et al*. [20] where the plane of separation in majority of the cases was sub epidermal. But in many other studies the plane of separation was intraepidermal specifically, supra basal [1, 4, 6, 17, 18] This variation might be because of the genetic, environmental factors.

The predominant type of inflammatory infiltrate in the bullous cavity was mixed infiltrate in our study which is in correlation with the study by Anupama *et al*. [17] Eosinophilic infiltrate was seen predominantly in the study by Heena *et al*, [19] and neutrophilic infiltrate was predominant and in other studies [4, 17, 18]

Immunofluorescence (IF) tests have redefined the understanding of many immune-mediated skin diseases, especially autoimmune blistering diseases [22] Immunofluorescence (IF) is a histochemical technique employed to detect antibodies bound to antigens in the tissue or in the circulation. It acts as a valuable adjunct to clinical and histopathological diagnosis, especially in vesiculobullous and connective tissue disorders [23]. IF has been extensively used to understand and classify various disorders where immune mechanisms play a role and has become an essential investigation in the diagnosis and management of autoimmune vesiculobullous disorders [22].

All the cases of Pemphigus group showed intraepidermal bullous lesions The plane of separation in Pemphigus vulgaris was in the suprabasal region. Subcorneal plane of separation was seen in pemphigus foliaceous and subcorneal pustular dermatosis. Cases of Bullous pemphigoid, Linear IgA dermatosis, EBA showed sub epithelial plane of separation. DIF is essential in such cases where histological features are overlapping and aids in making accurate diagnosis for proper patient management.

Bullous Pemphigoid showed linear IgG and C3c deposition in the Basement Membrane Zone (BMZ) and Pemphigus Vulgaris showed lace like deposits in the Intercellular Squamous Region (ISR). Out of the three cases of EBA one case was inconclusive and the other two showed linear deposits of IgG and C3c in EBMZ on the dermal side. The gold standard for diagnosis of Epidermolysis Bullosa Acquisita (EBA)vs Bullous Pemphigoid is the identification of immune deposits on the dermal side ('floor') for EBA or the epidermal side ('roof') for Bullous Pemphigoid in DIF [24]. In our study all the cases of linear IgA dermatosis showed IgA deposition in EBMZ.

Two cases were inconclusive even after IF and possible diagnosis was given in these cases. Inconclusive DIF findings were reported by Heena *et al*. [17], Anupama *et al*. [19] Thejasvi *et al*. [20] also. All the cases of pemphigus group had 100% sensitivity which correlated with many studies. [1, 4, 6, 17, 19]

DIF had a sensitivity of 875% in cases of BP in our study. The studies of Kabir *et al*. [25] and Mahmood *et al*. [26] had 100% sensitivity of DIF in their studies. The findings of our study are supported by study conducted by Deepthi *et al*. [4] and Heena *et al*. [19] which had lower sensitivity. The sensitivity of DIF for pemphigus group of lesions in our study was 100%. Anupama concluded from her study that the sensitivity of Pemphigus group of lesions was 90.09% and in the case of Bullous pemphigoid it was 77.7% [17]. Kumar SS concluded a sensitivity of 78% from his study [6].

The cases of Subcorneal Pustular Dermatosis, and drug induced bullous lesions which had overlapping histological features with other immunological bullous lesions were confirmed as non immune after negative DIF.

Conclusion

Vesiculobullous disorders represent a heterogeneous group of dermatoses with protean manifestations. Punch biopsy of the skin is a simple, inexpensive, safe OPD procedure, causing minimal discomfort to the patient. Basic histomorphological evaluation is very essential in skin vesiculobullous lesions and DIF studies are an adjunct to the histomorphology. Though light microscopy may be used to give the probable diagnosis in resource poor settings, and is as good as DIF, still DIF is always the gold standard and sensitive test in making a definitive diagnosis. DIF aids in distinguishing non immune lesions from immune mediated lesions which pose a diagnostic dilemma both clinically and histologically. An integrated approach of clinical findings in conjunct with histomorphology and DIF assist in accurate diagnosis and proper patient management.

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