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To Correlate Clinical Diagnosis with Histopathology and DIF Pattern of Autoimmune Based Vesiculobullous Disorders In A Tertiary Teaching Hospital

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Abstract: *Many skin diseases present with blisters but only in some of them blisters occur as the primary event. It is this group of disorders, which is traditionally termed the "vesiculobullous disorders". Vesicle is a raised lesion filled with tissue fluid of size <5mm where as bullae is a raised lesion filled with tissue fluid of size >5mm. Definition of vesiculobullous disorders is some what arbitrary, because many other skin disorders which are definite entities may also exhibit blisters as one of the features of their presentation. Eg:. Eczema, impetigo, fungal and viral infections, EMF, insect bites, etc., Skin diseases grouped under the term "vesiculobullous" are considered to have an autoimmune basis with demonstrable antibodies directed against specific components of the skin. Excluded from this group of disorders is congenital epidermolysis bullosa which are due to specific gene defects.* ***Aim:*** *To correlate clinical / histopathological / immunological findings.* ***Results:*** *A total of 20 cases clinically diagnosed as Autoimmune Bullous dermatoses in both sexes attending the department of dermatology, in teaching hospital of Fathima Institute of Medical Sciences, kadapa, were selected for this study.* ***Summary****: Autoimmune Bullous dermatosis was more common in females. Around 30% of patients belonged to 51-60 age group. The mean age being 42.6. Bullous pemphigoid constituted 55% of all the Autoimmune Bullous Disorders studied, followed by Pemphigus vulgaris 25%.*

Keywords: *Vesiculobullous, Direct immunofluorescence, Kadapa, Histopathology, Tzanck smear.*

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I. Introduction:

Many skin diseases present with blisters but only in some of them blisters occur as the primary event. It is this group of disorders, which is traditionally termed the "vesiculobullous disorders". Vesicle is a raised lesion filled with tissue fluid of size <5mm where as bullae is a raised lesion filled with tissue fluid of size >5mm [1].

Definition of vesiculobullous disorders is some what arbitrary, because many other skin disorders which are definite entities may also exhibit blisters as one of the features of their presentation. Eg:. Eczema, impetigo, fungal and viral infections, EMF, insect bites, etc.,

Skin diseases grouped under the term "vesiculobullous" are considered to have an autoimmune basis with demonstrable antibodies directed against specific components of the skin. Excluded from this group of disorders is congenital epidermolysis bullosa which are due to specific gene defects.

The autoimmune bullous diseases are characterized by pathogenic auto antibodies directed at target antigens whose function is either cell-cell adhesion within the epidermis or adhesion of stratified squamous epithelium to dermis or mesenchyme. These target antigens are components of desmosomes or of the functional unit of basement membrane zone known as adhesion complex [1], while the fluid filled sacs- bullae may involve any area of the skin or mucous membrane, histologically the location of bulla could be epidermis or subepidermis. So depending on the level of the blistering, the autoimmune bullous disorders are classified into intra-epidermal and sub epidermal.

The diagnosis of the bullous skin diseases is based on the typical skin manifestations, which may be objectified by the Nikolsky sign and characteristic Direct Immunofluorescence (DIF) patterns in skin biopsies. The presence of skin-specific autoantibodies in the circulation will further add to the diagnosis [2].

AIM:

1. To correlate clinical / histopathological / immunological findings.

II. Material & Methods

A total of 20 cases clinically diagnosed as Autoimmune Bullous dermatoses in both sexes attending the department of dermatology, in teaching hospital of Fathima Institute of Medical Sciences, kadapa were selected for this study.

Study period: This study was done from 01-09-2014 to 30-08-2017.

All patients were subjected to detailed history taking and clinical examination. Their particulars regarding age, sex, occupation, Personal and family history, presenting complaints, duration, general condition and findings on clinical examination were recorded in the proforma. Routine haematological and biochemical investigations were done and reports recorded. Tzanck smear was done at bedside, Skin biopsy specimens for histopathological study were collected in 10% formalin and for immunological study, the specimens were collected in the Michel's media, sent to Apollo hospitals, Hyderabad. Their histological features, Immunological features were studied and noted.

TZANCK SMEAR

Tzanck smear is very simple and rapid technique. It is a routinely done bed side diagnostic test for the diagnosis of vesiculo-bullous disorders. This type of cytology was first used in cutaneous disorders by tzank in 1947 [3]. These cytological findings are only suggestive of a disease, and this in turn need to be confirmed by histopathology.

In the cases of blistering disorders the intact roof of a blister is opened, by pricking with a needle or by scissors. The roof is folded back and the floor gently scraped. The material thus obtained is smeared on to a microscopic slide, allowed to air dry and stained with Giemsa stain. The Giemsa stain solution is diluted 1:10 with distilled water. The diluted solution is poured over the smear and kept for 15 minutes.

Tzanck test is very useful for the diagnosis of pemphigus vulgaris, particularly in the early stages of oral pemphigus, where a biopsy is uncomfortable to the patient and of little help in clinching the diagnosis.

It reveals multiple acantholytic cells (Tzanck cells). A typical Tzanck cell is a large round Keratinocyte with a hypertrophic nucleus, hazy or absent nucleoli and abaundant basophilic cytoplasm.

The basophilic staining is deeper peripherally on the cell membrane due to the cytoplasms tendency to get condensed at the periphery, leading to a perinuclear halo.

In Pemphigus vegetans, the cytologic features are identical but there are usually more inflammatory cells, particularly eosinophils. The acantholytic cells in pemphigus foliaceus and pemphigus erythematosus often have a hyalinized cytoplasm that corresponds to the dyskeraktosis seen in tissue sections. In Bullous pemphigoid, Tzanck smear is non specific and there are no acantholytic cells, the smear only serves to readily rule out pemphigus. Bullous pemphigoid shows scarcity of epithelial cells and an abaundance of leukocytes, particularly eosinophils with leucocyte adherence.

Biopsy:

Site of Biopsy: The early blister was chosen for the histopathological study and perlesional skin was taken for immunological study.

Type of Biopsy:

Biopsy was performed taking an adequate bit of the skin with intact early bullous lesion by exision biopsy.

The area selected is cleaned with spirit and infiltrated with 2% xylocaine locally after a test dose was done previously. An early lesion with intact bullae is removed by exision biopsy immediately placed in a labeled bottle containing 10% formalin solution. And the perilesional skin lesion also taken with 5 mm punch, immediately placed in Michel's medium.

The specimen along with requisition farm, containing the particulars of the patient, chief complaints of the patient, the site of the biopsy and nature of the biopsy along with the probable clinical diagnosis was transported through courier to Apollo hospitals, Hyderabad for Immunological and Histopathological study.

III. Results

Data collected from 20 cases clinically diagnosed as Bullous dermatoses. This study was done from 01-09-2014 to 30-08-2017 in the teaching hospital of Fathima Institute of Medical Sciences, kadapa.

TABLE-1: Distribution of patients according to sex

Sex	No.	Percentage
Male	9	45%
Female	11	55%
Total	20	100%

Out of 20 cases of Autoimmune bullous dermatoses studied,9 patients(45%) were males and 11(55%) were females.

TABLE-2: Distribution of patients according to age

Age Group (in yrs)	No.	Percentage
1-10	1	5%
11-20	2	10%
21-30	4	20%
31-40	1	5%
41-50	4	20%
51-60	6	30%
61-70	2	10%
Total	20	100%

TABLE-3: Distribution of patients according to different types of auto immune bullous disorders

Type	No.	Percentage
Bullous pemphigoid	11	55%
Pemphigus vulgaris	5	25%
Pemphigus foliaceus	1	5%
Linear IgA dermatosis	1	5%
Dermatitis herpetiformis	1	5%
Bullous SLE	1	5%
Total	20	100%

Out of 20 cases of Autoimmune bullous disorders, there were 11 cases of Bullous pemphegoid (BP) diagnosed clinically, contributing 55% in this study. 5 cases were diagnosed as Pemphigus vulgaris (PV) contributing 25% of the study group. Pemphigus foliaceus (PF), Linear IgA dermatoses (LAD), Dermatites herpetiformis (DH) and Bullous SLE (BSLE) constitute 1 case i,e 5% each.

TABLE-4: Distribution of different types of subepidermal bullous disorders

Sl.No.	Type	No.	Percentage
1.	Bullous pemphigoid	11	78.57%
2.	Linear IgA Dermatosis	1	7.14
3.	Dermatitis Herpetiforms	1	7.14
4.	Bullous SLE	1	7.14

Out of 14 Subepidermal Bullous disorders, 11 cases were Bullous pemphigoid(BP) which is most common accounting for 78.57%, remaining 3 cases were Linear IgA Dermatosis(LAD), Dermatitis Herpetiforms (DH) and Bullous SLE (BSLE) each accounting for 7.14%. Out of 6 intraepidermal bullous disorders pemphigus vulgaris constitute 5 cases i.e. 83.33%, Pemphigus Foleaceus 1 case i,e 16.67%.

TABLE-5: Mucosal involvement in different diseases

Mucosal Involvement	Present	Not Present
Auto Immune Bullous Dematoses	9	11
Pemphigus vulgaris	4	1
Bullous Pemphigoid	5	6

11 patients out of 20 cases of auto immune bullous dermatoses do not have mucosal involvement accounting for 55% and 9 patients(45%) have mucosal involvement. Out of 5 patients diagnosed as Pemphigus Vulgaris, mucosal involvement is present in 4 patients i.e. 80% and absent in 1 patient i.e. 20%. 6 patients out of 11 cases of Bullous pemphigoid do not have mucosal involvement accouting for 54.54% and 5 patients (45.45%) have mucosal involvement.

TABLE-6: Nikolsky's sign in different diseases

Nikolsky's Sign	Positive	Negative
Autoimmune Bullous Disorders	4	16
Pemphigus vulgaris	4	1

Out of 20 patients Nikolsky's Sign in positive in 4 patients, which account for 20%, Negative in 16 patients which account of 80%.

TABLE-7: Tzanck smear in auto immune bullous disorders

Tzanck smear	No.	Percentage
Positive	4	20%
Negative	16	80%
Total	**20**	**100%**

Out of 20 patients of auto immune bullous dermatoses, 4 patients (20%) shows acantholytic cells ie positive tzanck smear and 16 patients (80%) shows on acantholytic cells ie negative tzanck smear

TABLE-8 Direct Immunofluoroscene study in autoimmune bullous disorders (DIF)

DIF	No.	Percentage
Positive	17	85%
Negative	3	15%
Total	**20**	**100%**

Out of 20 patients on whom Direct immune fluorescence study was carried out, it was found to be positive in 85% and negative in 3 patients i.e. 15%. Out of 3 patients in whom DIF is negative. One patient was 75 years male patient who had clinically large tense bullae with mucosal involvement and histopathology showed subepidermal bullae with dense infiltrate of eosinophils in dermis – so diagnosed as Bullous pemphigoid. Another had flaccid bullae with mucosal involvement and histopathology showed intraepidermal bullae – so diagnosed as pemphigus vulgaris.

Third patient in whome DIF is negative, clinically had intensly itchy vesicles present on elbow, buttocks, scalp and face with no mucosal involvement. Histopathology showed subepidermal blister with neutrophils and showed dramatic response to Dapsone, so diagnosed as Dermatitis Herpetiformis.

IV. Discussion

Pemphigus vulgaris is the most common autoimmune blistering disease in eastern countries such as India (Adam BA 1992) [4] and the most commonly occurring Pemphigus diseases is Pemphigus vulgaris followed by other variants (Willson C, etal 1994). In the present study, out of 20 cases of autoimmune bullous diseases diagnosed, there were 11 cases of Bullous pemphigoid which accounts for 55% followed by Pemphigus vulgaris 25% which coincide with the studies conducted by SM Lagan, etal [5].

Pemphigus vulgaris affects both the Sexes (Korma NJ 1990)73. According to study conducted by Kumar KA [6], female preponderance in Pemphigus vulgaris was noted. In the present study out of 20 cases, 5 cases diagnosed as Pemphigus vulgaris, with distribution among females were 4 (80%) and 1 male case (20%), which correlates with the above study.

In Bullous pemphigoid, there is equal incidence in both the sexes (Stanley JR 1999) [7]. In the present study there is higher incidence of bullous pemphigoid noticed in 6 male patients (54.5%) out of 11 cases, and 5 were female patients (45.4%).

Out of 20 cases of Autoimmune bullous dermatoses studied 11 patients (55%) were females and 9 patients (45%) were males. There is 1 case of Pemphigus foliaceus, which was found in 1 male patient. And 1 case of Linear IgA Dermatosis noticed in a female child, 1 case of DH noticed in a male patient.

The majority of the patients were in the age group of 51-60 yrs, which constitutes 30%. The age groups of 41-50 yrs and 21-30 yrs constituted 20% each. The age groups 11-20 yrs and 61-70 yrs constitute 10% each and the age groups 1-10 and 31-40 constitute 5% each. The mean age is 42.6. Bullous pemphigoid mainly occurs in the elderly, typically between 60 and 80 yrs of age. In the present study, 6 of the total 11 patients with Bullous pemphigoid were in the age group of 51 – 60 yrs constituting 54.5%. Pemphigus is a disease of middle age, but patients are younger at presentation in India than in Western Countries [8]. In the present study 3 of the 6 patients are in the age group 41-50 yrs.

Nikolsky's sign is present in 4 of the 5 patients of Pemphigus vulgaris, which accounts for 80%24. Bullae spread sign accounts for 60%. In this study, out of 11 cases of bullous pemphigoid studied, bullae spread sign is positive in 9 patients i.e. 81.81% and negative in 2 patients i.e. 18.18%.

Among 5 patients diagnosed as Pemphigus vulgaris, 4 patients i.e. 80% showed acantholytic cells having hyperchromatic large nucleus with peripheral condensation of cytoplasm [3].

Histopathological findings of Bullous pemphigoid shows a sub epidermal blister with intact epidermis and perivascular infiltrate of eosinophils, mononuclear cells and some neutrophils. Histopathological findings of pemphigus vulgaris shows formation of bullae in the suprabasal zone, with acantholytic cells present in the bullae cavity. Perivascular infiltrates composed of lymphocytes and histiocytes. These findings correlate with the studies mentioned in the literature.

Of the 6 cases of Pemphigus, 5 cases were pemphigus vulgaris which account for 83.33% and one case was pemphigus foliaceus. Of the 5 cases diagnosed as Pemphigus vulgaris, 4 were females. These findings coincide with the study conducted by Kumar KA in Thrissur District, South India [6].

Of the 14 subepidermal bullous disorders 11 cases i.e. 78.57% were Bullous pemphigoid. So Bullous pemphigoid is most common subepidermal bullous disorders which coincides with studies of National skin center and Bernard P etal in 1995 [9].

Other three subepidermal bullous disorders were, Chronic bullous disease of childhood in 8 years female child with clinical, histological and immunofluorescence study correlated with linear IgA dermatosis. Other is Bullous SIE in 18 yrs female patient with tense bullae, vesicles more on exposed sites with arthralgias, photosensitivity, positive ANA titres and Immunofluorescence showed granular deposition of IgA and IgG along BMZ . The third patient diagnosed as Dermatitis Herpetiformis is a 21 yrs old male patient with intensly itchy vesicles present on elbow, buttocks, scalp and face with no mucosal involvement. Histopathology showed subepidermal blister with neutrophils and showed dramatic response to Dapsone.

Out of 20 patients in whom DIF study was carried out, it was found to be positive in 85%, and negative in 3 patients i.e. 15%. Out of 3 patients in whom DIF is negative, one was 75 yrs old male patient to had clinically large tense bullae with mucosal involvement and histopathology showed subepidermal bullae with dense infiltrate of eosinophils in dermis – so diagnosed as Bullous pemphigoid. Another had flaccid bullae with mucosal involvement and histopathology showed intraepidermal bullae – so diagnosed as pemphigus vulgaris. Third patient in whom DIF is negative, clinically had intensely itchy vesicles present on elbow, buttocks, scalp and face with no mucosal involvement. Histopathology showed subepidermal blister with neutrophils and showed dramatic response to Dapsone, so diagnosed as Dermatitis Herpetiformis. As in the reference cited in Beutner EH etal 1987 [10], all patients with active Pemphigus vulgaris shows IgG deposition in the intercellular spaces, 50% patients shows C3 deposition. In the Bullous pemphigoid patients also, there is there is deposition of C3 in all patients and IgG in 90% cases along BMZ (Hadi SM, et al 1998) [11].

In the present study Pemphigus vulgaris patients with positive DIF showed Intercellular IgG 80%, C3 in 60%. Bullous Pemphigoid patients with positive DIF showed C3 in 90% cases, IgG in 81.8%, IgA+ C3 and IgM each 9% which correlated with the above studies.

V. Summary And Conclusion

Autoimmune Bullous dermatosis was more common in females. Around 30% of patients belonged to 51-60 age group. The mean age being 42.6. Bullous pemphigoid constituted 55% of all the Autoimmune Bullous Disorders studied, followed by Pemphigus vulgaris 25%. Among Subepidermal bullous disorders, Bullous pemphigoid is most common constituted 78.57%. Mucosal involvement is present in 45% of cases of autoimmune Bullous Dermatoses. Mucosal involvement is present in 45.45% of cases of Bullous pemphigoid. Nikolsky's sign positive in 80% of pemphigus vulgaris patients. Bullae spread sign is positive in 60% of cases of Autoimmune Bullous disorders. Tzanck smear is positive in 20% of Autoimmune bullous dermatoses. Bullae were located subepidermally in 70% of patients and for 30% of patients they were located intraepidermally according to histopathology. Direct Immunofluorescence study was found to be positive in 85% of Autoimmune Bullous dermatoses patients. In Bullous pemphigoid C3 was present in 90%, IgG in 81.8%, IgA and IgM each in 9%. In Pemphigus vulgaris intercellular IgG was present in 80% and C3 in 60%.

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