PREVALENCE AND CORRELATION BETWEEN CLINICAL, HISTOPATHOLOGICAL AND DIRECT IMMUNOFLUORESCENCE PATTERN OF AUTOIMMUNE BLISTERING DISEASE- A RETROSPECTIVE STUDY

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ABSTRACT

BACKGROUND

Autoimmune blistering diseases are caused by autoantibodies which act against the target antigens present in the dermoepidermal junction. Immunofluorescence studies are considered as the most important diagnostic tool in the diagnosis of autoimmune blistering disease. Supplementing it with the clinical and histopathological diagnosis helps in arriving at the accurate diagnosis and management of autoimmune blistering disease.

MATERIALS AND METHODS

The study includes various autoimmune blistering diseases diagnosed by clinical, histopathological and direct immunofluorescence (DIF) pattern over a period of 5 years from January 2011 to December 2015.

RESULTS

Male to female ratio was 1: 1.54. Pemphigus vulgaris was the most common (59%), followed by bullous pemphigoid which was seen in 25% of the cases. Chronic bullous disease of childhood, pemphigus foliaceous and lichen planus pemphigoides constituted 8%, 6% and 2% of the cases respectively.

CONCLUSION

Direct immunofluorescence (DIF) pattern is important in histopathological investigation and helps in reaching confirmatory diagnosis of autoimmune blistering disease.

KEYWORDS

Autoimmune Blistering Disease, Direct Immunofluorescence (DIF), Pemphigus Vulgaris, Bullous Pemphigoid.

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BACKGROUND

Skin is the single largest organ of the body and the involvement of skin and mucous membranes manifest along with various diseases. Autoimmune blistering diseases are a group of heterogeneous disorders in which autoantibodies target structures which are important for integrity of skin and mucous membrane and lead to blister formation. They have dramatic impact on the patient and their family and have severe economic consequences for the family and health services. Intensive investigations have been carried out regarding autoimmune blistering diseases in recent years.[1]

In today's scenario the treating dermatologist pose great challenges to Autoimmune blistering diseases as they are a significant group of dermatoses. Most epidemiological studies have focused on a single autoimmune blistering disease. Few surveys have described the complete spectrum of various autoimmune blistering diseases in a region.[2]

The presence of vesicles or bullae at any level within the epidermis or at the dermoepidermal junction is characterized by the vesiculobullous reaction pattern.[3] It includes intraepidermal disorders like pemphigus vulgaris (PV) and pemphigus foliaceus (PF) and subepidermal disorders like bullous pemphigoid (BP), cicatricial pemphigoid (CP), linear IgA disease (LAD), dermatitis herpetiformis, pemphigoid gestationis, lichen planus pemphigoides (LPP), epidermolysis bullosa acquisita and bullous systemic lupus erythematosus.[4] Depending on the location of the bulla, these disorders can be classified as intraepidermal and subepidermal.

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Systematic analysis of blisters involves the pathological evaluation, which includes the blister separation plane, the blister formation mechanism and the character of the inflammatory infiltrate, indicating its absence or presence.[5]

Investigative dermatology has created new horizons over the past 10 years. Over the last two decades, great advances have been made in understanding the clinical behaviour and molecular nature of autoimmune diseases. To achieve the diagnosis, histopathological examination is necessary, but a definitive diagnosis can be achieved only using immunofluorescence study. Research techniques such as immunoelectron microscopy and immunoblotting may help in achieving the accurate diagnosis in the individual patient but do not replace the clinician.[1]

Large doses of Corticosteroids remain the mainstay treatment of autoimmune blistering diseases, which have eventually led to downfall in the mortality of the disease. Combination of corticosteroids and immunosuppressive drugs with dapsone have given satisfactory results.[3]

Western as well as Indian literature have highlighted on a particular entity only in the disease spectrum but a comparative prevalence of the disease with clinical and histopathological study has been attempted by very few people.

This study is done to analyse the clinical, histopathological and immunofluorescence features of autoimmune blistering diseases and to evaluate the diagnostic significance of immunofluorescence in these disorders.

Objectives of The Study

- To study the prevalence of various entities in patients with autoimmune blistering disease at KLES Dr Prabhakar Kore Hospital and MRC, Belagavi.
- To correlate the Direct immunofluorescence (DIF) patterns with clinical and histopathological findings.

MATERIALS AND METHODS

This is a Hospital based retrospective study where in a total of 816 biopsies were reported over a period of 05 years from the Department of Dermatology, Venereology and Leprosy and were reviewed (January 2011 to December 2015). Clinical diagnosis was collected from the files of department of Dermatology and the same was evaluated for the reports of histopathological and direct immunofluorescence diagnosis. For histopathological examination (HPE) a typical small early blister was chosen. It was cleaned using 70% alcohol. The area was then anaesthetized by infiltrating 2% lignocaine subcutaneously. An elliptical incision was made around the lesion. With a part of the normal skin through to the subcutaneous fat, the intact blister was removed. This was transferred to 10% formalin for histopathological examination.

For processing after fixation in 10% formalin for 12 – 24 hours, at Pathology Laboratory, the tissue was subjected to processing and paraffin embedding. The biopsy was oriented such that the sections obtained passed vertically through the skin. Sections of 3-5 mm were cut and stained with Haematoxylin and eosin, cleared and mounted.

The separation plane, whether subcorneal, intraepidermal, suprabasal, subepidermal or intradermal was observed. The character of the inflammatory infiltrate, its presence or absence, its pattern, the type of inflammatory cell infiltrate in the blister or in the dermis was separately recorded. In the dermis, localization of the inflammatory infiltrates whether in the superficial dermis, perivascular or periadnexal location was also observed.

For direct immunofluorescence (DIF), Perilesional skin, from an end of an elliptical biopsy taken for histopathology was biopsied. Tissue specimens in Michel's transport medium were then sent to the Department of Skin and STD, Kasturba Medical College, Manipal for direct immunofluorescence.

In the laboratory, the tissue specimens were washed with phosphate buffered saline (PBS) to remove any residual blood proteins. The tissue was then snap frozen in a cryostat at -16 to -18 degree Celsius. A drop of optical clear transparent (OCT) embedding compound was added. The skin biopsy was then correctly oriented in the central fluid portion, covered with further OCT. DIF is a one-step procedure. Tissue sections are incubated with Fluorescein isothiocyanate (FITC) – conjugated goat antihuman IgG antisera and viewed under fluorescent microscope.

RESULTS

The present study is a five-year retrospective study which included 61 patients who attended the OPD of Dermatology, Venereology and Leprosy in KLES Dr Prabhakar Kore Hospital and MRC, Belgaum, from January 2011 to December 2015. The age group ranged from 5 years (A female diagnosed with BP) to 85 years (a male diagnosed with BP) with a mean age of 50.16 years. The male to female ratio was 1:1.54.

Type	Number of Cases	% of Total
Pemphigus Vulgaris	36	59
Pemphigus Foliaceous	4	6
Bullous Pemphigoid	15	25
CBDC	5	8
LPP	1	2
Total	61	100

Table 1. Types of Autoimmune Blistering Disease

In the present study, pemphigus vulgaris constituted the most common Autoimmune Blistering Disease constituting 59% (36 out of 61 cases), followed by bullous pemphigoid constituting 25% of the cases. Chronic bullous disease of childhood, Pemphigus foliaceous and Lichen planus pemphigoides constituted 8%, 6% and 2% of the cases respectively.

Itching and burning sensation of the eroded skin were the common symptoms Vesicles, bullae, erosions and crusting were the most common clinical findings observed. Patients of PV, PF, and BP had lesions over the trunk, involvement of extremities, face and scalp was also seen with PV and PF.

DIF findings were positive in 60 patients. Whereas 57 histopathological findings were seen positive in almost all cases except in 4 biopsy reports.

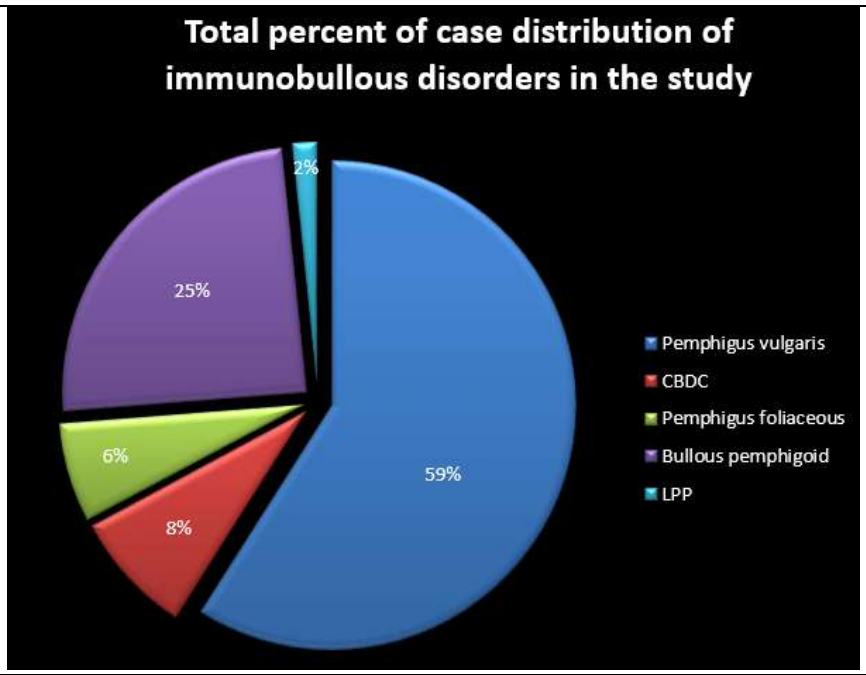
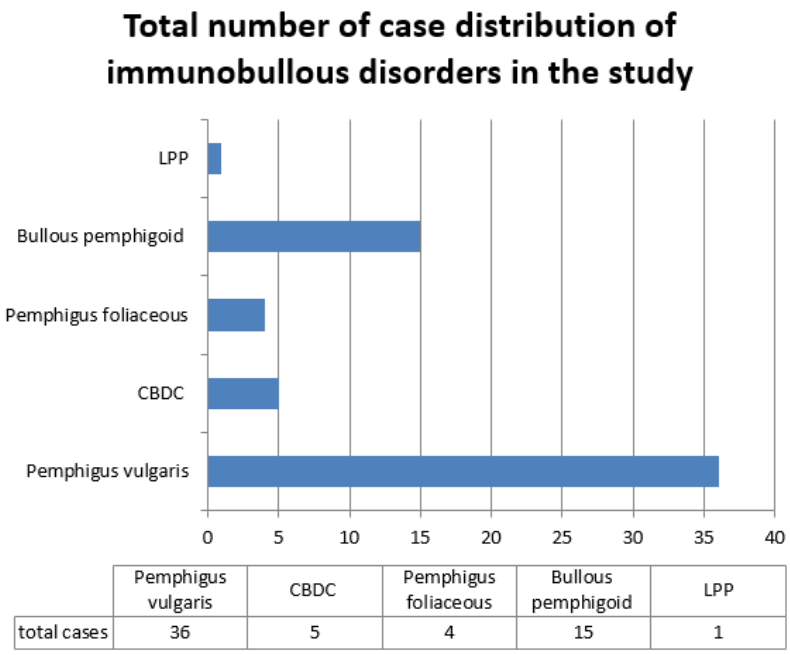


Figure 1

On histopathology all cases of Pemphigus Vulgaris showed the floor of the blister is lined by basal keratinocytes denoting the blister to be suprabasal upper dermis has lymphocytic infiltration with neutrophils. Cases of Bullous pemphigoid showed small fragments partly covered with stratified squamous epithelium with dermis and subepidermal bullae seen with destruction of bullous layer. The bullae show scanty eosinophilic infiltrate along with a few lymphocytes. In patients with LPP thinned epidermis with mild hyperkeratosis with pigment incontinence and melanophages are seen in upper dermis.



	Pemphigus vulgaris	CBDC	Pemphigus foliaceous	Bullous pemphigoid	LPP
total cases	36	5	4	15	1

Figure 2

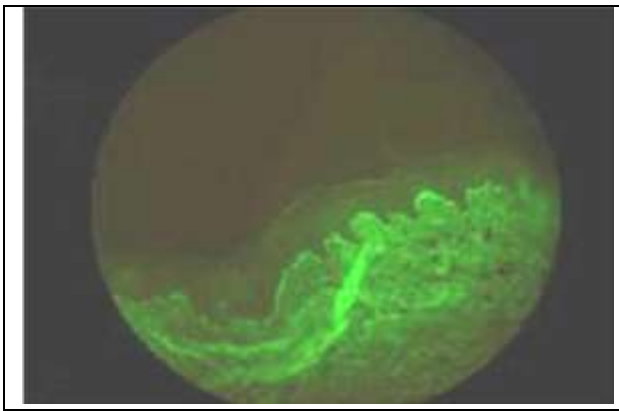


Image 1. Direct Immunofluorescence – Bullous Pemphigoid – Showing Continuous Linear Deposition of IgG and C3 Along Basement Membrane Zone

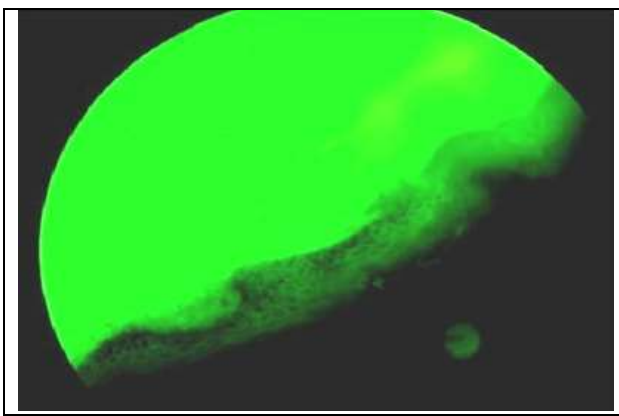


Image 2. Direct Immunofluorescence – Pemphigus Vulgaris - Showing Deposition of IgG in Squamous Intercellular Substance in the Epidermis in a Fishnet Pattern

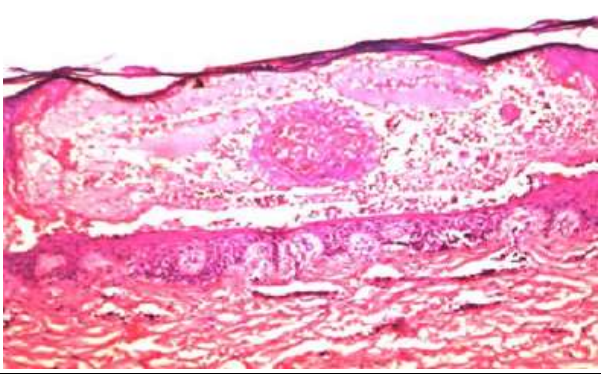


Image 3. Microscopy: Pemphigus Vulgaris – Suprabasal Bulla – Roof, Floor, Contents (Acantholytic Cells and Inflammatory Cells) of the Bulla, Dermis (100X, H and E)

On DIF examination 25 cases out of 36 showed intercellular space (ICS) deposition of IgG and C3 in all the cases of pemphigus vulgaris. 15 cases out of 36 showed only C3 deposition at the ICS.

All cases (i.e. 15 out of 15 cases) of BP showed linear IgG and C3 deposition in the basement membrane zone (BMZ).

Here 4 out of 5 patients of CBDC (chronic bullous disease of childhood) showed linear deposition of IgA along the (BMZ). In case of lichen planus pemphigoides it showed a linear deposition of IgA at the BMZ. All except two clinically suspected cases of DH were not confirmed either by histopathology (HPE) or direct immunofluorescence (DIF). The final diagnosis was based on Clinical Diagnosis, histopathology and DIF.

DISCUSSION

The presence of vesicles or bullae at any level within the epidermis or at the dermoepidermal junction is characterized by the vesiculobullous reaction pattern.[3] It includes intraepidermal disorders like pemphigus vulgaris (PV) and pemphigus foliaceus (PF) and subepidermal disorders like bullous pemphigoid (BP), cicatricial pemphigoid (CP), linear IgA disease (LAD), dermatitis herpetiformis, pemphigoid gestationis, lichen planus pemphigoides (LPP), epidermolysis bullosa acquisita and bullous systemic lupus erythematosus.[4] DIF demonstrates the amount of immunoglobulin and complement deposited within the skin biopsy specimen it can differentiate the type of Autoimmune blistering disease depending on the type of immune deposits and the pattern of deposition.

Male to female ratio in this study was 1:1.54 which was comparable to other studies done by Shamim et al[6] and Baum S[7] In our current study, Pemphigus (65%) group of disorders was the most common autoimmune blistering disease and PV (59%) was the most common by PF (6%) and this was in accordance with several other studies.[8,9,10,11]

On histopathology, all cases of PV (100%) showed suprabasal bulla with lymphocytic infiltration with neutrophils.[12,13]

Disease	Clinical Diagnosis	HPE Diagnosis	DIF Diagnosis
PV	36	36	40
PF	4	4	1
BP	15	15	15
CBDC	5	2	3
LPP	1	1	1

Table 2. Number of Cases Diagnosed by Clinical, Histopathological and Direct Immuno-fluorescence Test of Various Autoimmune Blistering Disease

Whereas all cases of BP (100%) showed subepidermal bullae with destruction of bullous layer having eosinophilic infiltrate.

Out of 5 cases, Of CBDC in 3 cases (3%) showing features of a subepidermal bulla and a neutrophil rich infiltrate. 1 case of LPP (100%) showed thinned epidermis with mild hyperkeratosis having melanophages was seen in upper dermis.

On DIF, all PV (100%) patients showed IgG deposition in intercellular space and C3 deposition in intercellular space in 70% of the cases in contrast to the similar studies.[8,14,15,16]

DIF findings of a linear deposition of IgG and C3 along the BMZ was matched with the clinical diagnosis in all cases of BP (100%).

5 cases of CBDC were clinically diagnosed out of which only 3 cases showed the linear IgA deposition at BMZ on DIF findings.

On analysis, out of 61 cases, 55 (90%) cases of autoimmune blistering diseases showed a positive similarity between clinical, histopathological and DIF findings. In the remaining 6 cases, the clinical diagnosis was different from that of histopathology and DIF pattern.

CONCLUSION

Clinical examination and cytology are the initial steps in making a diagnosis of autoimmune blistering disease. Histopathological examination and direct immunofluorescence are required for making a definitive diagnosis in autoimmune blistering disease. Direct immune-fluorescence is helpful in confirming the diagnosis. However, it is not a substitute for histopathology, but rather additional investigative procedure to arrive at diagnosis.

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