# COMPARISON OF DIRECT IMMUNOFLUORESCENCE OF ORAL MUCOSA AND PLUCKED HAIR IN PATIENTS WITH PEMPHIGUS

**Dissertation submitted to**

**The Tamil Nadu Dr. M.G.R Medical University, Chennai**

**In fulfilment of the requirements for the award of the degree of**

**Doctor of Medicine in Dermatology, Venereology and Leprology**

**Under the guidance of**

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**THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI, TAMILNADU**

**MAY 2018**

# CERTIFICATE

This is to certify that the thesis entitled **"COMPARISON OF DIRECT IMMUNOFLUORESCENCE OF ORAL MUCOSA AND PLUCKED HAIR IN PATIENTS WITH PEMPHIGUS"** is a bonafide work of **Dr. Ryan Raju** done under the direct guidance and supervision of **Dr. Reena Rai, MD** in the Department of Dermatology, Venereology and Leprology, and **Dr.Uma Maheswari, MD,** in the department of Pathology**,** PSG Institute of Medical Sciences and Research, Coimbatore in fulfilment of the regulations of Dr.MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

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# DECLARATION

I hereby declare that this dissertation entitled **"COMPARISON OF DIRECT IMMUNOFLUORESCENCE OF ORAL MUCOSA AND PLUCKED HAIR IN PATIENTS WITH PEMPHIGUS"** was prepared by me under the direct guidance and supervision of **Dr. Reena Rai, MD** and **Dr. Uma Maheswari, MD,** PSG Institute of Medical Sciences and Research, Coimbatore. The dissertation is submitted to The Tamil Nadu Dr. MGR Medical University in fulfilment of the University regulation for the award of MD degree in Dermatology, Venereology and Leprology. This dissertation has not been submitted for the award of any other Degree or Diploma.

**Dr. Ryan Raju**

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## CERTIFICATE – II

This is to certify that this dissertation work titled **COMPARISON OF DIRECT IMMUNOFLUORESCENCE OF ORAL MUCOSA AND PLUCKED HAIR IN PATIENTS WITH PEMPHIGUS** of the candidate **Dr. Ryan Raju,** with registration Number **201530352** for the award of **Doctor of Medicine** in the branch of **Dermatology**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** of plagiarism in the dissertation.

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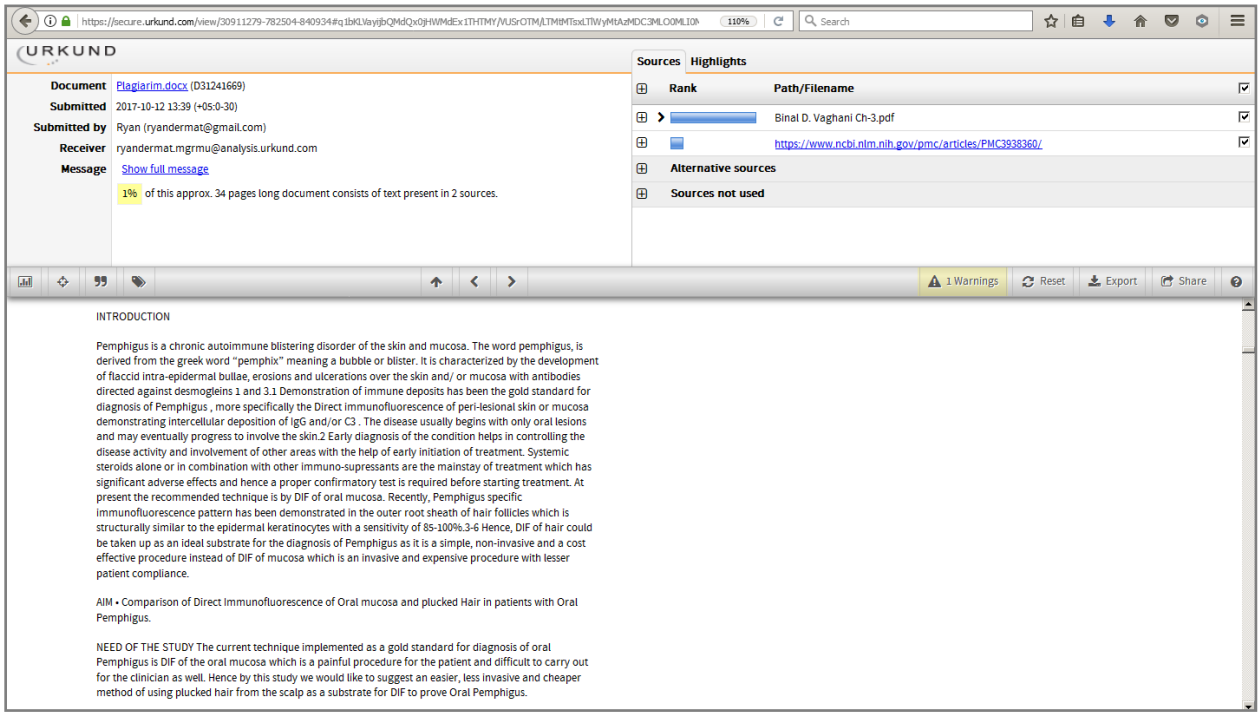

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# INTRODUCTION

Pemphigus is a chronic autoimmune blistering disorder of the skin and mucosa. The word pemphigus, is derived from the greek word “pemphix” meaning a bubble or blister. It is characterized by the development of flaccid intra-epidermal bullae, erosions and ulcerations over the skin and/ or mucosa with antibodies directed against desmogleins 1 and 3.[1] Demonstration of immune deposits has been the gold standard for diagnosis of Pemphigus, more specifically the Direct immune fluorescence of peri-lesional skin or mucosa demonstrating intercellular deposition of IgG and/or C3.

The disease usually begins with only oral lesions and may eventually progress to involve the skin.[2] Early diagnosis of the condition helps in controlling the disease activity and involvement of other areas with the help of early initiation of treatment.

Systemic steroids alone or in combination with other immuno-supressants are the mainstay of treatment which has significant adverse effects and hence a proper confirmatory test is required before starting treatment. At present the recommended technique is by DIF of oral mucosa.

Recently, Pemphigus specific immunofluorescence pattern has been demonstrated in the outer root sheath of hair follicles which is structurally similar to the epidermal keratinocytes with a sensitivity of 85-100%.[3-6]

Hence, DIF of hair could be taken up as an ideal substrate for the diagnosis of Pemphigus as it is a simple, non-invasive and a cost effective procedure instead of DIF of mucosa which is an invasive and expensive procedure with lesser patient compliance.

# AIM

- Comparison of Direct Immunofluorescence of Oral mucosa and plucked Hair in patients with Oral Pemphigus.

## NEED FOR THE STUDY

The current technique implemented as a gold standard for diagnosis of oral Pemphigus is DIF of the oral mucosa which is a painful procedure for the patient and difficult to carry out for the clinician as well.

Hence by this study we would like to suggest an easier, less invasive and cheaper method of using plucked hair from the scalp as a substrate for DIF to prove Oral Pemphigus.

# REVIEW OF LITERATURE

## History

The term pemphigus was first used in the period 460-370 B.C by Hippocrates. He enumerated different types of fevers and mentioned a pemphigoid type of fever.[7]

Pemphigus was first described in the year 1777 by McBride and Wichmann in 1791. Wichmann applied the term "pemphigus" to patients who had flaccid bullae and painful oral ulcers.[7]

In 1844 - Cazenave first described pemphigus foliaceous as a superficial, rapidly spreading form of pemphigus [8]

In 1868, Ferdinand von Hebra stated that pemphigus was a chronic disease and was the first to coin the term pemphigus vulgaris.[7-8]

In 1886, Neumann described a disease with "warty granulations" as pemphigus vegetans.[9]

In 1881- disruption of epidermal cells in patients with pemphigus, was first described by Auspitz.[10]

In 1925, Senear and Usher described pemphigus erythematosus[8]. In 1943, Civatte delineated acantholysis as histo-pathologic hallmark in pemphigus. He described acantholysis and intraepithelial bulla formation in pemphigus vulgaris, pemphigus foliaceous and pemphigus vegetans. These findings distinguished pemphigus from other blistering disorder of the skin.[11]

In 1953, Walter Levers distinguished pemphigus vulgaris and pemphigoid bullosus, by both clinical and histological parameters. He described pemphigus vulgaris as a life-threatening disease, characterized by intra-epidermal blisters and acantholysis with usually a lethal outcome.[12]

In 1964, Beutner and Jordon demonstrated auto-antibodies on the cell surface of keratinocytes by direct immunofluorescence.[13]

In 1976- Schiltz and Michel, by human skin organ culture demonstrated that auto-antibodies in pemphigus cause the blister formation[14]

In 1982, Anhalt et al demonstrated the auto-antibodies in pemphigus by means of passive transfer of antibodies to neonatal mice.[15]

In 1980s, pemphigus target antigens were identified by immune-precipitation and immune-blotting methods.[16]

In the early 1990s, isolation of cDNA for pemphigus antigens revealed the desmogleins as the target antigens in pemphigus.[17]

## EPIDEMIOLOGY

### Worldwide:

Pemphigus Vulgaris prevalence ranges from 0.18 to 6.96 case per million population all over the world.[18]

In the United Kingdom the incidence was 0.58 to 0.80 per 100,000 person years.[19]

In France, the prevalence was 1.7 cases per million population per year.[20]

In Tunisia, the prevalence was 6.7 cases per million population per year.[20]

In Iran, the mean incidence was 0.67 cases per million population per year.[21]

### India:

A survey conducted in Kerala showed 4.4 per million population incidence of pemphigus vulgaris per year. This was higher than United Kingdom, France, Germany & Iran but lesser than Tunisia.[22]

Among the dermatology outpatients in India, the incidence varies from 0.09 to 1.8%.[23]

Among pemphigus patients, the majority are diagnosed to have pemphigus vulgaris, the proportion being as high as 75- 92% [22] followed by pemphigus foliaceous, pemphigus erythematosus and pemphigus vegetans in decreasing order of frequency.[24]

**Gender:**

In general, both sexes are equally affected though various studies have shown contrasting results.[22]

**Age:**

In India, majority of the patients have been younger than 40 years of age. This is in contrast to other parts of the world where pemphigus occurs in the 5$^{th}$ and 6$^{th}$ decade of life.[25]

**Race:**

More prevalent among Jewish and Mediterranean population.[22]

**Genetic Factors:**

Pemphigus is a polygenic disorder, i.e the disease depends on the simultaneous presence of several genes. Higher incidence of Pemphigus vulgaris and earlier age at onset for pemphigus seen in Indian population have been attributed to higher frequency of DSG3*TCCCC in Indian population.[22]

HLA DRB1 *0402, 1401/04, HLA DQB1 * 0503 has been associated with increased susceptibility to PV, HLA DRB1 *04 associated with P.F (both sporadic and endemic form) and HLA DRB1 *0102, 0404 & 1402/06 associated with endemic P.F.[26-27]

An inherited predisposition is further proved by the following observations:

a. Difference in clinical profile of Pemphigus among various ethnic groups.
b. Ashkenazi Jews are more commonly affected.
c. Familial cases have been reported.
d. 40-60% of 1st degree relatives of patients with P.V have shown circulating anti- desmoglein antibodies.
e. The first-degree relatives of patients with pemphigus have an increase prevalence of auto-immune diseases.

**DISEASE ASSOCIATIONS**

A number of diseases have been described in association with pemphigus and these include cryoglobulinemia and cold agglutinin disease,[29] renal cell carcinoma, [30] hyperprolactinemia, [31] brain abscesses,[32] SLE, Myasthenia gravis, thymoma and lymphoproliferative diseases.[33]

EB virus, Herpes simplex, HHV 6 & 8 have been demonstrated in the skin of patients with Pemphigus.[34-35] Patients with pemphigus having co existing HIV infection have also been reported.[36]

**Pathogenesis**

The cornerstone of pemphigus is the presence of IgG auto-antibodies which act against desmoglein 3 and/or desmoglein 1.

These antibodies play an important role in loss of cell to cell adhesion of keratinocytes which further results in blister formation.

**Desmosomes**:

Desmosomes are the major adhesion complex in the epidermis, anchoring keratin intermediate filaments to the cell membrane and bridging adjacent keratinocytes and allowing cells to withstand trauma.

They are also seen in myocardium , meninges and cortex of lymph nodes.

The main components of desmosomes in the epidermis, consist of products of three gene super-families-

1. the desmosomal cadherins (desmogleins & desmocollins)
2. the armadillo proteins (plakoglobin, plakophilin)
3. plakins (desmoplakin, etc)

Among these desmogleins and desmocollins are the major components.[37]

Cadherins are a family of calcium dependent cell-cell adhesion molecules that play an important role in formation and maintainence of complex tissue integrity.[38]

They have two major subgroups –

1. classic cadherins
2. desmosomal cadherins.

The desmosome has desmosomal cadherins as its transmembrane components and plakoglobin, plakophilin and desmoplakin as its cytoplasmic components.

All the cadherins contain repeated amino acid sequences, called cadherin repeats, which have calcium-binding motifs in their extracellulardomains. Like the classic cadherins, desmogleins have four cadherin repeats in their extracellular domain, but with an extra carboxy-terminal domain with repeats of 29 ± 1 aminoacid residues in their intracellular domain.[38]

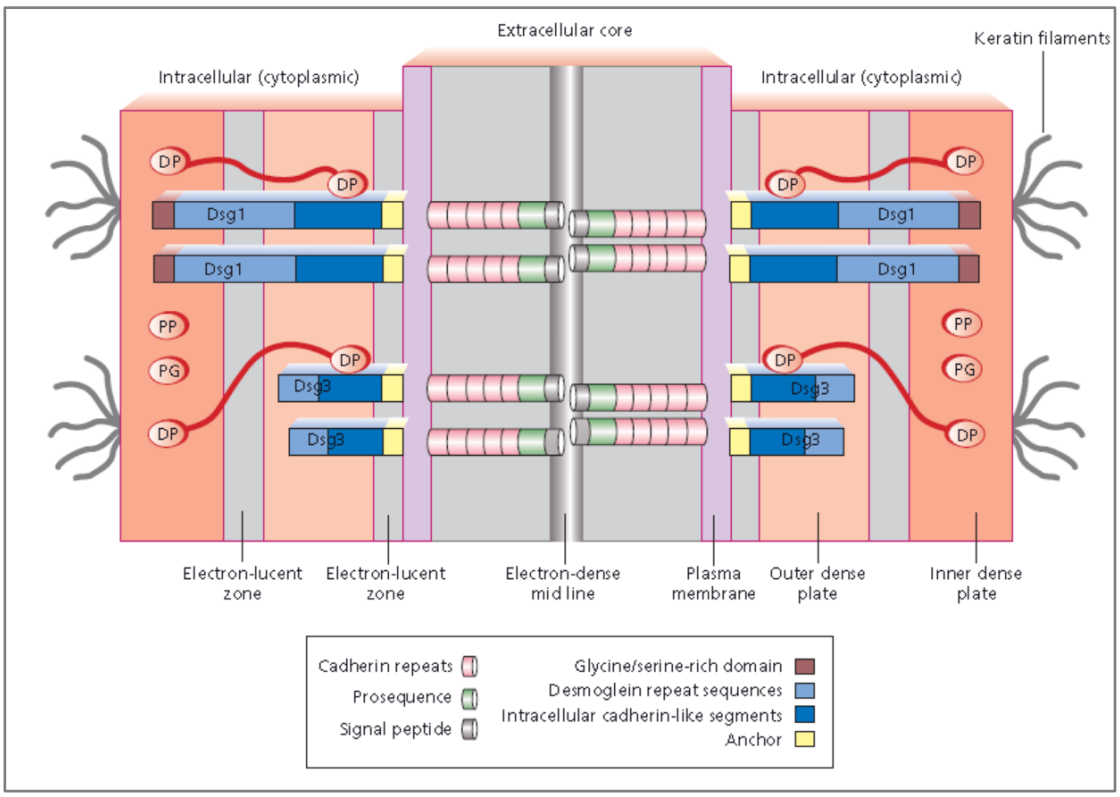



**Fig 1: Molecular components of desmosomes**

## DISTRIBUTION OF DESMOGLEINS IN THE SKIN

Desmogleins have four isoforms- Dsg 1 to 4 . Desmoglein 1 and 3 is basically restricted to the stratified squamous epithelia while Desmoglein 2 is expressed in all desmoglein possessing tissues like simple epithelia and myocardium. Desmoglein 4 is seen primarily in the hair follicles and in the granular layer.[38]

The presence of desmogleins in the skin depends on the differentiation and also the pattern of expression in mucosa differs from that in the skin. 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 differentiated cells, i.e, in the upper layers.[39,40]

Pemphigus IgG antibody binds to the extracellular domain on the amino-terminal region of dsg-3 and here it has a direct effect on the function of the desmogleins.[41]

The pathogenicity of Desmoglein antibodies depends on their titre and subclass.

Patients in whom the disease is active, both IgG1 and IgG4 subclass antibodies are present, but the IgG4 is more specific and pathogeneic.[42]

The pathogenicity of desmoglein antibodies is supported by,

a) Studies showing a correlation between titre of antibody in patient's serum and the disease activity.[43]
b) Transient bullae in the newborn may be caused by Transplacental transfer of maternal PV antibodies.
c) PV IgG antibodies causes suprabasal acantholysis in neonatal mouse model.[44]
d) Prior absorption of antibodies of the pemphigus vulgaris prevents blister formation.[45]
e) Desmoglein-3 antibodies can induce acantholysis can in mice which can be enhanced by adding desmoglein-1 antibodies.[46]

## Distribution of Desmogleins in Oral Mucosa

The oral mucosa, which has a characteristic compact lamellar stratum corneum, desmoglein 3 is expressed throughout the epithelium. It is also seen that the expression of desmoglein 1 is much lower than that of desmoglein 3.

## DISTRIBUTION OF DESMOGLEINS IN THE HAIR

### Desmoglein 1

It is expressed in the differentiating cells. The distribution is similar to that found in the epidermis on the inner root sheath and in the infundibulum of the hair follicle. At the level of the bulge it is confined to the suprabasal layers and is absent in the basal layer. Desmoglein 1 distribution gradually becomes confined to the inner most layers of ORS towards the base. This eventually disappears in the lower most part of the hair follicle ORS.[48]

### Desmoglein 2

It is abundantly expressed in the cells with least differentiation such as the basal layers of the bulge region of hair follicle and bulb matrix. In the lower part of the hair follicle, it is present in the basal cells of the ORS.[48]

### Desmoglein 3

Desmoglein 3 is present on all layer of the ORS, more so in the basal layers. At the level of infundibulum it is expressed predominantly in the basal layers. It is

also expressed in the cyst walls in the areas of trichilemmal keratinisation, medulla of the hair shaft, in the suprabasal matrix and the precortical cells.[48] Desmoglein 3 also acts in anchoring the telogen hair to the ORS of the hair follicle.[49]

**Desmoglein 4**

Dsg 4 is present in the Inner root sheath, pre-cortex and the matrix.[50]

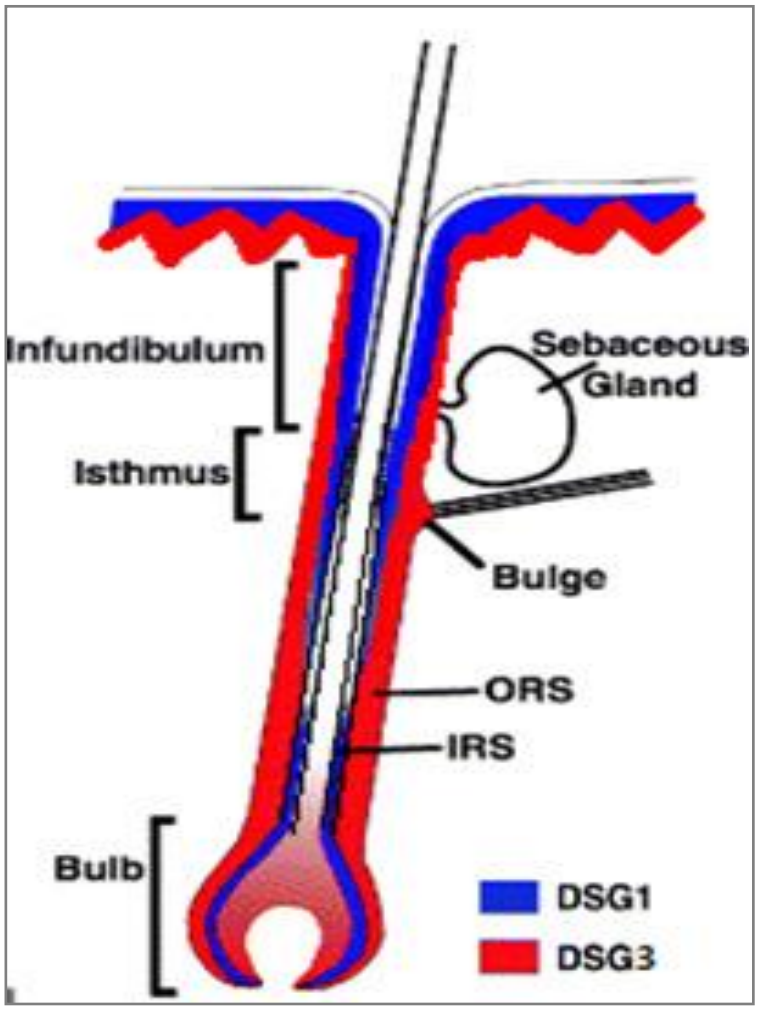



**Fig 2: Distribution of desmoglein 1 & 3 in the hair follicle**

**Table 1: Summary of Dsg expression in hair follicle[39]**

| Region | Dsg 1 | Dsg 2 | Dsg 3 |
|---|---|---|---|
| Basal cells of infundibulum | +/- | + | ++ |
| Suprabasal cells of infundibulum | +++ | - | + |
| Isthmus- Suprabasal cells of ORS | ++++ | + to - | +++ to - |
| Suprabasal cells of ORS from suprabulbar region to bulge | -To ++ | ++ to - | +++ to ++ |
| Bulge region | - | +++ | + |
| Basal cells of ORS below the bulge region | - | ++ to +++ | +/- |
| Precortical cells | - | + | + |
| Medulla | + | - | +++ |
| Inner root sheath | +++ | - | - |
| Matrix | - | ++ | +/- |

**Desmoglein Compensation Theory**

Desmoglein 1 and Desmoglein 3 compensate their adhesive function when expressed together on the same cell. The characteristic blisters' distribution and localization in PV and PF patients can be accounted for by the distribution and levels of expression of Desmoglein 1 and 3[51]

The anti-desmoglein autoantibody profile defines the clinical phenotype of pemphigus.[52] Patients with PV have either anti-Dsg3 IgG or have both anti-Dsg3 and anti-Dsg1 IgG. Patients with Pemphigus Foliaceous have only anti-Dsg1 IgG.

On the basis of the above findings, the level of blister formation in pemphigus can very well be explained. In fact, Pemphigus Foliaceous anti-Dsg1 IgG causes blisters in the superficial layers of the epidermis but not in the deep epidermis or mucosa, where the expression of Dsg3 compensates for the antibody-induced functional loss of Dsg1.

Similarly, in Mucosal PV, anti-Dsg3 IgG causes acantholysis in the deepest layers of the mucous membranes where Dsg1 expression is minimal but not in the skin where epidermal integrity is guaranteed by the high expressivity of Dsg1.[53] Hence only oral erosions are seen without apparent skin involvement in mucosal dominant Pemphigus Vulgaris. Moreover, in Mucocutaneous PV, both anti-Dsg1 and antiDsg3 antibodies are present, hence in the epidermis 'low acantholysis' occurs as well .

It is not clear why the split occurs just above the basal layer instead of the whole epithelium falling apart. However it is speculated that the cell–cell adhesion between the basal and the immediate suprabasal layers might be weaker than the other parts of the epidermis, because there are fewer desmosomes. In addition, the lower part of the epidermis might have better access for auto-antibodies which penetrate from the dermis. That may explain why the splits become suprabasilar in Muco-cutaneous PV.[54]

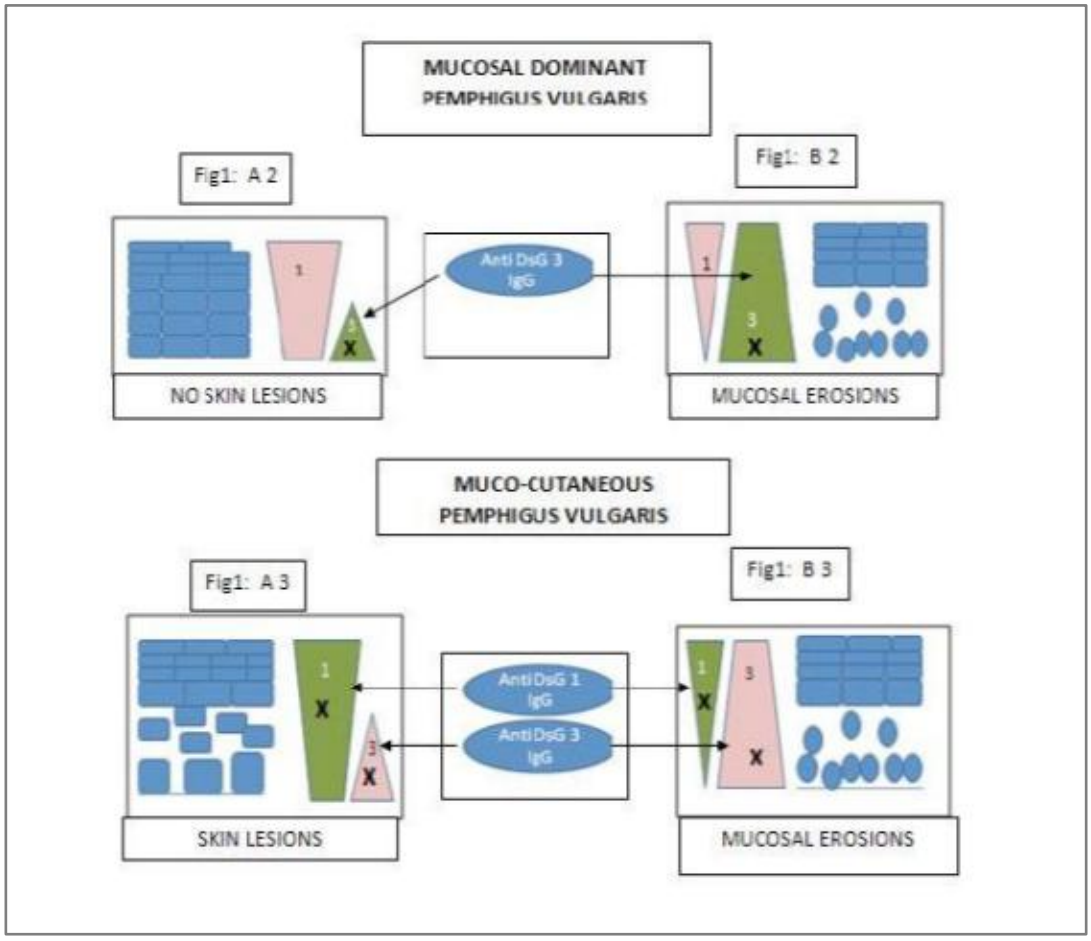



**Fig 3: Desmoglein compensation theory**

The coloured triangles represent the distribution of desmoglein (Dsg 1) and desmoglein 3(Dsg 3) in the skin and mucous membrane. Sera containing only anti – Dsg 3 IgG causes no or only limited blisters in the skin because Dsg 1 compensates for the loss of Dsg 3 mediated adhesion (A 2) ; however, these sera induce separation in the mucous membranes, where the low expression of Dsg 1 will not compensate for the loss of Dsg3 mediated adhesion (B2). When sera contains both anti Dsg 1 and anti Dsg3 IgG, the function of both Dsgs is compromised and blisters occur in both the skin and mucous membranes (A 3 and B 3)

In spite of acantholysis being well accepted as the basic patho-mechanism in Pemphigus, the exact mechanism that causes disruption of the adhesion between keratinocytes occurs to be not fully understood. The possible mechanisms are as follows:

1. **Steric Hindrance**

   Auto antibodies bind to their specific antigens which in-turn disrupt adhesion of the bound antigens by means of steric hindrance.[55] PV-IgG interfering directly with homophilic Dsg3 transinteraction can be demonstrated by means of atomic force microscopy. Neither PV-IgG nor PF-IgG directly interacts with the homophilic Dsg1. The Dsg1 transinteraction is reduced indirectly via cellular mechanisms.[56]

2. **Basal Cell Shrinkage Theory**:

It appears that PV IgG-induced phosphorylation of adhesion molecules and structural proteins leads to weakening of intercellular junctions and collapse of the cytoskeleton, respectively. This in turn results in reorganization of the keratinocyte cytoskeleton, and hence cellular shrinkage and separation of keratinocytes.

Numerous classical and modern clinical and experimental studies in Pemphigus demonstrated that desmosomes separate when the intercellular spaces are already widened. Desmosomes do not split and disappear until late in acantholysis when keratinocytes are almost completely separated from each other. Hence, disruption of intercellular bridges results from ripping intact desmosomes off the plasma membrane of collapsing keratinocytes by shearing forces. The intact desmosomes ripped off from neighbouring cells present in the intercellular space give rise to scavenging autoantibodies.[57]

3. **Apoptolysis Hypothesis**

The apoptolysis hypothesis links the basal cell shrinkage to suprabasal acantholysis and cell death, and emphasizes that apoptotic enzymes contribute to acantholysis in terms of both molecular events and chronologic sequence. Binding of pathogenic auto-antibodies to keratinocytes via a receptor –ligand interaction leads to activation of signalling elements and

elevation of intracellular $Ca^{2+}$, which in turn, initiate cell death enzymatic cascades/ caspases. Suprabasal acantholysis starts when basal cells shrink due to re-organization of cortical actin filaments, collapse and retraction of tonofilaments cleaved by executioner caspases. Acantholysis advances due to continued degradation and massive collapse of structural proteins, thus separating the collapsing cells and stimulating production of secondary antibodies.[58] Although the pool of anti-keratinocyte antibodies thus produced contains anti-Dsg1 and /or Dsg3 antibodies, studies indicate that non-Dsg antibodies are the major contributors to early signalling events.[56]

## 4. Multiple Hit Hypothesis

Involvement of multiple autoantibody specificities in Pemphigus pathogenesis is explained through the 'multiple hit' hypothesis. Antibodies against keratinocytes acetylcholine receptors – AchR trigger acantholysis by weakening the cohesion between neighbouring keratinocytes leading to the affected keratinocytes to shrink, causing Desmosomes to be sloughed in the intercellular space. These sloughed Desmosomes present in the intercellular space stimulate a reciprocal production of scavenger antibodies that in turn saturate epidermis thus preventing nascent desmosome formation by steric hindrance. Severity of the disease and exact clinical picture depends on the ratio of different kinds of auto-antibodies in each particular patient.[59]

## 5. Role of T cells

The role of T- lymphocytes in the pathogenesis of PV is not clear, but auto-reactive T-cell responses to Dsg-3 may be critical in its pathogenesis.[60] T-cell responses to Dsg3 have been detected in PV patients and in healthy donors carrying major HLA class 2 alleles identical or similar to those highly prevalent in PV. These auto-reactive CD4+ T cells preferentially produce Th2 cytokines such as IL-4 and IL-10. Further, auto-antibodies of the Th2-dependent IgG4 subclass are preferentially seen in active PV, while Th1-dependent IgG1 auto-antibodies are predominant during remission. Dsg3-specific, auto-reactive T-cells may thus provide the targets to eventually modulate the T-cell dependent production of pathogenic auto-antibodies in PV and PF.

A disturbance in the regulatory mechanisms of Dsg3-specific T cells that leads to loss of tolerance at the B-cell level leading to the production of auto-antibodies has also been demonstrated.[61]

## Non Desmoglein Antibodies

Pemphigus vulgaris sera may also contain auto-antibodies to desmocollins.[62-63] Antibodies to cadherin have also been detected, some but not all of which crossreact with desmoglein-1.[64]

Antibodies to non-cadherin antigens have also been reported. Antidesmoplakin in addition to antidesmoglein antibodies have been reported in severe pemphigus vulgaris .[65]

Antibodies to cholinergic receptors have been observed in pemphigus sera[66] and cholinergic agonists can inhibit acantholysis induced by Pemphigus sera *in vitro* and have an apparent steroid-sparing effect *in vivo* in pemphigus.[66] The relative contribution and significance of all the various antibodies described in pemphigus remain to be elucidated .[66-67]

**DRUG INDUCED PEMPHIGUS**

The causes of drug induced Pemphigus can be divided into 2 groups on the basis of the chemical structure.

1. Thiol/ SH group- penicillamine, captopril, piroxicam, etc
2. Non thiol group- penicillin, ampicillin, amoxicillin, rifampicin, propanolol, phenytoin, phenobarbitone.

Among these penicillamine is the most commonest cause. Upto 7% Patients treated with it for more than 6 months acquire Pemphigus.

Thiol group of drugs often induced Pemphigus, whereas the non thiol drugs trigger the disease in a predisposed individual.[68]

## CLASSIFICATION OF PEMPHIGUS

1. Pemphigus vulgaris
2. Pemphigus Vegetans
3. Pemphigus Foliaceous
4. Pemphigus Erythematosus
5. Pemphigus Herpetiformis
6. Induced pemphigus
7. Intercellular IgA dermatosis
8. Paraneoplastic Pemphigus

## CLINICAL FEATURES

Pemphigus vulgaris can be further classified into 2 types:

1.) Mucosal type- Dominant mucosal lesions with minimal skin lesions.
2.) Muco-cutaneous type- i.e mucosal involvement along with extensive bulla and erosions in skin.

## MUCOSAL MANIFESTASTION

The occurrence of oral lesions in Pemphigus vulgaris is seen in all patients and a majority of them present initially with them. Cutaneous lesions may develop only months later or sometimes oral lesions may be the only manifestation.[69] Intact bullae are rarely seen in the oral cavity. Usually patients have ill-defined, irregularly shaped buccal or palatal erosions, which take time to heal. There is peripheral

extension of the erosions with shedding epithelial shedding.[70] The conjunctiva, nasal, pharynx , larynx, oesophagus , urethra, vulva and cervix are the other mucosal surfaces that may be involved.

**CUTANEOUS INVOLVEMENT**

Most patients develop cutaneous lesions. Involvement may rarely remain localized to one site [71] but more commonly becomes widespread. The disease has a predilection for the scalp, face, axilla, groins and pressure points. It usually presents as blisters which are flaccid in nature containing clear fluid on normal skin or an erythematous base. The contents then become turbid or the blisters rupture, resulting in painful erosions which tend to extend at the edges as there is more loss of epidermis.

Healing occurs without scarring but pigmentary change and acanthomas may occur in resolving lesions .[72]

Lesions in skin folds readily form vegetating granulations, and flexural pemphigus vulgaris merges with its variant Pemphigus vegetans.

Nail dystrophies, acute paronychia and subungual haematomas have been observed in Pemphigus.[73]

**Complications**

The most commonly encountered complication in Pemphigus vulgaris is secondary infection, which if not treated could lead to sepsis and then death.

Complications are also seen as a result of long term treatment with immune-supressants and steroids. These include hypertension, adrenal axis suppression, fluid retention, osteoporosis, diabetes mellitus, cataracts, glaucoma, increased susceptibility to infections and reactivation of tuberculosis.

## DIFFERENTIAL DIAGNOSIS

Patients with mucosal lesions present to dental surgeons, oral surgeons and gynaecologists. Erosions may simulate acute herpetic stomatitis, erythema multiforme, aphthous ulcers or bullous lichen planus. Bullae are transient in the mouth and biopsies of erosions may not be diagnostic.

The diagnosis is less difficult when the patients have cutaneous blisters or erosions.

Blisters in pemphigoid are tense and may be haemorrhagic. The diagnosis of pemphigoid is confirmed histologically by showing subepidermal bullae with immunoreactants in the basement membrane zone.

Acute erythema multiforme is a short-lived disorder that may blister, but is easily differentiated from pemphigus histologically.

Blistering in dermatitis herpetiformis is subepidermal and direct immunofluorescence of involved and uninvolved skin shows a granular deposition of IgA in the basement membrane zone.

The histological differential includes Darier's disease, Hailey–Hailey disease (benign familial chronic pemphigus) and transient acantholytic dermatosis (Grover's disease). These conditions have distinctive clinical features in addition to negative immunofluorescence studies.

Eosinophilic spongiosis may be an early histological manifestation of either pemphigus or bullous pemphigoid.[74]

**Course and prognosis**

During the past few decades, survival of patients with Pemphigus has been continually improving. Prior to the advent of corticosteroids, Pemphigus was usually fatal. The average mortality rate used to be 29% and since 1970s, the rate has fallen to 5%. This has been attributed to the use of immunosupressive agents, timely initiation of therapy and improved treatment of complications of steroid therapy. The most common causes of death are septicaemia and pulmonary embolism.

The various prognostic factors that have been identified for Pemphigus are:[75]

1.) The type of Pemphigus:

Patients with PV and Paraneoplastic pemphigus have the worst prognosis.

2.) Age of the patient at disease onset:
Elderly patients have poorer prognosis.[76]

3.) Disease progression before beginning therapy:
Patients with minimal disease activity for prolonged periods have a better prognosis than patients with rapidly worsening disease.

4.) Dose of steroids required to control the disease:

Patients who require a daily dose of >180mg/day of prednisolone, have a higher risk of mortality.[77]

5.) Initial involvement of mucosa or skin:

Patients who initially have only cutaneous lesions have a better prognosis than those with mucosal involvement.[78]

6.) Time of institution of therapy:

Better prognosis is seen in patients in whom systemic steroid therapy was started within 6 months of appearance of disease.[79]

**PEMPHIGUS VEGETANS**

This is a variant of Pemphigus vulgaris that is characterized by vegetating erosions affecting predominantly the flexures.

Two subtypes are recognized: Neumann Pemphigus vegetans

Hallopeau pemphigus vegetans.

In Pemphigus vegetans, tumid, vegetating papillomatous and hypertrophic plaques are formed in the intertriginous areas and the flexural surfaces .

Local moisture, heat and friction are important factors in their development.

Characteristic glossal changes termed “cerebriform tongue” may be seen.[80] Exuberant vegetating granulations may occur in the oral cavity as well.[81]

## SCORING SYSTEMS IN PEMPHIGUS

As Pemphigus can present with a wide variety of symptoms, it is of importance to devise certain objective parameters for evaluation of the disease progression or its response to therapy. As a result various scoring systems have been used such as , Pemphigus Area and Activity Score, Pemphigus Activity score, Pemphigus Disease Activity Index (PDAI), Autoimmune Bullous Skin Disorder Intensity Score(ABSIS) etc.[82]

Among these, the scoring systems commonly used are the PDAI score and ABSIS score. The PDAI scoring method combines mucosal and cutaneous disease and also helps to assess the size and number of lesions with scoring for post inflammatory hyperpigmentation as well.[82]

The advantage of the ABSIS score over the rest is that it is a quality and quantity based score for cutaneous lesions and oral mucosa. It also monitors the clinical status of the patients over time.

### Signs in Pemphigus

1. **Nikolsky's sign**:

   The sign is positive when a firm tangential pressure with a finger over a bony prominence will produce an erosion by separate normal looking epidermis from the dermis.

   A positive Nikolsky sign indicates severe disease activity.

2. **Bulla Spread sign**:

   The sign is considered positive when unidirectional pressure applied by a finger causes peripheral extension of the bulla beyond the marked margin.[83]

3. **Asboe Hansen sign**:

   Pressure is applied to the center of the bulla, which causes peripheral extension beyond the marked margins.

## INVESTIGATIONS

1. **Tzanck Smear**

   It is the most useful bed side test for Pemphigus. The intact roof of a blister is opened on one side and the floor is gently scraped with a glass slide or blunt edge of a scalpel. The material obtained is smeared onto a glass slide, allowed to air dry and then stained with giemsa stain.

   The smear shows multiple acantholytic cells or tzanck cells. It is a large round keratinocyte, with a large nuclear: cytoplasmic ratio and a rim of eosinophilic cytoplasm. The staining is deeper and more basophilic peripherally on the cell membrane ("mourning edged" cells) due to cytoplasm's tendency to get condensed at the periphery leading to a peri-nuclear halo.[84]

In PF the acantholytic cell is smaller, less rounded, or cuboidal shaped with a small nucleus and abundant cytoplasm. The cells may have keratohyaline granules and show keratinization.

## 2. SKIN BIOPSY AND HISTOPATHOLOGY

Biopsy for H&E staining should be preferably taken from an early intact bulla or vesicle. In the absence of intact vesicle or bulla, biopsy can be done from the edge of an erosion.[85]

The earliest change in PV is eosinophilic spongiosis or just spongiosis of the lower epidermis, which is considered the earliest manifestation of acantholysis.[85]

Acantholysis leads first to the formation of clefts and then to blisters in a predominantly suprabasal location. The intraepithelial acantholysis may extend into the adnexal structures or occasionally be higher in the stratum spinosum. The basal keratinocytes, although separated from one another through the loss of attachment remain firmly attached to the dermis like a "row of tombstones." Within the blister cavity, the acantholytic keratinocytes, singularly or in clusters, have rounded condensed cytoplasm about an enlarged nucleus with peripherally palisaded chromatin and enlarged nucleoli.[85]

There is usually little inflammation but if present it is sparse lymphocytic perivascular infiltrate accompanied by dermal edema.

In the late stage of the bulla several changes occur. There is mixed inflammatory infiltrate of neutrophils, lymphocytes, eosinophils and macrophages in the dermis.[85]

The bulla ruptures to form an erosion or an ulceration with the base showing acantholytic cells.

An older bulla may show several layers of epidermis at the base as a result of keratinocyte migration and proliferation. Lastly, there may be down growth of the epidermis giving rise to villi.[85]

In case of an oral mucosal biopsy it is difficult to demonstrate an intact

bulla. Hence, only erosions and ulcerations of the mucosa is detected.

Biopsy is taken from the edge of the erosion with intact adjacent mucosa in order to demonstrate the typical pathological findings.[85]

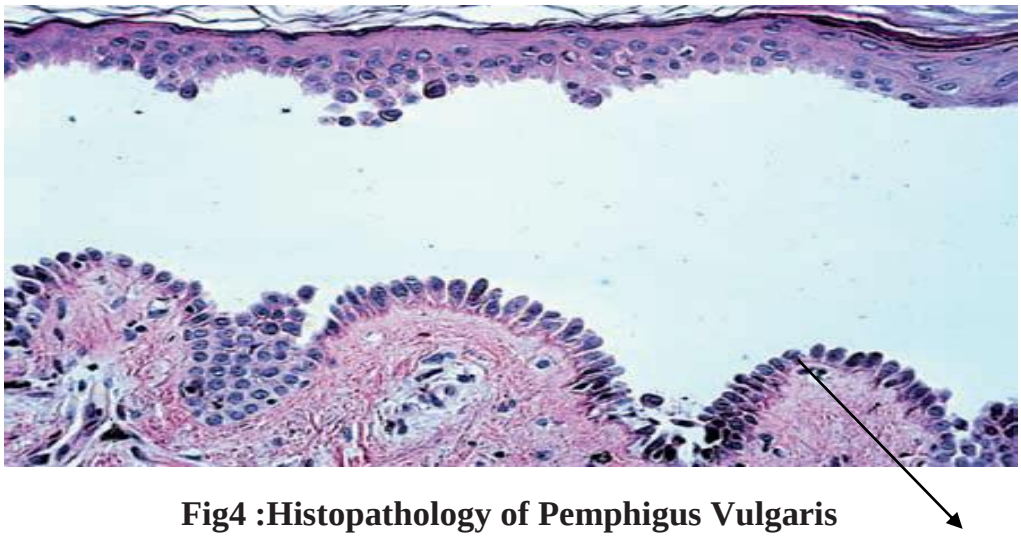



**Fig4 :Histopathology of Pemphigus Vulgaris**

**Pemphigus Vegetans**

Histopathology shows a suprabasal cleft similar to PV but the picture is dominated by papillomatosis and acanthosis having pseudo-epitheliomatous picture with occasional formation of intra-epidermal eosinophilic abscesses. Villi formation and downward proliferation of epithelial strands are more marked. The papillary and reticular dermal infiltrate is composed predominantly of eosinophils.

3. **Immunofluorescence**

   Immunofluorescence is a laboratory staining technique used to demonstrate antibodies or antigens in the tissue or blood or other body fluids. It is a technique essential to supplement clinical and histo-pathological findings in the diagnosis of immunobullous disorders.

   They play an important role in early diagnosis, treatment and subsequent monitoring of disease activity in patients having these disorders.

Both direct and indirect immunofluorescence can be utilized for the diagnosis of pemphigus. The gold standard in the diagnosis of pemphigus is considered to be the detection of direct immunofluorescence of skin or mucosa for the detection of antidesmoglein 3 and / or 1 antibodies.

## History

In 1941, Coons et al first developed the immunofluorescence technique with a blue fluorescing compound, β-anthracene, which made it possible to visualize the microscopic antigens, antibodies and other elements in tissue sections or cell smears.[86]

In 1963, diagnostic immunopathology in dermatology began with the demonstration of complement and immunoglobulin deposition in the dermo-epidermal junction of skin - Lupus band test in SLE.[87]

Beutner and Jordon demonstrated in the year 1964 the antibodies in the sera of Pemphigus patients by indirect immunofluorescence.[88]

In 1971, Jordon *et al.* demonstrated the deposition of IgG antibodies at the inter-cellular spaces in the epidermis by direct immunofluorescence of the lesional and peri-lesional skin.[89]

**Principle Of Fluorescence**

Fluorescence is defined as the light emitted by the singlet state of a molecule on returning to its ground state, following absorption of photon from an external source.

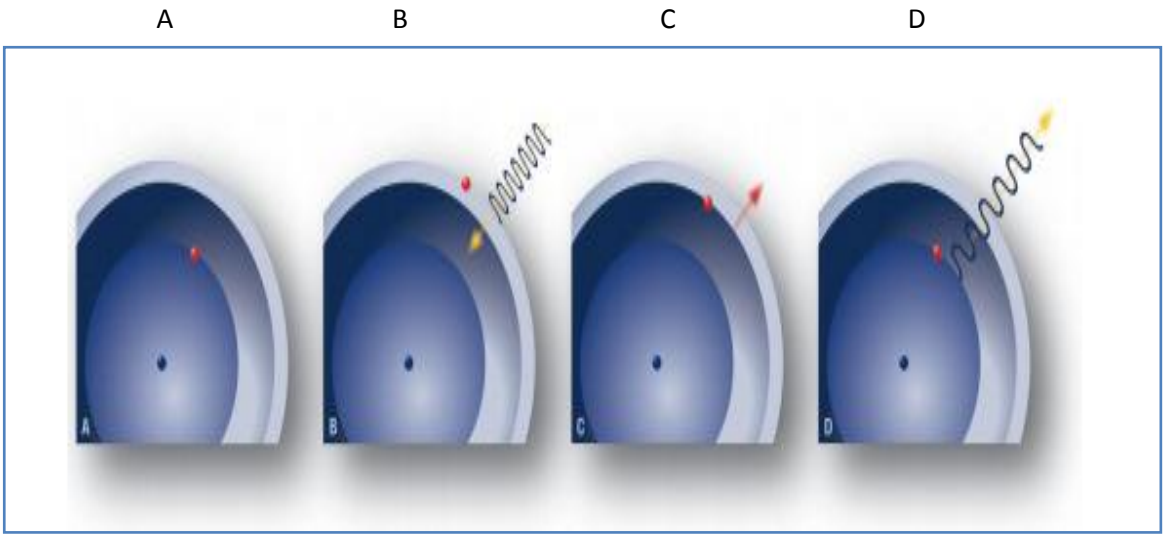



**Fig 5: Principle of Fluorescence**

A) Electron in its ground state in a molecule.

B) Electron excited by a high energy light- UV light and attains a higher energy state.

C) Electron unable to maintain its high energy state and drops to its lowest singlet energy state by losing energy as heat.

D) The electron then spontaneously returns to its original ground state by emitting the remaining energy as light with longer wavelength and lesser energy in the form of fluorescence

## BASIS OF IMMUNOFLUORESCENCE

In this procedure, the antibodies, antigens or complexes of both are stained with corresponding antibodies tagged with a fluorochrome and then viewed under a fluorescent microscope with a mercury vapour or xenon light source and appropriate exciter and barrier filters.[90]

Fluorochromes are defined as substances which have the ability to absorb light of a particular wavelength and reach an unstable higher energy state. Following which, on spontaneously returning to their original state, light with a longer wavelength is re-emitted.[91]

The commonly used flurochromes are[92]

- Fluorescein isothiocyanate (FITC)
- Tetramethylrhodamine isothiocyanate (TRITC)
- Phycoerythrin

FITC produces an apple green fluorescence, while TRITC and phycoerythrin produce red fluorescence.

## TYPES OF IMMUNOFLUORESCENCE

1. Direct Immunofluorescence

2. Indirect Immunofluorescence

3. Complement Indirect Immunofluorescence

### Direct Immunofluorescence

This is a single step method, where the antibody specific to the target molecule is tagged with a fluorescent dye.

In Immuno-bullous disorders FITC tagged tagged anti-immunoglobulin antibodies are used for the detection of in-vivo antibodies which are bound to the target antigen.

A specimen for direct immunofluorescence needs to be transported to the laboratory in cold normal saline either immediately or if placed in an ice box, within few hours of biopsy. The specimen can also be transported in michels medium where it can be stored for upto one month at 4 -8$^{o}$C.[93,94]

**Indirect Immunofluorescence**

This is a 2 step procedure where the circulating antibodies in the patient's serum are detected.

In this technique the antibody specific to the target molecule- the primary antibody is unlabeled, and a second anti-immunoglobulin antibody called the secondary antibody is directed towards the constant portion of the first antibody- which is tagged with the fluorescent dye.[93,94]

In autoimmune blistering disorders, first the substrate is incubated with the patient's serum and then the FITC tagged anti-immunoglobulin antibodies are added to it for detection of the pathogenic antibodies.[91]

**Complement Indirect Immunofluorescence**

This is a 3 step IIF technique in which the patient's serum is incubated with the substrate, then complement is added.

Presence of complement in the tissue is then detected by adding fluorescein labelled anti-complement antibodies. This test is done to detect complement fixing antibodies.[95]

## ROLE OF DIRECT IMMUNOFLUORESCENCE IN PEMPHIGUS

Direct immunofluorescence is considered to be the gold standard in the diagnosis of pemphigus with a sensitivity of 95-100%.[95,96] DIF shows deposition of IgG and/ or C3 against desmoglein 3 and / or 1 in the epidermal intercellular spaces. This is described as ‘lace-like’ or ‘chicken-wire’ or ‘fishnet’ pattern. In late lesions when the acantholytic cells are well developed the classical ‘fish-net’ pattern of immunofluorescence may become dot-like, corresponding to the aggregation of desmosomes on the cell surface.[96]

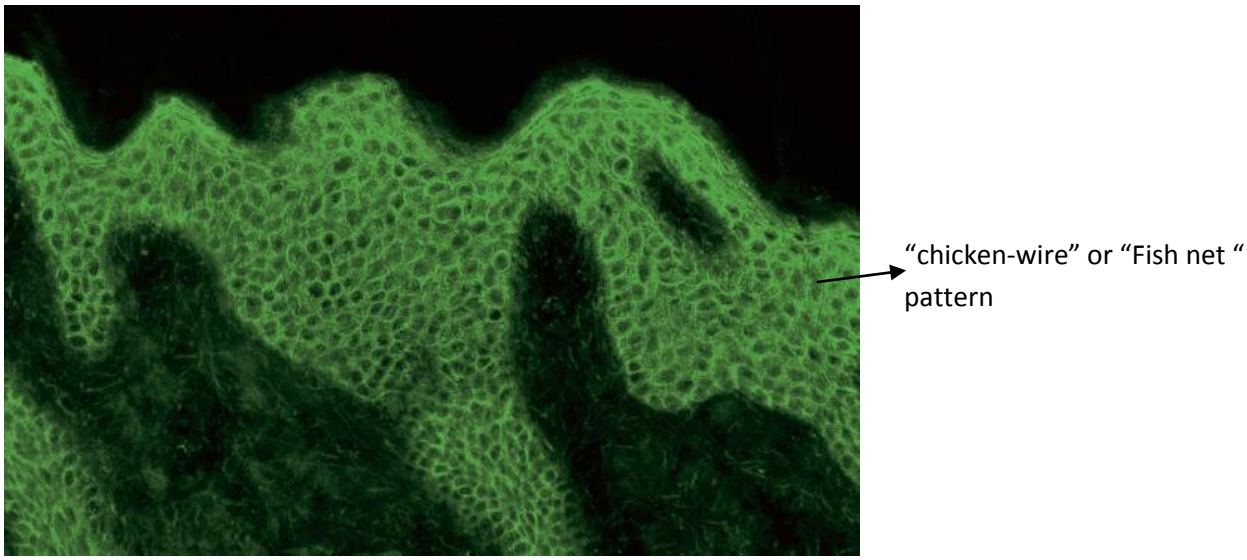



**Fig - 6: DIF of Pemphigus Vulgaris**

The DIF staining shows IgG antibodies in 100 % of positive cases and C3 in 50-100%.[95] IgA and IgM may be present, but less frequently.

Patients with active P.V have both IgG1 and IgG4 subclasses of antibodies but the IgG4 is pathogenic.[42]

The intensity of DIF staining correlates with the disease activity.

However, in few patients it may be positive even when the patient is in clinical remission.[95]

In pemphigus vulgaris patients, negative DIF may be an indicator of immunological remission. And repeated negative DIF during clinical remission may be considered as a possible sign for apparent cure of the disease, and treatment may be discontinued in such group of patients.[97]

**False positive DIF**

It is very rare, but non-specific intercellular staining can be seen in psoriasis, spongiotic dermatitis, bullous impetigo, and epidermis adjacent to ulcers secondary to any cause may have squamous intercellular substance IgG as the intercellular space may contain serum.[96]

## DIRECT IMMUNOFLUORESCENCE OF HAIR

The scalp is a commonly involved site in Pemphigus. Wilson et al, demonstrated that, in the scalp, the outer root sheath hair follicle and the dermal bulb matrix cells is rich in the target antigens of pemphigus. This may be the reason for scalp involvement in Pemphigus.[98]

Recently, it has been shown that outer root sheath of hair follicle which is structurally analogous to the epidermal keratinocytes also shows positive direct immunofluorescence findings with a sensitivity of 85-100%.[3-6]

Schaerer and Trueb in the year 2003, for the first time, demonstrated pemphigus specific DIF pattern in the ORS of the hair follicle of the plucked hair. They demonstrated positive DIF findings in 100% of their patients.[3]

Similarly another study demonstrated acantholysis in the hair follicle and also immune deposits specific to Pemphigus in the outer root sheath and matrix of hair follicle in the biopsy specimens.[98]

## INDIRECT IMMUNOFLUORESCENCE IN PEMPHIGUS

In this method circulating IgG antibodies are demonstrated in 80-90% of pemphigus cases.[96] The substrates commonly used for IIF include guinea pig oesophagus, monkey oesophagus and normal human skin. Of which money oesophagus is considered as the ideal substrate.[96]

IIF has been widely used for monitoring of the serological activity of pemphigus patients. It has been demonstrated that the antibody titres in the patients sera in many instances, correlates with the disease severity.[99,100]

However, other studies analyzing the serial titres by IIF, showed that the antibody titres do not always correlate with disease severity and hence, cannot be used as a guide to prognosis or monitoring the disease activity.[101.102]

Judd and Lever found that administration of a high dose of daily steroids resulted in clinical improvement as well as showed a marked fall in the titre of circulating antibodies. However, there was no predictable correlation between the disease activity and antibody titre by IIF when the patients were not receiving a high dose of steroids.[103]

**False positive IIF**

False positive immunofluorescence staining can be seen in burns, penicillin allergy, toxic epidermal necrolysis, bullous pemphigoid, myasthenia gravis, SLE, lichen planus, cicatricial pemphigoid and in patients with antibodies against blood group A and B.[133]

## ADVANTAGES OF IMMUNOFLUORESCENCE

1.) Various auto immune disorders with similar clinical picture are classified using immunofluorescence.
2.) Confirmation of diagnosis in cases where the clinical picture is atypical or non specific.
3.) Circulating antibody level detected by IIF can be used as a prognostic marker and also as a marker of disease activity and response to treatment in patients diagnosed with pemphigus.
4.) Antigen mapping can be done, which play an important role in classification of various form of hereditary epidermolysis bullosa.

## DISADVANTAGES OF IMMUNOFLUORESCENCE

1.) Expensive procedure and requires a lab with cryostat for frozen sections and a deep freezer for the storage of these specimens, with a well trained technician and a pathologist proficient in the performance and interpretation of the results of immunofluorescence.
2.) DIF stained slides cannot be stored for long-term, as the fluorescent stained slides quenches rapidly on exposure to sun light.
3.) False positive DIF and IIF can occur.

## LIMITATIONS OF IMMUNOFLUORESCENCE TECHNIQUES

### 1. Photobleaching

It refers to the photochemical reaction which causes reactive oxygen species mediated destruction of a fluorochrome in the specimen. It can be reduced by decreasing intensity and duration of excitation light, using a low concentration of a fluorochrome and addition of singlet oxygen scavengers.

### 2. Autofluorescence

It is due to flavin coenzymes and reduced pyridine nucleotides. Fixation with aldehydes, particularly glutaraldehyde, can increase autofluorescence.

### 3. Fluorescence Overlap

The emission signals may sometimes over lap if more than one colour fluorescence is emitted.

### Enzyme-Linked Immunosorbent Assay

Utilizing the recombinant ectodomains of Dsg1 and Dsg3 highly sensitive and specific enzyme linked immunosorbent assay (ELISA) have been developed to detect the titres of Dsg3 and Dsg1 antibodies in serum. These assays are highly sensitive and specific for PV. Anti-desmogleins ELISA assay has shown that 95% of PV patients have desmoglein-3 antibodies and around 50% have desmoglein-1

antibodies. ELISA titers correlate well with disease activity and are useful to in following up the patient.[43]

## OTHER INVESTIGATIONS

Other than the diagnostic investigations in Pemphigus, investigations such as complete blood count, urine routine, Liver function test, Renal function test, fasting & post prandial blood sugar, chest x-ray, mantoux test should be done as baseline prior to starting the treatment.

## TREATMENT OF PEMPHIGUS

Pemphigus vulgaris if left untreated can be a fatal disease with mortality as high as 73% before the advent of corticosteroids.[104]

At present, the first-line treatment of pemphigus is considered to be systemic corticosteroids with or without adjuvant immunosuppressive agents.

The end goal of treatment is maintenance of remission with the least dosage of steroids or other immunosuppressives, in order to limit adverse effects of treatment.

Before initiating specific treatment measures, it is important to assess the general condition and extent and severity of the disease.

Particular attention should be paid to assessing nutrition status, electrolyte imbalance and presence of secondary infection.

Supportive care options required are:

1. **Nursing care**: Periodic cleaning and dressing of erosions without extensive desloughing until re-epithelisation. This can be done by dressings with sterile petrolatum/ antibiotic gauze. Measures should be adapted for prevention of bed sores. And finally maintenance of proper oral hygiene.
2. **Nutrition**: Soft, protein rich and high calorie diet should be provided as there may be loss of proteins and also as patient may be unable to swallow due to severe oral ulcerations. If patient is not able to take oral feeds, patient may require a feeding tube or parenteral nutrition.

3. **Control of secondary infection**: Antibiotics should be given preferably following a culture and sensitivity report since infection remains the bugbear for treatment and if necessary anti-fungals need to be added.
4. **Correction of fluid and electrolyte imbalances**: There is increased loss of fluids and electrolytes from the erosions and adequate supplementation by intravenous route should be provided.

**Topical Therapy**

Mild localized relapse cases can sometimes be treated with topical therapy alone. This can be achieved with either topical clobetasol[105] propionate or topical tacrolimus.[106]

Topical epidermal growth factor has shown to reduce the healing time of skin lesions significantly in Pemphigus patients.[107]

**SYSTEMIC CORTICOSTEROIDS**

Systemic corticosteroids are the mainstay of treatment of Pemphigus. Prednisolone is the most widely used and time-tested drug hence it is the preferred drug. Deflazacort, dexamethasone and betamethasone has also been used.

For mild to moderate disease, the starting dose of prednisolone is of 60-80mg/day and for a severe disease 80-120mg/day. The dose can be incremented if there is no clinical improvement in a week by upto 50% until the disease activity is controlled.[104]

Once 80-90% of the lesions have healed, the dose can be tapered by 50% every 2 weeks till a dosage is reached to maintain clinical remission which is usually alternate day therapy.

Although corticosteroids may provide rapid resolution of lesions, they alone are not effective in a significant number of cases. Relative contraindications to the

use of steroids, serious side effects due to corticosteroids, or if a reduction of dosage is not possible due to disease activity, concomitant immunosupressives or other adjuvants are advocated.

## DEXAMETHASONE CYCLOPHOSPHAMIDE PULSE THERAPY

As the side effects of long term corticosteroids itself could contribute to the morbidity and mortality, pulse therapy was introduced.

Pulse therapy means administering bolus dose of a drug at a suprapharmacologic dosage over a short period of time and then withdrawing it completely till next dose.

Dexamethasone and cyclophosphamide pulse therapy for pemphigus was first proposed in the year 1982 by Parisch et al.[108]

Advantages are :

1.) Faster healing of lesions
2.) Faster control of disease activity in extensive disease
3.) Reduction in total cumulative dosage of CS.
4.) Cure with minimal CS induced side effects. [109]

Standard DCP therapy consists of 4 phases: [108]

**Phase 1**

Dexamethasone 100mg is given intravenously dissolved in 500ml of 5% dextrose over 3 hours on 3 consecutive days. Cyclophosphamide 500mg is dissolved in the same infusion on the second day. The same cycle is repeated again every 28 days until all lesions have completely healed and patient is off daily CS.

**Phase 2**

DCP is continued for another 9 months even though lesions have completely healed.

**Phase 3**

Monthly DCPs are stopped and cyclophosphamide 50 mg is continued for another 9 months and then stopped.

**Phase 4**

This is the period of observation where the patient is drug & disease free and under follow up to look for any recurrences.

## ADJUVANT IMMUNOSUPRESSANTS

The two main adjuvants are azathioprine and cyclophosphamide for most of the patients. Other adjuvants include Mycophenolate Mofetil, Cyclosporine, Dapsone and Methotrexate.

## AZATHIOPRINE

One of the most commonly used adjuvants for Pemphigus. It is given at a dose of 1-3 mg/kg/day. Ideally the dosage should be adjusted according to the TPMT levels.

The therapeutic effect of azathioprine is seen usually after 3-5 weeks. In terms of mortality and remission, Prednisolone with azathioprine is more effective than Prednisolone alone.[110]

## CYCLOPHOSPHAMIDE

Cyclophosphamide has been used as an adjuvant to CS and is usually given at a dose of 1-3 mg/kg body weight. Monthly IV cyclophosphamide in DCP pulse therapy with daily oral. Cyclophosphamide in low doses has been used with success.[108,111]

However, it should be used with caution in women of child bearing age and in patients who have not yet completed their family, as it's known to cause secondary infertility due to amenorrhoea and azospermia, on long-term administration.[112]

A study has shown that remission in pemphigus can be maintained with low dose of Cyclophosphamide alone.[109]

## MYCOPHENOLATE MOFETIL

MMF is usually given at a dose of 2-2.5g/day as a steroid sparing agent. One randomized controlled trial found MMF to be a less effective than azathioprine as a steroid sparing agent.[113]

## METHOTREXATE

It can be considered as an adjuvant, if the more commonly used steroid sparing agents cannot be used for the patient. Earlier studies with high dose methotrexate showed high mortality rate.[114] However, a recent study has shown that methotrexate can be useful and well tolerated in pemphigus patients with a considerable steroid sparing effect.[115]

## CYCLOSPORIN

Initial there were case reports that cyclosporine was a useful adjuvant with considerable steroid-sparing effects in PV.[116-118] However, a recent trial has found that cyclosporine as an adjuvant therapy has no benefit over steroids alone.[119]

## DAPSONE

Dapsone at a dose of 100-200mg/ day has been tried as an adjuvant in pemphigus.[120] It has been found to be effective as a steroid sparing agent in few studies.[121]

## RITUXIMAB

It is a chimeric monoclonal anti CD 20 antibody. Its effect, is mainly on the B cells. Two studies, have provided valuable data regarding the safety and efficacy of rituximab. A study conducted by Cianchini et al , showed that 86 % of patients treated with Rituximab achieved clinical remission and discontinued steroid within 6 months.[122] And a study by Reguiai et al with 13 Pemphigus patients treated with Rituximab achieved clinical remission within the first 3 months.[123]

**Rituximab can be administered by two different protocol:** [123]

- The lymphoma protocol- 375mg/$m^2$ BSA IV weekly for 4 weeks.
- The rheumatoid arthritis protocol- 1g IV at an interval of 15 days.

However, the major concern for rituximab is its adverse effects such as neutropenia, increased susceptibility to infections, sepsis, DVT.

**IVIG**

IVIG at a dose of 2g/kg body weight divided over 3 days has been tried, this cycle is repeated every 4 weeks.[120] A study showed that a minimum of 3 cycles of IVIG produced beneficial effects in 81% of patients with refractory pemphigus. Cost is the major limiting factor.

**Table 2: Side effects of Corticosteroids [124]**

| CATEGORY | ADVERSE EFFECTS |
|---|---|
| Glucocorticoid effect | Hyperglycemia, increased appetite and weight gain |
| Mineralocorticoid effects | Hypertension, congestive heart failure, arrhythmias secondary to hypokalemia, weight gain |
| Cutaneous | Steroid induced acne, rosacea, increased susceptibility to cutaneous infections, delayed wound healing, striae, telogen effluvium, hirsutism, fat atrophy |
| Bone | Osteoporosis, osteonecrosis, indirect hypocalcemia |
| Gastrointestinal | Peptic ulcer disease, bowel perforation, fatty liver, esophageal reflux, nausea, vomiting |
| Lipid effects | Hypertriglyceridemia, cushingoid habitus, menstrual irregularity |
| Ocular | Cataract, glaucoma, infection especially staphylococcus |

| | |
|---|---|
| Psychiatric | Psychosis, agitation, personality changes,depression |
| Muscular | Myopathy |
| Neurologic | Pseudotumor cerebri, epidural lipomatosis, peripheral neuropathy |
| Infections | TB reactivation, oppurtunistics infections like deep fungal, etc. |
| Pediatric | Growth impairement |
| Pulse therapy | Immediate flushing of face, hiccups, muscle weakness, asthenia,electrolyte shifts, cardiac dysarrythmias, seizures.<br>The long term side effects are similar to daily steroid administration. Though its comparatively lower with pulse therapy |

# MATERIALS AND METHODS

- It is a hospital - based prospective study.
- The study was done with patients diagnosed as a case of Pemphigus vulgaris, attending the outpatient clinic in the Department Of Dermatology, Venereology, Leprosy, PSG IMS & R, Coimbatore.
- The study was conducted over a period of one year after obtaining institutional ethics committee approval prior to the commencement of the study.
- Informed and written consent was obtained from all the patients and from the histopathology department, PSG IMS & R, where the investigations were done.
- The patients were then tested for direct immunofluorescence of oral mucosa and scalp hair.

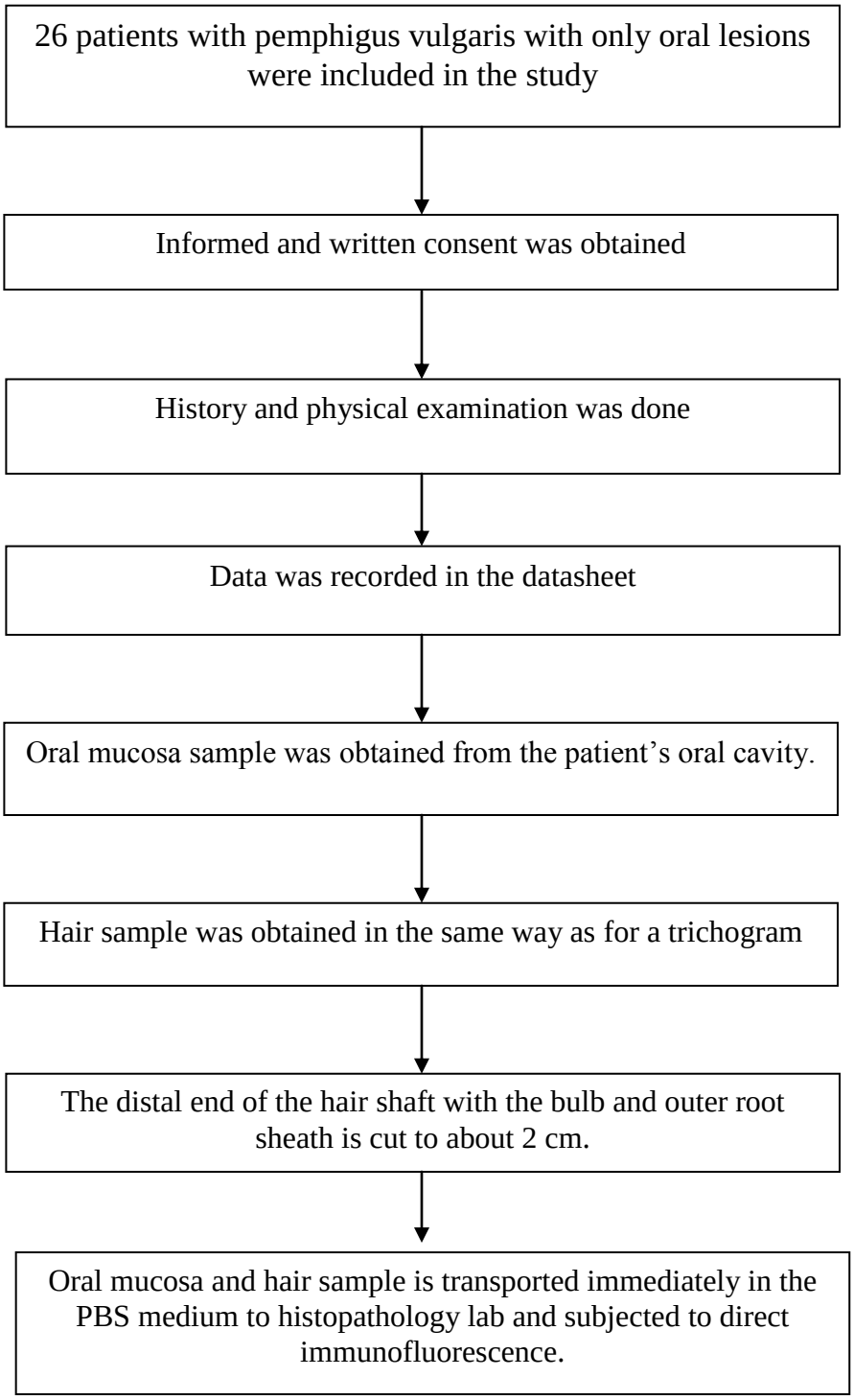
## INCLUSION CRITERIA

- Patients diagnosed with Pemphigus Vulgaris by biopsy or/and DIF.
- The patients had new or non-healing oral mucosal lesions.

## EXCLUSION CRITERIA

- Patients with new or non healing skin lesions in the preceding 6 months.
- Patients with other bullous disorders.

**TABLE 3: METHODOLOGY FLOWCHART**



## METHOD OF DIRECT IMMUNOFLUORESCENCE OF ORAL MUCOSA

1. A punch Biopsy specimen from the Oral mucosa is received in the PBS medium.
2. Specimen is then snap frozen in the cryostat.
3. 5 frozen sections of 5μm thickness each is cut using a cryotome and placed on the slide.
4. Fan dry the section for 10 minutes.
5. The section is then washed in PBS at 7.4 pH for 10 minutes.
6. Fan dry the section for 10 minutes.
7. Each of the slide is then incubated in room temperature for 1 hour with one of the following FITC-labeled antisera - IgG & Fibrinogen each diluted 1:200 in PBS & IgA, IgM, C3 each Diluted 1:100 in PBS (The PBS used in the above steps contains Propidium iodide which is a Counter-stain ).
8. Wash the slides 3 times with PBS for 10 minutes each.
9. Fan dry the sections
10. Mount in buffered glycerol
11. Examine under fluorescent microscope.

## METHOD FOR DIRECT IMMUNOFLUORESCENCE OF HAIR

1. The hair sample is received in PBS medium to the histopathology lab.
2. Hair sample is placed over a Glass slide
3. Washed three times with PBS medium for 10 minutes each
4. Then the specimen is fan dried.
5. Each of the slide is then incubated in room temperature for 1 hour with one of the following FITC-labeled antisera - IgG & Fibrinogen each diluted 1:200 in PBS & IgA, IgM, C3 each Diluted 1:100 in PBS (The PBS used in the above step contains Propidium iodide which is a Counter-stain)
6. Wash the slides 3 times with PBS for 10 minutes each
7. Fan dry the sections
8. Mount in buffered glycerol
9. Examine under fluorescent microscope.

Based on the presence or absence of immunofluorescence deposits in the specimen, the results were interpreted as positive or negative.

## Statistical Analysis

The data collected from the patients is tabulated using Microsoft Excel. The data has been reported in the tables as Percentages. The data was analyzed using Chi-square test and Fisher's exact test. Chi-square test has been used for categorical variables and Fishers exact test is applied for 2*2 way table. Statistical analysis for the data collected was done using SPSS (Statistical Package for Social Sciences) with the version of 16.0. The p value was tested at 5% level of significance.

- **Sensitivity** is the ability of a test to correctly classify an individual as ′diseased′.
- **Specificity** is the ability of a test to correctly classify an individual as 'disease- free'.
- **Positive predictive value** is the probability that subjects with a positive screening test truly have the disease.
- **Negative predictive value** is the probability that subjects with a negative screening test truly don't have the disease.

# RESULTS

## TABLE 4: PATIENTS CHARACTERISTICS

| | |
|---|---|
| Total No. of patients (n) | 26 |
| Age (years) Mean±SD | 43 ± 13.87 |
| Sex - female:male | 21 : 5 |
| Phenotype of Mucosal disease (n) | 26 |
| History of scalp involvement | 0 |
| Duration of Disease (months) | 32 ± 21.16 |
| DIF positive with either substrate | 13 |
| No. of positive hair DIF | 19 |
| No of positive Oral Mucosa DIF | 15 |

## AGE DISTRIBUTION

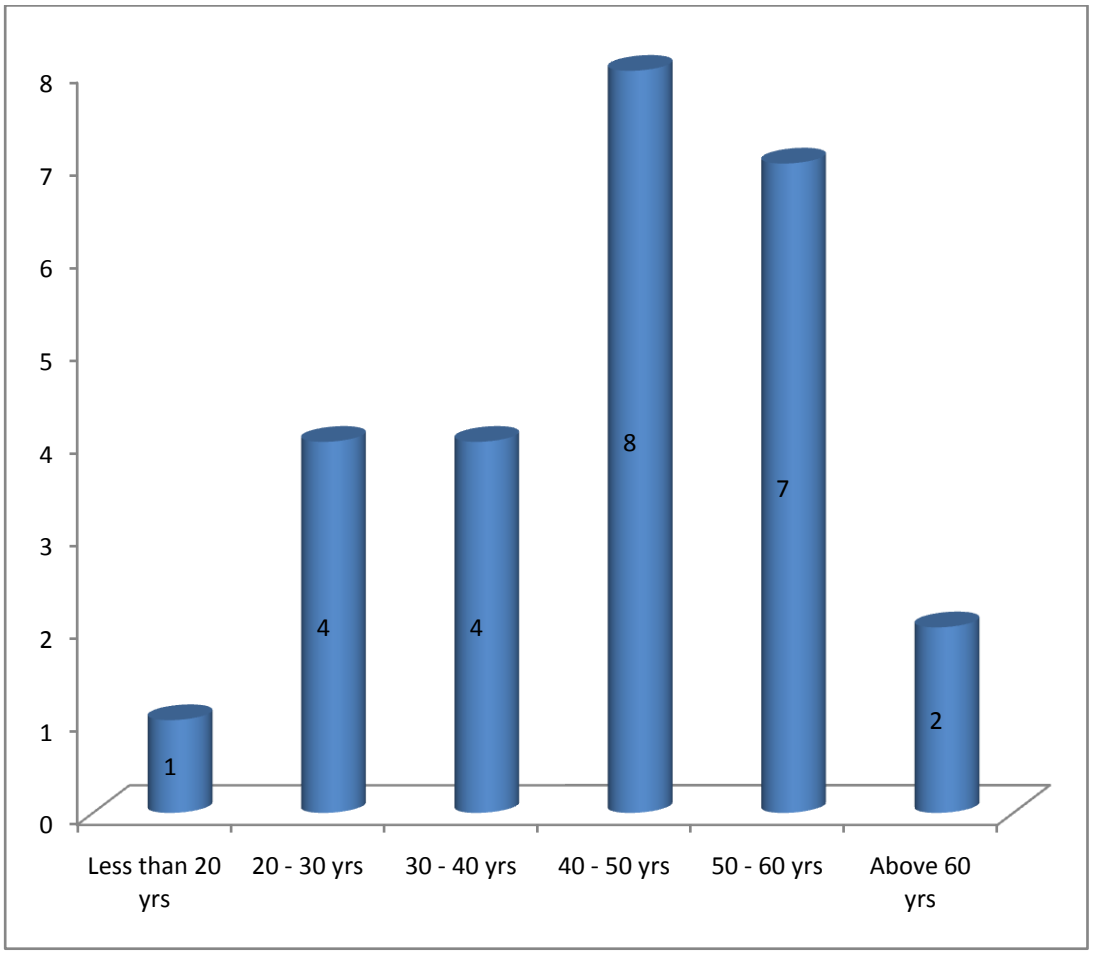



**Fig 7- No. of patients in the various age groups**

- The age of patients in our study ranged from 17-66 years with a mean age of 43.69 years.
- Majority (30.8%) of the patients in our study were in the age group of 41-50 years.

## TABLE 5: GENDER DISTRIBUTION

| Gender | Frequency | Percent |
|---|---|---|
| Male | 5 | 19.2 |
| Female | 21 | 80.8 |
| Total | 26 | 100.0 |

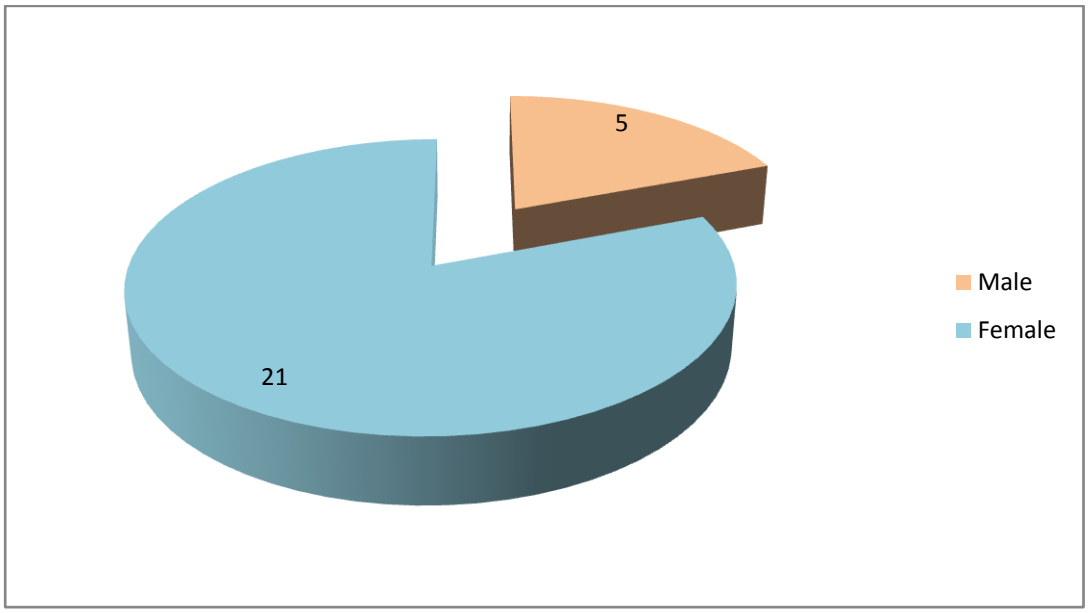



**Fig 8- Pie chart showing gender ratio**

- Our study showed a female preponderance with a male-female ratio of 1:4.2

## DURATION OF THE DISEASE

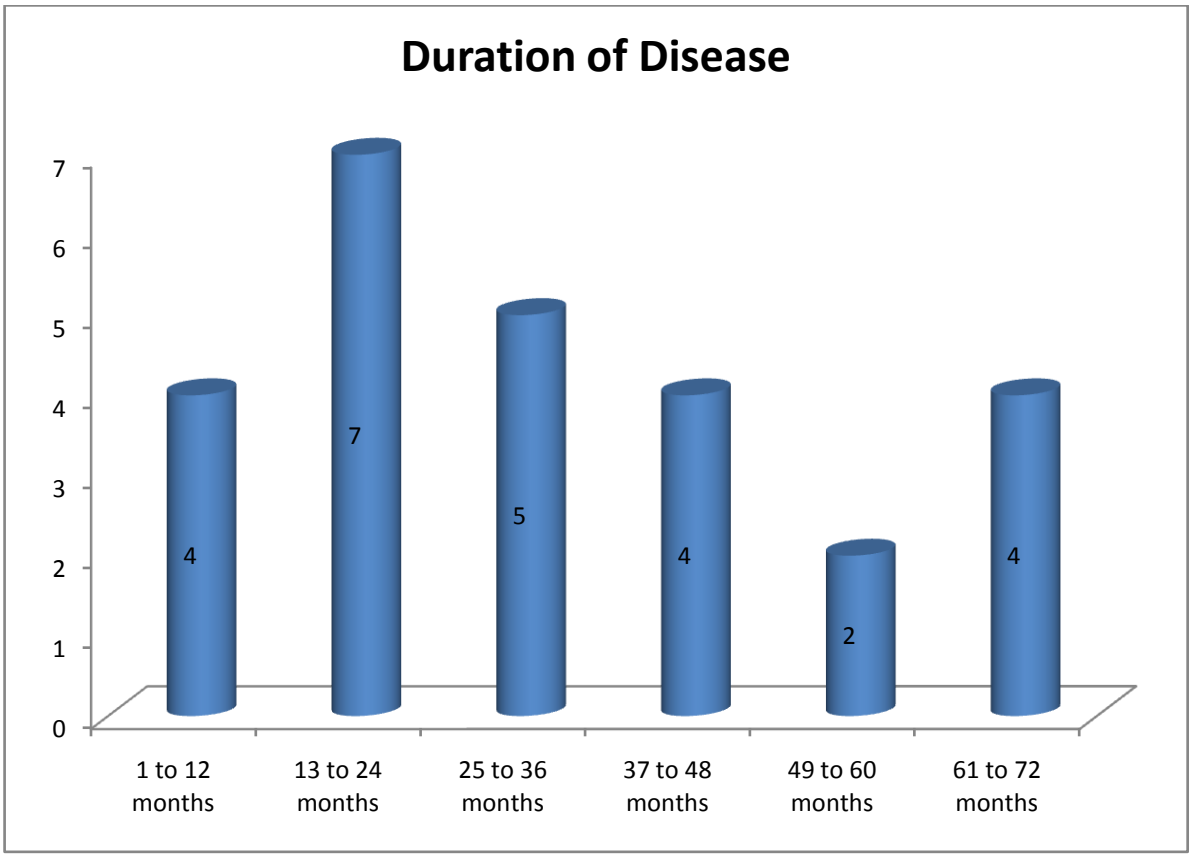



**Fig 9- Duration of the disease**

- Mean duration of the disease in the patients taken up for the study was 32.61 months.

## TABLE 6: ORAL MUCOSA DIF FINDINGS

| | Frequency | Percent |
|---|---|---|
| Positive | 15 | 57.7 |
| Negative | 11 | 42.3 |
| Total | 26 | 100.0 |

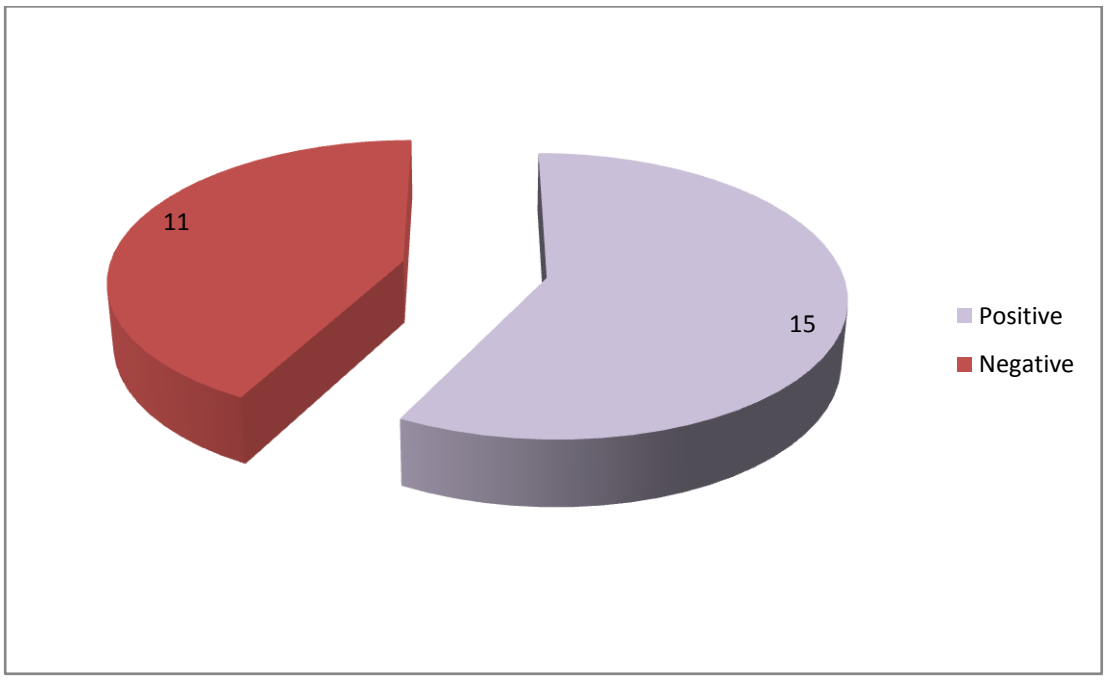



**Fig10-DIF findings of oral mucosa**

- 15 out of the 26 patients showed DIF positivity of the oral mucosa.

## TABLE 7: SCALP HAIR DIF FINDINGS

| | Frequency | Percent |
|---|---|---|
| Positive | 19 | 73.1 |
| Negative | 7 | 26.9 |
| Total | 26 | 100.0 |

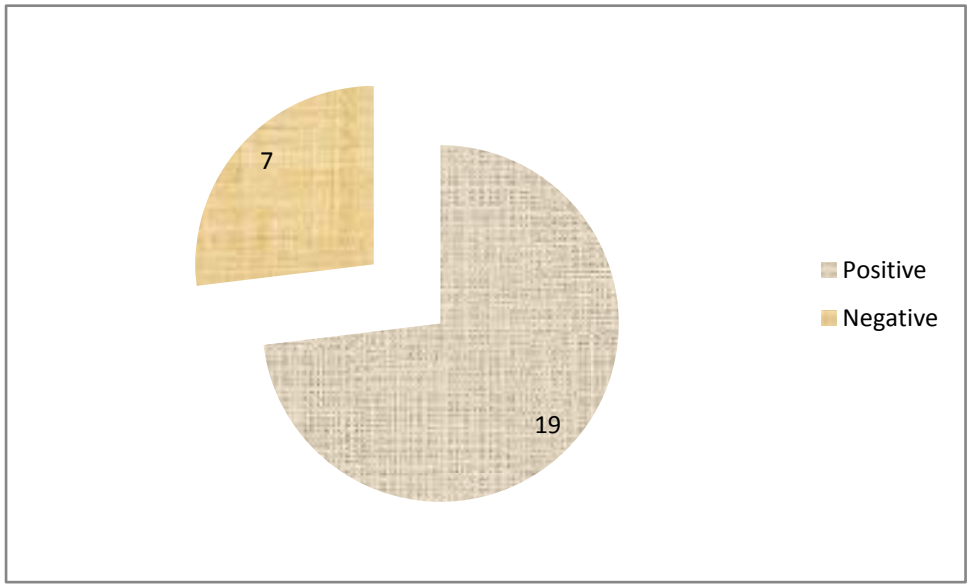



**Fig 11- DIF findings of scalp hair**

- 19 out of the 26 patients showed DIF positivity in plucked scalp hair.

**Oral Mucosa DIF results in different age groups**

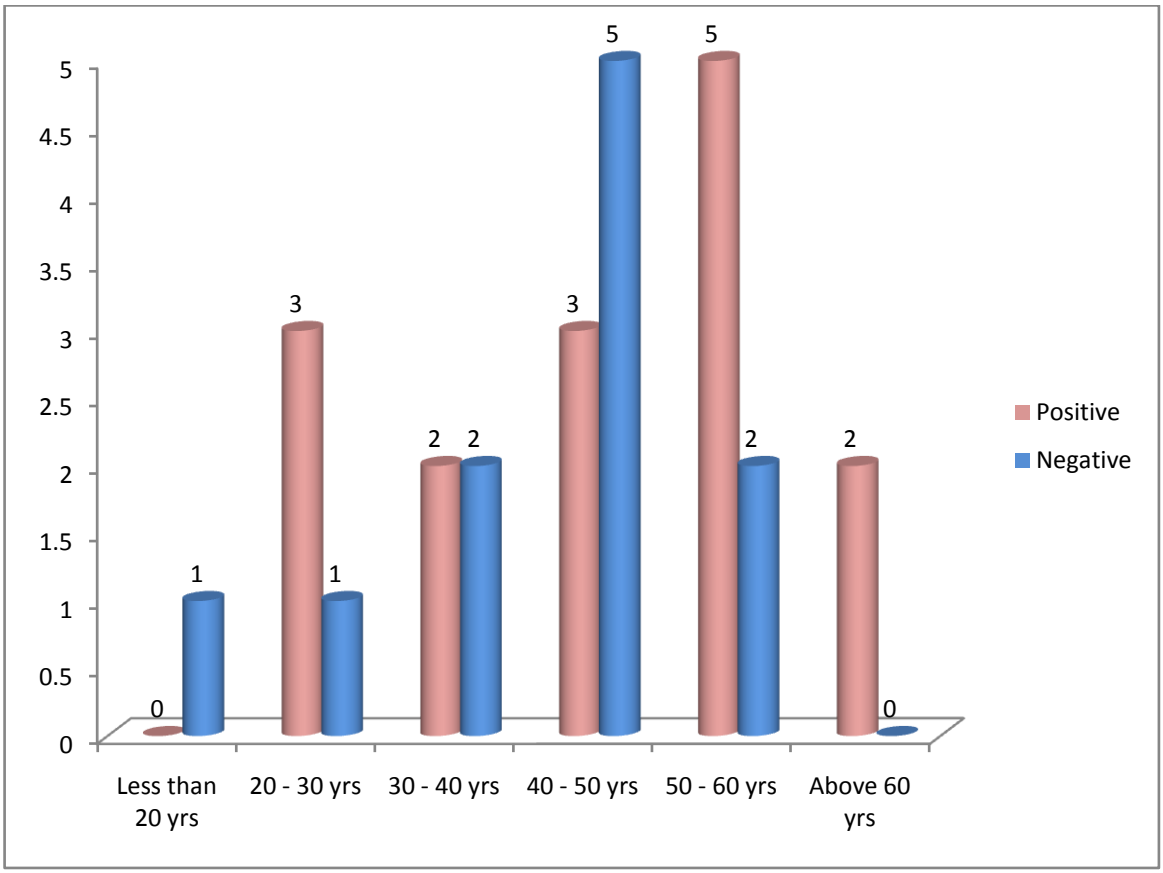



**Fig 12- DIF results of Oral mucosa in various age groups**

**Scalp Hair DIF results in different age groups**

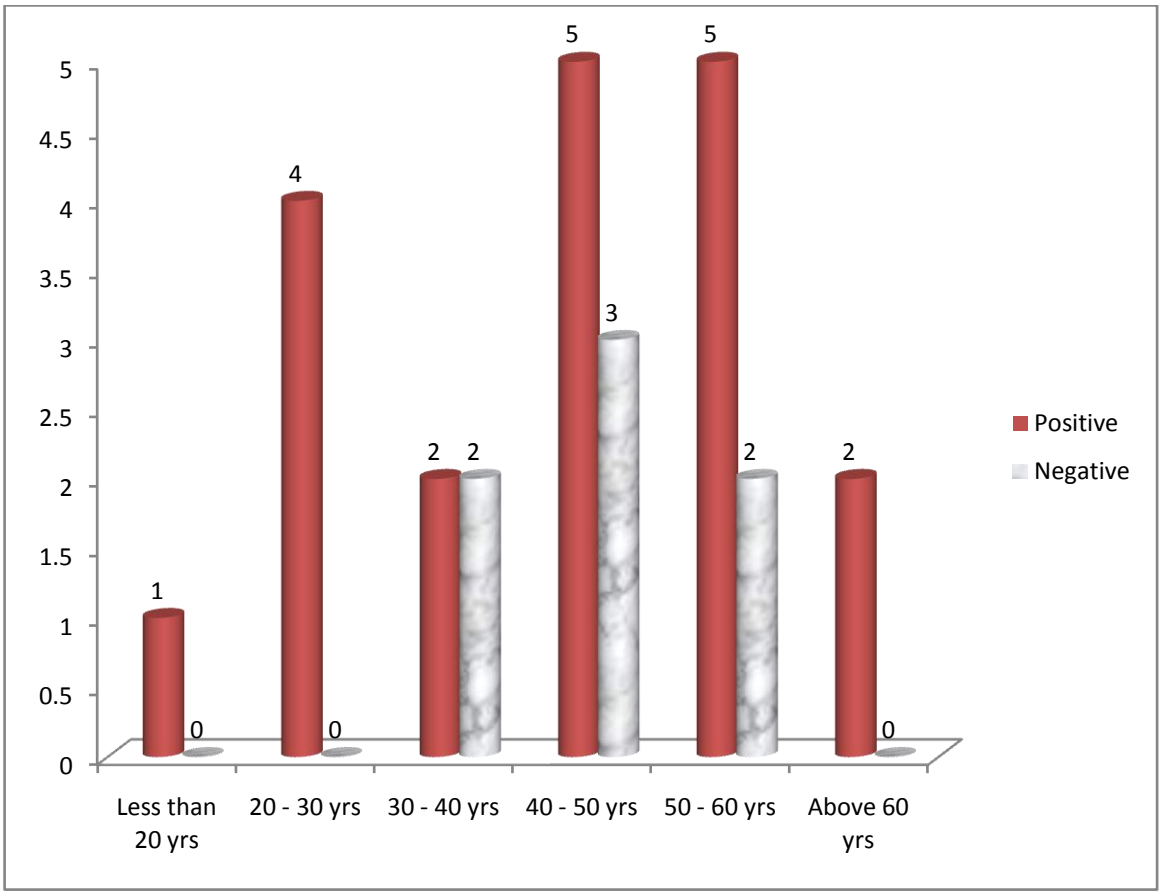



**Fig 13- DIF results of Scalp hair in various age groups**

**Hair DIF Results with respect to Gender**

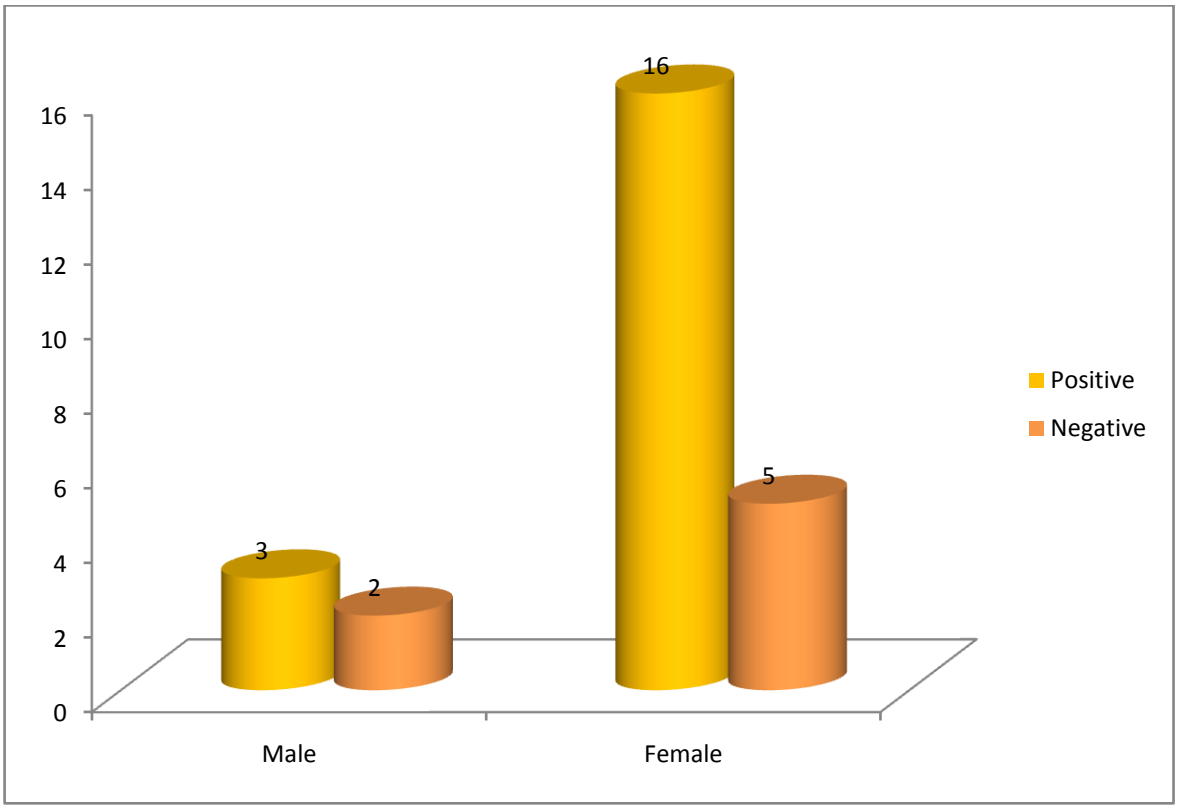



**Fig 14- DIF results of Scalp hair in male and female**

- Scalp Hair DIF positivity was seen in 16 out of 21 females and 3 out of 5 male patients.

## TABLE 8: FREQUENCY OF HAIR AND ORAL MUCOSA DIF

| Hair DIF | | Oral mucosa DIF | | Total | P value | Sensitivity | Specificity | Predictive value |
|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | |
| **Positive** | No. | 13 | 6 | 19 | 0.078 | 86.7 | 45.4 | Positive predictive value 68.4% |
| | % | 68.4% | 31.6% | 100.0% | | | | |
| **Negative** | No. | 2 | 5 | 7 | | | | Negative predictive value 71.4% |
| | % | 28.6% | 71.4% | 100.0% | | | | |
| **Total** | No. | 15 | 11 | 26 | | | | |
| | % | 57.7% | 42.3% | 100.0% | | | | |

- With a ‘p value ’ of 0.078 , there is no statistically significant difference between skin and hair DIF
- Hair DIF showed a positive predictive value of 68.4% and negative predictive value of 71.4%
- The sensitivity of Hair DIF was 86.7% while the specificity was 45.4%.

## TABLE 9: CORRELATIONS

| Correlations | | Oral mucosa DIF | Hair DIF |
|---|---|---|---|
| Oral mucosa DIF | Pearson Correlation | 1 | .358 |
| | Sig. (2-tailed) | | .073 |
| | N | 26 | 26 |
| Hair DIF | Pearson Correlation | .358 | 1 |
| | Sig. (2-tailed) | .073 | |
| | N | 26 | 26 |

- With the significant value of 0.073, there exists no correlation among Oral mucosa DIF and Hair DIF.

# DISCUSSION

Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies against desmogleins 3 and / or 1.[1]

In about 70–90% of the cases, the first sign of the disease appears on the oral mucosa. Lesions may occur anywhere on the oral mucosa, but the buccal mucosa is the most commonly affected site followed by involvement of the palatal, lingual and labial mucosa. [125]

The oral cavity may be the only site of involvement for a year or so, and this may lead to delayed diagnosis and inappropriate treatment. If oral pemphigus vulgaris can be recognized in its early stages, treatment may be initiated to prevent the progression of the disease to skin involvement. Diagnostic delays of greater than 6 months are common in patients with oral pemphigus vulgaris. [126]

The gold standard in the diagnosis of Pemphigus vulgaris is the demonstration of IgG and /or C3 by DIF in skin or oral mucosa and is also used to detect remission.

Among the other modalities of confirmation available like antidesmoglein ELISA titres and indirect Immunofluorescence, DIF is preferred as anti desmoglein ELISA titres are expensive and not available in all clinical centres.

Wilson et al. in 1991, demonstrated that the human hair follicle is rich in the target antigens of pemphigus.[98] As the outer root sheath (ORS) of the hair follicle is structurally analogous to epidermal keratinocytes, pemphigus antigens are distributed throughout the ORS and in the dermal bulb matrix cells.

Schaerer L, Trüeb RM in 2003, first reported the positive DIF findings in the Outer root sheath of plucked hair in 100% of their patients and hence, suggested that hair DIF could be a suitable and non-invasive alternative to skin DIF.[3]

Similarly a study of 50 patients with active pemphigus by Kumaresan, Rai R, et al in 2010, also demonstrated 100% positivity of hair DIF.[6]

Another study done by Daneshpazhooh et al in 2010 showed 81.8% of the cases showing Hair DIF positivity.[127]

Rao R et al in 2012, conducted a study to assess the role of hair DIF in monitoring the disease activity in pemphigus, they suggested that, in patients in clinical remission, DIF of hair could be an ideal substrate for assessment of immunological remission as it is simple and non- invasive.[128]

Another study done by Rai et al recommended that DIF of hair can be used as an additional procedure for the assessment of immunological remission in patients with pemphigus vulgaris.[129]

However, till date, there are only limited studies available, comparing the role of hair DIF in pemphigus patients with only mucosal lesions.

With this background we conducted a study in our department with 26 patients with Pemphigus Vulgaris, who had only oral mucosal lesions on presentation and no skin lesions. None of the patients had previous history of scalp involvement.

- Majority (30.8%) of the patients in our study were in the age group of 41-50 years, However, western studies have reported the common age of onset as 50-60 years.[22]

- Our study showed a female preponderance with male - female ratio of 1: 4.2, which was similar to the gender ratio seen in a study done by Kanwar et al in North India and Mascarenhas MF et al in Goa.[130] To our knowledge, there is only one study done by Daneshpazhooh et al done in Iran,which has compared DIF of oral mucosa to Scalp hair DIF.[5]

- In their study the hair sample was immediately frozen and sectioned while in our study the hair samples were directly mounted onto the slide for DIF without sectioning.

- The sensitivity of Hair DIF in our study was 86.7% which was similar to the sensitivity of Hair DIF in their study which was was 90.9%.

Our study showed a positive predictive value of 68.4%.The positive predictive value refers to the probability that the patients with positive results, truly have the disease.

In our study, among the 26 patients who had oral lesions, DIF positivity was seen in both the substrate in 13 patients (50%).

6 patients (23%) had Hair DIF positivity while oral mucosa being negative and this is of significance as it showed that hair DIF can be positive despite DIF findings not being demonstrable in the oral mucosa. This can be explained by the findings in other studies as well were the sensitivity of Oral DIF may not reach 100% and false negative results may occur.[5]

5 patients (19%) had DIF negativity of both substrates and this was important as it showed that the findings correlated in these patients as well.

Hence, The findings of hair and oral mucosa DIF correlated with each other in 18 patients.

The p value was tested at 5% level of significance and there was no statistically significant difference between oral mucosa and hair DIF with the ‘p value’ of 0.078.

Even though skin or mucosa is considered as the ideal substrate for DIF which is the gold standard for diagnosis of Pemphigus patients, we would like to highlight the fact that 6 patients in our study had positive hair DIF even though oral DIF was negative. Had the clinician relied only on the oral DIF finding, the diagnosis would not be confirmed and hence treatment delayed as well.

Pemphigus patients with oral lesions present with painful erosions and often secondary candidal infection as well, which causes pain and difficulty in even opening of the mouth. This poses a problem to the clinician as well as the patient to obtain the oral mucosa sample for DIF.

Replacing this method with a much easier and non-invasive technique of plucking scalp hair is advantageous to both clinician as well as the patient.

# CONCLUSION

Our study was done to assess the role of hair DIF in the diagnosis of Pemphigus in patients with only oral lesions. The sensitivity of Hair DIF was high enough and comparable with other studies done which recommend DIF of oral mucosa as the gold standard for diagnosis of Pemphigus.

The positivity of Hair DIF in the event of oral mucosa DIF being negative cannot be disregarded as well.

Hence we suggest that plucked scalp hair can be used as a substrate for DIF in cases with oral Pemphigus, which is a simple, non invasive & cost effective procedure.

## LIMITATIONS OF THE STUDY

Although the study has reached its aim, the unavoidable limitation was that the study was conducted on a small size of patients, because of the time limit.

Therefore we suggest that similar studies with much larger sample sizes are required before DIF of plucked hair can replace DIF of oral mucosa as a diagnostic tool.

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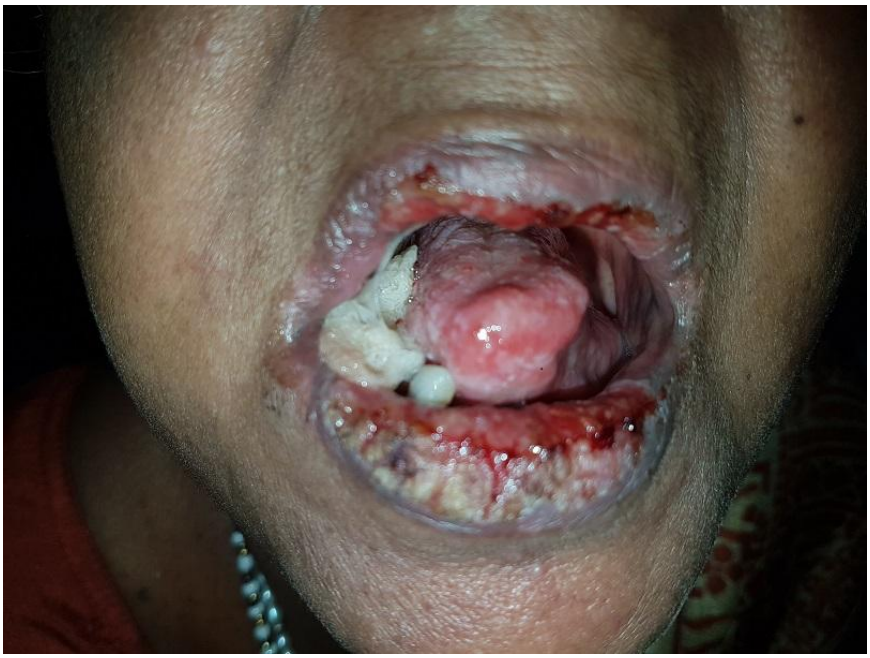
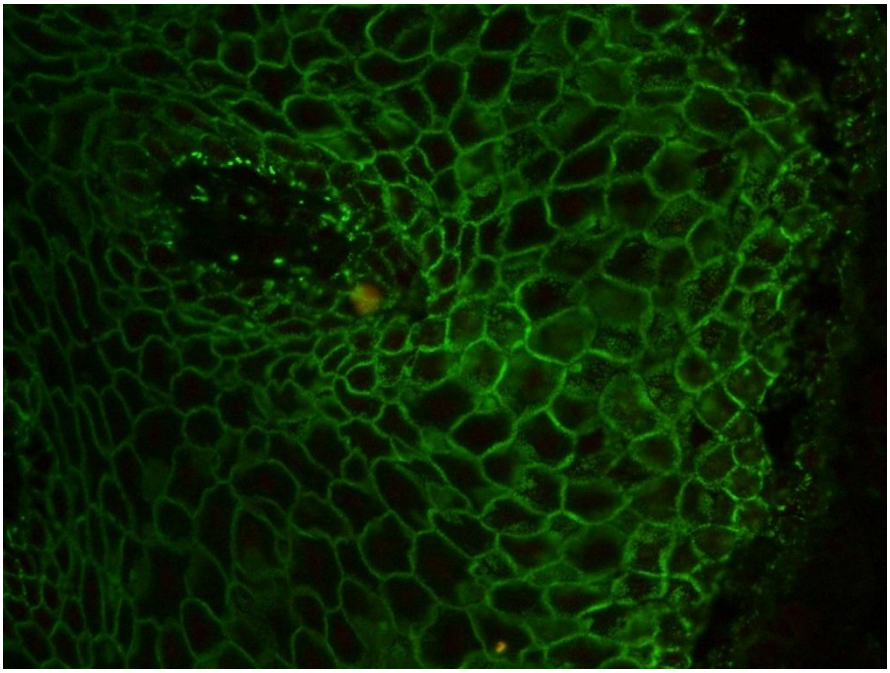
# CLINICAL PHOTOGRAPHS

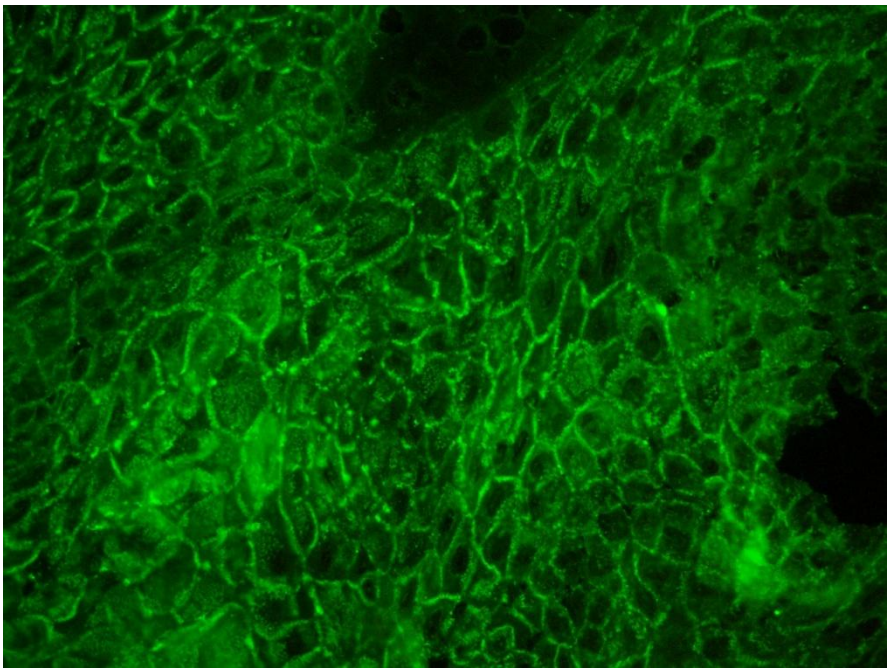


**PATIENT WITH ORAL EROSIONS FOR ORAL BIOPSY**

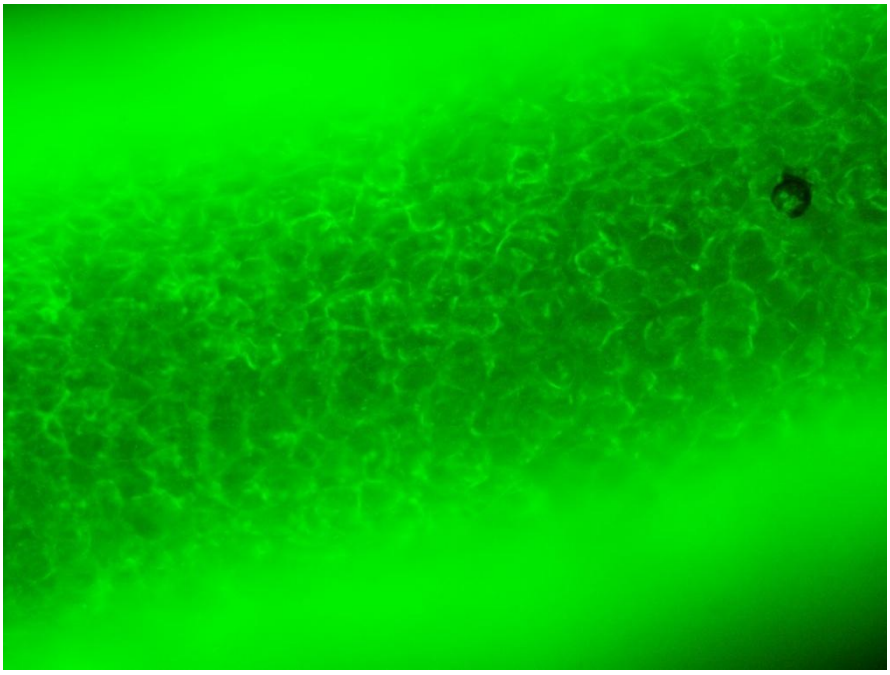
**PLUCKING OF SCALP HAIR**



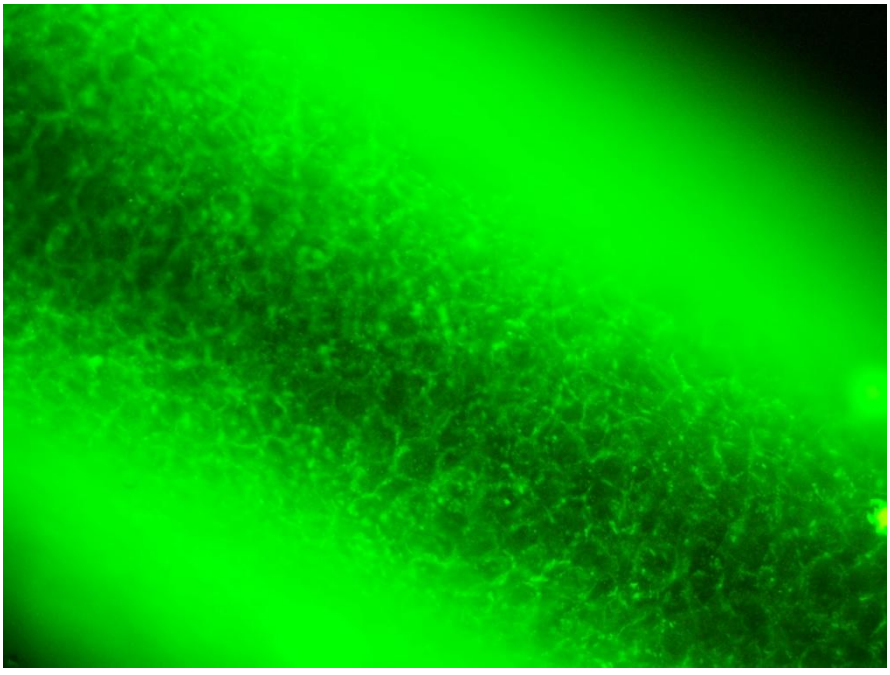
**POSITIVE DIF OF ORAL MUCOSA**



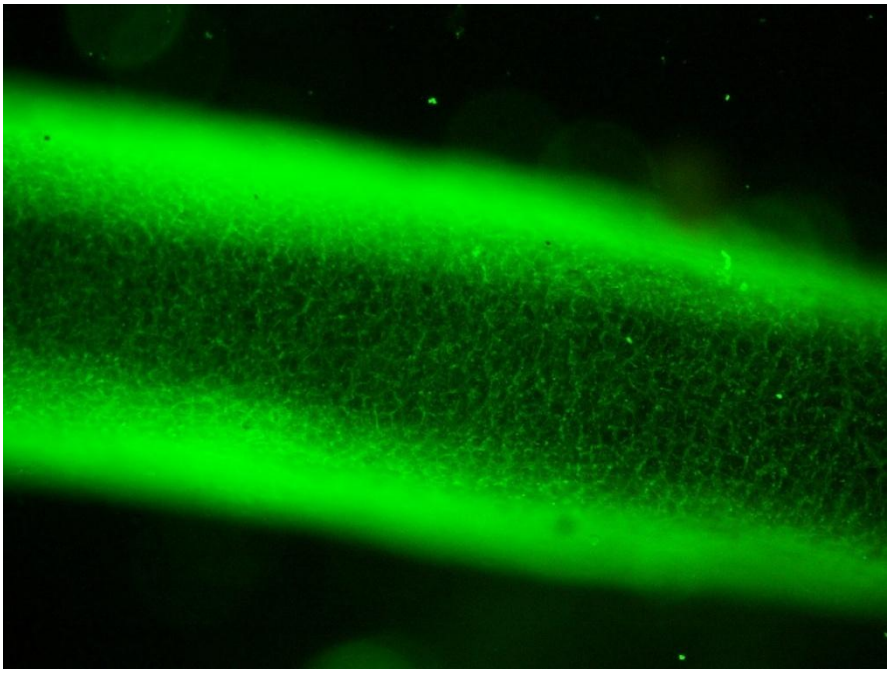
**ORAL MUCOSAL DIF SHOWING  STRONG POSITIVITY**



**ORS WITH POSITIVE DIF**



**STRONG DEPOSITS OF IgG IN HAIR FOLLICLE**



**POSITIVE DIF IN HAIR FOLLICLE OUTER ROOT SHEATH**

# PSG Institute of Medical Sciences & Research
## Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in


To
Dr Ryan Raju
Postgraduate
Department of Dermatology
**Guides:** Dr Reena Rai / Dr G Uma Maheswari
PSG IMS & R
Coimbatore

**Ref:** Project No.15/419

**Date:** December 30, 2015,

Dear Dr Ryan Raju,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 22.12.2015 to conduct the research study entitled *"Comparison of direct immunoflorescence of oral mucosa and plucked hair in patients with pemphigus"* during the IHEC review meeting held on 28.12.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Version 1 dated 22.12.2015)
3. Informed consent forms (Version 1 dated 22.12.2015)
4. Data collection tool (Version 1 dated 22.12.2015)
5. Permission letter from concerned Head of the Department
6. Current CVs of Principal investigator, Co-investigator
7. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 28.12.2015 at Research Conference Room, PSG IMS & R between 10.00 am and 12.30 pm:

| Sl. No. | Name of the Member of IHEC | Qualification | Area of Expertise | Gender | Affiliation to the Institution Yes/No | Present at the meeting Yes/No |
|---|---|---|---|---|---|---|
| 1 | Mrs Y Ashraf | MPT | Physiotherapy | Female | Yes | Yes |
| 2 | Dr. S. Bhuvaneshwari (Member-Secretary, IHEC) | MD | Clinical Pharmacology | Female | Yes | Yes |
| 3 | Mr Gowpathy Velappan | BA., BL | Legal Advisor | Male | No | No |
| 4 | Dr A Jayavardhana | MD | Clinician (Paediatrics) | Male | Yes | Yes |
| 5 | Mr P Karuppuchamy | M Phil in PSW | Social Scientist | Male | Yes | Yes |
| 6 | Mrs G Malarvizhi | M Sc | Nursing | Female | Yes | Yes |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | Mr. R. Nandakumar (Chairperson, IHEC) | BA., BL | Legal Expert | Male | No | Yes |
| 8 | Dr. Parag K Shah | DNB | Clinician (Ophthalmology) | Male | No | No |
| 9 | Dr. G. Rajendiran | DM | Clinician (Cardiology) | Male | Yes | Yes |
| 10 | Mrs P Rama | M Pharm | Non-Medical (Pharmacy) | Female | Yes | Yes |
| 11 | Dr. Seetha Panicker (Vice-chairperson, IHEC) | MD | Clinician (Obstetrics & Gynaecology) | Female | Yes | Yes |
| 12 | Dr R Senthil Kumar | MD | Clinician (Endocrinology) | Male | Yes | Yes |
| 13 | Dr. S. Shanthakumari | MD | Pathology, Ethicist | Female | Yes | Yes |
| 14 | Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC) | MD | Public Health, Epidemiology, Genetics, Ethicist | Female | Yes | Yes |
| 15 | Mrs. Swasthika Soundararaj | MBA | Lay person | Female | No | Yes |
| 16 | Dr. D. Vijaya | M Sc, Ph D | Basic Medical Sciences (Biochemistry) | Female | Yes | Yes |

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented

# PSG Institute of Medical Sciences & Research
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e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented

f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review

7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004

20.12.15

**Dr S Bhuvaneshwari**
**Member - Secretary**
**Institutional Human Ethics Committee**

# PROFOMA

Name: Gender:

Age: Occupation:

Address and telephone number:

Complaints with duration of illness:

Type of Pemphigus:

Sites of involvement:

History of scalp involvement:

Current treatment:

Duration of treatment:

**PSG Institute of Medical Science and Research, Coimbatore**
**Institutional Human Ethics Committee**
**INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS**

*(strike off items that are not applicable)*

I Dr. Ryan Raju, am carrying out a study on the topic: COMPARISON OF DIF OF ORAL MUCOSA AND PLUCKED HAIR IN PATIENTS WITH PEMPHIGUS.

as part of my research project being carried out under the aegis of the Department of Dermatology

My / our research guide is: Dr. Reena Rai

The justification for this study is: Obtaining a sample for Direct Immunofluorescence (oral) biopsy imposes an invasive and unpleasant procedure to the patient. Hence finding a less invasive technique for collecting a suitable substrate would be of much help.

**The objectives of this study are:**

Primary Objective: Comparison of DIF of oral mucosa and plucked hair in patients with pemphigus.

**Sample size**: 20 patients.

**Study volunteers / participants** are (specify population group & age group): above 18 years of age.

**Location**:Patients presenting to the dermatology OPD at PSGIMS & R , Coimbatore.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

**Initial interview** (specify approximate duration) 15 minutes.

Data collected will be stored for a period of 10 years. We will / will not use the data as part of another study.

**Benefits** from this study: Based on the results of this study , to perfom only hair DIF & to avoid invasive oral biopsy procedure in patients with pemphigus.

**Risks** involved by participating in this study: Infection, bleeding, scarring or pigmentation at the site of biopsy Risk of allergic reaction to the local anaesthetic.

How the **results** will be used: Based on these results DIF of hair alone can be done as it is non invasive and simple procedure. And oral mucosa biopsy which is an invasive procedure can be avoided.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

**Consent:** The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI: 9995051845

Contact number of Ethics Committee Office: During Office hours: 0422 2570170 Extn.: 5818
After Office hours: 9865561463

**ஒப்பதல் படிவம்**

தேதி

**ரயன் ராஜ்** ஆகிய நான் PSG மருத்துவக்கல்லூரியின் **தோல் பால்வினை மற்றும் தொழுநோய்** துறையின் கீழ் **"எதிர் அணுவினால் உண்டாகக்கூடிய கொப்பள நோயின் செயல்பாடு தன்மையை கண்டறிய, டி.ஐ.எப் என்னும் மருத்துவ பரிசோதனையில் தலைமுடி மற்றும் வாய் ஐவ்வில் இருந்து பரிசோதிப்பதில் எது சிறந்தது என்பதை கண்டறியும் ஆய்வு "** என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்

**என் ஆய்வு வழிகாட்டி** : மரு. ரீனாராய்

**ஆய்வு மேற்கொள்வதற்கான அடிப்படை**

தோலில் கொப்பள நோய் உண்டாவதற்கு நமது உடலில் உள்ள எதிர்ப்பு சக்தி தோலில் இயற்கையாக உள்ள பசை போன்ற ஒரு பொருளுக்கு எதிர் அணுவை பல்வேறு காரணங்களால் உருவாக்குகிறது. இந்த பசை போன்ற பொருளில் செயல்திறன் குறையும் பொழுது ஒரு செல் மற்றொரு செல்லிலிருந்து பிரிகிறது. இதுவே கொப்பளங்களாக வெளிப்படுகிறது. இந்த எதிர் அணுக்களை குறைப்பதற்கு மருந்துகள் வழங்கப்படும். ஆனால் என்று மருந்தை நிறுத்தவேண்டும் என்பதை தீர்மானிக்க தோல் மற்றும் முடியில் உள்ள வேர்களில் எதிர்ப்பு அணு உள்ளதா என்பதை பரிசோதிக்க 6 மாதங்களுக்கு ஒரு முறை தோல் மற்றும் முடியை டி.ஐ. எப். என்ற பரிசோதனையை மேற் கொள்ள வேண்டும். இதனை வைத்து நோயின் செயல்பாடு தன்மையை கண்டறிய இயலும்.

**ஆய்வின் நோக்கம்**

மேற்கூறிய டி.ஐ.எப் என்னும் மருத்துவ பரிசோதனையில்_தலைமுடி மற்றும் வாய் ஐவ்வில் இருந்து பரிசோதிப்பதில் எது சிறந்தது என்பதை கண்டறியும் ஆய்வு.

**ஆய்வில் பங்கு பெறும் நபர்களின் எண்ணிக்கை** : 20 நபர்கள்

**ஆய்வில் பங்கு பெறுவோர் மற்றும் வயது** : 18 வயதிற்கு மேல் உள்ள ஆண்களும் பெண்களும்.

**ஆய்வு மேற்கொள்ளும் இடம்** : பி.எஸ்.ஜி மருத்துவமனை,
தோல் பால்வினை மற்றும் தொழுநோய் துறை

நீங்கள் தயவு செய்து எங்களின் ஆய்வுக்கு ஒத்துழைக்க வேண்டும் என்று பணிவுடன் கேட்டுக் கொள்கிறேன். நாங்கள் சில தகவல்களை இந்த ஆய்விற்காக சேகரிக்க உள்ளோம்.

**ஆய்வு செய்யப்படும் முறை**

**முதன்மை நோ்காணல்** : 15 நிமிடங்கள்

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 10 வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்கு பயன்படுத்தப்படமாட்டாது.

**ஆய்வில் பங்கு பெறுவதால் ஏற்படும் பலன்கள்** :

பொதுவாக DIF பரிசோதனைக்கு சதை எடுப்பது நோயாளிக்கு அச்சுறுத்தலை ஏற்படுத்தும். ஆகையால் இந்த ஆய்வின் மூலம் முடியை வைத்து மிக எளிமையான முறையில் பரிசோதனை செய்யபடும்.

**ஆய்வினால் ஏற்படும் அசௌகரியங்கள் / பக்க விளைவுகள்** :

நோயாளிகளுக்கு சதை எடுக்கும்பொழுது சிலருக்கு உதிரப்போக்கு ஏற்படலாம். அதுவும் ஒரு தையல் மூலம் (தேவைப்பட்டால்) சரி செய்யலாம். கிருமிகள் தொற்றும் அபாயம் மிகவும் குறைந்த அளவில் உள்ளது. எங்களது கிருமி நாசி செயல் மூலம் இதுவும் தவிர்க்கப்படும். சதை எடுத்த பகுதியில் சிலருக்கு காயம் ஏற்படலாம். சிலருக்கு மரத்துப்போகும் மருந்தினால் அலர்ஜி ஏற்படலாம்.

**ஆய்வின் முடிவுகள் எந்த முறையில் பயன்படுத்தப்படும்**

எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது. இவை இரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக் கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்கு கிடைக்காது.

எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை உங்களுக்கு உண்டு.

ஆய்விலிருந்து விலகிக் கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கப்படும் / சில இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுக்கப்படும்.

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை.

நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

**ஆய்வுக்குட்படுபவரின் ஒப்புதல்**

இந்த ஆய்வைப் பற்றிய மேற்கூறிய தகவல்களை நான் படித்து அறிந்து கொண்டேன் / ஆய்வாளர் படிக்க கேட்டுக் கேட்டுத் தெரிந்து கொண்டேன். ஆய்வினைப் பற்றி நன்றாகப் புரிந்து கொண்டு இந்த ஆய்வில் பங்கு பெற ஒப்புக் கொள்கிறேன். இந்த ஆய்வில் பங்கேற்பதற்கான எனது ஒப்புதலை கீழே கையொப்பமிட்டு / கை ரேகை பதித்து நான் தெரிவித்துக் கொள்கிறேன்.

பங்குபெறுபவரின் பெயர், முகவரி :

பங்குபெறுபவரின் கையொப்பம் / கைரேகை / சட்டபூர்வ பிரதிநிதியின் கையொப்பம் :

தேதி :

ஆய்வாளரின் கையொப்பம் :

தேதி :

மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண். 0422-2570170 Extn. 5818

## ABBREVIATIONS

| | | |
|---|---|---|
| PV | - | Pemphigus Vulgaris |
| PF | - | Pemphigus Foliaceous |
| Dsg | - | Desmogleins |
| ORS | - | Outer Root Sheath |
| IRS | - | Inner root sheath |
| DIF | - | Direct Immunofluorescence |
| IIF | - | Indirect Immunofluorescence |
| IgG | - | Immunoglobulin G |
| FITC | - | Fluorescent isothiocyanate |
| CS | - | Corticosteroids |
| DCP | - | Dexamethasone Cyclophosphamide pulse therapy |
| TPMT | - | Thio-purine Methytransferase |
| DVT | - | Deep vein thrombosis |
| BSA | - | Body surface area |
| H&E | - | Hematoxylin and Eosin |
| IVIg | - | Intravenous immunoglobulin |
| SLE | - | Systemic Lupus Erythematosus |
| EBV | - | Ebstein Barr Virus |
| HHV | - | Human Herpes Virus |
| ELISA | - | Enzyme-Linked Immunosorbent Assay |
| HIV | - | Human Immunodeficiency Virus |