# "A ONE YEAR CROSS-SECTIONAL CLINICO-HISTOPATHOLOGICAL AND IMMUNOFLUORESCENCE STUDY OF AUTOIMMUNE VESICULOBULLOUS DISORDERS IN PATIENTS ATTENDING K.L.E.S DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"

By

DR. JULIE LEISHANGTHEM

*Dissertation*

**Submitted to the**

**KLE University**

**Belgaum, Karnataka**

**In partial fulfilment**

**of the requirements for the degree of**

**DOCTOR OF MEDICINE (M.D)**

**in**

**DERMATOLOGY, VENEREOLOGY AND LEPROSY**

**Under the Guidance of**

DR. A. M. PANDIT M.D

**DEPARTMENT OF DERMATOLOGY, VENEREOLOGY AND LEPROSY**
**J.N.MEDICAL COLLEGE, NEHRU NAGAR,**
**BELGAUM-590010.**

**MAY 2010**

# KLE UNIVERSITY, BELGAUM

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# ACKNOWLEDGEMENT

*With a deep sense of gratitude and respect, I humbly express my indebtedness to my beloved teacher and guide,* ***Dr A.M. Pandit****, Professor, Department of Dermatology, Venereology and Leprosy, J.N. Medical College, Belgaum for his excellent and able guidance, constant encouragement, advice and continuous supervision during the course of this study.*

*I express my sincere thanks to my co – guide, Dr* ***R.D. Mastiholimath,*** *Professor, Department of Pathology, for his guidance and valuable advice throughout this study.*

*I am grateful and would like to express my heartfelt thanks to my beloved teachers,* ***Dr B. Siddaramappa****, Professor and Head,* ***Dr B.S. Manjunathswamy****, Professor and* ***Dr Shilpa Dastikop****, Associate Professor, Department of Dermatology, Venereology and Leprosy for their guidance, affection and encouragement throughout this study.*

*I take this opportunity to thank* ***Dr P.R. Malur****, Professor and Head, Department of Pathology, for his ready co – operation and priceless help during this study.*

*I am also thankful to the* ***Principal of J.N. Medical College****, Belgaum and* ***the Medical Director of K.L.E.S. Dr Prabhakar Kore Hospital and MRC****, Belgaum for their kind permission to undertake this study.*

*I thank* ***Mr. M.D. Mallapur,*** *Statistician, J.N. Medical College for his valuable suggestions regarding statistical analysis of this study.*

*I sincerely thank all my postgraduate colleagues, who have been a source of constant help and unending co – operation to me.*

*I am grateful to all my patients, whose co – operation was vital for the execution and completion of this study.*

*I thank* ***Mr. Satish*** *of* ***Sheegra****, for his co – operation and courtesy, and for his help in typesetting and printing this dissertation.*

*Above all, I am indebted to* ***my parents*** *and* ***my brother****, who are my pillars of strength, and without whose unconditional love, encouragement and sacrifice, I would not be where I am today. Thank you so much for always believing in me and standing by me.*

Place :

Date :

**DR JULIE LEISHANGTHEM**

# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ARA | – | American Rheumatism Association |
| BM | – | Basement membrane |
| BP | – | Bullous pemphigoid |
| C | – | Complement |
| CBDC | – | Chronic bullous disease of childhood |
| DC | – | Differential count |
| DCP | – | Dexamethasone cyclophosphamide pulse therapy |
| DEJ | – | Dermo-epidermal junction |
| DIF | – | Direct immunofluorescence |
| ELISA | – | Enzyme linked immuno sorbent assay |
| ESR | – | Erythrocyte sedimentation rate |
| Hb | – | Haemoglobin |
| HIV | – | Human immunodeficiency virus |
| HLA | – | Human leucocyte antigen |
| IF | – | Immunofluorescence |
| Ig | – | Immunoglobulin |
| IIF | – | Indirect immunofluorescence |
| KLES | – | Karnataka Lingayat Education Society |
| LLL | – | Left lower limb |
| LUL | – | Left upper limb |
| MRC | – | Medical Research Centre |
| OPD | – | Outpatient Department |
| PF | – | Pemphigus foliaceous |
| PUVA | – | Psoralen + UVA |
| PV | – | Pemphigus vulgaris |
| PVE | – | Pemphigus vegetans |
| RBS | – | Random blood sugar |
| RLL | – | Right lower limb |
| RUL | – | Right upper limb |
| SCPD | – | Subcorneal pustular dermatoses |
| SLE | – | Systemic lupus erythematosus |
| TC | – | Total count |

# ABSTRACT

**Background and objectives**: The aim of the study was to study the various clinical features, histopathological findings and direct immunofluorescence findings in patients with autoimmune vesiculobullous disorders.

**Materials and methods**: The present study was a one-year cross sectional study from November 2007 to December 2008. All new clinically diagnosed cases of autoimmune vesiculobullous disorders were included in the study. The patient's demographic data, age of onset, duration of disease, symptoms, location and types of lesions and associated systemic diseases were noted in a pre- tested and pre-designed proforma. Routine investigations, Tzanck smear (cytology), skin biopsy and direct immunofluorescence were done in all the patients after informed consent and counseling.

**Results**: A total number of 20 cases were studied. The incidence of autoimmune vesiculobullous disorders in our hospital was 0.11%. Pemphigus vulgaris constituted the most common type (55%), followed by bullous pemphigoid (20%). Pemphigus was most common in the $5^{th}$ decade and bullous pemphigoid in the $7^{th}$ decade. The sex ratio of the study population was 1:1. Pemphigus vulgaris and pemphigus foliaceous showed female predominance while bullous pemphigoid and chronic bullous disease of childhood showed male predominance. Trunk was the most common site involved. All pemphigus patients had flaccid blisters, while all patients with bullous pemphigoid and chronic bullous disease of childhood had tense blisters. Oral mucosa was involved in pemphigus vulgaris (90.9%) and bullous pemphigoid (50%). Nikolsky sign, Bulla spread sign and Tzanck smear for acantholytic cells were positive in all pemphigus patients. Histopathology showed suprabasal blisers in all pemphigus vulgaris cases, subcorneal blisters in all pemphigus foliaceous cases and subepidermal blisters in all cases of bullous pemphigoid and chronic bullous disease of childhood. DIF was positive in all the cases.

**Conclusion**: Clinical examination and cytology are helpful in making a provisional diagnosis in autoimmune vesiculobullous disorders. Histopathological examination and direct immunofluorescence are required for making a definitive diagnosis. Direct immunofluorescence is helpful in confirming the diagnosis. It is however not a substitute for histopathology, but rather complementary to it.

**Key words**: Pemphigus vulgaris, pemphigus foliaceous, bullous pemphigoid, chronic bullous disease of childhood

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# INTRODUCTION

Skin is the single largest organ of the body and various diseases along with its manifestations can commonly involve the skin and mucous membranes. Vesiculobullous disorders represent a heterogenous group of dermatoses with protean manifestations.

They have dramatic impact on the patient and their family and have severe economic consequences for the family and health services. The diseases have been the subject of intensive investigation in recent years.[1]

Autoimmune bullous diseases are a significant group of dermatoses that pose great challenges to the treating dermatologist. Most epidemiological studies have focused on a single autoimmune bullous disease. Few surveys have described the whole spectrum of various autoimmune bullous diseases in a region.[2]

The vesiculobullous reaction pattern is characterized by the presence of vesicles or bullae at any level within the epidermis or at the dermoepidermal junction.[3]

Pathologic evaluation of blisters involves systematic analysis, which includes the blister separation plane, the mechanism of blister formation and the character of the inflammatory infiltrate, including its presence or absence.[4]

Recent advances in investigative dermatology have created new horizons. Over the last two decades, great advances have been made in understanding the clinical behaviour and molecular nature of autoimmune diseases.[2]The most important techniques for the investigation of patients with immunobullous disease are conventional histopathology, confirmative tests like direct and indirect

immunofluorescence. Research techniques such as immunoblotting and immunoelectron microscopy may refine the diagnosis in the individual patient but do not replace the clinician.[1]

Corticosteroids remain the mainstay of treatment of autoimmune bullous diseases, though often, fairly large doses of the drug are required to control the disease. The mortality has markedly fallen following the use of corticosteroids. Successful results have been obtained, with dapsone, combination of corticosteroids and immunosuppressive drugs.[3]

Studies including both Western and Indian literature on these diseases have highlighted on particular entity or some specific aspect of it, but a detailed clinical and histopathological study has been attempted by very few people, especially in this part of India.

This study is undertaken to evaluate the clinical features, histopathology and immunofluorescence findings of various autoimmune vesiculobullous disorder of the skin for their diagnostic potential.

# OBJECTIVES

- To study the various clinical features, histopathological findings and direct immunofluorescence findings in patients with autoimmune vesiculobullous disorders at KLES Dr Prabhakar Kore Hospital and MRC, attached to Jawaharlal Nehru Medical College, Belgaum.

# REVIEW OF LITERATURE

## Historical aspects:

The term pemphigus was most likely in use in the ancient world, but the first recorded instance was by Hippocrates (460-370 BC) who described pemphigoid fever as "pemphigoides pyertoi."

Galen (AD 131-201) named a pustular disease of the mouth as "febris pemphigoides."

In 1637, Zacutus again used the term "febris pemphigoides" to describe patients with blisters of short duration.

DeSauvages (1760) described patients with high fever and blisters of short duration as having "pemphigus major."

None of the above conditions is considered to be true pemphigus, as their disease was of short duration and all the patients recovered.

The first recorded case that probably represented true pemphigus was by McBride (1777) and Wichmann (1791). Two of McBride's cases died of "bloody ichor" and "putrid ulcers."

Wichmann applied the term "pemphigus" to his patients and accurately described flaccid bullae and painful oral ulcerations.[6] He described it as "chronic bullous disease". William in 1808 called it "Pompholyx diutinum."[7]

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

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

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

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

Senear and Usher in 1926, described pemphigus erythematosus, combining features of both pemphigus and lupus erythematosus.[10]

Civatte, in 1943, clearly delineated the histopathologic hallmark and labelled it acantholysis. He described acantholysis (loss of cohesion) and intraepidermal bulla formation in pemphigus vulgaris, pemphigus vegetans, and pemphigus foliaceus. These important pathologic findings clearly separated pemphigus from other blistering skin diseases.[9]

In 1953, Lever defined the disease entity bullous pemphigoid both clinically and histopathologically, clearly distinguishing it from pemphigus. This "pemphigus-like" disease affected primarily elderly patients and was characterized by subepidermal bulla formation.[11]

In 1964, Beutner and Jordon reported autoantibodies in the sera of pemphigus patients, reactive with an "intercellular substance" of skin and mucosa, by using indirect immunofluorescence (IIF). They later showed these same autoantibodies fixed in pathologic sections using direct immunofluorescence (DIF) methods.[12]

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

In 1968, Emmerson RW et al described eosinophilic spongiosis in pemphigus.[13]

In 1979, Chorzelskin et al first described linear IgA bullous dermatosis as an entity distinct from dermatitis herpetiformis (DH) or bullous pemphigoid (BP) on the basis of the immunopathologic finding of linear IgA deposits in the basement membrane zone (BMZ) on direct immunofluorescence (DIF).[14]

In 1984, Nishioka K et al described eosinophilic infiltration of the upper dermis and peripheral eosinophilia.[15]

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

In 1990, Anhalt GJ et al described paraneoplastic pemphigus.[16]

In 1996, Hoss DM et al described neutrophilic spongiosis in pemphigus.[17]

## BASIC FACTS ABOUT THE STRUCTURE OF SKIN

The skin covers the entire external surface of the body. It is continuous with the mucosae of the alimentary, respiratory and urogenital tracts at their respective orifices, where the specialized skin of 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 - 4 mm, these variations reflect maturation, aging and regional specializations.[18]

**THE EPIDERMIS :** The epidermis is derived from the surface ectoderm. It is made up of nonvascular layer that consists of cornified stratified squamous epithelium. Two types of cells constitute the epidermis, 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.

**The stratum malpighii**: It consists of three layers:

**1. Stratum basale (stratum germinativum):** The stratum basale consists of a single layer of columnar cells, which are placed perpendicular to the basement membrane (BM). They have a deeply basophilic cytoplasm and a dark staining oval or elongated nucleus. 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. Most of the mitotic activities are confined to this layer.

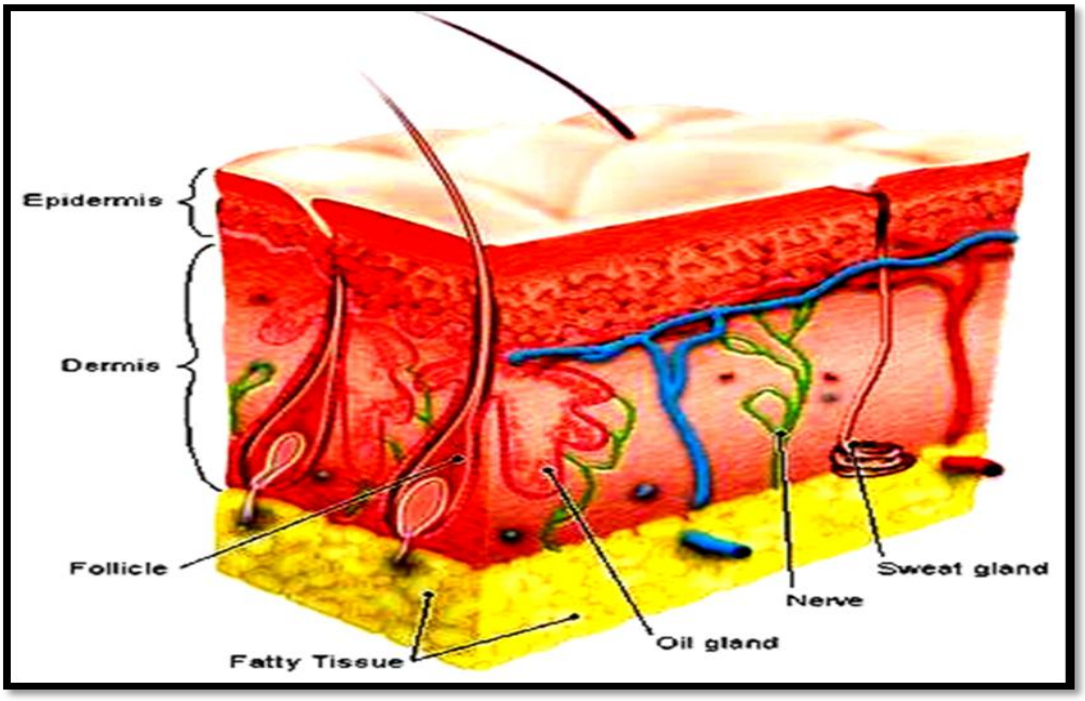



**Fig. 1 : Structure of skin**

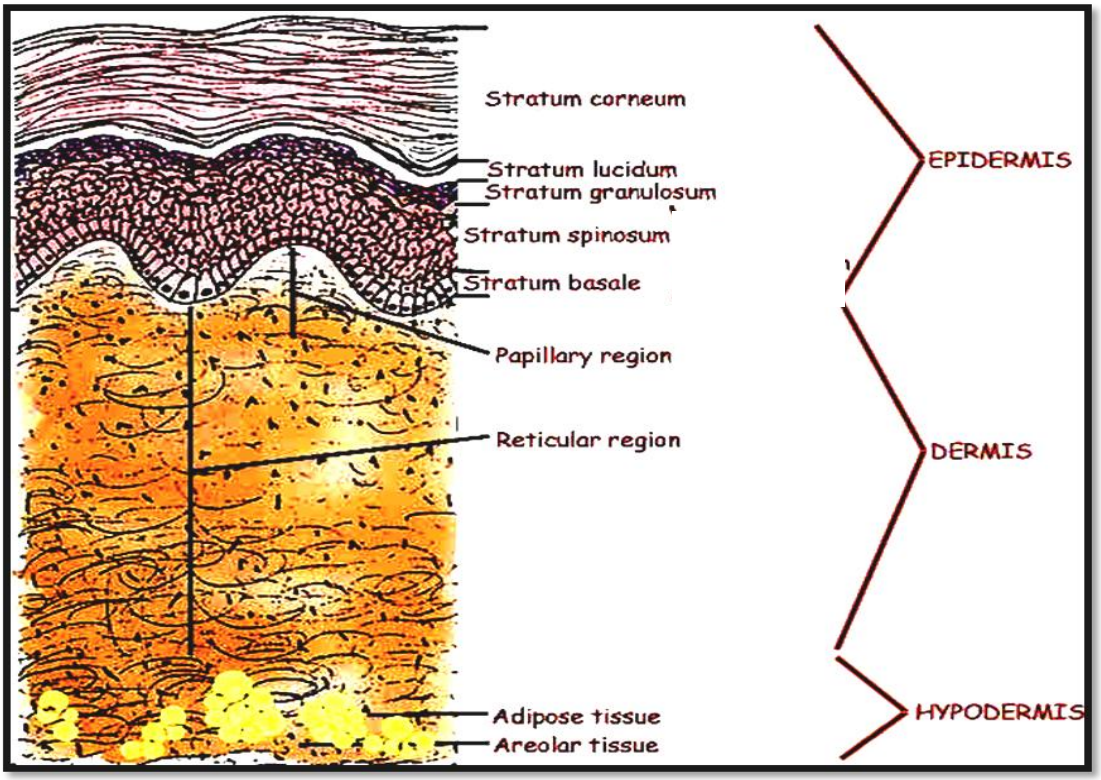



**Fig. 2 : Layers of the skin**

**2. Stratum spinosum (prickle cell layer):** This layer is composed of several strata of polyhedral cells which becomes somewhat flattened in the outermost layers. It is 5-6 cell layers thick with their long axes arranged parallel to the skin surface. The cells are separated by spaces that are traversed by intercellular bridges**.**

**3. Stratum granulosum :** This layer consists of 3-5 layers of flattened cells and their cytoplasm is filled with keratohyaline granules that are deeply basophilic and irregular in size and shape. The thickness of the granular layer in normal skin is generally proportional to the thickness of the horny layer. It represents the keratogenous zone of the epidermis.

**Stratum corneum (zona cornea):** It consists of closely packed layers of flattened polyhedral squames. The cells lack nucleus and membrane organelles and consist solely of a dense array of keratin filaments.

**Stratum lucidum (clear layer):** It appears as a thin homogenous eosinophilic zone. It is more refractile optically and contains nuclear debris. Ultrastructurally, the cells resemble transitional cells, which are incompletely keratinized cells. This zone is more pronounced in areas where the horny layer is thick, especially in the palms and soles.

**Dendritic cells:** There are three types

1. **Melanocytes:** These are found wedged between the basal cells of epidermis. The melanocytes synthesize melanin which protects the germinative cells from the adverse effects of ultraviolet radiation. These are clear cells with small dark staining nuclei.

2. **Langerhans cells:** They are specialized representative of the 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 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 two zones, a narrow superficial papillary layer and a deeper reticular layer.

**Papillary layer:** It lies immediately beneath the epidermis and extends into it in the form of dermal papillae. It is relatively loose and highly vascular with fine interlacing collagen fibers. Most of the collagen present here is type III.[18, 19]

**Reticular layer:** It forms a major bulk of the dermis and consists of dense bundles of collagenous fibers. Collagen in the reticular layer is type I with a small amount of type III.

**The structure and molecular aspects of epidermal cell attachment molecules:** It is of paramount importance to comprehend the adhesive forces and structures maintaining cell-to-cell and epidermal-dermal integrity despite constant movement.The autoantibody-mediated interactions against specific constituents of these structures result in disruption of the normal adhesive integrity leading to blister formation.[1]

## INTERCELLULAR ADHESION MOLECULES OF EPIDERMIS

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

- Desmosomes (maculae adherens)
- 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. Desmogleins subfamily comprises atleast three related proteins i.e. desmoglein 1, desmoglein 2, and desmoglein 3. The desmocollins are a family of proteins which exists in more than one isoform. Desmosomal cadherins (desmogleins and desmocollins) are linked to intracytoplasmic intermediate filaments (tonofilaments) by plakoglobin and desmoplakin. The tonofilaments loop through the cell and around the nucleus forming a network of filaments extending from one desmosome to another and also extends to hemidesmosomes on the basilar pole of epidermal cells.

Interactions between desmoplakins and keratin intermediate filaments stabilize cytoskeleton.

Pathological significance of adhesion molecules:

1. Desmoglein 1 is recognized by sera from patients with pemphigus foliaceus and some with pemphigus vulgaris.
2. Desmoglein 3 is the target antigen in pemphigus vulgaris.
3. Desmocollins are recognized by sera from patients with endemic pemphigus foliaceus (PF).
4. Autoantibodies to desmoplakins are present in the blistering disease, paraneoplastic pemphigus.[1]

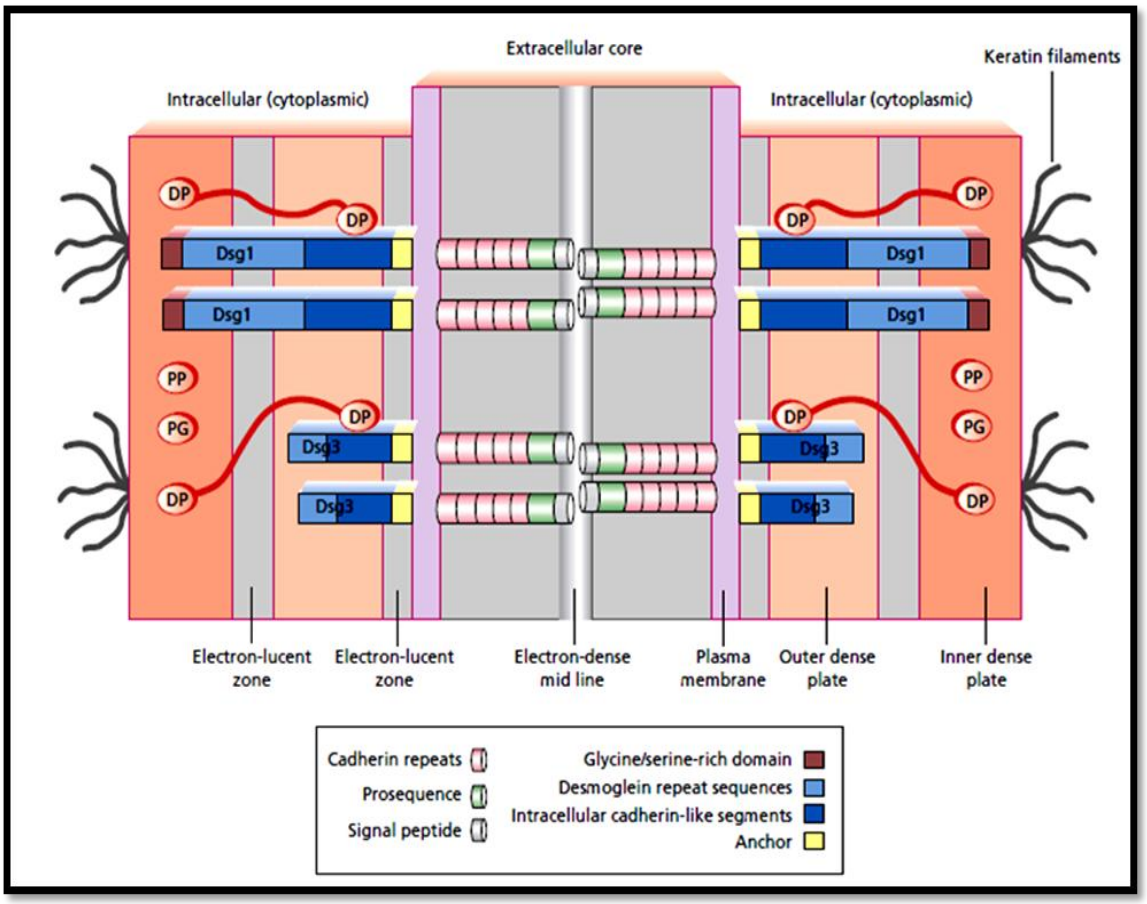



**Fig. 3 : Molecular components of the desmosome[1]**

**Dsg, desmoglein; DP, desmoplakin; PP, plakophillin; PG, plakoglobin**

## DERMO-EPIDERMAL JUNCTION (DEJ)

It consists of the following structures from above downwards:

- Plasma membrane and hemidesmosomes of basal cells of epidermis
- Lamina lucida
- Lamina densa/ basal lamina
- Sublamina densa

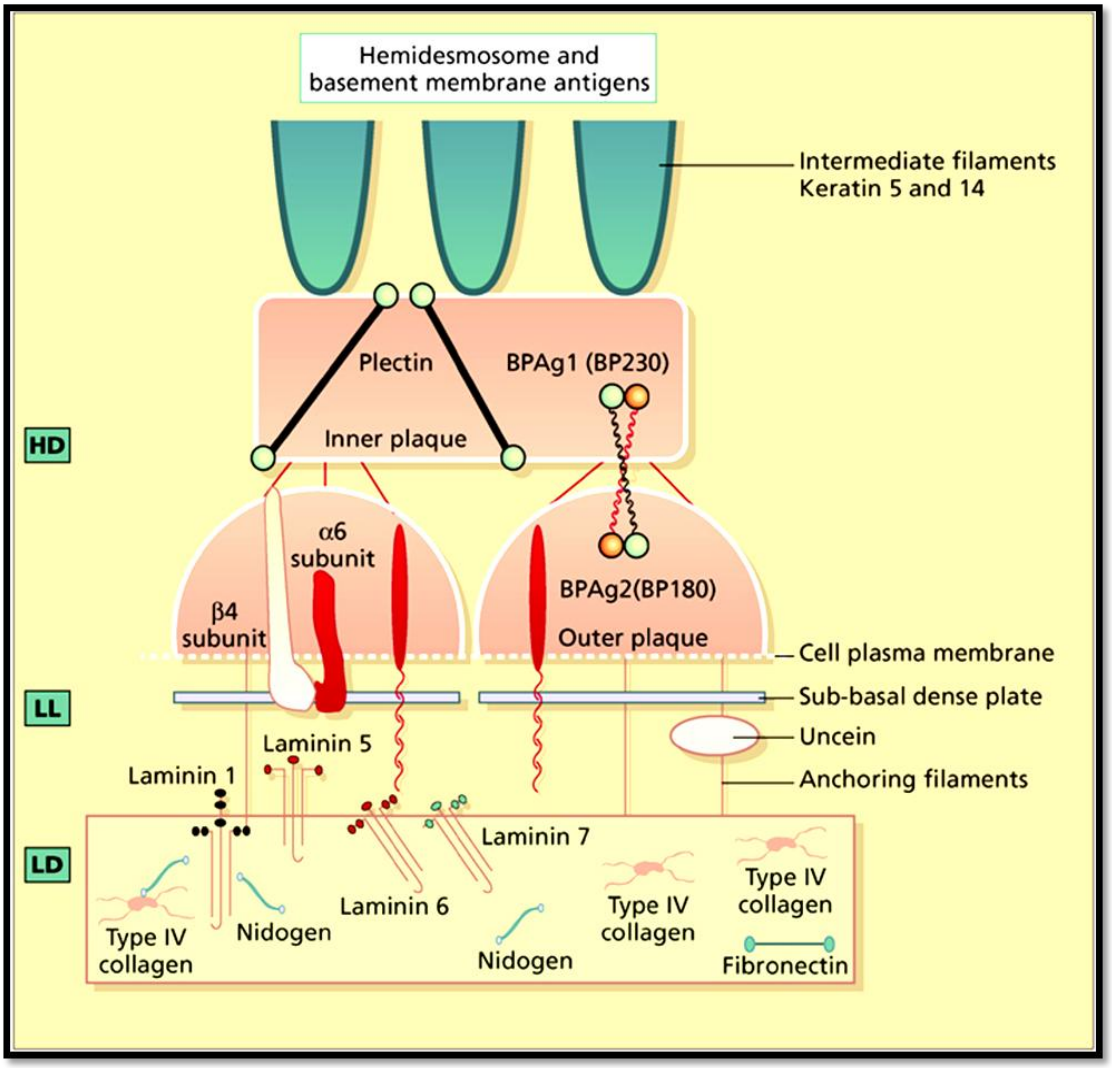



**Fig. 4 : Molecular structure of Dermo-Epidermal junction.[1] HD, hemidesmosome; LL, lamina lucida; LD, lamina densa**

## BASIS FOR 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).[4]

**Mechanism of blister formation:**

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

**Acantholysis:** Results from the loss of attachment between keratinocytes. It may result from damage to the intercellular connections (primary acantholysis) or secondary to other processes such as ballooning degeneration (secondary acantholysis). Histologic evidence of acantholysis includes the presence of rounded keratinocytes with condensed cytoplasm and large nuclei with peripheral condensation of chromatin and prominent nucleoli.

**Reticular degeneration:** It results from ballooning degeneration with secondary rupture of the keratinocytes (intracellular edema).

**Cytolysis:** It is the disruption of keratinocytes.

**Basement membrane zone destruction:** This results from primary structural deficiencies and from humoral and cellular immunologically mediated damage.[4]

**Table 1: Diseases and histological features of blister formation[4]**

| **Histological features** | **Diseases** |
|---|---|
| Spongiosis | Eczematous dermatitis, Pemphigus (early) |
| Acantholysis | Pemphigus, Hailey-Hailey disease, Darier's disease |
| Reticular degeneration | Viral infections |
| Cytolysis | Epidermolysis bullosa simplex, erythema multiforme |
| Basement membrane zone destruction | Bullous pemphigoid, cicatricial pemphigoid, linear IgA dermatosis, dermatitis herpetiformis, epidermolysis bullosa acquisita, epidermolysis bullosa dystrophica |

**Table 2: Mechanisms of blister formation[4]**

| **Mechanism** | **Disease – Examples** |
|---|---|
| Antibody mediated | Pemphigus vulgaris |
| Antibody-, complement-, and inflammatory cell-dependent | Bullous pemphigoid, cicatricial pemphigoid, acquired epidermolysis bullosa |
| Toxin mediated | Staphylococcal scalded skin syndrome |
| Inherent structural fragility, excessive degradative enzyme levels, and mechanical trauma | Recessive dystrophic epidermolysis bullosa |
| Other less-defined mechanisms | Erythema multiforme, dermatitis herpetiformis |

**Table 3 : Selected diseases with specific separation planes[4]**

**I. <u>Intraepidermal:</u>**

**1. Subcorneal/granular**

Pemphigus foliaceus and variants

IgA pemphigus

Subcorneal pustular dermatosis

**2. Spinous**

Spongiotic dermatitis

IgA pemphigus

Hailey-Hailey disease

**3. Suprabasal**

Pemphigus vulgaris and variants

Paraneoplastic pemphigus, Darier's disease

**II. <u>Subepidermal:</u>**

**1. Basal keratinocyte necrosis, cytolysis, or damage**

Epidermolysis bullosa simplex

Erythema multiforme

Herpes gestationis

**2. Epidermal basement membrane zone destruction or disruption**

**2.1 Lamina lucida**

Bullous pemphigoid

Cicatricial pemphigoid

Herpes gestationis

Dermatitis herpetiformis

Linear IgA dermatosis

Epidermolysis bullosa lethalis

**2.2 Sublamina densa**

Bullous SLE

Epidermolysis bullosa acquisita

Linear IgA dermatosis

Epidermolysis bullosa dystrophica

**III. <u>Dermal</u>**: Penicillamine-induced blisters

**Table 4: Principle infiltrating inflammatory cells in selected vesiculobullous dermatoses[4]**

| **Dermatosis** | **Principle cell type** | **Infiltrate** |
|---|---|---|
| Bullous pemphigoid (cell-poor) | Eosinophils | minimal |
| Erythema multiforme | Lymphocytes | present |
| Bullous pemphigoid (cell-rich) | Eosinophils | present |
| Herpes gestationis | Eosinophils | present |
| Dermatitis herpetiformis | Neutrophils | present |
| Linear IgA dermatosis | Neutrophils | present |
| Epidermolysis bullosa acquisita | Neutrophils or mixed neutrophils and eosinophils | present |
| Bullous SLE | Neutrophils, interface dermatitis | present |
| Cicatricial pemphigoid | Mixed neutrophils and eosinophils, lymphocytic, band-like (mucosa only) eosinophils | present |
| Paraneoplastic pemphigus | Lymphocytes-interface dermatitis | present |

**Immunobullous diseases[1]**

**<u>Intraepidermal immunobullous diseases</u>**

1. Pemphigus vulgaris

    Variant: Pemphigus vegetans - subtypes - Hallopeau and Neumann

2. Pemphigus foliaceus

    Variant: Pemphigus herpetiformis

    Variant: Pemphigus erythematosus

    Variant: Endemic Pemphigus foliaceus

3. Drug induced pemphigus
4. Intercellular IgA dermatosis
5. Paraneoplastic pemphigus
6. Subcorneal pustular dermatoses

**Subepidermal immunobullous diseases**

1) Bullous pemphigoid

   Variant: Pemphigoid nodularis, localized pemphigoid, localized vulvar pemphigoid, pemphigoid vegetans, lichen planus pemphigoides

2) Mucous membrane pemphigoid

   Variant: Oral pemphigoid, Brunsting - Perry pemphigoid

3) Pemphigoid gestationis

4) Linear IgA disease

   Variant: Chronic bullous disease of childhood, linear IgA disease of adults, dermal associated linear IgA disease, linear IgA mucous membrane pemphigoid, mixed immunobullous disease.

5) Epidermolysis bullosa acquisita

6) Bullous systemic lupus erythematosus

7) Dermatitis herpetiformis.

## INTRAEPIDERMAL AUTOIMMUNE VESICULOBULLOUS DISEASES

### PEMPHIGUS GROUP:

**Definition:** It refers to a group of autoimmune blistering diseases of skin and mucous membranes that are characterized histologically by intraepidermal blisters due to acantholysis and immunopathologically by in vivo bound and circulating IgG autoantibodies directed against the cell surface of keratinocytes.[21]

**Pemphigus vulgaris (PV):**

It is the most common form of pemphigus and is generally seen in the fourth or fifth decade of life.[20]

Pemphigus vulgaris affects all races and both sexes, but the patients are younger at presentation in India than in western countries.[1] The average annual incidence for the adult population (over the age of 20 years) was 0.42 cases per million per year with an increased incidence of 0.61 per million per year in Jews, among whom Askenazi Jews have the highest incidence of 2.7 per million per year.[22] Pemphigus vulgaris accounts for approximately 70% of all cases of pemphigus and is the most common autoimmune blistering disease in eastern countries such as India, Malaysia, China and the Middle East.[1]

**Etiology:** The precise etiologies of all forms of pemphigus and mechanisms that initiate the antibody production are essentially unknown. Predisposition to 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.[23] Certain major histocompatibility complex (MHC) class II genotypes, in particular alleles of HLA-DRB1*04 and DRB1*14 subtypes, are common in patients with pemphigus vulgaris across racial barriers.[1] HLA DRW4 was found in Jewish patients with pemphigus vulgaris.[24]

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 with or without thymoma has been reported.[23]

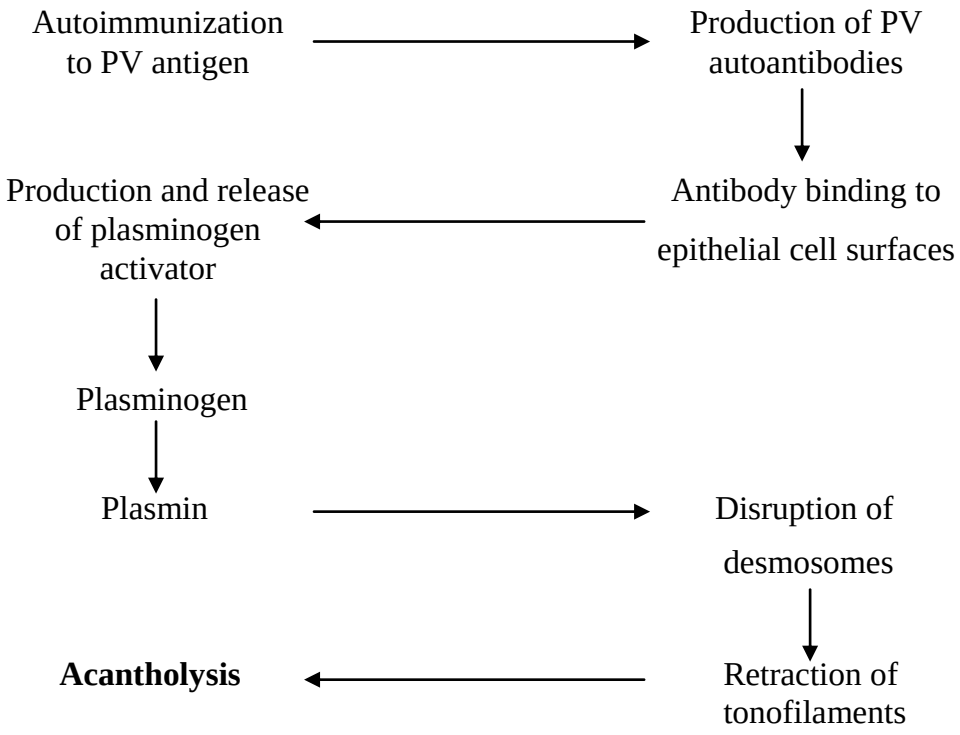
**Pathogenesis:** The pemphigus vulgaris (PV) antigen, known as desmoglein 3, is a desmosomal cadherin, involved in mediating intercellular adhesion in the epidermis. The antibody binds to an extracellular domain on the amino-terminal region of desmoglein 3.[1] Desmoglein 3 is detected primarily in lower epidermis, buccal mucosa and scalp skin. Pemphigus vulgaris patients with only desmoglein 3 antibodies tend to have lesions limited to the mucous membrane, while those with both desmoglein 3 and desmoglein 1 antibodies develop wide spread mucocutaneous blistering with suprabasal blisters.[1]

**Antibodies:** Pemphigus antibody has been found among all four IgG subclass and rarely in IgA and IgM classes.[12] Patients with active disease have antibodies of both IgGl and IgG4 subclasses, but the IgG4 antibodies are pathogenic.[1]

Antibody titer appears to be directly proportional to the severity of the disease.[12]

**Mechanism of blister formation[20]:**

Pemphigus is an autoimmune disorder characterized by production of IgG autoantibodies against polypeptide complexes present in the intercellular cement of the epidermis. The antibodies are deposited in the intercellular area and induce the keratinocytes to release serine proteinases which are enzymes which dissolve the intercellular substance. The keratinocytes then separate from adjoining cells and this process is called acantholysis.




**Clinical features** :

Pemphigus vulgaris (PV) presents with oral lesions in 50-70% of patients. Oral ulcerations at times misdiagnosed as desquamative gingivitis, may be the presenting feature and these lesions may persist for a period of time before characteristic flaccid bullae occur on non-mucosal skin.[20] Other mucosal surfaces involved include the conjunctival, nasal, pharyngeal, larynngeal, oesophageal, urethral, vulval and cervical.[1, 25]

Most patients develop cutaneous lesions. Involvement may be localized or generalized, but the disease has a predilection for the scalp, face, axillae, groins and pressure points. Flaccid blisters filled with clear fluid arise on normal skin. The contents soon become turbid or the blisters rupture producing painful erosions which extends beyond the edges as more epidermis is lost. A shearing pressure applied to the normal appearing skin or to an already formed blister may produce

new blister formation or the extension of a previously formed blister. This is called Nikolsky sign.[4]

"Bulla spread" sign or Asboe - Hansen sign, shows extension of the bulla when pressure is applied to the center of the bulla.[1]

Healing occurs without scarring, but pigmentary changes and acanthomas may occur in resolving lesion. Nail changes are characterized by dystrophic changes, discoloration, pitting, transverse lines, paronychia and onychomadesis.[26]

Pregnancy, in cases of pemphigus vulgaris, seems to cause a mild flare up of the lesions without having much adverse effect on the fetus.[27]

**Cytology:** Cytological examination using a Tzanck preparation is useful for the rapid demonstration of acantholytic epidermal keratinocytes in the blisters of pemphigus vulgaris. These cells are rounded with condensed cytoplasm around an enlarged nucleus with peripherally palisaded chromatin and enlarged nucleoli.[4]

**Histopathology:**

- The earliest changes consist of intercellular edema with loss of intercellular attachment in the basal layer.
- Suprabasal epidermal cells separate from the basal cells to form clefts and blisters.
- Basal cells remain attached to the basement membrane and stand like a 'row of tombstones' on the floor of the blister.
- Blister cavities contain some acantholytic cells. Clefting may extend in to the walls of adnexae.

- The superficial dermis has a mild superficial mixed inflammatory infiltrate which includes some eosinophils.
- Although acantholysis is the initial and the most distinctive change, preacantholytic inflammatory change like eosinophilic spongiosis is seen in some cases.[13]

**Immunoelectronmicroscopy (IEM):** IEM has shown that widening of the intercellular space is followed by splitting of the desmosomal junctions. When the cells separate from each other the intracellular cytokeratin tonofilaments retract around the nucleus and the desmosomal plaques disappear. The attachment of basal cells to the basement membrane is not affected.[1]

The diagnosis of pemphigus is confirmed by direct immunofluorescence (DIF) which shows IgG deposited on the surface of keratinocytes throughout the epidermis (fish net appearance).[1] DIF testing is a very reliable and sensitive diagnostic test for pemphigus vulgaris in that it demonstrates IgG in the squamous intercellular surface area in up to 95% of cases, including early cases and those with few lesions, and in up to 100% of cases with active disease.[4]

Circulating pemphigus autoantibodies are detected by indirect immunofluorescence (IIF) in over 80% of patients. Esophageal substrate is preferred for the detection of desmoglein 3 antibodies. ELISA can be used to monitor disease activity. Pemphigus-like circulating intercellular antibodies have been reported in condition such as thermal burns, toxic epidermal necrolysis, penicillin reactions, first degree relatives of pemphigus patients, bullous pemphigoid, cicatricial pemphigoid, SLE, myasthenia gravis, lichen planus,[1] blood group AB and tricophyton infections.[20]

**Pemphigus vegetans (PVE):**

This is an uncommon variant of pemphigus vulgaris, comprising only 1% to 2% of cases.[4] Two subtypes are recognized, the Hallopeau type and the Neumann type.[28]

**Clinical features:** Both subtypes have common clinical, histologic and immunopathologic features, however they differ in their course and prognosis.

They may be considered to form a clinical spectrum, from the severe Neumann type to the mild Hallopeau type.[28] The disease starts at a slightly earlier age than pemphigus vulgaris. Involvement of oral mucosa is almost invariable.[1]

**Neumann type:** Vesicles and bullae rupture to form hypertrophic granulating erosions which bleed easily. The lesions evolve into vegetating masses exuding serum and pus. The edges are studded with small pustules.[1]

**Hallopeau type** (syn: pyodermite vegetante, pyoderma vegetans): Pustules, rather than vesicles, are the characteristic early lesions but they soon progress to vegetating plaques.[1]

Neumann type has relapses and remissions like pemphigus vulgaris, but Hallopeau type has few, if any relapses and usually remains in remission.

Cutis verticis gyrata 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. This sign may antedate the skin lesions.[28]

**Histopathology**: In the Neumann type, as the early lesions revolve, there is formation of villi and verrucous epidermal hyperplasia. Numerous eosinophils are present within the epidermis and dermis, producing both eosinophilic spongiosis and eosinophilic pustules. In the Hallopeau type, the early lesions consist of pustules arising on the normal skin with acantholysis and formation of small clefts in a suprabasal location. The clefts are filled with numerous eosinophils and degenerated acantholytic epidermal cells. Early lesions may reveal more eosinophilic abscesses than in the Neumann type.[4]

DIF findings of perilesional skin in pemphigus vegetans are identical to those in pemphigus vulgaris. A correlation exists between the disease activity and the demonstrable anti- intercellular space antibody titers.[28]

**Treatment of pemphigus vulgaris and pemphigus vegetans**:

1. **Monitoring activity of the disease:** DIF studies of normal skin have been recommended to predict remission or relapse.
2. **Topical therapy:** Potent topical or intralesional steroids, potassium permanganate and topical antibiotics.
3. **Systemic therapy:** Prednisolone with an adjuvant is the preferred treatment for pemphigus vulgaris.[1]

**Table 5 : Therapeutic ladder for pemphigus vulgaris[29]**

| | |
|---|---|
| **Standard treatment** | |
| Oral prednisone: | 1.0 mg/kg/day as the initial dose (usually 60 mg/day) |
| **Aggressive Treatment** | |
| Immunosuppressive agents in combination with prednisone: | |
| Azathioprine: | 2-4 mg/kg/day (usually 100 to 300 mg/day) |
| Cyclophosphamide: | 1-2 mg/kg/day (usually 50 to 200 mg/day) |
| Mycophenolate mofetil: | 2-3 g/day |
| Cyclosporine: | 5 mg/kg/day |
| Pulse methyl prednisolone: | 1 g/day over a period of 2-3 hours for 3-5 consecutive days |
| Plasmapheresis | |
| High dose intravenous immunoglobulin | |

Monthly intravenous pulses of dexamethasone (100mg) for three consecutive days, with cyclophosphamide (500mg), single dose added to the drip any one day, followed by low dose of oral cyclophosphamide (50mg) for four weeks (DCP therapy) started by Pasricha JS et al[30] is highly effective.

Tetracyclines with prednisolone, oral or intramuscular gold with steroid, cyclosporine 5mg/kg/day with steroids are other options.

Dapsone is used as an adjunct in mild disease. Methotrexate 10-17.5 mg/week permitted withdrawal of prednisolone in steroid - dependent patients.

For resistant cases, unproven therapies like Psoralen Ultraviolet A therapy, extra corporeal photopheresis, high dose intravenous immunoglobulin, acetretin with prednisolone can be used.[1]

**Pemphigus foliaceus (PF):**

Pemphigus foliaceus, also referred to as superficial pemphigus manifests as recurrent shallow erosions, accompanied by erythema, scaling and crusting. Blistering occurs high in the epidermis, either in the granular layer or just beneath the stratum corneum.[1]

**Aetiology:** Pemphigus foliaceus is less common worldwide than pemphigus vulgaris, and accounts for only 10-20% of the cases of pemphigus. Pemphigus foliaceus is more common than pemphigus vulgaris in Mali, Libya, rural Tunisia and in South Africa.[1]

**Pathogenesis:**

Pemphigus foliaceous antigen : Sera from all patients with pemphigus foliaceus recognize epitopes located in the extra cellular aminoterminal domain of desmoglein 1, a 160kDa desmosomal cadherin. Pemphigus foliaceous antigen is expressed more strongly in skin from upper torso than from lower torso or scalp. Desmoglein 1 is present, but only weakly expressed in mucosae, accounting for the lack of mucosal involvement in pemphigus foliaceous.[1]

Antibodies : The pathogenic antibodies in all forms of pemphigus foliaceous are predominantly of the IgG4 subclass.[1]

**Clinical features:** Pemphigus foliaceus is less severe than pemphigus vulgaris. Insidious onset with scattered scaly lesions involving the 'seborrhoeic' areas like the scalp, face, chest and upper back.[1] Patients present with small flaccid bullae that rupture easily, usually arise on an erythematous base or as scaly patches without evident blisters.[4] Erosions are both painful and offensive with characteristic musty odour. The general health is not affected. Nikolsky sign is positive. It may affect the whole skin surface resembling exfoliative dermatitis.[31] Oral lesions are uncommon.[1]

**Clinical variants of pemphigus foliaceus:**

1. Pemphigus resembling dermatitis herpetiformis

Jablonska first described a rare and atypical variant of pemphigus that resembles dermatitis herpetiformis in its early phase (Pemphigus herpetiformis (PH)).[1] Patients present with pruritic, erythematous, vesicular or papular lesions, often in herpetiform pattern.[4] DIF demonstrates intercellular IgG deposition indistinguishable from pemphigus. In pemphigus herpetiformis, neutrophilic spongiosis may occur in the absence of intra epidermal IgA deposition and in the presence of intra epidermal IgG deposition.[17]

2. Pemphigus erythematosus (PE)

Pemphigus erythematosus is a variant of pemphigus foliaceus originally described by Senear and Usher.[1] It usually develops insidiously, the initial lesions appearing on the face, scalp, back or chest. Scaling, crusting and oozing lesions are usually predominant. The lesions of the face often resemble the scaling plaques of lupus erythematosus. Co-existence of pemphigus and lupus erythematosus is known as Senear-Usher syndrome. Oral lesions are unusual.[10] Patients have immunological features of both lupus erythematosus and pemphigus (granular IgG and C3 at the BMZ (positive lupus band test), intercellular IgG and C3 in the epidermis and circulating antinuclear antibodies).[1]

3. Endemic pemphigus foliaceus

(Syn. Fogo Selvagem (wild fire), Brazilian PF)

It is clinically, histologically and immunologically indistinguishable from pemphigus foliaceous. It develops in those who visit areas close to rivers and streams in Brazil.[3]Incidence of the disease is greatest at the end of the rainy season

when insects are most abundant. Black fly (Simulium pruinosum) bites are a risk factor for the disease.[1] Sunlight may exacerbate the condition. It differs from classical pemphigus foliaceus by the age of those affected, by the geographic distribution and by the presence of familial cases in genetically related persons. It has not been reported in Indian literature.[32]

Endemic pemphigus foliaceus affects children and young adults with a peak incidence in the second and third decades where other forms of pemphigus foliaceus affects middle aged adults. Patients presents with flaccid bullae, which rupture easily leaving superficial erosions. Nikolsky sign is positive. The burnt appearance and burning sensation, particularly on sun exposure gives the disease its popular name, fogo selvagem, Portuguese for 'wild fire'. Oral mucosa is spared.[1]

**Cytology:** Tzanck smear reveals few acantholytic keratinocytes.[4]

**Histopathology:** The earliest change consists of acantholysis in the upper epidermis, within or adjacent to the granular layer, leading to a subcorneal bulla. The histologic features of pemphigus foliaceus may have 3 patterns:

a. Eosinophilic spongiosis

b. Subcorneal blister, often with few acantholytic keratinocytes.

c. Subcorneal blister with dyskeratotic granular keratinocytes, diagnostic for this disorder.

The character of the inflammatory infiltrate is variable and depends on the age of the lesion.[4]

**Immunofluorescence Testing:** DIF testing of perilesional skin is positive in the majority of cases. Two patterns of pemphigus antibody deposition have been described. In most cases, there is full thickness squamous intercellular substance deposition of IgG. Rarely, IgG may be localized only to the superficial portion of the epidermis.[4]

**Treatment:** These superficial forms of pemphigus respond to potent topical or intralesional steroids. If control is inadequate, prednisolone 20-40 mg/day is effective. Azathioprine, cyclophosphamide are effective adjuncts to oral steroids.

Hydroxychloroquine 200 mg twice daily is also used. Intravenous immunoglobulin is used in resistant cases. Dapsone 100-300 mg/day is the treatment of choice for pemphigus with dermatitis herpetiformis-like lesions (pemphigus herpetiformis). Retinoids are used as second line of therapy.

Mycophenolate mofetil is used as a steroid sparing agent.

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

**Drug-induced pemphigus:**

Drug-induced pemphigus was first described in 1969 by Degos in patients using D-penicillamine. Drugs may exacerbate or induce pemphigus.[1] Drugs 'at risk' for pemphigus are sulphydryl (SH) - group containing drugs, known as thiol - drugs such as penicillamine and captopril. Pemphigus can also be attributed to non-thiol drugs including ACE inhibitors, nifedipine, penicillins, cephalosporins, pyrazolon derivatives and rifampicin. Drug-induced pemphigus and drug-triggered pemphigus are considered to be separate entities.[33] In drug-induced pemphigus,

exogenous, non-autoimmune factors play a major role, and the disease regresses when the offending drug is discontinued.[34] In drug - triggered pemphigus, the drug only stimulates a predisposition (endogenous and genetic factors) to develop active autoimmune disease. Drug - triggered pemphigus is known to be refractory to therapy if the offending drug is not stopped immediately.

Pemphigus has also been induced by radiotherapy and thermal burns. Dietary factors containing chemical compound resembling drugs, such as thiols (garlic, onion, celery), isothiocynates (mustard, horse radish), phenols (mango, cashew) and tannins (cassava, red chillis, tea, red wine) are also mentioned as exogenous factors known to trigger pemphigus in genetically predisposed persons.[35]

**Etiopathogenesis:** Thiol - drugs provoke acantholysis in vitro, possibly by increasing the activity of plasminogen activators.[1]

**Clinical features:** Pemphigus foliaceus or pemphigus erythematosus are the most common patterns induced by drugs. Drug - induced pemphigus vulgaris and pemphigus vegetans are rare.[1]

The earliest clinical manifestation is that of nonspecific morbilliform or urticarial eruptions.[1]

**Histopathology:** The findings in the early eruptions are non specific, consisting of spongiosis, parakeratosis and a variable dermal infiltrate. Well-developed lesions are essentially identical to those of pemphigus foliaceus or pemphigus vulgaris. Eosinophilic spongiosis may be prominent.[4]

**Paraneoplastic pemphigus (PNP):**

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 pemphigus foliaceus.[16]

There are approximately 150 reported cases of paraneoplastic pemphigus. Paraneoplastic pemphigus is seen almost exclusively in association with B-cell lymphoproliferative disorders. The malignancies most commonly associated with paraneoplastic pemphigus include Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Castleman's disease, sarcomas and thymomas. The age range for paraneoplastic pemphigus is from 7-83 years, although majority of the patients are between the ages of 45 and 70 years. There appears to be a male predominance.[36]

**Clinical features:** The most characteristic feature of paraneoplastic pemphigus is intractable stomatitis causing erosions and ulcerations in the oral mucosa, extending to the vermilion border of the lips. The mucocutaneous lesions of paraneoplastic pemphigus consist of a variable mixture of blisters, erosions and target lesions.[17] The trunk and proximal extremities are most commonly involved.[36]

**Histopathology:**

*Major findings*

- Epidermal acantholysis
- Suprabasal cleft or blister
- Dyskeratotic keratinocytes
- Basal vacuolation
- Epidermal exocytosis of inflammatory cells

*Minor findings*

- Acantholysis and dyskeratosis of follicles and eccrine epithelia
- Lymphocytic satellitosis
- Polymorphic superficial perivascular infiltrate
- Melanin incontinence, melanophages
- Necrotic blister roof
- Absence of apoptotic keratinocytes in dermis
- Epidermal regeneration from adnexal epithelium.[37]

Paraneoplastic pemphigus represents a syndrome defined by five criteria,[37] as described by Anhalt et al (1990):

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 (BMZ).
4) Serum antibodies detected by IIF that bind cell surfaces of stratified squamous epithelia as well as simple, columnar and transitional epithelia
5) Serum immunoprecipitation with a complex of four proteins (250, 230, 210 and 190 kDa). A 170 kDa was subsequently found to be involved.

Camisa and Helm (1993) have proposed a revised set of criteria for the diagnosis of paraneoplastic pemphigus:[38]

*Major criteria*

- Polymorphous mucocutaneous eruption
- Concurrent internal neoplasia
- Characteristic serum immunoprecipitation findings

*Minor Criteria*

- Histologic evidence of acantholysis
- DIF showing intercellular and basement membrane staining
- IIF staining with rat bladder

All three major or two major and two minor criteria are required to diagnose paraneoplastic pemphigus.

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

## Intercellular IgA dermatosis:

**Syn:** IgA pemphigus foliaceus, intraepidermal neutrophilic IgA dermatosis, IgA herpetiform pemphigus, intercellular IgA vesiculopustular dermatosis

Two types are distinguished based on the level of pustule formation and IgA deposition - the subcorneal pustular dermatosis type (subcorneal pustules) and the intraepidermal neutrophilic type (intraepidermal pustules).[1]

**Pathology:** A neutrophil-rich polymorphonuclear infiltrate in the epidermis and microabscesses at various levels depending upon the age of the lesion. Acantholysis is sparse or absent.[1]

**Clinical features:** The disease chiefly affects adults. Patient with both types of the disorder have flaccid vesicles or pustules. The lesions may be pruritic and show a circinate or annular configuration with central clearing, and evolving to crusted or scaly erythematous macules. The sites of predilection are the axillae and groins. Flaccid pustules may resemble subcorneal pustular dermatoses (SCPD) of Sneddon-Wilkinson or pemphigus foliaceus or pemphigus herpetiformis.[1]

**Associated diseases:** Monoclonal IgA gammopathy, HIV infection, Crohn's disease, gluten-sensitive enteropathy, rheumatoid arthritis and intake of thiol drugs.[1]

**Treatment:** Patients respond well to dapsone and poorly to steroid. Other therapies include etretinate, isotretinoin, PUVA, immunosuppressive drugs, plasmapheresis and colchicine.[1]

## SUBEPIDERMAL AUTOIMMUNE VESICULOBULLOUS DISEASES

### Bullous pemphigoid (BP):

**Definition:** Bullous pemphigoid is an immunobullous disorder characterized by the deposition of autoantibodies at the basement membrane zone (BMZ).[39] Lever in 1953, described bullous pemphigoid and differentiated it from pemphigus vulgaris.[11]

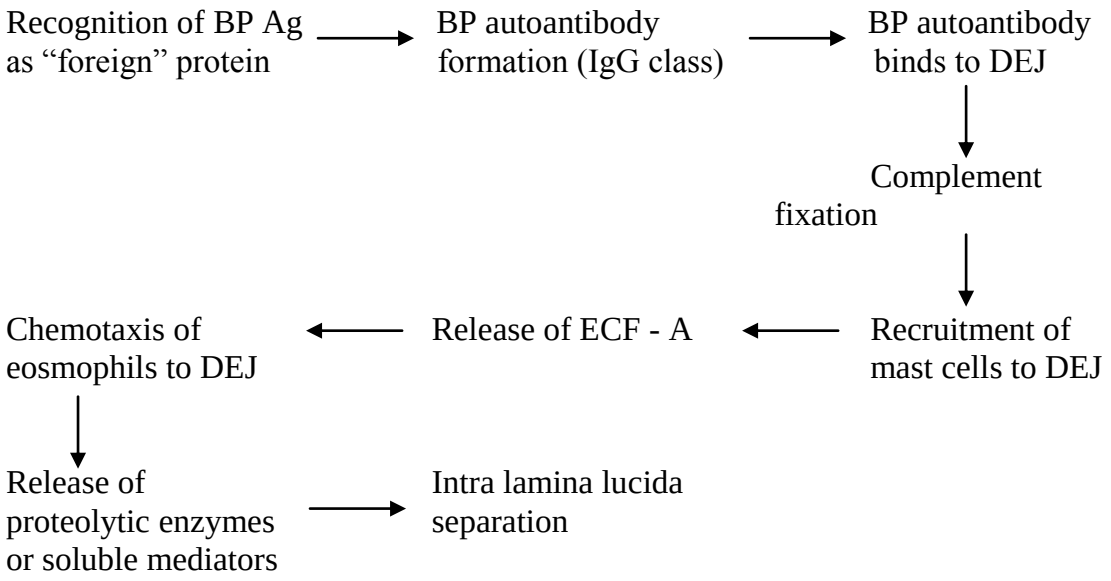
**Epidemiology:** Bullous pemphigoid is the major autoimmune subepidermal bullous disease in the West, with the estimated incidence rate of 6.62, 7 and 7.6 new cases per million people per year in France,[40] Central Germany[41] and

Singapore.[42] The incidence of bullous pemphigoid was highest in the Indians of Malaysian origin.[43] It occurs most frequently in people older than 65 years.[44] It has a peak between 40 and 80 years.[43] Men and women are equally affected,[44] but it is slightly more common in women.[43] The incidence in children is rare and the youngest patient described with childhood bullous pemphigoid is a 2.5 months old female infant.[45]

**Etiopathogenesis:** The two important bullous pemphigoid antigens located within the epidermal hemidesmosomes, are $BPAG_1$ or BP230Ag and $BPAG_2$ or BP180Ag with molecular weight 230 kDa and 180 kDa respectively.

Hemidesmosomes mediate the linkage of the intermediate filament proteins to the basement membrane proteins. BP230Ag is an intracellular protein that is one of the plakins. BP180Ag is a unique transmembrane protein (with a short non-coilagenous ectodomain that lies adjacent to the plasma membrane, and a long collagenous ectodomain that interacts with the anchoring proteins of the basement membrane).[46]

**Mechanism of blister formation:[20]**



**Clinical Features:** The lesions of bullous pemphigoid are 1 to 3 cms tense blisters, that occur on healthy skin or an erythematous base (complement involvement). Blisters typically are found on the flexor surfaces of the arms and legs, axillae, groin and abdomen. Oral lesions are rare and are usually transient. Patients may present with urticarial plaques that may evolve into blisters.[48] Pruritus is a common feature; it may be mild to quite severe. Non-traumatized lesions tend to heal without scarring. It is typically a chronic disease characterized by periods of exacerbation and partial remission.[47]

Vaillant et al (1998) derived a set of diagnostic criteria for bullous pemphigoid[49:]

- Age >70 years
- Absence of atrophic scars
- Absence of mucosal involvement (ocular, nasal, oral, anal or genital)
- Absence of head and neck blisters

The above 4 criteria allow the diagnosis of bullous pemphigoid.

Several clinical presentations of bullous pemphigoid have been reported. Polymorphous lesions-like, generalized bullous, erythrodermic, urticarial, vesicular, localized, papular, nodular (hypertrophic), vegetating (verrucous), dyshidrosiform, atypical and subclinical.[50]

Bullous pemphigoid may present with generalized pruritus but without blistering lesions (pruritic pemphigoid), also known as bullous pemphigoid sine bullae or a prodrome of classical bullous pemphigoid. Thus an elderly patient with severe or persistent unexplained generalized pruritus merits DIF and IIF studies to exclude bullous pemphigoid as the cause of generalized pruritus.[50]

**Cutaneous diseases associated with bullous pemphigoid[50]**

- Pemphigus vulgaris, dermatitis herpetiformis, lichen planus, psoriasis
- Bullous pemphigoid, vitiligo and primary biliary cirrhosis constituting a triad of three autoimmune diseases

**Systemic diseases associated with bullous pemphigoid[51]**

- Lillicrap in 1963 first reported a case of bullous pemphigoid with rheumatoid arthritis. Bullous pemphigoid and SLE, bullous pemphigoid with polymyalgia rheumatica and hyperthyroidism have been described.
- Obasi and Sarin described bullous pemphigoid with pernicious anaemia.
- Peck and Lefkowitz described bullous pemphigoid with polymyositis.
- Been et al described bullous pemphigoid with ulcerative colitis.
- Downham and Chapel described (preexistent) adult onset diabetes mellitus with bullous pemphigoid.
- Bullous pemphigoid has been found in patients with several different types of malignancies.

Drugs implicated in the causation of bullous pemphigoid are D-penicillamine, captopril, furosemide, ampicillin and phenacetin.[44]

**Histopathology:** In the early lesions, papillary dermal edema in combination with a cell-poor or cell-rich perivascular lymphocytic and eosinophilic infiltrate is present. The blister arises at the DEJ. In the cell rich pattern, the blisters arise on erythematous skin.[4] Eosinophilic papillary microabscesses may develop with numerous perivascular and interstitial eosinophils intermingled with lymphocytes and neutrophils in the superficial and deep dermis. Eosinophlic spongiosis may be seen.[15] The cell-poor pattern is observed when blisters develop on relatively normal skin.[4]

**Immunoelectron microscopy (IEM):** IEM reveals autoantibodies that are deposited in the lamina lucida which binds C3 along the DEJ (Pemphigoid band test). The antibodies are directed against BP 180 and BP 230 and are mainly of IgG subclass ($IgG_1$ and $G_4$).[1]

**DIF**: DIF of perilesional skin demonstrates the deposition of all classes of IgG along the BMZ. Properdin and properdin factor B have also been detected along BMZ.[20]

**IIF**: IgG (frequently), IgA and IgM anti-BMZ antibodies are detected in 80% of patients. Serum IgE levels are frequently elevated.[20]

**Treatment:** Topical and systemic corticosteroids are the mainstay of treatment. Topical corticosteroids aid control and reduce the dosage of systemic agents. Prednisolone 20 mg/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 50-70 mg/day or 0.75-1 mg/kg/day in severe diseases. Corticosteroid dosage is reduced over a course of few weeks.

Dapsone, tetracyclines and nicotinamide are used in mild to moderate disease.

Immunosuppressants used are azathioprine and methotrexate.[1]

**Mucous membrane pemphigoid (MMP):**

**Syn:** Cicatricial pemphigoid, benign mucosal pemphigoid, ocular pemphigoid, scarring pemphigoid

**Definition:** It is a chronic blistering disease of the mucosa, which may involve the skin, and usually results in permanent scarring of the affected area, particularly the conjuctiva. Earlier called cicatricial pemphigoid, recently the entity has been defined on the basis of clinical picture and renamed as mucous membrane pemphigoid.[52]

**Etiology:** The incidence of mucous membrane pemphigoid is 0.85 and 1.16 new cases per million people annually in Central Germany[41] and France[40] respectively, and probably less in China and the East.[40] It most commonly affects females, with onset of the disease usually occurring in the 6th or 7th decade.[53]

There is an association with HLA-DQ7 (DQB1*0301) in all types of mucous membrane pemphigoid.[53]

**Clinical Features:** The striking features are recurrent blisters on either a mucous membrane or an area of skin often near one of the orifices, together with the tendency for scars formation at these sites. The initial site may be any mucous membrane, including the conjunctiva, oral mucosa, nose, larynx, pharynx, oesophagus, penis, vulva, vagina and anus,[9] glabrous skin, urethra, bladder and rectum may also be involved.[54] Oral lesions occur in majority of the patients.[9]

Two types of skin lesions may occur, the most common one being a generalized bullous eruption, such that an initial diagnosis of bullous pemphigoid is made. The second type of lesion is a localized erythematous plaque, which becomes the site of recurrent blisters with subsequent scarring and hyperpigmentation. In one variant of this disease, scalp may be involved resulting in scarring alopecia.[9]

**Associated diseases:** There is an association with autoimmune disease, both organ and non-organ specific. Malignancies are associated with laminin 5 mucous membrane pemphigoid.[1]

**Histopathology:** In cutaneous lesions, a subepidermal blister develops which may extend down the adnexa. Neutrophils, lymphocytes and histiocytes predominate in the inflammatory infiltrate. Eosinophils may or may not be numerous. Lamellar fibrosis beneath the epidermis is a hallmark, but may not be present in the initial lesions.[4]

**Antigens:** There are multiple target antigens including BP180, BP230, α6β4 integrin, laminin5 and collagen VII,[52] explaining the variation of IF patterns.

**Antibodies:** IgG autoantibodies are most commonly detected and are usually of IgG1 and IgG4 sub types.

**DIF findings**: Linear BMZ IgG or C3, and less commonly IgA and IgM in lesional and perilesional skin in approximately 80% of the cases.[1, 4]

**IIF**: The serum in mucous membrane pemphigoid does not always contain detectable circulating autoantibodies. The use of salt-split skin increases the sensitivity as the majority of sera bind to the epidermal aspect of the split skin.[1]

**IEM:** Antigens of mucous membrane pemphigoid are within the lamina lucida or associated with the lamina densa. Oral and cutaneous lesions possess an intact BMZ, at the base of the blisters, and are destroyed according to another study.[4]

**Treatment:** Local treatment modalities are crucial and may be sufficient to control the disease to an acceptable level. Systemic treatment is required for severe mucosal and laryngeal lesions. Cyclophosphamide, dapsone and sulfamethoxypyridazine, tetracyclines and nicotinamide and intravenous immunoglobulins are used.[1]

## Pemphigoid gestationis (PG):

**Syn:** Herpes gestationis

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

**Etiology:** Pemphigoid gestationis is a rare condition that may affect 1 in 10,000 to 1 in 60,000 pregnancies.[1] Incidence is 0.4, 0.54 and 1.83 per million people per year in France,[40] Central Germany[41] and Kuwait[2] respectively.

The disease arises only in the presence of paternal derived tissue, the fetus, and rarely hydatiform mole, the tissue expressing HLA antigens from the father. The mothers frequently manifest the autoimmune haplotype, HLA-B8, -DR3 and -DR4.[1]

**Clinical Features:** Pemphigoid gestationis may begin at any time between 4 weeks' gestation and 5 weeks' postpartum, with the majority presenting in the second and third trimester.[1] It may initially present as intense pruritus only, or along with an urticarial, papulovesicular eruption, beginning on the umbilicus, then spreading around the abdomen and thighs. The palms, soles, chest, back, face and extremities may also be affected. Oral cavity involvement is rare.[55]

Neonatal pemphigoid gestationis may occur in 3% of pregnancies, which results from transfer of antibodies (HG factor) across the placenta.[1, 55] Foetal mortality is very rare.[35]

**Associated Diseases:** Pemphigoid gestationis is associated with other autoimmune diseases, particularly Grave's disease, hypothyroidism, vitiligo, alopecia areata and autoimmune thrombocytopenia.[1]

**Histopathology:** Subepidermal bulla, shaped like a teardrop is seen with eosinophilic infiltration of the dermis. Epidermal edema and papillary dermal edema are also seen.[55]

**Antibodies:** The autoantibodies are directed at the same hemidesmosomes target antigens as in bullous pemphigoid, namely BP180, and less commonly BP230. The extra cellular region of BP180 adjacent to the transmembrane portion, the NC16A domain is the target for pathogenic autoantibodies.[1]

**IF Testing:** DIF testing reveals linear deposition of C3 in the perilesional skin in virtually all patients.[4] IgG1 is found in some cases.[1]

IIF demonstrate binding of C3 to the BMZ, this serum factor was known as Herpes gestationis factor.[1]

The autoantibodies bind to the epidermal side of salt-split skin.[1]

**Treatment:** In mild cases, topical potent steroids are used, often combined with a systemic antihistamine. Moderate disease responds to prednisolone 20-30 mg/day. Severe disease requires prednisolone 40-80 mg/day, which is then tapered fairly rapidly. Plasmapheresis is considered in the most severe cases.

**Linear IgA disease :**

**Syn:** Chronic bullous disease of childhood (CBDC), juvenile dermatitis herpetiformis, juvenile pemphigoid, linear dermatitis herpitiformis, linear IgA bullous dermatosis (LABD), IgA pemphigoid

**Definition:** It is a chronic acquired subepidermal disease of children and adults, with cutaneous and mucosal involvement, characterized by IgA basement membrane antibodies.[1]

Two main clinical syndromes are distinguished.

- Childhood onset LABD or CBDC
- Adult onset LABD

**Etiology:** It affects all ages, from babies of a few months to the elderly.

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 estimated incidence of 0.23, 0.26, 0.48 and 0.69 per million people per year in Germany[41], Singapore[42], France[40] and Kuwait[2] respectively.

There is a strong association between LABD and the extended autoimmune haplotype HLA-B8, DQW1, DR3.[56]

**Clinical features:** Linear IgA disease may be diagnosed based on the following 3 criteria.[14]

- The presence of vesicular or bullous eruption, usually confined to the skin; mucous membrane may be involved.
- The presence of subepidermal vesicle with a predominantly neutrophilic infiltrate on histology of lesional skin. The presence of BMZ - specific IgA antibody deposited in a linear pattern in the absence of other Igs on DIF of perilesional skin.

The clinical features of linear IgA disease may resemble those of dermatitis herpetiformis, with a primarily pruritic papulovesicular eruption involving extensor surfaces symmetrically. More commonly, the clinical features are more like those of bullous pemphigoid with tense vesicles and bullae developing denovo or on an urticarial base. The bullae of LABD may be linear or “sausage” like in shape. Generalized blistering of the body can be present occasionally. Mucous membrane involvement is seen in 80% of the patients.[14]

Skin lesions in this disease are variable, and can be papular, vesicular, bullous, erythematous or oedematous, or can resemble erythema multiforme.[57]

Two main clinical differences between adult onset linear IgA disease and CBDC[14]:

- CBDC occurs in children, linear IgA in adults
- In CBDC, there 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.

The distribution of linear IgA disease may have one of two distinct patterns:

i) Involvement of extensor surfaces with grouped papulovesicles, similar to the clinical picture of dermztitis herpetiformis, or

ii) Flexural and truncal involvement with scattered vesicles and bullae similar to bullous pemphigoid.

**Diseases associated with linear IgA dermatosis:**

Hodgkins disease, B-cell lymphoma, dermatitis herpetiformis, bladder cancer, esophageal cancer, SLE, ulcerative colitis, multiple sclerosis, dermatomyositis, Crohn's disease, hydatidiform mole, rheumatoid arthritis.[14]

**Drugs associated with linear IgA disease:**

Vancomycin, lithium carbonate, diclofenac, amiodarone, ampicillin, captopril, iodine, phenytoin, piroxicam, rifampicin, sodium hypochloride, pencillinG.[58]

**Histopathology:** Subepidermal bullae are present with extensive basal vacuolization, admixed with neutrophils at the epidermal BMZ.

**Antibodies:** In LABD, specific antibody binding has been found to various antigens. The most well characterized of these antigens is a 97kDa Ag found in a modified epidermal extract.[14]

IgA autoantibodies bind in the lamina lucida and recognize a 97-120 kDa antigen that appears to be a proteolytic cleavage segment representing the extracellular domain of the 180 kDa BPAg, also known as BPAg2 or type XVII collagen. IgA antibodies in LABD sera have been reported to bind to the 230 kDa BPAg (BPAg1), 100 kDa, 110-120 kDa, 145 kDa, 160-180 kDa, 200 kDa, 220 kDa, 255 kDa and 285 kDa.[60]

IF: The sine qua non for the diagnosis of LABD is the presence of BMZ - specific IgA class antibody in a linear distribution on DIF of perilesional skin in the absence of other Igs. Complement may occasionally be present at the BMZ in addition to IgA.[60]

Direct salt splitting of the biopsies show antibodies binding to the epidermal side, dermal side, or both.[60]

**Treatment:** Topical steroid is used for mild disease. Erythromycin is tried as a first line treatment in children. Dapsone 0.5 mg/kg is increased slowly to 1 mg/kg without significant side effects. Sulphonamides and sulfamethoxypyridazine are alternatives, Azathioprine, cyclosporin, colchicines, tetracyclines and nicotinamide are also used.[1]

**Dermatitis herpetiformis (DH):**

**Syn:** Duhring Brocq disease

**Definition:** It is a rare intensely pruritic, chronic recurrent, autoimmune papulovesicular disease that usually occurs in young adults and is characterized by the presence of granular deposits of IgA within the upper papillary dermis of the skin.[21]

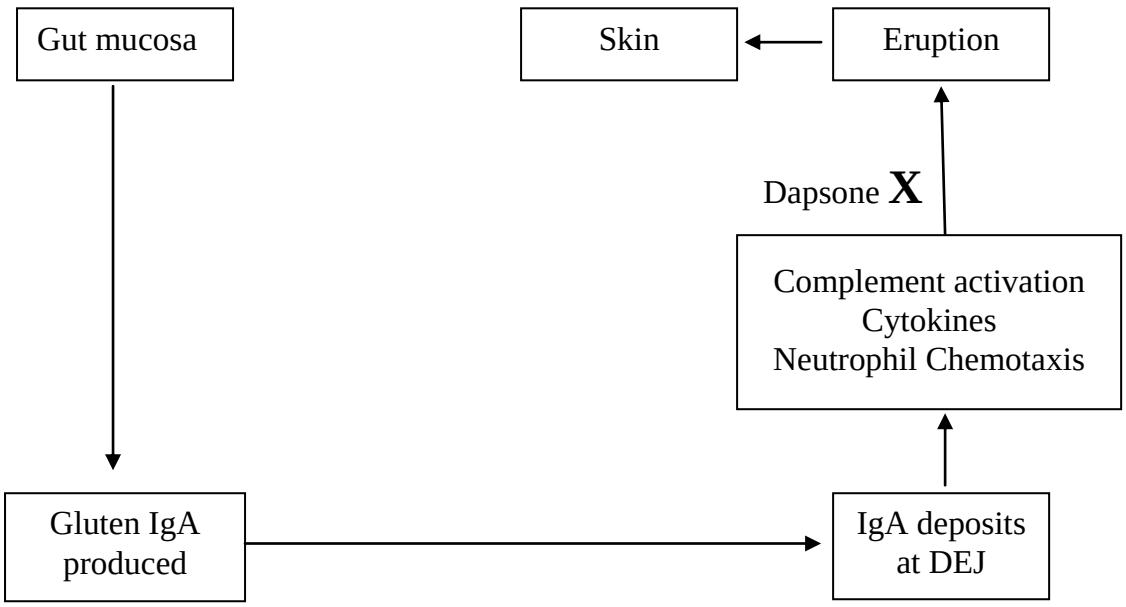
**Historical features:** The disease was named dermatitis herpetiformis by Duhring in 1884, was distinguished histologically from bullous pemphigoid by Civatte in 1943, and was found to be associated with the presence of dermal IgA deposits by Vander Meerin l969.[21]

**Epidemiology:** Dermatitis herpetiformis affects men slightly more frequently than women. In a majority of cases its onset is between the second and fourth decades of life. It can appear in late years and even in childhood.[61]

**Etiopathogenesis:** Dermatitis herpetiformis is a familial disease in 10.5% of patients, in which the first-degree relatives can be affected both with dermatitis herpetiformis and celiac disease (CD). It is strictly associated with Class II histocompatibility locus antigens HLA DR3 and DQW2 and especially with the DQ alleles Al* 0501 and Bl *0201 in the short arm of chromosome 6.[62]

Most patients with dermatitis herpetiformis have asymptomatic gluten – sensitive enteropathy i.e., celiac disease.

**Dermatitis herpetiformis from gut to skin[61]:**



The characteristic finding is deposition of IgA in a granular pattern in the papillary dermis, although IgM, IgG and C3 may be found. The IgA deposits are gluten - dependent, and are slowly cleared from the skin once gluten is removed from the diet.[1]

**Clinical features:**

**Cutaneous manifestations:** The onset may be acute or gradual, and pruritus is usually the first and predominant symptom. Early lesions on the skin are erythematous papules, urticarial wheals or groups of small vesicles, often excoriated.[1] They are symmetrically distributed over extensor surfaces, especially the elbows, knees, shoulders, sacrum, buttocks, and posterior nuchal area. Lesions may involve the scalp, face and groin. Lesions heal usually without scarring; occasionally mild scarring may be seen. Post inflammatory pigmentary changes do occur.[61]

**Oral manifestation:** Mucous membrane involvement is rare. Oral involvement consisted of erythematous, pseudovesicular, purpuric, and erosive lesions. Cutaneous IgA deposits pathognomonic for dermatitis herpetiformis were found on uninvolved buccal mucosa in patients with dermatitis herpetiformis but not in those with Celiac disease.

**Childhood dermatitis herpetiformis :** It is rare and occurs more frequently between ages 2 and 7 years, but has been reported as early as age 10 months. Clinically, the skin lesions resemble those of adult dermatitis herpetiformis.[61]

**Associated diseases:** There are often associated autoimmune diseases, particularly thyroid disease, pernicious anemia and diabetes.[1]

**Histopathology:** Skin biopsy of an erythematous papule typically shows the characteristic neutrophilic microabscesses within the dermal papillae in association with few eosinophils, fibrin, leukocytoclastic debris and edema. As micro abscesses form, a separation develops between the tips of the dermal papillae and the overlying epidermis. The early blisters are multiloculated. Within one to two days, the rete ridges lose their attachment to the dermis, and the blisters then become unilocular. Perivascular infiltrate composed of lymphocytes, neutrophils and eosinophils may be apparent. Apoptotic keratinocytes above the papillary micro abscesses may be seen.[61]

**Pathogenesis:** Epidermal transglutaminase (TG3) is the major auto antigen recognized in the skin lesion of dermatitis herpetiformis. IgG autoantibodies against tissue transglutaminase (TTG) cross-react with TG3 of skin.

Antireticulin, endomysial antibodies are associated with these antibodies and require TTG to bind to tissues. Cutaneous IgA deposits are polyclonal and predominantly IgAl.[61]

**IF testing:** The characteristic finding is granular deposits (80-90%) of IgA within the dermal papillae in both lesional and non lesional skin, although IgM, IgG and C3 may be found.[1] Linear deposits of immunoglobulins are seen in 10% of cases.

Circulating IgA antibodies that react against reticulin, smooth muscle endomysium, the dietary antigen gluten, bovine serum albumin, and beta-lactoglobulin may be present. Only the presence of IgA endomysial antibodies is of diagnostic importance.[4]

**Diagnosis of Dermatitis herpetiformis:** There are 3 basic criteria for the diagnosis of dermatitis herpetiformis. Atleast 2 of these should be fulfilled before embarking on any treatment program[63]:

1. Clinical characteristics consisting of involvement of extensor surfaces with a pruritic papulovesicular eruption.
2. Histologic changes of involved skin including vesicle formation at the DEJ and infiltration of dermal papillary tips with neutrophils.
3. Immunopathologic finding of granular IgA at the DEJ on DIF of perilesional skin. DIF is the single best way to confirm the diagnosis of dermatitis herpetiformis.

Dapsone is so uniformly effective that the therapeutic response to it was considered for many years as the major diagnostic criterion. Symptoms may abate in as few as 3 hours after the drug is taken.

Patch test with potassium iodide can elicit dermatitis herpetiformis-like skin lesions 24-48 hours after application in patients with the disease (Halogen test).[31]

**Treatment:** Dapsone 100-200 mg/day is the most widely used treatment. Sulfapyridine 1.5 g/day, sulfamethoxypyridazine 0.5-1.5 g/day are other alternatives. Topical steroids may be helpful. Heparin, with or without tetracyclines in combination with nicotinamide is advocated for patient who cannot tolerate dapsone or sulfonamides.

A gluten-free diet is the treatment of choice in the long term.[1]

## Epidermolysis bullosa acquisita (EBA)

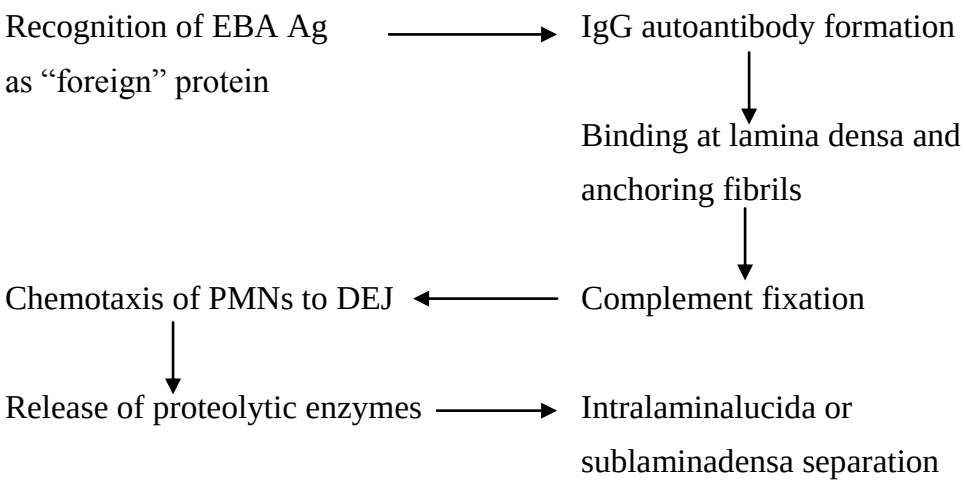
**Syn:** Dermolytic pemphigoid, acquired epidermolysis bullosa.

**Definition:** Epidermolysis bullosa acquisita is an acquired sub epidermal blistering disease in which autoantibodies are produced to type VII collagen, an epithelial basement membrane protein.[55]

**Etiopathogenesis:** Epidermolysis bullosa acquisita can occur at any age. It has been described in children and adults. It has equal prevalence in both genders. Estimated annual incidence is 0.17-0.26, 0.22, and 0.5 per million people in France, Germany and Singapore respectively.[40, 41, 42] There is an association with HLA - DR2.[1]

Collagen type VII, the major component of anchoring fibrils, is the target antigen recognized by autoantibodies from patients with epidermolysis bullosa acquisita.[64] The autoantibodies are IgG, and are of all the IgG isotypes, although the IgG1 and IgG4 isotypes may be predominant in the chronic mechanobullous forms.[1]

**Mechanism of blister formation in EBA**[20]



**Clinical features:** Two blistering entities may present the first is a generalized inflammatory eruption clinically indistinguishable from bullous pemphigoid, and the second is a pattern of non-inflammatory acral blistering resulting in scarring and milia formation. Nevertheless, all patients with epidermolysis bullosa acquisita can be clinically classified by a combination of milia, blisters, erosions, scars and skin depigmentation.[55]

Blisters may be haemorrhagic or serous and localized in areas of trauma, especially on the extensor regions of the extremities. The nails may become dystrophic, and scarring alopecia may be seen. Mucosal involvement is variable; it may be severe and can affect the oral cavity, eyes, urogenital tract, larynx, and oesophagus. Hence the two main phenotypes are the bullous pemphigoid phenotype and the cicatricial pemphigoid phenotype.[55]

Induced epidermolysis bullosa acquisita and associated diseases: conditions associated with epidermolysis bullosa acquisita are inflammatory bowel disease, ulcerative colitis, Crohn's disease, multiple myeloma, amyloidosis, lymphoma, and SLE. Drug induced epidermolysis bullosa acquisita has been described.[65]

**Histopathology:** The bullous pemphigoid - like presentation is the most common form of epidermolysis bullosa acquisita. The subepidermal blisters are inflammatory. The predominant infiltrating cells are lymphocytes and neutrophils in perivascular and focal interstitial assay. Eosinophils are present in variable numbers. In the classic form, the subepidermal blisters are noninflammatory, and fibrosis and milia formation are often present.[4]

**Electron microscopy:** Has shown dermolyis and cleavage in the upper dermis with preservation of the basal lamina, but no reduction in the number of anchoring fibrils. Amorphous material has been found beneath the basal lamina.[1]

**IF testing:** Routine DIF cannot reliably distinguish between bullous pemphigoid and epidermolysis bullosa acquisita. IgG is by for the most common Ig found, but IgM and IgA may be present. The presence of linear C3 at the DEJ alone favours bullous pemphigoid over epidermolysis bullosa acquisita.

IIF reveals circulating anti-BMZ antibodies in up to 50%. The antibodies in epidermolysis bullosa acquisita have specificity for the globular carboxyl terminus of the type VII collagen and are deposited beneath the lamina densa. Therefore, on salt split skin studies, IgG is on the floor and not on the roof of the split.[4]

**Treatment:** Steroid with dapsone or sulphonamides.

Azathioprine, ciclosporin, vitamin E, gold, intravenous immunoglobulins and photopheresis are used.[1]

**Bullous Systemic Lupus Erythematosus (bullous SLE):**

**Syn: Vesiculobullous SLE**

**Definition:** An autoimmune blistering condition, often transient, that occurs in the setting of SLE.[1]

**Etiology:** It affects young adults, chiefly women[1]. Estimated annual incidence is 0.21 and 0.26 per million people in France and Singapore respectively. There is an association with HLA - DR2.[1]

**Pathogenesis:** The bullous disease is mediated by autoantibodies to the BMZ, as demonstrated by positive DIF at the BMZ. Both IgG and IgA autoantibodies are involved. No circulating autoantibodies were found in few cases.[1]

**Clinical features:** The onset of disease is usually in patients with established SLE. There is widespread blistering, all cutaneous sites are involved. Mucosal lesions are uncommon. Post inflammatory hyperpigmentation may occur.[1]

**Associated diseases:** Pemphigus, Bullous pemphigoid, linear IgA disease, erythema multiforme and toxic epidermal necrolysis.[1]

**Histopathology:** Three histologic patterns have been identified in such lesions. The first is striking basal layer vacuolization with subsequent blister formation. The second is vasculitis with subepidermal blister and pustule formation. The third and most common is a dermatitis herpetiformis-like histologic pattern. Histologic features more routinely identified with lupus erythematosus are not present.[4]

**IF testing:** DIF shows linear band of IgG, IgA, IgM and C3 in the BMZ. With IIF, circulating antibodies when present, may bind to the BMZ.[1] A salt split skin using patient serum reveals localization to the split floor as in epidermolysis bullosa aquisita.[4]

**IEM:** Reveals electron dense deposits of IgG at the lower edge of the basal lamina and immediately subjacent dermis.[1]

Camisa and Sharma (1983) proposed the following criteria for the diagnosis of the bullous eruption of SLE.[66]

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 a diagnosis of dermatitis herpetiformis.
4. Negative IIF for circulating BMZ antibodies.
5. DIF revealing IgG and/or IgM, often, IgA at the BMZ.

**Subcorneal pustular dermatosis (SCPD):**

**Syn :** Sneddon - Wilkinson disease

**Definition:** A chronic relapsing pustular lesion, mainly involving the trunk, which affects women over 40 years of age.[1]

**Pathogenesis:** The pathogenesis is obscure. The salient feature is subcorneal accumulation of neutrophils. Culture of the pustule is sterile.[1]

The squamous intercellular substance IgA leads to neutrophilic infiltration.[4] Excess production of TNF-alpha has been implicated in the pathogenesis of neutrophil activation.

**Clinical features:** It is characterized by sterile pustules that have a predilection for flexural surfaces and the axillary and inguinal folds. It usually spares the face and mucous membranes. The pustules develop in an annular or serpiginous arrangement. Pus characteristically accumulates in the lower half of large pustules (horizontal level of pus).[4]

**Associated conditions:** Monoclonal gammopathy (IgA), IgA myeloma, benign paraproteinemia, pyoderma gangrenosum, inflammatory bowel disease, rheumatoid arthritis.[1]

**Histopathology:** Biopsy from early lesions show a perivascular inflammatory infiltrate with neutrophils and occasional eosinophils. Neutrophils migrate through the epidermis, without forming spongiform pustules, to collect beneath the stratum corneum in subcorneal pustules. A few acantholytic cells may be found in old lesions.[1]

**IF Testing:** Both DIF and IIF studies are negative in classic cases, but many cases have squamous intercellular IgA, indicating that they are best considered as IgA pemphigus.[4]

**IEM:** The edge of the pustules shows cytolytic changes in the granular layer of epidermis. Dissolution of the plasma membrane and of the cytoplasm of the cells causes the formation of a subcorneal split.[1]

**Treatment:** Dapsone 50-150 mg/day is the treatment of choice, sulfapyridine and sulfamethoxypyridine are alternatives. Steroids are ineffective even in high doses. Etretinate, acitretin has been used. Broadband UVB, narrow-band UVB, PUVA and Re-PUVA are effective. Colchicines and topical tacalcitol has been recommended.[1]

**Table 6 : Target antigens in pemphigus[29]**

| Diseases | Auto antibodies | Antigens | MW (kDa) |
|---|---|---|---|
| Pemphigus vulgaris | | | |
| Mucosal dominant type | IgG | desmoglein 3 | 130 |
| Muco cutaneous type | IgG | desmoglein 3 | 130 |
| | | desmoglein 1 | 130 |
| Pemphigus foliaceus | IgG | desmoglein 1 | 160 |
| Paraneoplastic Pemphigus | IgG | desmoglein 3 | 130 |
| | | desmoglein 1 | 160 |
| | | Plectin | 500 |
| | | desmoplakin I | 250 |
| | | desmoplakin II | 210 |
| | | BPAG 1 | 230 |
| | | envoplakin | 210 |
| | | periplakin | 190 |
| | | ? | 170 |
| Drug-induced Pemphigus | IgG | desmoglein 3 | 130 |
| | | desmoglein 3 | 160 |
| IgA Pemphigus | | | |
| SPD type | IgA | desmocollin 1 | 110/100 |
| IEN type | IgA | ? | ? |

**Table 7 : Major autoantigens of subepidermal autoimmune-mediated blistering diseases[67]**

| Disease | Target antigen(s) | MW (kDa) | Morphologic structures |
|---|---|---|---|
| Bullous pemphigoid (BP) | BP180/BPAG2 | 180 | Hemidesmosomal plaque/anchoring filaments |
| | BP230/BPAG1 | 230 | Hemidesmosomal plaque |
| Gestational pemphigoid | BP180/BPAG2 | 180 | Hemidesmosomal plaque/anchoring filaments |
| | BP230/BPAG1 | 230 | Hemidesmosomal plaque |
| Cicatricial pemphigoid | BP180/BPAG2 | 180 | Hemidesmosomal plaque/anchoring filaments |
| | BP230/BPAG1 | 230 | Hemidesmosomal plaque |
| | Laminin-5 ($\alpha 3\beta 3\gamma 3$) | 165,140,105 | Anchoring filaments |
| | Laminin-5 ($\alpha 3\beta 3\gamma 3$) | 165,220,200 | Anchoring filaments/extracellular matrix |
| | Integrin$\beta 4$ subunit3 | 200 | Hemidesmosomal plaque |
| Linear IgA bullous dermatosis (LAD) | LADF antigen4 | 97/120 | Anchoring filaments |
| | BP180/BPAG2 | 180 | Hemidesmosomal plaque/anchoring filaments |
| | BP230/BPAG1 | 230 | Hemidesmosomal plaque |
| | Type VII collagen | 290/145 | Anchoring fibrils |
| Epidermolysis bullosa acquisita | Type VII collagen | 290/145 | Anchoring fibrils |
| Bullous systemic lupus erythematosus | Type VII collagen | 290/145 | Anchoring fibrils |

**Table 8 : Direct immunofluorescence findings in the autoimmune bullous diseases[20]**

| Disease | Tissue bound immunoreactants | | |
|---|---|---|---|
| | **Type of immune reactants** | **Deposition pattern** | **Ultra structural site** |
| Pemphigus | IgG, C3 | "Intercellular space" (Epidermis) | Keratinocyte cell surface |
| Bullous pemphigoid | IgG, C3 | DEJ (Linear, homogenous) | Intra-Iamina lucida |
| Cicatricial pemphigoid | IgG, Other Igs (IgA), C3, Fibrin | DEJ (Linear, homogenous) | Intra-lamina lucida |
| Epidermolysis bullosa acquisita | IgG, Other Igs (IgA), C3, Fibrin | DEJ (Linear, homogenous) | Within or bekieath lamina densa |
| Dermatitis herpetiformis | IgA, rarely tra.ce IgM or C3 | Upper papillary dermis (granular; focal or continuous) | Sub-lamina densa |
| Linear IgA dermatosis (Adult & childhood) | IgA, rarely trace amount of other Igs or C3 | DEJ (Linear, homogenous) | Sub-lamina densa or Intra-lamina densa |
| Bullous eruption of SLE | IgG, other Igs (IgA), C3, Fibrin | DEJ (Linear, homogenous, granular or fibrillar) | Within or beneath lamina densa |
| Herpes gestationis | IgG, C3 | DEJ (Linear, homogenous) | Intra-lamina densa |

# METHODOLOGY

**Source of Data :** The present study is a one-year cross sectional descriptive study from November 2007 to October 2008. The source of data includes all cases of autoimmune vesiculobullous disorders attending dermatology OPD and referred cases from other departments, at KLES Dr Prabhakar Kore Hospital and MRC, Belgaum.

**Inclusion criteria :** All new clinically diagnosed cases of autoimmune vesiculobullous disorders.

**Exclusion criteria :** Old cases of autoimmune vesiculobullous disorders were excluded from the study. Cases of Toxic Epidermal Necrolysis / Steven Johnson Syndrome, and other blistering disorders that are not of autoimmune etiology were excluded. Patients who were not willing to undergo tests and who were not willing to enroll themselves in the study were also excluded.

A sample size of 20 was selected and this was calculated by taking 80% of the average yearly number of cases of autoimmune vesiculobullous disorders over the last three years attending the dermatology OPD at KLES Dr Prabhakar Kore Hospital and MRC, Belgaum.

Patient's demographic data, age of onset, duration of disease, symptoms, location and types of lesions, associated systemic disease and complications were noted in a pre- tested and pre-designed proforma after taking informed and written consent. Diagnosis was established by history and clinical examination.

Routine haematological and urine investigations such as Hb%, TC, DC, ESR, RBS, Urine routine and microscopy were done in all patients. Tzanck

smears were done in all patients. In all cases, skin biopsy and direct immunofluorescence were done after taking informed consent and counseling.

This was a descriptive study in which various clinical presentations, histological and direct immunofluorescence findings were examined. Analysis was done in the form of tables and noting the proportions and percentages. As this was a descriptive study, no tests of statistical significance were required.

**PROCEDURE**

**TZANCK SMEAR PREPARATION**

An early blister was selected and cleaned with 70% alcohol. The blister was gently deroofed with No.15 scalpel. The base was gently scraped with the blunt end of the scalpel. The cellular material so obtained was spread over a clean glass slide, air-dried and stained with May-Grunwald Giemsa stain, cleared and mounted. Tzanck test was considered positive when the smear showed characteristic acantholytic cells which are usually rounded and almost uniform in size with a relatively large hyperchromatic nucleus, and cytoplasm showing peripheral condensation, appearing usually discrete and occasionally in clusters. Inflammatory cells like eosinophils, neutrophils and lymphocytes may be seen mixed with these cells. (Annexure-III)

**BIOPSY**

A typical small early blister was chosen. It was cleaned using 70% alcohol. The area was then anaesthetized by infiltrating 2% lignocaine subcutaneously. An elliptical incision was made around the lesion. With a part of the normal skin through to the subcutaneous fat, the intact blister was removed. This was transferred to 10% formalin for histopathological examination.

**PROCESSING**

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

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

**DIRECT IMMUNOFLUORESCENCE (DIF)**

Immunofluorescence techniques are essential to supplement clinical findings and histopathology in the diagnosis of the immunobullous disorders. DIF studies require a biopsy of the patient's skin or the mucosa. Biopsies of the lesions themselves are not satisfactory as immunoreactants and tissue structures may be altered, making interpretation difficult. Perilesional skin, from an end of an elliptical biopsy taken for histopathology was biopsied. Tissue specimens in Michel's transport medium were then sent to the Department of Skin and STD, Kasturba Medical College, Manipal for direct immunofluorescence.

In the laboratory, the tissue specimens were washed with phosphate buffered saline (PBS) to remove any residual blood proteins. The tissue was then

snap frozen in a cryostat at -16 to -18 degree Celsius. A drop of optical clear transparent (OCT) embedding compound was added. The skin biopsy was then correctly oriented in the central fluid portion, covered with further OCT. DIF is a one step procedure. Tissue sections are incubated with Fluorescein isothiocyanate (FITC) – conjugated goat antihuman IgG antisera and viewed under fluorescent microscope (Annexure III).

# RESULTS

The present study is a one-year cross-sectional descriptive study and included 20 patients who attended the OPD of Dermatology, Venereology and Leprosy in KLES Dr Prabhakar Kore Hospital and MRC, Belgaum, from November 2007 to October 2008

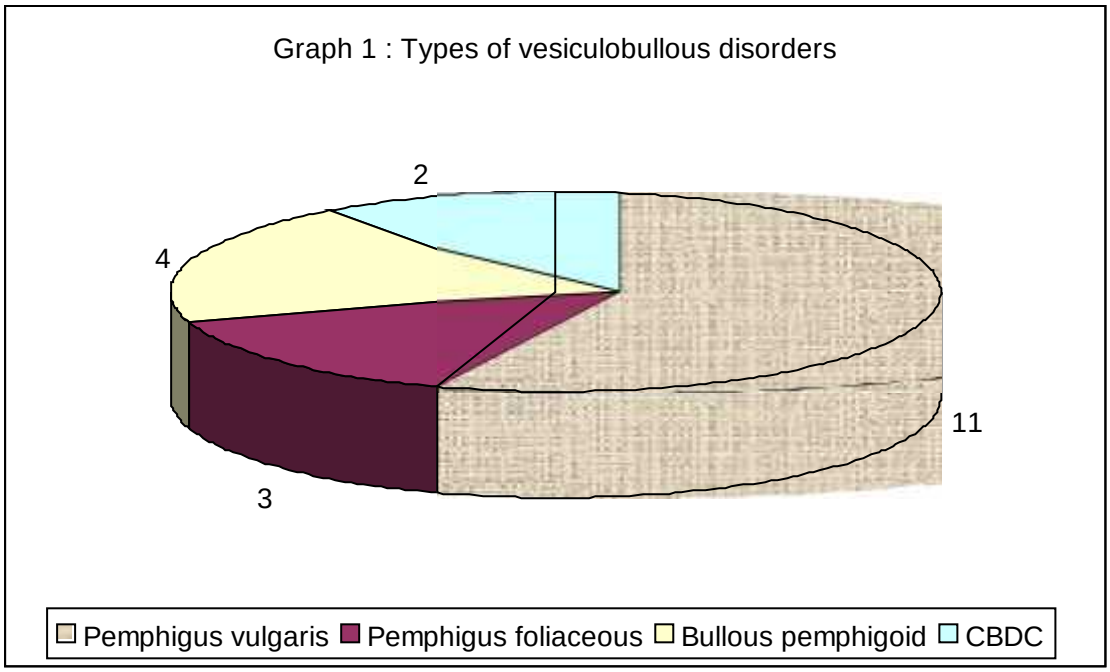
**Incidence**

The present study was conducted over a period of 12 months from November 2007 to October 2008 in the OPD of Dermatology, Venereology and Leprosy in KLES Dr Prabhakar Kore Hospital and MRC, Belgaum. A total number of 21,071 patients attended the OPD during this period, of which 24 were cases of autoimmune vesiculobullous disorders. However 4 patients were excluded from the study as they were not willing to participate. The incidence of autoimmune vesiculobullous disorders in our hospital works out to be 0.11%.

**Table 9 : Types of vesiculobullous disorders**

| Type | Number of cases | % of total |
|---|---|---|
| Pemphigus vulgaris | 11 | 55 |
| Pemphigus foliaceous | 3 | 15 |
| Bullous pemphigoid | 4 | 20 |
| CBDC | 2 | 10 |
| **Total** | **20** | **100** |

In the present study, pemphigus vulgaris constituted the most common vesiculobullous disorder constituting 55% (11 out of 20 cases), followed by bullous pemphigoid constituting 20% of the cases. Pemphigus foliaceous and chronic bullous disease of childhood constituted 15% and 10% of the cases respectively.

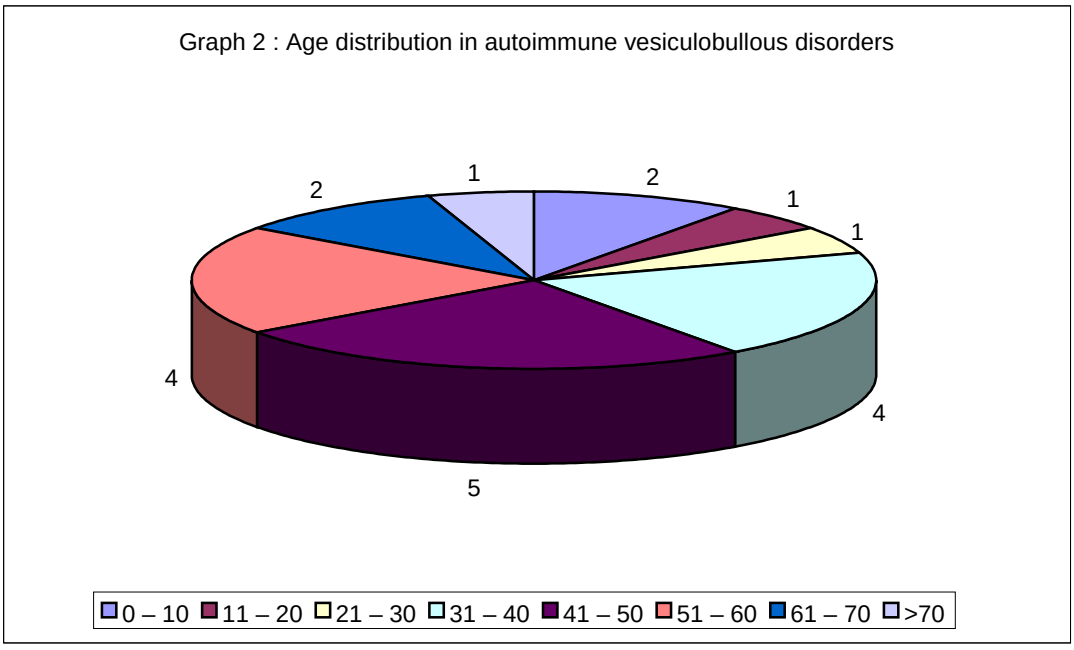


## Age distribution

**Table 10 : Age distribution in autoimmune vesiculobullous disorders**

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

In the present study, the youngest patient was 2 years old and the oldest patient was 85 years old. The maximum number of cases were seen in the age group 41-50 years (25%) followed by 31-40 years (20%) and 51-60 years (20%) age groups which had equal number of cases. The mean age of the study population was 42.55

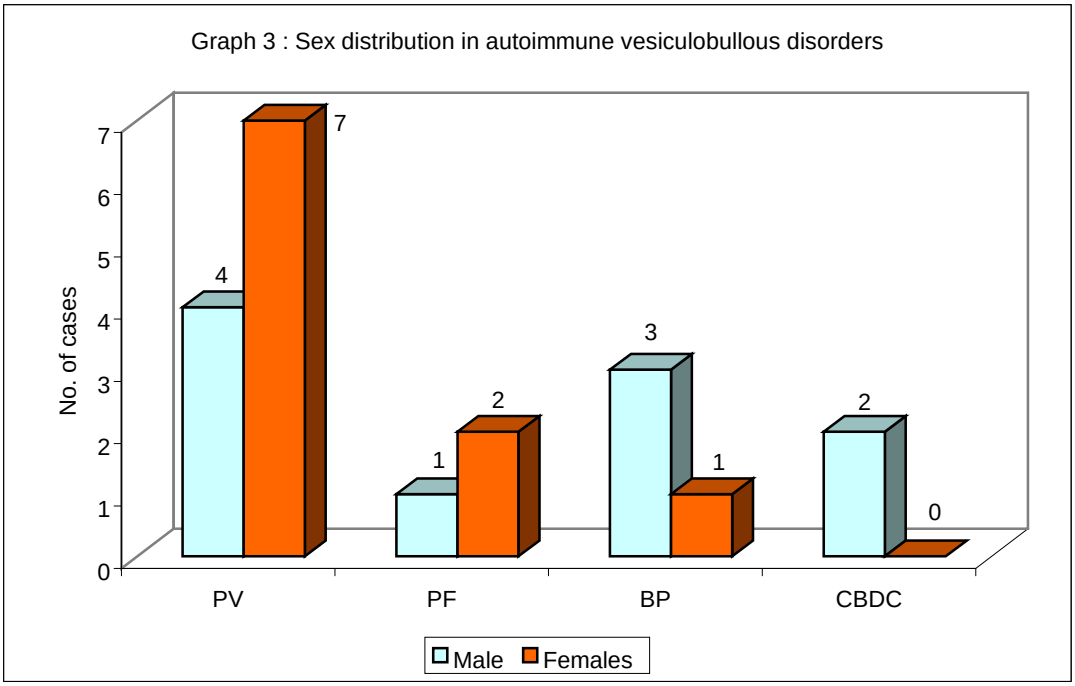


**Sex distribution**

**Table 11 : Sex distribution in autoimmune vesiculobullous disorders**

| Sex | PV | % | PF | % | BP | % | CBDC | % | Total |
|---|---|---|---|---|---|---|---|---|---|
| **Male** | 4 | 36.4 | 1 | 33.3 | 3 | 75 | 2 | 100 | 10 |
| **Female** | 7 | 63.6 | 2 | 66.7 | 1 | 25 | 0 | 0 | 10 |
| **Total** | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 | 20 |
| **M:F ratio** | 1:1.75 | 0 | 0.5:1 | 0 | 1:0.3 | 0 | - | 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 group of disorders had female predominance, pemphigus vulgaris showing a M:F ratio of 1:1.75 and pemphigus foliaceous showing a M:F ratio of 0.5:1.Bullous pemphigoid (M:F=1:0.3) and chronic bullous disease of childhood showed male predominance.

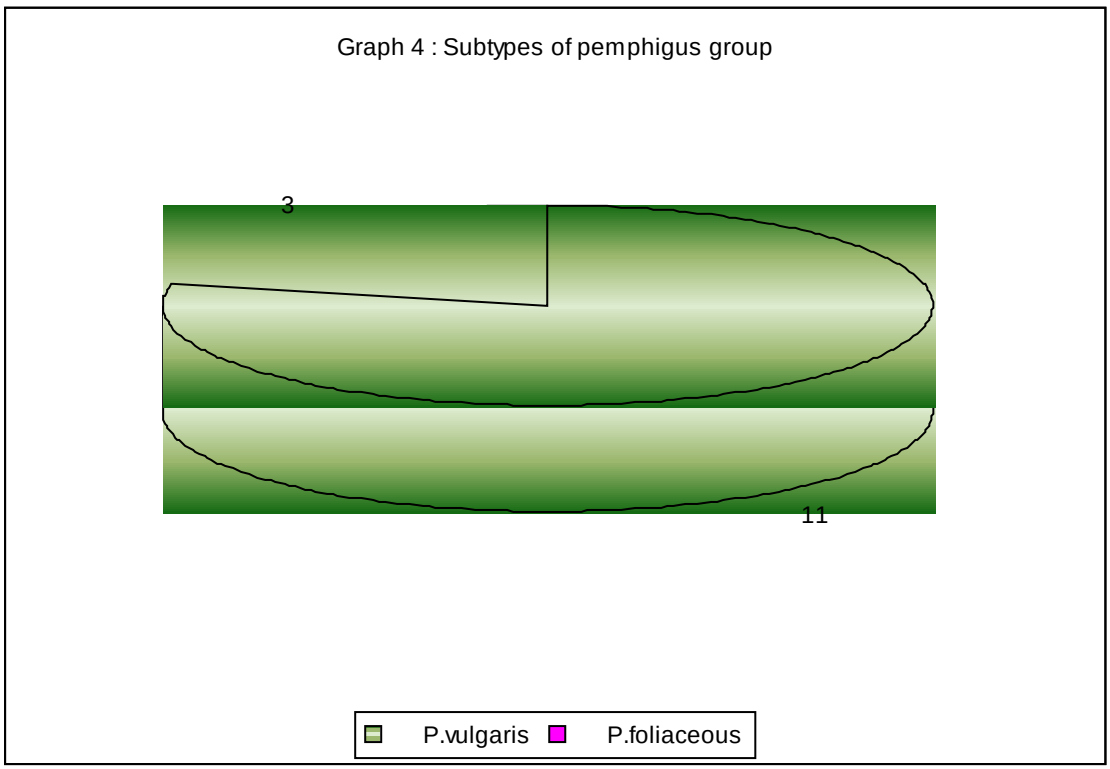


**Subtypes of pemphigus group**

**Table 12: Subtypes of pemphigus group**

| Subtype | Number | Percentage |
|---|---|---|
| P.vulgaris | 11 | 78.6 |
| P.foliaceous | 3 | 21.4 |
| **Total** | **14** | **100** |

In the present study, there were 11 cases of pemphigus vulgaris and 3 cases of pemphigus foliaceous in the pemphigus group. Pemphigus vulgaris (78.6%) was the most common subtype followed by pemphigus foliaceous (21.4%).



**Age distribution in pemphigus group**

**Table 13: Age distribution in pemphigus group**

| Age (years) | PV | % | PF | % |
|---|---|---|---|---|
| < 21 | 0 | 0 | 0 | 0 |
| 21 - 30 | 1 | 9 | 0 | 0 |
| 31 – 40 | 4 | 36.4 | 0 | 0 |
| 41 - 50 | 4 | 36.4 | 1 | 33.3 |
| 51 - 60 | 2 | 18.2 | 2 | 66.7 |
| Total | 11 | 100 | 3 | 100 |

In the present study, maximum numbers of patients with pemphigus vulgaris were in the age group 31-40 years (36.4%) and 41-50 years (36.4%). Pemphigus foliaceous presented commonly in the $6^{th}$ decade.

**Age distribution in bullous pemphigoid**

**Table 14: Age distribution in bullous pemphigoid**

| Age group | Number of patients | Percentage |
|---|---|---|
| 1 - 10 | 1 | 25 |
| 11 – 60 | 0 | 0 |
| 61 – 70 | 2 | 50 |
| 71 – 90 | 1 | 25 |
| **Total** | **4** | **100** |

In the present study, maximum number of patients with bullous pemphigoid belonged to the age group 61-70 years (50%). The youngest patient was 2 years old and the oldest patient was 85 years old.

**Clinical features**

**Table 15 : Clinical features of vesiculobullous disorders**

| Clinical features | PV | % | PF | % | BP | % | CBDC | % |
|---|---|---|---|---|---|---|---|---|
| **Symptoms** | | | | | | | | |
| Itching | 3 | 27.3 | 2 | 66.7 | 4 | 100 | 1 | 50 |
| Burning | 7 | 63.6 | 3 | 100 | 2 | 50 | 2 | 100 |
| Pain | 9 | 81.8 | 2 | 66.7 | 1 | 25 | 1 | 50 |
| **Lesions at presentation** | | | | | | | | |
| Oral lesions | 10 | 90.9 | 0 | 0 | 2 | 50 | 0 | 0 |
| Fluid filled lesions | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 |
| Erosions /crusting | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 |
| Red lesions/ rash | 1 | 9.1 | 0 | 0 | 4 | 100 | 2 | 100 |
| Others | 2 | 18.2 | 0 | 0 | 0 | 0 | 1 | 50 |

In the present study, pemphigus patients predominantly presented with fluid filled lesions, erosions, crusting (100%). Oral lesions were present in 90.9% of pemphigus vulgaris patients at the time of presentation, but absent in pemphigus foliaceous patients. Pain (81.8%) and burning sensation (63.6%) were the most common symptoms in pemphigus vulgaris while burning, itching and pain were the most common symptoms in pemphigus foliaceous. Bullous pemphigoid patients presented commonly with red lesions/rash followed by fluid filled lesions, erosions and crusting (100%). Oral lesions were present in 50% of the cases at the time of presentation. Itching (100%) was the most common symptom. Chronic bullous disease of childhood patients also presented commonly with red lesions/ rash, followed by fluid filled lesions, erosions and crusting. The patients had burning sensation (100%) as the most common symptom.

**Duration of illness**

**Table 16: Duration of illness in autoimmune vesiculobullous disorders**

| Duration (months) | PV | % | PF | % | BP | % | % | CBDC | % |
|---|---|---|---|---|---|---|---|---|---|
| 1-6 | 7 | 63.6 | 2 | 66.7 | 3 | 75 | 50 | 1 | 50 |
| 7-12 | 3 | 27.3 | 1 | 33.3 | 1 | 25 | 50 | 1 | 50 |
| 13-24 | 1 | 9.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| > 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

In the present study, most cases of pemphigus vulgaris (63.6%), pemphigus foliaceous (66.7%) and bullous pemphigoid (75%) had illness for 1–6 months before presentation. 50% of the cases of CBDC had illness for 1–6 months duration and 50% for 7–12 months before presentation.

**Associated conditions**

**Table 17 : Distribution of cases based on associated condition**

| Associated diseases | PV | % | PF | % | BP | % | CBDC | % |
|---|---|---|---|---|---|---|---|---|
| **Diabetes mellitus** | 1 | 9.1 | 0 | 0 | 2 | 50 | 0 | 0 |
| **Hypertension** | 2 | 18.2 | 1 | 33.3 | 3 | 75 | 0 | 0 |
| **Oral candidiasis** | 3 | 27.3 | 1 | 33.3 | 0 | 0 | 0 | 0 |
| **None** | 5 | 45.5 | 1 | 33.3 | 1 | 25 | 2 | 100 |

In the present study, bullous pemphigoid had the highest association with hypertension (75%) and diabetes mellitus (50%). Association with oral candidiasis was present in 27.3% of the cases of pemphigus vulgaris. Pemphigus foliaceous showed association with hypertension (33.3%) and oral candidiasis (33.3%).

**Types of lesions**

**Table 18: Morphology of lesions in vesiculobullous disorders**

| Lesions | PV | % | PF | % | BP | % | CBDC | % |
|---|---|---|---|---|---|---|---|---|
| **Vesicles** | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 |
| **Bullae** | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 |
| **Crusting** | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 |
| **Erosion** | 11 | 100 | 3 | 100 | 4 | 100 | 2 | 100 |
| **Pustule** | 2 | 18.2 | 0 | 0 | 0 | 0 | 1 | 50 |

In the present study, the most predominant lesions were vesicles, bullae, erosions and crusting in all the autoimmune vesiculobullous disorders. Pustules were present in 18.2% of the cases of pemphigus vulgaris and in 50% of the cases of chronic bullous disease of childhood.

**Consistency of blisters**

**Table 19 : Consistency of blisters in autoimmune vesiculobullous disorders**

| Consistency of blisters | PV | % | PF | % | BP | % | CBDC | % |
|---|---|---|---|---|---|---|---|---|
| **Tense** | 0 | 0 | 0 | 0 | 4 | 100 | 2 | 100 |
| **Flaccid** | 11 | 100 | 3 | 100 | 0 | 0 | 0 | 0 |

In the present study, flaccid blisters were present in all the cases of pemphigus, while tense blisters were present in all the cases of bullous pemphigoid and chronic bullous disease of childhood.

**Distribution of lesions**

**Table 20: Distribution of lesions in autoimmune vesiculobullous disorders**

| Sites | Pemphigus group | % of total | BP | % of total | CBDC | % of total |
|---|---|---|---|---|---|---|
| **Scalp** | 13 | 92.8 | 0 | 0 | 0 | 0 |
| **Face** | 12 | 85.7 | 1 | 25 | 2 | 100 |
| **Neck** | 10 | 71.4 | 3 | 75 | 2 | 100 |
| **Axilla** | 6 | 42.8 | 1 | 25 | 1 | 50 |
| **Chest** | 13 | 92.8 | 3 | 75 | 2 | 100 |
| **Back** | 14 | 100 | 4 | 100 | 2 | 100 |
| **Abdomen** | 11 | 78.6 | 4 | 100 | 2 | 100 |
| **Groin** | 6 | 42.8 | 1 | 25 | 2 | 100 |
| **R UL** | 12 | 85.7 | 4 | 100 | 2 | 100 |
| **L UL** | 12 | 85.7 | 4 | 100 | 2 | 100 |
| **R LL** | 13 | 92.8 | 4 | 100 | 1 | 50 |
| **L LL** | 13 | 92.8 | 4 | 100 | 1 | 50 |

In the present study, back (100%) was the most common site of distribution of lesions in the pemphigus group of patients followed by scalp (92.8%), chest (92.8%) and lower limbs (92.8%). The most common site of involvement in bullous pemphigoid was back, abdomen and all the four limbs (100%). The most common site of involvement in chronic bullous disease of childhood was face, neck, trunk, groin and the upper limbs (100%).

**Site of onset of lesion**

**Table 21 : Site of onset of lesions**

| Site of onset | No. of Pemphigus cases | No. of BP cases | No. of CBDC cases |
|---|---|---|---|
| **Oral mucosa** | 6 | 0 | 0 |
| **% of total** | 42.86% | 0 | 0 |
| **Scalp** | 1 | 0 | 0 |
| **% of total** | 7.14% | 0 | 0 |
| **Cutaneous** | 7 | 4 | 2 |
| **% of total** | 50% | 100% | 100% |
| **Total** | **14** | **4** | **2** |

In the present study, the most common site of onset of lesion in the pemphigus group was skin (50%), followed by the oral mucosa (42.86%). The most common site of onset of lesions was skin in bullous pemphigoid (100%) and chronic bullous disease of childhood (100%) also.

**Mucous membrane involvement**

**Table 22 : Mucosal involvement in autoimmune vesiculobullous disorders**

| Mucous membrane | PV | % of total | PF | % of total | BP | % of total | CBDC | % of total |
|---|---|---|---|---|---|---|---|---|
| **Oral** | 10 | 90.9% | 0 | 0 | 2 | 50% | - | - |
| **Conjunctival** | - | - | - | - | - | - | - | - |
| **Nasal** | 3 | 27.3% | - | - | - | - | - | - |
| **Genital** | - | - | - | - | - | - | - | - |
| **Total** | **13** | **-** | **0** | **-** | **2** | **-** | **-** | **-** |

In the present study, the most common site of mucosal involvement was oral mucosa, which was involved in 90.9% of the cases of pemphigus vulgaris and 50% of the bullous pemphigoid cases. 3 patients (27.3%) with pemphigus vulgaris also had involvement of the nasal mucosa in addition to involvement of the oral mucosa. There was no mucosal involvement in pemphigus foliaceous and chronic bullous disease of childhood.

**Oral lesions**

**Table 23: Oral lesions in autoimmune vesiculobullous disorders**

| Oral lesions | PV | PF | BP | CBDC |
|---|---|---|---|---|
| **Present** | 10 | 0 | 2 | 0 |
| **% of total** | 90.9 | 0 | 50 | 0 |
| **Absent** | 1 | 3 | 2 | 2 |
| **% of total** | 9.1 | 100 | 50 | 100 |
| **Total no. of pts** | **11** | **3** | **4** | **2** |

In the present study, oral lesions were present in 90.9% of the cases of pemphigus vulgaris and in 50% of the bullous pemphigoid cases. There was no oral involvement in pemphigus foliaceous and chronic bullous disease of childhood.

**Site of oral lesions**

**Table 24: Common sites of involvement of the oral mucosa**

| Site | Pemphigus vulgaris | Bullous pemphigoid |
|---|---|---|
| **Buccal mucosa** | 10 | 2 |
| **% of total** | 90.9 | 50 |
| **Hard palate** | 3 | 1 |
| **% of total** | 27.3 | 25 |
| **Tongue** | 1 | 0 |
| **% of total** | 9.1 | 0 |
| **Lips** | 9 | 0 |
| **% of total** | 81.8 | 0 |
| **Oropharynx** | 1 | 0 |
| **% of total** | **9.1** | **0** |

In the present study, the most common site of oral lesions in pemphigus vulgaris was buccal mucosa (90.9%) followed by the lips (81.8%). The most common site of oral lesions was the buccal mucosa (50%) followed by the hard palate (25%) in bullous pemphigoid.

**Signs**

**Table 25: Nikolsky's sign and Bulla spread sign**

| Signs | PV | % of total | PF | % of total | BP | % of total | CBDC | % of total |
|---|---|---|---|---|---|---|---|---|
| **Nikolsky's sign +ve** | 11 | 100 | 3 | 100 | 0 | 0 | 0 | 0 |
| **Bulla spread sign +ve** | 11 | 100 | 3 | 100 | 0 | 0 | 0 | 0 |

In the present study, Nikolsky sign and Bulla spread sign were positive in all the cases of pemphigus and negative in all the cases of bullous pemphigoid and chronic bullous disease of childhood.

**Investigations**

**Table 26: Tzanck smear**

| Type of cells | PV | % of total | PF | % of total | BP | % of total | CBDC | % of total |
|---|---|---|---|---|---|---|---|---|
| **Acantholytic cells** | 11 | 100 | 3 | 100 | 0 | 0 | 0 | 0 |
| **Eosinophils** | 2 | 18.2 | 1 | 33.3 | 3 | 75 | 1 | 50 |
| **Neutrophils** | 6 | 54.5 | 2 | 66.7 | 4 | 100 | 2 | 100 |
| **Other cells** | 3 | 27.3 | 1 | 33.3 | 2 | 50 | 2 | 100 |

Acantholytic cells were present on tzanck smear in all the patients with pemphigus group of disorders. There was also presence of inflammatory cells, predominantly neutrophils in pemphigus vulgaris (54.5%) and pemphigus foliaceous (66.7%). Tzanck smear showed absence of acantholytic cells and presence of inflammatory cells in all patients with bullous pemphigoid and chronic bullous disease of childhood, with predominance of neutrophils (100%) in bullous pemphigoid and predominance of neutrophils (100%) and other cells (100%) in chronic bullous disease of childhood.

**Table 27 : Findings of histopathological examination**

| Findings | PV | % of total | PF | % of total | BP | % of total | CBDC | % of total |
|---|---|---|---|---|---|---|---|---|
| **Epidermal changes** | | | | | | | | |
| Subcorneal cleft | 0 | 0 | 3 | 100% | 0 | 0 | 0 | 0 |
| Suprabasal cleft | 11 | 100% | 0 | 0 | 0 | 0 | 0 | 0 |
| Row of tombstone appearance | 6 | 54.5 % | 0 | 0 | 0 | 0 | 0 | 0 |
| Acantholytic cells | 11 | 100% | 3 | 100% | 0 | 0 | 0 | 0 |
| Spongiosis | 2 | 18.2% | 1 | 33.3% | 1 | 25% | 0 | 0 |
| Lymphocytes | 6 | 54.54% | 2 | 66.67% | 3 | 75% | 1 | 50% |
| Eosinophils | 4 | 28.57% | 1 | 33.33% | 4 | 100% | 2 | 100% |
| Neutrophils | 3 | 21.43% | 0 | 0 | 3 | 75% | 2 | 100% |
| **Dermal changes** | | | | | | | | |
| Subepidermal cleft | 0 | 0 | 0 | 0 | 4 | 100% | 2 | 100% |
| Papillary edema | 0 | 0 | 0 | 0 | 1 | 25% | 0 | 0 |
| Predominantly neutrophilic infiltration | 3 | 27.3% | 2 | 66.7% | 1 | 25% | 2 | 100% |
| Predominantly eosinophilic infiltration | 1 | 9% | 1 | 33.3% | 2 | 50% | 0 | 0 |
| Papillary microabscesses | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

In the present study, histopathology showed presence of subcorneal blisters with acantholytic cells and few inflammatory cells in the epidermis in all patients with pemphigus foliaceous. Suprabasal cleft with acantholytic cells were present in all patients and inflammatory cells in the epidermis were present in patients with pemphigus vulgaris. Row of tombstone appearance was seen in majority of the cases of pemphigus vulgaris (54.5%). Subepidermal cleft, and eosinophils in the epidermis were present in all bullous pemphigoid patients. Subepidermal cleft and inflammatory cells in the epidermis was present in all patients with chronic bullous disease of childhood. Dermis showed inflammatory cell infiltrate in all the subtypes of autoimmune vesiculobullous disorders, with predominance of neutrophils in pemphigus and CBDC and predominance of eosinophils in bullous pemphigoid.

**Direct immunofluorescence**

**Table 28 : Findings of Direct immunofluorescence in autoimmune vesiculobullous disorders**

| No. | Findings | | PV (n=11) | PF(n=3) | BP(n=4) | CBDC(n=2) |
|---|---|---|---|---|---|---|
| 1 | IgG deposition | Intercellular | 11 (100%) | 3 (100%) | 0 | 0 |
| | | BMZ | 0 | 0 | 4(100%) | 0 |
| 2 | IgA deposition | Intercellular | 0 | 1(33.3%) | 0 | 0 |
| | | BMZ | 0 | 0 | 1(25%) | 2(100%) |
| 3 | IgM deposition | Epidermis | 0 | 0 | 0 | 0 |
| | | Dermis | 0 | 0 | 0 | 0 |
| 4 | C3 deposition | Intercellular | 5 (45.4%) | 2(66.7%) | 0 | 0 |
| | | BMZ | 0 | 0 | 4(100%) | 0 |
| 5 | Fibrinogen | Intercellular | 0 | 0 | 0 | 0 |
| | | BMZ | 0 | 0 | 1(25%) | 0 |

On DIF examination, intercellular deposition of IgG was present in all the cases of pemphigus vulgaris which is a classical finding. Deposition of C3 in the intercellular space in the lower epidermis was seen in 45.4% of the cases of pemphigus vulgaris.

All the cases of pemphigus foliaceous also showed intercellular deposition of IgG in upper epidermis, while C3 deposition in upper epidermis was seen in 66.7% of the cases. Intercellular deposition of IgA was seen in one patient (33.3%).

All the bullous pemphigoid cases showed linear deposition of IgG and C3 in the basement membrane zone. Additional findings were deposition of IgA in basement membrane zone in one patient (25%) and deposition of fibrinogen in the basement membrane zone in another patient (25%).

Both the patients with CBDC showed linear deposition of IgA in the basement membrane zone.

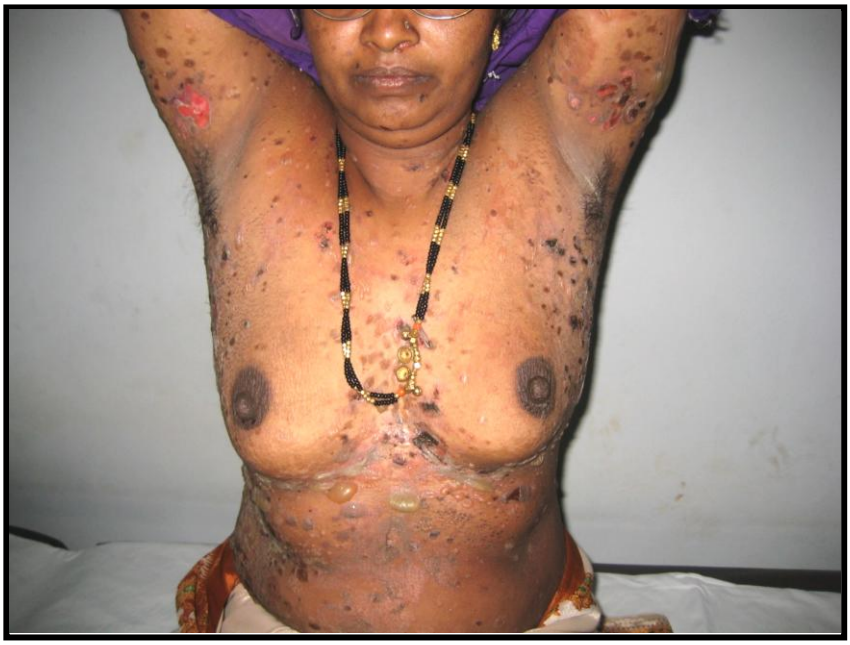


**Fig. 5 : Flaccid vesicles, bullae, erosions and crusted plaques on the trunk in a patient with pemphigus vulgaris**

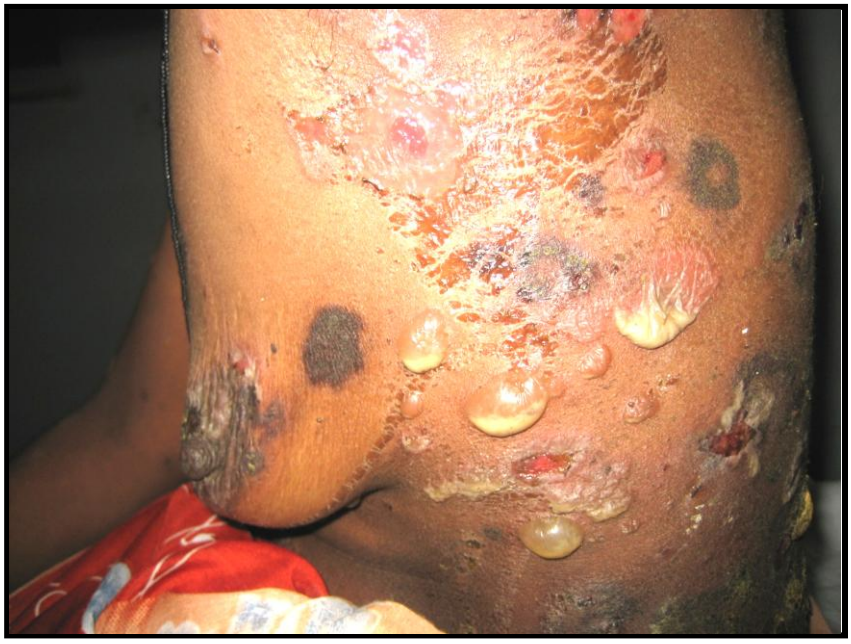


**Fig. 6 : Large flaccid bullae filled with pus, erosions and crusted plaques in a patient with pemphigus vulgaris**

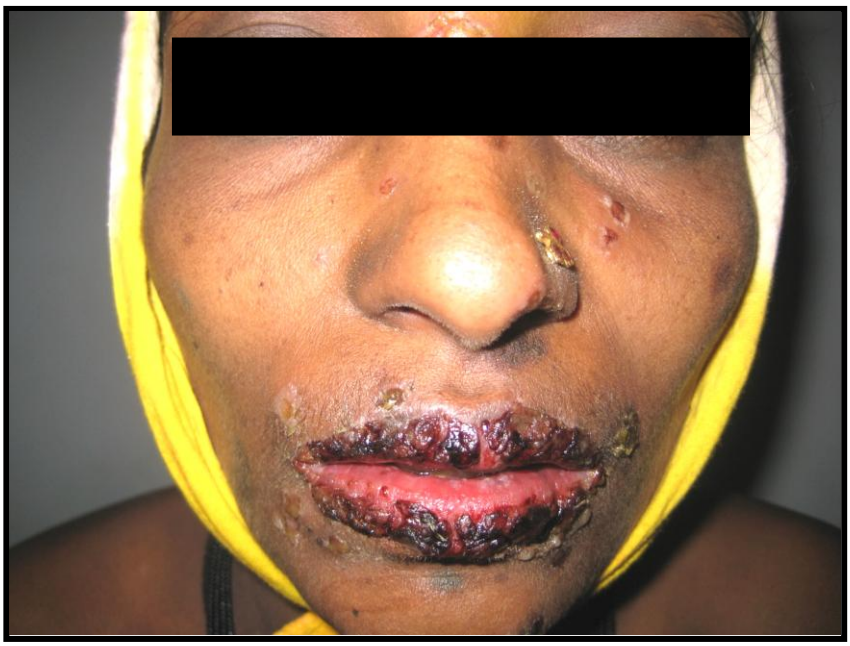


**Fig. 7 : Crusted plaques and erosions on the lips and crusted plaques on the face in a patient with pemphigus vulgaris**

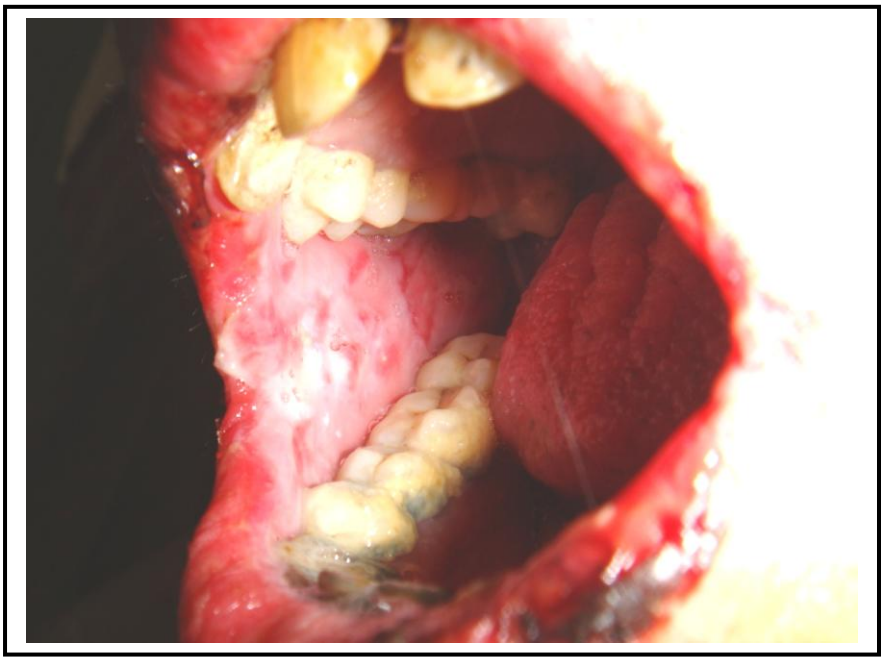


**Fig. 8 : Erosions in the buccal cavity and on the lips in a patient with pemphigus vulgaris**

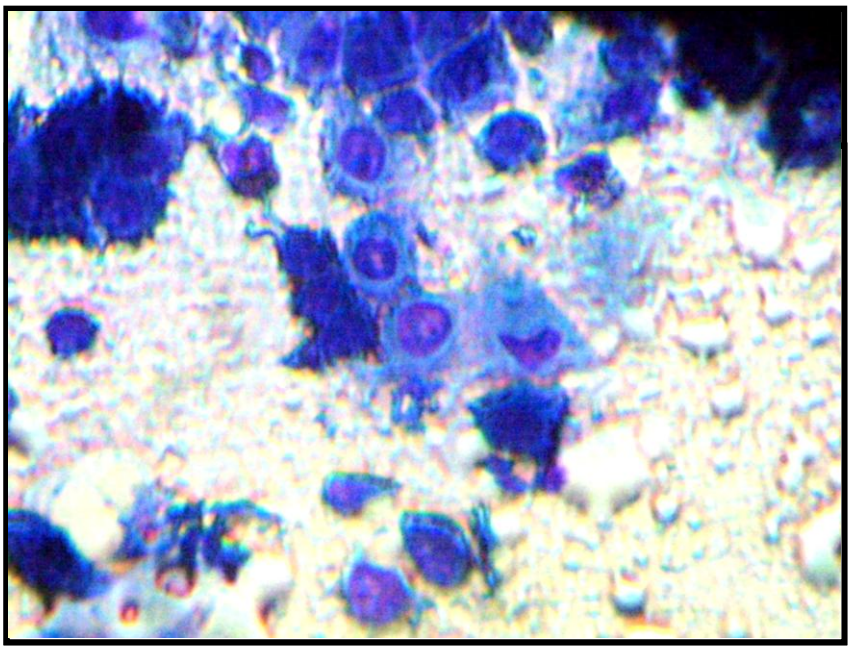


**Fig. 9 : Microscopy – Tzanck smear showing acantholytic cells in pemphigus vulgaris**

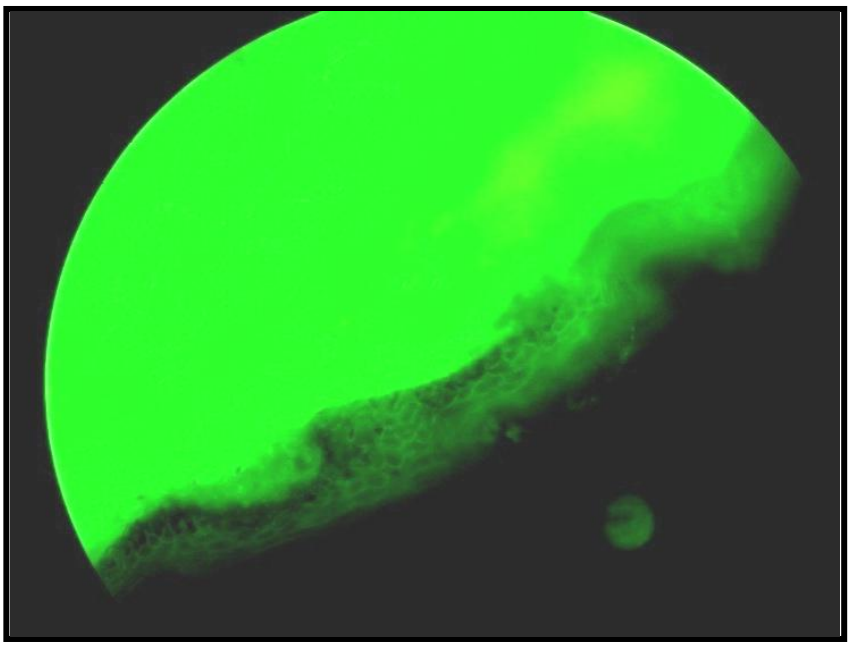


**Fig. 10 : Direct immunofluorescence – pemphigus vulgaris - showing deposition of IgG in squamous intercellular substance in the epidermis in a fishnet pattern**

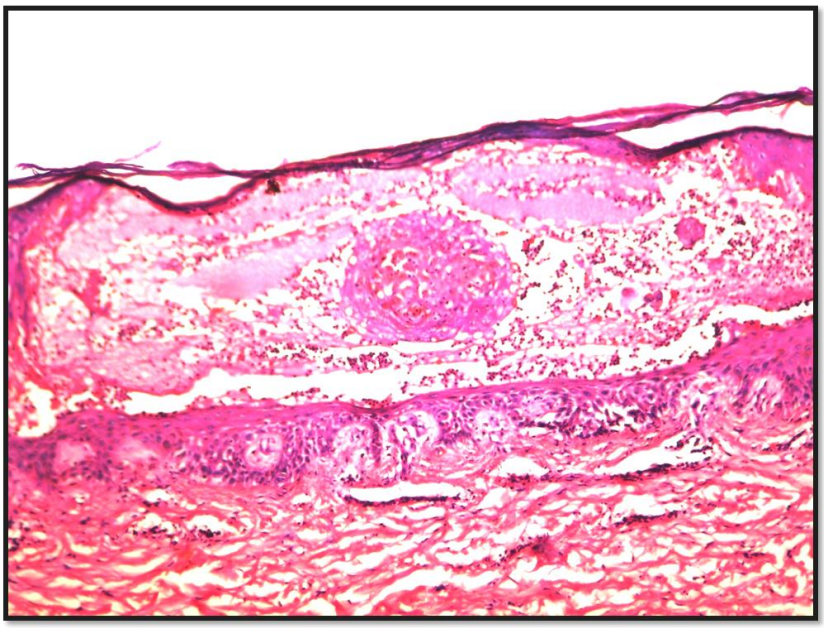


**Fig. 11 : Microscopy: Pemphigus vulgaris – suprabasal bulla – roof, floor, contents (acantholytic cells and inflammatory cells) of the bulla, dermis (100X, H and E)**

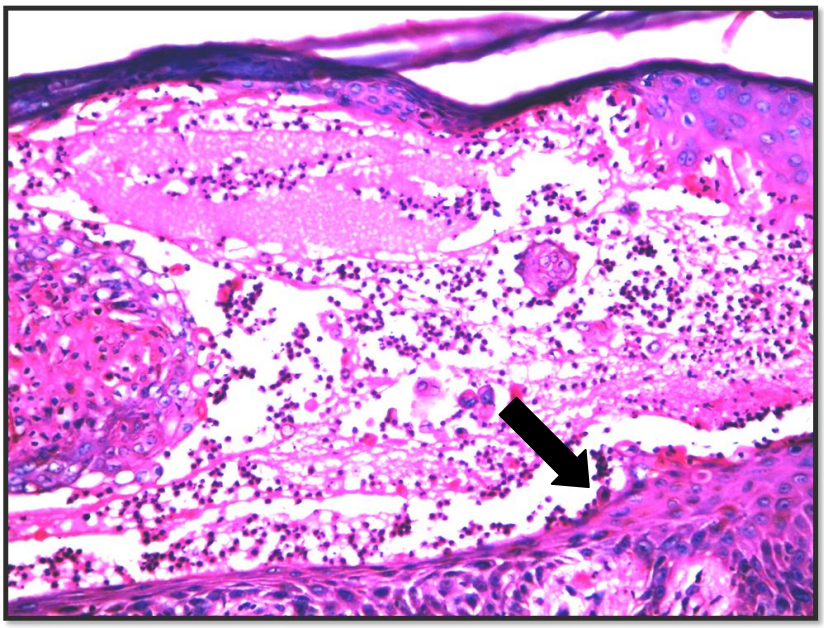


**Fig. 12 : Microscopy – pemphigus vulgaris – suprabasal bulla and "row of tombstone" appearance in the basal layer of the epidermis (200X, Hand E)**

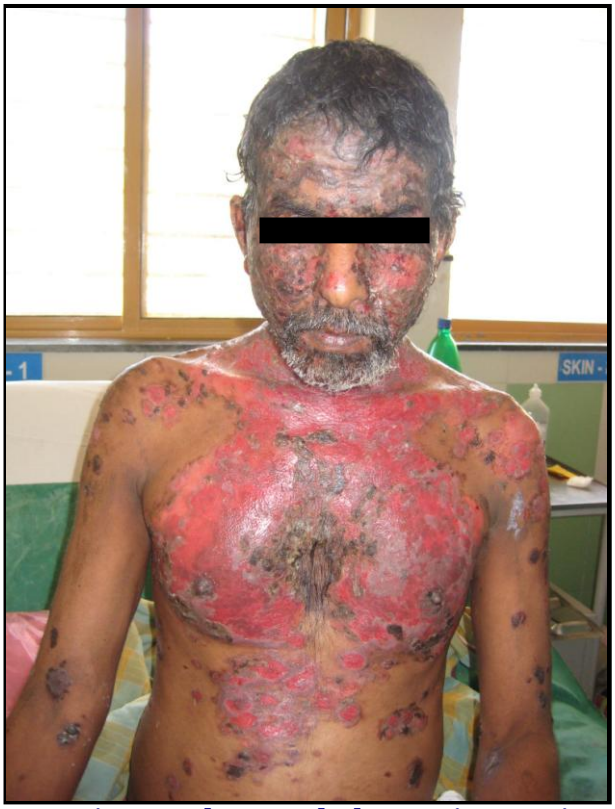



**Fig. 13 : Extensive erosions and crusted plaques in a patient with pemphigus foliaceous**

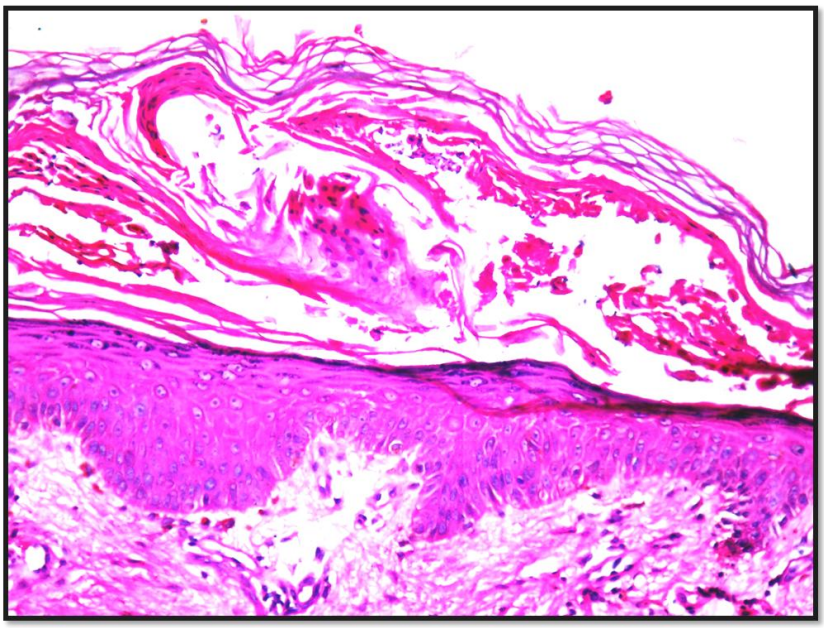


**Fig. 14 : Microscopy – pemphigus foliaceous – subcorneal bulla – roof, floor and contents (acantholytic cells and inflammatory cells) of the bulla**

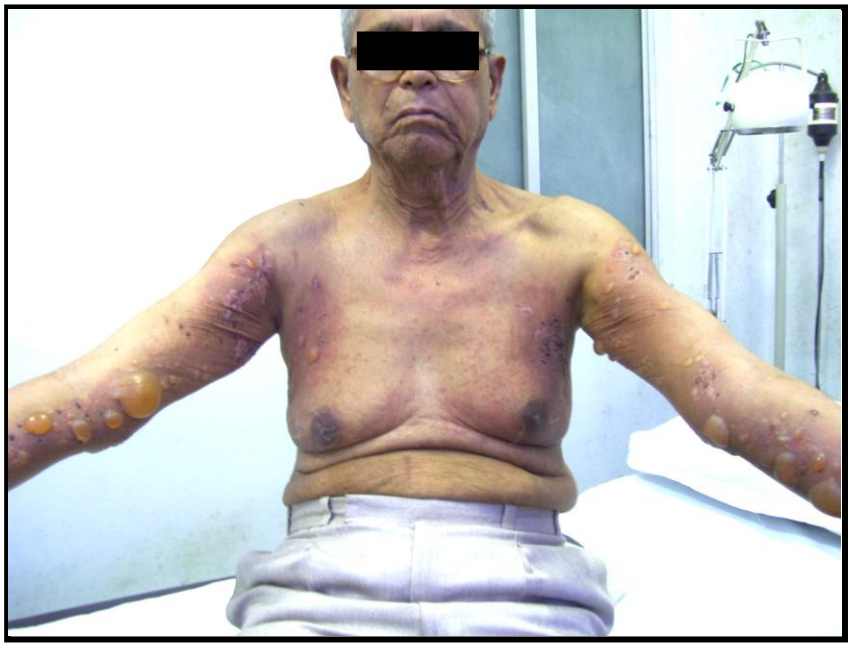


**Fig. 15 : Tense vesicles and bullae on erythematous base, erosions and crusts, mainly on the flexures in a patient with bullous pemphhigoid**

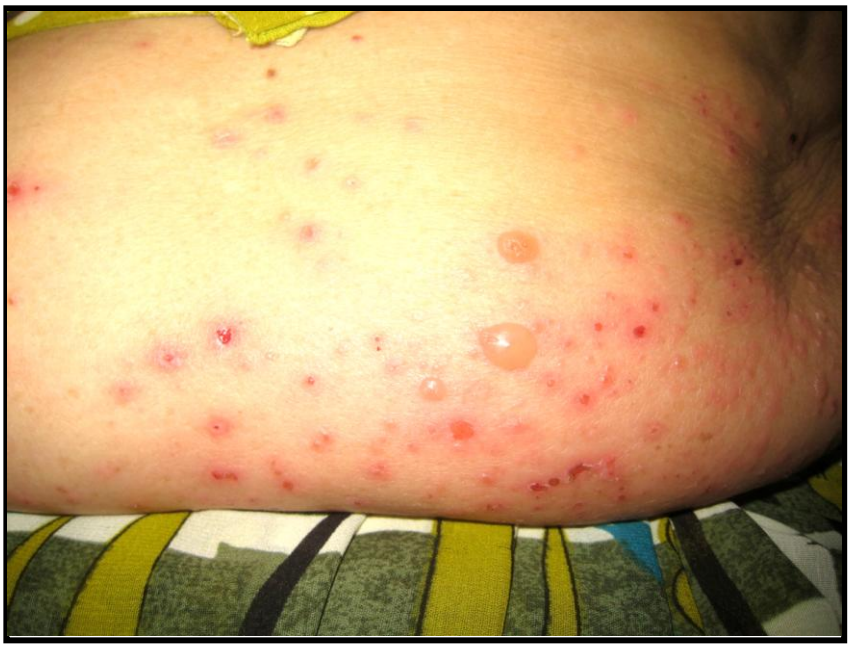


**Fig. 16 : Tense vesicles and bullae, erythema and urticarial lesions on the abdomen in a patient with bullous pemphigoid**

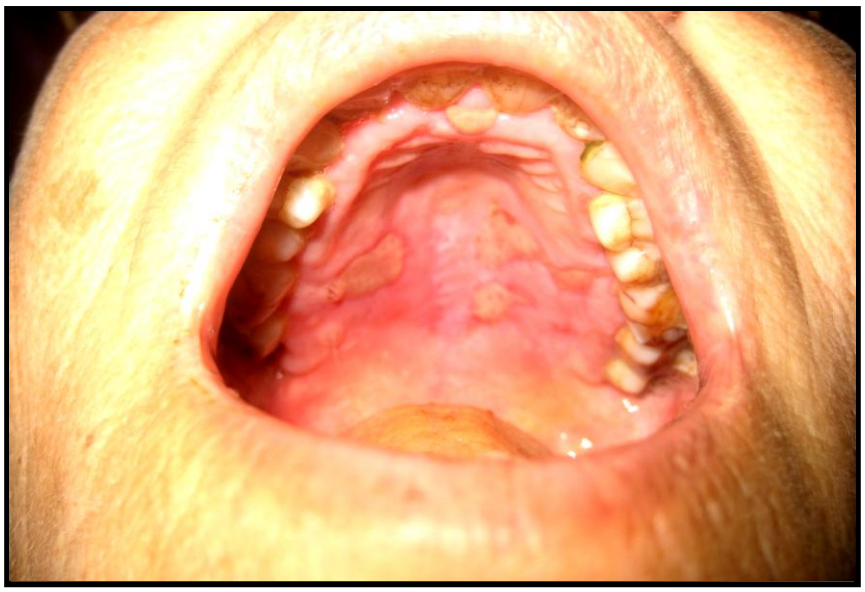


**Fig. 17: Erosions in the hard palate in a patient with bullous pemphigoid**

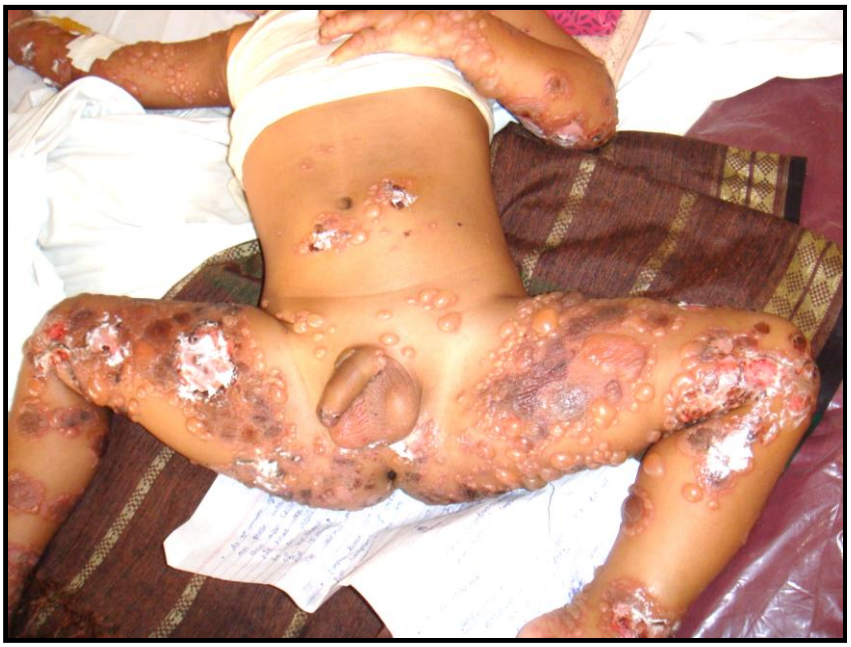


**Fig. 18 : Multiple tense vesicles, bullae, erosions and crusted plaques present all over the body in a 2 year old boy with bullous pemphigoid**

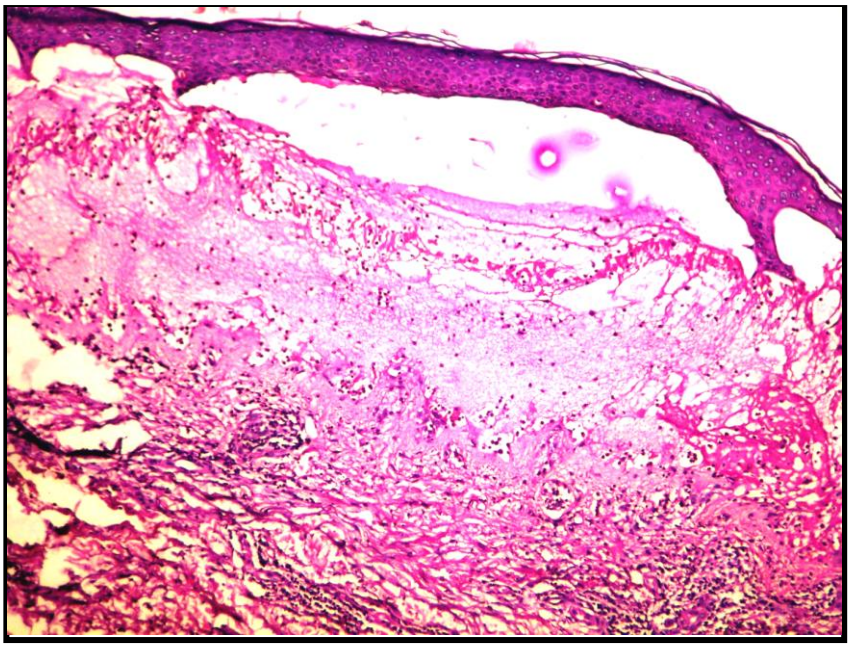


**Fig. 19 : Microscopy – bullous pemphigoid – subepidermal bulla – roof , floor and contents (eosinophils, fibrin) of the bulla, eosinophilic infiltrate in the dermis (200X, Hand E)**

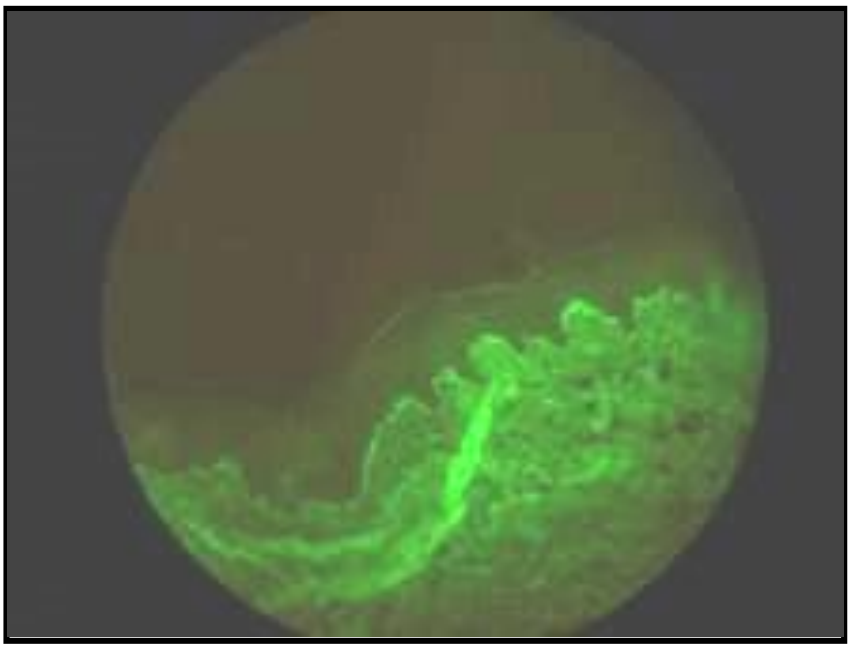


**Fig. 20 : Direct immunofluorescence – bullous pemphigoid – showing continuous linear deposition of IgG and C3 along basement membrane zone**

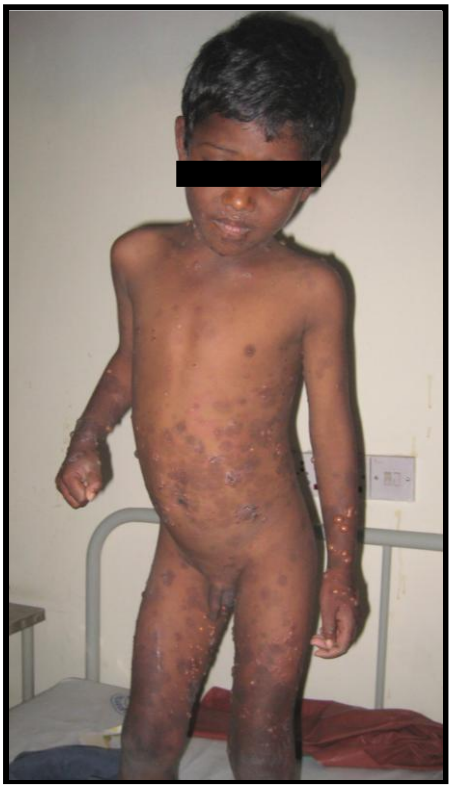


**Fig. 21 : Tense vesicles, bullae and crusted plaques in a patient with chronic bullous disease of childhood**

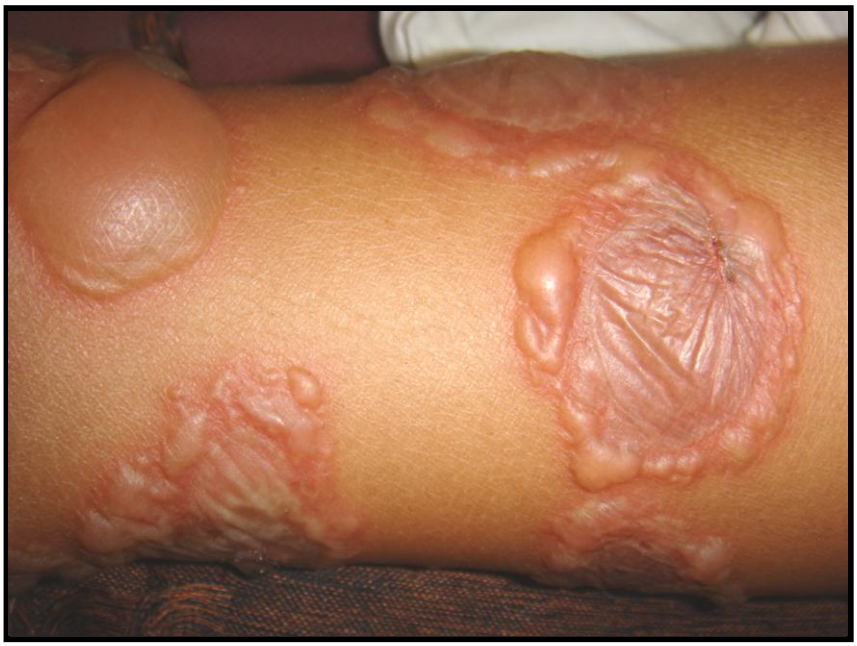


**Fig. 22 : Cluster of jewel appearance of the lesions in chronic bullous disease of childhood**

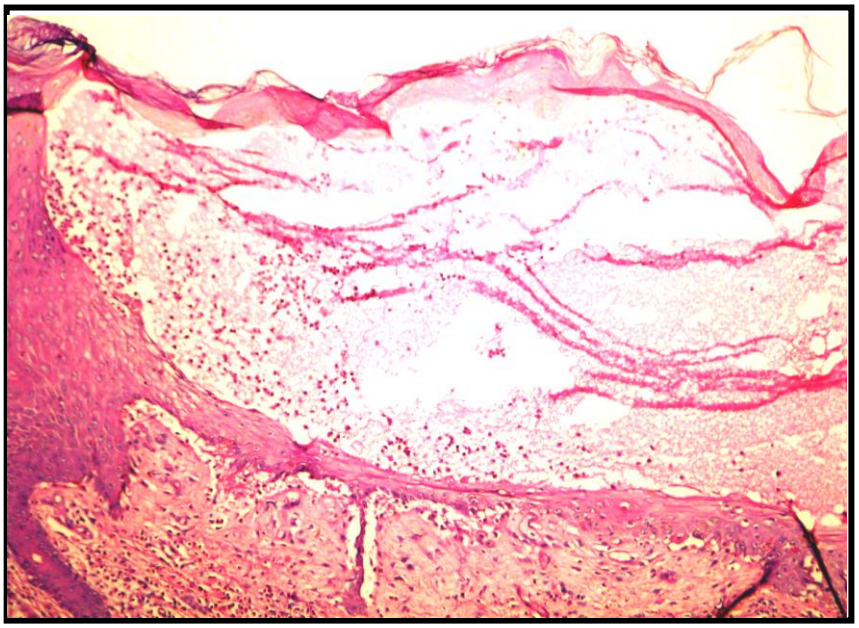


**Fig 23 : Microscopy – chronic bullous disease of childhood – subepidermal bulla – roof, floor and contents (eosinophils and few neutrophils) of the bulla (100X, Hand E)**

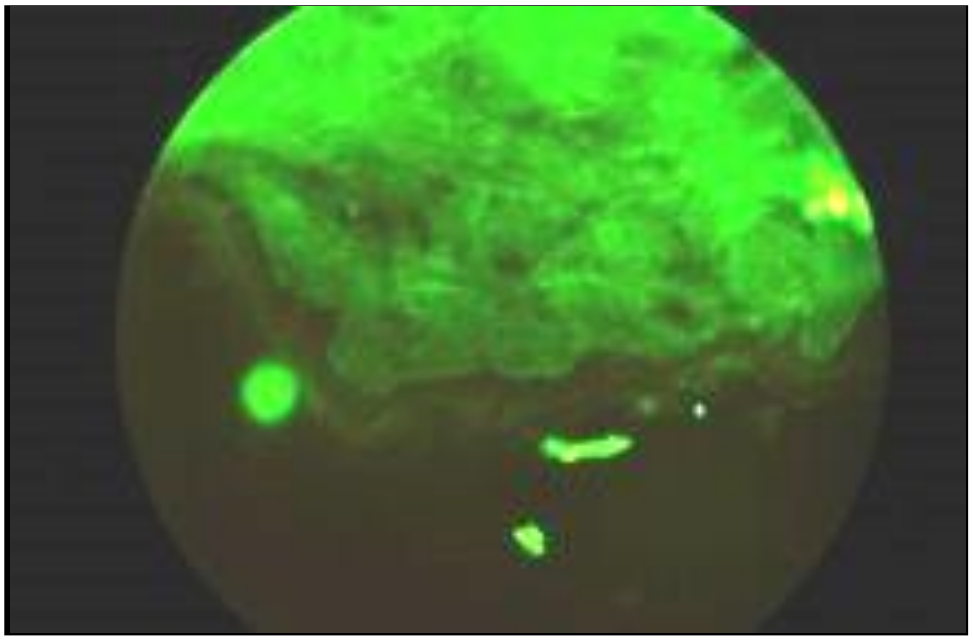


**Fig 24 : Direct immunofluorescence – chronic bullous disease of childhood – showing linear homogenous deposition of IgA along basement membrane zone**

# DISCUSSION

## Incidence of autoimmune vesiculobullous disorders

The present study was conducted over a period of 12 months from November 2007 to October 2008 in the OPD of Dermatology, Venereology and Leprosy in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

A total number of 21,071 patients attended the Dermatology OPD during this period out of which 24 were cases of autoimmune vesiculobullous disorders. So the incidence of autoimmune vesiculobullous disorders among the OPD cases in one year works out to be 0.11%. Another study done by Kanwar AJ et al[68] showed an incidence of 0.3% which is comparable.

However, it must be stated that ours is a hospital-based study and so the above number does not reflect the true incidence of autoimmune vesiculobullous disorders in the community.

## Types of autoimmune vesiculobullous disorders

**Table 29 : Analysis of types of autoimmune vesiculobullous disorders**

| No. | Studies | Year | PV | PF | PVE | BP | DH | CBDC | SCPD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Kanwar AJ et al[68] | 1987 | 24 (36.3%) | - | - | 21 (31.8%) | 8 (12.12%) | 6 (9.1%) | 2 (3.03%) |
| 2 | Micali G et al[69] | 1998 | 63 (75%) | 2 (2%) | 2 (2%) | - | - | - | - |
| 3 | Arya SR et al[70] | 1999 | 43 (61.4%) | 2 (2.9%) | 2(2.9%) | - | - | - | - |
| 4 | Tsankov N et al[71] | 2000 | 57 (77.03%) | - | - | - | - | - | - |
| 5 | Ljubojevic et al[72] | 2002 | 154 (76%) | 5 (2%) | 5 (2%) | - | - | - | - |
| 6 | Nanda A et al[2] | 2004 | 48% | - | - | 27% | - | - | - |
| 7 | Chams-Davatchi et al[73] | 2005 | 1111 (91.9%) | 33(2.7 %) | 33(2.7% ) | - | - | - | - |
| 8 | Present study | 2008 | 11(55%) | 3 (15%) | - | 4 (20%) | - | 2 (10%) | - |

In the present study, pemphigus vulgaris was the most common vesiculobullous disorder constituting 55% (11 out of 20 cases) followed by bullous pemphigoid constituting 20% (4 out of 20 cases) of the total cases of autoimmune vesiculobullous disorders. Pemphigus foliaceous constituted 15% of the cases. The pattern of frequency of different types of autoimmune vesiculobullous disorders correlates closely to Nanda A et al study.[2] The incidence of chronic bullous disease of childhood was 10% in our study which correlates closely with Kanwar AJ et al study.[68] Other diseases like dermatitis herpetiformis and subcorneal pustular dermatoses were not found during the present study, unlike Kanwar et al study, which showed few cases of the above mentioned studies.

**Age and sex distribution of autoimmune vesiculobullous disorders**

**Table 30 : Mean age of onset and sex distribution of vesiculobullous disorders in different studies including present study**

| No. | Studies | Year | PV | | PF | | PVE | | BP | | CBDC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Age | M:F | Age | M:F | Age | M:F | Age | M:F | Age | M:F |
| 1. | Micali G et al[69] | 1998 | 56 | 1:2.2 | - | - | 22.5 | - | - | - | - | - |
| 2. | Aboobaker J et al[74] | 2001 | 48 | 1:1.7 | 43 | 1:1.4 | - | - | - | - | - | - |
| 3. | Present study | 2008 | 41.9 | 1:1.75 | 51.5 | 0.5:1 | - | - | 54.5 | 1:0.3 | 9 | 2:0 |

The mean age of onset for pemphigus vulgaris was 41.9 years and M: F ratio was 1:1.75 in the present study which is almost similar to the results of Aboobaker J et al study.[74] Pemphigus foliaceous presented in the early 6$^{th}$ decade in the present study as compared to early 5$^{th}$ decade in Aboobaker J et al study.

Females had a slightly higher incidence of pemphigus vulgaris and pemphigus foliaceous compared to males in the present study. The above studies[69, 74] also show similar results.

The mean age of onset of bullous pemphigoid was 54.5 years with a higher incidence in males in the present study. The mean age of onset of chronic bullous disease of childhood was 9 years with both the patients being male patients in the present study.

## PEMPHIGUS VULGARIS

**Table 31: Incidence, age group and clinical feature of pemphigus vulgaris in different studies including present study**

| No. | Features | | Present study | Handa F et al study[27] | Arya SR et al study[70] |
|---|---|---|---|---|---|
| 1. | Year | | 2008 | 1973 | 1999 |
| 2. | No. of cases | | 11 (55% ) | 83 (97.6%) | 43 (61.4%) |
| 3. | No. of cases in age group 21-60years | | 11(100%) | 73 (87.9%) | 88.4% |
| 4. | Site of onset of lesions | Mucosal | 6(54.54%) | 83% | 37.2% |
| | | Cutaneous | 5(45.54%) | 17% | 62.8% |
| 5. | Type of lesions: Flaccid vesiculobullous | | 11(100%) | Majority | 100% |
| 6. | Mucous membrane involvement | | 10(90.9%) | 71(85.5%) | 72.1% |
| 7 | Nikolsky sign | | 11(100%) | Majority | 97.2% |

In the present study, pemphigus vulgaris constituted 55% of the total number of cases of autoimmune vesiculobullous disorders, which is slightly lesser than that in Arya SR et al study.[70] The incidence of pemphigus vulgaris is relatively lesser than that in the other studies. This could be because of the small sample size taken in the present study. The age of the patients ranged from 30 to 56 years. All cases were in the age group 21-60 years, which is in agreement with other Indian studies listed in the table above.[27, 70]

The site of onset of lesion was mucosal in majority (6 out of 11 cases, 54.54%) of the cases in the present study which is in agreement with Handa F et al study.[27]

All the patients had classical features of pemphigus vulgaris, i.e. flaccid vesiculobullous lesions. Mucosal involvement was present at one time or the other during the disease course in 90.9% of the cases. Nikolsky sign was positive in all the cases. Similar findings have been reported by other Indian studies mentioned above.

Bulla spread sign or Asboe – Hansen sign (a part of Nikolsky sign, which is due to acantholysis) was also present in all the cases of pemphigus vulgaris.

**Table 32: Specific sites of mucosal involvement in pemphigus vulgaris in different studies**

| No. | Study | Year | Oral mucosa | Conjunctival mucosa | Nasal mucosa | Genital mucosa | Total |
|---|---|---|---|---|---|---|---|
| 1. | Micali G et al[69] | 1998 | 40 | 7 | - | 4 | 51 |
| 2. | Present study | 2008 | 10 | - | 3 | - | 13 |

Oral mucosa was the most common site of mucosal involvement in pemphigus vulgaris in the present study (10 out of 11 cases) which is similar to Micali G et al study.[69] 3 patients had involvement of the nasal mucosa. Involvement of the conjunctival and genital mucosa was not seen in the present in the present study. This finding is different from that of the study mentioned above. Changing trend in the pattern of the disease or the patients receiving prior treatment could be the possible reason for this.

**Table 33: Duration of illness at the time of presentation**

| No. | Duration of illness (months) | Present study | Handa F et al 1973[27] |
|---|---|---|---|
| 1. | 1 – 6 | 7 | 43 |
| 2. | 7 – 12 | 3 | 30 |
| 3. | 13 - 24 | 1 | 6 |
| 4. | > 24 | 0 | 4 |

In the present study, most cases of pemphigus vulgaris had illness for 1–6 months before the presentation similar to Handa et al study.[27]

**Investigations**

**Table 34 : Tzanck smear findings in pemphigus vulgaris**

| No. | Study | Year | Tzanck smear for acantholytic cells |
|---|---|---|---|
| 1. | Present study | 2008 | 11 (100%) |
| 2. | MM Huda et al study[75] | 1998 | 42 (100%) |

Tzanck smear observations showed acantholytic cells in 100% of the cases. This is in concordance with the study conducted by MM Huda et al.[75] The main inflammatory infiltrate was of neutrophils and few eosinophils and lymphocytes.

The main histopathologic features observed in cases of pemphigus vulgaris was acantholysis with suprabasal bulla which was seen in all the cases. Acantholysis is the hallmark and a prerequisite for the diagnosis of pemphigus. This was originally described by Lever and later confirmed by many other authors like Fernandez JC et al,[76] Handa et al[27] and Arya S.R. et al[70]

**Table 35 : Histopathological findings in pemphigus vulgaris**

| No. | Features | Present study | Arya SR et al[70] 1999 |
|---|---|---|---|
| 1. | Suprabasal bulla | 100% | 81.4% |
| 2. | Acantholysis | 100% | 93% |
| 3. | Row of tombstone appearance | 54.5% | 41.8% |
| 4. | Inflammatory infiltrate in the bullous cavity | 54.5% | 53.5% |

The present study showed acantholysis and suprabasal bulla in all cases of pemphigus vulgaris which closely relates to the finding in Arya SR et al[70] study. Row of tombstone appearance of basal cells is another histological feature favouring the diagnosis of pemphigus vulgaris which is present in 54.5% of the cases which is slightly higher than the finding in the above study. Other findings were presence of inflammatory infiltrate in the bullous cavity (54.5%) which correlates the finding in the above study.

**Table 36 : Direct immunofluorescence findings in pemphigus vulgaris**

| No. | Studies | No. of patients with PV | DIF done | DIF positive |
|---|---|---|---|---|
| 1. | Present study | 11 | 11(100%) | 11(100%) |
| 2. | Kumar S et al study[78] 1995 | 20 | 20(100%) | 20(100%) |

Direct immunofluorescence was done in all the patients with pemphigus vulgaris and the findings were suggestive of pemphigus vulgaris in all the cases. This is similar to the finding of Kumar S et al study.[78]

**PEMPHIGUS FOLIACEOUS**

The disease was seen in 3 cases constituting 15% of the total cases of autoimmune vesiculobullous disorders. This correlates closely with the findings of Fernandez J et al study[77]

**Table 37: Incidence and age distribution in pemphigus foliaceous**

| No. | Features | Present study | Fernandez J et al[76] 1970 | Arya SR et al[70] 1999 |
|---|---|---|---|---|
| 1. | No. of cases | 3 (15%) | 18 (18%) | 25 (35.7%) |
| 2. | Age 21 – 60 years | 3 (100%) | 94.4% | 80% |

The incidence is however lesser than that in Arya SR et al study[70], probably because of the lesser sample size in the present study. All the cases in the present study were in the age group 21 – 60 years, which closely correlates with the above study.

**Table 38: Clinical features and histopathological findings in pemphigus foliaceous**

| No. | Features | Present study | Fernandez J et al[76] 1970 | Arya SR et al[70] 1999 |
|---|---|---|---|---|
| 1. | Types of lesion: Flaccid vesiculobullous lesions | 3(100%) | 100% | 100% |
| 2. | Mucous membrane involvement | 0 | Nil | 20% |
| 3. | Nikolsky's sign | 3(100%) | 100% | 94.7% |
| 4. | Subcorneal bulla | 3(100%) | 80% | 60% |
| 5. | Acantholysis | 3(100%) | 100% | 96% |

The patients presented predominantly with extensive flaccid vesiculobullous lesions (100%). The other predominant lesions seen were extensive crusts and erosions. Nikolsky sign was positive in all the cases. These findings were similar to the above studies.

Mucous membrane involvement was absent in the present study and Fernandez J et al study,[76] which differs from the findings of Arya S.R. et al[70] study where mucous membrane was involved in 20% of the cases. This variation could possibly be due to decreased involvement of mucous membrane in pemphigus foliaceous as compared to pemphigus vulgaris.

Tzanck smear was positive for acantholytic cells in all the cases. The salient histopathological features of pemphigus foliaceous observed in our study were acantholysis (100%) and subcorneal bulla (100%), identical to that observed by the above mentioned studies.

**Table 39: Direct Immunofluorescence findings in pemphigus foliaceous**

| No. | Studies | No. of patients with PF | DIF done | DIF positive |
|---|---|---|---|---|
| 1. | Present study | 3 | 3 | 3 (100%) |
| 2. | Chams-Davatchi C et al study[73] 2005 | 89 | 34(38.2%) | 30 (88%) |

Direct immunofluorescence was done in all cases of pemphigus foliaceous and the findings were suggestive of pemphigus foliaceous in all the cases. This finding is almost identical to that of Chams-Davatchi C et al[73] study

**BULLOUS PEMPHIGOID**

**Table 40 : Frequency of bullous pemphigoid**

| No. | Features | No. of cases of bullous pemphigoid | % of total |
|---|---|---|---|
| 1. | Present study | 4 | 36.4% |
| 2. | Nanda A et al study[2] 2004 | 43 | 27% |

In our study, 4 cases of bullous pemphigoid were observed, of which 3 were adults and one was a child. Bullous pemphigoid constituted 36.4% of the total cases of autoimmune vesiculobullous disorders, which is almost similar to the finding of Nanda A et al study.[2]

The patients presented predominantly with itching and urticarial lesions followed by the appearance of fluid filled lesions.

**Table 41: Mean age of onset and sex ratio in bullous pemphigoid**

| No. | Studies | Mean age (years) | M: F ratio |
|---|---|---|---|
| 1. | Present study 2008 | 54.5 (2 – 85) | 1:0.33 |
| 2. | Bernard P et al study 1995[38] | 82.4 | 1:1.48 |
| 3. | Wong et al study 2002[40] | 77 (51 – 101) | 1:2 |
| 4. | Nanda A et al study 2004[2] | 65.97 | 1:5.75 |

The mean age of onset among the adult patients of bullous pemphigoid was 72 years in the present study which is similar to Wong et al study[40].

There was a male predominance in the present study with a male to female ratio (M: F) of 1: 0.33, which is against the observations made in the above studies.

**Table 42: Distribution of lesions in bullous pemphigoid**

| No. | Site of lesions | Present study | Budimir J et al study[79] 2008 |
|---|---|---|---|
| 1. | Trunk | 4(100%) | 14 (82%) |
| 2. | Extremities | 4(100%) | 18 (100%) |

Trunk and extremities were the most common sites of involvement (100%) which is similar to the finding observed by Budimir J et al.[79] All the patients presented with itching or urticarial lesions as a prodromal symptom, followed by the appearance of tense vesicles and bullae. The lesions were present over an erythematous base in 75% of the cases.

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**Table 43: Oral mucosal involvement in bullous pemphigoid**

| No. | Study | Number of patients with oral mucosal involvement |
|---|---|---|
| 1. | Present study | 2 (50%) |
| 2. | Kanwar AJ et al study[68] 1987 | 7 (70%) |

Involvement of oral mucosa was seen in 50% of the cases in the present, which is lower than that reported by the above study. Mucous membranes can get involved in bullous pemphigoid, occasionally as mucous pemphigoid, but the frequency is lesser than that seen in the pemphigus group. Tzanck smear examination revealed only inflammatory cells. No acantholytic cells were identified in any of the cases studied.

**Table 44: Histopathological findings in bullous pemphigoid**

| No. | Features | Present study | Nishioka K et al study[15] 1984 |
|---|---|---|---|
| 1. | Subepidermal blister | 4 (100%) | All cases |
| 2. | Predominant neutrophilic infiltrate in dermis | 1 (25%) | 2 (8%) |
| 3. | Predominant eosinophilic infiltrate in the dermis | 2 (50%) | 8 (32%) |
| 4. | Papillary microabscesses | - | - |

Histopathological examination revealed subepidermal bulla in all the cases. Predominant eosinophilic infiltration in the dermis was more common than predominant neutrophilic infiltration, which corresponds to the above study.

**Table 45: Direct Immunofluorescence findings in bullous pemphigoid**

| No. | Studies | No. of cases of BP | DIF done | DIF positive |
|---|---|---|---|---|
| 1. | Present study | 4 | 4 (100%) | 4 (100%) |
| 2. | Kippes W et al 1999[80] | 115 | 115 (100%) | 115 (100%) |
| 3. | Cozzani E et al 2000[81] | 32 | 32 (100%) | 32 (100%) |

Direct immunofluorescence was done in all the 4 cases of bullous pemphigoid and the features were suggestive of bullous pemphigoid in all the cases. Similar findings are seen in the studies mentioned above.

**CHRONIC BULLOUS DISEASE OF CHILDHOOD (CBDC)**

We observed two cases of CBDC in the present study which constituted 10% of the total number of cases of autoimmune vesiculobullous disorders. One patient was 6 years old and the other was 12 years old. Both were male patients and both presented with multiple tense fluid filled lesions on the trunk, face and extremities. The characteristic “string of pearls” appearance of the lesions was seen in one patient. There was no mucosal involvement.

Tzanck smear examination revealed a good number of neutrophils, lymphocytes and eosinophils. Histopathological examination revealed subepidermal bulla with neutrophils and eosinophils in the bullous cavity similar to that observed by Wojnarowska F et al[82]

Direct immunofluorescence showed deposition of IgA in the basement membrane zone in both the patients which is the classical DIF finding in CBDC.

# CONCLUSION

Incidence of autoimmune vesiculobullous disorder was 0.11% of the total number of patients attending the skin OPD. Pemphigus vulgaris constituted the most common subtype of autoimmune vesibulobullous disorder in this study, followed by bullous pemphigoid. Pemphigus group of diseases were most common in the $5^{th}$ decade while bullous pemphigoid was most common in the $7^{th}$ decade. Histopathological examination showed features typical of each subtype of autoimmune vesiculobullous disorder. Direct immunofluorescence was positive in all the patients.

Clinical examination and cytology are the initial steps in making a diagnosis of autoimmune vesiculobullous disorders. Histopathological examination and direct immunofluorescence are required for making a definitive diagnosis in autoimmune vesiculobullous disorders. Direct immunofluorescence is helpful in confirming the diagnosis. However, it is not a substitute for histopathology, but rather complementary to it.

# SUMMARY

The present study is a one-year cross-sectional descriptive study from November 2007 to October 2008. The source of data includes all cases of autoimmune vesiculobullous disorders attending dermatology OPD and referred cases from other departments, at K.L.E.S Dr. Prabhakar Kore Hospital and MRC, Belgaum.

The objective of the study was to observe the various clinical features, histopathological and immunofluorescence findings in patients with autoimmune vesiculobullous disorders.

- There were 24 cases of autoimmune vesiculobullous disorders out of 21,071 patients who attended the OPD. The incidence of autoimmune vesiculobullous disorders in our hospital is 0.11%.
- In the present study, pemphigus vulgaris constituted the most common type of autoimmune vesiculobullous disorder, constituting 55% of the cases, followed by bullous pemphigoid, constituting 20% of the cases.
- The youngest patient was 2 years old and the oldest patient was 85 years old. The maximum number of patients were in the age group 41 – 50 years.
- Pemphigus vulgaris and pemphigus foliaceous were most common in the $5^{th}$ decade. Bullous pemphigoid was most common in the $7^{th}$ decade.
- The overall M: F ratio of autoimmune vesiculobullous disorders in the study was 1: 1.
- Pemphigus vulgaris had a higher incidence in females than in males (M: F= 1: 1.75). Pemphigus foliaceous also showed female predominance (M: F= 0.5: 1),

while bullous pemphigoid and chronic bullous disease of childhood showed male predominance. M: F ratio was 1: 0.3 in bullous pemphigoid

- Trunk was the most common site of involvement in all types of autoimmune vesiculobullous disorders.
- Overall, skin was the most common site of onset of lesions in autoimmune vesiculobullous disorders (65% of the cases).
- Vesicles, bullae, erosions and crusted lesions were the predominant lesions in all the patients
- All pemphigus patients had flaccid blisters, while all patients with bullous pemphigoid and chronic bullous disease of childhood had tense blisters.
- Oral mucosa (78.57%) and scalp (92.85%) were involved predominantly in the pemphigus vulgaris.
- Buccal mucosa (90.9%) was the most common site of oral mucosal involvement in the pemphigus vulgaris patients followed by lips (81.8%).
- 50% of the cases of bullous pemphigoid had involvement of the oral mucosa. Buccal mucosa was the most common site of involvement of oral mucosa in these patients.
- Mucosal involvement was not seen in chronic bullous disease of childhood.
- Nikolsky sign and Bulla spread were positive in all the pemphigus patients and negative in all the patients of bullous pemphigoid and chronic bullous disease of childhood
- Tzanck smear showed acantholytic cells and few inflammatory cells in all the pemphigus patients.

- Tzanck smear showed inflammatory cells and absence of acantholytic cells in patients with bullous pemphigoid and chronic bullous disease of childhood
- Level of blister was suprabasal in all the cases of pemphigus vulgaris and subcorneal in the pemphigus foliaceous patients.
- Acantholytic cells, lymphocytes and few eosinophils and neutrophils were present in the blister cavity of pemphigus vulgaris and foliaceous.
- All cases of bullous pemphigoid and chronic bullous disease of childhood showed subepidermal blisters on histopathology. Eosinophils were the predominant cells in all the cases of bullous pemphigoid. Both eosinophils and neutrophils were the predominant cells in all cases of chronic bullous disease of childhood.
- DIF showed intercellular deposition of IgG in all the cases of pemphigus vulgaris which is a classical finding. Deposition of C3 in the intercellular space in the lower epidermis was seen in 45.4% of the cases.
- All cases of pemphigus foliaceous also showed intercellular deposition of IgG in the upper epidermis and few cases showed intercellular C3 deposition (66.7%) in the upper epidermis on DIF. Intercellular deposition of IgA was seen in one out of the three patients.
- All cases of bullous pemphigoid showed linear deposition of IgG and C3 in the basement membrane zone.
- Both the patients with CBDC showed linear deposition of IgA in the basement membrane zone on DIF.

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## ANNEXURE-I

## Proforma

**A one year cross sectional clinico - histopathological and immunofluorescence study of autoimmune vesiculobullous disorders in patients at Dr. Prabhakar Kore's KLE Hospital and Medical Research Centre, Belgaum**

| | | | |
|---|---|---|---|
| **NAME** | CASE # | **DATE** | |
| AGE | OP/IP # | DOA | |
| SEX | MARITAL STAT | DOD | |
| RELIGION | | | |
| OCCUPATION | INCOME | | |
| ADDRESS | | | |

**PRESENTING COMPLAINTS**

**HISTORY OF THE PRESENT ILLNESS**

**Skin lesions**

| | | | | |
|---|---|---|---|---|
| Type | Vesicular O | Bullous O | Pustular O | Erosion O |
| | Hemorrhagic O | Scaling O | Crusting O | |
| Onset | Acute O | Gradual O | | |
| Progress | | | | |
| Site of onset | | | | |
| Preceding lesions | Erythematous O | Urticarial O | Eczematous O | |
| Lesion | | Heals O | Spreads O | |
| How long does the blister stay? | | | | |
| Itching O | Present O | Absent O | | |

**Mucous membrane involvement**

| | | | | |
|---|---|---|---|---|
| Oral O | Genitalia O | Conjunctiva O | Nasal O | Rectal O |
| Burning sensation O | Discomfort O | Difficulty in eating O | | |
| Hoarseness of voice O | | | | |

**Other history**

| | | |
|---|---|---|
| Remissions and exacerbations | Present O | Absent O |
| Photosensitivity | Present O | Absent O |
| Trauma | Present O | Absent O |
| Diarrhoea | Present O | Absent O |
| Bleeding PR | Present O | Absent O |
| Weight loss / Appetite loss | Present O | Absent O |
| Seasonal Variation | Present O | Absent O |
| Drug Intake | Present O | Absent O |
| Sexual exposure | Present O | Absent O |
| Patchy hair loss | Present O | Absent O |

**Past History**

| | | | | |
|---|---|---|---|---|
| Diabetes O | Hypertension O | Asthma O | Epilepsy O | Other O |
| Other skin disease O | | | | |

**Family History**

| | | | | |
|---|---|---|---|---|
| Similar complaints | | Present O | Absent O | |
| Diabetes O | Hypertension O | Asthma O | Epilepsy O | Other O |

**Personal History**

| | | | | |
|---|---|---|---|---|
| Diet | Veg O | Mixed O | | |
| Appetite | Normal O | Poor O | | |
| Bowel/Bladder | Normal O | Disturbed O | | |
| Sleep | Normal O | Disturbed O | | |
| Habits | Smoking O | Alcohol O | Tobacco O | |
| Obstetric/Gynecological History | | G P L A | Cycles | |

**EXAMINATION**

**General Physical Examination**

| | | | | |
|---|---|---|---|---|
| Nourishment | Poor O | Moderate O | Well O | |
| Build | Poor O | Moderate O | Well O | |
| Vitals | Pulse | BP | Temperature | Weight |
| Pallor O | Icterus O | Cyanosis O | Edema O | |
| Lymphadenopathy O | Odour O | | | |

**Cutaneous Examination**

| | | | | |
|---|---|---|---|---|
| Morphology | Vesicular O | Bullous O | Pustular O | Erosion O |
| | Vegetative O | Hemorrhagic O | Crusting O | Scaling O |
| Blister | Flaccid O | Tense O | | |
| Distribution | Localized O | Generalized O | | |
| | Grouped O | Discrete O | Confluent O | |
| | Symmetrical O | Asymmetrical O | | |
| Sites involved | Face O | Trunk O | UL O | LL O |
| Underlying skin | Normal O | Affected O | | |

**Mucosal Examination**

| | | | | |
|---|---|---|---|---|
| Oropharynx O | Vagina O | Rectum O | Conjunctiva O | Nose O |
| Nails | Discolouration O | Ridging O | Splitting O | Shedding O |
| Beau's Line | Hemorrhages O | Paronychia O | Pterygium O | |
| Hair involvement | Present O | Absent O | | |
| **Scalp involvement** | Present O | Absent O | | |
| **Pigmentary changes** | Hyper O | Hypo O | None O | |

**Special Signs**

| | | | | |
|---|---|---|---|---|
| Nikolsky's Sign | Present O | Absent O | | |
| Bulla Spread Sign | Present O | Absent O | | |
| Milia | Present O | Absent O | | |

**Other conditions**

| | | | | |
|---|---|---|---|---|
| **Systemic Examination** | CVS | RS | PA | CNS |

**Investigations**

| | | | | |
|---|---|---|---|---|
| Hb | TC | DC | ESR | Urine |
| Urea | Creatinine | Total Bilirubin | RBS | |
| **Tzank smear** | Acantholysis O | Giant cells O | Neutrophils O | Eosinophils O |
| **Skin Biopsy** | Histopathology O | DIF O | | |

**Other investigations**

**PROVISIONAL DIAGNOSIS**

**Signature of Investigator** **Signature of Guide** **Signature of Co-Guide**

# ANNEXURE II

# INFORMED CONSENT FORM

**A clinico-histopathological and immunological study of autoimmune vesiculobullous disorders**

**Purpose of the Study:**

The purpose of this study is to study the various clinical presentations of autoimmune vesiculobullous disorders, common sites of involvement, risk factors and demographic characteristics and associated conditions. You are being asked to participate in this research because you have been clinically diagnosed as suffering from the disease. All patients attending the outpatient department, who are diagnosed to have this disease, will be requested to participate in this study during the period of one year. This study will be conducted by Dr. Julie Leishangthem of the J N Medical College, Belgaum.

**Procedure and Treatment:**

Should you choose to participate, you will be asked to give a detailed history of your disease, undergo a physical examination, and consent to a few routine blood and urine investigations. You will undergo a Tzanck test, skin biopsy for histopathological examination and direct immunofluorescence.

**Risks and Benefits**:

You may undergo a slight degree of discomfort during the process of investigations, which may include a slight amount of pain and bleeding. However, all necessary steps and precautions will be taken to ensure your safety. The result of you taking part in this research would help health care providers towards a better understanding of this disease, and thus we will be able to provide improved patient care.

**Alternatives:**

If you decide not to participate in this study, you will still be receiving the usual standard care for your disease.

**Privacy and confidentiality:**

Your privacy will be respected and all information collected about you during the course of this study will be kept confidential. Your identity will remain undisclosed.

**Institutional Policy:**

The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in its projects. In the event you believe that you have suffered any physical injury as the result of your participation in this study, you may contact Dr. Julie leishangthem, Telephone No. 9844587085 or Dr. A.M. Pandit, Telephone No. 9448133475. In the event of an emergency, you should contact KLES Hospital and MRC on Telephone No. 95 831 473777.

**Financial Incentives:**

You shall not be receiving any payment or any financial incentives for participating in this study.

**Authorization to publish results:**

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will be maintained confidential at all times.

**Voluntary participation:**

Your participation in this study is voluntary. Your decision whether or not to participate will neither affect the care of your current disease, nor your future relations with the doctor or the hospital. You are free to discontinue participation in this study at any time and for any reason. In case you need further information regarding your rights as a study participant, you may please contact Dr. V D Patil, Principal and Chairman of the Ethical Committee, J N Medical College, Belgaum on telephone no. 95 831 473777.

**Statement of Consent:**

I volunteer and consent to participate in this study. I have read the consent or it has been read to me. The study has been fully explained to me and I may ask questions at any time.

Participant's name:

Signature or left thumb print of participant:

Witness' name:

Signature of witness:

Signature of Doctor:

Date:

If the participants are Minors (Under 18), the parents or guardian sign the form, rather than the participants.

# PROCEDURE-1

## MAY-GRUNWALD-GIEMSA STAIN[83]

**SOLUTION**

**May - Grunwald reagent**

Stock solution

| | |
|---|---|
| Eosin - Methylene blue | 1.0gm |
| Absolute methanol | 100ml |

**Working solution**

| | |
|---|---|
| May - Grunwald stock | 40ml |
| Absolute methanol | 20ml |

**Giems stain**

Stock solution

| | | |
|---|---|---|
| Azure II - Eosin | 0.6gm | Incubate at $37^0$C For 3 hrs |
| Azure II | 0.16gm | |
| Glycerine | 150ml | |
| Absolute methanol | 100ml | |

Working solution

| | |
|---|---|
| Giemsa stock | 10ml |
| Distilled water | 90ml |

**STAINING PROCEDURE**

| | | |
|---|---|---|
| 1. | May - Grunwald solution | 5mins |
| 2. | Running water | 1min |
| 3. | Giemsa solution | 15mins |
| 4. | Running water | 1-2mins |
| 5. | Air dry | |

**RESULTS**

Nuclei - Blue

Cytoplasm - Pink to rose

# PROCEDURE - 2

## HARRIS HAEMATOXYLIN AND EOSIN STAIN (REGRESSIVE STAIN)[84]

### SOLUTION

**Harris Haematoxylin**

| | | |
|---|---|---|
| Haematoxylin crystals | ----- | 5.0gm |
| Alcohol, 100% | ----- | 50.0ml |
| Ammonium or potassium alum | ----- | 100.0gm |
| Distilled water | ----- | 1000.0ml |
| Mercuric oxide (red) | ----- | 2.5gm |

Dissolve the haematoxylin in alcohol, the alum in hot water. Remove from heat Mix the two solutions together and heat to boiling. Remove from heat and add mercuric oxide slowly. Reheat until it becomes dark purple, Remove from heat immediately and plunge the vessel into a basin of cold water until it is cool. Add 2-4m1 of glacial acetic acid per 100 m1 of solution and the stain is ready for use,

**Acid Alcohol**

| | | |
|---|---|---|
| Alcohol, 70% | --- | 1000.0ml |
| Hydrochloric acid, conc, | --- | 10.0 m1 |

**1% Stock alcohol Eosin**

| | | |
|---|---|---|
| Eosin Y, water soluble | --- | 1.0ml |
| Distilled water | --- | 20.0 m1(Dissolve and add) |
| Alcohol, 95% | --- | 80.0 m1 |

**Working Eosin solution**

| | | |
|---|---|---|
| Eosin solution | --- | 1 part |
| Alcohol, 80% | --- | 3 parts |

Just before use, add 0.5 m1 of glacial acetic to each 100 m1. of stain and stir.

**STAINING PROCEDURE**

1. Remove paraffin wax with xylene for 5 mins.
2. Treat with absolute alcohol, 2 changes for 1 min. each.
3. Wash in water.
4. Stain with Harris haematoxylin for 45 seconds.
5. Wash in water
6. Differentiate in 1 % acid alcohol, till only the nuclei remain blue.
7. Wash in water.
8. Blue in running water.
9. Rinse in water
10. Stain in 1% Eosin Y for 3 mins.
11. Wash in running water for 1 min.
12. Dehydrate in 3 changes of Absolute alcohol.
13. Clear in 2 changes of xylene
14. Mount in synthetic resin.

**RESULTS**

| | |
|---|---|
| Nuclei | - blue black |
| Cytoplasm | - varying shades of pink |
| Muscle fibers | - deep pinky red |
| Red | - orange red |
| Fibrin | - deep pink |

# PROCEDURE-3

**Direct immunofluorescence**[85]

1. Phosphate buffer saline (PBS)
2. Fluorescein isothiocyanate (FITC) – conjugated Goat antihuman IgG antisera
3. Optical clear transparent media (OCT)

**Procedures :**

DIF is a one step procedure

1. Cut two tissue sections of 5 micro meter on cryotome and place it on a slide and dry for 15 – 20 minutes
2. Wash in PBS for 30 minutes (3 washings, 10 minutes each) at pH 7.4
3. Keep the slide on water bath
4. Incubate with optimal concentration of FITC - labelled antisera for 45 minutes at $37^0$ C
5. Repeat second step
6. Stained sections are viewed under the fluorescence microscope

**Results :** Immunoreactants are deposited in two main patterns

1. In the epidermal intercellular space – throughout the epidermis or restricted to certain layers
2. Along the basements membrane zone – smooth and linear, granular and discontinuous or a combination of the two

## MASTER CHART

| S.No | Name | IPD | Age (yrs) | Sex | Duration of illness (months) | ITC | BUR | PN | OL | FFL | ER/CR | RL | CS | Site of onset | Treatment history | Family history | Associated conditions | VS | BL | C | E | PS | Consistency of blisters | Sites involved | MMI | Site of oral MI | Nikolsky's sign | Bulla spread sign | Tzanck smear | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ACNC | E | N | OC |
| 1 | Jaisingh | 254531 | 32 | M | 2 | - | + | + | + | + | + | - | - | Oral muscosa | + | - | DM | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext | Oral | BCM, LIPS | + | + | + | - | - | + |
| 2 | Basawa G | 258934 | 35 | F | 3 | - | + | + | + | + | + | - | - | Cutaneous | - | - | - | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext | Oral, Nasal | BCM, LIPS | + | + | + | - | + | - |
| 3 | Kamlakar | 254957 | 85 | M | 3 | + | + | + | + | + | + | + | - | Cutaneous | - | - | DM, HTN | + | + | + | + | - | Tense | Trunk, Ext | Oral | BCM | - | - | - | + | + | - |
| 4 | Suman K | 257480 | 42 | F | 1 | + | - | - | - | + | + | - | - | Scalp | - | - | - | + | + | + | + | - | Flaccid | Scalp, Trunk | - | - | + | + | + | + | - | - |
| 5 | Basawa D | 289677 | 30 | F | 2 | - | + | + | + | + | + | - | - | Oral muscosa | - | - | Oral Candidiasis | + | + | + | + | + | Flaccid | Scalp, Face, Trunk, Ext, Gn | Oral | BCM, HP, TG, LIPS | + | + | + | - | + | - |
| 6 | Suresh Bhairu | 260612 | 60 | M | 8 | + | - | - | - | + | + | + | + | Cutaneous | - | - | HTN | + | + | + | + | - | Tense | Trunk, Ext | - | - | - | - | - | + | + | + |
| 7 | Irappa Budi | 289266 | 53 | M | 10 | - | + | + | - | + | + | - | - | Cutaneous | + | - | Oral Candidiasis, HTN | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext, Gn | - | - | + | + | + | + | + | + |
| 8 | Irfan Mulla | 261944 | 6 | M | 8 | - | + | + | - | + | + | + | + | Cutaneous | + | - | - | + | + | + | + | + | Tense | Face, Trunk, Ext, Gn | - | - | - | - | - | + | + | + |
| 9 | Sangappa | 283346 | 56 | M | 7 | + | + | + | + | + | + | - | - | Cutaneous | + | - | HTN | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext | Oral, Nasal | BCM, LIPS | + | + | + | + | - | - |
| 10 | Renuka Patil | 250271 | 45 | F | 3 | - | + | - | - | + | + | - | - | Cutaneous | - | - | - | + | + | + | + | - | Flaccid | Face, Trunk, Ext | - | - | + | + | + | - | - | + |
| 11 | Krishna | 285812 | 55 | M | 9 | - | + | + | + | + | + | - | - | Oral muscosa | + | - | - | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext | Oral, Nasal | BCM, LIPS | + | + | + | - | + | - |
| 12 | Ramabai | 939521 | 66 | F | 2 | + | + | - | + | + | + | + | - | Cutaneous | - | - | HTN, DM | + | + | + | + | - | Tense | Trunk, Ext | Oral | BCM | - | - | - | - | + | - |
| 13 | Prema | 278791 | 35 | F | 3 | - | - | + | + | + | + | - | + | Cutaneous | - | - | Oral Candidiasis | + | + | + | + | + | Flaccid | Scalp, Face, Trunk, Ext, Gn | Oral | BCM, HP, LIPS | + | + | + | + | - | - |
| 14 | Iranna U | 283678 | 32 | M | 5 | - | + | + | + | + | + | - | + | Cutaneous | + | - | - | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext | Oral | BCM, LIPS | + | + | + | - | + | - |
| 15 | Sharabee | 284877 | 58 | F | 2 | - | + | + | - | + | + | - | - | Cutaneous | - | - | - | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext | - | - | + | + | + | - | + | - |
| 16 | Rukamavva | 286081 | 55 | F | 8 | - | - | + | + | + | + | + | - | Oral muscosa | + | - | Oral Candidiasis, HTN | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext, Gn | Oral | BCM, H,P LIPS | + | + | + | - | - | + |
| 17 | Mugutma | 297408 | 42 | F | 6 | - | + | + | + | + | + | - | - | Oral muscosa | - | - | - | + | + | + | + | - | Flaccid | Scalp, Face, Trunk, Ext, Grn | Oral | BCM | + | + | + | - | + | - |
| 18 | ustaquee | 297381 | 2 | M | 2 | + | - | - | - | + | + | + | - | Cutaneous | - | - | - | + | + | + | + | - | Tense | Face, Trunk, Limbs, Gn | - | - | - | - | - | - | + | + |
| 19 | Iravva | 298835 | 50 | F | 15 | + | - | - | + | + | + | - | - | Oral muscosa | + | - | - | + | + | + | + | - | Flaccid | Scalp, Trunk, Ext, Gn | Oral | BCM, LIPS | + | + | + | - | + | - |
| 20 | Dundesh | 299602 | 12 | M | 2 | - | + | - | - | + | + | + | - | Cutaneous | - | - | - | + | + | + | + | - | Tense | Face, Trunk, Ext, Gn | - | - | - | - | - | - | + | + |

| CLD | Histopathological examiantion | | | | | | | | | | Histological diagnosis | Direct immunofluorescence | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Level of blister | Findings in Epidermis | | | | | Findings in Dermis | | | | | IgG | | IgA | | IgM | | C3 | | FIBN | |
| | | Row of Tomstone | ACN | L | E | N | PE | PNI | PEI | PM | | IC | BMZ | IC | BMZ | EP | D | IC | BMZ | IC | BMZ |
| PV | Suprabasal | + | + | + | - | + | - | + | - | - | PV | + | - | - | - | - | - | + | - | - | - |
| PV | Suprabasal | + | + | - | + | - | - | - | + | - | PV | + | - | - | - | - | - | - | - | - | - |
| BP | Subepidermal | - | - | + | + | - | + | - | + | - | BP | - | + | - | - | - | - | - | + | - | - |
| PV | Suprabasal | - | + | + | - | - | - | - | - | - | PV | + | - | - | - | - | - | + | - | - | - |
| PV | Suprabasal | - | + | - | + | - | - | - | - | - | PV | + | - | - | - | - | - | - | - | - | - |
| BP | Subepidermal | - | - | - | + | + | - | - | + | - | BP | - | + | - | + | - | - | - | + | - | + |
| PF | Subcorneal | - | + | + | + | - | - | + | + | - | PF | + | - | + | - | - | - | + | - | - | - |
| CBDC | Subepidermal | - | - | + | + | + | - | + | - | - | CBDC | - | - | - | + | - | - | - | - | - | - |
| PV | Suprabasal | + | + | + | - | - | - | - | - | - | PV | + | - | - | - | - | - | + | - | - | - |
| PF | Subcorneal | - | + | - | - | - | - | - | - | - | PF | + | - | - | + | - | - | - | - | - | - |
| PV | Suprabasal | + | + | + | - | + | - | + | - | - | PV | + | - | - | - | - | - | + | - | - | - |
| BP | Subepidermal | - | - | + | + | + | - | - | - | - | BP | - | + | - | - | - | - | - | - | + | - |
| PV | Suprabasal | - | + | - | + | - | - | - | - | - | PV | + | - | - | - | - | - | - | - | - | - |
| PV | Suprabasal | - | + | - | - | - | - | - | - | - | PV | + | - | + | - | - | - | + | - | - | - |
| PF | Subcorneal | - | + | + | - | - | - | + | - | - | PF | + | - | + | - | - | - | + | - | - | - |
| PV | Suprabasal | + | + | + | + | - | - | - | - | - | PV | + | - | - | - | - | - | - | - | - | - |
| PV | Suprabasal | - | + | - | - | + | - | + | - | - | PV | + | - | - | - | - | - | - | - | - | - |
| BP | Subepidermal | - | - | + | + | + | - | + | - | - | BP | - | + | - | - | - | - | - | + | - | - |
| PV | Suprabasal | + | + | + | - | - | - | - | - | - | PV | + | - | - | - | - | - | - | - | - | - |
| CBDC | Subepidermal | - | - | - | + | + | - | + | - | - | CBDC | - | - | - | + | - | - | - | - | - | - |

# KEY TO MASTER CHART

| | | |
|---|---|---|
| M | : | Male |
| F | : | Female |
| ITC | : | Itching |
| BUR | : | Burning |
| PN | : | Pain |
| OL | : | Oral lesions |
| FFL | : | Fluid filled lesions |
| ER | : | Erosions |
| CR | : | Crusting |
| RL | : | Red lesions |
| CS | : | Constitutional symptoms |
| VS | : | Vesicles |
| BL | : | Bullae |
| PS | : | Pustule |
| MMI | : | Mucous membrane involvement |
| MI | : | Mucous involvement |
| ACN | : | Acantholysis |
| ACNL | : | Acantholytic cell |
| E | : | Eosinophil |
| N | : | Neutrophil |
| PE | : | Papillary Eoema |
| PNI | : | Predominantly neutrophilic infiltrate |
| PEI | : | Predominantly eosinophilic infiltrate |
| DM | : | Papillary microabscess |
| IC | : | Intercellular |
| BMZ | : | Basement membrane zone |
| EP | : | Epidermis |
| D | : | Dermis |
| FIBN | : | Fibrinogen |
| PV | : | Pemphigus vulgaris |
| PF | : | Pemphigus foliaceous |
| BP | : | Bullous pemphigoid |
| CBDC | : | Chronic bullous disease of childhood |