Dissertation on

CLINICAL AND IMMUNOHISTOPATHOLOGICAL STUDY OF DERMATOLOGICAL LESIONS IN A TERTIARY CARE CENTRE

Submitted in partial fulfilment for the Degree of

M.D PATHOLOGY BRANCH – III

THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY
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MAY – 2020

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This is to certify that this dissertation entitled **"CLINICAL AND IMMUNOHISTOPATHOLOGICAL STUDY OF DERMATOLOGICAL LESIONS IN A TERTIARY CARE CENTRE"** is the original work of **Dr. MOHANAPRIYA.L,** in partial fulfilment of the requirement for M.D(Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2020.

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The Institutional Ethics Committee has considered your request and approved your study titled **"CLINICAL AND IMMUNOHISTOPATHOLOGICAL STUDY OF DERMATOLOGICAL LESIONS IN A TERTIARY CARE CENTRE" NO.07032018**

The following members of Ethics Committee were present in the meeting held on **27.03.2018** conducted at Madras Medical College, Chennai 3

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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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LIST OF ABBREVIATIONS

ACLE	-	Acute cutaneous lupus erythematosus
BP Ag	-	Bullous pemphigoid antigen
BMZ	-	Basement membrane zone
CCLE	-	Chronic cutaneous lupus erythematosus
DEJ	-	Dermoepidermal junction
DIF	-	Direct immunofluorescence
DLE	-	Discoid lupus erythematosus
EB	-	Epidermolysis bullosa
FITC	-	fluorescein isothiocyanate
HPE	-	Histopathological examination
HSP	-	henoch schonlein purpura
ICS	-	Intercelluar space
IF	-	Immunofluorescence
IIF	-	Indirect immunofluorescence
Ig	-	Immunoglobulin
LAD	-	Linear IgA dermatosis
LE	-	Lupus erythematosus
PCT	-	Porphyria cutanea tarda
SLE	-	Systemic lupus erythematosus

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INTRODUCTION

Skin is the largest organ of our body, accounting for about 15% of the total body weight [1] and the integrity of the epidermis, which serves to protect humans from the external environment. In past few decades great progress has been made in our understanding of the biology of skin. This has led to more accurate classification and diagnosis which is essential for proper management. Understanding of the immunologic basis of bullous diseases has greatly improved in a era of modern medicine. New diseases have been defined and continue to be defined. Newly defined diseases during the past 5 decades include bullous pemphigoid (BP), mucosal or cicatricial pemphigoid , linear IgA disease, IgA pemphigus, and paraneoplastic pemphigus (PNP) etc. The main reason for the continued identification of new dermatological disorder is that better understanding of immunological and molecular properties of the diseases inaddition to histopathological finding.

It is essential to make a rapid and accurate diagnosis in order to classify the disease, to plan a proper treatment protocols and also to predict the prognosis for the patients. But in many patients due to heterogeneous symptoms, atypical appearance of lesions , accurate diagnosis in dermatological disorders remain challenging for the dermatologist and physicians .In such circumstance immunofluorescence is a very useful tool to aid an accurate diagnosis.

Immunofluorescence has been used for past few decades which helps to understand the pathophysiology of skin disorders and to help physicians in the diagnosis of various cutaneous disorders, especially bullous diseases and connective tissue diseases. It can also provide the indirect measure of disease activity , monitor the response to treatment and also useful in predicting relapse. Even a negative direct immunofluorescence [DIF] results also exclude the immunological disorder and indirectly helps in diagnosis.

REVIEW OF LITERATURE

Skin is the outer covering and also the largest organ of our body, accounting for about 15% of the total body weight [1]. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, and as well as prevention of excess water loss from the body and plays a pivotal role in thermoregulation.

Adequate knowledge about the normal histology of skin is very essential to understand the pathophysiology of the dermatological disorders. Skin is embryologically derived from ectoderm [2,3] and is divided into three layers.they are,

- The epidermis
- The dermis
- The subcutis or hypodermis

EPIDERMIS

The epidermis is the outermost layer of skin and is composed of four types of cells.

They are

1. Keratinocytes
2. Melanocytes

3. Langerhans cells
4. Merkel cells

The predominant cell type are keratinocytes which constitute about atleast 80% of epidermal cells .They are large cells with stainable cytoplasm and intercellular bridges. The keratinocytes are arranged in five layers. They are [from bottom to top]

1. Stratum basalis [basal cell layer]

   This layer is the deepest one and also is the closest to the dermis. It is mitotically active and contains melanocytes, a single row of keratinocytes, and stem cells. Melanocytes are responsible for producing melanin, a substance that gives colour to skin . Keratinocytes from this layer evolve and mature as they travel upward to create the remaining layers.

2. Stratum spinosum [squamous cell layer]

   This layer constitute most of the epidermis and contains several layer of cells connected by desmosomes. These desmosomes allow cells to remain tightly bound to one another and resemble "spines" architecturally.

3. Stratum granulosum [granular layer]

This layer contains several layers of cells that contain lipid-rich granules. In this layer, cells begin to immortalize and lose their nuclei, as they move away from the nutrients located in the deeper tissue.

4. Stratum Lucidum

This layer exists only in the thick skin like palms and soles , it consists of mostly immortalized cells.

5. Stratum corneum [horny layer]

This keratinized layer is the outermost layer of the epidermis and serves as a protective overcoat .Due to keratinization and lipid content, this layer plays an important role in the regulation of water loss by preventing internal fluid evaporation[4].

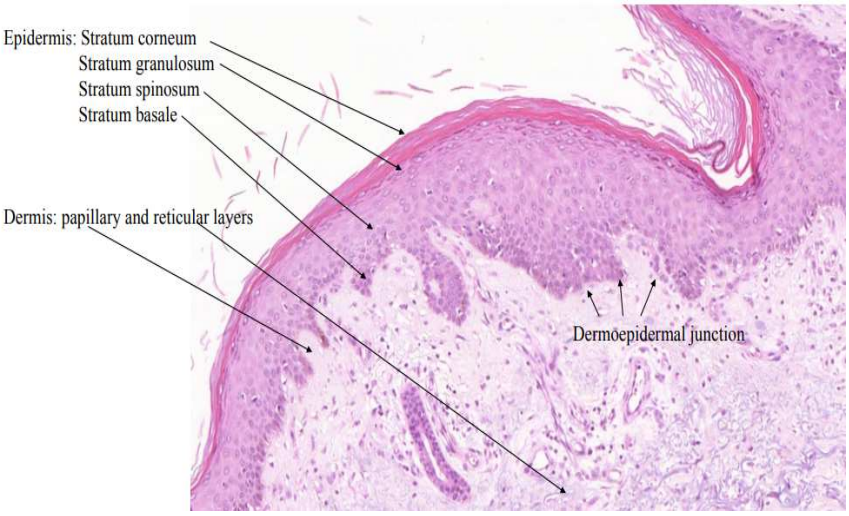


FIGURE 1: Normal histology of skin

DERMIS

Dermis is a thick layer of connective tissue consisting of collagen and elastin which is responsible for skin's strength and flexibility respectively. The dermis also contains nerve endings, blood vessels, and adnexal structures such as hair shafts, sweat glands, and sebaceous glands.

Dermis has two layers

- Papillary dermis
- Reticular dermis

The apical layer of dermis folds to form papillae that extend into the epidermis like tiny finger-like projections and is referred to as the papillary dermis, while the lower layer of the dermis is referred to as the reticular dermis.

SUBCUTIS

This is the deepest layer of skin and consisting mainly of adipose tissue which is divided into lobules by fibrous septae. It also contains nerve bundles and small to medium sized blood vessels.

FUNCTIONS OF SKIN

The main functions of skin are as follows [5]

1. **Sensation:** The skin contains many types of different receptors that sense pain, temperature, pressure, and touch.

2. **Thermoregulation:** Hair and sweat glands helps in the regulation of body temperature to maintain the homeostasis..
3. **Protection:** The skin act as a barrier between inside and outside of the body against infection, chemical stress, thermal stress, and UV light
4. **Metabolism:** Adipose tissue in the hypodermis has a pivotal role in the production of Vitamin D and lipid storage.

SKIN BIOPSY TECHNIQUES

The choice of skin biopsy technique is mainly depends on the site , size and nature of the lesion, clinical differential diagnosis and general condition of the patient. Various methods are available for skin biopsies. They are ,

- Punch biopsy,
- Shave biopsy [superficial and deep],
- Deep incisional biopsy,
- Complete excision, and
- Curettage.

LABORATORY INVESTIGATIONS

Laboratory investigations available for the diagnosis of dematological disorders are,

- Histopathology

- Frozen section study
- Histochemistry
- Polariscopic examination
- Immunofluorescence
- Immunohistochemistry
- Electron Microscopy
- Serological tests

VESICULO BULLOUS DISORDERS

The vesiculobullous disorders are defined by the presence of vesicles or bullae at any level within the epidermis or at the dermoepidermal junction. Fluid filled lesions which are less than 0.5 cm are called vesicles ,more than 0.5 cm are called bullae.

Adequate knowledge about the mechanisms involved in normal epidermal keratinocytic cell to cell adhesion is very very essential to under the pathophysiology of blister formation and which is must needed for the classification of the disease and thereby selection of the appropriate treatment for the patient.

MECHANISMS OF BLISTER FORMATION

There are various underlying mechanisms which are responsible for blister formation. They are,

- Spongiosis
- Reticular degeneration
- Cytolysis
- Acantholysis
- Disruption of basement membrane zone

SPONGIOSIS

Excessive accumulation of extracellular fluid within the epidermis is called spongiosis. This will lead to the separation of the keratinocytes subsequently results in the disruption of desmosomes and blister formation. It is a passive phenomenon and associated with increased vascular permeability. Microscopically lymphocytic infiltration around the blood vessels as well as within the epidermis is also present. Conditions associated with spongiosis are Eczematous dermatitis , Early stages of Miliaria Pemphigus ,Transient acantholytic dermatosis .

RETICULAR DEGENERATION

Intracellular edema along with secondary rupture and death of the keratinocytes will lead to reticular degeneration and blister formation. Conditions associated with reticular degeneration are some viral infections and late stages of eczematous dermatitis .

CYTOLYSIS

Cytolysis is the disruption of the keratinocytes due to the physical agents like heat and friction. Friction may cause shearing of the keratinocytes from one another which will lead to the formation of clear fluid filled blisters. Conditions associated with cytolysis are epidermolysis bullosa simplex , epidermolytic hyperkeratosis , friction blister , erythema multiforme and some cases of Irritant dermatitis .

ACANTHOLYSIS

This is caused by the presence of autoantibodies against the antigens which are responsible for the cell to cell adhesion and cell to matrix adhesion , which can be congenital or acquired. Acanthocytes are the viable cell and microscopically they are round cells with condensed cytoplasm having large nuclei with prominent nucleoli which differs from stellate shaped keratinocytes in spongiosis. Conditions associated with acanthosis are Pemphigus group of disorders , transient acantholytic dermatosis , Hailey-Hailey disease , Darier's disease and some cases of Irritant dermatitis .

DISRUPTION OF BASEMENT MEMBRANE ZONE

This can be due to immunologically mediated damage of the basement membrane zone[BMZ]. This disruption either in the following structures at the basement membrane zone,

- The basal keratinocytes
- The lamina lucida,
- The lamina densa, composed principally of type IV collagen
- The sublamina densa zone.

CLASSIFICATION OF VESICULO BULLOUS DISORDERS BASED ON PLANE OF BLISTER FORMATION

- Epidermal
  - Intra epidermal
    - Suprabasal
    - suncorneal
  - subepidermal
- Dermal

CAUSES OF SUPRABASAL VESICULO BULLOUS DISORDERS :

- Pemphigus vulgaris and its variants
- Paraneoplastic pemphigus
- Darier's disease

CAUSES OF SUBCORNEAL VESICULO BULLOUS DISORDERS :

- Pemphigus foliaceus and its variants
- IgA pemphigus
- Miliaria crystalline

- Staphylococcal scalded skin syndrome
- Bullous impetigo
- Hailey-Hailey disease
- Subcorneal pustular dermatosis
- EAcropustulosis of infancy
- Erythema toxicum neonatorum
- Transient neonatal pustular melanosis
- Spongiotic dermatitis Friction blister
- Miliaria rubra
- Incontinentia pigmenti
- Epidermolytic hyperkeratosis

CAUSES OF SUBEPIDERMAL VESICULO BULLOUS DISORDERS :

- Epidermolysis bullosa simplex
- Thermal injury
- Erythema multiforme
- Herpes gestationis
- Bullous pemphigoid Cicatricial pemphigoid
- Dermatitis herpetiformis
- epidermolysis bullosa [EB]
- Suction blister
- Bullous SLE

- Epidermolysis bullosa aquista [EBA]
- Linear IgA dermatosis [LAD]
- Epidermolysis bullosa dystrophica
- Porphyria cutanea tarda / Pseudoporphyria

CAUSES OF BLISTER IN THE DERMAL PORTION OF THE SKIN:

- Drug induced blisters [eg. Penicillamine]
- Bullous amyloidosis

HISTORY

The term pemphigus was first used by Hippocrates in the period 460-370 B.C. He enumerated different types of fevers and mentioned a pemphigoid type of fever. [6]

The term Pemphigus was first described in the year of 1777 by McBride. In 1791 this concept was also described by Wichmann . Wichmann first applied the term "pemphigus" to patients with flaccid bullae and painful oral erosions. [6]

Ferdinand von Hebra et al was the first to coin the term pemphigus vulgaris in 1868 and also found that pemphigus vulgaris is the chronic disease state.[6,7]

In pemphigus disruption of epidermal cells which is responsible for blister formation was first described by Auspitz et al. [9]

Cazenave et al first use the word pemphigus foliaceous as a rapid and superficial spreading condition which is the subtype of pemphigus group of disorders in 1844 [12].

In pemphigus vegetans Neumann et al demonstrate the warty granulations in 1886 [8].

Senear et al and Usher et al described pemphigus erythematosus in 1925 [7]

Civatte et al in 1943 explained in detail about the mechanism of blister formation in pemphigus group of disorders and also described acntholysis is the hallmark feature of pemphigus in histopathology . In addition he discovered that pemphigus is the separate group of disorder which differs from other vesiculo bullous disorders [10].

Walter Levers et al discovered that pemphigus vulgaris and bullosus pemphigoid are separate entities both by clinical as well as by histopathology In 1953.Also he described pemphigus vulgaris as a life-threatening disorder, characterized by intra-epidermal blisters and acantholysis having lethal outcome.[11]

Schiltz et al and Michel et al demonstrated the auto-antibodies in pemphigus cause the blister formation in 1976 by human skin organ culture. [12]

Anhalt et al demonstrated the auto-antibodies in pemphigus by passive transfer of antibodies to neonatal mice In 1982. [13]

Pemphigus target antigens were identified In 1980s, by immune-blotting methods and immune-precipitation methods.[14]

Isolation of cDNA for pemphigus antigens revealed the desmogleins as the target antigens in pemphigus in 1990s.[15]

EPIDEMIOLOGY

IN INDIA

In India the incidence of pemphigus ranges from 0.09%- 1.8% based on out patient stastistics.[16,17]

In a Study conducted in Thrissur Kerala, has shown the incidence of pemphigus to be 4.4 per million population/year.[18]

In a review article by Sehgal et al, said that Pemphigus Vulgaris was the most commonest form of vesiculobulllous disorders followed by pemphigus foliaceous, pemphigus erythematosus, pemphigus Vegetans in a decreasing order of frequency.[19]

WORLD WIDE

Incidence of pemphigus Vulgaris is more common in Ashkenazi jews, Indians and Japanese. [19,20]

The incidence of pemphigus Vulgaris in Tunisia is about 2.5 cases per million population/ year.[21]

The incidence of pemphigus Vulgaris in france is 1.3 cases per million population/year. [21]

The prevalence of pemphigus Vulgaris in finland is 0.76 cases per million population.[22]

The prevalence of Pemphigus Vulgaris ranges from 0.18 to 6.96 case per million population all over the world.[23,24]

In a study by Wilson CL et al showed that, the proportion of Pemphigus Vulgaris and pemphigus foliaceous was almost equal in patients from UK, while Pemphigus Vulgaris was the most common type in Indian population [25].

AGE

In the western population most common age group affected by pemphigus Vulgaris was between 50-60 years.[26] In contrast in India it affected younger population about 30-40 years [16.19,27]

PATHOPHISIOLOGY OF PEMPHIGUS GROUP OF DISORDERS

The main target antigens of pemphigus are located in the desmosomes. Desmosomes are the major adhesion complex located in the epidermis, act as a

anchoring keratin intermediate filaments to the cell membrane and bridging adjacent keratinocytes and allowing cells to withstand mechanical stress like trauma. They are also seen in myocardium , meninges and cortex of lymph nodes.

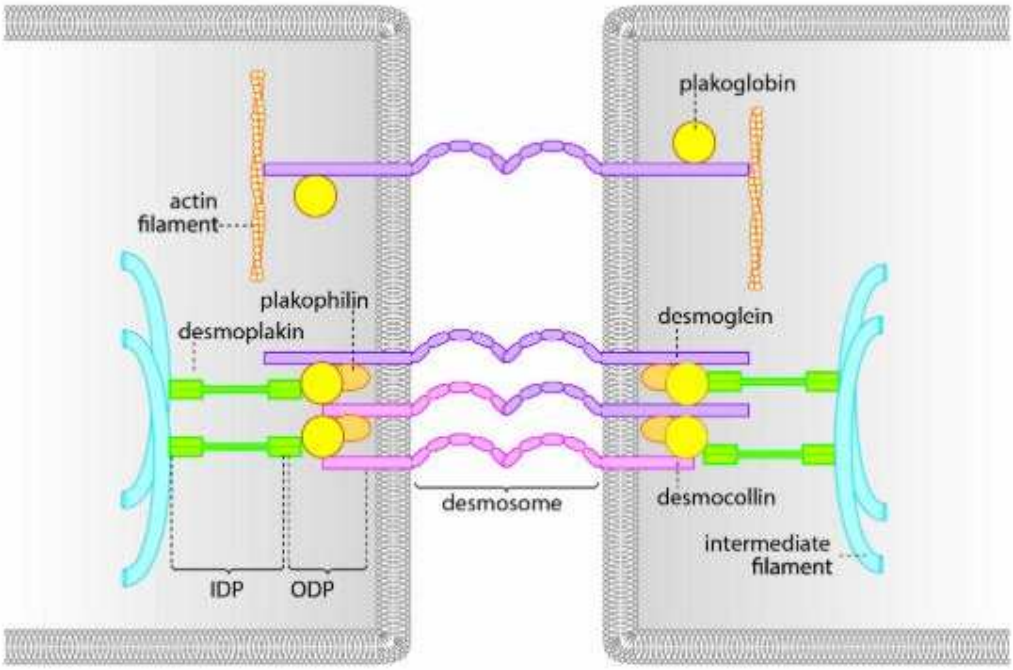
Desmosome complex have two components

- Transmembrane components
- Cytoplasmic components

Desmogleins and desmocollins are the transmembrane components which are more important in the pathogenesis of pemphigus group of disorders. Plakoglobin, plakophilin, and desmoplakin are the cytoplasmic components of desmosome complex. Desmogleins and desmocollins are the members of cadherin supergene family .[28,29]

Cadherins are group of calcium dependent cell to cell adhesion molecules which play an important role in the formation and maintenance of complex tissue integrity. [30]

FIGURE 2: Structure of desmosome



TYPES OF DESMOGLEINS

Desmogleins have four isoforms. They are,

- Desmoglein 1
- Desmoglein 2
- Desmoglein 3
- Desmoglein 4

DISTRIBUTION OF DESMOGLEINS IN THE SKIN

The presence of desmogleins in the skin depends on the differentiation and maturation. Desmoglein-3 expression is restricted to the basal and suprabasal layers of the epidermis, whereas desmoglein-1 is present in the

entire thickness of the epidermis but more in the upper layers i.e., in the more differentiated cells.[31,32]

Desmoglein 1 and 3 are usually restricted to the stratified squamous epithelia while Desmoglein 2 is expressed in all desmoglein possessing tissues like myocardium and simple epithelia. Desmoglein 4 is seen primarily in the hair follicles and in the granular layer of the epidermis.[30]

THE PATHOGENICITY OF DESMOGLEIN ANTIBODIES IS SUPPORTED BY,

- ✓ Many studies showing a correlation between amount of antibody in patient's serum and the disease activity.[33]
- ✓ Transient bullae formation in the neonates may be caused by the transplacental transfer of antibodies from the mother.
- ✓ Pemphigus vulgaris IgG antibodies causes suprabasal bullae in the neonatal mouse model.[34]
- ✓ Prior absorption of the antibodies of pemphigus vulgaris can prevents blister formation.[35]
- ✓ Acantholysis induced by Desmoglein-3 antibodies can be enhanced by adding desmoglein-1 antibodies in mice.[36]

DESMOGLEINS DISTRIBUTION IN THE ORAL MUCOSA

The pattern of expression of desmoglein in oral mucosa is usually different from the skin. In oral mucosae, desmoglein -1 expression is weak, whereas, desmoglein -3 is strongly expressed in the entire thickness.[37]

Desmoglein 3 is a 130-kD molecule that localizes primarily in the deeper layer of epidermis and mucous membrane. It is the target antigen in pemphigus vulgaris (38) . Desmoglein-1 is 160-kD molecule and is the target antigen in pemphigus foliaceus which is usually present in the upper layer of epidermis. Since mucosal epithelium expresses mainly desmoglein 3 but skin expresses both desmoglein 1 and 3, damage by antibodies to desmoglein 3 alone results in oral lesions with or without skin lesions. If both desmoglein 3 and desmoglein 1 antibodies are present, cutaneous lesions as well as mucosal lesions appear, and the disease tends to be more severe [39].

Desmocollins are less recognised in the pathogenesis of pemphigus but in some cases of IgA pemphigus can have autoantibodies against the desmocollins.

TABLE 1: Target antigens in pemphigus group of disorders

Target Antigens in Pemphigus			
Diseases	**Autoantibodies**	**Antigens**	**Location of antigens**
Pemphigus vulgaris Mucosal mainly Mucocutaneous	 IgG IgG	 Desmoglein 3 (130 kD) Desmoglein 3 (130 kD) Desmoglein 1 (160 kD)	Desmosomes
Pemphigus foliaceus	IgG	Desmoglein 1 (160 kD)	Desmosomes
Paraneoplastic pemphigus	IgG	Desmoglein 1 (160 kD) Desmoglein 3 (130 kD) Desmoplakin I (250 kD) Envoplakin (210 kD) Periplakin (190 kD) Plectin (500 kD) BPAg1 (230 kD) √-Catenin (plakoglobin-82 kD)	Desmosomes or hemidesmosomes
Drug-induced pemphigus (autoimmune variant)	IgG	Desmoglein 3 (130 kD) Desmoglein 1 (160 kD)	Desmosomes
Drug-induced pemphigus (toxic variant)	—	—	—
IgA pemphigus SPD type IEN type	 IgA IgA	 Desmocollin 1 (110/100 kD) Desmoglein 1 (160 kD) Desmoglein 1 (160 kD)	Desmosomes

SUBEPIDERMAL BULLOUS DISORDERS

This group of disorders having different clinical presentations, histologic findings, and pathogenesis. This could be due to inherited and alterations in key adhesion proteins at or in the dermal–epidermal junction which results in blister formation.

To understand pathogenesis of subepidermal bullous disease, it is essential to have adequate knowledge about the epidermal basement membrane zone and associated various target proteins. From the epidermis to the dermis, there are four distinct structural components present in the dermal epidermal basement membrane zone. They are,

- The intermediate filaments, hemidesmosomal plaques, and plasma membranes of the basal keratinocytes. Major intracellular components of hemidesmosomes is 230-kD bullous pemphigoid antigen (BPAg1) and plectin. Another transmembrane components of the hemidesmosomes include α6 β4 integrin and the 180-kD bullous pemphigoid antigen (BPAg2) or collagen XVII [40]. Antibodies to the BPAg1 are of the most common autoimmune subepidermal bullous disease like bullous pemphigoid. BPAg2 autoantibodies present in patients with bullous pemphigoid, pemphigoid gestationis, cicatricial pemphigoid, and a subgroup of linear IgA bullous dermatosis.
- The lamina lucida is the weakest link in the dermal– epidermal junction contains delicate anchoring filaments which connecting the hemidesmosomes in basal keratinocytes to the underlying lamina densa. Multiple antigens are associated with the lamina lucida include laminin 5, laminin 6, uncein, nidogen, and P200.[41,42].

- The lamina densa is an electron-dense layer which provides the basement membrane with much of its strength because of the presence of type VII collagen. Other antigenic components in the lamina densa are laminin 1, nidogen, and heparan sulfate proteoglycans.
- The sublaminar densa region, which contains anchoring fibrils (such as type VII collagen), anchoring plaques, and filamentous proteins of the papillary dermis. Type VII collagen is the major component of anchoring fibrils. Autoantibodies against type VII collagen have been identified in EBA, bullous SLE, and some variants of linear IgA disease.

FIGURE 3: Structure of basement membrane zone

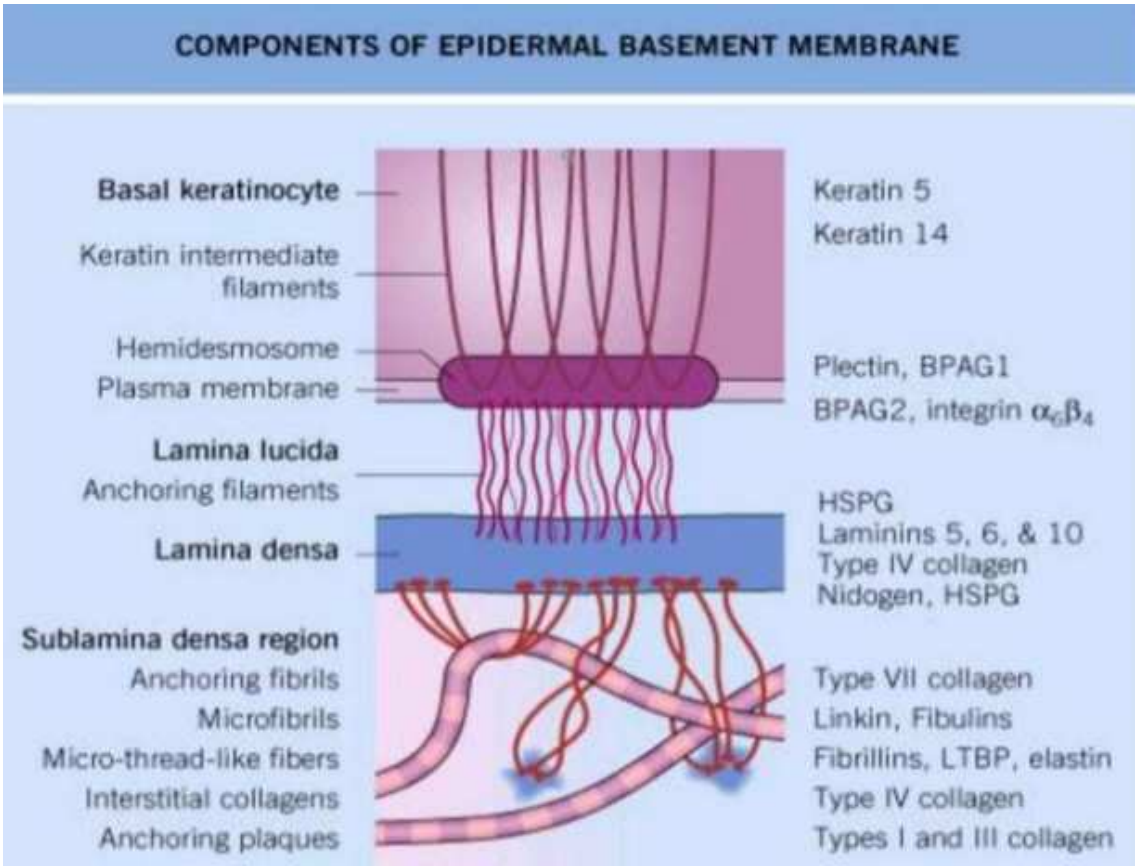


TABLE 2:Principal Inflammatory infiltrate in various Vesiculobullous disorders

Disease condition	Principal cell type
Porphyria Cutanea tarda	Absent
EBA (classic)	Absent
Bullous pemphigoid (cell poor)	Eosinophils
Spongiotic dermatitis	Lymphocytes
Erythema multiforme	Lymphocytes
Bullous pemphigoid (cell rich)	Eosinophils
Herpes gestationis	Eosinophils
Dermatitis herpetiformis	Neutrophils
Linear IgA dermatosis	Neutrophils
EBA (inflammatory)	Neutrophils or Mixed neutrophils and eosinophils
Anti-p200 pemphigoid	Neutrophils or Mixed neutrophils and eosinophils
Bullous SLE	Neutrophils Interface dermatitis
Cicatricial pemphigoid	Mixed neutrophils and eosinophils Lymphocytic, bandlike (mucosa only) Eosinophils
Paraneoplastic pemphigus	Lymphocytes—interface dermatitis (lichen planus or EM-like)

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus [SLE] is an autoimmune disorder. It has multiorgan involvement and skin is the second most common organ affected by SLE after joint . Its clinical spectrum ranges from mild skin involvement at one end to the fatal conditions from the systemic manifestations of LE like nephritis, central nervous system disease, or vasculitis [43] .

The term lupus is coined in the 13th century because it ate away parts with the rapidity of a wolf [44].

Lupus erythematosus (LE) is caused by the development of autoantibodies against molecular constituents of nucleosomes and ribonucleoproteins.

SYSTEMIC LUPUS INTERNATIONAL COLLABORATING CLINICS CLASSIFICATION CRITERIA FOR SYSTEMIC LUPUS ERYTHEMATOSUS -2012[45].

The Systemic Lupus International Collaborating Clinics (SLICC) developed 17 criteria for the diagnosis of SLE in 2012. This criteria has higher sensitivity but lower specificity and this system consist of clinical and immunological criteria.

CLINICAL CRITERIA

1. Acute cutaneous lupus -this consist of lupus malar rash, bullous lupus, toxic epidermal necrolysis variant of SLE, maculopapular rash, photosensitive lupus rash in the absence of dermatomyositis; or subacute cutaneous lupus SCLE
2. Chronic cutaneous lupus – this consist of Classic discoid rash discoid lupus erythematosus [DLE], hypertrophic(verrucous) lupus, lupus

panniculitis (profundus), mucosal lupus, lupus erythematosus tumidus, chilblain lupus, discoid lupus/lichen planus overlap

3. Oral ulcers – involving palate, buccal, tongue or nasal ulcers in the absence of other causes.
4. Non-scarring alopecia- diffuse thinning or hair fragility with broken hairs excluding other causes
5. Synovitis - involving two or more joints with effusion or swelling or tenderness and at least 30 minutes of morning stiffness
6. Serositis - pleurisy or pericarditis, more than one day duration of pleural/pericardial effusions or pleural/pericardial rub
7. Renal disorder - persistent proteinuria (>0.5 μg/day) or cellular casts
8. Neurological disorder - seizures, psychosis, mononeuritis multiplex, myelitis or acute confusional state in the absence of other causes
9. Hemolytic anemia
10. Leukopenia (less than 4000/mm3 at least one occasion) or lymphopenia (less than 1000/mm3)
11. Thrombocytopenia (less than 1 lakh /mm3 at least once)

IMMUNOLOGICAL CRITERIA

1. Anti nuclear antibodies above the level of laboratory reference range
2. Anti-ds DNA antibody above the laboratory reference range (or more than two fold the reference range if tested by ELISA)

3. Anti-Smith antibody: presence of antibody to Sm nuclear antigen
4. Positive for Antiphospholipid antibody
5. Low complement level (low C3, C4 or CH50)
6. Direct Coombs' test in the absence of haemolytic anaemia

CRITERIA FOR DIAGNOSIS

A patient must satisfy at least four criteria, including one clinical criterion and one immunological criterion

[or]

The patient must have biopsy-proven lupus nephritis in the presence of Anti nuclear antibodies or anti-double-stranded DNA (anti-dsDNA) antibodies.

SYSTEMIC INVOLVEMENT

Systemic involvement occur due to deposition of antigen and antibody complexes in systemic organs. Commonly affected organs are skin,joint , blood vessels, kidney and choroid plexus of brain.

- Arthritis is more common.
- Renal involvement is the most common cause of death in SLE patients.
- Serositis – occurs in epicardium, pleura and peritoneum.
- Periarterial fibrosis in spleen.

- In Heart - Libman sacks endocarditis also known as verrucous endocarditis, which forms vegetations along the valve leaflets.
- Antiphospholipid antibody syndrome – 10% of SLE patients have lupus anticoagulants. Thrombosis and its complications, recurrent fetal abortions and thrombocytopenia are some common manifestations.
- Anti cardiolipin antibody – it is more common than lupus anticoagulant. It is associated with thrombosis, essential hypertension and abnormalities in the valves, livedo reticularis, disseminated intravascular coagulation [DIC] and ulcers.

Some other conditions associated with SLE are Rheumatoid arthritis, Myasthenia gravis, Systemic sclerosis, Sjogren's syndrome, Polymyalgia, Hashimoto's thyroiditis, Lymphoma, Von willebrand's disease, Pernicious anemia, Leukemia, Monoclonal gammapathy and Multiple myeloma, Ulcerative colitis, Pemphigus, Pemphigoid, Dermatitis herpetiformis etc (54).

EPIDEMIOLOGY

In India Prevalence of SLE is approximately 30 per million population. In a study conducted in rural northen India showed the prevalence to be 14 to 60 per 100,000 population[46]. Females are more commonly affected with the female male ratio of 7:1 to 15:1 [47]. Regarding age distribution SLE is most common in 4th decade with the peak age of onset of symptom in female is about 38 years of age and in men its about 44 years of age [46]. In all age groups the

manifestations of the disease are remain same, except serositis and Sjögren syndrome are more common disease manifestations in the elderly. [48] SLE associated renal damage, skin manifestations ,cytopenias ,neurological involvement , thrombosis, vasculitis and serositis are more common in male gender.

CLINICAL PRESENTATION

It is divided into two groups

- LE [Lupus erythematosus]specific
- NON LE[non Lupus erythematosus] specific

LE [Lupus erythematosus] SPECIFIC FEATURES

- Butterfly rash
- Subacute cutaneous LE[Lupus erythematosus]
- Scarring DLE[Discoid Lupus erythematosus] alopecia
- Chronic DLE [Discoid Lupus erythematosus]

LE [Lupus erythematosus] NON SPECIFIC

- Non-scarring alopecia
- Chilblain lupus
- Mouth ulceration
- Bullous eruptions

- Photosensitivity
- Episcleritis
- Cheilitis
- Raynaud's phenomenon
- Chronic urticaria (for more than 36 hours)
- Cutaneous vasculitis
- Livedo reticularis
- Facial oedema

LE[LUPUS ERYTHEMATOSUS] SPECIFIC CHANGES

The LE-specific changes are further divided into three groups based on duration of the manifestation

1. Acute cutaneous LE (ACLE)
2. Subacute cutaneous LE (SCLE)
3. Chronic cutaneous LE (CCLE)

ACUTE CUTANEOUS LE

ACLE indicates the active stage of SLE disease and these features are more common in sun exposed areas. This includes

- Lupus malar rash
- Bullous lupus

- Toxic epidermal necrolysis
- Maculopapular rash
- Photosensitive lupus rash

SUBACUTE CUTANEOUS LE

The incidence of SCLE in SLE is approximately 50% among which 10-15% of patients having serious organ damage [49]. This category includes ,

- Annular
- Psoriasiform variants

CHRONIC CUTANEOUS LE [CCLE]

The features of CCLE are ,

- Localized and generalized DLE
- Hypertrophic LE
- Lupus profundus/panniculitis
- Lupus tumidus

NAIL CHANGES IN SLE [50]

1. Nail fold erythema
2. Splinter haemorrhages
3. Red lunula
4. Nail ridging

5. Onycholysis
6. Onychomadesis
7. Blue-black nail pigmentation
8. Nail fold hyperkeratosis

HAIR CHANGES IN SLE [51]

Diffuse non scarring alopecia is the most common non-specific skin manifestation of SLE otherwise known as telogen effluvium. Rarely Alopecia areata and Permanent scarring alopecia can be present .

VASCULAR CHANGES [51,52]

- Vasculitis - Arterioles and venules of any size can be affected . Usually leukocytoclastic type of vasculitis is present and these patients clinically presented with petechiae and palpable purpura commonly involved lower limbs
- Narrowing of the vessel lumen or vessel wall due to thromboembolic disease .

BULLOUS OR BLISTERING LESIONS IN SLE [53]

This manifestation is uncommon in Systemic lupus erythematosus and it can be divided in to three groups. They are,

I. Subepidermal bullae due to separation of the epidermis and dermis as a result of severe liquefaction and degeneration of the basal layer of the epidermis and dermal oedema .

II. SLE-associated autoimmune bullous disease including dermatitis herpetiformis, pemphigus vulgaris (so-called pemphigus erythematosus), pemphigus foliaceus, paraneoplastic pemphigus, bullous pemphigoid, pseudoporphria, epidermolysis bullosa acquisita and IgA disease.

III. Bullous SLE , a separate subset, is a distinct type of nonspecific, autoantibody-mediated, cutaneous SLE that results in a subepidermal blister formation.

CRITERIA FOR BULLOUS SLE

Patients should fulfil the following criteria for the diagnosis of bullous SLE.

- Patient should be a known case of SLE .
- Presence of vesiculobullous lesions but not limited to sun-exposed skin .
- neutrophilic infiltration in the upper dermis and histopathologically subepidermal blisters and
- With direct immunofluorescence immunoglobulin and complement deposition at the basementmembrane zone

HISTOPATHOLOGICAL FEATURES OF SLE

In early stages of SLE the histopathological changes are mild as well as non specific. Histological changes are more pronounced in well established lesions only. The changes are ,

- Hyperkeratosis with follicular plugging
- Fibrinoid necrosis at dermoepidermal junction with liquefactive [hydrophic] degeneration and atrophy of epidermis
- Fibrinoid material is deposited in the dermis around capillary blood vessels, on collagen and in the interstitium
- Inflammatory cell infiltrates around adnexal structure and blood vessels and leukocytoclastic type of vasculitis
- Basement membrane thickening
- In Bullous SLE there are two histologic inflammatory patterns. First is neutrophilic type which simulates dermatitis herpetiformis or linear IgA bullous disease with the formation of papillary microabscesses and the other one is mononuclear infiltration associated with subepidermal blistering. The second category is more common in chronic cases.

DIRECT IMMUNOFLUORESCENCE

Usually positive for two or more immunoreactants [Ig G ,Ig A ,IgM, C3c] . The positivity is granular deposition along the dermal–epidermal junction [basement membrane zone] called band like positivity.

VASCULITIS

Vasculitis is an inflammatory process , and thus the absence of inflammation precludes the diagnosis even though vascular alterations may be present but in late stages of vasculitis inflammation can be minimal in number. Inflammatory cells can be neutrophils, lymphocytes, eosinophils or histiocytes The type of inflammatory infiltrate can give a clue in the diagnosis. Vasculitis can affect the small- or medium-sized vessels of the skin.

CRITERIA FOR DIAGNOSIS

Microscopically vasculitis should have the following features.

1. An inflammatory cell infiltrate around blood vessels and
2. Evidence of vascular injury

VASCULAR INJURY [55]

This can be primary or secondary

PRIMARY VASCULAR INJURY[56,57]

Primary vascular injury indicates that the vascular insult is the predominant disease process .This consist of vasculitis and vasculopathy.

FEATURES OF VASCULITIS

- Perivascular inflammatory infiltration composed of neutrophilis, eosinophilis, lymphocytes, histiocytes, or mixed inflammation .
- Fibrinoid necrosis [deposition of fibrinoid material in the blood vessel wall].
- Edema
- Extravasation of RBCs
- Leucocytosis
- Vessel wall inflammatory infiltration
- Swelling of endothelial cells and
- Intra luminal thrombosis.

VASCULOPATHY

This term is used to describe certain degrees of vascular alteration and injury that fail to satisfy the criteria for vasculitis. Fibrinoid deposition and thrombosis of the vessel present without inflammatory Infiltrates. Also minimal leukocytoclasis of tissue infiltrate with minimal alteration of vessel

like swelling of endothelial cells in the absence of fibrinoid necrosis are also present.

SECONDARY VASCULAR INJURY

It is otherwise called incidental vascular injury. It also indicates that the primary pathologic process is present outside the vessels . Microscopically it is difficult to differentiate secondary vascular injury from primary vascular injury.

CLINICAL FEATURES

- Palpable purpura,
- Petechiae,
- Urticaria,
- Livedo reticularis, and
- Skin nodules and ulcers.

CLASSIFICATION OF VASCULITIS [58,59]

Based on the size of the vessel vasculitis can be divded into three categories.

I. Large vessel vasclitis
II. Medium vessel vasclitis
III. Small vessel vasclitis

This type of classification is having some clinical correlation that is patients with small vessel injury are usually present with purpura, palpable purpura, urticaria, vesicles and bullae, and splinter hemorrhages. But patients with medium size vessel injury clinically present with Cutaneous nodules, ulcers, livedo reticularis, and digital gangrene. most of the cutaneous vasculitides affect primarily the small vessels of the dermis and the subcutis.

IMMUNOFLUORESCENCE TESTING

Immunofluorescence (IF) is a histochemical technique employed to detect antibodies bound to antigens in the tissue or in the circulating body fluids. It acts as a valuable adjunct to clinical and histopathological diagnosis, especially in vesiculobullous and connective tissue disorders [60].

Demonstration of IF technique was first done by Coons in 1940s to demonstrate the microorganism in the infected tissue. However, its application in dermatopathology came much later; in 1963, when these techniques were used to demonstrate the deposition of immunoglobulins and complement at the dermoepidermal junction in systemic lupus erythematosus (SLE) .[61] One year later, in 1964, Beutner and Jordon using indirect IF (IIF) successfully demonstrated the circulating antibodies in the sera of pemphigus patients.[62] Since then, it has been extensively used to understand and classify various disorders where immune mechanisms play a role. Thus, IF has become an

essential investigation in the diagnosis and management of vesiculobullous, vasculitis, autoimmune, and connective tissue disorders.[60]

Beutner and Jordon demonstrated auto-antibodies on the cell surface of keratinocytes by direct immunofluorescence [DIF] In 1964.[84]

TYPES OF IMMUNOFLUORESCENCE

There are two types of IF techniques[61]. They are

- Direct immunofluorescence method .
- Indirect immunofluorescencemethod.

DIRECT IMMUNOFLUORESCENCE

Direct immunofluorescence testing has a pivotal role in the diagnosis of several dermatological disorders like autoimmune and inflammatory mucocutaneous diseases, including autoimmune-mediated blistering diseases, dermatitis herpetiformis, Henoch-Schoenlein purpura [Ig A vasculitis], and cutaneous lupus erythematosus[63] in which probes for immunoreactants localized in patients' skin or mucous membranes.

BIOPSY PROCEDURE

A 3–4 mm punch biopsy is optimum for DIF study; to get a maximum yield, it is important to take biopsy from an appropriate site.

An ideal site of biopsy in all autoimmune blistering diseases (AIBDs) is the perilesional skin.Because DIF microscopy may be negative if the biopsy is taken from lesional skin as the in vivo-bound autoantibodies are consumed by the inflammation.

In cases of vasculitis, a freshly erupted purpuric spot in the most proximal part of the limb is preferred as IgA deposits may undergo degradation in older lesions.

Lesional biopsy is also preferred in cases of discoid lupus erythematosus (DLE), amyloidosis, and lichen planus (LP).

In porphyria cutanea tarda , biopsy should be taken preferably from the lesional skin; a second biopsy from the perilesional, normal skin may be considered, especially if the patient has an intact blister.

Even in asymptomatic dermatitis herpetiformis , wide-shaved specimens from the elbows or any other classically affected area will still show the typical IgA deposits at the tips of dermal papillae in direct immunofluorescence [64].

PRECAUTION

It is important to avoid contamination of biopsy samples with formalin which render the skin specimen unsuitable for DIF study. Common scenario in clinical settings where formalin contamination of biopsy sample occurs is when two biopsies are planned for routine histopathology and DIF. In a situation like

this, the first biopsy is taken for histopathology and the same forceps are used to pick up the second biopsy (for DIF) specimen leading to formalin contamination. Therefore, when two biopsies are planned, the first biopsy should always be taken for DIF rather than histopathology examination.

TRANSPORTATION OF BIOPSY SAMPLES

Skin biopsies for immunofluorescence studies should be kept fresh and moist until it is quickly frozen. Skin specimens can be kept on saline-moistened gauze in a small Petri dish for 24 hours [65] . If the facility for IF is not available locally, biopsy sample can be transported to the test centre in Michel's medium .

Michel's transport medium contains [66]

- 5% ammonium sulfate,
- N-ethylmaleimide,
- potassium citrate buffer [ph 7.25],
- magnesium sulfate, and distilled water.

It probably preserves immunoantigenicity of the specimen by its ability to precipitate macromolecules while inhibiting proteolytic enzymes [67]. Immunoreactants may be demonstrable by DIF even at 6 months, indicating the reliability of this medium in long-term preservations of skin biopsies. [68]

Biopsy specimen received in Michel's medium is washed in phosphate bufferd saline [PBS] to remove all the ammonium sulphate from the tissue ,

preferably in a rotator at 4°C. It is then oriented and embedded in optimal cutting temperature compound and then the specimen snap frozen. Sections of 4–6 µm thickness are then cut using a cryostat. The frozen sections are incubated with antihuman antibodies to IgG, IgA, IgM, C3,C5b-9, and fibrinogen. These antibodies are linked to a fluorescent label such as fluorescein isothiocyanate to allow visualization using a fluorescence microscope [69].

INTERPRETATION OF DIRECT IMMUNOFLUORESCENCE

The DIF test is analyzed based on the following four parameters:

(1)Nature of immune deposits: IgG, IgA, IgM, C3c

(2) Site of immune deposits: Dermoepidermal junction/intercellular spaces (ICS) in epidermis/blood vessels/hair shaft/cytoid bodies

(3) Semiquantitative grading of strength of fluorescence: + to ++++

(4) Pattern of immune complex deposits: granular or linear .

IF STAINING PATTERNS

INTERCELLULAR SPACE STAINING

This pattern of deposition of IgG and C3c in intercellular space staining (ICS) has been referred to as “chicken-wire” or “fish-net”pattern.[70] .Autoantibodies in pemphigus are directed against desmosomal proteins, called desmoglein 1 and desmoglein 3 which are responsible for the cell-to-cell adhesion in the epidermis results in fishnet pattern of staining.

IgG STAINING IN INTERCELLULAR SPACE STAINING

This pattern is seen in all types of pemphigus except IgA pemphigus. The staining pattern is identical in pemphigus vulgaris and pemphigus foliaceus , but at times, the fluorescence may be localized to or more intense along the upper layers of epidermis in pemphigus foliaceus .[71] C3c deposition follows the same pattern as IgG, but it is less intensely stained compared to IgG and usually detected in patients with active disease.[71] .

IgA STAINING IN INTERCELLULAR SPACE STAINING

It is characteristically seen in IgA pemphigus; two types of IgA pemphigus have been recognized - subcorneal pustular dermatoses type and intraepidermal neutrophilic type[71]. In subcorneal pustular dermatoses type, IgA deposition is seen predominantly in the upper epidermal layers, whereas in intraepidermal neutrophilic type, it is seen throughout the epidermis or restricted to the lower epidermis.[72] Basement Membrane Zone Staining Deposition of immunoreactants at the dermoepidermal junction occurs in a diverse group of conditions such as subepidermal autoimmune blistering disorders and connective tissue diseases such as lupus erythematosus. This may be linear deposition or granular deposition.

LINEAR BASEMENT MEMBRANE ZONE [BMZ] STAINING

The deposition of IgG, C3c, or both in a linear fashion along the BMZ is seen in Bullous pemphigoid, mucous membrane pemphigoid , Pemphigus gestationalis, epidermolysis bullosa aquistia (EBA), and recently described anti-p200pemphigoid.[73,74,75,76,77,78]

Relative intensity of the staining with IgG and C3c may sometimes help to subcategorize these conditions. For example, a more intense staining of C3c when compared to IgG at basement membrane zone indicates the diagnosis of pemphigoid group of disorders (BP, MMP, and PG).

Linear deposition IgA along the BMZ is a pathognomonic feature of linear IgA disease (LAD) . Occasionally, C3c or IgG deposition can be seen, but it is less intense when compared to IgA.[79]

GRANULAR BASEMENT MEMBRANE ZONE STAINING

Granular positivity along the BMZ [band like positivity] is present in SLE. It is usually seen with IgM but may be seen with all other immunoreactants as well. In fact, presence of 3 or more immunoreactants deposition in BMZ is highly suggestive of SLE. In addition, colloid bodies (staining frequently with IgM) in the papillary dermis and epidermal nuclear staining with IgG (epidermal “ANA”) may be seen in SLE.

INTERCELLULAR AND BASEMENT MEMBRANE ZONE STAINING

This type of dual staining pattern of epidermal intercellular and basement membrane zone occurs in two conditions, namely, pemphigus erythematosus [PE] and paraneoplastic pemphigus[PNP] [71].

Pemphigus erythematosus is a variant of pemphigus foliaceus characterized by immunopathological coexistence of pemphigus foliaceus and lupus erythematosus [80]. DIF in pemphigus erythematosus reveals ICS in a "fish-net" pattern; in addition, there is granular BMZ staining with IgG resembling "lupus band." Occasionally, these patients may have circulating antinuclear antibodies in their blood.

PNP is characterized by autoantibodies against desmosomal (Dsg 1 and 3, desmoplakin, envoplakin, and periplakin) as well as basement membrane zone protein. So, DIF in PNP reveals intercellular staining and linear BMZ staining with IgG and C3c. Intercellular staining in PNP tends to be weak, diffuse, and nonspecific, while deposition along BMZ is almost identical to that of BP.[71]

BASEMENT MEMBRANE AND BLOOD VESSEL WALL STAINING

DIF microscopy in porphyrias (PCT, pseudo–PCT, and erythropoietic protoporphyria) is characterized by a homogeneous deposition of IgG, IgA, and less frequently C3 along the BMZ as well as within superficial blood vessel

walls. The density of these reactants is quite considerable and extends onto the surrounding dermis.

PAPILLARY DERMAL STAINING

Granular deposits of IgA in the papillary dermis are the diagnostic feature of dermatitis herpetiformis (DH) .sometimes, a similar pattern may be seen with other immunoreactants (C3 and fibrinogen) [81]. Occasionally, in atypical DH a fibrillar pattern of IgA deposition along BMZ may be seen [82].

EXCLUSIVE BLOOD VESSEL WALL STAINING

This is the characteristic feature of cutaneous small vessel vasculitis. The primary site of immune deposition is within the walls of the post capillary venules in the superficial dermis[85].the most common immune deposits are C3c and fibrinogen but in Henoch–Schönlein purpura the classical feature is the granular staining of IgA within the vessel wall with or without other immune deposition.

TABLE 3:DIF findings in various vesiculobullous disorders

Direct Immunofluorescence in Vesiculobullous disorderd			
Dermatosis	**Principal immunoreactant**	**Site**	**Pattern**
Pemphigus, all variants except IgA pemphigus & PNP	IgG	ICS	Lacelike,dotlike
IgA pemphigus	IgA	ICS	Lacelike,dotlike
Paraneoplastic pemphigus [PNP]	IgG C3, IgG C3, IgG	ICS BMZ BMZ	Lacelikedotlike Linear Granular
Bullous pemphigoid	C3, IgG	BMZ	Linear
Cicatricial pemphigoid	C3, IgG	BMZ	Linear
Anti-p200 pemphigoid	C3, IgG	BMZ	Linear
Herpes gestationis	C3	BMZ	Linear
EBA	C3, IgG	BMZ	Linear
Bullous SLE	C3, IgG C3, IgG	BMZ BMZ	Linear Granular
Dermatitis herpetiformis	IgA	BMZ	Granular
LAD	IgA	BMZ	Linear
EM	C3, IgM C3, IgM	BMZ Vessels	Granular Granular
Porphyria/Pseudoporphyria/Bullous dermatosis of hemodialysis	IgG	BMZ vessels	Glassy broad Glassy broad

SALT SPILT TECHNIQUE

This method was first demonstrated by Gammon et al to diagnose the vesiculobullous disorders with similar DIF features [83].

This technique cosist of thawing the frozen section specimen and incubating it in 1M sodium chloride [Nacl] for 48-72 hours and allowing for the seperation of epidermis from the dermis. This salt cleaves the basement

membrane zone through the lamina lucida because this is the weakest point in basement membrane zone, finally leaving the hemidesmosomes on the epidermal side and deap seated proteins like type VII collagenon the dermal side of the artificially induced blister.

AIMS AND OBJECTIVES

- To analyse clinical picture, histopathological finding with direct immunofluorescence[DIF]
- To access the role of direct immunofluorescence in the evaluation of dermatological disorders

MATERIALS AND METHODS

DATA SOURCE

The present study "CLINICAL AND IMMUNOHISTOPATHOLOGICAL STUDY OF DERMATOLOGICAL LESIONS IN A TERTIARY CARE CENTRE" was conducted in the Institute of Pathology ,Madras Medical College and Rajiv Gandhi Government General Hospital and Department of Dermatology, Rajiv Gandhi Government General Hospital, Chennai-03.

STUDY PERIOD: 1.5 years [May 2018 to October 2019]

STUDY DESIGN: Prospective study .

SAMPLE SIZE : 100 cases.

INCLUSION CRITERIA

Patients clinically diagnosed as immunobullous disorders, connective tissue disorder and vasculitis.

EXCLUSION CRITERIA

1. Patients with dermatological malignancy.

2. Patients with active bacterial and parasitic infections.

METHODOLOGY

SAMPLE COLLECTION METHOD

Two skin biopsies were collected from the patient.

1. One for histopathological examination with hematoxylin and eosin staining.
2. Other for DIF study by using fluorescein isothiocyanate(FITC)-Conjugated with rabbit antihuman immunoglobulin G (IgG) ,IgA, IgM,and C3c.

After obtaining informed consent and xylene test dose with universal aseptic precautions and under local anaesthesia incisional biopsy of skin was done. Totally two samples were taken one put into the 10% neutral buffered formalin for histopathological examination other one was put into the saline for DIF testing. The procedure was done by the clinician as a routine procedure for diagnosis and treatment. Both samples were processed in institute of pathology for histopathological and DIF studies.

SITE OF BIOPSY:

- For vesiculobullous disorders : biopsy from the perilesional area less than 1 cm from the bulla.
- For non bullous lesions : biopsy from an established skin lesion.

HISTOPATHOLGICAL EXAMINATION

The skin biopsy sample obtained in 10% neutral buffered formalin was kept in the fixative for 24 hours. Then processed by the automated tissue processor and finally embedded in the paraffin blocks. The block were cut into 3-4 µm thickness sections . After removing the wax ,the slides were stained with haematoxylin and eosin

HAEMATOXYLIN AND EOSIN STAINING

REQUIRED MATERIALS:

1. Harris haematoxylin.
2. 1% eosin (1gram of eosin + 100 ml of distilled water).
3. Xylol.
4. Absolute isopropyl alcohol I and II .
5. 90% isopropyl alcohol .
6. 1% acid alcohol (79 ml of isopropyl alcohol + 1 ml concentrated hydrochloric acid[HCL]+ 29 ml distilled water)

PROCEDURE:

1. Paraffin sections of thickness 2-4 µm were taken on an egg albumin coated slides.
2. Air dry the slides and dewax them at 62- 64ºc.

3. Then transfer the sections immediately to xylene I and II each for 30 minutes.
4. Sections are then transferred to absolute alcohol I and II each for 5 minutes.
5. Bring the sections to water.
6. Clean the slides around the sections.
7. Transfer the sections to harris haematoxylin for 5 to 10 minutes.
8. Drain the slides and wash them in tap water.
9. Dip the slides 1 to 2 times in 1% acid alcohol.
10. Wash the slides in tap water.
11. Keep the slides in washing tray (for blueing) for 10 to 15 minutes.
12. Slides are dipped 1 to 2 times in 1% eosin.
13. Wash the slides in several changes of water till the water becomes colourless.
14. Air dry and clear the sections using xylol
15. Sections are mounted with DPX mountant.

RESULTS :

- Cytoplasm – shades of pink.
- Nuclei – Blue.

DIRECT IMMUNOFLUORESCENCE METHOD FOR SKIN BIOPSY

REQUIRED MATERIALS:

1. Glass microscopic slides
2. Coverslips in the size of 22x 22mm
3. Slide holding box
4. Cryostat with accessories
5. Distilled water
6. Phoshate buffer solution
7. Fluorescence microscope- 495 nm exciter filter ,515 nm barrier filter (blue filter- 450-490 nm, green filter- 510560 nm)

REQUIRED REAGENTS

1. Phosphate buffered saline
2. Antihuman IgG FITC
3. Antihuman IgM FITC
4. Antihuman IgA FITC
5. Antihuman C3c FITC
6. Fluorescein free glycerol [mounting medium]

SOLUTION PREPERATION

PHOSPHATE BUFFERED SALINE [PBS] STOCK

It contains the following components.

- Disodium hydrogen phosphate – 14.8 gms
- Potassium dihydrogen orthophosphate -4.3 gms
- Sodium chloride- 68 gm
- Distilled Water- 1000ml

WORKING PHOSPHATE BUFFERED SALINE

Dilute 1:10 of stock PBS in Distilled water for working dilution by adding 1 part of stock buffer to 9 parts of distilled water (100 ml stock PBS, 900 ml distilled water , PH 7-7.4)

FITC dilution

Dilute FITC conjugate 1: 20 with PBS 20 micro litre of FITC and 380 micro litre of PBS.

MOUNTING MEDIUM

Dilute 9 parts of flurorescin free glycerol with1 part of PBS

PROCEDURE

1. First snap freezing of the tissue was done with with liquid nitrogen .
2. Cut cryostat sections of skin tissue which has been frozen .The tissue sections should be 3µm in thickness .
3. Pick up the sections on a clean and dry slide & label the slide.
4. Place the slides in a slide holding box and leave it in the freezer at -20ºC Overnight.
5. Next day morning take the slides out from freezer and bring the slides to room temperature.
6. Select 4 slides and label them with assigned IF number and the name of immunoglobulin and the complement which is to be added on them .
7. Arrange the slides on a staining rack .
8. Flood the slides with PBS and leave it for 10 minutes .
9. After 10 min drain off the PBS and add fresh saline.
10. Repeat the washing three times each wash of 10 minutes each .
11. At the end of the last wash drain off the excess PBS and wipe dry around the sections .
12. Place the slides back on the staining rack .
13. Add the appropriate FITC dye diluted at 1: 20 on the slides as per the label on the slide .
14. Cover the slide rack with another rack .

15. Incubate the slides at room temperature (22- 25ºC) for 30 minutes.
16. At the end of the incubation drain off the unbound dyes.
17. Wash the slides three times with buffered saline of 10 minutes each.
18. At the end of the third wash drain off the excess saline wipe all around the section dry .
19. Mount the section with fluorescein free glycerol using a coverslip .
20. Immediately view the slides under a immunofluorescence microscope.

RESULT:

- Green fluorescence - Positive.
- Background – black.

OBSERVATION AND RESULTS

The present study was a prospective analytical study in skin biopsies of patients with vesiculobullous disorder , connective tissue , disorder and with vasculitis . This study was conducted over a period of 1.5 years from May 2018 to October 2019 . The total number of cases included for my study was 100. The biopsies were received from the Department of dermatology , Madras Medical College & Rajiv Gandhi Govt. General Hospital, Chennai.

Clinical picture , histopathological findings and direct immunofluorescence findings were analysed in this study and results were taken for the final conclusion . I divided the total cases into three groups based on clinical diagnosis for the easy analysis of the results.The three groups are,

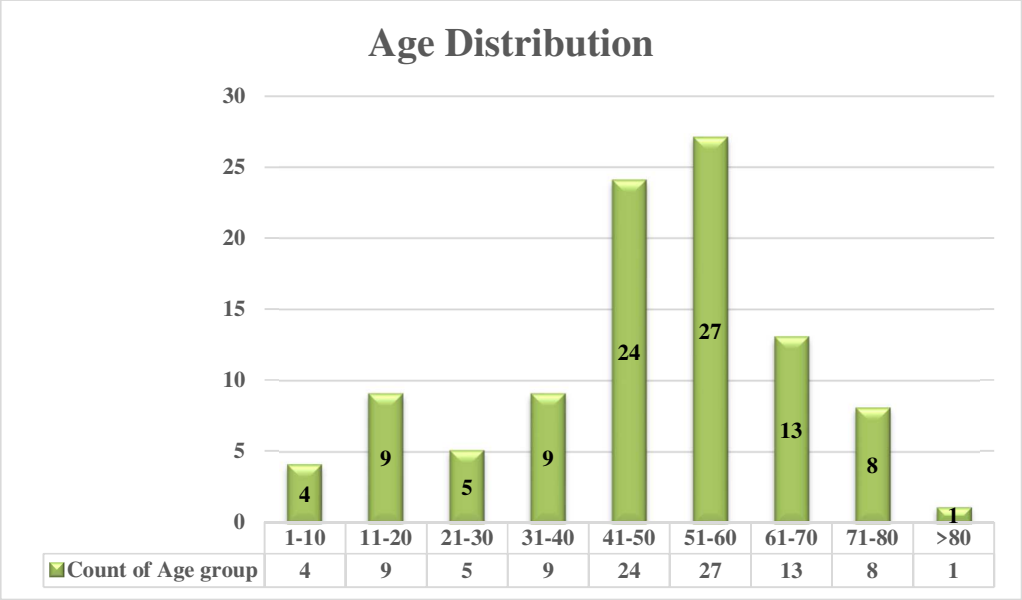
1. Patients with suspected immunobullous disorder[pemphigus & pemphigoid group of disorders -85 cases
2. Systemic lupus erythematosus -7 cases
3. Vasculitis -8 cases

AGE WISE DISTRIBUTION

In my present study maximum number of patients were belonging to the age group between 51-60 years which constitute about 27% followed by the age group 41-50 years. This study included four patients [4%] with less than 10 years of age. Least number of

the patients were present in the age group of more than 80 years which constitute about 1% of the total cases.

CHART 1: AGE WISE DISTRIBUTION



AGE DISTRIBUTION IN EACH GROUP

In the analysis of age distribution in each group the most common age group in immunobullous disorders were 51-60 years which is about 30.5% .

The most common age group for SLE was 11-20 years which is constitute about 57.14 % followed by 21-30 years that is 42.8%.

In vasculitis most of the patients are belong to the age between 11-20 years that is 37.5% .

CHART 2: AGEWISE DISTRIBUTION IN EACH GROUP

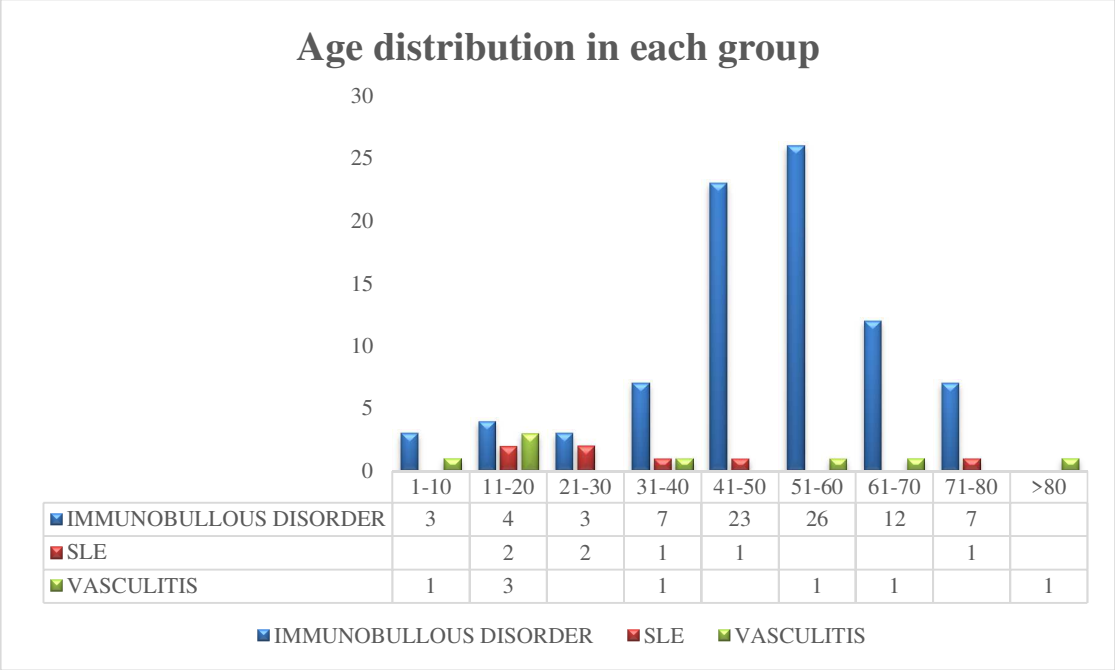


TABLE 4: GENDER WISE DISTRIBUTION OF CASES

SEX	FREQEUNCY	PERCENTAGE
MALE	**52**	**52%**
FEMALE	**48**	**48%**
TOTAL	**100**	**100%**

In the present study the male patients constitute about 52% and female patients constitute aboue 48% with male female ratio of 1.08:1 .

CHART 3:GENDERWISE DISTRIBUTION OF CASES

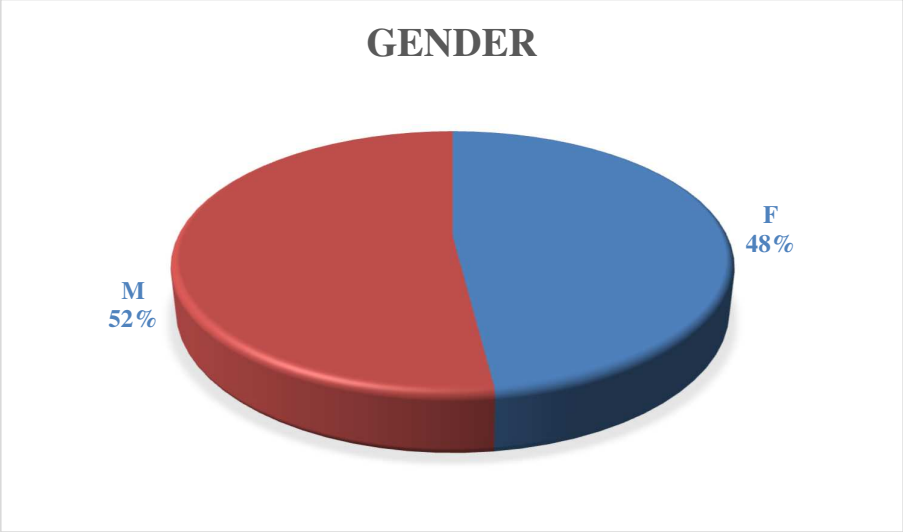
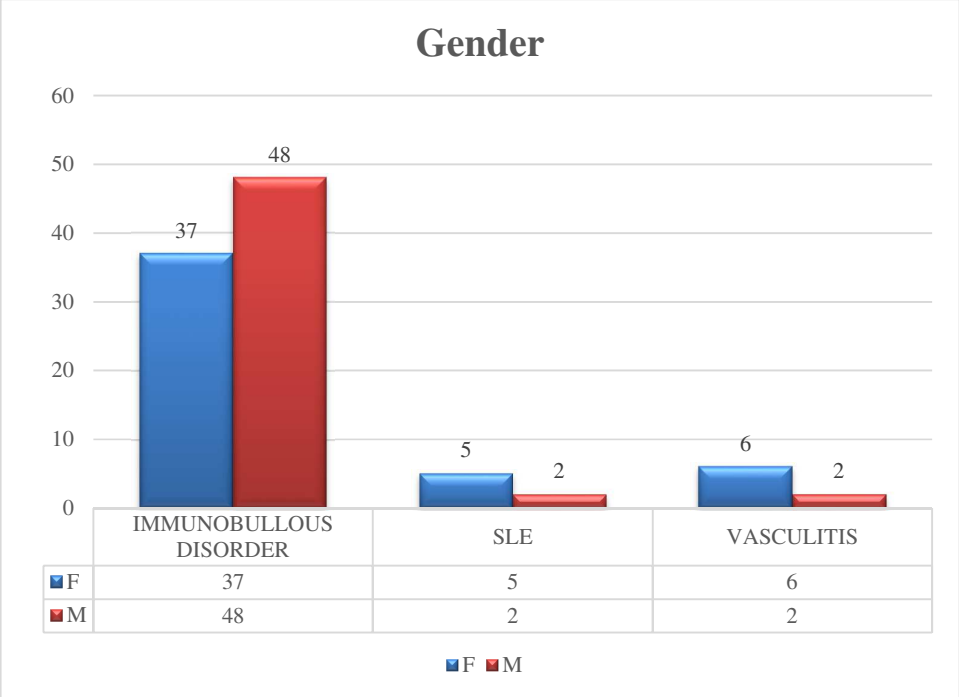


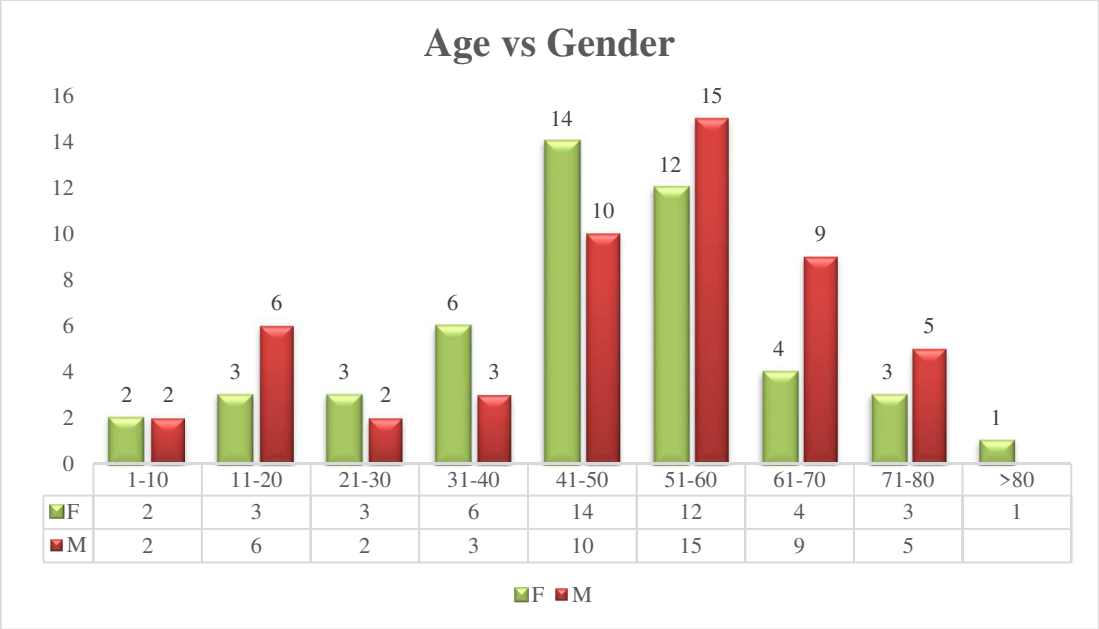
CHART 4: SEXWISE DISTRIBUTION OF CASES IN EACH GROUP



When gender wise distribution in each group was analysed SLE and vasculitis having female preponderance which is about 71.45 , 75%

respectively while immunobullous diaorder having male prepondarnce which is constituted about 56.4%.

CHART 5: AGE VS GENDER DISTRIBUTION OF CASES

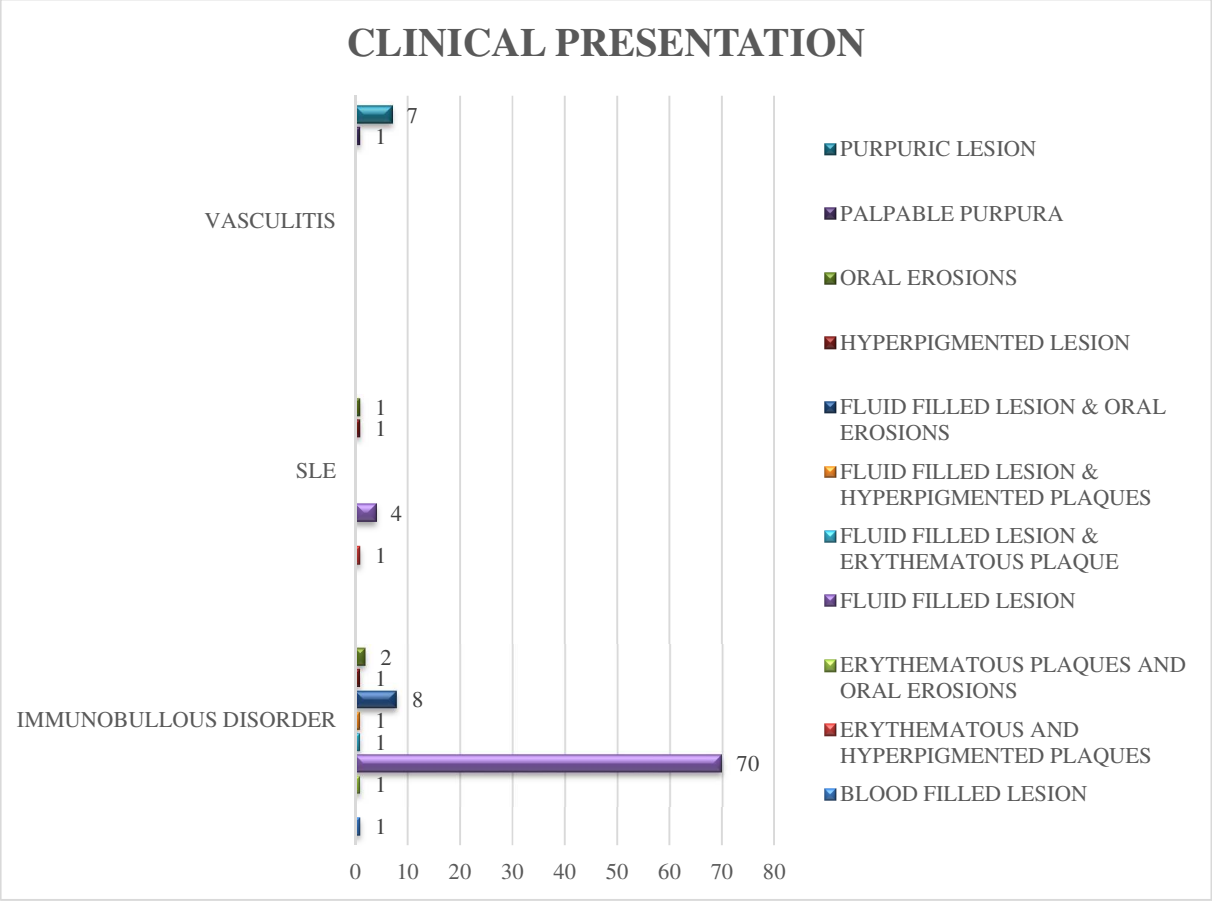


Majority of the patients in immunobullous disorder group were clinically presented with fluid filled lesions which is about 82.35%.Among the total 85 cases in this group 8 patients [9.4%] were associated with oral erosions along with fluid filled lesions.

In SLE group also the most common clinical presentation was fluid filled lesions which constituted about 57.14%. The other cutaneous manifestations of SLE in the present study were oral erosions, hyperpigmented lesion , erythematous and hyperpigmented plaques each constituted about 14.2%.

In vasculitis group 87.5% of the cases [7 cases] clinically presented with purpuric lesions. Only one case of vasclulitis [12.5%] was presented with palpable purpura.

CHART 6:CLINICAL PRESENTATION OF CASES



In 51.76 % [44 cases] of the immunobullous disorders the lesions were involved all over the body followed by trunk [23 cases] and upper limb [10 cases]. The other sites of involvement were buccal mucosa [2 cases], lower limb [2 cases] , scalp [2 cases] , face [1 case] , and scrotum [1 case] .

In SLE 2 cases were having upper limb involvement which constituted about 28.57 %. Other site like buccal mucosa, scalp ,face and trunk involvement in 1 cases each. Only one case was presented with all over the body involvement [14.28 %].

In vasculitis 7 out of 8 cases were have lower limb involvement which is about 87.5% . only one case of vasculitis was having trunk involvement. None of the vasculitis cases was having all over body involvement.

CHART 7:SITE OF INVOLVEMENT OF CASES

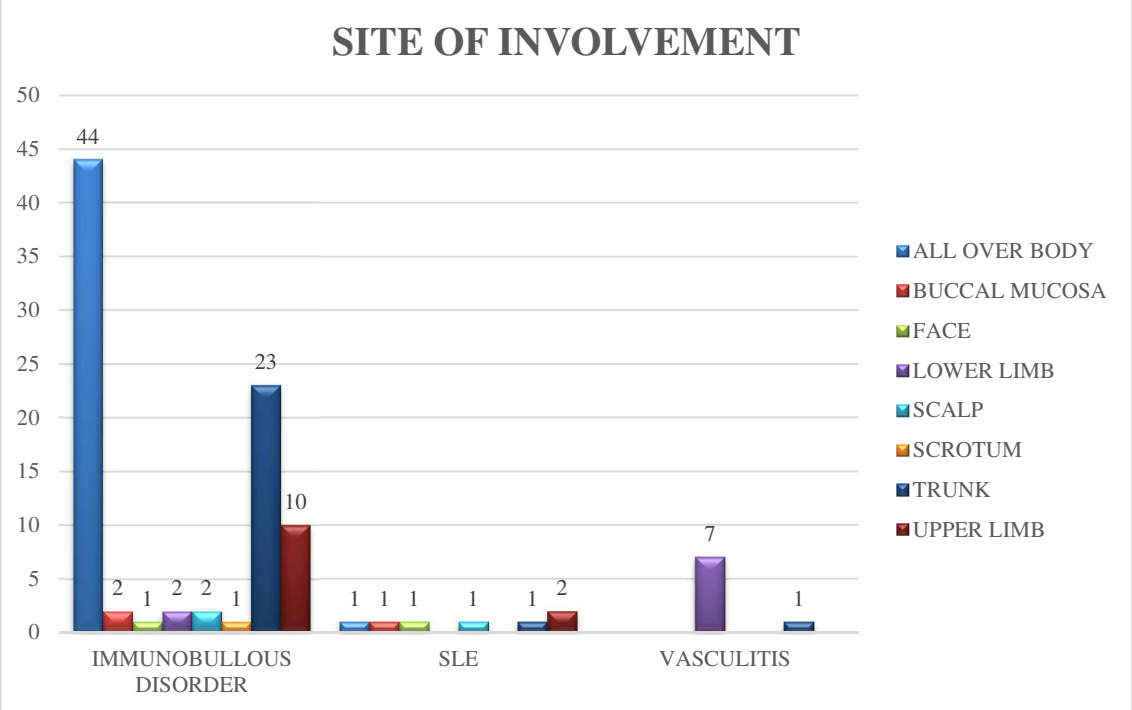


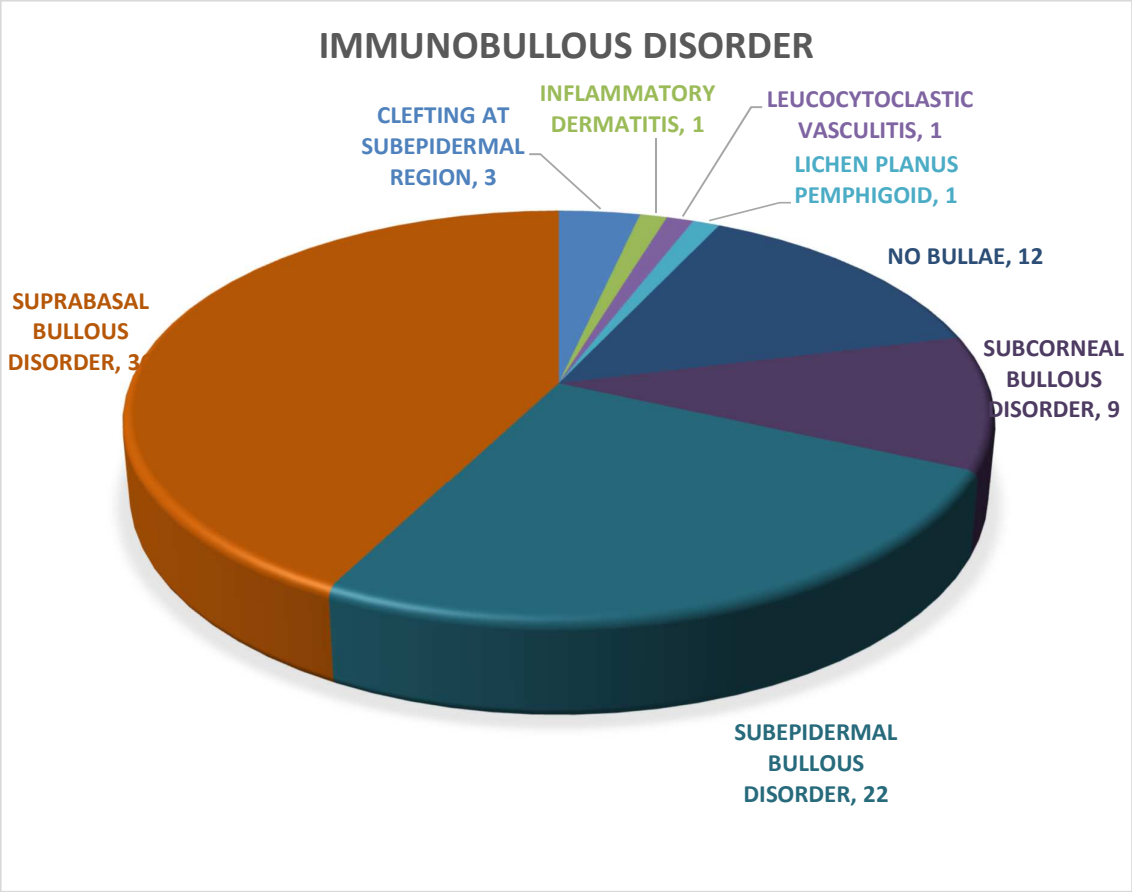
TABLE 5: HISTOPATHOLOGICAL FINDINGS IN PATIENTS SUSPECTED IMMUNOBULLOUS DISORDER

HISTOLOGICAL FINDING	TOTAL NO OF CASES	PERCENTAGE [%]
CLEFTING AT SUBEPIDERMAL REGION	3	4%
INFLAMMATORY DERMATITIS	1	1%
LEUCOCYTOCLASTIC VASCULITIS	1	1%
LICHEN PLANO PEMPHIGOID	1	1%
NO BULLAE	12	14%
SUBCORNEAL BULLOUS DISORDER	9	11%
SUBEPIDERMAL BULLOUS DISORDER	22	26%
SUPRABASAL BULLOUS DISORDER	36	42%

The most common histopathological finding in patients suspecting immunobullous disorder was suprabasal bullae which constituted about 42% [36 cases] followed by subepidermal bullae[22cases-26%]. The skin biopsy of 14 % of cases [12 patients] were showed no evidence of bullae. 11% [9 cases] of biopsies were showed subcorneal bullae , 3 biopsies [4%] were showed no definite bullae but subepidermal clefting was made out. Inflammatory dermatitis

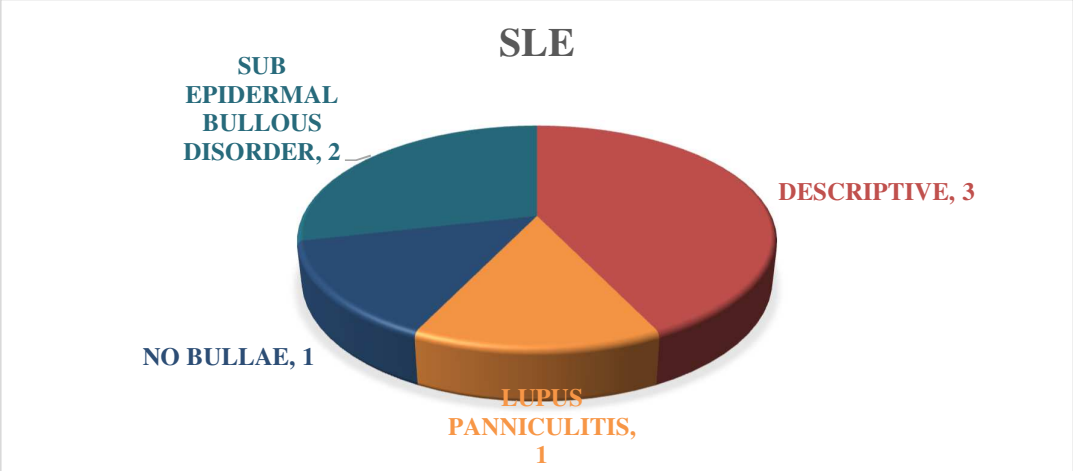
,lichenplano pemphigoid and leucocytoclastic vasculitis were formed 1% of cases each.

CHART 8:HISTOPATHOLOGICAL FINDINGS IN PATIENTS WITH SUSPECTED IMMUNOBULLOUS DISORDER



The histopathological findings of the patients with the clinical history of SLE were analysed. 2 cases [28.5%] were showed subepidermal bullae ,one case [14.2%] was showing features of lupus panniculitis and 3 cases [42.8%] were given description only without making any conclusive opinion. One case [14.2%] was showed no evidence of bullae.

CHART 9:HISTOPATHOLOGICAL FINDINGS IN SLE

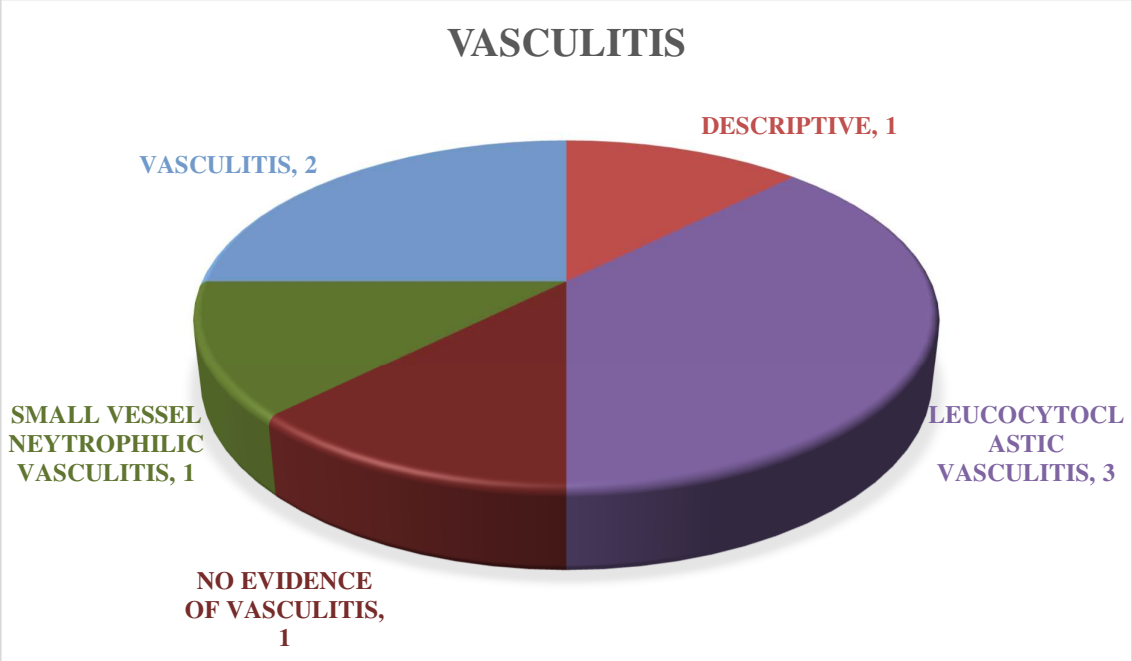


Among vasculitis cases leucocytoclastic vasculitis was the commonest hiatopathological findings which constitute about 37.5% . One case showed no evidence of vasculitis histopthologically.

TABLE 6: HISTOPATHOLOGICAL FINDINGS OF VASCULITIS

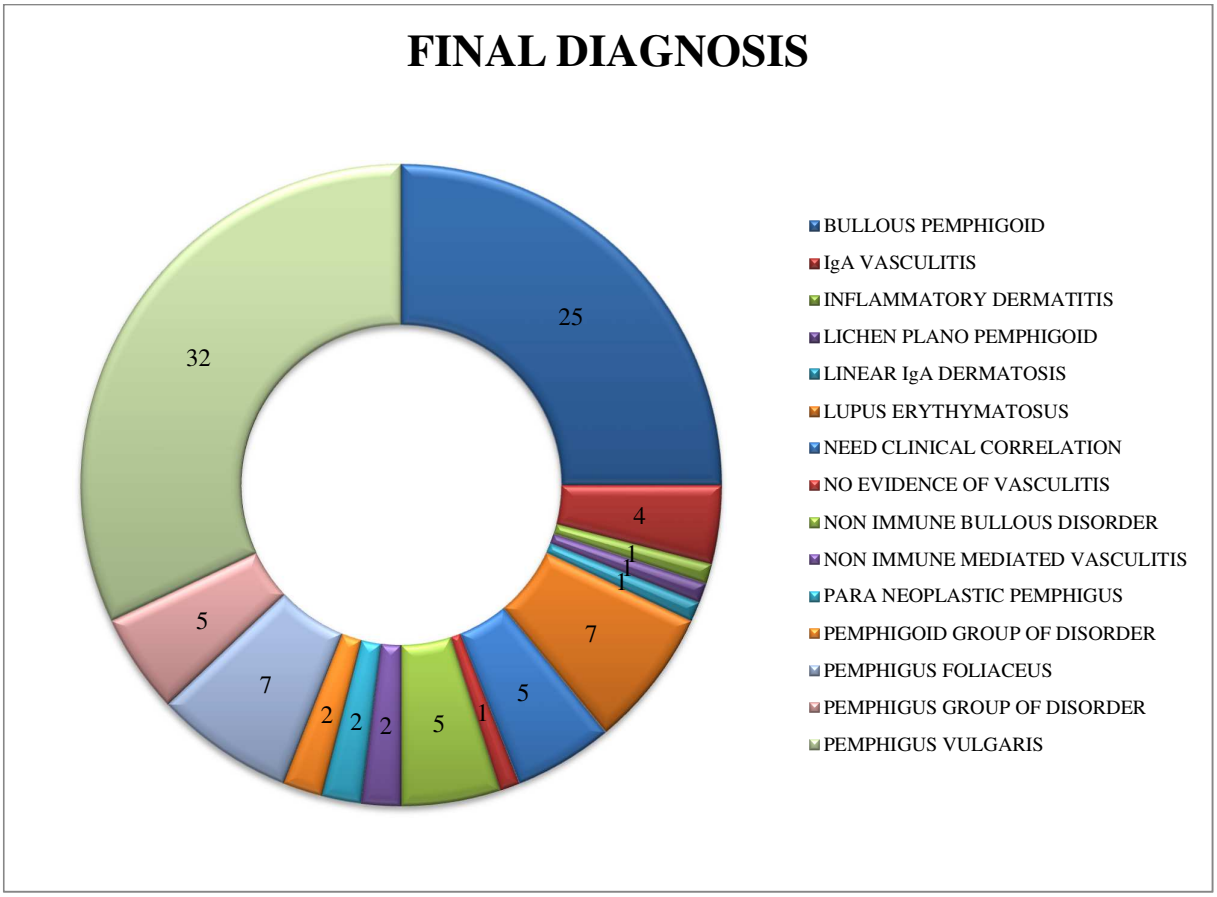
HPE FINDING	NO OF CASES	PERCENTAGE [%]
Leucocytoclastic vasculitis	3	37.5%
Vasculitis	2	25%
Small vessel neutrophilic vasculitis	1	12.5%
Descriptive	1	12.5%
No evidence of vasculitis	1	12.5%
TOTAL	8	100%

CHART 10:HISTOPATHOLOGICAL FINDINGS IN VASCULITIS



The most common diagnosis after DIF testing was pemphigus vulgaris which contributed about 32%[32/100 cases] followed by bullous pemphigoid which contributed about 25% [25/100 cases]. And the rare cases were lichen planus pemphigoid, paraneoplastic pemphigus, linear IgA dermatosis,.

CHART 11:FINAL DIAGNOSIS OF ALL CASES



Out of 7 vasculitis cases 4 were reported as IgA vasculitis which showed positivity in the superficial dermal vessels in DIF testing. Among SLE cases all were finally reported as lupus erythematosus[100%].

TABLE 7: DIF FINDINGS IN PATIENTS WITH SUSPECTED IMMUNOBULLOUS DISORDER

FINAL DIAGNOSIS	Total	IgG	IgA	IgM	C3c
Pemphigus vulgaris	32	32 [100%]	0	0	22[68.75%]
Bullous pemphigoid	25	23 [92%]	1 [4%]	1 [4%]	20 [80%]
Pemphigus foliaceus	7	7 [100%]	1 [14.28 %]	1 [14.28%]	4 [57.14%]
Pemphigus group of disorders	5	5 [100%]	0	0	2 [40%]
Pemphigoid group of disorders	2	2 [100%]	0	0	2 [100%]
Paraneoplastic pemphigus	2	2 [100%]	0	0	0
Linear IgA dermatosis	1	1 [100%]	1 [100%]	0	0
Lichenplano pemphigoid	1	1 [100%]	0	0	1 [100%]
Inflammatory dermatitis	1	0	0	0	0
Non immune bullous disorder	5	0	0	0	0
Need clinical correlation	5	0	0	0	0

Pemphigus vulgaris, Pemphigus foliaceus, Paraneoplastic pemphigus, Linear IgA dermatosis, Lichenplano pemphigoid showed 100% positivity with IgG.

DIF FINDINGS IN PATIENTS WITH SLE

Among the lupus erythematosus cases 100% of the cases were positive for IgG [7 cases] ,IgM was positive in 6 cases [85.7%] ,IgA was positive in one case [14.28%] and C3c was positive in 4 cases [57.14%].only one case was showed full house effect i.e positive for all [IgG , IgA ,IgM ,and C3c]. All the positivity were at the basement membrane zone [granular band like positivity]

DIF FINDINGS IN PATIENTS WITH VASCULITIS

Out of 8 cases 4 were positive for IgA [50%] which were finally diagnosed as IgA vasculitis . The fluorescent positivity was present in the small blood vessel in the superficial dermis. One case was positive for C3c in the superficial dermal vessels..

FINAL DIAGNOSIS OF CASES WITH NO BULLAE IN HPE

Totally 13 cases were found to be have no evidence of bullae in histopathological examination .Out of which 5 cases were diagnosed as pemphigus group of disorder ,3 cases were diagnosed as bullous pemphigoid.

Pemphigoid group of disorder and lupus erythematosus were contribute about one case each. 3 out of 13 cases were negative for both in DIF and histopathology.

DISCUSSION

The present study was done with 100 cases in patients with vesiculobullous disorder , connective tissue disorder and with vasculitis . The duration of the study was one and half years from May 2018 to October 2019 . In this study skin biopsies taken from the patients were subjected into histopathological examination and direct immunofluorescence examination .

In the present study Clinical findings , Histopathological picture and direct Immunofluorescence findings were analysed and correlated. Also the Results were compared with other studies.

TABLE 8: COMPARISON OF GENDER DISTRIBUTION WITH OTHER STUDIES

AUTHORS	YEAR OF STUDY	AGE RANGE INYEARS
Vijaya v.mysorekar et al [86]	2015	2-94
Keya basu et al[90]	2019	17-85
Kamal ahmed et al[89]	2006-2008	3-80
Present study	2018-2019	2-80

In the present study the most common age group was 51-60 years. But in most of the studies [Keya basu et al [90]& Rajeswari thivya dhanapalan et al[91]] the commonest age group was between 4th to 5th decade.

TABLE 9: COMPARISON OF GENDER DISTRIBUTION WITH OTHER STUDIES

AUTHOR	YEAR	MALE FEMALE RATIO
Ranjana walker minz et al[87]	2010	1:1.2
Rajeswari thivya dhanapalan et al[91]	2012-1013	1.08:1
Kamal ahmed et al[89]	2006-2008	0.73:1
Vijaya v.mysorekar et al[86]	2015	1:1.2
Present study	2018-2019	1.08:1

Analysis based on gender wise distribution showed overall slight male preponderance in the present study but in diseasewise SLE and vasculitis had female preponderance which was concordant with the study of Rajeswari thivya dhanapalan et al [91]. But in most of the studies showed female predominance [Ranjana walker mint et al[87] , Kamal ahmed et al[89] , Vijaya v.mysorekar et al [86]

The most common condition among vesiculobullous disorders in the present study was pemphigus vulgaris 37.6% which was concordant with many of the studies conduted in various places [Kamal ahmed et al[89] -40.6%,

Rajeswari thivya dhanapalan et al[91] -20.5%,khan WA et al [88]-33.33% , Keya Basu et al[90] -53%].

In the present study 5 cases [5.88%] were showing no bullae in histopathological examination and in the study of Kamal ahmed[89] et al no bullae in HPE was found in 15.25% of cases.

In the present study the most common age group affected by pemphigus vulgaris was 41-50 years with female preponderance. Majority of the patients [83cases-97.6%] clinically presented with fluid filled lesion and 50% of the patients having all over the body involvement.46.9% of the cases [15 /32 cases] clinical diagnosis were correlate with the final diagnosis. 100% of the cases DIF findings were correlate with the final diagnosis. All the 32 cases [100%] of pemphigus vulgaris were positive for IgG ,22 cases [68.75%] were positive for C3c and combined positivity with both IgG and C3c was observed in 22 cases [68.75%].

The second most common condition among vesiculobullous disorders was bullous pemphigoid which had concordance with the study by Vijaya v.mysorekar et al [86] which constitute about 29.4% [25 cases] in our study. The commonest age group affected was 41-50 years with male preponderance. Final diagnosis of 48% of the cases were correlate with the clinical diagnosis and 84% of the cases were correlate with histopathological findings. DIF studies showed 92% positivity for IgG ,80% positivity for C3c and 4% positivity for

IgA & IgM each. Combined positivity with both IgG and C3c was observed in 72% of cases.

Totally 7 cases [8.2%] were diagnosed as pemphigus foliaceus . Most common age group affected was 51-60 years with slight female preponderance. All the cases were clinically presented with fluid filled lesion and histopathological examination showed subcorneal bullae. In DIF testing 100% of cases positive for IgG , 57.14%[4 cases] of cases positive for C3c.IgA and IgM was positive in one case each. Combined IgG and C3c positivity observed in 57.14 % of cases.

We could not able to distinguish pemphigus vulgaris from pemphigus foliaceus by DIF findings alone as both the conditions were showing fishnet positivity in the epidermis ,and the correct diagnosis is possible only with the correlation of clinical picture and histopathological diagnosis with the DIF findings. In some cases of pemphigus foliaceus intensity of positivity is strong in the superficial part of epidermis ,it may give some clue to the diagnosis but not always.

Two cases of paraneoplastic pemphigus were reported in this study both cases had past history of malignancy ,one was a known case of carcinoma oesophagus and the another one was a known case of non Hodgkin lymphoma. Both cases were showed suprabasal bullae in histopathological examination and in DIF testing one case showed fishnet IgG positivity in the

epidermis and another case showed trace positivity in the basement membrane zone in addition to fishnet positivity in the epidermis .

A single case of lichen palnus pemphigoid was observed which is clinically presented with hyperpigmented plaques and in DIE testing showed fishnet positivity with IgG & C3c.

One case of linear IgA dermatosis was identified which showed subepidermal clefting in histopathological examination and in DIF testing showed 3+ linear positivity with IgA and 1+ linear positivity with IgG at the basement membrane zone.

One case which was clinically suspected as immunobullous disorder had negative DIF results and histopathologically showed features of inflammatory dermatitis finally diagnosed as inflammatory dermatitis.

Seven cases without any bullae in the histopathological examination and showed positive results in DIF testing. 5/7 cases showed fishnet positivity in the epidermis with IgG or with both IgG& C3c ,those cases were reported as pemphigus group of disorders and 2/7 cases showed linear positivity along basement membrane zone with both IgG& C3c,those cases were reported as pemphigoid group of disorders.

In histopathological examination bullae was observed without DIF positivity in five cases ,in those cases immune mediated disorders were ruled out and finally diagnosed as non immune bullous disorder.

In five cases no bullae were made out in the histopathology as well as showed negative DIF results. In such cases we need more clinical correlation regarding site of biopsy, durtation of the disease,past treatment history etc. One case showed evidence of vasculitis by hisopathology with negative DIF results and diagnosed as non immune mediated vasculitis.

In the present study mucosal involvement was observed in 11.76% of cases [10/85 cases] and the most common condition associated with mucosal involvement was pemphigus vulgaris [70%]. In a study by Kamal ahmed et al[4] Mucosal involvement was found in 57.6% of cases which was discordant with our present study.

In the present study among with vesiculobullous disorder patients DIF was negative in 12.9% of cases [11/85 cases] and positive in 87.1% of cases [74/85 cases] which is concordant with the study of kamal ahmed et al[89] in which the positive DIF results was about 93.2% of cases.

Totally 7 cases of SLE were included in this study . The common age group affected was 11-30 years with female preponderance. 2/7cases [28.5%] were showed subepidermal bullae.one case [14.2%] was showing features of

lupus panniculitis and 3 cases [42.8%] were given description only without any conclusive opinion. One case [14.2%] was showed no evidence of bullae. All the cases [100%] were positive for IgG [7 cases] ,IgM was positive in 6 cases [85.7%] ,IgA was positive in one case [14.28%] and C3c was positive in 4 cases [57.14%].

Only one case [14.28%] of SLE showed full house effect i.e positive for all [IgG , IgA ,IgM ,and C3c].In the study of ranjana walker minz et al[87] full house effect was observed in 23% of cases and most common Immunoglogulin positive was IgM where as in our study the most common Immunoglogulin was IgG. In the study by Vijaya v.mysorekar et al [86] full house effect was seen in 77.7 % of cases which was discordant with the present study.

Eight cases of vasculitis were included in the present study . Most of the cases were between 11-20 years of age with female Preponderance. The common site of involvement was lower limb [87.5%] and the most common hiatopathology finding was leucocytoclastic vasculiitis . All these findings were very well correlate with the study by Nandeesh B et al [92] . 4/7 cases showed IgA positivity in the blood vessel wall at superficial dermis and diagnosed as IgA vasculitis. Three cases showed evidence of vasculitis in histopathology among which one case C3c positivity was observed in dermal blood vessels and conclusive opinion was not made out. One case showed no evidence of vasculitis in histopathology as well as with negative DIF results.

In the present study out of 100 cases totally 12 cases were belonging to paediatric age group among which IgA vasculitis and pemphigus vulgaris were the common conditions and each disease constitute about 3 cases [25% each] followed by lupus erythematosus [2cases-16.6%]. Other conditions in paediatric age group were linear IgA dermatosis ,pemphigoid group of disorder,non immune mediated vasculitis each 1 cases. In one case final diagnosis was inconclusive.

Overall concordance between clinical diagnosis and final diagnosis was 47% and concordance between histopathological diagnosis and final diagnosis was 83%.

SUMMARY

The present study was conducted in the Institute of pathology, Madras medical college and Rajiv Gandhi Government General Hospital, Department of dermatology, Rajiv Gandhi Government General Hospital,Chennai-3, with 100 cases in patients with vesiculobullous disorder , connective tissue disorder and with vasculitis . The duration of the study was one and half years from May 2018 to October 2019 . In this study skin biopsies taken from the patients were subjected into histopathological examination and direct immunofluorescence examination .

The aim of the present study was to analyse the clinical picture histopathological finding with DIF finding. Relevant history were taken from the patients regarding presenting complaints, duration of illness, site of involvement, past history, clinical diagnosis and site of biopsy. These informations were analysed along with histopathological examination and DIF findings.

The common age group in this study was 51-60 years but SLE and vasculitis patients were clustered in younger age group [11-20 years].

Regarding sexwise distribution overall study was having slight male preponderance with male female ratio of 1.08:1 and in cases with SLE and vasculitis were having female preponderance.

Among vesiculobullous disorder pemphigus vulgaris was the most common condition followed by bullous pemphigoid.

Majority of the patients in vesiculobullous disorder were clinically presented with fluid filled lesions which constitute about 82.35% and 11.76% of cases associated with mucosal involvement.

The most common condition associated with mucosal involvement was pemphigus vulgaris.

13 cases were found no bullae among which 10 cases [76.92%] were with positive DIF results . In these cases final correct diagnosis was made only with the help of DIF study.

In 5 cases, bullae was observed in histopathological examination with negative DIF results. So it excluded the immune mediated etiology by this we could avoid many unneccesory toxic drug intake as well as this may avoid the prolonged duration of treatment thereby improve the patients general condition both by physically and economically.

Among SLE cases DIF was positive in 100% of cases with one case [14.28%] showed full house effect. All the cases were showing granular band like positivity along the basement membrane zone. 2/7 cases showed subepidermal bullae in histopthological examination.

The majority of the vasculitis patients clinically presented with purpuric lesion and commonest site of involvement was lower limb.

In 4/8 cases [50%] of vasculitis were finally diagnosed as IgA vasculitis with the help of DIF and one was diagnosed as non immune mediated vasculitis. False negative results are more common in vasculitis especially in HSP because as lesions get older, the IgA deposits get degraded and cleared and will lead to negative DIF results.

CONCLUSION

In dermatological disorders combined analysis of clinical picture , histopathological examination and direct immunofluorescence yield more accurate results.

Direct immunofluorescence [DIF] plays an important role in the diagnosis even when the histopathological results are inconclusive.

In every case of clinically suspected immunobullous disorder, DIF must be done for the classification of the condition thereby lead to proper treatment and better outcome.

COLOUR PLATES

CASE 1: PEMPHIGUS VULGARIS

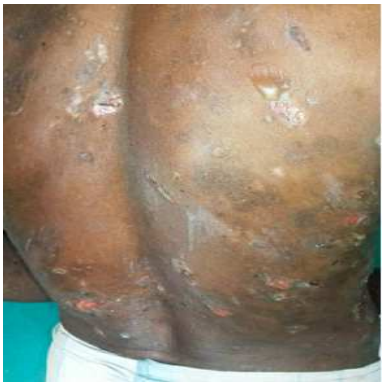


FIGURE 1:fluid filled lesions with ulceration

FIGURE 2:Oral erosions

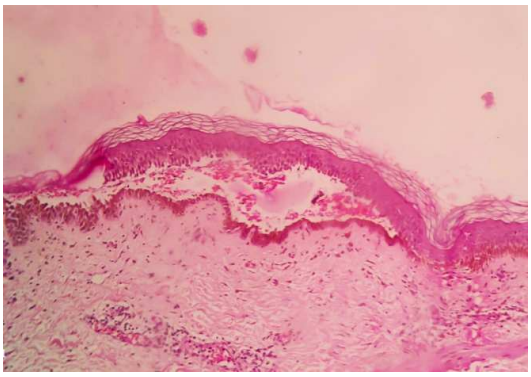


FIGURE 3: suprabasal bulla in HPE [400x]

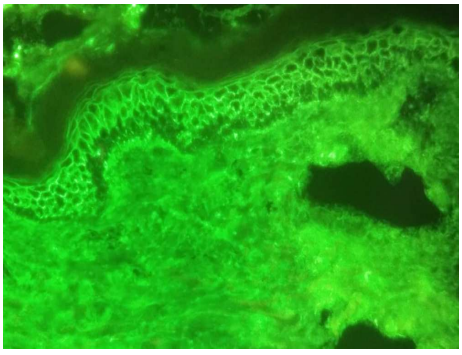


FIGURE 4:IgG fishnet positivity In epidermis [400x]

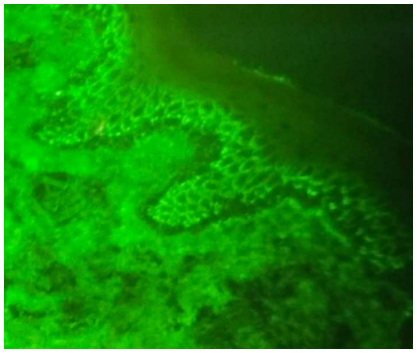


FIGURE 5:C3c fishnet positivity in epidermis [400x]

CASE 2: PEMPHIGUS FOLIACEUS

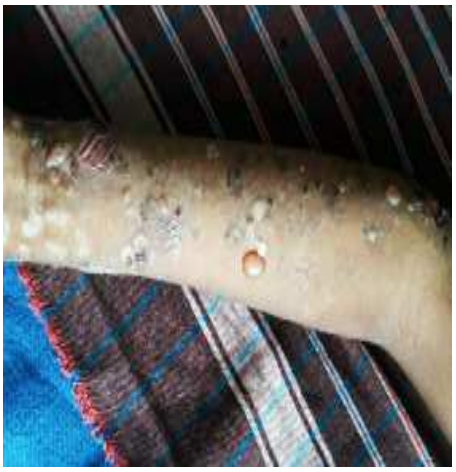


FIGURE 6:fluid filled lesions

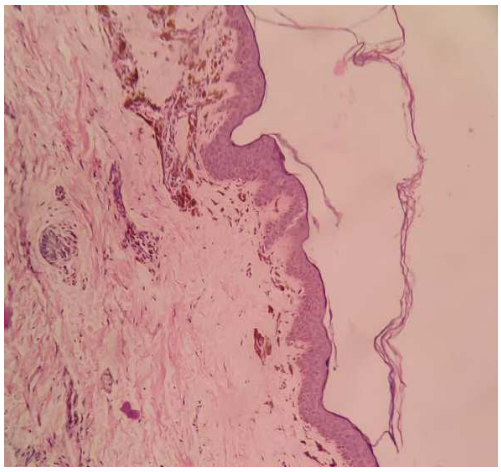


FIGURE 7: subcorneal bulla [400x]

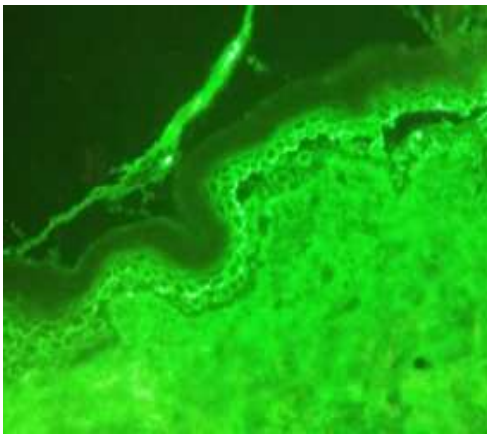


FIGURE 8: IgG fishnet positivity [400x]

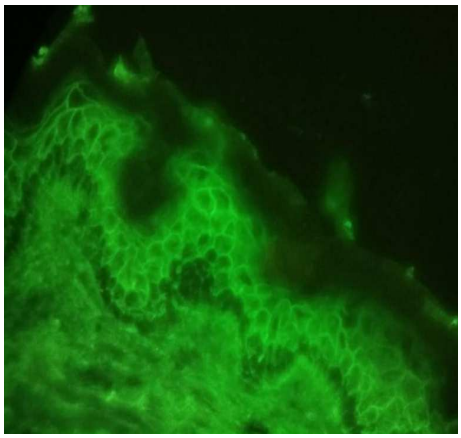


FIGURE 9: IgG fishnet positivity [400x]

CASE 3: BULLOUS PEMPHIGOID

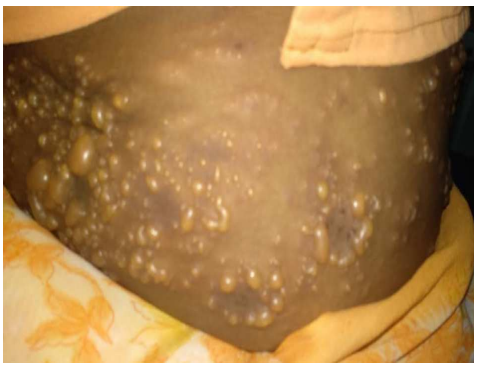


FIGURE 10:Fliuid filled lesions[tense bullae]

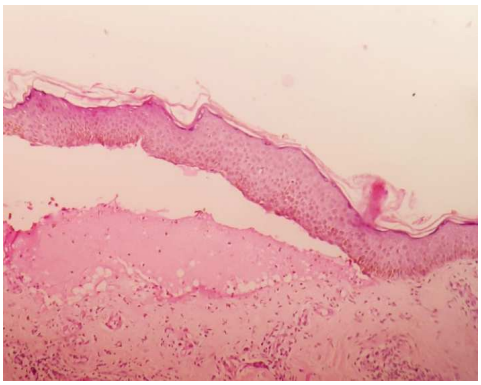


FIGURE 11:subepidermal bulla [400X]

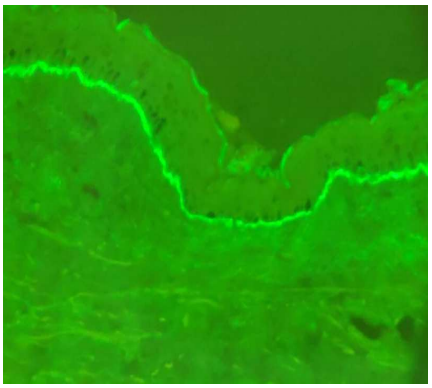


FIGURE 12:IgG linear positivity at BMZ [400x]

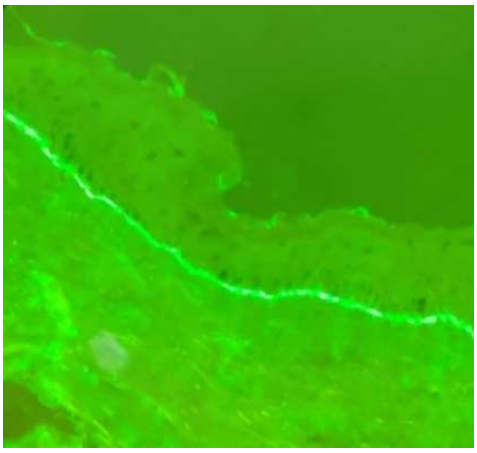


FIGURE 13:C3c linear positivity At BMZ[400x]

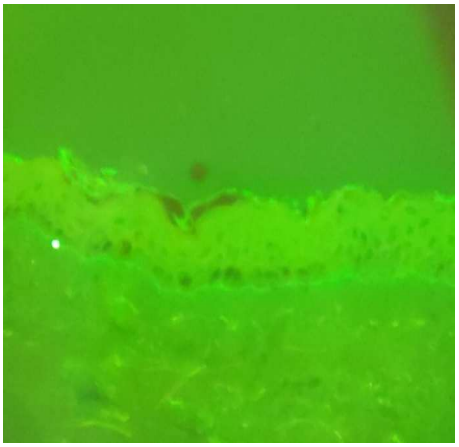


FIGURE 14: Negative IgM in DIF[400x]

CASE 4: **SYSTEMIC LUPUS ERYTHEMATOSUS**

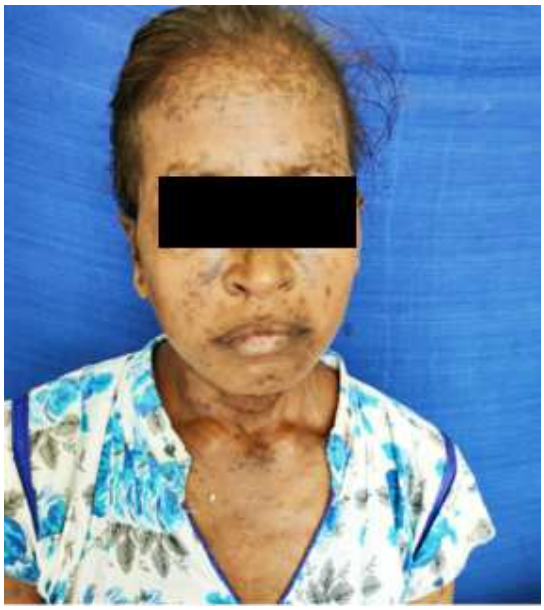


FIGURE 15: SLE patient showing malar rash with Sparing of naso labial folds

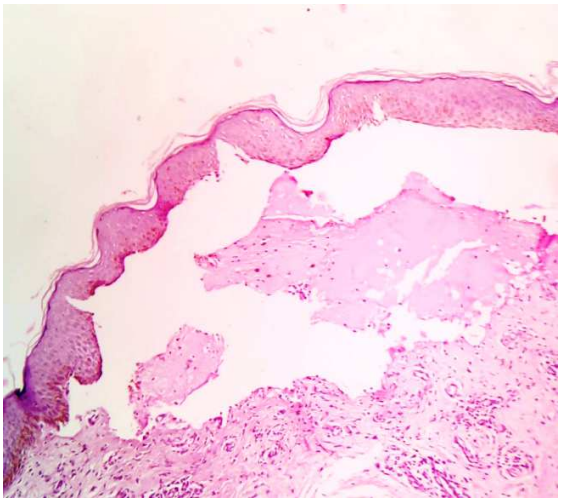


FIGURE 16: subepidermal bulla [400x]

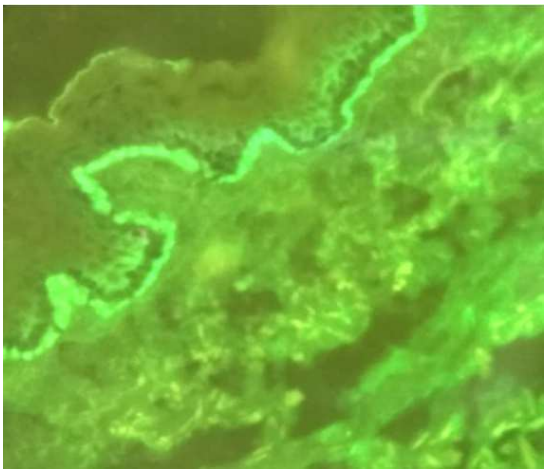


FIGURE 17:IgG band like positivity along BMZ [400x]

CASE 5: IgA VASCULITIS

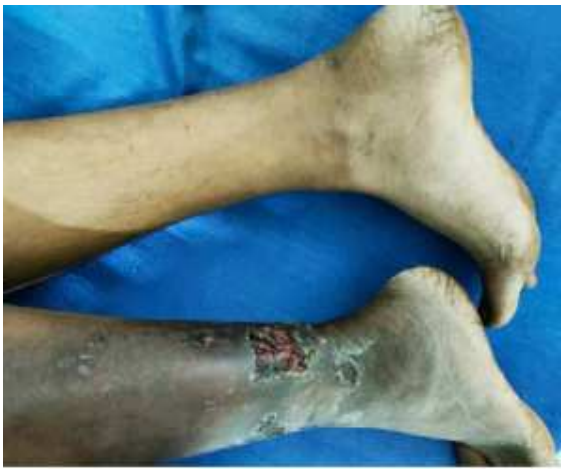


FIGURE 18: purpuric lesion with ulceration in lower limb

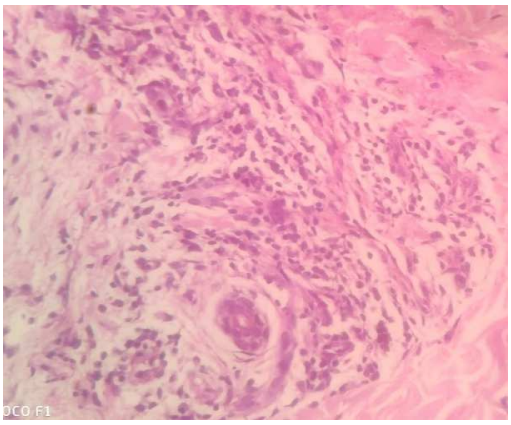


FIGURE 19:Leucocytoclastic Vasculitis [400x]

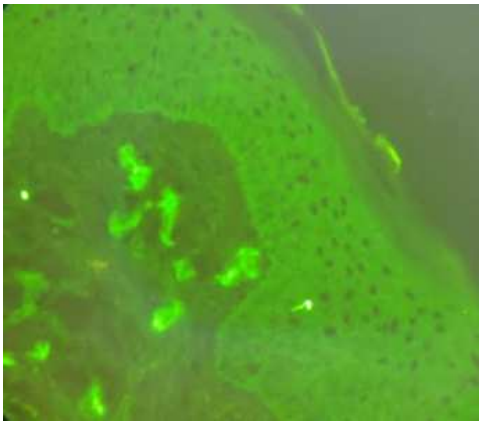


FIGURE 20:IgA granular positivity in superficial dermal vessels [400x]

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ANNEXURE -I

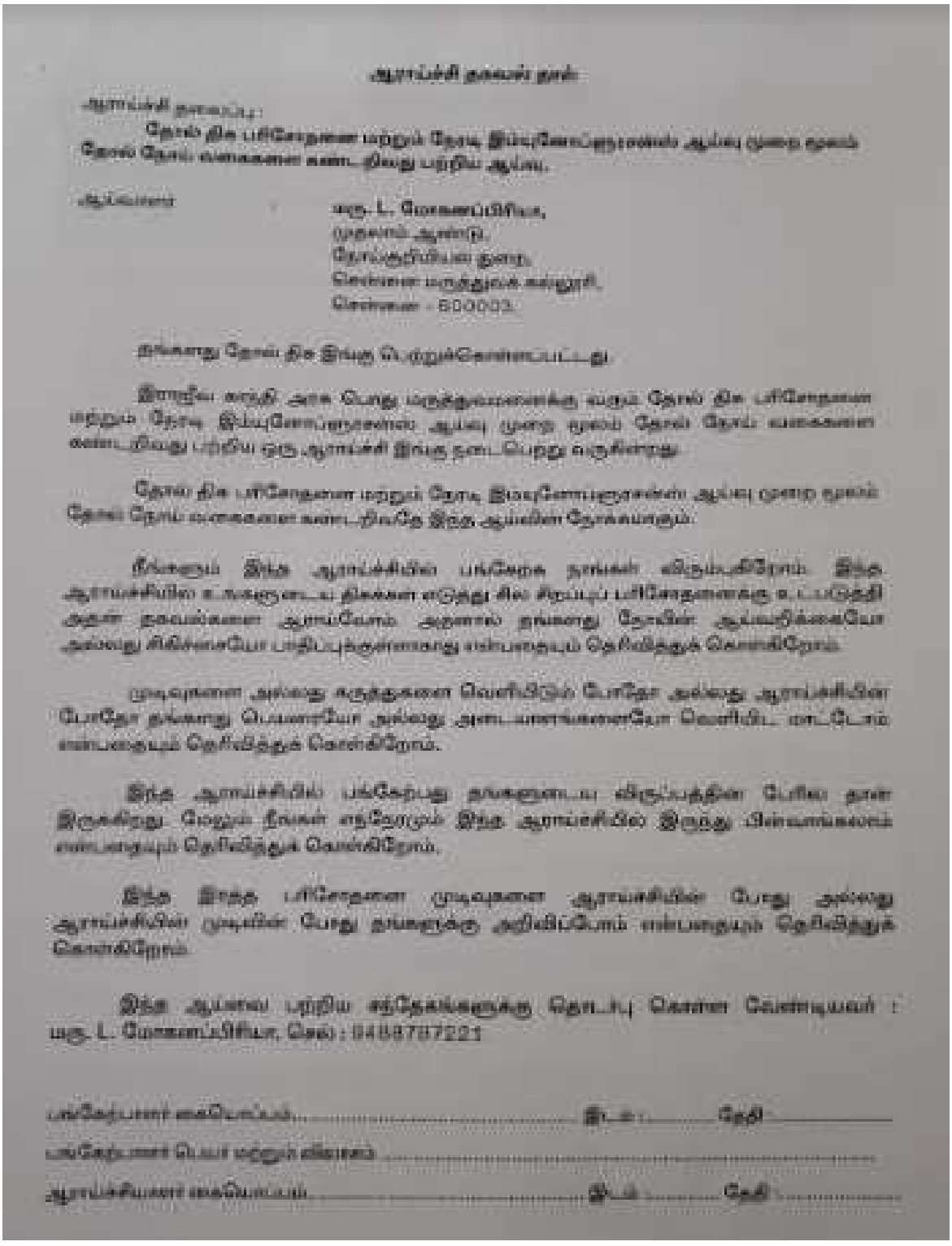
INFORMATION SHEET

- We are conducting a study on dermatological disorders in Government General Hospital, Chennai and for that your specimen may be valuable to us.
- The purpose of this study is to evaluate the role of DIF in diagnosis of dermatological disorders.
- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. Inthe event of any publication or presentation resulting from the research, no personallyi dentifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate inthis study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

ANNEXURE -II



ஆராய்ச்சி தலைப்பு தான்

ஆராய்ச்சி தலைப்பு:

தோல் திசு பரிசோதனை மற்றும் தோல் இம்யூனோஹிஸ்டோகெமிக்கல் ஆய்வு முறை மூலம் தோல் நோய் வகைகளை கண்டறிவது பற்றிய ஆய்வு.

ஆய்வாளர்

டாக்டர் L. மோகனப்பிரியா,
முதலாம் ஆண்டு,
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600003.

தங்களது தோல் திசு இங்கு பெற்றுக்கொள்ளப்பட்டது.

இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் தோல் திசு பரிசோதனை மற்றும் தோல் இம்யூனோஹிஸ்டோகெமிக்கல் ஆய்வு முறை மூலம் தோல் நோய் வகைகளை கண்டறிவது பற்றிய ஒரு ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

தோல் திசு பரிசோதனை மற்றும் தோல் இம்யூனோஹிஸ்டோகெமிக்கல் ஆய்வு முறை மூலம் தோல் நோய் வகைகளை கண்டறிவதே இந்த ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய திசுவை எடுத்து சில சிறப்புப் பரிசோதனைகளை உட்படுத்தி அதன் முடிவுகளை ஆராய்வோம். அதனால் நமக்கு நேரடியாக ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புகளையோ ஏற்படாதென்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து விலகலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த இந்த பரிசோதனை முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வு பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : டாக்டர் L. மோகனப்பிரியா, செல் : 9488787221

பங்கேற்பாளர் கையொப்பம்..... இடம்:..... தேதி .....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் ..... தேதி .....

ANNEXURE -III

INFORMED CONSENT FORM

Title of the study:A study on **CLINICAL AND IMMUNOPATHOLOGICAL STUDY OF DERMATOLOGICAL LESIONS IN TERTIARY CARE CENTRE.**

Name of the Participant :Dr.L.Mohanapriya

Name of the Principal(Co-Investigator) :

Name of the Institution :Madras Medical College

Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read tome). I was free to ask any questions and they have been answered. I am over 18 years of age and,exercising myfree power of choice, hereby give my consent to be included as a participant in **CLINICAL AND IMMUNOPATHOLOGICAL STUDY OF DERMATOLOGICAL LESIONS IN TERTIARY CARE CENTRE**

I have read and understood this consent form and the information provided to me.

1. I have had the consent document explained to me.
2. I have been explained about the nature of the study in which the skin biopsies will be subjected to histopathological examination and DIF..
3. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
4. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
5. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

6. I have understood that my identity will be kept confidential if my data are publicly presented
7. I have had my questions answered to my satisfaction.
8. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant

incompetent)

Name _____ Signature_____
Date_____

Name and Signature of impartial witness (required for illiterate patients):

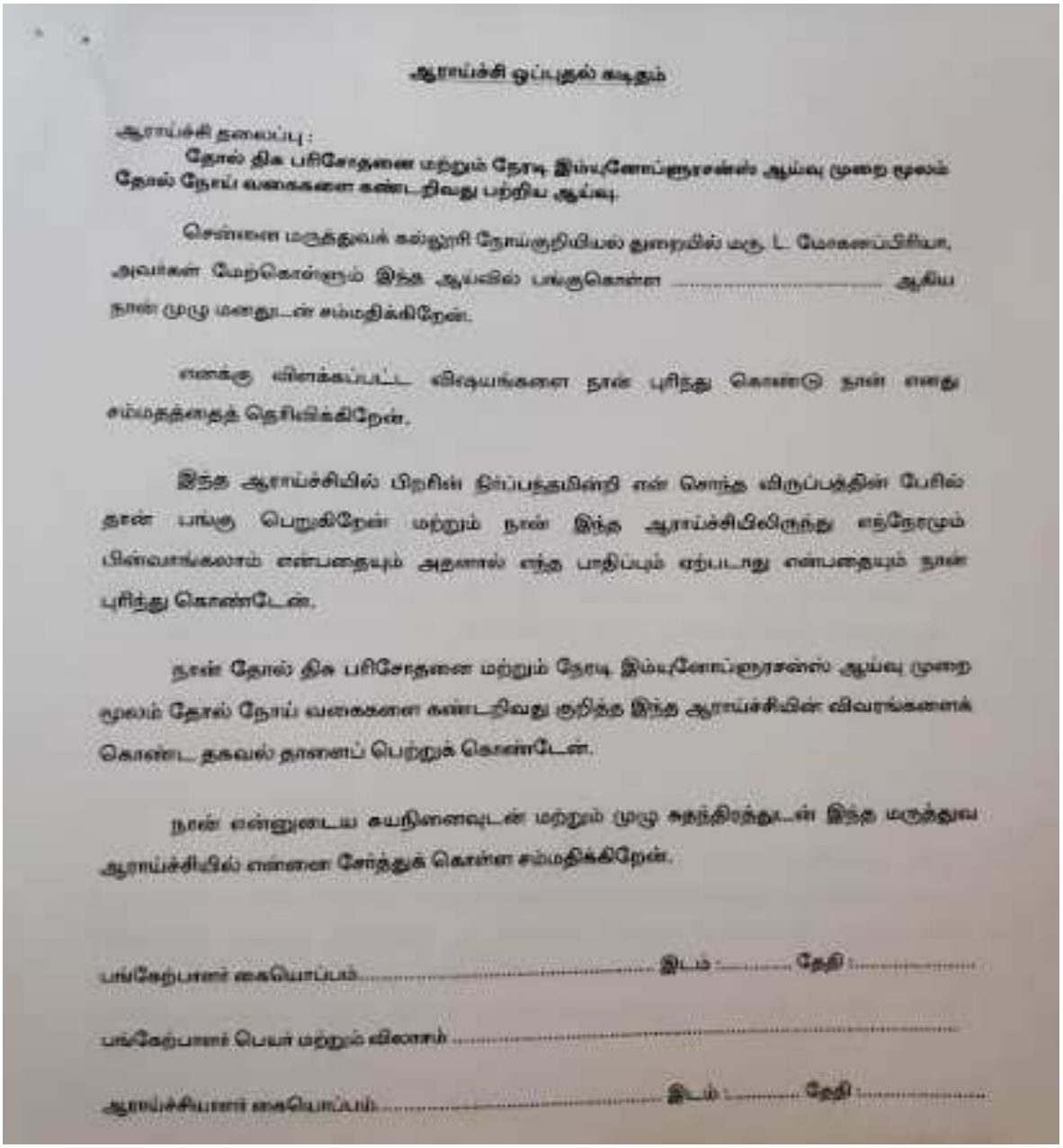
Name _____ Signature_____
Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____
Date_____

ANNEXURE –IV



ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு :

தோல் திசு பரிசோதனை மற்றும் நோடி இம்யுனோப்ராசஸ்ஸ் ஆய்வு முறை மூலம் தோல் நோய் வகைகளை கண்டறிவது பற்றிய ஆய்வு.

சென்னை மருத்துவக் கல்லூரி நோயுறியியல் துறையில் டாக்டர் மோகனப்பிரியா, அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் விலகிக்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் தோல் திசு பரிசோதனை மற்றும் நோடி இம்யுனோப்ராசஸ்ஸ் ஆய்வு முறை மூலம் தோல் நோய் வகைகளை கண்டறிவது குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம் ..... இடம் : ..... தேதி : .....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம் ..... இடம் : ..... தேதி : .....

IF NO	NAME	AGE	SEX	CILICAL PRESENTATION	SITE	DURATION	CLINICAL DIAGNOSIS	SITE OF BIOPSY	HPE DIAGNOSIS
34/18	MANI	60	M	FLUID FILLED LESION	UPPER LIMB	2 WEEKS	IMMUNOBULLOUS DISORDER	FOREARM	SUPRABASAL BULLOUS DISORDER
37/18	SUNDAR RAJ	45	M	FLUID FILLED LESION	TRUNK	3 MONTH	IMMUNOBULLOUS DISORDER	BACK	NO BULLAE
37/18	KANNAN	50	M	FLUID FILLED LESION	TRUNK	2 MONTHS	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER
38/18	RUTHRA MOORTHY	47	M	FLUID FILLED LESION	UPPER LIMB	2 MONTHS	IMMUNOBULLOUS DISORDER	ARM	SUBEPIDERMAL BULLOUS DISORDER
39/18	PADMAVALLI	70	F	FLUID FILLED LESION	TRUNK	6 MONTHS	IMMUNOBULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
42/18	SUBRAMANI	55	M	FLUID FILLED LESION	ALL OVER BODY	6 MONTHS	BULLOUS PEMPHIGOID	BACK	NO BULLAE
43/18	RAJU	57	M	FLUID FILLED LESION	UPPER LIMB	2 WEEKS	IMMUNOBULLOUS DISORDER	FOREARM	NO BULLAE
44/18	PARVATHI	55	F	FLUID FILLED LESION	ALL OVER BODY	4 YEARS	IMMUNOBULLOUS DISORDER	NAPE OF NECK	SUPRABASAL BULLOUS DISORDER
45/18	DENIS MARY	48	F	FLUID FILLED LESION	LOWER LIMB	5 YEARS	PEMPHIGUS VULGARIS	THIGH	SUPRABASAL BULLOUS DISORDER
47/18	MAHALINGAM	6	M	FLUID FILLED LESION	TRUNK	1 MONTH	PEMPHIGUS VULGARIS	BACK	SUPRABASAL BULLOUS DISORDER
48/18	SHANTHI	50	F	FLUID FILLED LESION	ALL OVER BODY	5 MONTH	IMMUNOBULLOUS DISORDER	FOREARM	SUPRABASAL BULLOUS DISORDER
50/18	GUNASEKARAN	50	M	FLUID FILLED LESION	TRUNK	3 MONTH	IMMUNOBULLOUS DISORDER	CHEST	SUBEPIDERMAL BULLOUS DISORDER
51/18	SRINIVASAN	52	M	FLUID FILLED LESION	ALL OVER BODY	1 WEEK	PARA NEOPLATIC PEMPHIGUS	ARM	SUPRABASAL BULLOUS DISORDER
52/18	RADHA BAI	75	F	FLUID FILLED LESION	TRUNK	1 MONTH	PEMPHIGUS VULGARIS	BACK	SUPRABASAL BULLOUS DISORDER
53/18	KUMARI	60	F	FLUID FILLED LESION	TRUNK	1 MONTH	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER
54/18	SELVARAJ	75	M	FLUID FILLED LESION	UPPER LIMB	2 WEEKS	BULLOUS PEMPHIGOID	ARM	SUBEPIDERMAL BULLOUS DISORDER
55/18	ANNAMAL	45	F	FLUID FILLED LESION	ALL OVER BODY	4 MONTHS	IMMUNOBULLOUS DISORDER	FOREARM	SUPRABASAL BULLOUS DISORDER
56/18	KULLAMMAL	70	F	FLUID FILLED LESION	ALL OVER BODY	2 MONTHS	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER
57/18	GOWRI	51	F	FLUID FILLED LESION & ORAL EROSIONS	ALL OVER BODY	20 DAYS	IMMUNOBULLOUS DISORDER	FOREARM	SUBEPIDERMAL BULLOUS DISORDER
58/18	PALANI	55	M	FLUID FILLED LESION	ALL OVER BODY	2 MONTHS	PEMPHIGUS VULGARIS	SHOULDER	SUPRABASAL BULLOUS DISORDER
60/18	JAYASHANKAR	45	F	FLUID FILLED LESION	ALL OVER BODY	3 MONTH	PEMPHIGUS VULGARIS	FOREHEAD	SUBEPIDERMAL BULLOUS DISORDER
61/18	VIJAYALAKSHMI	49	M	FLUID FILLED LESION & ORAL EROSIONS	ALL OVER BODY	6 MONTHS	PEMPHIGUS VULGARIS	SHOULDER	SUPRABASAL BULLOUS DISORDER
62/18	MANI	60	M	ORAL EROSIONS	BUCCAL MUCOSA	1 WEEK	PEMPHIGUS VULGARIS	BUCCAL MUCOSA	SUPRABASAL BULLOUS DISORDER
64/18	VENNILA	21	F	FLUID FILLED LESION	UPPER LIMB	2 DAYS	SLE	FOREARM	LUPUS PANNICULITIS
65/18	EZHILRANI	43	F	FLUID FILLED LESION & ORAL EROSIONS	TRUNK	6 MONTHS	IMMUNOBULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
66/18	MARIYAMMAL	55	F	FLUID FILLED LESION	UPPER LIMB	2 YEARS	IMMUNOBULLOUS DISORDER	BACK	SUBCORNEAL BULLOUS DISORDER
67/18	RAJAGOPAL	23	M	FLUID FILLED LESION	UPPER LIMB	1 MONTH	IMMUNOBULLOUS DISORDER	FOREARM	NO BULLAE
68/18	GOVINDHAMMAL	37	F	FLUID FILLED LESION	TRUNK	3 DAYS	IMMUNOBULLOUS DISORDER	LEG	SUPRABASAL BULLOUS DISORDER
70/18	KASINATHAN	70	M	FLUID FILLED LESION	UPPER LIMB	1 MONTH	BULLOUS PEMPHIGOID	ARM	SUBEPIDERMAL BULLOUS DISORDER
72/18	SAI NIKIL	12	M	FLUID FILLED LESION & ORAL EROSIONS	ALL OVER BODY	3 MONTH	PEMPHIGUS VULGARIS	ABDOMEN	SUPRABASAL BULLOUS DISORDER
73/18	ARUNAGIRI	68	M	FLUID FILLED LESION	ALL OVER BODY	4 MONTHS	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER
74/18	SELVI	46	F	ORAL EROSIONS	BUCCAL MUCOSA	3 MONTH	PEMPHIGUS VULGARIS	LOWER LIP	SUPRABASAL BULLOUS DISORDER
75/18	HEMAVATHY	67	F	FLUID FILLED LESION	ALL OVER BODY	4 DAYS	PEMPHIGUS ERYTHYMATOSUS	BACK	SUPRABASAL BULLOUS DISORDER

IF NO	IgG	IgA	IgM	C3c	FINAL DIAGNOSIS
34/18	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE BULLOUS DISORDER
37/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	NEGATIVE	BULLOUS PEMPHIGOID
37/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
38/18	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
39/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
42/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS GROUP OF DISORDER
43/18	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEED CLINICAL CORRELATION
44/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
45/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
47/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
48/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
50/18	NEGATIVE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
51/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PARA NEOPLASTIC PEMPHIGUS
52/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
53/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
54/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
55/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
56/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
57/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
58/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
60/18	NEGATIVE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
61/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
62/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
64/18	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	LUPUS ERYTHEMATOSUS
65/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
66/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	1+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS FOLIACEUS
67/18	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	PEMPHIGOID GROUP OF DISORDER
68/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
70/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
72/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
73/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE		NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
74/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
75/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS

IF NO	NAME	AGE	SEX	CILICAL PRESENTATION	SITE	DURATION	CLINICAL DIAGNOSIS	SITE OF BIOPSY	HPE DIAGNOSIS
76/18	DEVI	37	F	FLUID FILLED LESION	ALL OVER BODY	5 DAYS	PEMPHIGUS VULGARIS	BACK	SUBCORNEAL BULLOUS DISORDER
77/18	RISHWANTH BASHA	14	M	FLUID FILLED LESION	ALL OVER BODY	2 DAYS	BULLOUS SLE	BACK	SUBEPIDERMAL BULLOUS DISORDER
81/18	PRASANTH	64	M	FLUID FILLED LESION	TRUNK	2 MONTHS	IMMUNOBULLOUS DISORDER	ARM	NO BULLAE
83/18	MAHALAKSHMI	53	F	FLUID FILLED LESION	ALL OVER BODY	5 MONTH	PEMPHIGUS VULGARIS	FOREARM	SUBCORNEAL BULLOUS DISORDER
84/18	CHELLADURAI	48	M	FLUID FILLED LESION	ALL OVER BODY	5 YEARS	IMMUNOBULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
85/18	SIDDIKA PARVEEN	32	M	FLUID FILLED LESION	ALL OVER BODY	3 MONTH	IMMUNOBULLOUS DISORDER	THIGH	SUPRABASAL BULLOUS DISORDER
86/18	SOBANA	25	F	FLUID FILLED LESION	UPPER LIMB	1 MONTH	BULLOUS SLE	FOREARM	SUBEPIDERMAL BULLOUS DISORDER
87/18	NEHRU	52	M	FLUID FILLED LESION	ALL OVER BODY	1 MONTH	IMMUNOBULLOUS DISORDER	HAND	SUBEPIDERMAL BULLOUS DISORDER
88/18	VALLIAPAN	60	M	FLUID FILLED LESION	TRUNK	20 DAYS	PEMPHIGUS FOLIACEUS	CHEST	SUBCORNEAL BULLOUS DISORDER
89/18	RATHINASAMY	80	M	FLUID FILLED LESION	SCROTUM	6 MONTHS	DERMATITIS HERPITIFORMIS	SCROTUM	NO BULLAE
90/18	MALLLIGA	75	F	FLUID FILLED LESION	ALL OVER BODY	10 DAYS	BULLOUS PEMPHIGOID	FOREARM	SUBEPIDERMAL BULLOUS DISORDER
91/18	DILISH WARANAV	2	M	FLUID FILLED LESION	TRUNK	8 MONTHS	LINEAR IgA DERMATOSIS OF CHILDHOOD	BACK	CLEFTING AT SUBEPIDERMAL REGION
92/18	SRIDEVI	28	F	FLUID FILLED LESION	SCALP	3 YEARS	PEMPHIGUS VULGARIS	SCALP	SUPRABASAL BULLOUS DISORDER
93/18	KOLLANDARAJ	55	M	FLUID FILLED LESION	TRUNK	1 YEAR	IMMUNOBULLOUS DISORDER	ABDOMEN	NO BULLAE
94/18	DIVYA SRI	6	F	PURPURIC LESION	LOWER LIMB	1 MONTH	VASCULITIS	LEG	VASCULITIS
95/18	PARASAKTHI	53	F	FLUID FILLED LESION	ALL OVER BODY	2 WEEKS	IMMUNOBULLOUS DISORDER	THIGH	SUPRABASAL BULLOUS DISORDER
1/19	GOPIKA	15	F	PURPURIC LESION	LOWER LIMB	2 WEEKS	HSP	THIGH	DESCRIPTIVE
2/19	FAHIMULLA BARG	13	M	PURPURIC LESION	LOWER LIMB	2 WEEKS	HSP	THIGH	LEUCOCYTOCLASTIC VASCULITIS
3/19	KUMARI	47	F	ORAL EROSIONS	BUCCAL MUCOSA	2 WEEKS	BULLOUS SLE	LOWER LIP	DESCRIPTIVE
4/19	HINU KOUSAN	72	M	FLUID FILLED LESION	SCALP	2 MONTHS	BULLOUS SLE	SCALP	DESCRIPTIVE
5/19	VARATHAN	70	M	FLUID FILLED LESION	TRUNK	3 MONTH	BULLOUS PEMPHIGOID	CHEST	CLEFTING AT SUBEPIDERMAL REGION
6/19	RIYAZ MOHAMMED	14	M	FLUID FILLED LESION	ALL OVER BODY	1 MONTH	LINEAR IgA DERMATOSIS	FOREARM	CLEFTING AT SUBEPIDERMAL REGION
7/19	MURALI SEKAR	58	M	FLUID FILLED LESION	TRUNK	10 DAYS	IMMUNOBULLOUS DISORDER	ABDOMEN	SUBCORNEAL BULLOUS DISORDER
8/19	SELVI	42	F	FLUID FILLED LESION	TRUNK	2 MONTHS	PEMPHIGUS VULGARIS	BACK	SUPRABASAL BULLOUS DISORDER
9/19	SAKUNTHALA	45	F	FLUID FILLED LESION	ALL OVER BODY	6 MONTHS	IMMUNOBULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
10/19	SAMVEEN BEGUM	15	F	PALPABLE PURPURA	LOWER LIMB	3 MONTH	SMALL VESSEL VASCULITIS	LEG	LEUCOCYTOCLASTIC VASCULITIS
11/19	JAYA	67	F	PURPURIC LESION	LOWER LIMB	3 WEEKS	VASCULITIS	LEG	LEUCOCYTOCLASTIC VASCULITIS
12/19	VALARMATHI	40	F	FLUID FILLED LESION	ALL OVER BODY	4 MONTHS	PEMPHIGUS ERYTHYMATOSUS	BACK	SUBEPIDERMAL BULLOUS DISORDER
16/19	GOWTHAM	11	M	FLUID FILLED LESION & ORAL EROSIONS	LOWER LIMB	2 MONTHS	IMMUNOBULLOUS DISORDER	THIGH	LEUCOCYTOCLASTIC VASCULITIS
17/19	ABIRAMI	60	F	FLUID FILLED LESION	ALL OVER BODY	1 MONTH	PEMPHIGUS VULGARIS	NAPE OF NECK	NO BULLAE
19/19	MANI	71	M	FLUID FILLED LESION	TRUNK	3 YEARS	IgA PEMPHIGUS	BACK	SUPRABASAL BULLOUS DISORDER

IF NO	IgG	IgA	IgM	C3c	FINAL DIAGNOSIS
76/18	3+ FISHNET POSITIVITY IN EPIDERMIS	1+ FISHNET POSITIVITY IN EPIDERMIS	1+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	PEMPHIGUS FOLIACEUS
77/18	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	2+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	2+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	LUPUS ERYTHEMATOSUS
81/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS GROUP OF DISORDER
83/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS FOLIACEUS
84/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
85/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
86/18	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	LUPUS ERYTHEMATOSUS
87/18	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE BULLOUS DISORDER
88/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS FOLIACEUS
89/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
90/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
91/18	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	PEMPHIGOID GROUP OF DISORDER
92/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
93/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS GROUP OF DISORDER
94/18	NEGATIVE	2+ GRANULAR POSITIVITY IN SUPERFICIAL DERMAL VESSELS	NEGATIVE	NEGATIVE	IgA VASCULITIS
95/18	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
1/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEED CLINICAL CORRELATION
2/19	NEGATIVE	3+ GRANULAR POSITIVITY IN SUPERFICIAL DERMAL VESSELS	NEGATIVE	NEGATIVE	IgA VASCULITIS
3/19	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	2+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	LUPUS ERYTHEMATOSUS
4/19	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	LUPUS ERYTHEMATOSUS
5/19	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
6/19	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	LINEAR IgA DERMATOSIS
7/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS FOLIACEUS
8/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	3+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
9/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE		PEMPHIGUS VULGARIS
10/19	NEGATIVE	3+ GRANULAR POSITIVITY IN SUPERFICIAL DERMAL VESSELS	NEGATIVE	NEGATIVE	IgA VASCULITIS
11/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE MEDIATED VASCULITIS
12/19	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
16/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE MEDIATED VASCULITIS
17/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS GROUP OF DISORDER
19/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS

IF NO	NAME	AGE	SEX	CILICAL PRESENTATION	SITE	DURATION	CLINICAL DIAGNOSIS	SITE OF BIOPSY	HPE DIAGNOSIS
21/19	PRAMESHWARI	17	F	ERYTHEMATOUS AND HYPERPIGMENTED PLAQUES	FACE	6 MONTHS	SLE	BACK	DESCRIPTIVE
22/19	RAJAKUMARI	45	F	FLUID FILLED LESION	UPPER LIMB	10 DAYS	PEMPHIGUS VULGARIS	FOREARM	NO BULLAE
24/19	SUBRAMANI	60	M	FLUID FILLED LESION	ALL OVER BODY	5 MONTH	IMMUNOBULLOUS DISORDER	THIGH	SUBEPIDERMAL BULLOUS DISORDER
25/19	SHGEBA BEGUM	53	F	FLUID FILLED LESION	ALL OVER BODY	2 MONTHS	LINEAR IgA DISEASE	FOREARM	SUBEPIDERMAL BULLOUS DISORDER
26/19	CHINNAPAPPA	55	F	BLOOD FILLED LESION	ALL OVER BODY	1 MONTH	PEMPHIGUS VULGARIS	CHEST	SUPRABASAL BULLOUS DISORDER
28/19	SHANTHI	35	F	FLUID FILLED LESION	ALL OVER BODY	1 WEEK	IMMUNOBULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
30/19	MANIMARAN	52	M	FLUID FILLED LESION & ORAL EROSIONS	TRUNK	1 WEEK	PEMPHIGUS VULGARIS	BACK	SUPRABASAL BULLOUS DISORDER
31/19	RAVI	28	M	ERYTHEMATOUS PLAQUES AND ORAL EROSIONS	SCALP	10 MONTHS	IMMUNOBULLOUS DISORDER	FOREARM	INFLAMMATORY DERMATITIS
33/19	SARANYA	8	F	FLUID FILLED LESION	ALL OVER BODY	1 YEAR	CHRONIC BULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
34/19	KANNIYAPPAN	53	M	FLUID FILLED LESION	ALL OVER BODY	1 MONTH	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER
35/19	CHARULATHA	40	F	FLUID FILLED LESION	UPPER LIMB	1 WEEK	PEMPHIGUS VULGARIS	FOREARM	SUPRABASAL BULLOUS DISORDER
36/19	DHANALAKSHMI	75	F	FLUID FILLED LESION & HYPERPIGMENTED PLAQUES	UPPER LIMB	1 MONTH	LICHEN PLANUS PEMPHIGOID	FOREARM	LICHEN PLANUS PEMPHIGOID
37/19	THIRUNAVUKARASU	61	M	FLUID FILLED LESION	ALL OVER BODY	2 YEARS	PARA NEOPLATIC PEMPHIGUS	SHOULDER	SUPRABASAL BULLOUS DISORDER
40/19	LINGESHWARAN	57	M	FLUID FILLED LESION	TRUNK	2 WEEKS	IMMUNOBULLOUS DISORDER	BACK	SUPRABASAL BULLOUS DISORDER
41/19	KRISHNAN	80	M	FLUID FILLED LESION	ALL OVER BODY	3 YEARS	IMMUNOBULLOUS DISORDER	ARM	SUBEPIDERMAL BULLOUS DISORDER
42/19	NATRAJ	70	M	FLUID FILLED LESION	FACE	3 MONTH	PEMPHIGUS FOLIACEUS	BACK	SUBCORNEAL BULLOUS DISORDER
43/19	SUBULAKSHMI	58	F	FLUID FILLED LESION & ERYTHEMATOUS PLAQUE	ALL OVER BODY	3 MONTH	BULLOUS PEMPHIGOID	BACK	SUPRABASAL BULLOUS DISORDER
44/19	ANNAMAL	47	F	FLUID FILLED LESION	ALL OVER BODY	2 MONTHS	IMMUNOBULLOUS DISORDER	FOREARM	NO BULLAE
56/19	SUBRAMANI	40	M	FLUID FILLED LESION	ALL OVER BODY	6 MONTHS	IMMUNOBULLOUS DISORDER	BACK	SUBEPIDERMAL BULLOUS DISORDER
58/19	ILAKIYAN	61	M	FLUID FILLED LESION	TRUNK	2 WEEKS	BULLOUS PEMPHIGOID	CHEST	NO BULLAE
59/19	MANKANI	19	M	FLUID FILLED LESION	ALL OVER BODY	4 MONTHS	PEMPHIGUS GESTATIONALIS	BACK	SUBCORNEAL BULLOUS DISORDER
60/19	SAMPOORANAM	42	F	FLUID FILLED LESION	ALL OVER BODY	2 WEEKS	BULLOUS PEMPHIGOID	ARM	SUPRABASAL BULLOUS DISORDER
62/19	VIJAYA	55	F	PURPURIC LESION	TRUNK	1 WEEK	VASCULITIS	BACK	NO EVIDENCE OF VASCULITIS
63/19	MUTHU	65	M	FLUID FILLED LESION & ORAL EROSIONS	ALL OVER BODY	3 MONTH	BULLOUS PEMPHIGOID	BACK	SUPRABASAL BULLOUS DISORDER
64/19	VALLIVITTAN	50	M	FLUID FILLED LESION & ORAL EROSIONS	TRUNK	3 YEARS	PEMPHIGUS VULGARIS	CHEST	SUPRABASAL BULLOUS DISORDER
65/19	USHARANI	57	F	FLUID FILLED LESION	TRUNK	3 MONTH	PEMPHIGUS VULGARIS	BACK	SUBCORNEAL BULLOUS DISORDER
66/19	KANNAN	50	M	HYPERPIGMENTED LESION	ALL OVER BODY	6 MONTHS	BULLOUS PEMPHIGOID	FOREARM	SUBCORNEAL BULLOUS DISORDER
71/19	GOVINDARAJ	50	M	FLUID FILLED LESION	ALL OVER BODY	6 MONTHS	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER
74/19	AVASTEN	48	M	FLUID FILLED LESION	ALL OVER BODY	2 WEEKS	BULLOUS PEMPHIGOID	BACK	SUBEPIDERMAL BULLOUS DISORDER

IF NO	IgG	IgA	IgM	C3c	FINAL DIAGNOSIS
21/19	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	LUPUS ERYTHEMATOSUS
22/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS GROUP OF DISORDER
24/19	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
25/19	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
26/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
28/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	1+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
30/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
31/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	INFLAMMATORY DERMATITIS
33/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	1+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
34/19	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
35/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	1+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
36/19	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	LICHEN PLANUS PEMPHIGOID
37/19	1+ FOCAL FISHNET POSITIVITY IN EPIDERMIS & TRACE POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	NEGATIVE	PARA NEOPLASTIC PEMPHIGUS
40/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	1+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
41/19	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
42/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS FOLIACEUS
43/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	1+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
44/19	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	NEGATIVE	BULLOUS PEMPHIGOID
56/19	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
58/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEED CLINICAL CORRELATION
59/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE BULLOUS DISORDER
60/19	1+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
62/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO EVIDENCE OF VASCULITIS
63/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE BULLOUS DISORDER
64/19	2+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	NEGATIVE	PEMPHIGUS VULGARIS
65/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS FOLIACEUS
66/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NON IMMUNE BULLOUS DISORDER
71/19	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	NEGATIVE	BULLOUS PEMPHIGOID
74/19	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	NEGATIVE	BULLOUS PEMPHIGOID

IF NO	NAME	AGE	SEX	CILICAL PRESENTATION	SITE	DURATION	CLINICAL DIAGNOSIS	SITE OF BIOPSY	HPE DIAGNOSIS
76/19	ILANKANI	44	F	FLUID FILLED LESION	TRUNK	3 MONTH	PEMPHIGUS VULGARIS	BACK	SUPRABASAL BULLOUS DISORDER
77/19	DURGA RAMAN	54	M	FLUID FILLED LESION	ALL OVER BODY	3 MONTH	IMMUNOBULLOUS DISORDER	THIGH	SUBEPIDERMAL BULLOUS DISORDER
79/19	LALITHA	42	F	FLUID FILLED LESION	ALL OVER BODY	2 MONTHS	BULLOUS PEMPHIGOID	FOREARM	SUBEPIDERMAL BULLOUS DISORDER
80/19	ANBALAGAN	63	M	FLUID FILLED LESION	ALL OVER BODY	1 MONTH	DERMATITIS HERPITIFORMIS	CHEST	NO BULLAE
82/19	MURUGESAN	38	M	PURPURIC LESION	LOWER LIMB	1 MONTH	HSP	LEG	VASCULITIS
85/19	MALLIGA	34	F	HYPERPIGMENTED LESION	TRUNK	4 MONTHS	SLE	FOREARM	NO BULLAE

IF NO	IgG	IgA	IgM	C3c	FINAL DIAGNOSIS
76/19	3+ FISHNET POSITIVITY IN EPIDERMIS	NEGATIVE	NEGATIVE	2+ FISHNET POSITIVITY IN EPIDERMIS	PEMPHIGUS VULGARIS
77/19	3+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	1+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	BULLOUS PEMPHIGOID
79/19	2+ LINEAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	NEGATIVE	NEGATIVE	BULLOUS PEMPHIGOID
80/19	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEED CLINICAL CORRELATION
82/19	NEGATIVE	2+POSITIVITY IN SUPERFICIAL DERMAL VESSELS	NEGATIVE	NEGATIVE	IgA VASCULITIS
85/19	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	NEGATIVE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	3+ GRANULAR POSITIVITY IN BASEMENT MEMBRANE ZONE	LUPUS ERYTHEMATOSUS