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Original Article

Pathology Section

A Study of Vesiculobullous Lesions of Skin

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ABSTRACT

Introduction: Bullous dermatoses are a wide variety of blistering diseases some of which can be extremely debilitating and even fatal. This study shows the importance of histopathology over immunofluorescence studies in the diagnosis of the same.

Aim: To analyse the histopathological patterns of non infectious vesiculobullous lesions seen in patients attending Dermatology Department of Kottayam Medical College, for a period of 2 years. Also to correlate the histopathology and immunofluorescence diagnosis in auto-immune blistering diseases.

Materials and Methods: Skin biopsies of 100 clinically diagnosed vesiculobullous cases were processed and paraffin embedded. Histologic examination of the H&E stained sections of the same were done. The level of split of lesions, mechanism of blistering and nature of infiltrate noted. Meanwhile perilesional skin of the suspected autoimmune diseases were sent in Michel's medium for immunofluorescence study and their patterns correlated with histological diagnosis.

Results: Of the 100 cases studied, autoimmune blistering diseases comprised the major portion of cases. Pemphigus vulgaris headed the list among all the individual lesions, second being bullous pemphigoid. Among all, histopathological diagnosis correlated with clinical diagnosis in 87% cases. Of all the IF proven cases, histopathological diagnosis correlated with immunofluorescence in 77.77% cases. The positive predictive value of histopathological examination in diagnosing pemphigus group of diseases which are proven by immunofluorescence was 93.75%, sensitivity was 90.09%. The positive predictive value of histopathological examination in diagnosing bullous pemphigoid lesions which are proven by immunofluorescence was 93.33%, sensitivity was 77.77%.

Conclusion: This study emphazises that morphological diagnosis by histopathology is as good as immunoflourescence in the diagnosis of vesiculobullous lesions and shows the diversity of histomorphological patterns in the various blistering diseases encountered. The systematic approach to the histopathological sections could help in correct diagnosis at the earliest.

Keywords: Bullous pemphigoid, Histopathology, Immunofluorescence, Pemphigus vulgaris

INTRODUCTION

There is a wide variety of cutaneous blistering diseases, some of which can be extremely debilitating and even fatal. According to the recent advances in investigations, the most important techniques for the diagnosis of vesiculobullous disease are conventional histopathology and confirmative tests like direct and indirect immunofluorescence [1]. Biopsies obtained from the cutaneous blisters tend to have characteristic light microscopic and immunofluorescence patterns which are of great help in reaching an accurate diagnosis at the earliest.

When blisters are encountered microscopically, the critical assessments to be approached in sequence are as follows:

1) Blister separation plane

2) The mechanism of blister formation

3) The character of inflammatory infiltrate.

The blister separation plane can be epidermal, subepidermal or dermal. Epidermal can again be classified into subcorneal, spinous or suprabasal. The majority of vesiculobullous skin disorders are immune mediated. The main pathogenic mechanism involved is the binding of antibody to the antigen which may directly interfere with desmosomal function [1-4]. This results in spongiosis, acantholysis, reticular degeneration, cytolysis, basement membrane zone destruction with blister formation. The nature of inflammatory infiltrate vary in different vesiculobullous lesions making it diagnostically significant. However ageing of lesion, superadded infections and treatment can alter the inflammatory response.

The main pathogenic mechanism involved is the binding of antibody to the antigen which may directly interfere with desmosomal function [1-5]. This results in spongiosis,

acantholysis, reticular degeneration, cytolysis, basement membrane zone destruction with blister formation. The plane of cleavage depends upon the antigen involved.

The accumulation of extracellular fluid within the epidermis is called spongiosis, which when pronounced leads to disruption of desmosomes and subsequent blister formation.

Acantholysis results from the loss of keratinocyte cell - cell contract, as evidenced by rounded keratinocytes. Reticular degeneration results from ballooning degeneration with secondary rupture of keratinocytes. Cytolysis is the disruption of keratinocytes.

MATERIALS AND METHODS

This was a cross-sectional study. The study group included 100 patients having vesiculobullous lesions of skin, attending the Dermatology OPD of Medical College Hospital, Kottayam, India, for the duration of two years (2009-2011). The study excludes all infectious bullous lesions and cases with no definite bullae for the proper histopathological evaluation under light microscopy.

The skin biopsies were taken by the treating Dermatologist, undertaking the standard precautions and practices followed in the institution. Informed consent was obtained from each of the patients included in the study prior to the biopsy by the Dermatologist.

These biopsies for histopathology and immunofluorescence were taken as a part of the necessary diagnostic work-up needed for that particular condition, provided there were no contraindications.

Biopsy was taken from the site of lesion, including the intact vesicle.

An elliptical piece of skin was taken from the site of lesions. A comparatively fresh intact vesicle was excised in toto. The specimen was sent in 10% buffered formalin for routine histological analysis to Histopathology Laboratory of Pathology Department of Medical College, Kottayam. It was processed and paraffin embedding done. Serial sections were taken using a rotory microtome, which were then deparaffinised and stained with Haematoxylin and Eosin. Histologic examination was then done with the aid of light microscope. The level of split of lesions, the presence of acantholytic cells and nature of infiltrate were noted.

For direct immunofluorescence study, part of the biopsy material from the peri-lesional area was cut and put in Michel's medium (transport medium containing ammonium sulfate, N-ethyl maleimide and magnesium sulfate in a citrate buffer). The specimen was then sent for immunofluorescence study

Biopsy specimens were rinsed in Phosphate Buffered Saline (PBS) at pH 7.4 for 10 minutes. Snap freezing with embedding medium was carried out in the cryostat at -20^0 C and 3-4 micron section obtained. A drop of specific reagent-Fluorescein Isothiocyanate (FITC) labeled antihuman IgG, IgM, IgA, complement C3 and fibrinogen were used. Reading was done under the fluorescence microscope (Nikon model HB 1010 AF). The pattern of distribution and type of immunoreactants deposited in the intercellular space were noted.

RESULTS

Age distribution: In our study, the maximum number of patients were in the age group of 46-60 years (40%), followed by elderly age group of >60 years (27%). The youngest patient in the series was a male child of 2 years with chronic bullous disease of childhood and the oldest one was a female, 87 years with bullous pemphigoid.

In our study, there were 3 cases with age <15 years. One case was chronic bullous disease of childhood, one bullous pemphigoid, and one pemphigus foliaceus.

Sex distribution: Of the 100 patients, 48 were males and 52 were females. Male:female ratio was 0.93.

Histopathology: The critical assessments approached in our study were as follows .

1) Blister separation plane [Table/ Fig-1],

2) Mechanism of blistering [Table/ Fig-2],

3) Character of inflammation [Table/ Fig-3],

A few cases of pemphigus and spongiotic dermatitis had subcorneal and suprabasal bullae due to the extension of the lesion. Those with suprabasal and subepidermal planes of separation could be explained due to the re-epithelisation of the blister.

Acantholysis was the major mechanism of blister formation in all the 100 cases. In 11 cases, spongiosis supervened with acantholysis and/or basement membrane destruction. This could be explained by the superadded infection on the already existing lesion.

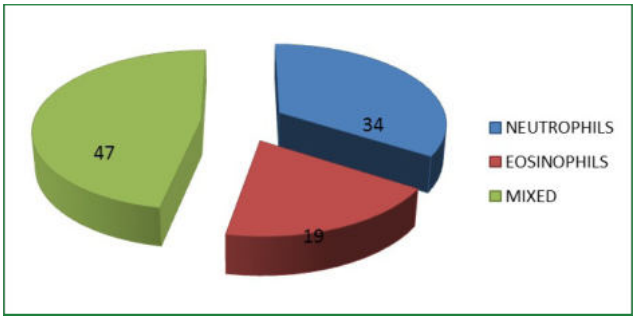
Histopathological diagnosis of the cases given in [Table/ Fig-4]. Of 100 cases, 13 cases showed discrepancies between clinical and histopathological diagnosis i.e., 87% positive correlation was obtained [Table/Fig-5].

Among those cases in which no specific diagnosis was possible, majority had bullous pemphigoid pattern on

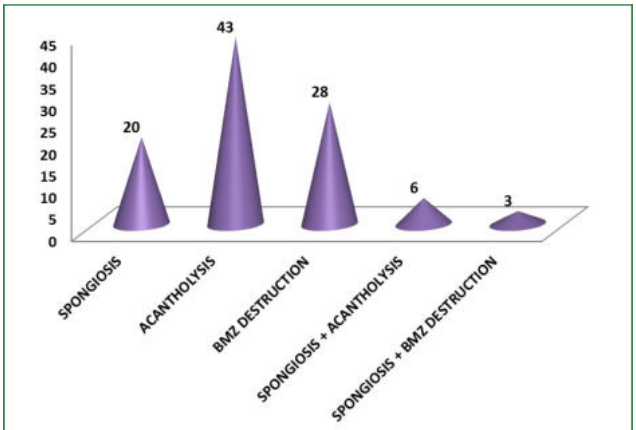
Plane of Separation	No. of Cases
Subcorneal	13
Suprabasal	44
Subepidermal	28
Dermal	0
Subcorneal + Suprabasal	9
Suprabasal + Subepidermal	4
Subcorneal + Subepidermal	1
Dermal + Subepidermal	1

[Table/Fig-1]: Blister separation plane in the skin.

immunofluorescence examination. On retrospective slide analysis, it was found that this could be attributed to the lack of the characteristic eosinophilic inflammatory infiltrate and presence of superadded infection, masking the typical features of bullous pemphigoid.



[Table/Fig-2]: Character of inflammatory infiltrate.



[Table/Fig-3]: Mechanism of inflammation.

Diagnosis	No. of Cases
Pemphigus Vulgaris	36
Bullous Pemphigoid	20
Spongiotic Dermatitis	8
Pemphigus Foliaceous	4
Subcorneal Pustular Dermatosis	4
Lichen Planus Pemphigoides	3
Drug Reaction	2
Dariers Disease	2
Hailey Hailey	2
Inconclusive/Description*	7
Other Single Cases**	**
Total	100

[Table/Fig-4]: Histopathological diagnosis profile.
*The term inconclusive/description is given to all those cases where the definite features of a particular lesion could not be found out. There were 7 such cases.
**There was one case each of Bullous impetigo, Bullous lichen planus, Chronic bullous disease of childhood, Dermatitis herpetiformis, Epidermolysis bullosa acquisita, Grovers disease, Herpes gestationis, Linear IgA bullous dermatosis, Bullous lupus erythematosus, Pemphigus vegetans, Pustular psoriasis and Toxic epidermolytic necrosis.

Among 33 cases of pemphigus group of diseases, 30 cases showed positive immunofluorescence correlation, among 18 immunofluorescence proven cases of bullous pemphigoid, 14 showed positive correlation [Table/Fig-6,7]. Each case of lichen planus pemphigoides, chronic bullous dermatosis of childhood, linear IgA, showed positive correlation with immunofluorescence pattern.

Clinical Diagnosis	Histopathological Diagnosis	Immunofluorescence Diagnosis
Linear IgA Bullous Dermatosis	Bullous Pemphigoid	Bullous Pemphigoid
Pemphigus Foliaceous	Description	Bullous Pemphigoid
Sle + IgA Pemphigus	Description	Lupus Band
IgA Pemphigus	Description	Pemphigus
Para-neoplastic Pemphigus	Pemphigus Vulgaris	Pemphigus
Grovers Disease	Subcorneal Pustular Dermatosis	-
Subcorneal Pustular Dermatosis	Drug Reaction	No Features of Immunobullous
Pemphigus Vulgaris	Bullous Pemphigoid	Bullous Pemphigoid
Pemphigus Vulgaris	Spongiotic Dermatitis	Bullous Pemphigoid
Dermatitis Herpetiformis	Description	Bullous Pemphigoid
Bullous Pemphigoid	Description	Bullous Pemphigoid/ Epidermolysis Bullosa Acquisita
Pemphigus Vulgaris	Description	Bullous Pemphigoid
Epidermolysis Bullosa Acquisita	Description	Epidermolysis Bullosa Acquisita

[Table/Fig-5]: Discrepancies between clinical and histopathological diagnosis*.
*Of the 13 discrepant cases, 7 were given as description, the diagnosis of which were confirmed by immunofluorescence.

DISCUSSION

In this study, the maximum number of patients fall under the age group 46-60 years (40%) followed by >60 years (27%). Handa F et al., showed prevalence of pemphigus in younger age groups [6]. Of the 100 patients, 48 were males and 52 were females. The male to female ratio was 0.93 In our study, pemphigus vulgaris was seen in almost equal proportions among males and females with a slight female predominance. According to Brenner S et al., exposure to pesticides and metal vapour increases the risk of pemphigus [7]. Wohl Y et al., showed that occupational exposure to UV

Immunofluorescence Diagnosis	No. of Patients
Pemphigus	33
Bullous Pemphigoid	18
Bullous Pemphigoid/Epidermolysis Bullosa Acquisita	1
Chronic Bullous Dermatosis of Childhood	1
Linear IgA Bullous Dermatosis	1
Epidermolysis Bullosa Acquisita	1
Lichen Planus Pemphigoides	1
Bullous Lupus Erythematosus	1
Lupud Band	1
No Features of Immunobullous Disease	4
Total	62

[Table/Fig-6]: Immunofluorescence positive cases.

Disease	No. of Positive Correlation	No. of Negative Correlation	No. of Cases Reported as Description
Pemphigus (33)	30	2	1
Bullous Pemphigoid (18)	14	1	3
Epidermolysis Bullosa Acquisita/Bullous Pemphigoid (1)	0	0	1
Other Single Cases*	4*	0	0
Lupus Band (1)	0	0	1
No Features of Immuno Bullous Disease (5)	1	4	0
Epidermolysis Bullousa Acquisita (1)	0	0	1
Total (63)	49	7	7

[Table/Fig-7]: Analysis and comparison of histopathological and immunofluorescence examination in all proven cases.
*other cases with positive correlation - Chronic bullous disease of childhood (1), Linear IgA bulous disease (1), Lichen planus pemphigoides (1), Bullous lupus erythematosus (1)

radiation was said to induce pemphigus. The duration of illness at the time of presentation varied from 10 days to 10 years [8]. In our study, most of the patients presented early probably because of the accessibility to medical care as well as increased awareness

Pisanti S et al., reported that in 50-70% of patients with pemphigus vulgaris [9], the disease originated in the oral cavity. Out of all, 64% of patients in our study showed oral lesions. Of these, 56 were diagnosed as pemphigus group, 6 cases were suffering from bullous pemphigoid, one had TEN and one had bullous LE. Predominant sites of involvement of vesiculobullous lesions in our study were the trunk and extremities (36%), followed by abdomen and back (33%). Trunk and extremities were frequently involved sites in most of the cases which was also observed by Shafi M et al., [10].

In our study, 43% cases were having acantholysis as the major mechanism, basement membrane zone destruction in 28% cases, and spongiosis in 20% cases. In spongiosis, desmosomal stretching occurs prior to cell separation, while in acantholysis, cell separation occurs without stretching [11].

The plane of cleavage was suprabasal in 44% cases, subepidermal in 28% cases and subcorneal in 13% cases. The occurrence of lesions with multiple level blisters, could be attributed to the re-epithelisation phenomenon expected in bullous lesions, the features of which were noted in the respective cases.

Arya SR et al., and Fernandez JP et al., observed suprabasal bullae with neutrophils, eosinophils and acantholytic cells [12,13]. We had a case of pemphigus vegetans with a typical eosinophilic spongiosis and another case of drug hypersensitivity reaction with exclusively eosinophilic infiltrate. Lymphocytes were seen in older lesions.

There is strong evidence that pemphigus antibodies are pathogenic because:-

1. In most patients with pemphigus, the antibody titers as measured by indirect immunofluorescence study correlate with disease activity [13].
2. Plasmapheresis induces short term remissions through reduction in pemphigus antibody titers [14]
3. Pemphigus has developed in neonates born to mothers with active pemphigus secondary to transplacental transfer of maternal IgG [15].
4. Pemphigus antibody produces positive DIF findings in human skin organ cultures with suprabasal acantholysis produced after 24 hours [16].
5. The partially purified IgG fraction from pooled sera of patients with pemphigus vulgaris has the same in vitro effect as whole serum [17].
6. Intraperitoneal injections of IgG fractions from patients with pemphigus vulgaris into neonatal mice caused blisters with the histological, ultra structural and immunofluorescence features of pemphigus vulgaris in 39 of 55 mice (71%) [18].
7. Pemphigus vulgaris IgG Fab fragments from humans result in acantholysis in neonatal mice [19].
8. Indeed, the distribution of lesions correlates with the distribution pattern in IF studies of pemphigus vulgaris antigen, where experience is greatest in buccal mucosa [3].

The transition from PF to PV is less common than that of PV to PF [3]. One possible mechanism is an epitope-spreading

phenomenon which is considered as a primary autoimmune or inflammatory process that causes tissue damage by exposing immunologically hidden protein to the immune system, thereby evoking a secondary autoimmune reponse. In cases of PV transforming from PF, anti-Dsg 1 autoantibodies were initially present This leads to the production of anti-Dsg 3 autoantibodies and a change in the phenotype [3,5,20,21]. In our study, histopathology correlated with clinical diagnosis in 87% cases. According to Korman, DIF testing demonstrates IgG in the squamous inter cellular substance in 80-95% cases of pemphigus vulgaris, including early cases and those with very few lesions, and in upto 100% cases with active disease [25]. Study reported that 30-60% of patients had IgM and IgA and upto 50% had C3 deposits in the intercellular spaces. DIF testing showed a full thickness squamous intercellular substance deposition of IgG in 100% of pemphigus cases. But in our study, DIF was a failure in distinguishing between the subtypes of pemphigus. May be the phenomenon of epitope spreading might have contributed to the same.

Thirty out of 33 cases of pemphigus and 14 out of 18 cases of bullous pemphigoid showed positive immunoflourescence correlation with histopathology. From the analysis made, we could find that the positive predictive value of histopathological examination in diagnosing pemphigus group of diseases [Table/Fig-8-11], which are proven by immunofluorecence was 93.75%, sensitivity was 90.09%. As in the case of bullous pemphigoid it was 93.33% positive predictive value and 77.77% sensitivity.

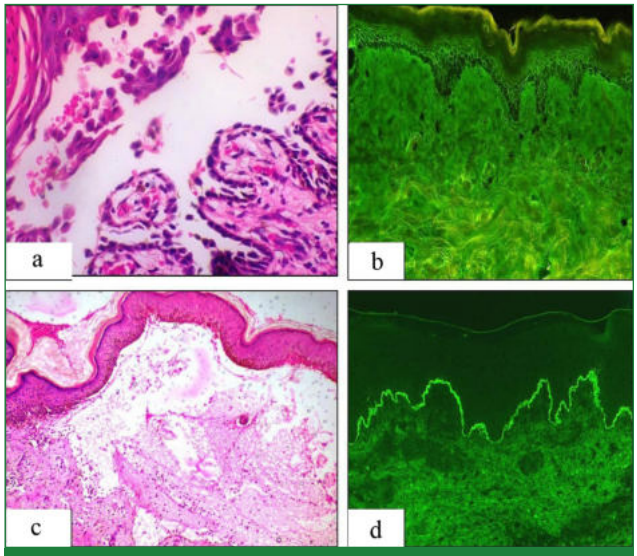
To sum up, the role of the basic histomorphological evaluation of skin biopsies in diagnosing the bullous dermatoses has to be emphasized. Along with the fact that, in diagnosing autoimmune blisters, histopathology is as good as and cost effective than the immune fluorescence pattern analysis. This study has been submitted under ethical clearance committee and had been cleared ethically uneventfully.

Disease	Immuno-fluorescence Positive	Immuno-fluorescence Negative	Total
HP Positive	30	2	32
HP Negative	3	0	3
Total	33	2	35

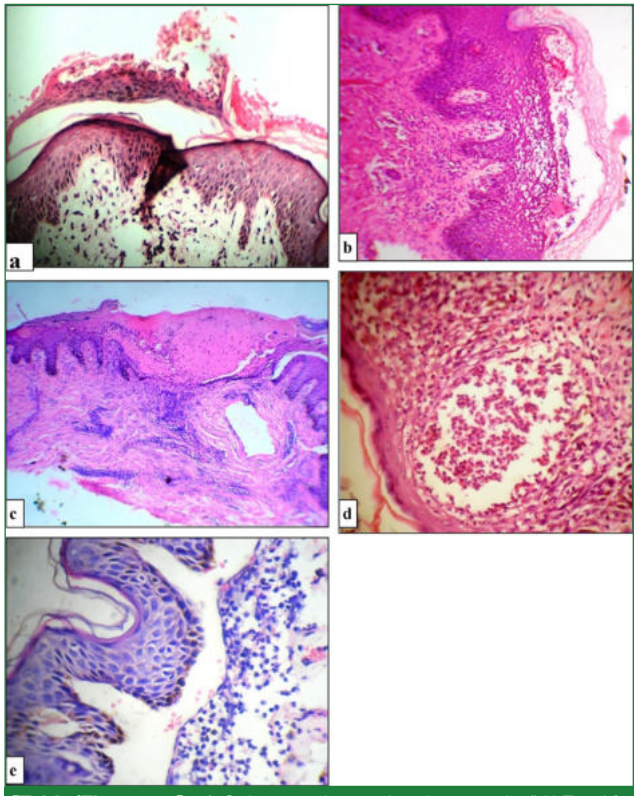
[Table/Fig-8]: Analysis of histopathology and immunofluorescence results in pemphigus group.
Note: Positive predictive value, 30/32= 93.75%; Sensitivity, 30/33= 90.09%

	Immuno-fluorescence Positive	Immuno-fluorescence Negative	Total
HP Positive	14	1	15
HP Negative	4	0	4
Total	18	1	19

[Table/Fig-9]: Analysis of histopathology and immunofluorescence results in bullous pemphigoid.
Note: Positive predictive value, 14/15= 93.33%; Sensitivity, 14/18= 77.77%



[Table/Fig-10a-d]: a) Pemphigus vulgaris showing row of tomb stone appearance (H&E 40X); b) Immunofluorescence pattern of pemphigus vulgaris; c) Bullous pemphigoid (H&E 10X); d) Immunofluorescence pattern of bullous pemphigoid.



[Table/Fig-11a-e]: a) Subcorneal pustular dermatosis (H&E,10X); b) Pustular psoriasis (H&E,10X); c) Spongiotic bullous (H&E,10X); d) Pemphigus vegetans (H&E,40X); e) Chronic bullous disease of childhood (H&E,40X).

LIMITATION

The limitation of the study is that we have not included the infectious bullous lesions and only the two most dominant lesions-pemphigus vulgaris and bullous pemphigoid have

been analysed with respect to positive predictive value and sensitivity.

CONCLUSION

Pemphigus vulgaris was the most common vesiculobullous lesion seen in patients seeking the institution, next being Bullous pemphigoid. Histopathology correlated with clinical diagnosis in 87% cases.

Among the immunoflourescence proven cases, histopathological diagnosis had a positive correlation in 77.77% cases. DIF was not effective in sub-classifying the various sub groups of pemphigus. The PPV of histopathological examination in diagnosing pemphigus group of diseases which are proven by immunofluorescence was 93.75% , sensitivity was 90.09%. The positive predictive value of histopathological examination in diagnosing bullous pemphigoid lesions which are proven by immunofluorescence was 93.33%, sensitivity was 77.77%.

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