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A CLINICAL, HISTOPATHOLOGICAL AND DIRECT IMMUNOFLUORESCENCE CORRELATION OF AUTOIMMUNE BULLOUS DISORDERS

Dermatology

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ABSTRACT

Background: Autoimmune vesiculobullous disorders are group of blistering dermatoses in which pathogenic autoantibodies are present against various antigens against adhesion molecules in epidermis and dermo-epidermal junction which leads to blister formation.
Aims: To study various clinical patterns and morphology of autoimmune bullous disorders and their histopathological findings along with direct immunofluorescence findings and to correlate between clinical, histopathological and immunofluorescence findings.
Materials and Methods: A cross sectional study was done on 70 patients in OPD of dermatology department, detailed clinical history, examinations and routine investigations were done and recorded. Two biopsies from lesion and perilesional skin were done for histopathology and immunofluorescence respectively.
Results: The clinical, histopathological and direct immunofluorescence concordance for cases ranged from 0% to 100%.The concordance was 81.39% in pemphigus vulgaris, 72.73% in pemphigus foliaceus, 62.5% in bullous pemphigoid and, 40% in dermatitis herpetiformis. We had two cases of linear IgA bullous dermatitis and only one case of Pemphigoid gestationalis. The percentages of concordance in these cases were 100%, for both. Out of 70 cases of autoimmune vesiculobullous diseases clinical, histopathological and immunological correlation was present in 54 cases (77.14%).

KEYWORDS

Autoimmune, vesiculobullous diseases, histopathology, direct immunofluorescence, correlation

Introduction

Autoimmune vesiculobullous disorders represent a diverse group of dermatoses with variable manifestations. They have dramatic impact on the patient and their family and have severe economic consequences for the family and health services. The diseases have been investigated intensively in recent years.[1]

The immunological diseases are classified largely by their clinical presentation, histopathology and immunopathology. The immunobullous diseases are characterized by pathogenic autoantibodies directed at target antigens whose function is either cell-to-cell adhesion within the epidermis or adhesion of stratified squamous epithelium to dermis or mesenchyme. These target antigens are components of desmosomes or the functional unit of the basement membrane zone known as the adhesion complex.[1]

Histopathology and direct immunofluorescence are the most important techniques for investigations of patients with immunobullous diseases. Direct immunofluorescence technique most valuable for confirmation of diagnosis made by clinical and histopathological examination. This is especially true for subepidermal immunobullous disorders, which have overlap in clinical and histopathological findings.[2]

Methods

A cross-sectional, descriptive hospital based study of clinical ,histopathological and DIF features of vesiculobullous diseases was conducted on patients attending department of dermatology of tertiary care hospital over a period of two year.

After obtaining institutional ethical committee clearance and written consent, all the patients attending out-patient department of dermatology were screened for presence of vesiculobullous lesions. In patients with vesiculobullous lesions, detailed history and clinical examination was done with particular reference to age, gender, morphology of lesions, site of involvement and clinical tests such as Nikolsky's sign and Bulla spread sign. The patients with clinical features suggestive of immunobullous disorders were included in the study as these disorders show varied clinical manifestations. Histopathology and DIF in these disorders help in the final diagnosis, exclusion of differential diagnosis and determining course of the disease and their response to treatment.

In all the patients, punch biopsy from the lesional skin preferably including intact vesicle was performed for histopathological study and another biopsy from perilesional normal looking skin was taken for DIF. Of the two biopsies, one was sent in cold normal saline for DIF and the other in 10% neutral buffered formalin for hematoxylin and eosin staining (H and E).

Results

The present study was conducted over a period of 24 months from November 2013 to October 2015 in the OPD of Dermatology, Venereology and Leprosy in a tertiary care hospital. In the present study, pemphigus vulgaris (Figure 1) constituted the most common vesiculobullous disorder constituting 61.4% (43 out of 70 cases), followed by pemphigus foliaceus constituting 15.7% of the cases. Bullous pemphigoid (Figure 2) and dermatitis herpetiformis constituted 11.4% and 7.2% of the cases respectively.

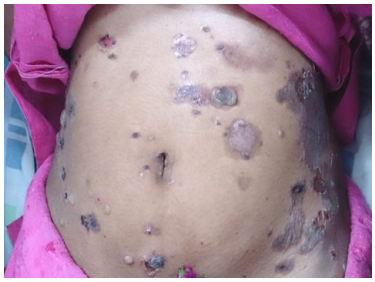


Figure 1: Flaccid vesicles, bullae, erosions and crusted plaques on the trunk in a patient with pemphigus vulgaris



Figure 2: Tense vesicles and bullae on erythematous base, erosions and crusts, on legs in a patient with bullous pemphigoid

Linear IgA disease was found in 2.9% of cases and pemphigoid gestationis constituted 1.4% of total cases as shown in [Table 1].The youngest patient was 09 years old and the oldest patient was 75 years old. The maximum numbers of cases were seen in the age group 31-40 years (28.6%) followed by 41-50 years (20%), 51-60 years and 21-30 years (14.3%) which had equal number of cases. The mean age of the study population was 42.25 years. Most cases of pemphigus vulgaris (23.26%), pemphigus foliaceus (36.37%) and bullous pemphigoid (62.5%) had illness for 1–6 months before presentation. In cases of dermatitis herpetiformis, 40% had illness for 1-6 months and 40% had disease of duration 7-12 months. The 50% of the cases of LAD had illness for 25–36 months and 50% for 7–12 months before presentation.

Table – 1: Distribution of types of vesiculobullous disorders

Type	Number of cases	Percentage
Pemphigus vulgaris	43	61.4%
Pemphigus foliaceus	11	15.7%
Bullous pemphigoid	08	11.4%
Linear IgAdisease	02	2.9%
Dermatitis herpetiformis	05	7.2%
Pemphigoid gestationis	01	1.4%
Total	70	100

It was noticed that the most predominant lesions were fluid filled lesions (Vesicles/bullae) in all autoimmune vesiculobullous disorders. Erosions/crusting were present in 100% of cases of pemphigus, linear IgA disease and pemphigoid gestationis and 75% patients of bullous pemphigoid and 60% of dermatitis herpetiformis patients. Oral lesions were present in 79.07% of pemphigus patients and 50% patients of bullous pemphigoid and linear IgA disease. The commonest site of mucosal involvement was oral mucosa, which was involved in 79.07% of the cases of pemphigus vulgaris and 50% of the bullous pemphigoid cases. Out of all, 14 patients (32.56%) with pemphigus vulgaris also had involvement of the genital mucosa in addition to involvement of the oral mucosa. There was no mucosal involvement in pemphigus foliaceus, chronic bullous disease of childhood and pemphigoid gestationis as shown in [Table 2].

Table – 2: Distribution of mucosal involvement

Mucous membrane	PV	PF	BP	DH	LAD	PG
Oral	34 (79.07%)	0 (0%)	04 (50%)	0 (0%)	0 (0%)	0 (0%)
Conjunctiva l	02 (04.65%)	0 (0%)	01 (12.5%)	0 (0%)	0 (0%)	0 (0%)
Nasal	07 (16.28%)	0 (0%)	01 (12.5%)	0 (0%)	0 (0%)	0 (0%)
Genital	14 (32.56%)	0 (0%)	01 (12.5%)	0 (0%)	0 (0%)	0 (0%)

Acantholytic cells were present on Tzanck smear in all the patients with pemphigus group of disorders. There was also presence of inflammatory cells, predominantly neutrophils in pemphigus vulgaris (53.49%) and pemphigus foliaceous (54.55%). Tzanck smear showed absence of acantholytic cells and presence of inflammatory cells in all patients with bullous pemphigoid and linear IgA disease, with predominance of neutrophils (100%) in bullous pemphigoid and predominance of neutrophils (100%) and other cells (100%) in Linear IgA disease.

Histopathology showed presence of subcorneal blisters with acantholytic cells and few inflammatory cells in the epidermis in all patients with pemphigus foliaceus. Suprabasal cleft with acantholytic cells were present in all patients and inflammatory cells in the epidermis were present in patients with pemphigus vulgaris. Row of tombstone appearance was seen in majority of the cases of pemphigus vulgaris (58.14%) (Figure 3).

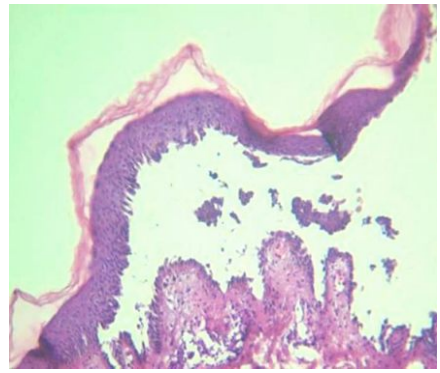


Figure 3: Pemphigus vulgaris – suprabasal bulla and "row of tombstone" appearance in the basal layer of the epidermis

In epidermal changes, acantholysis was present in 90.07% of pemphigus vulgaris cases and in 100% of cases of pemphigus foliaceus. Other changes like villi were present in 41.86% and hyperkeratosis in 27.43% of cases of pemphigus vulgaris. Subepidermal cleft, and eosinophils in the epidermis were present in all bullous pemphigoid patients. Subepidermal cleft and inflammatory cells in the epidermis was present in all patients with linear IgA disease as shown in [Table 3].

Table – 3:Distribution according to epidermal changes on histopathology

Final diagnosis	Epidermal changes					
	Row of tombstone appearane	Villi	Hyperkeratosis	Dyskeratosis	Acanthosis	Acantholysis
PV	25 (58.14%)	18 (41.86%)	12 (27.43%)	0 (0%)	06 (13.95%)	39 (90.07%)
PF	0 (0%)	02 (18.18%)	0 (0%)	02 (18.18%)	02 (18.18%)	11 (100%)
BP	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
DH	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
LAD	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PG	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

The dermis showed inflammatory cell infiltrate in all the subtypes of autoimmune vesiculobullous disorders. Pemphigus vulgaris and pemphigus foliaceus showed predominance of neutrophils in 27.91% and 36.37% respectively. Linear IgA disease also showed predominance of neutrophils. A characteristic papillary micro abscess was present in 60% cases of dermatitis herpetiformis. Predominantly eosinophilic infiltrate was present in 50% cases of bullous pemphigoid and in 100% cases of Pemphigoid gestationis as shown in [Table 4].

TABLE – 4: Distribution of dermal changes in histopathology

Final diagnosis	Dermal changes			
	Papillary oedema	Papillary microabscesses	Predominantly eosinophilic infiltrate	Predominantly neutrophillic infiltrate
PV	0(0%)	0(0%)	0(0%)	12(27.91%)
PF	0(0%)	0(0%)	1(0.91%)	4(36.37%)
BP	1(12.5%)	0(0%)	4(50%)	2(25%)
DH	3(60%)	3(60%)	1(20%)	1(20%)

LAD	1(50%)	0(0%)	0(0%)	2(100%)
PG	0(0%)	0(0%)	1(100%)	0(0%)

DIF pattern of deposition of various antibodies is shown in [Table 5 and figure 4&5]

TABLE – 5: Antibody and complement deposition pattern

S.NO.	FINDINGS		PV	PF	BP	DH	LAD	PG
1	IgG deposition	Intercellular	35	7	–	–	–	–
		BMZ	_		4	1	_	_
2	IgA deposition	Intercellular	_	2	–	–	–	–
		BMZ	_	_	1	1	2	1
3	IgM deposition	Intercellular	2	_	–	–	–	–
		BMZ	_	_	_	_	_	_
4	C3 deposition	Intercellular	17	5	_	_	_	_
		BMZ	_	_	2	_	1	0
5	Fibrinogen deposition	Intercellular	_	_	–	–	–	–
		BMZ	_	_	1	_	_	1

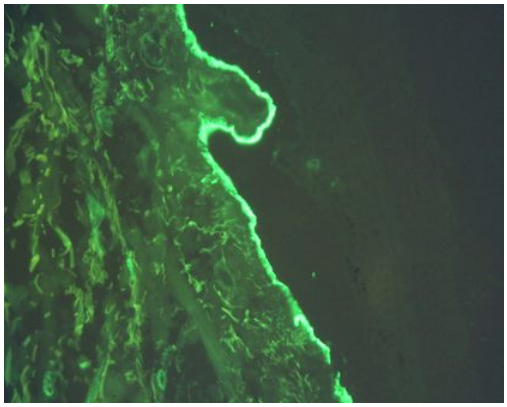


Figure 4: Bullous pemphigoid – direct immunofluorescence showing deposition of IgG and C3 in linear pattern at dermo-epidermal junction

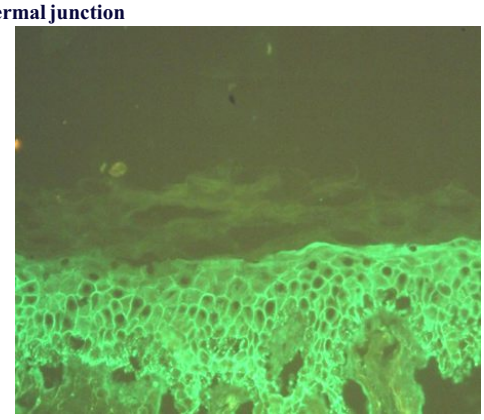


Figure 5: Pemphigus vulgaris – direct immunofluorescence showing deposition of IgG and C3 in 'fishnet pattern'

In our study there was an average concordance between clinical and histopathological diagnosis was 84.29% and discordance was 25.71%. Out of 43 cases of pemphigus 38 were histopathologically confirmed so that concordance in pemphigus vulgaris was 88.37%. The concordance in pemphigus foliaceus was 81.82% nearly similar to pemphigus. In bullous pemphigoid concordance was 75% and in dermatitis herpetiformis it was minimum (60%) of all the autoimmune vesiculobullous diseases. The concordance was highest in linear IgA disease and pemphigoid gestationis that was 100% may be due to less number of cases in both as shown in [Table 6].

TABLE – 6: Concordance between clinical, histopathological and DIF diagnosis various autoimmune vesiculobullous disorders

Name	Clinical diagnosis	Histo-pathologic diagnosis	DIF diagnosis	Concordance Clinico/ Histo-pathologic	Concordance Histo-pathologic/ DIF	Concordance Clinico-histopathologic/ DIF
PV	43	38	35	88.37%	92.1%	81.39%
PF	11	9	8	81.82%	88.89%	72.73%
BP	8	6	5	75.00%	83.33%	62.5%
DH	5	3	2	60.00%	66.67%	40%
LAD	2	2	3	100.00%	100%	100%
PG	1	1	1	100.00%	100%	100%
Total	70	59	54	84.29%	91.5%	77.14%

Discussion

The incidence of autoimmune vesiculobullous disorders among the OPD cases in two year works out to be 0.14% which is comparable to study done by Kanwar AJ et al[3]. Pemphigus vulgaris was the most common vesiculobullous disorder constituting 61.4% (43 out of 70 cases) followed by pemphigus foliaceus constituting 15.7% (11 out of 70 cases) of the total cases. Bullous pemphigoid constituted 11.4% of the cases. The pattern of frequency of different types of autoimmune vesiculobullous disorders correlates closely to Arya et al[4] study. The incidence of Linear IgA disease was 2.9% in our study which correlates closely with study by Kanwar AJ et al[3]. Dermatitis herpetiformis was present in 7.2% of cases. Also there was a case of pemphigoid gestationis like in study of Solanki A et al[5].

The mean age of onset for pemphigus vulgaris was 39.33 years and M: F ratio was 1:1.38 in the present study which is almost similar to the results of Javidi Z et al[6] study. Females had a slightly higher incidence of pemphigus vulgaris and pemphigus foliaceous compared to males in the present study. All of these studies Abobaker et al[7], Javidi Z et al[6], Micali G et al[8] and Deepti SP et al[9]also show similar results. The age of the patients ranged from 13 to 70 years. 32 (74.41%) cases were in the age group 21-60 years, which is in agreement with the studies done by Handa F et al[10]and Arya S et al[4]. Oral mucosa was the most common site of mucosal involvement in pemphigus vulgaris in the present study (34 out of 43 cases) which is similar to Micali G et al[8] study. Tzanck smear observations showed acantholytic cells in 100% of the cases. This is in concordance with various studies conducted by MM Huda et al[11], Leena JB et al[12] and Kambil SM et al[13]. The main inflammatory infiltrate comprising of neutrophils was seen in 53.49% cases in the present study as compared to 58.8% cases in Deepti SP et al[9]. The main histopathological features observed in cases of pemphigus vulgaris showed acantholysis in 90.69% cases which closely relates to the finding in Arya SR et al[4] and Arundhati S et al[14] study and suprabasal bulla was present in 74.42% which was less than above of the studies. "Row of tombstone" appearance of basal cells is another histological feature favouring the diagnosis of pemphigus vulgaris which is present in 58.14% of the cases in the present study which is slightly higher as compared to studies by Arya SR et al[4] (41.8%), Leena JB et al[12] (36.34%) and Arundhati S et al[14] (41.8%) but Deepti SP et al[9] showed this in 70.5% cases higher than the present study.

Pemphigus foliaceus constituted 15.7% of the total cases which correlates closely with the findings of the study by Fernandez J et al[15]. The salient histopathological features of pemphigus foliaceous observed in our study were acantholysis (100%) and subcorneal bulla (81.82%), identical to that observed by Arundhati et al[14].

Bullous pemphigoid constituted only 11.43% of the total cases of autoimmune vesiculobullous disorders, which is almost similar to the finding of study by Arundhati S et al[14]. The mean age of onset among the adult patients of bullous pemphigoid was 62.5 years in the present study which is similar to study by Nanda A et al[16]. There was a female predominance in the present study with a male to female ratio (M: F) of 1: 1.6, which is again similar to the observations made in studies Nanda et al[16], Bernard P et al[17], Wong SN et al[18], Arundhati SP et al[14] and Deepti et al[9]. Involvement of oral mucosa was seen in 50% of the cases in the present study, which is higher than that reported by Deepti et al[9] and Budimir J et al[19]. Histopathological examination revealed subepidermal bulla in all the cases in the present study. Predominant eosinophilic infiltration (50%) in the dermis was more common than predominant neutrophilic infiltration (25%) in the present study which

correlates with the studies done by Nishioka et al[20], Arundhati et al[14] and Deepti SP et al[9]. Papillary edema was present in 15.15% cases in the present study which is less as compared to 29.41% observed in Arundhati S et al[14] and more than 7.8% observed in Deepti SP et al[9].

There were 5 cases of dermatitis herpetiformis which constituted 7.2% of total cases in our study. These were from age group 35- 50 years of with female preponderance with M:F ratio 1:4 which was different from earlier studies. Subepidermal bulla were present all the cases showing papillary microabscesses and neutrophilic and eosinophilic infiltrates as in Deepti SP et al[9] and BanuLebi et al [21].

The total clinicohistopathological concordance in our study was 84.29% which was near to Solanki et al[5] 85%. It was more than Arundhati S et al[14] 68.85% and less than Kambil et al[13] 88.88%. The concordance in Pemphigus vulgaris was 88.37% which comparable to Solanki et al[5]. The concordance of Bullous pemphigoid was also similar to Solanki et al[5]. The concordance was lowest in Dermatitis herpetiformis 60% which is near to Solanki et al[5]. Total clinicohistopathological correlation was 70% as compared to 60% in case Solanki et al[5]. The tabular comparison for the above is mentioned in displayed in Table 7.

Table – 7: Comparison of clinicohistopathological concordance of Autoimmune Vesiculobullous Disorder with Various Studies

No.	Studies	Year	PV	PF	BP	DH	LAD	PG
1	Arundhati S et al	2013	72.22%	66.66%		0%		100%
2.	Kambil SM et al[83]	2014	100%	-	100 %	_	66.6%	_
3	Solanki A et al[85]	2015	100%	100%	75%	50%		100%
4.	Present study	2015	88.37%	81.82%	75%	60%	100%	100%

Limitations:

The number of patients were less and salt split technique for DIF and indirect immunofluorescence were not used.

Conclusion:

Incidence of autoimmune vesiculobullous disorder was 0.11% of the total number of patients attending the skin OPD. Pemphigus vulgaris constituted the most common subtype of autoimmune vesiculobullous disorder in this study, followed by pemphigus foliaceus. Pemphigus group of diseases were most common in the 4th decade while bullous pemphigoid was most common in the 7th decade.

Histopathological examination showed features typical of each subtype of autoimmune vesiculobullous disorder.

Clinical examination is the initial step in making a diagnosis of autoimmune vesiculobullous disorders. Next step are various bed side tests Nikolsky's sign, Bulla spread sign and Tzanck smear. Histopathological features were conclusive in diagnosis of most of autoimmune vesiculobullous disorders. The subtle light microscopic features apart from classical diagnostic features help in diagnosis of difficult cases. Direct immunofluorescence microscopic study was done in few cases but it is required in diagnosis of LAD and DH. However, it is not a substitute for histopathology, but rather complementary to it.

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