"1 YEAR CROSS-SECTIONAL STUDY OF DIRECT IMMUNOFLUORESCENCE IN THE DIAGNOSIS OF IMMUNOBULLOUS DISORDERS."

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Endorsement

This is to certify that the dissertation entitled **"1 YEAR CROSS-SECTIONAL STUDY OF DIRECT IMMUNOFLUORESCENCE IN THE DIAGNOSIS OF IMMUNOBULLOUS DISORDERS."** is a bonafide research work done by **REG NO. BT0116003.**

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ABBREVIATIONS

PV	Pemphigus vulgaris
PF	Pemphigus foliaceus
PNP	Paraneoplastic pemphigus
BP	Bullous pemphigoid
BPAG	Bullous pemphigoid antigen
MMP	Mucous membrane pemphigoid
CBDC	Chronic bullous dermatosis of childhood
LABD	Linear Iga bullous dermatosis
DH	Dermatitis herpetiformis
TG3	Transglutaminase 3
EBA	Epidermolysis bullosa acquisita
BMZ	basement membrane zone
HPE	histopathological examination
DIF	direct immunofluorescence
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
C3	complement 3

ABSTRACT

Objectives:

The aim of the study is to study the clinical, histopathological and direct immunofluorescence features and to evaluate the sensitivity of direct immunofluorescence in diagnosis of autoimmune vesiculobullous disorders.

Materials and methods:

The present study was a one-year cross sectional study from January 2017 to December 2017. Newly diagnosed cases of autoimmune vesiculobullous disorders were included in the study. The patient's demographic data, age of onset, duration of disease, symptoms, types and distribution of lesions and other diseases were noted in a pre-designed proforma. Routine investigations, bedside Tzanck smear and two specimens of skin biopsy, a lesional specimen for histopathology and a perilesional specimen of direct immunofluorescence were done in all patients after informed consent was taken.

Results:

In the present study, pemphigus vulgaris consisted of the most common vesiculobullous disorder composing 50% of the study group followed by 35% by bullous pemphigoid, 10% by pemphigus foliaceus and 5% by chronic bullous disease of childhood. Most of the cases presented in age group of 41-60 years with a female preponderance. All of the cases of pemphigus vulgaris showed suprabasal clefting with acantholytic cells. 1 of the 2 cases of pemphigus foliaceus showed subcorneal cleft. 4 of the 7 cases of bullous pemphigoid and the only CBDC case showed cleft at

dermo-epidermal junction. 100% of cases showed positive findings on DIF. In 4 cases out of 20, HPE findings were discordant with clinical and DIF findings.

Conclusion:

Clinical and histopathological findings have to be correlated with DIF findings to reach a definitive diagnosis and hence direct immunofluorescence is a gold standard for the diagnosis of autoimmune vesiculobullous disorders.

Key words: Autoimmune vesiculobullous disorders, pemphigus, pemphigoid, histopathology, direct immunofluorescence.

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INTRODUCTION

The auto-immune vesiculobullous disorders are group of blistering disorders characterized by pathogenic auto-antibodies 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.[1]

Autoimmune vesiculobullous disorders are a cause for significant morbidity in patients[2] and disorders like pemphigus vulgaris carry a significant mortality.[3]

These disorders pose a great challenge to a dermatologists with respect to arriving at a confirmatory diagnosis and management of the disease owing to the long duration of treatment as well as the comorbidities associated with the disease and its treatment.

Corticosteroid administration for variable but a long duration remains as the mainstay in the management of autoimmune bullous disorders. Antibiotics with anti-inflammatory property or steroid sparing immunesuppressants are added to reduce the adverse effects associated with long term corticosteroid therapy. Newer modalities developed in these regards are biological agents which precisely target the pathogenic auto-antibodies or their production. However their use is limited owing to the lack of information provided by conventional diagnostic methods in regards of the auto-antibodies involved as well as the high cost of these medications.

Pathological examination is done to locate the level of the blister in the skin as well as presence of inflammatory cells or lack thereof. However they do not provide a clue of the auto-antibodies involved.

Recent advances that complement histopathological examination are direct and indirect immunofluorescence. These tests are useful to precisely locate the types, location and pattern of the blister producing auto-antibody deposition. They are regarded as more accurate and prompt in diagnosis and management of autoimmune vesiculobullous disorders.[4]

Hence this study is undertaken to evaluate the clinical, histopathological and direct immunofluorescence findings and to evaluate the sensitivity of direct immunofluorescence so as to arrive to an accurate and prompt diagnosis of auto-immune vesiculobullous disorders.

OBJECTIVES

To study clinical, histopathological and direct immunofluorescence findings and determine the sensitivity of direct immunofluorescence in the diagnosis of autoimmune vesiculo-bullous disorders at KLES Dr. Prabhakar Kore hospital attached to Jawaharlal Nehru medical college, Belagavi.

REVIEW OF LITERATURE

History

Hippocrates (460-370 BC) described pemphigoid as “pemphigoides pyertoi.

”Galen (131-201 AD) called a pustular disease in mouth as “febris pemphigoides.”

In 1637, Zanctus described patients with blisters over body as febris pemphigoides. DeSauvages in 1760 described patients with high fever and blisters of short duration as “pemphigus major.”

These conditions were later not considered as true pemphigus as their duration was short with complete recovery.

The first case recorded and considered true pemphigus was by McBride (1777) Wichmann used the term pemphigus for his patients to describe flaccid bullae and painful oral ulcers he found in them[6]. He described it as ‘chronic bullous disease and William in 1808 called it “Pompholyx diutinum.”[7]

Pemphigus foliaceus was first described by Cazenave in 1844, as a superficial spreading form of pemphigus.[7]

In 1884, Louis Adolphus Duhring of Philadelphia described dermatitis herpetiformis.[8]

Neumann, in 1886 described a form of disease with warty lesions as pemphigus vegetans.[7]

In 1911, Thorst distinguished mucous membrane pemphigoid from other forms of pemphigus and suggested the name "benign mucous membrane pemphigoid" in 1918.[9]

Senear and Usher in 1926 described pemphigus erythematosus for diseases they found to have features of botho pemphigus and lupus erythematosus.[10]

Civatte, in 1943, described the histopathologic features in such diseases as "acantholysis." He described acantholysis and intraepidermal bulla formation in pemphigus vulgaris, pemphigus foliaceus and pemphigus vegetans. These findings separated pemphigus from other blistering diseases.[9]

Lever, in 1953 differentiated bullous pemphigoid from pemphigus both clinically and histopathologically. This blistering disorder according to him affected elderly patients and characterized by subepidermal bulla formation.[11]

In 1964, Beutner and Jordon reported autoantibodies in serum of pemphigus patients reacting with "intercellular substance" in skin and mucosa. They demonstrated these autoantibodies with direct immunofluorescence.[12]

In 1967, they demonstrated autoantibodies in sera and skin specimens from patients with bullous pemphigoid but reactive with basement membrane zone. These latter findings distinguished bullous pemphigoid from pemphigus and established it as a distinct bullous disease.[12]

In 1979, chorzelskin et al first described linear IgA bullous dermatosis as an entitiy distinct from dermatitis herpetiformis or bullous pemphigoid on the basis of immunopathologic findings of linear IgA deposits in basement membrane zone on direct immunofluorescence.[13]

In 1984, Nishioka K et al described eosinophilic infiltration of upper dermis and peripheral eosinophilia.[14]

In 1986, IgA antibodies were found to be specific for dermatitis herpetiformis and celiac disease.[8]

In 1990, Dr Anhalt first recognized an atypical pemphigus with associated neoplasia.[15]

In 2001, Nguyen proposed the term paraneoplastic autoimmune multi-organ syndrome because of the recognition that the condition affects multiple organ systems.[16]

Structure of skin

The skin spans entire external body surface and is continuous with mucosae of alimentary, respiratory and urogenital tracts at their orfices where a specialized mucocutaneous junction is present. Skin forms 8% of total body mass, and its surface area varies with height and weight. Its thickness ranges from 1.5- 4mm and this varies with region and ageing.

EPIDERMIS:

The epidermis is derived from surface ectoderm. It is a nonvascular layer consisting of cornified stratified squamous epithelium. Two types of cells constitute epidermis viz. keratinocytes and dendritic cells. The layers of epidermis are divided into the living stratum malpighii which rests on the dermis and the dead horny superficial stratum corneum.

Layers of skin:

1. Stratum basale or germinativum: The stratum basale consists of single layer of columnar cells which are placed perpendicular to basement membrane. They have a deeply basophilic cytoplasm and a dark staining elongated oval neucleus. They are connected to each other by desmosomes and to the basement membrane by hemidesmosomes which in turn is anchored to the dermis by short filaments. Mitotic activity is confined to this layer.
2. Stratum spinosum or prickle cell layer: This layer is composed of several strata of polyhedral cells which become somewhat flattened in the outermost layers. It is 5-6 cell layer thick with their long axes arranged parallel to the skin surface.
3. Stratum granulosum: This later consists of 3-5 layers of flattened cells and their cytoplasm is filled with keratohyalin granules which are deeply basophilic and irregular in size and shape. The thickness of granular layer in normal skin is generally proportional to the thickness of horny layer. It represents the keratogenous zone of epidermis.
4. Stratum lucidum: It appears as a thin homogenous eosinophilic zone. It is more refractile optically. Ultrastructurally, the cells resemble transitional cells which are incompletely keratinized cells. These zone is more pronounced in area were the horny layer is thick, especially in palms and soles.
5. Stratum corneum: It consists of closely packed layers of flattened polyhedral cells. The cells lack neucleus and membrane organelles and consists of dense keratin filaments.

Dendritic cells:

1. Melanocytes: These are found wedged between the basal cells of epidermis. The melanocytes synthesize melanin which protects the germinative cells from adverse effects of ultraviolet radiation. These are clear cells with small dark staining neuclei.
2. Langerhans cells: They are specialized representative of macrophage-monocyte system which are the antigen presenting cells in the skin.
3. Merkel cells: they are immature dendritic antigen presenting cells. They are associated with free nerve endings in thick skin and are presumed to serve as sensory receptors.

THE DERMIS:

It is derived from the mesenchyme. It is an irregular moderately dense connective tissue with a matrix composed of an interwoven collagenous and elastic network in an amorphous ground substance.

The dermis can be divided into 2 layers, a superficial papillary layer and a deeper reticular layer.

Papillary layer:

It lies below the epidermis and projects into it to form dermal papillae. It is relatively loose and highly vascular with fine interlacing collagen fibers. Most of the collagen found here is type III.[17 18]

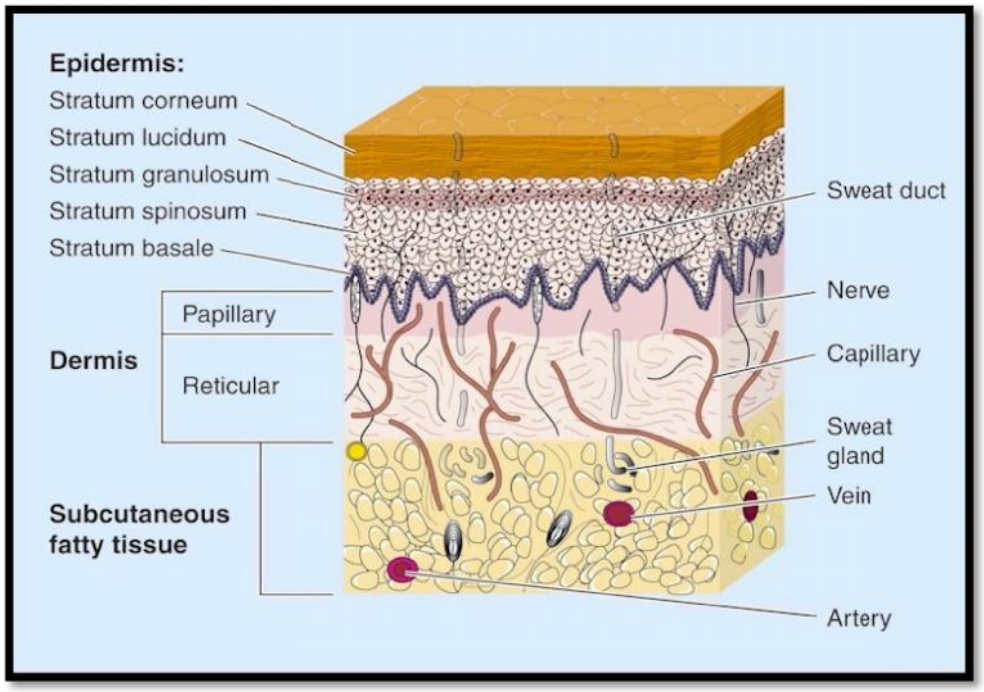
Reticular layer:

It forms major bulk of dermis and consists of dense bundles of collagen fibers. Collagen is of type I with small amount of type III.

The structure and molecular aspects of epidermal cell attachment molecules:

It is important to understand the structures that maintain cell-to-cell and epidermal to dermal integrity despite of constant movement and external forces. The autoantibody mediated interactions against specific constituents of these structures result in disruption of integrity leading to blister formation.[1]

Figure 1: Structure of skin



INTERCELLULAR ADHESION MOLECULES OF EPIDERMERIS

Adhesion between keratinocytes is mediated predominantly by cell-to-cell adhesion molecules of cadherin family localized in two specialized intercellular adhesion molecules. They are:

1. Desmosomes (maculae adherens)
2. Intermediate or adherens junction (zonulae adherens)

Desmosomes

They are specialized regions of plasma membrane which link cells to each other, providing a stabilizing network across epidermis. They correlate with intercellular bridges.

Two families of cadherins have been described in desmosomes. They are desmogleins and desmocollins. Desmoglein subfamily comprises of three proteins- Desmoglein 1, 2 and 3. Desmocollins are a family of proteins which exists in more than isoform. These desmosomes are linked to intracytoplasmic intermediate filaments (tonofilaments) by plakoglobin and desmoplakin. The tonofilaments loop through the cell and around the neucleus forming a network of filaments extending from one desmosome to another, extending ino hemidesmosomes on basal pole of epidermal cells.

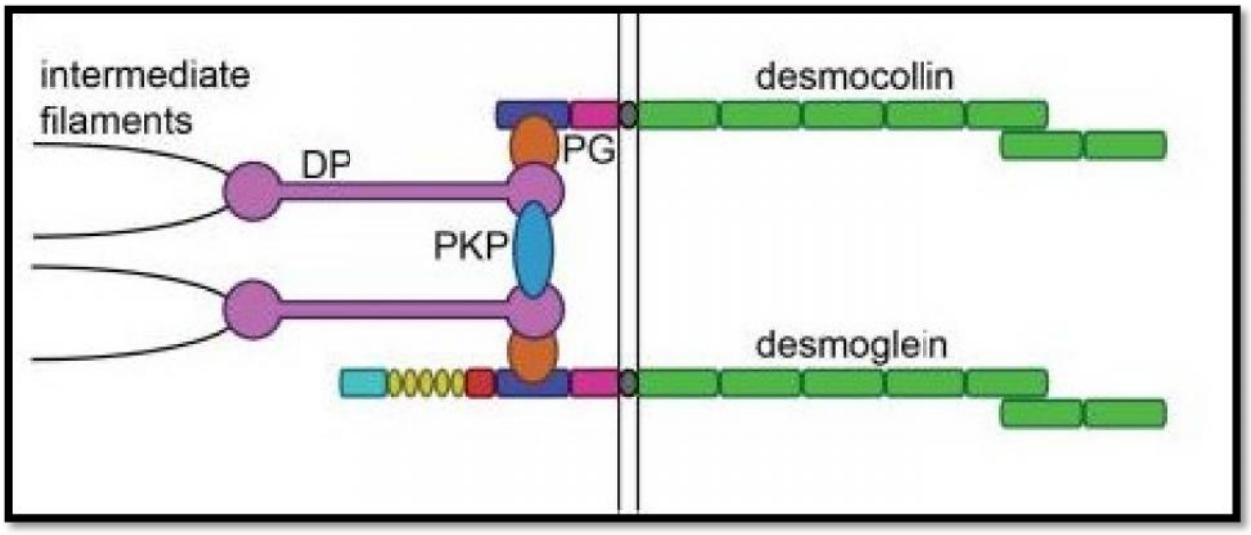
Interactions between desmoplakins and keratin intermediate filaments stabilize cytoskeleton.

Pathological significance of adhesion molecules:

1. Antibodies against desmoglein 1 are found in patients with pemphigus foliaceus and pemphigus vulgaris.
2. Desmoglein 3 along with desmoglein 1 are the target antigens in pemphigus vulgaris.
3. Desmocollins are targeted by autoantibodies in patients with endemic pemphigus foliaceus.

4. Paraneoplastic pemphigus presents with antibodies in sera against desmoplakins in epidermis as well as several antigens in basement membrane as well[1]

Figure 2 : Desmosomes [19]



DERMO-EPIDERMAL JUNCTION

It consists of the following structures from above downwards:

1. Plasma membrane of basal keratinocytes and hemidesmosomes.
2. Lamina lucida
3. Lamina densa
4. Sublamina densa/ Lamina fibroreticularis

Components of dermo-epidermal junction[1]:

1. Plasma membrane of basal keratinocytes and hemidesmosomes.

- Tonofilaments/ Keratin intermediate filaments: They traverse through basal cells and insert into hemidesmosomes. They comprise of keratin 5 and 14.
- Hemidesmosomes: It is a transmembrane complex extending through the basal cell membrane till lamina lucida. It comprises of BPAG1/BP230 and pectin

forming the intracellular hemidesmosomal plaque region and BPAG2/BP180, α6β4 integrin form the transmembrane portion.

2. Lamina lucida

- It is a less electron dense region and comprises of anchoring filaments, laminins, α6β4 integrin and BP180 NC16a.
- Anchoring filaments: They are fine thread like structures traversing through lamina lucida connecting α6β4 integrin in hemidesmosomes to type VII collagen in anchoring fibrils. They are composed of mainly by Laminin 5 (laminin 332) and other major antigenic component is LAD-1.
- Laminins: They extend from basal membrane to lamina densa. They mediate binding of basal cell membrane to the underying basement membrane with the help of α6β4 integrin. They are cruciform structures formed of three polypeptide subunits α-, β- and γ-chains as follows

TABLE 1: Chain composition of Laminins

Type	Chain composition	Distribution in basement membrane
Type 111	α1β1γ1	Blood vessels and lamina densa
Type 332/ laminin 5	α 3β3γ2	Lamina lucida and densa
Type 311/ laminin 6	α3β1γ1	Lamina lucida and densa
Type 511/ laminin 10	α 5β1γ1	Lamina lucida and densa

3. Lamina densa: It is an electron dense layer comprised mainly by Type VII collagen. The other components include Laminin 5, laminin 6, laminin 10, nidogen, perlecan and heparin sulphate
4. Sublamina densa: It is formed by anchoring fibrils, microfibrils and anchoring plaques. Anchoring fibrils are U-shaped structures extending from lower margin of lamina densa to reticular dermis. They are synthesized by keratinocytes as well as dermal fibroblasts and are mainly formed of collagen VII. Microfibrils form a mesh and are comprised of elastic fibres. Both anchoring fibrils and microfibrils insert into anchoring plaques

Figure 3: Dermo-epidermal junction

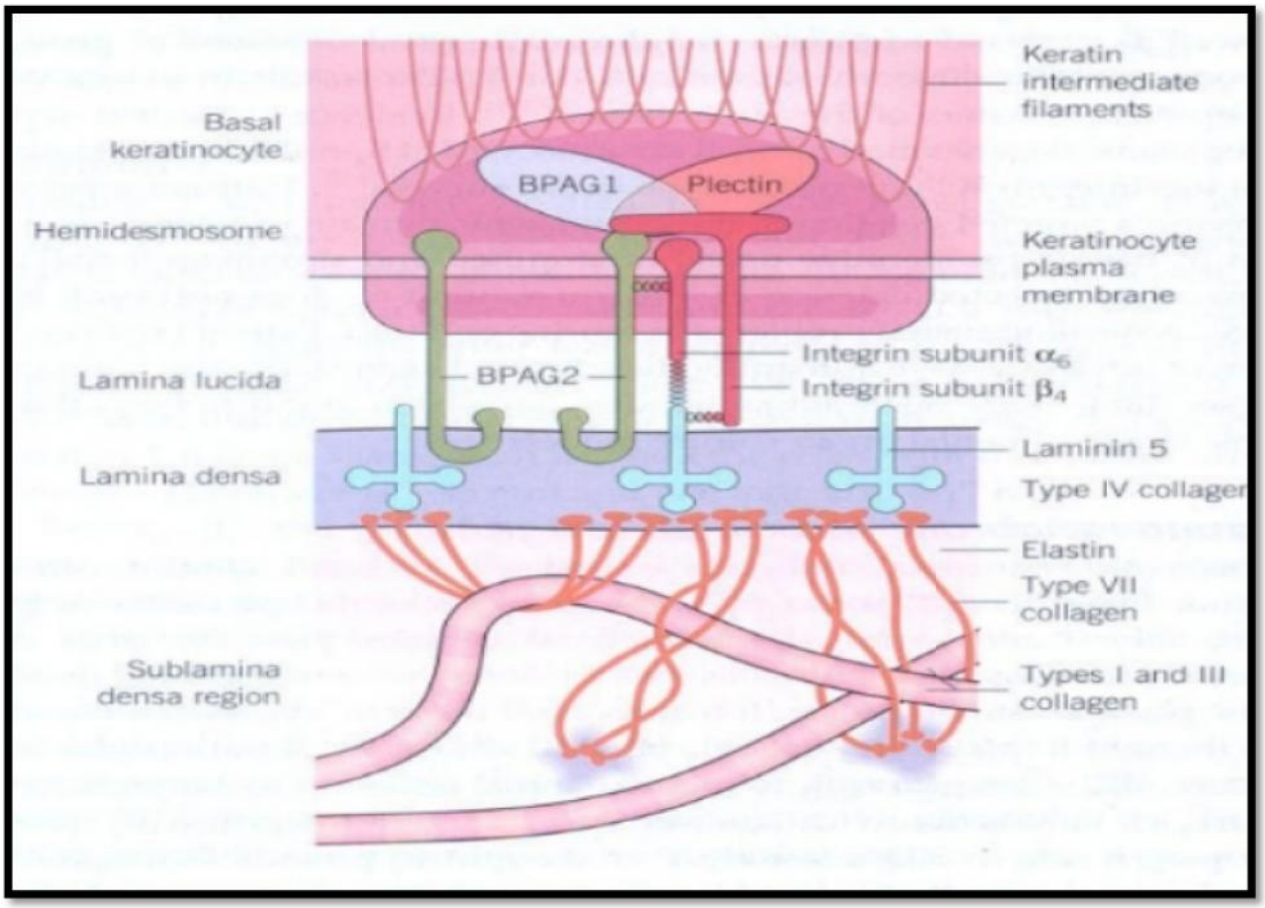


Table 2: Diseases with specific separation planes [5]

Intraepidermal	
Subcorneal/ granular layer	Pemphigus foliaceus IgA pemphigus Subcorneal pustular dermatosis
Spinous layer	Eczema IgA pemphigus Hailey-hailey disease
Suprabasal	Pemphigus vulgarisParaneoplastic pemphigus Darier's disease
Subepidermal	
Lamina lucida	Bullous pemphigoid Cicatricial pemphigoid Herpes gestationis Dermatitis herpetiformis Linear IgA dermatosis Epidermolysis bullosa Erythema multiforme Herpes gestationis
Sublamina densa	Bullous SLE Epidermolysis bullosa acquisita Linear IgA dermatosis Dystrophic epidermolysis bullosa

CLASSIFICATION OF VESICULOBULLOUS LESIONS

A blister is defined as a fluid filled cavity formed within or beneath the epidermis. Blisters are categorized into vesicles (<0.5cm in diameter) and bullae. (>0.5cm in diameter) [5]

Mechanism of blister formation:

Spongiosis: It is an accumulation of extracellular fluid within the epidermis with resultant separation of keratinocytes (intercellular edema).

Acantholysis: It results from the loss of attachment between keratinocytes. It may result from damage to intercellular connections (primary acantholysis) or secondary to other processes such as ballooning degeneration (secondary acantholysis). As a result acantholytic cells are formed which are rounded keratinocytes with condensed cytoplasm, large neuclei, prominent neucleoli and peripheral condensation of chromatin.

Reticular degeneration: It results from ballooning degeneration with secondary rupture of keratinocytes

Cytolysis: It is the disruption of keratinocytes.

Basement membrane zone destruction: This results from primary structural deficiencies and from humoral and cellular immunopathologically mediated damage.[5]

Table 3: Diseases and histological features of blister formation [5]

Histological features	Disease examples
Spongiosis	Eczema, miliaria
Acantholysis	Pemphigus, Hailey-Hailey disease, Darier's disease
Reticular degeneration	Viral infections
Cytolysis	Epidermolysis bullosa simplex, epidermolytic hyperkeratosis, friction blister, erythema multiforme
Basement membrane zone destruction	Bullous pemphigoid, cicatricial pemphigoid, linear IgA dermatosis, dermatitis herpetiformis, epidermolysis bullosa aquisita, lethalis and dystrophica

Table 4: Mechanisms of blister formation [5]

Mechanism	Disease examples
Antibody mediated	Pemphigus vulgaris
Antibody, complement and inflammatory cell dependent	Bullous pemphigoid, cicatricial pemphigoid, epidermolysis bullosa acquisita
Toxin mediated	Staphylococcal scalded skin syndrome
Inherent structural fragility and mechanical trauma	Epidermolysis bullosa
Other less-defined mechanisms	Erythema multiforme

Table 5: Principle infiltrating inflammatory cells in selected vesiculobullous dermatoses [5]

Dermatosis	Principle cell type
Bullous pemphigoid	Eosinophil
Erythema multiforme	Lymphocytes
Herpes gestationis	Eosinophils
Dermatitis herpetiformis	Neutrophils
Linear IgA dermatosis	Neutrophils
Epidermolysis bullosa acquisita	Neutrophils and eosinophils
Bullous SLE	Neutrophils and lymphocytic interface dermatitis
Cicatricial pemphigoid	Mixed neutrophils, eosinophils and lymphocytes
Paraneoplastic pemphigus	Lymphocytic interface dermatitis

Classification of immunobullous diseases [1]

Intraepidermal immunobullous diseases

1. Pemphigus vulgaris
    - Pemphigus vegetans with subtypes: Hallopeau and Neumann
2. Pemphigus foliaceus
    - Pemphigus herpetiformis
    - Pemphigus erythematosus
    - Endemic pemphigus foliaceus
3. Drug induced pemphigus
4. Paraneoplastic pemphigus

5. IgA pemphigus
    - Subcorneal pustular
    - Intraepithelial neutrophilic

Subepidermal immunobullous diseases

1. Bullous pemphigoid
2. Mucous membrane pemphigoid
3. Cicatricial pemphigoid
4. Anti-p200/ laminin γ1 pemphigoid
5. Pemphigoid gestationis
6. Linear IgA bullous dermatosis
7. Dermatitis Herpetiformis
8. Epidermolysis bullosa acquisita
9. Bullous systemic lupus erythematosus

Intraepidermal autoimmune vesiculobullous disorders

PEMPHIGUS GROUP

It is a group of diseases characterized clinically by flaccid bullae over body and erosions over body and mucosae; histologically by intraepidermal clefts due to acantholysis and immunohistologically by IgG antibodies bound to cell surface of keratinocytes.[20]

Pemphigus vulgaris:

It is the most common type of pemphigus seen generally in 4^{th} to 5^{th} decade of life.[21] Pemphigus vulgaris affects all races and both sexes equally. [1] The average annual incidence for adult population was 0.42 cases per million /year with a higher

incidence of 0.61 per million /year. Askenazi jews have the highest incidence of 2.7 per million /year.[22] Pemphigus vulgaris accounts for 80% of all cases of pemphigus.

Etiology: The precise etiologies of all forms of pemphigus and mechanisms that initiate the antibody production are essentially unknown. Predisposition to development of pemphigus is linked to genetic factors. First-degree relatives of patients with pemphigus vulgaris are more susceptible to the development of autoimmune diseases than controls.[1] Certain major histocompatibility complex (MHC) class II genotypes, in particularly HLA-DRB1*04 and DRB1*14 subtypes are common in patients with pemphigus vulgaris. HLA DRW4 was found in jewish patients with pemphigus vulgaris.[1]

Pemphigus occurs in patients with other disorders characterized by immunological disturbances.[1] The association of pemphigus vulgaris with other autoimmune diseases like rheumatoid arthritis, Sjogren's syndrome pernicious anemia, systemic lupus erythematosus, scleroderma, Hashimoto's thyroiditis, Addison's disease, bullous pemphigoid and myasthenia gravis has been reported.[23]

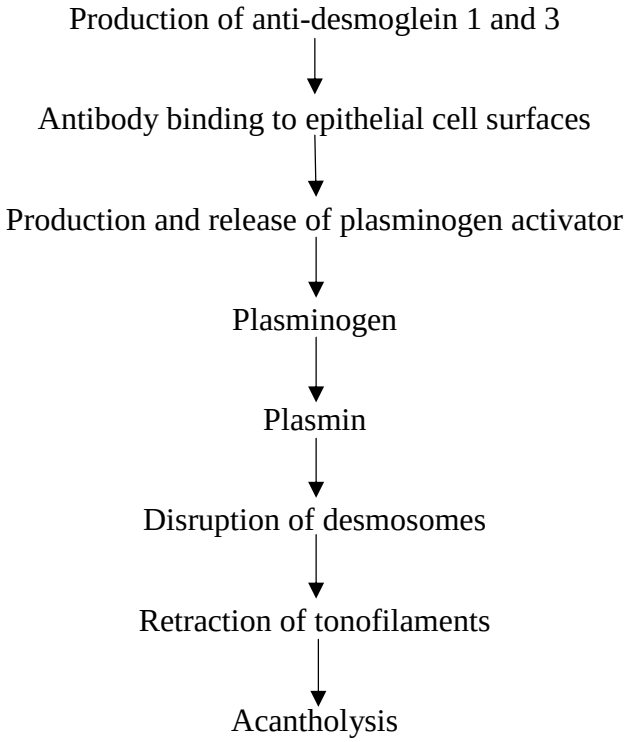
Pathogenesis: Pemphigus vulgaris is characterized by antigens against desmoglein 1 and 3 which are involved in intercellular adhesion in epidermis. Disease can be caused by presence of anti-desmoglein 3 only with the disease limited to mucous membrane or both anti-desmoglein 1 and 3 leading to widespread mucocutaneous disease. [1] This finding can be explained by desmoglein compensation theory and differing distribution of these two proteins different epithelia. In skin there is expression of desmoglein 1 in all layers where as there is a high level of desmoglein 3 in basal and suprabasal layer. Where as in mucosal epithelium, desmoglein 3 is expressed through all layers whereas desmoglein 1 is present only in lower layers.

Desmoglein 1 and 3 act cooperatively to produce widespread mucocutaneous disease. Presence of only anti-desmoglein 3 will not be able to produce cutaneous disease due to maintenance of epithelial integrity by desmoglein 1 and only mucosal lesions develop.

Antibodies: Antibodies in pemphigus vulgaris have been found of all four IgG subclasses and rarely IgA and IgM.[12] But in patients with active disease IgG and C3 are pathogenic with antibody titres being directly proportional to disease severity.[1]

Mechanism of blister formation[1]

Pemphigus is an autoimmune disorder characterized by production of IgG autoantibodies against adhesion proteins present in intercellular cement of epidermis. These antibodies cause steric hinderance[1], protease activation[1] and disruption of intracellular signaling pathways[1]. Keratinocytes then separate from adjoining cells and this process is called acantholysis.



Clinical features:

Nearly all of patients (50-70%) have oral and genital mucosal erosions that precede cutaneous lesions by months or may be the sole disease manifestation. Blisters are not seen mucosa, but are erosions which show no tendency to heal or which may spread peripherally. Other mucosal surfaces include conjunctival, laryngeal, pharyngeal, oesophageal, urethral and cervical surfaces.

Pemphigus of the skin may be localized or generalized with sites showing predilection being scalp, face; friction prone intertriginous axillae, inframammary and inguinal folds and pressure bearing back, buttock areas. Lesions begin as flaccid blisters with clear fluid arising over a normal skin. Following which the blisters rupture leaving behind painful erosions which may heal spontaneously, not heal spontaneously or further extend beyond the edges depending on the disease severity.[1 25]

Shearing force applied to skin over the edge of a blister leads to extension of blister; this is called marginal Nikolsky's sign.[1]

Whereas shearing force over normal skin, away from a blister over a bony prominence induces a fresh blister formation. This is called direct Nikolsky's sign.[1]

Healing occurs without scarring but hyperpigmentation may occur in resolving lesion. Nail changes involve dystrophy, discoloration, beau's lines, paronychia and onychomadesis.[26]

Aggravating factors:

In pregnancy, pemphigus vulgaris has mild flare up of lesions without having adverse effects on fetus.[27]

Pemphigus can be induced by radiotherapy, thermal burns.[1]

Dietary factors that are mentioned as exogenous factors known to trigger pemphigus in genetically predisposed persons are:

Thiols (garlic, onion, celery),

Isothiocynates (mustard, horse raddish),

Phenols (mango, cashew)

Tannins (cassava, red chillies, tea, red wine) [1]

Cytology:

Cytological examination using a Tzanck smear is useful for demonstration of acantholytic cells in the blisters of pemphigus vulgaris. These cells are rounded keratinocytes with condensed cytoplasm around an enlarged nucleus with peripherally palisaded chromatin and enlarged nucleoli.[5]

Histopathology[5]:

The earlist changes consists of intercellular edema with loss of intercellular attachment in basal cell layer.

Suprabasal epidermal cells separate from the basal cells to form clefts containing acantholytic cells..

Basal cells remain attached to the basement membrane and give appearance of 'row of tombstones' on the floor of the blister.

Mild superficial mixed inflammatory infiltrate is seen in the superficial dermis.

Immunoelectronmicroscopy (IEM)

Direct immunofluorescence shows deposition of IgG and C3 on the intercellular surface of keratinocytes giving a fish net appearance. [1]

Circulating pemphigus autoantibodies are detected by indirect immunofluorescence. Monkey esophagus is preferred for detection of desmoglein 3 antibodies. It can be used to monitor disease activity and response to treatment.

Pemphigus vegetans

This is an uncommon variant of pemphigus vulgaris, comprising only 1-2% of cases.[5] Two subtypes are recognized- Hallopeau type and Neumann type.[28]

Clinical features: Both subtypes have common clinical, histological and immunopathological features, however they differ in their course and prognosis

They may be considered to form a clinical spectrum from severe Neumann type to mild Hallopeau type.[28]

Neumann type: Vesicles and bullae rupture to form hypertrophic granulating erosions in intertriginous areas which bleed easily. The lesions progress to form vegetating masses exuding serum and pus with their edges studded with small pustules.[1]

Hallopeau type/ Pyoderma vegetans: Pustules rather than vesicles are the characteristic early lesions which progress to vegetating plaques.[1]

Cutis verrticis gyrate can occur in both types of pemphigus vegetans.

Neumann type of pemphigus vegetans may have cerebriform tongue, which is characterized by a pattern of sulci and gyri on the dorsum of the tongue.[28]

Histopathology:

In the Neumann type, as the early lesions, there is formation of villi and verrucous epidermal hyperplasia. Numerous eosinophils are present within the epidermis and dermis, producing eosinophilic spongiosis. In Hallopeau type, the early lesions consists of pustules arising on normal skin with acantholysis and formation of small clefts in suprabasal location. The clefts are filled with numerous eosinophils and degenerated acantholytic cells. Early lesions may reveal more eosinophilic abscesses than in the Neumann type.[5]

Direct immunofluorescence: Findings in perilesional skin are identical to those in pemphigus vulgaris.[28]

Treatment of pemphigus vulgaris and pemphigus vegetans:

1. Topical therapy: Potent topical or intralesional steroid, potassium permagnate and topical antibiotics.
2. Systemic therapy:

Table 6: Systemic therapeutic ladder for pemphigus vulgaris[29]

Standard treatment
Oral prednisolone: 1 mg/kg/day as initial dose
Aggressive treatment
Immunosupressive agents are given in combination with prednisolone
Azathioprine: 2-4 mg/kg/day
Cyclophosphamide: 1-2 mg/kg/day
Mycophenolate mofetil: 2-3 mg/kg/day
Cyclosporine: 5 mg/kg/day
Intravenous immunoglobulin:
Rituximab: Rheumatoid arthritis regimen
Plasmapheresis

Dexamethasone-cyclophosphamide pulse therapy (DCP):[30]

DCP therapy was introduced by Pasricha for pemphigus and was first used in 1981 with the aim of reducing the toxicity of corticosteroids and also to achieve better therapeutic results.

The entire therapy is divided into four phases-

1. Phase I:

Patient is given monthly doses of 100 mg of dexamethasone dissolved in 500 mL of 5% dextrose by slow intravenous infusion over 2 hour on three consecutive days along with 500 mg of cyclophosphamide in the infusion on day 2. In between, the patient is given 50 mg of oral cyclophosphamide daily except on the 2nd day of DCP.

2. Phase II:

Patients were in remission but monthly DCP therapy and daily oral cyclophosphamide were continued for 9 months.

3. Phase III:

Only oral cyclophosphamide 50 mg was given to patients for an additional 9 months.

4. Phase IV:

All treatments were withdrawn and patient is followed-up for relapse, if any.

Rituximab[31]:

Ritximab is a monoclonal chimeric IgG1 antibody targeting the B cell specific cell-surface antigen CD20. CD 20 is expressed on the surface of B cell lineage from pre-B-cell through to memory B-cell stages but not on the stem cells or plasma cells. Rituximab leads to depletion of mature B lymphocytes which would transform into antibody producing short lived plasma cells but stem cells are not affected as they do not express CD20 cell surface molecule and these cells reconstitute the B cell population after 6 months to 1 year.

Indication:

- First line treatment especially in patients with severe disease.
- Patients who fail to respond adequately to conventional treatment modality,
- Systemic corticosteroids and/or other immunosuppressants are contraindicated or cause severe adverse effects requiring its discontinuation.

Dosage:

Rituximab is given in two different schedules

1. Lymphoma regimen: 375mg/m^2 given intravenously every weekly for 4 weeks.

2. Rheumatoid arthritis regimen: 1gm given intravenously, 2 doses given with an interval of 15 days in between them.

Pemphigus foliaceus:

Epidemiology: Pemphigus foliaceus is less common worldwide than pemphigus vulgaris and accounts for only 10-20% cases of pemphigus.

Pathogenesis:

Pemphigus foliaceus antigen recognize epitopes located in the extra cellular amino-terminal domain of desmoglein 1, a 160kD desmosomal cadherin. Pemphigus foliaceus antigen is expressed more strongly in skin from scalp and upper torso than from lower torso. Desomogelin 1 is present in mucosa but weakly expressed accounting for lack of mucosal involvement in pemphigus foliaceus.[1]

Antibodies:

The pathogenic antibodies in all forms of pemphigus foliaceus are predominantly IgG4 subclass.[1]

Clinical features:[1]

Pemphigus vulgaris is less severe than pemphigus vulgaris. Insidious in onset with scattered scaly lesions involving the seborrheic areas like scalp, face, chest and upper back. Patients present with small flaccid bullae that rupture easily forming erosions and scaly plaques. Erosions have an offensive musty odor. Nikolsky's sign is positive. It may affect the entire skin surface resembling exfoliative dermatitis.[32]

Clinical variants:

1. Pemphigus herpetiformis:

Jablonska first described a rare and atypical variant of pemphigus that resembles dermatitis herpetiformis in its early phase.[1] Patients presents with pruritic, erythematous popular lesions in closely grouped pattern.[5] In pemphigus herpetiformis, neutrophilic spongiosis is seen histopathologically and presence of intra epidermal IgG and C3 deposition on direct immunofluorescence similar to classical pemphigus foliaceus.[5]

2. Pemphigus erythematosus:

Pemphigus erythematosus is a variant of pemphigus foliaceus originally described by Senear and Usher.[1] It usually develops insidiously, the initial lesions appear on the face, scalp, back and chest. Scaling, crusting and oozing lesions are usually predominant. The lesions of the face often resemble scaly plaques of lupus erythematosus. Co-existance of pemphigus and lupus erythematosus is known as Senear-Usher syndrome. Patients have immunological features of both lupus erythematosus and pemphigus. (granular IgG and C3 at basement membrane zone i.e. positive lupus band test and intercellular IgG and C3 in the epidermis and circulating antineuclear antibodies)[1]

3. Endemic pemphigus foliaceus (syn. Fogo Sevagem, Brazilian pemphigus foliaceus)

It is clinically, histologiocally and immunologicall similar to classical pemphigus foliaceus. It develpps in those who visit areas close to rivers and stream is brazil.[1] Incidence of the disease is the greatest at the end of rainy season when insect black fly

(Similium prunosum) bites are frequent. Sunlight may exacerbate the disease. It has not been reported in Indian literature.

Endemic pemphigus foliaceus affects children and young adults wiwth peak incidence in second and third decades where other forms of pemphigus foliaceus affects middle aged adults. Patients present with flaccid bullae which rupture easily leaving superficial erosion. Nikolsky's sign is positive. The burnt appearance and burning sensation over sun exposed sites gives the diseas fogo selvage (Portugese for 'wild fire')[33]

Cytology: Tzanck smear reveals few acantholytic keratinocytes.[5]

Histopathology: the earlist change consists of acantholysis in the upper epidermis, within or adjacent to the granular layer, leading to subcorneal bulla.

The histological features of pemphigus foliaceus may have 3 patterns

1. Eosinophilic spongiosis
2. Subcorneal cleft, often with few acantholytic keratinocytes
3. Subconeal cleft with dyskeratotic keratinocytes, diagnostic for pemphigus foliaceus

The inflammatory infiltrate is variable.[5]

Immunofluorescence:

Direct immunofluorescence test of perilesionsal skin reveals in most cases full thickness intercellular deposition of IgG but rarely it may be localized only to the superficial portion of epidermis. [5]

Treatment:

Pemphigus foliaceus responds to potent topical or intralesional steroids. It control is inadequate, prednisolone 20-40mg/day is effective. Azathioprine, cyclophosphamide, mycofenolate mofetil are used as adjunt steroid sparing drugs.

Nicotinamide 1.5g/day combined with tetracycline 500mg 4 times per day or minocycline 100mg/day is also effective. [1]

Drug- induced pemphigus:

Drug-induce pemphigus was first described by Degos in 1969 in patients using D-penicillamine. Drugs may exacerbate or induce pemphigus. [1] Drugs 'at risk for pemphigus are sulphydryl (SH) i.e. thiol group containing drugs such as penicillamine and captopril. Pemphigus can also be attributed to non-thiol drugs including ACE inhibitors, nefedipine, penicillin, cephalosporins and rifampicin. Drug induced pemphigus and drug- triggered pemphigus are considered to be separate entities.[34]

In drug-induced pemphigus, exogenous, non-autoimmune factors play a major role and the disease regresses when the offending drug is discontinued.[35] In drug-triggered pemphigus, the drug only stimulates a predisposition to develop pemphigus.[36]

Etiopathogenesis:

Thiol- drugs provoke acantholysis by increasing activity of plasminogen activators. [1]

Clinical features:

Pemphigus foliaceus is the most common pattern induced by drugs. Drug- induced pemphigus vulgaris and pemphigus vegetans are rare with morbilliform or urticarial eruption the early finding. [1]

Histopathology:

The findings in early eruption are non-specific consisting of eosinophilic spongiosis, parakeratosis and variable dermal infiltrate. Well-developed lesions are identical to pemphigus vulgaris and foliaceus.[5]

Paraneoplastic pemphigus:

Paraneoplastic pemphigus is a distinctive form of pemphigus described in association with a variety of underlying neoplasms. It is clinically distinct from pemphigus vulgaris and foliaceus.[16]

Table 7: Relative frequencies of neoplasms associated with paraneoplastic pemphigus.[37]

Associated malignancy	Occurrence in %
Non-Hodgkin lymphoma	39
Chronic lymphocytic leukemia	18
Castleman disease	18
Carcinoma	9
Thymoma	6
Sarcoma	6
Waldenström's macroglobulinemia	1
Hodgkin lymphoma, monoclonal gammopathy, and melanoma	<1

Clinical features:

The most characteristic feature of paraneoplastic pemphigus is intractable stomatitis causing erosions and ulceration in oral mucosa, extending to vermillion border of lips. The mucocutaneous lesions of paraneoplastic pemphigus consists of variable mixture of blisters, erosions and target lesions. The trunk and proximal extremities are most commonly involved sites.

Histopathological findings:[5]

Major findings

1. Epidermal acantholysis
2. Suprabasal cleft or blister
3. Dyskeratotic keratinocytes
4. Basal cell vacuolation
5. Epidermal exocytosis of inflammatory ells

Minor findings

1. Acanthosis and dyskeratosis of follicles and eccrine epithelia
2. Lymphocytic satellitosis
3. Poloymorphic superficial perivascular infiltrate
4. Melanin incontinence, melanophages
5. Necrotic blister roof
6. Absence of apoptotic keratinocytes in dermis
7. Epidermal regeneration from adnexal epithelium

Paraneoplastic pemphigus represents a syndrome defined by five criteria as described by Anhalt et al in 1990:[38]

1. Painful mucosal erosions and a polymorphous skin eruption
2. Histopathologic features of intraepidermal acantholysis, dyskeratosis and vacuolar interface dermatitis.
3. DIF findings of intercellular epidermal IgG and complement with or without granular linear complement deposition along the basement membrane zone.
4. Serum antibodies detected by indirect immunofluorescence that bind cell surfaces of stratified squamous epithelia as well as simple columnar transitional epithelia.
5. Serum immunoprecipitation with a complex of four proteins (250, 230, 210 and 190 kDa)

Camisa and helm in 1993 have proposed revised set of criteria for the diagnosis of paraneoplastic pemphigus:[38]

Major criteria

1. Polymorphous mucutaneous eruption
2. Concurrent internal neoplasia
3. Characteristic serum immunoprecipitation findings

Minor criteria

1. Histological evidence of acantholysis
2. Direct immunofluorescence showing intercellular and basement membrane staining
3. Indirect immunofluorescence staining with rat bladder

All the three major and two major criteria are required for diagnosis of paraneoplastic pemphigus

Treatment:

Paraneoplastic pemphigus is generally refractory to treatment. Oral steroids, azathioprine, cyclosporine, mycophenolate mofetil and plasmapheresis have been tried. Cases with benign or low-grade neoplasia may remit after surgical removal of neoplasm.[1]

IgA pemphigus:

Two types are distinguished based on the level of pustule formation and IgA deposition-

1. Subcorneal pustular type
2. Intraepidermal neutrophilic type

Pathology:

A neutrophilic polymorphoneuclear infiltrate in the epidermis and microabscesses at various levels depending upon the age of the legion is seen with sparse or absent acantholysis.[1]

Clinical features:

The disease chiefly affects adults. Patients with both types of disorder have flaccid vesicles or pustules. The lesions may be pruritic and show a circinate or annular configuration with central clearing, progressing to crusted or scaly plaques. The sites of predilection are the axillae and groins. Flaccid pustules may resemble subcorneal pustular dermatosis of Sneddon-Wilkinson.

Associated diseases: Monoclonal IgA gammopathy, HIV infection, Chron's disease, gluten-sensitive enteropathy, rheumatoid arthropathy and intake of thiol containing drugs predispose to development of IgA pemphigus.

Treatment:

Patients respond well to dapsone and poorly to corticosteroids. Second line therapies include acitretin, isotretinoin, PUVA and colchicine.[1]

Subepidermal autoimmune vesiculobullous diseases

Pemphigoid diseases and dermatitis herpetiformis comprise subepidermal autoimmune blistering disorders. Antibodies against structural components of dermo-epidermal junction are seen in pemphigoid diseases whereas autoantigen transglutaminase is found in dermatitis herpetiformis.[40]

Table 8: Classification of pemphigoid diseases and respective bmz antigens targeted [1]

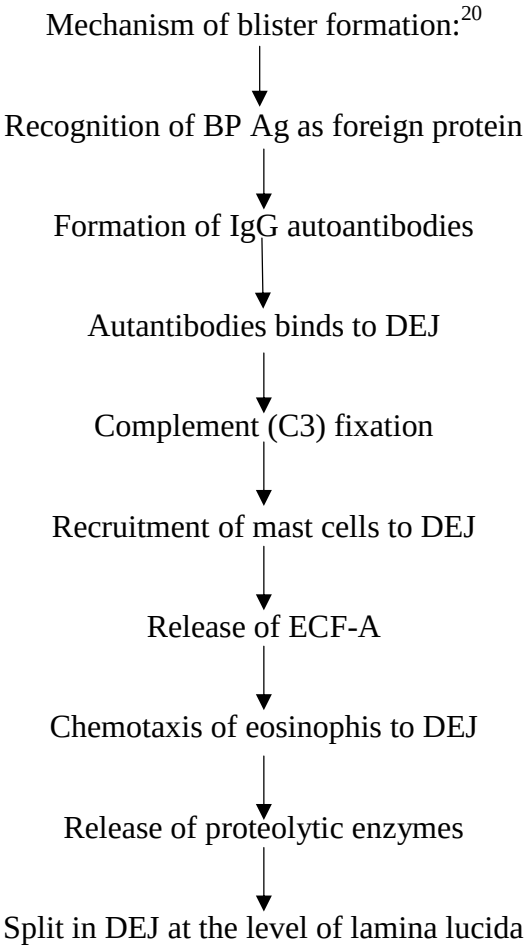
Disease	Auto-antibody
Bullous pemphigoid	BP180 NC16A, BP230
Mucous membrane pemphigoid	BP180, Laminin332, BP230, α6β4 integrin, collagen VII
Cicatricial pemphigoid	BP180, BP230, Laminin332
Pemphigoid gestationis	BP180 NC16A, BP230
Anti-p200/ laminin γ1 pemphigoid	P200 antigen laminin γ1
Epidermolysis bullosa acquisita	Type VII collagen
Bullous systemic lupus erythematosus	BP180, BP230, Laminin332
Lichen planus pemphigoides	BP180 NC16A, BP230
Linear IgA disease	LAD-1, BP230

BULLOUS PEMPHIGOID

Introduction: Bullous pemphigoid is the most common disorder of the sub-epidermal immunobullous disorders affecting mainly the elderly population although younger patients may also be affected.[1]

Epidemiology: BP is the most common type of subepidermal autoimmune blistering disorder in western population, with an incidence of about 25 cases per million persons per year in Europe.[41] and 7 cases per million in Germany. [42] The incidence was highest in Indians of Malaysian origin.[43] It occurs in patients older than 65 years.[44] Both sexes are equally affected with a slightly greater preponderance in females. It is a rare entity in childhood with youngest patient being 2.5 months old female infant.[46]

Etiopathogenesis: The antigens BP180 and BP230 involved in pathogenesis of bullous pemphigoid are located over hemidesmosomes that mediate the linkage of keratin intermediate filaments to basement membrane. BP230 or BPAG1 is an intracellular intracellular 230kD molecular weight protein of plakin type. BP180 or BPAG2 or type XVII collagen is a transmembrane 180kD protein with a short extracellular non collagenous domain- BP180 NC16A and an intracellular BP180 N-terminal.[47]



Clinical features:

The lesions of bullous pemphigoid are 1 to 3 cm tense blisters that occur on healthy skin or an erythematous or urticarial plaque.[48] Blisters typically are found on the flexor surfaces of the arms, legs, axillae, groin and abdomen. Pruritus is mild to severe intractable pruritus to conventional anti-pruritic therapy. Lesions heal without scarring. It is a chronic disease characterized by periods of exacerbation and partial remission.[49] Oral lesion are present but rarely found.[1]

Nikolsky's sign is negative and bulla spread sign is positive.[1]

Villant et al (1998) had set diagnostic criteria for bullous pemphigoid:[50]

1. Age > 70 years
2. Absence of atrophic scars
3. Absence of mucosal involvement
4. Absence of head and neck blisters

Cutaneous diseases associated with bullous pemphigoid:

Bullous pemphigoid is associated with neurological and psychiatric disorders including cognitive impairment, Parkinson's disease, stroke, epilepsy, multiple sclerosis and uni-polar and bi-polar disorder. These can be explained by the fact that BP180 and BP230 are expressed in central nervous system.[1]

Other autoimmune disorders associated with bullous pemphigoid include rheumatoid arthritis and pernicious anemia.[1]

Bullous pemphigoid has been found associated with several malignancies.[51 52]

Histopathology[5] :

In early lesions, papillary dermal edema in combination with a perivascular infiltrate with lymphocytes and eosinophils is present. The infiltrate may be cell-poor or cell-rich.

In cell-rich pattern, eosinophilic spongiosis is seen and consequently blisters arise on an erythematous skin whereas in cell-poor pattern blister arises on normal skin.

Split is seen subepidermally at dermo-epidermal junction.

Direct immunofluorescence:

Direct immunofluorescence reveals deposition of IgG autoantibodies and C3 along the basement membrane.

Salt split technique:

This technique was introduced in 1984 to differentiate in between anti-lamina lucida and anti-sublamina densa antibodies by Gammon et al. This is now termed as indirect salt split technique.[53]

Direct salt split technique is done by incubating the DIF sample in 0.1M normal saline for 24 hours to differentiate bullous pemphigoid from epidermolysis bullosa acquisita. Salt split technique allows determining the deposition of bound autoantibodies either on the blister roof i.e. epidermal side as in bullous pemphigoid or on the blister floor i.e dermal side as in epidermolysis bullosa acquisita by a split induced within the BMZ.[54]

Indirect immunofluorescence:

IgG (frequently), IgE, IgA and IgM anti-BMZ antibodies are detected in 80% of the patients.[20]

Treatment:

Topical and systemic corticosteroids are the mainstay of treatment. Topical corticosteroid help in control of disease and to reduce the dosage of systemic agents. Prednisolone 20mg/day or 0.3 mg/kg/day in localized or mild disease, 40 mg/day or 0.6 mg/kg/day in moderate disease and 60mg/day or 1mg/kg/day in severe disease. Corticosteroid dosage is reduced over a course of few weeks.

Antibiotics dapsone, tetracyclines and nicotinamide and immunomodulators like azathioprine and methotrexate are used as steroid sparing agents.

Mucous membrane pemphigoid (MMP):

Syn: Benign MMP, oral pemphigoid, ocular pemphigoid

Mucous membrane pemphigoid is a group of chronic, autoimmune subepithelial blistering diseases which predominantly involves predominantly the mucous membranes and occasionally the skin.[55] Previously the term cicatricial pemphigoid was used synonymously with MMP however cicatricial pemphigoid is a rare clinical variant in which mucous membranes are not involved and lesions develop on skin which heal with scarring.

Epidemiology:

Incidence in UK is 0.8 per million population[56], 1.3–2.0 per million per year in Germany.[57]

MMP predominantly affects women more often than men with a male to female ratio of 2:1[58] and mainly seen in the elderly population between 60 and 80 years of age.[59]

Genetic association:

The association of MMP with human leukocyte antigens of major histocompatibility class II HLA-DQB1*0301, DRB1*04 and DRB1*11 is found.[60]

Pathogenesis:

Circulating IgG and/or IgA autoantibodies against components of the basement membrane zone antigens mainly BP180 and also against BP230, laminin 322, α6β4 integrin and collagen VII are found[1]. Antigen-antibody binding results in the

expression of inflammatory mediators that induce migration of lymphocytes, eosinophils, neutrophils, and mast cells to the BMZ and An antibody-induced complement-mediated process results in sub epithelial epithelial detachment.[1]

Fibroblasts activated secondary to the production of inflammatory profibrotic factors such as TGF-β, heat shock protein 47, connective tissue growth factor, IL-4,5 and 13 induce fibrosis and collagen produced leads to scarring.[1]

Clinical features[1]:

MMP can affect multiple mucosal sites with an occasional skin involvement. It is a chronic, progressive condition which presents with

1. Oral mucosa (85%) as asymptomatic to extremely painful erosions and ulcers as well as chronic gingivitis.
2. Ocular conjunctiva (65%) starting unilaterally with subtle features as burning, dryness and foreign body sensation which progresses to scarring causing shortening of inferior fornix, symblepharon, trichiasis and finally blindness. Within 2 years the disease progresses to involve bilaterally.
3. Nasal mucosa (20–40%) as hemorrhagic crusts and epistaxis leading to septal perforation and disfiguring fibrosis.
4. Skin (25–30%) as tense bullae arising on an erythematous or urticarial plaques which heal with scarring and millia formation.
5. Anogenital area (20%) as erosions, scarring leading to labial fusion, introitus narrowing resembling lichen sclerosis et atrophicans.
6. Pharynx (20%) as odynophagia.
7. Larynx (5–15%) as hoarseness.
8. Esophagus (5–15%) as odynophagia, dysphagia and heartburn.

Histopathology:

Sub-epidermal split is seen which may extend down the adnexa. Neutrophils, lymphocytes and few eosinophils and mast cells predominate the inflammatory infiltrate. Lamellar fibrosis in the dermis is hallmark but may not be present in initial lesions.[5]

Direct immunofluorescence:

Linear deposition of IgG, IgA and C3 along the basement membrane is seen in U-serrated pattern.

Table 9: Diagnostic criteria for MMP

Clinical features	Chronic, inflammatory, blistering disease predominantly affecting any or all mucous membranes, with or without skin involvement and with or without identifiable scarring
Direct immunofluorescence	Continuous deposits of IgG, IgA and/or C3 in epithelial BMZ

Treatment[61]:

Those who have the disease affecting only the oral mucosa and/or the skin with less tendency of scarring with minimal clinical significance are defined as “low-risk” MMP patients. On the contrary, “high-risk” patients are those who have disease occurring in any of the following sites: ocular, nasopharyngeal, esophageal, laryngeal, and genital mucosa.

Low-risk patients:

1. Tetracycline (1500–2000 mg/day)
2. Minocycline (50–100 mg/day)
3. Dapsone: Initial dose of 50mg/day, increase by 25 mg every 7 days to 100–200 mg/day as needed and tolerated
4. Corticosteroids: Prednisone 0.5–1.0 mg/kg/day

High-risk patients:

1. Corticosteroids: Prednisone 1–2 mg/kg/day; or dexamethasone 100 mg/day for 3 days (pulse therapy); or intravenous pulse therapy at 0.5– 1.0 g for 3 days
2. Mycophenolate mofetil: 35–45 mg/kg/day
3. Azathioprine: initial dose 1–2 mg/kg/day; can be raised to 5 mg/kg/day
4. Intravenous immunoglobulins: 2 mg/kg/cycle every 4 weeks
5. Cyclophosphamide: 1–2 mg/kg/d for progressive, organ- or life-threatening patients
6. Methotrexate: 12.5–22.5 mg/wk
7. Cyclosporine: 100–250 mg/day
8. TNFα antagonist (etanercept): 25–50 mg, once or twice a week
9. CD-20 monoclonal antibody (rituximab): 375 mg/m2 in 4-week intervals or 1000 mg 2 weeks apart for two treatments total

Pemphigoid gestationis:

It is an intensely pruritic, bullous eruption that may develop in association with pregnancy, or rarely the trophoblastic, hyaditiform mole and choriocarcinoma.[1]

Epidemiology:

Pemphigoid gestationis is rare condition with incidence of 0.4 per million per year in france[41], 0.54 per million per year in central Germany[42]

It is associated with HLA-D8, HLA-DR3 and HLA-DR4.[1]

Clinical features:

Pemphigoid gestationis begins at any time between 4 weeks of gestation to 5 weeks postpartum, with majority presenting in the second and third trimester.[1] It may initially present as intense pruritus or along with urticarial or papulovesicular eruption beginning on the umbilicus and gradually spreading around the abdomen and thighs. The palms, soles, chest, back, face and extremities may also be affected. Oral cavity involvement is rare.[62]

Neonatal pemphigoid gestationis may occur in 3% of pregnancies resulting from transfer of HG factor across placenta.[1] Foetal mortality is very rare.[1, 62]

Histopathology:[5]

- Epidermal edema and papillary edema.
- Teardrop shaped subepidermal bulla.
- Eosinophilic infiltration of the dermis.

Antibodies:

Autoantibodies are directed at the same hemidesmosomal target antigens as in bullous pemphigoid i.e. BP180 and less commonly BP230. The extracellular region of BP180 antigen NC16A is the target for pathogenic autoantibodies.[1]

Direct immunofluorescence: Linear deposition of C3 and occationally IgG along the basement membrane zone

Indirect immunofluorescence: Reveals binding of Herpes gestationis (HG) factor i.e. C3 against the basement membrane zone

On salt spit technique, fluorescence is seen on the epidermal side of the split.

Treatment:[1]

Mild disease: Topical potent steroid + oral antihistamines.

Moderate disease: Oral prednisolone 20-30 mg/day.

Severe disease: Oral prednisolone 40-80 mg/day which is tapered rapidly. Plasmapheresis can be considered in very severe cases.

Linear IgA disease:

It is a chronic acquired subepidermal disease of children and adults with cutaneous and mucosal involvement, characterized by IgA autoantibodies against basement membrane zone.[1]

Two main clinical syndromes are distinguished

- Chronic bullous disease of childhood (CBDC)
- Adult onset linear IgA disease

Epidemiology:

CBDC occurs in children with a peak incidence of about 4.5 years. The age of peak incidence of this disease is 60-65 years, with a slight female predominance. It is an

uncommon dermatosis with an incidence of 0.48 per million per year in france[41], 0.23 per million per year in Germany[42], 0.26 per million per year in Singapore[43]

There is a strong association with halotypes HLA-B8, HLA-DQW1 and HLA-DR3[63]

Clinical features:

Skin lesions in this disease are variable and can be erythematous or oedematous papular, vesicular or bullous or can resemble erythema multiforme.[64]

The distribution and morphology of legions in linear IgA disease may have one or two distinct patterns:

1. Involvement of the extensor surfaces with grouped papulovesicles, similar to the clinical features of dermatitis herpetiformis.
2. Flexural and truncal involvement with scattered vesicles and bullae similar to bullous pemphigoid.

Mucous membrane involvement is seen in 80% of the patients.[14]

Main differences in clinical presentation between childhood and adult onset LABD is a typical localization of blisters on the lower abdomen and perineum, frequently occur in a configuration known as a “cluster of jewels” or “string of pearls” sign where new lesions occur at the periphery of older blisters.[1]

Drugs associated with linear IgA disease:

Vancomycin, ampicillin, pencillin G, rifampicin, diclofenac, piroxicam, lithium, phenytoin, amiodarone, captopril.[65]

Histopathology:[5]

1. Subepidermal cleft.
2. Infiltration of neutrophils in the papillary dermis forming micro abscesses.

Antibodies:

Major target antigens in LABD are the components of extracellular portion of BP180-NC16A domain. These components are 97 kDa protein- LABD97 and 120 kDa protein- LAD-1.[66]

Direct immunofluorescence:

Shows deposition of IgA autoantibodies and occasionally C3 in a linear distribution along the basement membrane zone in absence of other immunoglobulin classes.[66]

Direct salt split technique shows antibodies binding to either the epidermal or dermal side or both.[55]

Treatment [1]:

First line: Very potent corticosteroid + Dapsone 1mg/kg/day or Prednisolone 0.25- 0.5 mg/kg/day

Second line: Anti-inflammatory antibiotics like tetracycline, doxycycline + nicotinamide

Third line: Mycophenolate mofetil `1.5- 2 g/day, Azathioprine 1-2 mg/kg/day, Cyclosporine 3-6 mg/kg/day and colchicine 1mg/day

Dermatitis herpetiformis (DH):

Syn: Duhring-Brocq disease

It is an intensly pruritic, chronic recurrent, autoimmune papulovesicular disease that usually occurs in yound adults and is characterized by the presence of granular deposits of IgA within the upper papillary dermis of the skin.[22]

Epidemiology:

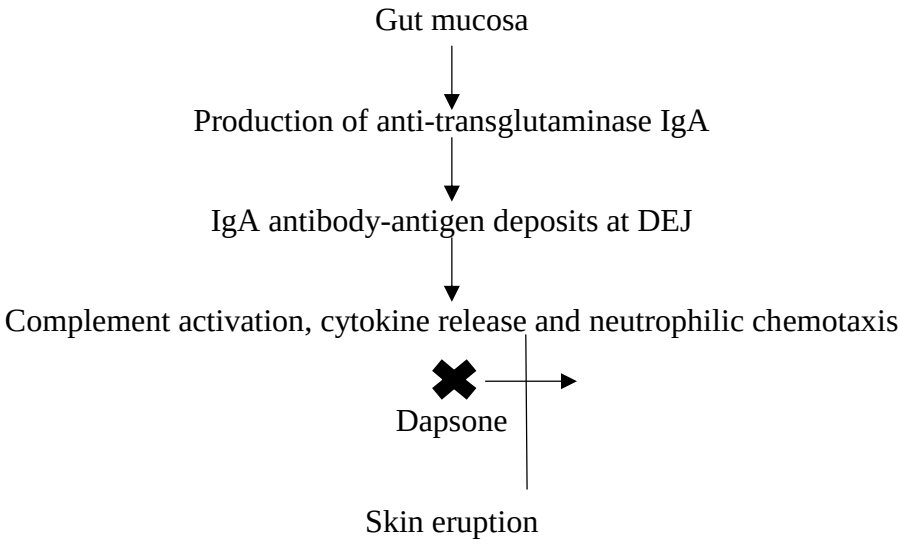
Dermatitis herpetiformis affects males more frequently than females. In a majority of cases its onset is between second and fourth decades of life. It can appear in the late years and rarely in childhood.[67]

Etiopathogenesis:

Dermatitis herpetiformis is a familial disease in which the first-degree relatives can be affected both with dermatitis herpetiformis and celiac disease. Most patients with dermatitis have asymptomatic gluten- sensitive enteropathy i.e. celiac disease.[67]

Epidermal transglutaminase (TG3) is the major autoantigen recognized in the skin lesion. IgA antibodies against tissue transglutaminase cross react with TG3. The IgA deposits are gluten- dependent and are slowly cleared from the skin once gluten is removed from the diet.[68]

It is strictly associated with class II histocompatibility antigens HLA DR3 and HLA DQW2.[1]



Clinical features:

Cutaneous manifestation: The onset may be acute or gradual and pruritus is the first and predominant symptom. Early lesions on the skin are erythematous papules, urticarial wheals with superimposed groups of small vesicles resembling herpes zoster. They are symmetrically distributed over extensor surfaces, especially over the elbows, knees, shoulders, sacrum, buttocks and neck. Lesions heal with post inflammatory hyperpigmentation and without scarring.[1]

Oral manifestation: Mucous membrane involvement is rare. Oral involvement consisted of purpuric or erosive lesions.[1]

Associated diseases: Autoimmune diseases particularly thyroid disease, pernicious anemia and type 1 diabetes mellitus are associated.[1]

Histopathology:

Skin biopsy of an erythematous papule typically shows characteristic neutrophilic microabscesses within the dermal papillae in association with few eosinophils, fibrin, leukocytoclastic debris and oedema. As microabscesses form, a separation develops

between the tips of dermal papillae and overlying epidermis. Perivascular infiltrate composed of lymphocytes, neutrophils and eosinophils is seen.[5]

Direct immunofluorescence:

Characteristic finding is granular deposits of IgA witin the dermal papillae. Linear deposits of immunoglobulins is found in 10% cases.[1]

Treatment:

Gluten free diet and Dapsone 100-200 mg/day are the treatment of choice for all patients.

In patients who cannot tolerate dapsone, G6PD deficiency or hemolytic anaemia topical very potent steroid, oral tetracyclines plus nicotinamide are alternatives.[1, 69]

Epidermolysis bullosa acquisita (EBA)

Syn: Dermolytic pemphigoid

EBA is an acquired sub epidermal blistering disease in which antibodies are produced against the epithelial basement mebrane protein type VII collagen.[62]

Epidemiology:

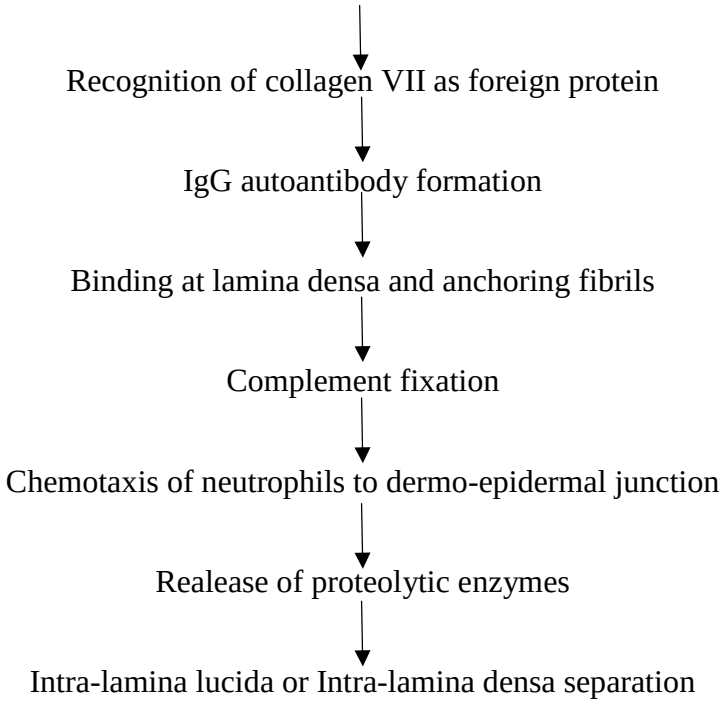
EBA occurs in any age group with peak age of onset being 44 to 54 years of age with 5% of disease present in children and adolescents.

Estimated annual incidence is 0.17-0.26 per million in france[41], 022 per million in Germany[42] and 0.5 per million in Singapore.[43]

Etiopathogenesis:

The target antigen is Collagen type VII which is a component of anchoring fibrils. IgG antibodies against this antigen is recognized in EBA.[70]

Mechanism of blister formation in EBA[20]:



Clinical features:

Two main clinical forms are seen

1. Classical or mechanobullous variant: It clinically resembles dystrophic epidermolysis bullosa in severe cases and porphyria cutanea tarda in mild cases. Clinical features seen are skin fragility, , tense blisters which heal with hypo or hyperpigmentation, scarring and milla, erosions and crusts seen predominantly over trauma-prone areas such as hands, knuckles, elbows, knees and toes. Scarring alopecia, nail dystrophy, shedding, nail bed scarring and anonychia may occur.
2. Inflammatory variant: It clinically resembles other pemphigoid disorders mainly bullous pemphigoid and less commonly mucous membrane pemphigoid (5-10%), linear IgA dermatosis (5-10%)

The diagnostic criteria for EBA as per Roenigk et. al. include[71]

i) Spontaneous or trauma-induced blisters resembling hereditary dystrophic EB,

ii) Adult onset,

iii) A negative family history for EB and

iv) The exclusion of all other bullous diseases.

Histopathology:

The bullous pemphigoid- like presentation is the most common form of EBA. The subepidermal blisters are inflammatory. The predominant infiltrating cells are lymphocytes and neutrophils in perivascular and interstitial areas. In classic form, subepidermal cleft is seen with no inflammatory infiltrate. [5]

Direct immunofluorescence:[1]

DIF shows linear deposition of IgG and C3 along the basement membrane zone.

Routine DIF cannot reliably distinguish EBA from bullous pemphigoid and direct salt split technique is employed in which fluorescence is seen on floor (dermal side) of the induced split.

Treatment: [1]

First line: Prednisolone 0.5-2 mg/kg/day (depending on disease severity) + colchicine 0.5-3 mg/day (highest dose that does not lead to dirrhoea) + dapsone 1.5 mg/kg day

Second line: Mycophenolate mofetil 1.5-2 g/day or cyclosporine 3-6 mg/kg/day

Third line: IVIg 2g/kg/day or Rituximab by rheumatoid arthritis regimen i.e. 2 doses of 1g, administered 15 days apart.

Bullous systemic lupus erythematosus (BSLE):

It is an autoimmune vesiculobullous disease occurring in the setting of SLE.[1]

Epidemiology:

Estimated annual incidence is 0.21 per million in France[41] and 0.26 per million in Singapore.[43]

It is most commonly seen in young adult females.[1]

It is associated with HLA- DR2

Clinical features:[1]

Primary lesions consists of tense vesicles and bullae with clear or hemorrhagic content arising over a normal or erythematous base. Lesions usually are seen in generalized distribution or localized to sun-exposed areas only. Distinguishing feature from other pemphigoid disorders is that face is relatively more affected and in some patients it is the only site of manifestation. Burning sensation instead of pruritus is more commonly seen.

Clinically BLSE may mimic dermatitis herpetiformis most commonly followed by EBA, LAD and bullous pemphigoid.

Histopathology:

Three histopathological patterns have been identified in such lesions.

1. Most commonly seen, dermatitis herpetiformis like pattern
2. Striking basal cell layer vacuolization with neutrophilic infiltration and subsequent subepidermal split.
3. Neutrophilic vasculitis and subepidermal split.

Direct immunofluorescence:

DIF shows linear band of IgG and/or IgM, C3, sometimes IgA and deposition along the basement membrane zone. Salt split technique shows fluorescence along the floor (dermal side) of the split.

Diagnostic criteria:

Camisa and Sharma in 1983 proposed the following criteria for the diagnosis of BSLE.[72]

1. The diagnosis of SLE based on ARA criteria.
2. Vesicles and bullae arising on, but not limited to, sun-exposed skin.
3. Histopathologic findings compatible with dermatitis herpetiformis.
4. DIF revealing IgG and/or IgM, often, IgA at the BMZ.

Treatment:

First line: Dapsone 1-1.5 mg/kg/day

Second line: Prednisolone 0.5-1 mg/kg/day + azathioprine 2.5 mg/kg/day or mycophenolate mofetil 1.5-2 g/day

Third line: Methotrexate 10-15 mg/week or Rituximab by rheumatoid arthritis regimen i.e. 2 doses of 1g give with 15 day interval in between.

Laminin γ1/ Anti-p200 pemphigoid:

It was first described in 1996 by Zillikens and Hashimoto[1].

P200 protein is an acidic non collagenous N-linked glycoprotein present in lamina lucida outside of hemidesmososmes. It is different from the major target antigens of

the dermoepidermal junction including BP180, BP230, Laminin332, α6β4 integrin, collagen VII [1]

Epidemiology:

It is a rare, recently discovered chronic autoimmune blistering disorder with 70 cases of anti-p200 pemphigoid have been reported in the literature[73] with 3 case reports in India[74]

Mean age of patients reported was 81.6 ± 6.5 years.

Clinical features:

Anti-p200 pemphigoid is characterized by tense blisters and vesicles, erosions, and urticarial plaques, closely resembling bullous pemphigoid and the inflammatory variant of epidermolysis bullosa acquisita.

Other presenting lesions include ezematous, dishydrosiform, and rosette-like herpetiform[1].

Mucosal involvement is frequently seen attributed to higher expression of Laminin γ1.

Histopathology[1]:

- Subepidermal cleft.
- Neutrophilic and eosinophilic microabscesses in dermal papillae
- Neutrophilic inflammatory infiltrate in superficial papillar dermis

Direct immunofluorescence:

Linear deposition of IgG and rarely (1 case reported) with IgA along the basement membrane in an N-serrated pattern.[1]

Treatment:

Disease shows prompt response with potent topical corticosteroids. [1] However relapses have been reported and systemic treatment was initiated with successful resolution of disease with oral prednisone, 0.5 to 1mg/kg/day , methotrexate, 12.5 mg/week and dapsone, 100 mg/day.[1, 73]

METHODOLOGY

Source of data: The present study is a 1-year cross sectional study from November 2007 to October 2008. The source of data includes all cases of autoimmune vesiculobullous disorders attending dermatology OPD at KLES Dr. Prabhakar Kore Hospital and MRC, Belagavi.

Inclusion criteria: All the newly diagnosed patients with vesiculobullous disorders in Dermatology OPD of KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

Exclusion criteria: All the patients who are currently on treatment and patients not consenting for the study.

A sample size of 20 was selected by calculating 80% of the average yearly number of cases of autoimmune vesiculobullous disorders in the previous three years attending dermatology OPD at KLES Dr. Prabhakar Kore Hospital and MRC, Belagavi.

Patient’s demographic data, age of onset, duration of disease, symptoms and morphology of lesions were noted in a pre-designed proforma after taking an informed and written consent. The diagnosis was established by clinical examination and was confirmed by histopathological and direct immunofluorescence findings.

Hematological and urine examination with CBC, LFTs, RFTs, RBS, urine routine and microscopy along with HIV and HbsAg were done in all patients. Bedside Tzanck smear was done in all patients after obtaining an informed consent. In all cases, two skin biopsies were taken, one from lesional skin for histopathological

examination and one from perilesional skin for direct immunofluorescence after counselling and informed and written consent.

This was a descriptive study in which clinical, histopathological and direct immunofluorescence findings were examined and their analysis was done in form of tables.

Procedure:

TZANCK SMEAR PREPARATION:

The skin having a fresh blister was cleaned with 70% alcohol. The blister was deroofed with no. 15 scalpel. The base was gently scraped with blunt end of the scalpel. The material obtained was smeared over a clean glass slide, air dried and stained with May-Grunwald Giemsa stain and mounted for microscopic examination. The Tzanck smear was positive when the smear contained discrete acantholytic cells. These cells are rounded keratinocytes with hyperchromatic neucleus and peripherally condensed cytoplasm. Inflammatory cells like eosinophils, neutrophils and lymphocytes may be seen.

BIOPSY:

An early blister was selected and cleaned using 70% alcohol. The area was then anaesthetized by infiltrating 2% lignocaine subcutaneously. The lesional and perilesional skin were biopsied by using a 3.5mm punch. The lesional biopsy specimen was transferred to a container with 10% formalin for histopathological examination. The perilesional biopsy specimen to a container with Michel’s medium and dispatched to Department of dermatology, Kasturba Hospital, Manipal for direct immunofluorescence.

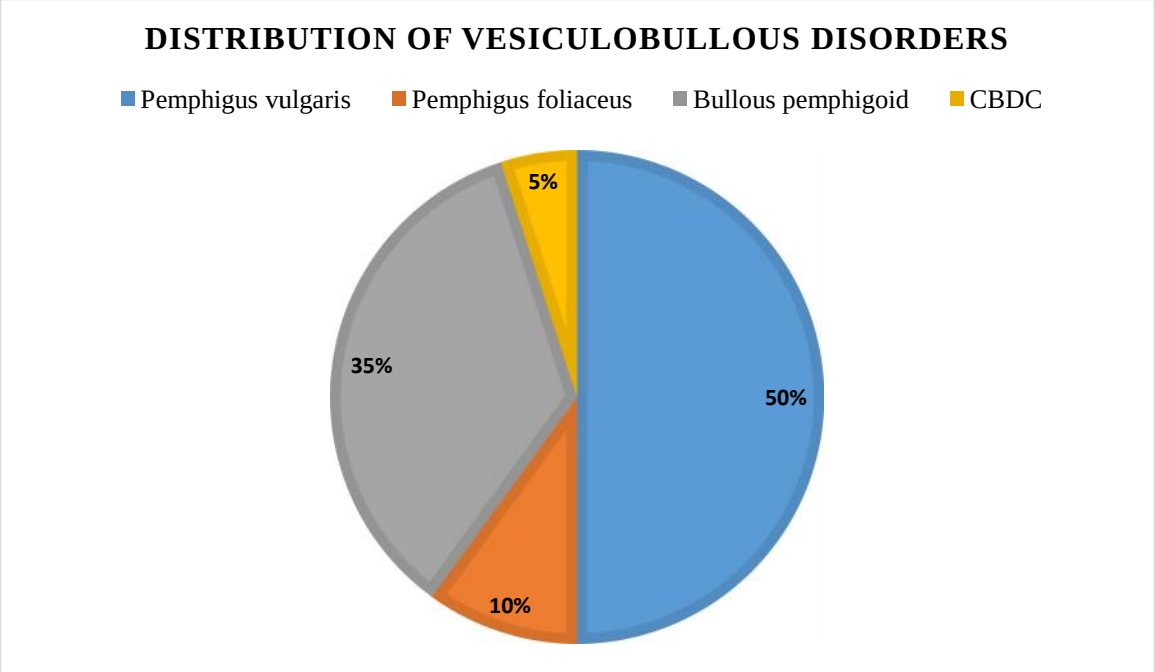
RESULTS

The current study is a one-year cross-sectional study of 20 patients who attended the OPD of Dermatology, venereology and leprosy in KLE Dr. Prabhakar Kore hospital and medical research center, Belagavi from Jan 2017 to Dec 2017.

TABLE 10: Distribution of vesiculobullous disorders

Type	Number of cases	% of total
Pemphigus vulgaris	10	50
Pemphigus foliaceus	2	10
Bullous pemphigoid	7	35
CBDC	1	5
Total	20	100

In present study pemphigus vulgaris constituted the most common vesiculobullous disorder constituting 50% of the cases, followed by bullous pemphigoid constituting 35% of the cases. Pemphigus foliaceus constituting 10% and chronic bullous disease of childhood constituting 5% of the cases.

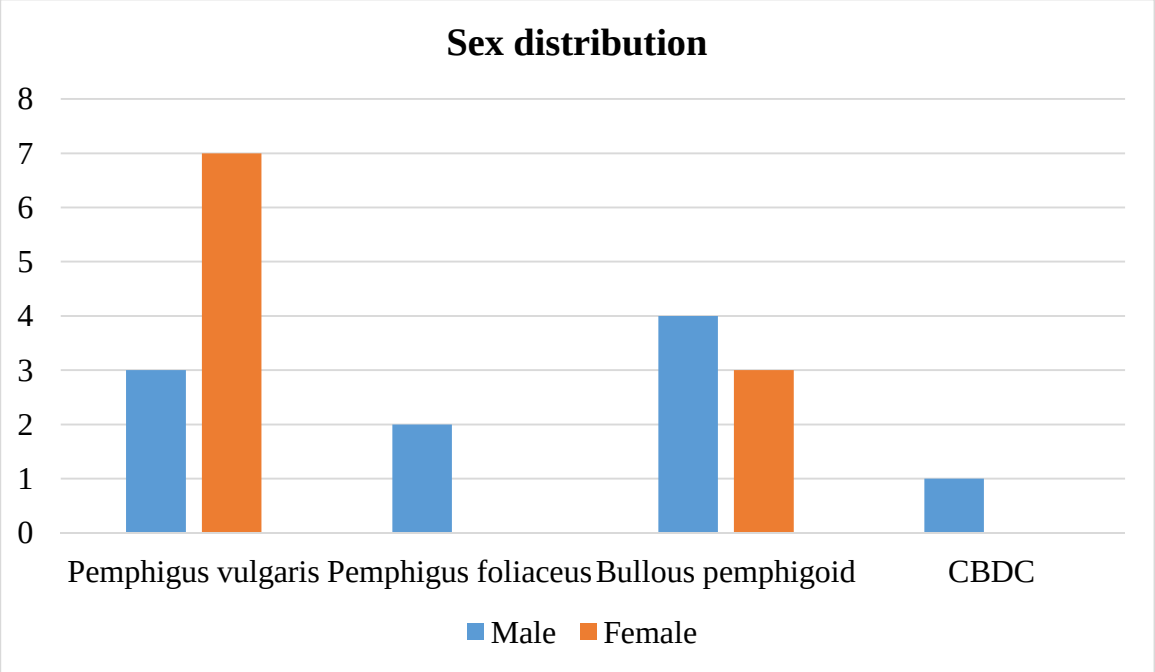


Sex distribution

TABLE 11: Sex distribution in autoimmune vesiculobullous disorders

Sex	PV	%	PF	%	BP	%	CBDC	%	Total
Male	3	30	2	100	4	57	1	100	10
Female	7	70	0	0	3	43	0		10
Total	10	100	2	100	7		1		20
M:F ratio	3:7		2:0		4:3		1:0		1:1

In the present study, there were totally 10 males and 10 females giving a M:F ratio of 1:1 for autoimmune vesiculobullous disorders. Pemphigus vulgaris had a female preponderance, showing a ratio of 3:7 bullous pemphigoid showing a M:F ratio of 4:3, pemphigus foliaceus and chronic bullous disease of childhood showing male preponderance. (M:F ratio of 2:0 and 1:0 respectively)

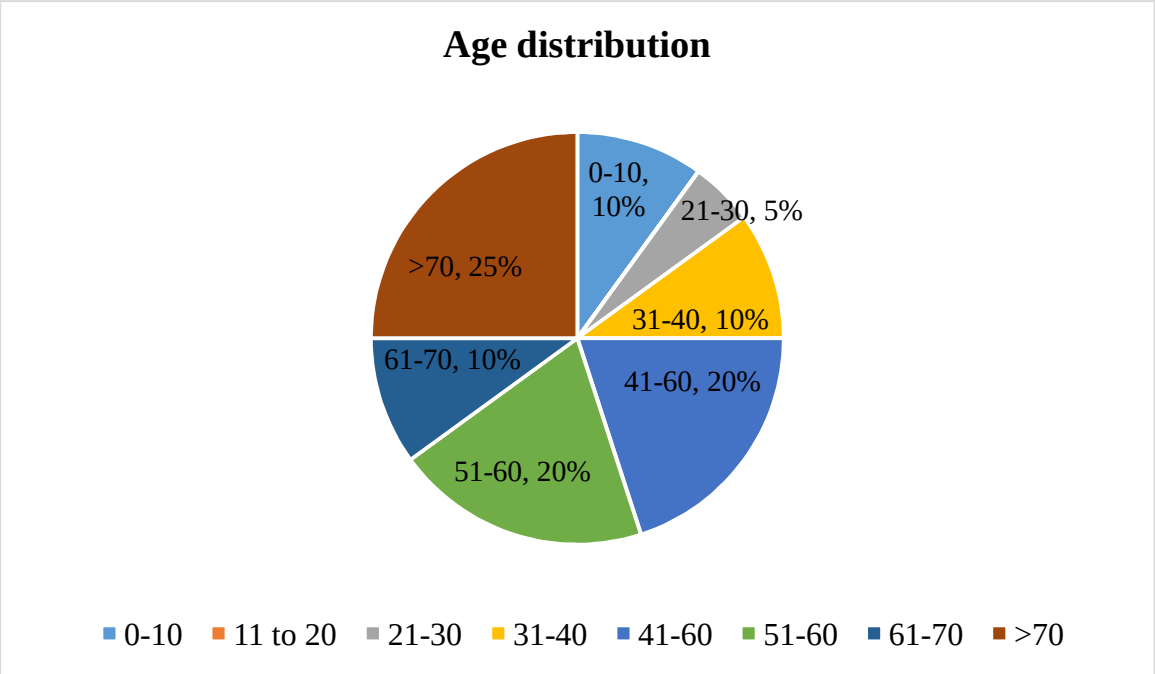


Age of distribution

TABLE 12: Age of distribution in autoimmune vesiculobullous disorders.

Age group	Number of patients	% of total
0-10	2	10
11-20	0	0
21-30	1	5
31-40	2	10
41-50	4	20
51-60	4	20
61-70	2	10
>70	5	25
Total	20	100

In the present study, the youngest patient was 4 months old and the oldest patient was 85 years old both belonging to bullous pemphigoid group. Maximum of cases were seen in the age group of >70 years (25%) belonging to bullous pemphigoid group followed by 51-60 and 41-50 years group (20% each). The mean age of the study population was 47.4 years.



Age distribution in pemphigus group

TABLE 13: Age distribution in pemphigus group

Age (years)	PV	%	PF	%
<21	0	0	0	0
21-30	1	10	1	50
30-40	2	20	0	0
41-50	4	40	0	0
51-60	3	30	1	50
Total	10	100	2	100

In the present study, maximum number of patients with pemphigus vulgaris were in age of 41-50 years group (40%)

Age distribution in bullous pemphigoid

TABLE 14: Age distribution in bullous pemphigoid

Age (years)	PV	Percentage
<10	1	14
11-60	1	14
61-70	2	29
71-80	2	29
81-90	1	14
Total	7	100

In the present study, maximum number of patients with bullous pemphigoid belonged to both 61-70 and 71-70 age group. The youngest patient was 4 months old.

Duration of illness in autoimmune vesiculobullous disorder

TABLE 15: Duration of illness in autoimmune vesiculobullous disorder

Duration in months	PV	%	PF	%	BP	%	CBDC	%
<1	2	20	0	0	1	14	0	0
1-6	7	70	1	50	3	43	1	100
7-12	0	0	1	50	1	14	0	0
12-24	1	10	0	0	0	0	0	0
>24	0	0	0	0	2	29	0	0

In the present study, most cases of pemphigus vulgaris (70%) and bullous pemphigoid (43%) had illness for 1-6 months before presentation. Two patients of pemphigus foliaceus had illness for 1-12 months and the patient of CBDC had illness for 4 months before presentation.

CLINICAL FEATURES OF VESICULOBULLOUS DISORDERS

TABLE 16: Symptoms and morphology of lesions at the time of presentation

Clinical features	PV	%	PF	%	BP	%	CBDC	%
Symptoms								
Itching	0	0	0	0	6	85	1	100
Pain	10	100	1	50	4	57	0	0
Lesions the time of presentation								
Vesicles	2	20	0	0	5	71	1	100
Bullae	7	70	0	0	5	71	0	0
Erosions	9	90	2	100	5	71	1	100
Crusting	4	40	2	100	4	54	1	100
Red raised lesions	0	0	0	0	7	100	1	100
Oral lesions	8	80	0	0	0	0	0	0
Genital lesions	2	20	0	0	0	0	0	0
Hyperpigmentation	8	80	2	100	7	100	1	100
Hypopigmentation	0	0	0	0	0	0	0	0

TABLE 17: Consistency of blisters in autoimmune vesiculobullous disorders

Consistency of blisters	PV	%	PF	%	BP	%	CBDC	%
Flaccid	7	70	0	0	0	0	0	0
Tense	0	0	0	0	5	71	1	100

Distribution of lesions

Table 18: Distribution of lesions in autoimmune vesiculobullous disorders

Sites	Pemphigus vulgaris	% of total	Pemphigus foliaceus	% of total	Bullous pemphigoid	% of total	CBDC	% of total
Scalp	3	30	2	100	0	0	0	0
Face	3	30	0	0	0	0	0	0
Neck	6	60	0	0	0	0	0	0
Chest	6	60	1	50	2	29	1	100
Upper back	7	70	0	0	0	0	0	0
Lower back	6	60	0	0	0	0	1	100
Axillae	5	50	1	50	0	0	1	100
Upper limbs	4	40	0	0	5	71	1	100
Hands	0	0	0	0	2	29	0	0
Palms	0	0	0	0	0	0	0	0
Abdomen	4	40	0	0	4	57	1	100
Buttocks	4	40	0	0	0	0	1	100
Genitals	2	20	0	0	0	0	0	0
Perineum	2	20	0	0	0	0	1	100
Lower limbs	2	20	0	0	3	43	1	100
Feet	0	0	0	0	1	14	1	100
Soles	0	0	0	0	0	0	0	0

Signs

Table 19: Signs in autoimmune vesiculobullous disorders.

Positive signs	Pemphigus vulgaris	% of total	Pemphigus foliaceus	% of total	Bullous pemphigoid	% of total	CBDC	% of total
Indirect Nikolsky's sign	9	90	1	0	0	0	0	0
Direct Nikolsky's sign	3	30	0	0	0	0	0	0
Bulla spread sign	0	0	0	0	5	71	0	0

In the present study, pain (100%) was the most common symptom in pemphigus vulgaris whereas itching (85%) was the most common symptom in bullous pemphigoid and CBDC.

Pemphigus vulgaris patients presented with fluid filled lesions (70%) which were flaccid in nature , erosions and crusting (90%) which healed with hyperpigmentation. Lesions were predominantly found over upper back (70%), neck, lower back and chest (60% each). Oral lesions were present in 80% of pemphigus vulgaris patients at the time of presentation. Indirect Nikolsky's sign and direct Nikolsky's sign was positive in 90% and 30% patients respectively.

Pemphigus foliaceus presented with crust predominantly over the scalp which resolved with hyperpigmentation (100%). Oral lesions were completely absent in pemphigus foliaceus patients and indirect nikolsky's sign was positive in only 1 case.

Bullous pemphigoid patients presented commonly with red raised lesions (100%) but fluid filled lesions which were tense in nature developed over the red plaques in only 71% cases which progressed to erosions and crusting which healed

with hyperpigmentation. Bulla spread sign was positive in all the patients who developed vesiculobullous lesions. Oral lesions were not found in any patient.

The chronic bullous disease of childhood patient also presented with red raised lesions followed by tense fluid filled lesions, erosions and crusting over upper limbs, axillae, chest, external surface of genitals, perineum, buttocks, lower back and lower limbs with positive bulla spread sign. Lesions healed with hypo- and hyperpigmentation. Mucosal lesions were absent in the patient.

Table 20: Findings in histopathological examination

Findings	PV	% of total	PF	% of total	BP	% of total	CBDC	% of total
EPIDERMAL CHANGES								
Subcorneal cleft	0	0	1	50	0	0	0	0
Suprabasal cleft	9	90	0	0	0	0	0	0
Row of tombstone	7	70	0	0	0	0	0	0
Acantholytic cells	9	90	0	0	0	0	0	0
Spongiosis	0	0	0	0	4	42	0	0
lymphocytes	0	0	0	0	0	0	0	0
Neutrophils	4	40	0	0	4	57	0	0
Eosinophils	0	0	0	0	3	42	0	0
DERMAL CHANGES								
Subepidermal cleft	0	0	0	0	4	57	1	100
Papillary oedema	0	0	0	0	2	29	1	100
Papillary microabscess	0	0	0	0	2	29	1	100
Neutrophilic infiltrate	0	0	0	0	4	57	1	100
Eosinophilic infiltrate	0	0	0	0	3	42	0	0

In present study, histopathological examination of lesional skin biopsy specimens suspected clinically with pemphigus vulgaris showed predominantly suprabasal cleft (90%) with acatholytic cells in 90% and lymphocytes in 40% of cases inside the blister cavity and row of tombstone appearance at the base of blister in 90% of cases. And in pemphigus foliaceus, histopathology showed subcorneal cleft in 100% cases and acantholytic cells in 50% of cases.

Bullous pemphigoid showed predominantly subepidermal cleft (71%). Dermal neutrophilic infiltrate (57%) and eosinophilic infiltrate (42%) as well as epidermal neutrophilic spongiosis (42%) was the next common finding.

CBDC specimen supepidermal cleft, papillary oedema and papillary neutrophilic microabscesses and dermal neutrophilic infiltrate was present

Direct immunofluorescence

Table 21: Findings of direct immunofluorescence in autoimmune vesiculobullous disorders

Number	Findings		PV (n=10)	PF (n=2)	BP (n=7)	CBDC (n=1)
1	IgG deposition	Intercellular	10	2	0	0
		BMZ	0	0	7	0
2	IgA deposition	Intercellular	0	0	0	0
		BMZ	0	0	0	1
3	IgM deposition	Intercellular	0	0	0	0
		BMZ	0	0	0	0
4	C3	Intercellular	10	2	7	0
		BMZ	0	0	0	0
5	Fibrinogen	Intercellular	0	0	0	0
		BMZ	0	0	0	0

On direct immunofluorescence examination, intercellular deposition of IgG and C3 was present in all cases of pemphigus vulgaris and pemphigus foliaceus which is a classical finding.

All cases of bullous pemphigoid cases showed linear deposition of IgG and C3 along the basement membrane zone

The patient with CBDC showed linear deposition of IgA along the basement membrane zone.

DISCUSSION

Types of autoimmune vesiculobullous disorders

Table 22: Analysis of types of autoimmune vesiculobullous disorders

No	Authors of study	Year of publication	Pemphigus vulgaris	Pemphigus Foliaceus	Bullous pemphigoid	CBDC	Dermatitis herpetiformis
1	Deepti et. al.[76]	2013	17/50 (34%)	4/50 (8%)	13/50 (26%)		2/50 (4%)
2	Ahmed et. al[78]	2014	24/59 (40%)	6/59 (10%)	10/59 (16.9%)	2/59 (3.45%)	1/59 (1.67%)
3	Mittal et. al.[77]	2017	53/110 (48.2%)	4/110 (2.7%)	30/110 (27.3%)	1 (0.9%)	9/110 (4.55%)
4	Archana et. al.[79]	2018	58/100 (58%)	12/100 (12%)	25/100 (25%)	-	2/100 (2%)
5	Chanabasayya et. al.[80]	2018	17/91 (18.68%)	10/91 (10.98%)	42/91 (46.15%)	-	5/91 (5%)
6	Present study	2018	10/20 (50%)	2/20 (10%)	7/20 (35%)	1/20 (5%)	0

In the present study, pemphigus vulgaris was the most common vesiculobullous disorder constituting 50% (10 out of 20 cases) followed by bullous pemphigoid constituting 35% (7 out of 20 cases) and pemphigus foliaceus constituting 10% of the cases. The pattern of frequency of autoimmune vesiculobullous disorders closely matches the study done by Mittal et al[77]. and Ahmed et. al[78]. Chronic bullous disease of childhood was 5% in our study which correlates with study done by Ahmed et. al[78]. Cases of dermatitis herpetiformis were not found in the present study unlike the study done by Chanabasayya et. al[80] and Mittal et. al[77] had dermatitis herpetiformis where it had an incidence of 5%.

Age and sex distribution of autoimmune vesiculobullous disorders

Table 23: Mean age and sex distribution of vesiculobullous disorders in different studies.

No	Studies	Year	Pemphigus vulgaris		Pemphigus foliaceus		Bullous pemphigoid		CBDC	
			Age	M:F	Age	M:F	Age	M:F	Age	M:F
1	Deepti et. al. [76]	2013	37.1	1:24	1:3	3:1	72.4	1:1.16	-	-
2	Mittal et. al.[77]	2017	42.04	1:1.6	46.25	3:1	60.17	1:1.1	5.00	1:0
3	Present study	2018	45.1	1:2.3	45	2:0	57	1.3:1	9	1:0

The mean age of patients of pemphigus vulgaris was 45.1 years with a greater female preponderance which was similar to the results of study conducted by Mittal et. al[77].

Pemphigus foliaceus had a mean age of 45 with a male preponderance similar to study done by Mittal et. al.

Mean age of patients of bullous pemphigoid was 57 years with a higher incidence in males in present study.

The mean age in chronic bullous disease of childhood group was 9 years in the single male patient.

PEMPHIGUS VULGARIS

Table 24: Comparison of incidence, age distribution and clinical features in pemphigus vulgaris.

No	Features	Present study	Mittal et. al.[77]	Deepti et. al.[76]
1	Year	2018	2017	2013
2	No of cases	10/20	53/110	17/50
3	Age of distribution	21-60	20-49	12-60
5	Cutaneous flaccid vesiculobullous lesions	90%	77.3%	94.2%
6	Mucous membrane involvement	80%	22.6%	88.2%
7	Positive Nikolsky's sign	90%	49.1%	88.2%

In the present study, pemphigus vulgaris constituted 50% of the total cases of autoimmune vesiculobullous disorders which is similar to the study conducted by Mittal et. al.[77]

The ages in which pemphigus vulgaris was 21-60 years and is consistent with similar Indian study done by Mittal et. al. and Deepti et. al.[76]

All the patients had classical features of pemphigus vulgaris as per literature with cutaneous flaccid vesiculobullous lesions, mucosal erosions and positive Nikolsky's sign confirmed by similar study done by Deepti et. al.[76]

Table 25: Comparison of histopathological findings in pemphigus vulgaris

No	Histopathological features	Present study	Mittal et. al.[77]	Deepti et. al. [76]
1	Suprabasal cleft	90%	56.6%	88.2%
1	Acantholytic cells	90%	84.9%	94%
3	Row of tombstone appearance	70%	45.3%	70.5%
4	Inflammatory cells in cleft	40%	86.7%	94%

The main histological features observed in cases of pemphigus vulgaris was acantholysis and suprabasal cleft which was seen in 90% of cases followed by row of tombstone appearance in 70% of cases. These histopathological features were first described by Lever and confirmed in study done by Mittal et al.[77] and Deepti et. al.[76]

Table 26: Comparison of direct immunofluorescence positivity in pemphigus vulgaris

no	Study	DIF done	DIF positive
1	Present study	10/10	100%
2	Mittal et. al.[77]	37/53	94.2%
3	Deepti et. al.[76]	17/17	94.11%

Direct immunofluorescence was positive in all the patients clinically diagnosed with pemphigus vulgaris showing staining of intercellular spaces with IgG and C3 in a fish net like pattern consistent with studies done by Mittal et. al.[77]

PEMPHIGUS FOLIACEUS

Table 27: Incidence, age distribution and clinical features in pemphigus foliaceus

No	Features	Present study	Mittal et. al.[77]	Deepti et. al.[76]
1	Year	2018	2017	2013
2	No of cases	2/20	4/110	4/50
3	Age of distribution	31-60	30-65	10-39
5	Crusts	100%	100%	100%
6	Mucous membrane involvement	0	0	25%
7	Nikolsky's sign	50%	100%	75%

The disease was seen in 2 of the 20 cases with incidence of 10%. This correlates closely with study done by Deepti et. al.[76]

The patients presented predominantly with crusts with Nikolsky's sign positive in one case

Mucous membrane involvement was absent in present study.

Table 28: Comparison of histopathological findings in pemphigus foliaceus

No	Histopathological features	Present study	Mittal et. al.[77]	Deepti et. al. [76]
1	Subcorneal cleft	50%	100%	100%
2	Acantholytic cells	50%	100%	100%
4	Inflammatory cells in cleft	0	100%	75%

The salient histopathological features suggestive of pemphigus foliaceus who were clinically diagnosed as pemphigus foliaceus were seen in 1 of the 2 patients in present study and was subcorneal cleft containing acantholytic cells, consistent with literature and confirmed by the study done by Deepti et. al.[76] and Mittal et. al.[77]

Table 29: Comparison of direct immunofluorescence positivity in pemphigus foliaceus

no	Study	DIF done	DIF positive
1	Deepti et. al.[76]	4/4	100%
2	Mittal et. al.[77]	3/4	100%
3	Present study	2/2	100%

Direct immunofluorescence was positive in all the patients clinically diagnosed with pemphigus foliaceus showing staining of intercellular spaces with IgG and C3 in a fish net like pattern consistent with studies done by Deepti et. al.[76] and Mittal et. al.[77]

BULLOUS PEMPHIGOID

Table 30: Incidence, age distribution and clinical features in bullous pemphigoid

No	Features	Present study	Deepti et. al.[76]	Mittal et. al.[77]
1	Year	2018	2013	2017
2	No of cases	7/20	13/50	30/110
3	Age of distribution	4 mths- 90 yrs	60-80 yrs	19-88 yrs
5	Erythematous plaques	7/7 (100%)	9/13 (69.3%)	25/30 (84%)
6	Cutaneous tense vesiculobullous lesions	5/7 (71%)	6/13 (35.3%)	22/30 (73%)
7	Mucous membrane involvement	0	3/13 (3%)	3/30 (10%)
8	Bulla spread sign	5/7 (100%)	4/13 (30%)	24/30 (80%)

Most of the cases were seen predominantly in age group of 60-80 years similar to studies done by Deepti et. al.[76]

Clinically patients presented predominantly with erythematous plaques in 100% cases followed by tense vesiculobullous lesions in 71% cases similar to study done by Mittal et. al.[77]

Mucous membrane involvement was absent in present study.

Table 31: Comparison of histopathological findings in bullous pemphigoid

No	Histopathological features	Present study	Deepti et. al. [76]	Mittal et. al.[77]
1	Subepidermal cleft	57%	92.3%	100%
2	Inflammatory cells in cleft	57%	92.3%	63.3%
3	Epidermal spongiosis	20%	-	42%
4	Dermal inflammatory infiltrate	57%	84.7%	63.4%

Compared to study done by Mittal et. al.[77] the salient histopathological features of bullous pemphigoid were seen in 4 of the 7 patients whereas 3 specimens were not suggestive of bullous pemphigoid.

Table 32: Comparison of direct immunofluorescence positivity in bullous pemphigoid

no	Study	DIF done	DIF positive
1	Deepti et. al.	12/13	100%
1	Mittal et. al.	30/30	92%
2	Present study	7/7	100%

Direct immunofluorescence was positive in all the patients clinically diagnosed with bullous pemphigoid showing staining of basement membrane zone with IgG and C3 in a linear pattern consistent with study done by Mittal et. al.[77] and Deepti et. al.[76]

CHRONIC BULLOUS DISEASE OF CHILDHOOD

In present study the patient diagnosed as CBDC was 9 month male clinically presenting predominantly with tense vesicles in an annular 'string of pearls' pattern progressing to erosions and crusts and healing with hyperpigmentation. Bulla spread sign was positive.

Histopathologically the specimen showed normal epidermis, supepidermal cleft, papillary neutrophilic microabscesses dermal oedema with neutrophilic infiltration.

Direct immunofluorescence showed deposition of IgA and C3 along the basement membrane in a linear pattern.

These findings were consistant with study done by Mittal et. al.[77]

Table 33: Clinical- histopathological- direct immunofluorescence finding correlation in autoimmune vesiculobullous diseases.

Disease spectrum	Clinical diagnosis	Histopathological diagnosis	Direct immunofluorescence	Sensitivity of Histopathology	Sensitivity of direct immunofluorescence
PV	10	9	10	100%	100%
PF	2	1	2	66.67%	100%
BP	7	4	7	70%	100%
CBDC	1	1	1	100%	100%
Total	20	16	20	80%	100%

The clinical findings in the pemphigus vulgaris group and CBDC group were concordant with histopathological and immunofluorescence findings. However discordant histopathological findings compared with clinical and immunofluorescence findings were seen in 3 of the 7 cases of bullous pemphigoid and 1 of the 2 cases of pemphigus foliaceus.

Table 34: Comparision of sensitivity of histopathology and DIF

Study	HPE sensitivity	DIF sensitivity
Mittal et. al.[77]	86.4%	94.5%
Present study	80%	100%

In the present study, histopathological examination had a 80% sensitivity and direct immunofluorescence a 100% sensitivity making DIF the gold standard for diagnosis of autoimmune vesiculobullous disorders.

CONCLUSION

The autoimmune vesiculobullous disorders are a group of blistering disorders characterized by pathogenic auto-antibodies 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.

They are broadly classified as intraepidermal and subepidermal blistering disorders with pemphigus and pemphigoid as their prototype diseases respectively.

In present study, pemphigus vulgaris constituted the most common disorder followed by bullous pemphigoid.

Patients suspected clinically to be suffering from this disorder are advised to undergo histopathological and direct immunofluorescence tests to confirm their diagnosis.

Direct immunofluorescence is the most sensitive test and has proven to be the gold standard in diagnosis of autoimmune vesiculobullous disorders.

Hence, for confirmation of the diagnosis of autoimmune vesiculobullous disorders, direct immunofluorescence should be done in addition to histopathology.

SUMMARY

The current study is a one-year cross-sectional study of 20 patients who attended the OPD of Dermatology, venereology and leprosy in KLE Dr. Prabhakar Kore hospital and medical research center, Belagavi from Jan 2017 to Dec 2017.

The objective of the study was to observe the various histopathological and immunofluorescence findings in patients with vesiculobullous disorders and to correlate the clinical and pathological findings with DIF findings.

Out of 20 cases of vesiculobullous disorders, pemphigus vulgaris constituted the most common form with 50% followed by bullous pemphigoid 35%. Most of the vesiculobullous lesions were in the age group of 40-49 yrs.

PV was common in age group of 41-50 years with a female preponderance. Patients with PV and PF presented predominantly by painful lesions. Morphologically the lesions of PV were flaccid bullous lesions, erosions, crust and hyperpigmentation whereas PF predominantly showed crusts. Oral mucosal surfaces were commoly involved in PV and never in PF. Nikolsky's sign was positive in majority of cases and bulla spread sign was negative.

BP was commonly seen in >70 year patients with a male predominance. Patients presented with itchy erythematous lesions and tense vesiculobullous lesions having positive bulla spread sign. Erosions and crusting was also seen with negative Nikolsky's sign. Oral mucosal lesions were never seen.

Histopathology showed suprabasal cleft in all of the PV specimens and subcorneal cleft in PF with acantholytic cells in the cleft in only 1 of the 2 cases. DIF

showed staining of intercellular spaces in a fish net pattern with IgG and C3. Histopathology showed a subepidermal cleft in and DIF showed deposition of IgG and C3 along the basement membrane in all of the bullous pemphigoid cases and CBDC case.

Subepidermal cleft was seen in 4 of the 7 cases but DIF showed deposition of IgG and C3 in all cases suspected clinically with BP and with IgA in the case of CBDC.

1 of the 2 cases of PF and 3 of the 7 cases of BP discordance of histopathological findings with DIF.

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ANNEXURE I: CONSENT FORM

CONSENT TO PARTICIPATE IN RESEARCH STUDY

"I voluntarily agree that I am to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicated that I have read this entire consent form or it has been read to me, and had all my questions answered. I will be given a copy of this consent form"

Signature of the Participant or legally authorized representative:

Participant Name: ….....

Signature/Left Thumb Impression: ….....

Name of legally authorized representative: .....

Signature/Left Thumb Impression: ….....

Witness Name: ….....

Signature/Left Thumb Impression: ….....

Investigators Name: ….....

Investigator Signature: ….....

Date: ..... Place: .....

QUESTION:

If any enquiries in the future or in case of study related injury or illness, you may contact following person:

If you still have any queries please contact:

Dr. GANGA PILLI MD (PATHOLOGY)

Chairperson,

Institutional Ethics Committee for Human Subjects Research,

Jawaharlal Nehru Medical College, Belagavi – 590010.

Phone no.- 0831-2473777

Mobile no.- 9448863866

ANNEXURE II: PROFORMA FOR HISTORY TAKING

Title of topic- "One year cross-sectional study of direct immunofluorescence in the diagnosis of immunobullous disorders"

Examiner: Dr.

Case number_____ OPD/IPD number_____

Date_____

Name_____

Age_____ Sex- Male/ Female

Occupation_____

Marital status- Unmarried/ Married/ Widowed

Address_____

Email Address_____

Chief complaints-

History of presenting illness

1. Onset of symptoms:
2. Painful lesions in mouth: yes/no
3. Blisters over the body: Present/ absent
4. Blisters are: flaccid/ tense

5. Blisters are filled with: clear fluid/ turbid fluid/ hemorrhagic fluid/ purulent fluid
6. Sites of blisters: Scalp/ face/ neck/ chest/ upper back/ lower back/ axillae/ upper limbs/ hands/ palms/ abdomen/ buttocks/ genitals/ perineum/ lower limbs/ feet/ soles
7. Itching prior to blister formation: Present/ absent
8. Urticarial papules or plaques: Present/ absent
9. Erosions form: crusts/ vegetation/ scales
10. Heal with hyperpigmentation: yes/ no
11. Heal with hypopigmentation: yes/ no
12. Heal with scarring: yes/ no

Past history:

1. Fever: yes/ no
2. Malaise: yes/ no
3. Trauma: yes/ no
4. Joint pain: yes/ no
5. Dirrhoea: yes/ no
6. Topical application yes/ no of _____
7. Drug intake: yes/ no of _____
8. Topical application: yes/ no
9. Insect bite: yes/ no
10. Painful grouped vesicles: yes/ no

Aggravating factors:

1. Sun exposure: yes/ no
2. Food containing: wheat/ rye/ barley: yes/ no
3. Salt/ fish: yes/ no
4. Garlic: yes/ no
5. Banana: yes/ no
6. Tomato: yes/ no

Personal history:

1. Known case of: DM/HTN/TB/Bronchial asthama/IHD
2. Drug allergy: yes/ no of_____
3. Alcoholism: yes/ no consumption of _____ ML every _____
4. Smoking: yes/ no _____ cigarette/ bidi per day
5. Tobacco chewing: yes/ no
6. Appetite: Normal/ increased/ decrease
7. Nourishment: well/ poorly
8. Built: Moderately/ poorly

Menstrual history:

Cycles of _____Days/ Amenorrhea since _____ with LMP on _____

_____ days of menstruation

Obstetric history

1. G_P_L_A_
2. Family complete: yes/ no

3. Contraceptive used: yes/ no
4. Tubal ligation done: yes/ no

General examination

1. Pulse: _____/min
2. BP: _____/_____mmHg
3. Temperature: _____C/F
4. Weight: _____KG
5. Pallor: Present/ absent
6. Icterus: Present/ absent
7. Cyanosis: Present/ absent
8. Clubbing: Present/ absent
9. Oedema: Present/ absent

Lymphadenopathy: Present/ absent

Cutaneous examination:

1. Distribution of lesions:

- Symmetrically/ asymmetrically distributed
- Over Scalp/ face/ neck/ chest/ upper back/ lower back/ axillae/ upper limbs/ hands/ palms/ abdomen/ buttocks/ genitals/ perineum/ lower limbs/ feet/ soles
- BSA involvement: ___%
- Morphology:
- Number of lesions: Solitary/ few/ multiple
- Type of blister: Tense/ flaccid
- Content of blister: clear fluid/ turbid fluid/ hemorrhagic fluid/ purulent fluid
- Size: Smallest: ___CM Largest: ___CM

- Skin over base of lesions: Normal/ erythematous
- Present over- Scalp/ face/ neck/ chest/ upper back/ lower back/ axillae/ upper limbs/ hands/ palms/ abdomen/ buttocks/ genitals/ perineum/ lower limbs/ feet/ soles
- Wheals: Present/ Absent

2. Raw areas:

- Number of lesions: Solitary/ few/ multiple
- Type of lesions: Erosion/ ulcer
- Crust: Present/ absent
- Scales: Present/ absent
- Odor: Nil/ foul/ musty/ mousy
- Size: Smallest: ____CM Largest: _____CM
- Present over: Scalp/ face/ neck/ chest/ upper back/ lower back/ axillae/ upper limbs/ hands/ palms/ abdomen/ buttocks/ genitals/ perineum/ lower limbs/ feet/ soles

3. Healed lesions: Present/ absent

- Hyperpigmentation: present/ absent
- Hypopigmentation: Present/ absent
- Scarring: Present/ absent
- Milia: Present/ absent

4. Signs:

- Direct Nikolsky's sign: Present/ absent
- Indirect Nikolsky's sign: Present/ absent
- Bulla spread sign: Present/ absent

5. Oral cavity

- Erosions: Present/ absent
- Involvement: Tongue/ buccal mucosa/ palate
- Oral hygiene: Good/ poor

6. Genitals

- Erosions: Present/ absent
- Scarring: Present/ absent

7. Eyes:

- Erosions: Present/ absent
- Scarring: Present/ absent

8. Nails

- Paronychia: Present/ absent, involving finger nails/ toe nails
- Scarring: Present/ absent, involving finger nails/ toe nails
- Onychomadesis: Present/ absent, involving finger nails/ toe nails
- Dystrophy: Present/ absent, involving finger nails/ toe nails

9. Hair: Normal/ abnormal

Investigations

CBC		LFT		RFT	
HB TLC DLC- N -L -E -M -B RCC Retic count Platelet count Blood group		Total bilirubin Direct bilirubin A/G ratio Total protein SGOT SGPT ALP		Urea Creatinine HIV	

Histopathology:

Direct immunofluorescence:

Provisional diagnosis:-

Final diagnosis:-

Signature of examiner

_____

Signature of guide

_____

ANNEXURE I: PHOTOGRAPHS

FIG 4: Flaccid bulla and erosion in a patient with pemphigus vulgaris

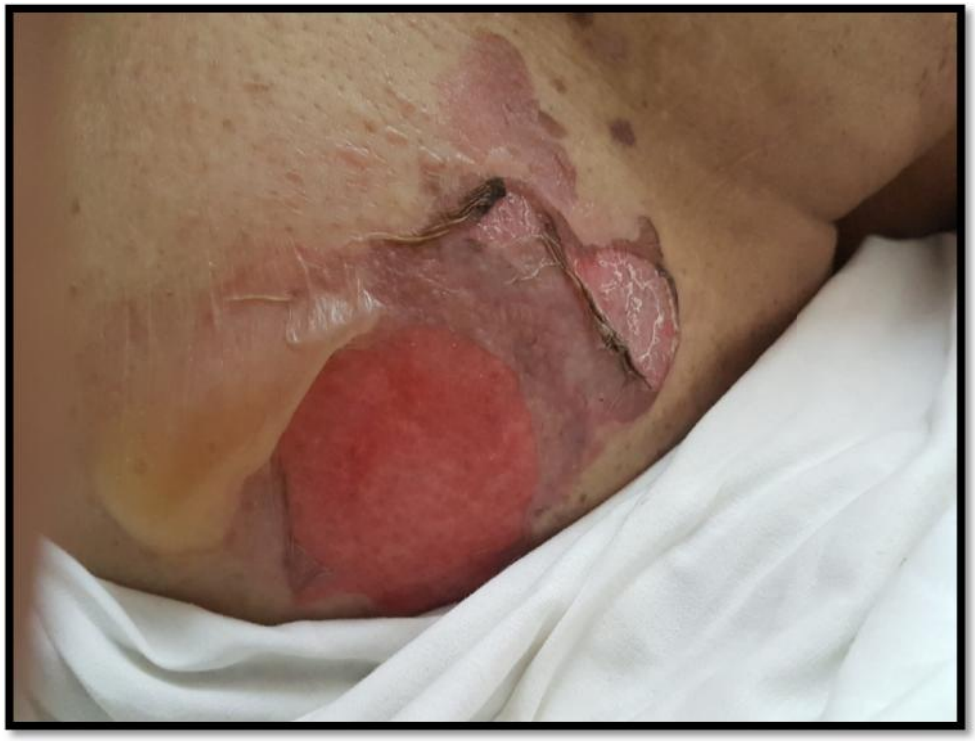


FIG 5: Erosions in a patient with pemphigus vulgaris

FIG 6: Histopathology of pemphigus vulgaris showing suprabasal cleft, acantholytic cells and row of tombstone appearance of basal layer

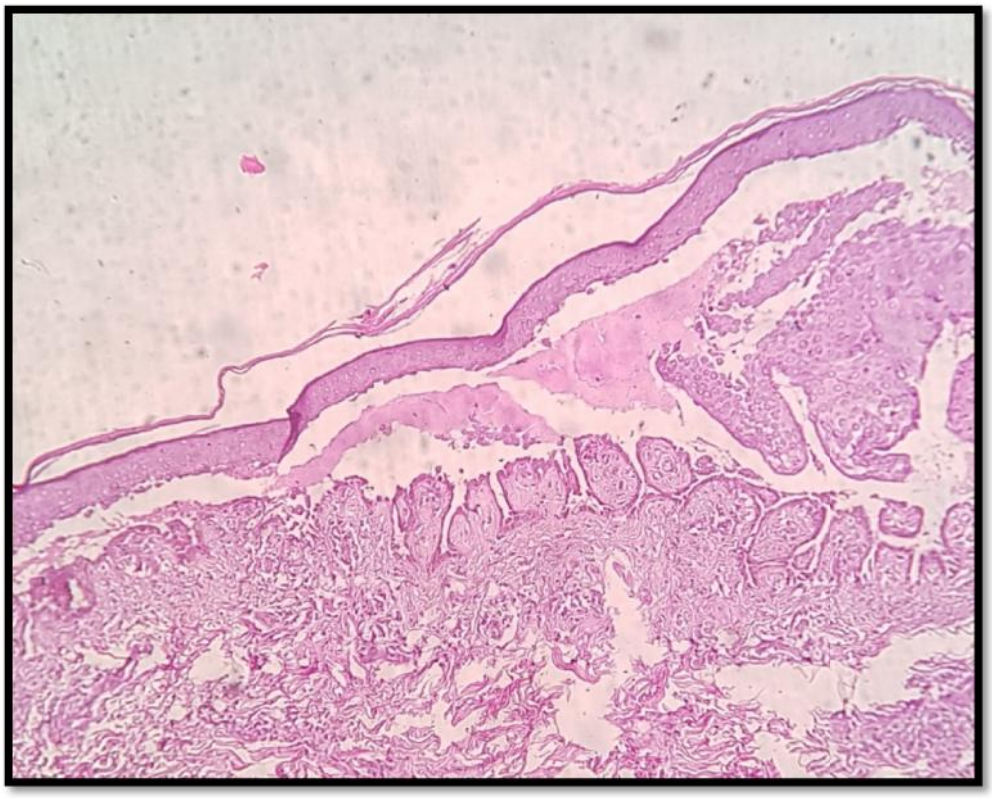


FIG 7: Direct immunofluorescence of pemphigus vulgaris showing deposition of IgG and C3 in intercellular spaces giving a fish net appearance

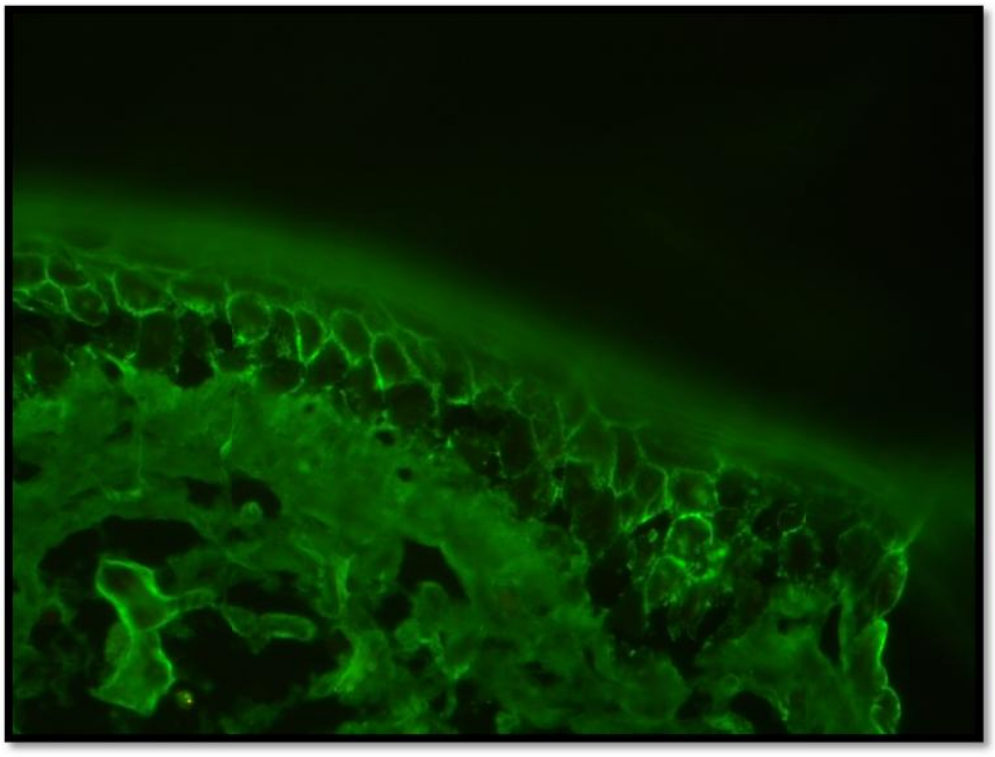


FIG 8: Tense bullae over normal as well as an erythematous base in a patient with bullous pemphigoid

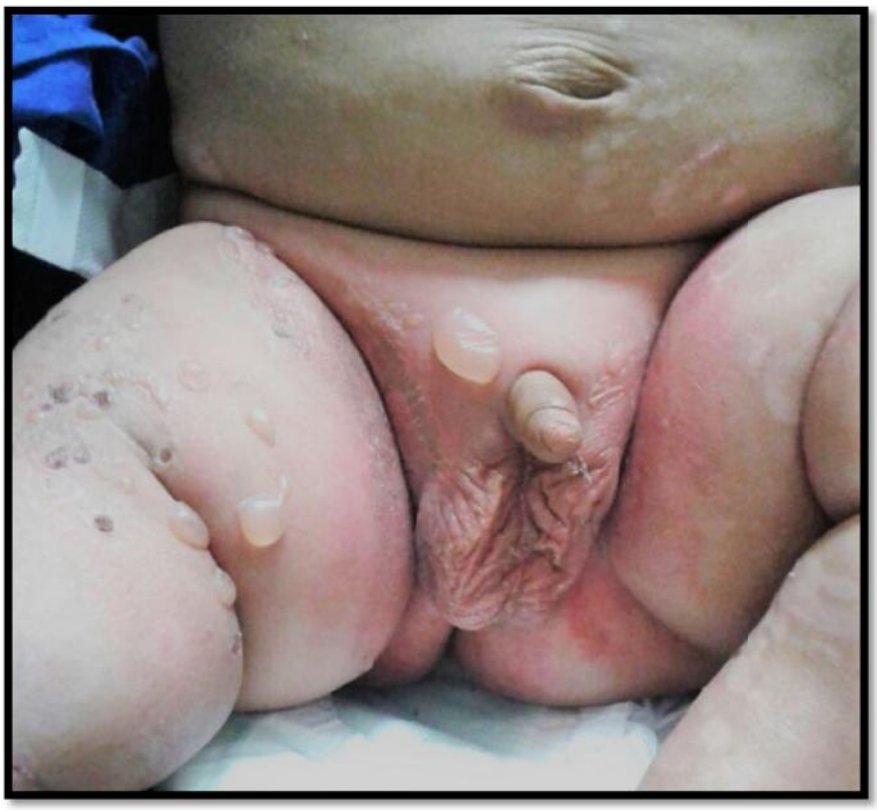


FIG 9: Histopathology of bullous pemphigoid showing sub-epidermal cleft.

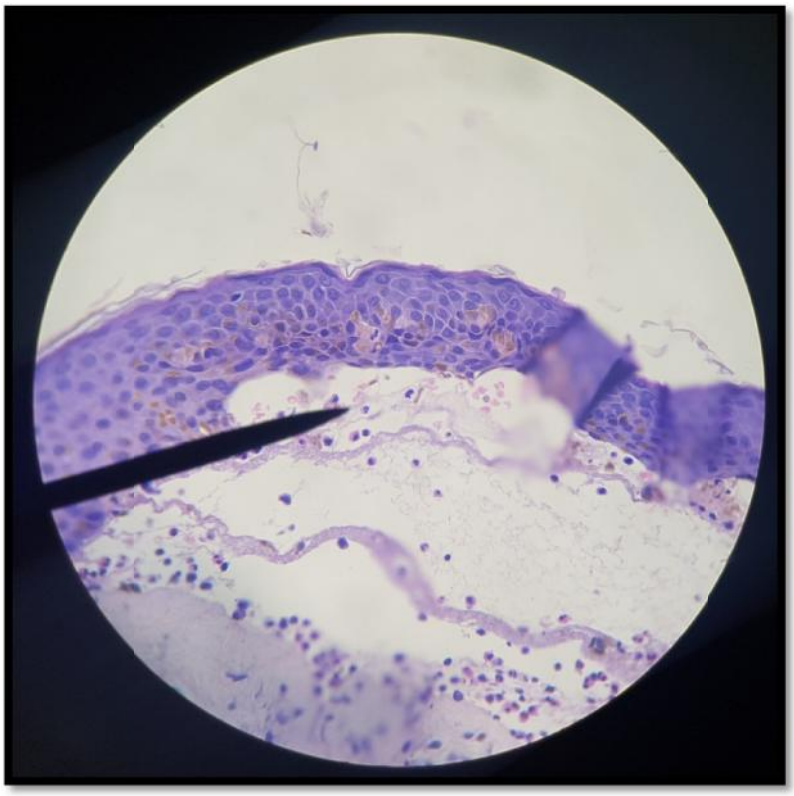


FIG 10: Direct immunofluorescence of bullous pemphigoid showing deposition of IgG and C3 along the basement membrane

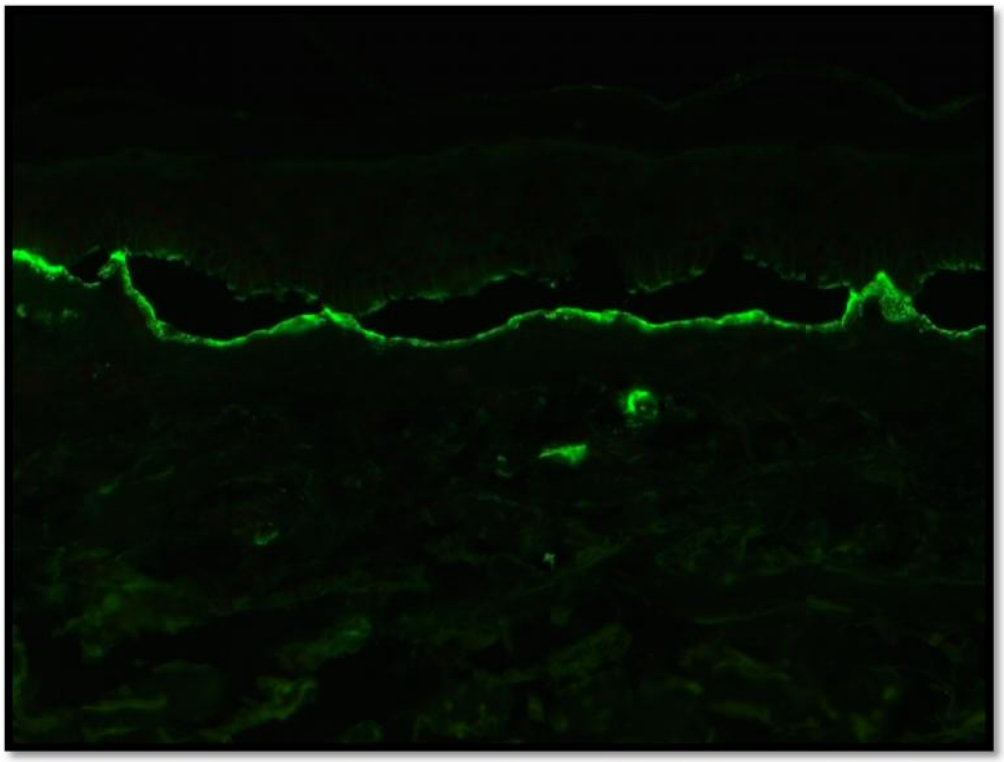


FIG 11: Tense vesicles in a patient with chronic bullous disease of childhood

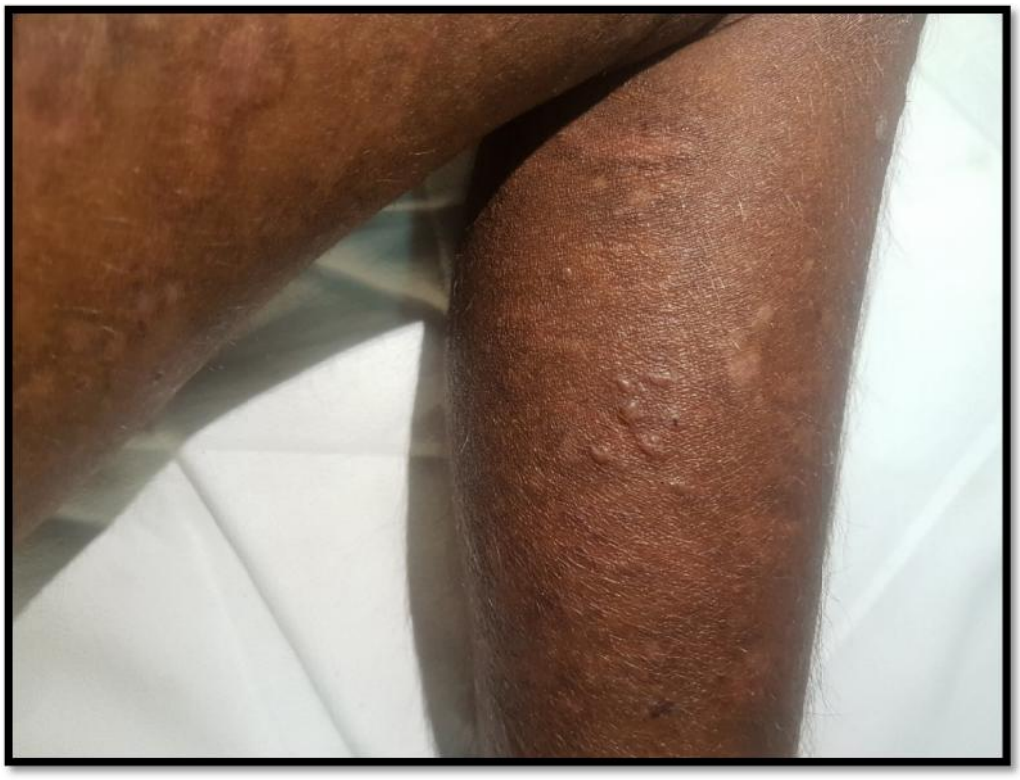


FIG 12: Erosions, crusts and hyperpigmentation in a patient with chronic bullous disease of childhood

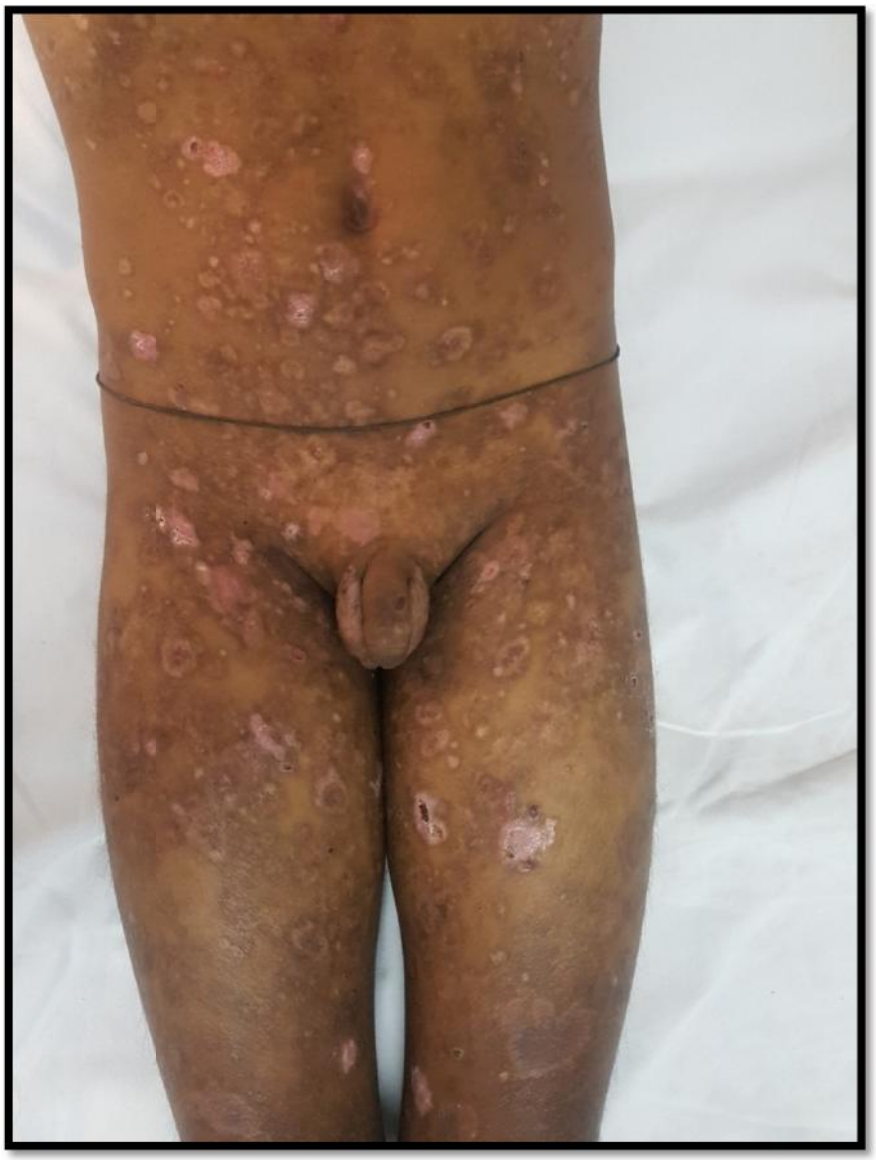


FIG 13: Histopathology of chronic bullous disease of childhood showing sub-epidermal cleft.

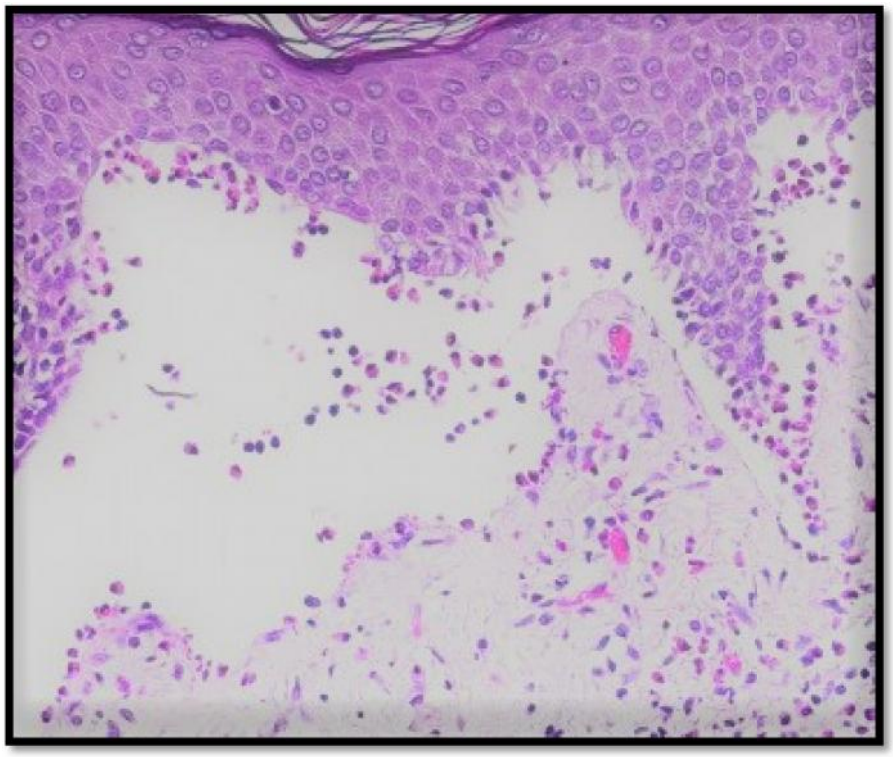
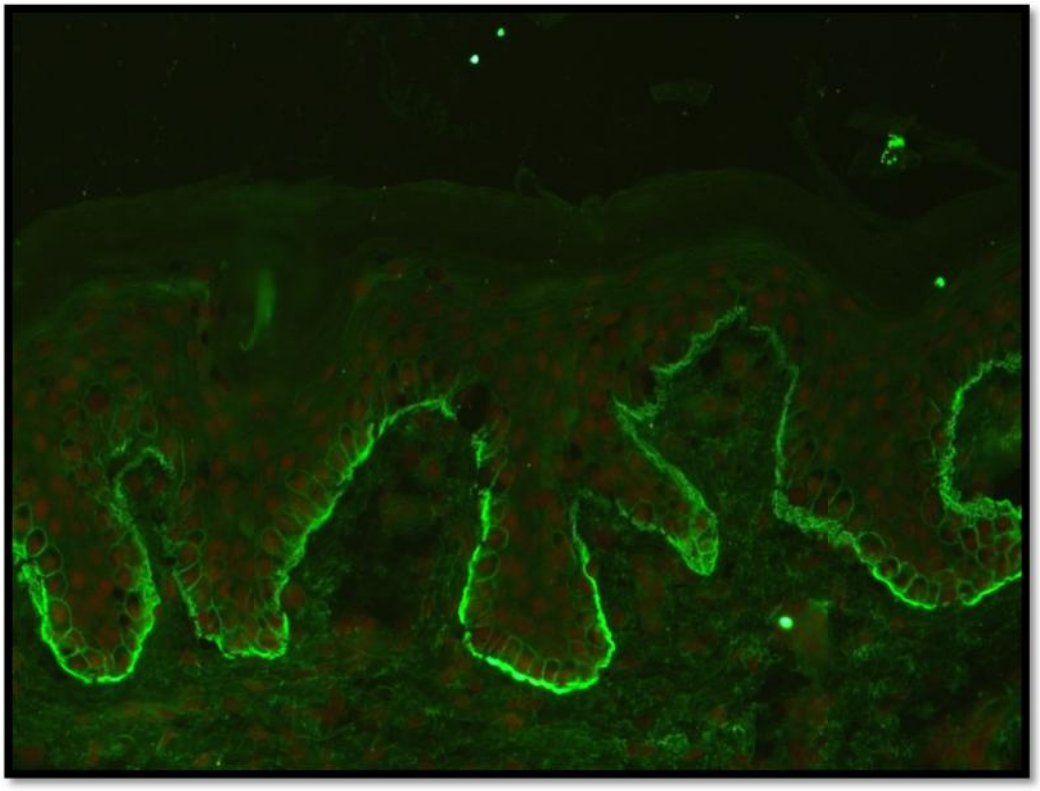


FIG 14: Direct immunofluorescence of pemphigus vulgaris showing deposition of IgA and C3 along the basement membrane.



ANNEXURE IV: KEY TO MASTER CHART

HPE	-	Histopathological examination
DIF	-	Direct immunofluorescence
PV	-	Pemphigus vulgaris
PF	-	Pemphigisfoliaceus
BP	-	Bullous pemphigoid
CBDC	-	chronic bullous disease of childhood
EBS	-	Epidermolysis bullosa simplex
A	-	Acantholytic cells
N	-	Neutrophils
E	-	Eosinophils

No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Name	Parvati	Ratnavva	Laxmi	Sarojawwa	Surekha	Umesh	Basawwa	Dnyaneshwar	Kasturi	Kasavva	Bambai	Prakash	Gangappa	Bharmappa	Sharifa	Bhimraya	Shourya	Sudha	Suhalata
Sex	F	F	F	F	F	M	F	M	F	F	F	M	M	M	F	M	M	F	F
Age(years)	65	60	48	52	40	60	48	30	45	40	62	25	55	9	70	38	4 mths	71	45
Duration of illness	3 yrs	2 mths	16 nths	3 mths	5 mths	7 mths	5 mth	4 mth	10 mth	25 days	6 mths	3 mths	12 days	4 mths	5 mths	1 mth	20 days	4 mths	1 mth
Clinical diagnosis	?BP ?ABCD ?ICD	?PV ?BP	?OPV ?RAS ?OLP	PV	PV	PF	PV	?PF	BP	PV	BP	PV	PV	CBDC	BP	PV	?BP	BP	PV
Tzanck smear	E	A	not done	A	A	not done	A	not done	N	A	N,E	A	A	N,E	N	A	N	N	A
Positive signs	BS	IN	not done	IN	IN	IN	IN	negative	BS	DN, IN	BS	DN, IN	IN	negative	BS	DN, IN	BS	BS	IN
HPE	dermatitis	PV	PV	PV	PV	PF	PV	PF	BP	PV	BP	PV	PV	CBDC	negative	PV	?BP, ?EBS, ?Viral vesicles	BP	PV
DIF	BP	PV	PV	PV	PV	PF	PV	PF	BP	PV	BP	PV	PV	CBDC	BP	PV	BP	BP	PV

20
Virupakshappa
M
85
3 yrs
BP
not done
not done
negative
BP

SL No	Name	Sex	Age(years)	Duration of illness	Clinical diagnosis	Tzanck smear	Positive signs	HPE	DIF
1	Parvati	F	65	3Yrs	?BP ?ABCD ?ICD	E	BS		BP
2	Ratnavva	F	60	2Mths	?PV ?BP	A	IN	PV	PV
3	Laxmi	F	48	16Mths	?OPV ?RAS ?OLP	Not done	Not done	PV	PV
4	Sarojawwa	F	52	3Mths	PV	A	IN	PV	PV
5	Surekha	F	40	5Mths	PV	A	IN	PV	PV
6	Umesh	M	60	7Mths	PF	Not done	IN	PF	PF
7	Basawwa	F	48	5Mths	PV	A	IN	PV	PV
8	Dnyaneshwar	M	30	4Mths	?PF	Not done	Negative	PF	PF
9	Kasturi	F	45	10Mths	BP	N	BS	BP	BP
10	Kasavva	F	40	25Days	PV	A	DN,IN	PV	PV
11	Bambai	F	62	6Mths	BP	N,E	BS	BP	BP
12	Prakash	M	25	3Mths	PV	A	DN,IN	PV	PV
13	Gangappa	M	55	12Mths	PV	A	IN	PV	PV
14	Bharmappa	M	9	4Mths	CBDC	N,E	Negative	CBDC	CBDC
15	Sharifa	F	70	5Mths	BP	N	BS	Negative	BP
16	Bhimraya	M	38	1Mths	PV	A	DN,IN	PV	PV
17	Shourya	M	4Mths	20Days	?BP	N	BS	?BP, ?EBS, ?Viral vesicles	BP
18	Sudha	F	71	4Mths	BP	N	BS	BP	BP
19	Suhalata	F	45	1Mth	PV	A	IN	PV	PV
20	Virupakshappa	M	85	3Yrs	BP	Not done	Not done	Negative	BP