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Clinical, Histopathological and Immunofluorescent Study of Vesicobullous Lesions of Skin

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ABSTRACT

BACKGROUND: An important class of dermatological conditions known as immunobullous illnesses is brought on by pathogenic autoantibodies that target antigens in the dermo-epidermal junction or intercellular material. The accurate diagnosis plays a major role in the management of many disorders. Among bullous illnesses, different clinical signs have different diagnostic specificities. Clinical overlap exists across several categories of bullous disorders. Early vesicles should ideally undergo histological evaluation in order to identify the place of formation as well as the amount, kind, and makeup of the inflammatory infiltrate. The combination of these findings leads to the creation of a differential diagnosis. Blisters appear to indicate that blistering is too generic or vague to be used in a clinical gross examination, at least initially. These conditions do, however, continue to be linked to high rates of morbidity, high rates of death, and poor quality of life. An accurate diagnosis of vesiculobullous lesions in the skin requires a review of immunofluorescence, histopathologic, and clinical data.

AIM: The aim was to evaluate the correlation between clinical, histopathological, and direct immunofluorescence (DIF) patterns.

MATERIAL AND METHOD: Patients undergoing treatment at the hospital's Department of Pathology participated in a cross-sectional, descriptive study designed to investigate the clinical, histological, and DIF characteristics of vesiculobullous illnesses. Following approval from the institutional ethics committee and signed consent, vesiculobullous lesions were checked for in all patients visiting the dermatology outpatient department. A thorough history and clinical examination were performed on patients with vesiculobullous lesions, paying special attention to factors such as age, gender, the morphology of the lesions, the place of involvement, and clinical tests like the Bulla spread sign and Nikolsky's sign. Since these conditions have a variety of clinical presentations, patients with clinical characteristics suggestive of immunobullous, mechanobullous, severe adverse cutaneous medication reactions, or metabolic disorders were included in the study.

RESULTS: The Department of Pathology carried out a cross-sectional hospital descriptive research for the current investigation. Out of the 60 instances examined in this study, 53.3% (32 cases) had a mean age of 44.12 years (4–70 years), indicating a male majority; the remaining 46.6% (28 cases) had a mean age of 50.17 years (20–70 years). The bulk of patients (30%) were between the ages of 51 and 60, with a mean age of 47.04 years and a slight 1.07:1 male preponderance. In the study, the oldest patient was seventy years old, while the youngest was four years old.

CONCLUSION: In the current investigation, Pemphigus foliaceus and Pemphigus vulgaris could be distinguished only based on histological analysis. In order to distinguish between epidermolysis bullosa acquisita and bullous pemphigoid, which have a similar histological appearance, direct immunofluorescence was helpful. This work demonstrates that for vesiculobullous illnesses, direct immunofluorescence is both diagnostic and confirming. The final diagnosis of vesiculobullous illnesses is aided by the mixture or combination of clinical, histological, and DIF characteristics. None of these techniques is always diagnostic when used alone.

KEYWORDS: Vesiculobullous disorders, Bullous Pemphigoid and Direct immunofluorescence

INTRODUCTION

In addition to serving as a protective layer, skin is a component of the body's immune system. Vesiculobullous illnesses are varied and encompass a wide range of conditions.[1,2] The presence of vesicles or bullae at any level within the epidermis or at the dermo-epidermal junction is what defines the vesiculobullous response pattern.[3] Vesiculobullous diseases are a diverse group of dermatoses with a range of clinical symptoms. In recent years, the disorders have been the focus of extensive research.[4] A class of illnesses known as vesiculobullous diseases are characterized by a primary lesion that is either a bulla or a vesicle on

the skin, mucous membrane, or both. Veniculobullous lesions rank among the most common clinical issues among the numerous dermatological disorders. It is among the classic skin reaction patterns to injury resulting from internal and exterior situations and forces.

A useful method that has made significant advancements in the diagnosis, management, and pathogenesis of vesiculobullous skin lesions is immunofluorescence. It is employed in clinical laboratories as well as scientific research. Immunofluorescence is an unavoidably powerful tool in the identification of bullous disorders due to its relative simplicity and accuracy.[5] Vesicobullous diseases are a diverse group of dermatoses characterized by a range of symptoms. They have a profound effect on the patients and their families and have negative financial repercussions. Blister development can be caused by five main mechanisms: pharmacological responses, physical, immunological, and inflammatory damage, and genetic derangement. The majority of vesiculobullous dermatological illnesses are caused by these immune reactions.[6] Since many of these blistering illnesses have clinical and histological similarities, immunofluorescence techniques are utilized in addition to standard histological investigation to diagnose vesicobullous skin lesions.[5]

Blister separation plane, blister formation mechanism, and the nature of the inflammatory infiltrate—including its presence or absence—are all part of the systematic investigation that goes into the pathologic evaluation of blisters.Six Discoveries in investigative dermatology have opened up new possibilities. The most crucial methods for studying patients with vesiculobullous disease include indirect and direct immunofluorescence as well as traditional histology for confirmation.[4] In recent years, vesiculobullous illnesses have been the subject of extensive research. These conditions do, however, continue to be linked to high rates of morbidity, high rates of death, and poor quality of life. The amount of documentation regarding the histologic changes of dermatitides under different therapy, as well as their temporal relationship, is inadequate to enable an ideal or even reliable diagnostic research.[7] Unna's pioneering work in dermatopathology provides a foundation for both contemporary dermatology and the application of immunofluorescence in skin immunopathology research.[8] The diagnosis, management, and understanding of the pathogenesis of vesiculobullous lesions of the skin have all benefited tremendously from immunofluorescence.[9] Positive direct immunofluorescence (DIF) results in individuals in remission indicate an early disease relapse, making it a valuable prognostic tool as well.[10] Research methods like immunoblotting and immunoelectron microscopy can help determine a patient's precise diagnosis. In poor nations like India, only a few locations even perform DIF. Most dermatologists may now obtain DIF because to the availability of transport media like Michel's medium. The objectives were to investigate and compare various forms of vesiculobullous lesions with respect to age and sex, to appraise the diagnostic utility of DIF, and to analyze the relationship between histological, clinical, and DIF patterns.

MATERIAL AND METHODS

Patients undergoing treatment at the hospital's Department of Pathology participated in a cross-sectional, descriptive study designed to investigate the clinical, histological, and DIF characteristics of vesiculobullous illnesses. Following approval from the institutional ethics committee and signed consent, vesiculobullous lesions were checked for in all patients visiting the dermatology outpatient department. A thorough history and clinical examination were performed on patients with vesiculobullous lesions, paying special attention to factors such as age, gender, the morphology of the lesions, the place of involvement, and clinical tests like the Bulla spread sign and Nikolsky's sign. Since these conditions have a variety of clinical presentations, patients with clinical characteristics suggestive of immunobullous, mechanobullous, severe adverse cutaneous medication reactions, or metabolic disorders were included in the study. In these conditions, histopathology and DIF aid in making the final diagnosis, ruling out alternative diagnoses, and figuring out how the illness progresses and how well it responds to therapy. A total of 60 patients with vesiculobullous lesions of both sexes who visited the Department of Dermatology between the ages of 3 and 70 were chosen, and their clinical, histological, and direct immunofluorescence (DIF) data were examined.

Inclusion criteria

- All skin biopsies from the cases with vesiculobullous disorders irrespective of age, sex, and associated diseases.

Exclusion criteria

- Blisters caused by mechanical, thermal, suction, or chemical agents; drug-induced blisters; bullous lesions resulting from infections; and other conditions such as irritant contact dermatitis and eczematous dermatitis were not

included in the study because they did not exhibit typical clinical features and histopathology or DIF were not the primary means of diagnosis.

Procedure:

For histological analysis, a punch biopsy was taken from the lesional skin or oral mucosa in each patient, ideally with an intact vesicle. A second sample was collected for DIF from the perilesional normal-looking skin or oral mucosa. Hematological and biochemical tests were performed on a routine basis, and the results were documented. In every instance, two 4 mm punch biopsies were performed: one from the perilesional area (sent in Michel's transport medium for DIF testing) and one from the vesicle (fixed in 10% buffered formalin for histological investigation). Following usual protocol, specimens collected in 10% formalin had a thorough examination and were given bits. Hematoxylin and eosin was used to stain sections of the tissue after it underwent standard processing. Clinical and immunofluorescence findings were correlated with the diagnosis of the histological sections. Based on the distinctive clinical characteristics, a clinical diagnosis was made. When clinical symptoms overlapped, differential diagnoses were retained rather than relying just on one diagnosis. After taking into account the clinical, histological, and DIF findings, a definitive diagnosis was made for each case. Proforma was followed in the recording and analysis of the histopathologic results.

A biopsy was collected from the oral mucosa or perilesional skin for Direct Immunofluorescence (DIF). The tissue was snap frozen, sections were cut, and polyclonal fluorescence isothiocyanate (FITC) conjugated antisera specific for IgG, IgA, IgM, C3, and fibrinogen were used to stain the tissue. Under a fluorescent microscope, the pattern and distribution of immune complex deposits were qualitatively examined, and the intensity was ascertained semi-quantitatively. Data was evaluated and findings from immunofluorescent staining and light microscopy were correlated.

STATISTICAL ANALYSIS

Data storage and analyses were performed using the SPSS Version 19.0. Chi-Square test and unpaired student test were used to get analysis. Data were analyzed both using an intention to treat philosophy and analysis by treatment principle. Descriptive statistics were obtained for demographic variables.

RESULT: -

The Department of Pathology carried out a cross-sectional hospital descriptive research for the current investigation. Out of the 60 instances examined in this study, 53.3% (32 cases) had a mean age of 44.12 years (4–70 years), indicating a male majority; the remaining 46.6% (28 cases) had a mean age of 50.17 years (20–70 years). The bulk of patients (30%) were between the ages of 51 and 60, with a mean age of 47.04 years and a slight 1.07:1 male preponderance. In the study, the oldest patient was seventy years old, while the youngest was four years old.

Table 1: Distribution of cases (n=60).

Type	Frequency	%
Pemphigus vulgaris	17	28.3
Pemphigus foliaceous	10	16.6
Bullous pemphigoid	10	16.6
Darier's disease	6	10
Hailey Hailey disease	4	6.6
Dermatitis herpetiformis	3	5
chronic bullous disorder of childhood (CBDC)	1	1.6
Epidermolysis bullosa acquisita	3	5
Bullous erythema multiforme	2	3.3
Lichen planus pemphigoid	2	3.3
Ichthyosis form erythroderma	2	3.3
Total	**60**	**100**

In the present study, pemphigus vulgaris constituted the most common vesiculobullous disorder constituting 28.3% (17 out of 60 cases), followed by Bullous pemphigoid and Pemphigus foliaceous, 16.6% each (10 out of 60 cases). Out of the remaining vesiculobullous diseases, Darier's disease constituted 10% (6 out of 60 cases), Hailey Hailey disease 6.6% (4 out of 60 cases), dermatitis herpetiformis and epidermolysis bullosa acquisita-5% each (3 out of 60 cases). The least common were, bullous erythema multiforme, lichen planus pemphigoid, and ichthyosis form erythroderma which constituted 3.3% each.

Table 2: Findings of direct immunofluorescence.

Antibody	Deposition	Type	No. of cases
IgG	ICS	PV	17
		PF	10
		BP	10
	BMZ	EBA	3
		LPP	1
IgM	BMZ	BEM	1
		BP	1
IgA	BMZ	DH	1
C3	ICS	PV	17
		PF	10
		BP	10
		EBA	3
	BMZ	BEM	1
		LPP	1
		DD	6
		HH	4
Negative		CBDC	1
		IE	1

Direct immunofluorescence analysis revealed that all Pemphigus vulgaris cases had intercellular deposition of IgG and C3 in a fishnet pattern. The top epidermis of every Pemphigus foliaceus case also displayed intercellular deposition of IgG and C3. The basement membrane in every bullous pemphigoid instance had linear IgG and C3 deposition. Along with IgG and C3, one bullous pemphigoid case also displayed IgA deposition along the basement membrane. The basement membrane in one lichen planus pemphigoid instance had IgG and C3 deposition. Of the two cases of dermatitis herpetiformis, only one had IgA deposition at the BMZ that was granular and compatible with the diagnosis, whereas the other case had IgA deposition that was linear and inconsistent with the diagnosis.

Table 3: Clinicopathological correlation and deferred cases (n=60).

Clinical diagnosis	No. ofcases	Correlatedwith HPE	Not correlatedwith HPE
Pemphigusvulgaris	19	15	4 (PF-3,BP-1)
Pemphigusfoliaceous	5	5	-
Pemphigusvegetans	1	-	1 (PV)
Pemphigus erythematosus	1	-	1 (PF)
Paraneoplasticpemphigus	1	-	1 (PV)
Bullous pemphigoid	9	6	3 (PF-1, BEM-1, LPP-1)
Darier's disease	7	7	-
Hailey Haileydisease	4	4	-
Dermatitis herpetiformis	7	4	3 (BP-3)
CBDC	1	1	-
Epidermolysis bullosa acquisita	3	3	-
Bullous erythemamultiforme	1	1	-
Ichthyosis form erythroderma	1	1	-
Total	**60 (100%)**	**47 (78.3%)**	**13 (21.6%)**

Out of 60 instances, a total of 47 cases (78.3%) had an established overall clinicopathological association. Of the 19 clinically diagnosed cases of pemphigus vulgaris, histological analysis identified 15 as pemphigus vulgaris, 3 as pemphigus foliaceous, and 1 as bullous pemphigoid. As a result, in 15 cases (78.94%) the clinicopathological correlation was found, and it was statistically significant. Histopathological evidence supported the bullous pemphigoid diagnosis in six of the nine

clinically diagnosed patients. Thus, in 66.6% of cases, there was a clinicopathological association seen. In one instance, pemphigus vulgaris was identified histopathologically in place of both the clinically diagnosed paraneoplastic pemphigus and pemphigus vegetans. Only four of the seven instances of dermatitis herpetiformis that were clinically diagnosed were found to be DH; the other three cases were identified as bullous pemphigoid. The current investigation found a statistically significant overall clinicopathological association in erythroderma caused by Ichthyosis form, CBDC, Hailey's illness, and Darier's disease.

DISCUSSION

On histopathological examination, vesiculobullous lesions might be difficult to diagnose. There are now new possibilities in investigative dermatopathology thanks to recent developments.[4] Significant progress has been achieved in the past 20 years in our understanding of the molecular basis and clinical behavior of autoimmune disorders. Clinical and immunofluorescence patterns play a major role in the classification of immunological diseases. Some of the effects of systemic corticosteroids may still be lethal even after they are introduced. Thus, early diagnosis and treatment of the illness are imperative.[11] It is imperative to perform biopsies on early lesions in order to provide a histological diagnosis. A vesiculobullous lesion may not always be accurately diagnosed once epidermal regeneration starts or subsequent alterations like ulceration or infection happen.[3]

Shafi M et al.1994[12] studied 109 cases of pemphigus from Tripoli, Libya, and found that the incidence of pemphigus in Libya is very high, with the predominant variant being pemphigus foliaceus. In their study, **Shafi M et al.1994**[12] also found out that males were more affected than females. 3 In the present study, pemphigus vulgaris was the most common clinically diagnosed disease accounting for 15 cases, out of which 12 cases were later confirmed by histology and by DIF. There was a difference in the diagnosis of 3 cases, out of which 2 cases were pemphigus foliaceus and one case was sub-corneal pustular dermatosis.

Nguyen et al.2001[13] introduced the concept of paraneoplastic autoimmune multiorgan syndrome (PAMS), highlighting the systemic nature of PNP. Usually, a neoplasm is detected before the onset of PNP.[14-16] However, PNP is the first clinical manifestation that leads to the detection of an occult tumor in about 30% of cases.[17] Bullous pemphigoid shows clinical similarity to pemphigus (hence its name) but the blisters are sub-epidermal, not intraepidermal.[14] It is most common in people over the age of 50 years, with male preponderance.[15] It is a commoner disease in Europe and North America as the average age of the population increases and is characterized by the presence of large, tense bullae, usually on the thighs, arms, and abdomen.[18] **Olbricht et al.1986**[19] in their study of 21 cases demonstrated papillary micro abscesses containing predominantly neutrophils in almost all the cases and they were common in males and the predominant age group was between 20-40 years.

Kabir et al.2008[20] where less than 50% of clinical diagnosis was in concordance with histopathology. This type of clinical histopathological discrepancy may result from secondary modifications or from the patient's prior therapy, which altered the morphology of the lesions. Making the right diagnosis can also depend on the biopsy site selection. This suggests that depending on clinicopathological association rather than just clinical findings is more significant. The study by **Javidi et al.2007**[21] showed mucosal involvement in 14% skin involvement in 21.7% and involvement of both the skin and mucosa in 64.3% of the patients. 50% patients of with PE showed only skin involvement whereas the other 50% showed involvement of both skin and mucosa. **Minz et al.2010**[1] in their study proved that 77% of their DIF diagnosis correlated with the clinical diagnosis whereas histopathology correlated with 70% of the clinical diagnosis. However, in the study conducted by **Kabir et al.2008**[20] less than 50% of cases showed concordance of histopathology with DIF diagnosis.

According to a study conducted by **Buch et al., 2014**[22] DIF is a very reliable diagnostic test for pemphigus, which becomes positive at an early stage and remains positive for a long period after clinical remission. A combination of clinical, histologic, and immunologic data is required to establish the definitive diagnosis of various immunobullous illnesses in the absence of the distinctive DIF pattern. Even while only a tiny percentage of persons develop "primary" vesiculobullous skin lesions, they have been linked to a high rate of morbidity and mortality. In order to manage and treat each of these entities effectively, it is critical to distinguish between them. Histopathological and DIF testing are essential since they validate the diagnosis and subtyping, even though clinical evaluation is important in and of itself. Even though the complete panel of DIF is advised as a standard approach, most cases can be accurately diagnosed using DIF in conjunction with an anti-G IgG FITC antibody and a histopathological investigation since these methods are more practical and appear to be superior

alternatives. The initial step in diagnosing vesiculobullous illnesses is a clinical examination. For a final diagnosis, a histopathological investigation and DIF are necessary. When histological or clinical findings are equivocal, DIF can be useful. As a result, the final diagnosis is reached by taking into account the combined effects of clinical, histological, and DIF aspects, since one of these approaches may not be diagnostic in every situation.

CONCLUSION:

The most prevalent vesiculobullous condition in the current study was pemphigus vulgaris, which was followed by pemphigus foliaceous and bullous pemphigoid. In the current investigation, Pemphigus foliaceus and Pemphigus vulgaris could be distinguished only based on histological analysis. When attempting to distinguish between epidermolysis bullosa acquisita and bullous pemphigoid, which share the same histological appearance, direct immunofluorescence proved to be helpful. There was demonstrated overall clinicopathological connection. In 78% of cases, a direct link between histopathology and immunofluorescence was revealed. This work demonstrates that for vesiculobullous illnesses, direct immunofluorescence is both diagnostic and confirming. To maximize its usefulness, larger studies utilizing well chosen examples and prudent application are required.

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