

## ORIGINAL ARTICLE

## HISTOPATHOLOGICAL SPECTRUM AND ROLE OF CLINICOPATHOLOGICAL CORRELATION IN THE DIAGNOSIS OF VESICULOBULLOUS LESIONS

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**Background:** Histopathology is an important diagnostic modality for vesiculobullous lesions, however the diagnosis may at times require use of Immunofluorescence techniques which are expensive and not widely available. The aim of this study was to determine the histopathological spectrum of vesiculobullous diseases and to determine the role of clinic-pathological correlation in diagnosing bullous lesions. **Methods:** This was cross sectional validation study conducted in a tertiary care hospital, over a period of 18 months. All the clinically diagnosed cases of bullous diseases were included and examined as histological sections by three histopathologists. **Results:** Out of 58 total cases, the most frequently diagnosed lesions included Pemphigus vulgaris (27%), Bullous pemphigoid (13.8%) and Pemphigus foliaceus (12.1%). Females comprised 55% of cases, age distribution was wide but most patient were in age bracket of 20–39 years. **Conclusion:** There was 89.6% correlation between clinical and histopathological diagnosis. Only 2 cases were sent for Immunofluorescence studies, as histopathology was inconclusive in those cases. Therefore, we conclude that histopathological examination along with clinical correlation is a very useful way of diagnosing vesiculobullous disorders.

**Keywords:** Vesiculobullous disorders, Blisters, Immunofluorescence.

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### INTRODUCTION

Histopathological examination is an essential modality involved in diagnosis of vesiculobullous skin lesions.<sup>1</sup> This is a group of mucocutaneous lesions characterized by formation of vesicles and bullae (i.e., blisters). Both vesicles and bullae are fluid filled lesions distinguished on the basis of size; vesicles are <5 mm while bullae are >10 mm. Each entity included in this group has distinct presentation, pathogenesis, prognosis and treatment.<sup>2</sup> Hence accurate diagnosis of vesiculobullous lesions is of utmost importance for subsequent management of patients. Wide variety of pathological mechanisms can lead to development of these eruptions on mucous membranes as well as skin, which may include inflammatory causes, infections, autoimmune mechanisms, drug induced and genetic factors.<sup>3</sup> Histopathological evaluation of blisters include study of blister separation plane, presence or absence of inflammatory cells and predominant type of inflammatory cells.<sup>4,5</sup> Various international studies have highlighted either one entity or a specific aspect of it. There are only few international studies which have discussed in detail the histo-morphological spectrum of vesiculobullous diseases.<sup>6</sup> As some vesiculobullous lesions especially the ones with autoimmune aetiology require histopathology followed by direct or indirect immunofluorescence studies for definitive diagnosis.<sup>7</sup> Immunofluorescence studies are highly specialized

investigations which are not available in many hospitals. Only few specialized centers offer these and cost of these tests is quite high as compare to routine histopathology. Hence histopathological evaluation is considered a valuable tool for diagnosis in Dermatology. Diagnostic accuracy increases more by correlating clinical and histopathological findings.<sup>8</sup> Histopathological evaluation requires skin punch biopsy which is a simple, inexpensive and safe out-patient procedure, causing little discomfort and scarring.<sup>9</sup> In our country especially in this part of our country not much work has been done on histopathological spectrum of vesiculobullous lesions. Since Pakistan belongs to a third world country where cost of medical investigations is a big issue and also most of the hospital setups do not offer specialized investigations like Direct Immunofluorescence, therefore how reliable histopathology alone can prove in diagnosing bullous disorders, is an area that needs to be evaluated. The objectives of this research are to study the histopathological spectrum of vesiculobullous disorders and to document the role of clinical and histopathological correlation in diagnosing various bullous disorders of skin.

### MATERIAL AND METHODS

It is a Cross-sectional validation study carried out in Pathology department of Benazir Bhutto Hospital, Rawalpindi Medical University. Sampling technique was consecutive sampling. Sample size was calculated using WHO sample size calculator, keeping Confidence level

90% with relative precision of 20%. We took population proportion 50% to get maximum sample size. Calculated sample size was 68 with 14% dropout rate, actual sample size for this study came out to be 58. Sampling time was between 1<sup>st</sup> Jan 2018 to 30<sup>th</sup> June 2019. The inclusion criteria for samples included all skin biopsies received in Pathology department from Dermatology OPD with a clinical diagnosis of vesiculobullous lesion. Suspected malignancies and patients who refused to undergo biopsy were excluded from study. Detailed clinical history of all the patients was documented. Skin biopsies were allowed to fix in 10% buffered formalin fixative for 6–12 hours before tissue processing. Subsequent paraffin embedding followed by 3µm thin cutting of tissue blocks was carried out. Tissue sections were stained with Harris Haematoxylin and Eosin stain.<sup>10</sup> The prepared slides were evaluated by two Histopathologists and histopathological ambiguities were addressed by a senior histopathologist. Histopathological evaluation of each biopsy sample included plane of blister separation, i.e., whether blister was below keratin layer (subcorneal), within the epidermis (intraepidermal) or below the epidermis (suprabasal or subepidermal). Associated findings of spongiosis or acantholysis and presence or absence of inflammatory infiltrate along with predominant cell type was documented. All the biopsies were also discussed with consultant Dermatologist in a Dermatopathology meeting. Degree of percent agreement between dermatological diagnoses with dermatopathological diagnosis was also documented.

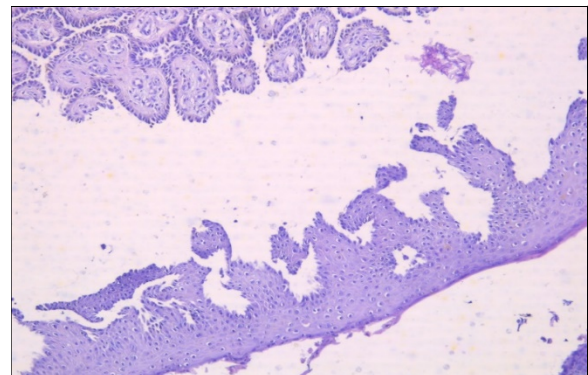
**RESULTS**

Total number of patients included in our study was 58 (n=58). Out of these, 16 (27%) were diagnosed as Pemphigus vulgaris. The other two frequent diagnoses included Bullous Pemphigoid and Pemphigus Foliaceus, accounting 8 cases (13.8%) and 7 cases (12.1%) respectively. Both male 26 (45%) and female 32 (55%) patients were included in the study. The age range of patients was between few months to 100+ years. Most of the patients (41%) lied in the age bracket of 20–39 years. The composition of inflammatory infiltrate varied among

different entities as tabulated in Table-1. Overall, on histopathologic examination, 51 cases showed inflammatory infiltrate, while 07 cases were free from inflammation. Out of 58 cases, 52 cases showed complete correlation between clinical and histopathological diagnosis. There were four cases in which diagnosis was established mainly on histopathology alone. While there were two cases in which definitive histopathological diagnosis could not be made with certainty and these were referred for immunofluorescence studies for final diagnosis. Correlation of clinical and histopathological diagnosis is shown in table-2.



**Figure-1: Clinical features of pemphigus vulgaris**



**Figure-2: Histology of pemphigus vulgaris with typical tomb stone appearance**

**Table-1: Entity-wise inflammatory cell composition of vesiculobullous disorders**

Cases	Neutrophils	Eosinophils	Lymphocytes	Mixed	Absent
Pemphigus Vulgaris	14	-	-	2	-
Bullous Pemphigoid	-	7	-	1	-
Pemphigus Foliaceus	7	-	-	-	-
Epidermolysis Bullosa	-	-	-	-	4
Erythema Multiforme	-	-	3	-	-
Dermatitis Herpetiformis	3	-	-	-	-
Linear IgA dermatosis	3	-	-	-	-
Sub-corneal Pustular Dermatitis	3	-	-	-	-
Allergic Contact Dermatitis	-	1	-	-	1
Bullous SLE	-	-	1	1	-
Chronic Bullous Dermatitis of Childhood	-	-	-	-	2
Pemphigus Gestationalis	-	-	1	-	-
Hailey-Hailey disease	-	-	-	1	-
Bullous Impetigo	1	-	-	-	-
Staphylococcal scalded skin syndrome	1	-	-	-	-
Viral Herpes	-	-	1	-	-
Total	32	8	6	5	7

**Table-2: Clinico-pathological correlation of vesiculobullous disorders (n=58)**

Correlations	No. of cases	Percentage
Histological confirmation of clinical diagnosis	52	89.6
Diagnosed only on histology, without any clinical suspicion of bullous disease	4	6.9
Histological ambiguous	2	3.4

## DISCUSSION

Vesiculobullous disorders comprise a group of cutaneous diseases having distinct aetiology, pathogenesis and histo-morphological features, which share common clinical symptom of mucocutaneous vesicle or bullous formation.<sup>11</sup> Entities included in this group have distinct clinical features. In our study, there were total 58 cases, out of which 26 (44.8%) were males and 32 (55%) were females. Literature also suggests that vesiculobullous lesions are more common in female patients as compare to males.<sup>12</sup> Vesiculobullous lesion can affect people of all ages, however, certain entities can have predilection for certain age groups. In our study, 41% of the patients belonged to age group of 20–40 years which is slighter younger age group as compare to most other studies.<sup>11–13</sup>

The most frequent entity in our study was Pemphigus vulgaris, comprising 27% of the cases. It is a rare Autoimmune blistering disease that comprises the most common type of Pemphigus, in which autoantibodies targeting Desmoglein (Dsg) adhesion proteins are found. The age range of this disease is wide with predominance between 50–60 years and being more common in females.<sup>14</sup> Most of the patients (61%) of this disease, in our study, belonged to age range of 20–39 years, which is different from literature.<sup>15</sup> Most of our patients were females (56%), as described by literature.<sup>13,15</sup> This disease is characterized clinically by painful ulcers, erosions and blisters on skin and mucous membranes (Figure-1). Histologically, Pemphigus vulgaris is characterized in its early stage by intercellular oedema with loss of intercellular attachments. The fully developed disease shows intraepidermal suprabasal clefting with intact basal cells on basement membrane giving the lesion a tomb stone appearance (Figure-2). The cleft shows occasional acantholytic cells along with mixed inflammatory cells.<sup>14</sup> In our study, all cases except one showed neutrophilic infiltrate. Pemphigus vulgaris with distinct histological picture can easily be diagnosed on histopathology alone. Several other studies also showed pemphigus vulgaris to be the most common blistering disorder.<sup>3,5,12,13,15</sup>

The second most frequent entity in our study was Bullous pemphigoid, comprising 13.8% of the cases. It is an autoimmune pruritic skin

disease, with autoantibodies directed to components of the basement membrane, particularly the BP antigens BP180 and BP230.<sup>16</sup> It preferentially effects elderly people.<sup>15</sup> Some studies report twice as more incidence in females as compared to males<sup>17</sup>, however, others report no gender predilection. In our study, most of the patients suffering from this disease were >50 years with an equal gender incidence. The disease is clinically characterized at an early stage by urticarial rash, but could also appear dermatotic, targetoid, lichenoid, nodular or even without visible rash (essential pruritus). Tense bullae eventually erupt. Any part of the skin surface can be involved with rare mucosa involvement. Histologically, the disease is characterized by subepidermal bullae with eosinophils and superficial dermal oedema. All cases except one, showed eosinophilic infiltrate in bullous cavity in our study. Direct IF studies typically show linear IgG (usually IgG4) and complement deposits at the basement membrane zone with non-serrated pattern. Clinical presentation along with histopathology is usually sufficient for definitive diagnosis of bullous pemphigoid.<sup>16</sup> In our study, almost all cases of bullous pemphigoid showed complete correlation among clinical and histopathological diagnosis.

The third most frequent disease in our study was Pemphigus foliaceus, comprising 12.1% of cases. Our study showed a wide age distribution from 20 to 100 years and a slight male predominance. Literature findings reveal wide age range of 30–60 years with no gender preferences.<sup>2</sup> All the cases of Pemphigus foliaceus in our study were diagnosed on the bases of clinical history and physical examination followed by histological confirmation.

The other relatively less frequent number of diseases included in our study were 4 cases of Epidermolysis bullosa, 3 cases of each Erythema multiforme, Dermatitis herpetiformis, Linear IgA Dermatitis and Subcorneal pustular dermatosis. There were two cases each of bullous SLE, Chronic bullous dermatosis of childhood and allergic contact dermatitis. Remaining less frequent entities are listed in Table-1.

There were 4 cases of vesiculobullous lesions which were clinically characterized as non-vesiculobullous dermatosis and were later

diagnosed as one of the types of vesicobullous lesions purely on the basis of histopathological features. There were two cases of early lesions of bullous pemphigoid. Both of these cases presented as nonspecific eruptive lesions with pruritis in dermatology OPD. Skin biopsy was done with clinical suspicion of urticaria/dermatitis. Subsequent histopathology revealed small bullae arising at the dermoepidermal junction along with eosinophilic infiltrate. Other studies also showed similar findings where early lesions of bullous pemphigoid were picked up by histopathology alone.<sup>2</sup> One of the cases that was diagnosed on histopathology alone was of Subcorneal pustular dermatosis, clinically labelled as pustular Psoriasis, other one was of Chronic bullous dermatosis of childhood.

In our study group there were two cases in which histopathological features were ambiguous and definitive diagnosis of a distinct entity could not be made with certainty. Therefore, these were referred for direct Immunofluorescence studies for final diagnosis. One of these cases proved to be of Linear IgA dermatosis. Linear IgA dermatosis has similar histopathological features as of Dermatitis Herpetiformis and requires Direct Immunofluorescence (DIF) for differentiation. DIF reveals linear IgA positivity in Linear IgA disease, whereas granular IgA positivity in Dermatitis Herpetiformis, along the basement membrane area.<sup>18</sup> The other case which required DIF for final diagnosis was of Epidermolysis bullosa Acquisita. In this case, histopathological examination showed subepidermal bulla containing neutrophilic and lymphocytic infiltrate & a differential diagnosis of Bullous pemphigoid (BP) was made with DIF testing suggestion.

The histopathological findings of BP and Epidermolysis Bullosa Acquisita (EBA) are similar but requires DIF for differentiation. DIF Salt split skin test (SSS) reveals IgG positivity along the roof of cleft in BP and along the floor of cleft in EBA.<sup>19</sup> In this case, final diagnosis of Epidermolysis bullosa acquisita was made on DIF.<sup>19</sup> Out of 58 cases, percent agreement between clinical and histopathological diagnosis is 89.6%. A previous study<sup>1</sup> showed 87% correlation among clinical and histopathological diagnosis while another study<sup>2</sup> showed 64.88% clinicopathological correlation.

#### Limitation:

The main limitation of our study was limited sample size which missed important vesiculobullous lesions like Darier's disease, Cicatricial pemphigoid and Paraneoplastic pemphigus.

## CONCLUSION

Hence our study concluded that histopathological findings alone can be used as the single diagnostic modality in diagnosing various subtypes of vesiculobullous lesions. The diagnostic accuracy of histopathology increases more when it is correlated with clinical history and findings.

## AUTHORS' CONTRIBUTION

SK: Concept, design, data acquisition & analysis. HS: Data analysis, publication draft and revision. AI: Concept and design. MM, NT, SS: Acquisition of data.

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