A CLINICOPATHOLOGICAL STUDY OF AUTOIMMUNE VESICULOBULLOUS DISEASES

Dissertation submitted

in partial fulfillment for the Degree of

DOCTOR OF MEDICINE

BRANCH – XII A

M.D., (DERMATO VENEROLOGY)

MARCH 2007

DEPARTMENT OF DERMATOLOGY

MADURAI MEDICAL COLLEGE

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – TAMILNADU

CERTIFICATE

This is to certify that this dissertation entitled **"A CLINICOPATHOLOGICAL STUDY OF AUTOIMMUNE VESICULOBULLOUS DISEASES"** submitted by **Dr.M. Subramania Adityan** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai is in partial fulfillment of the requirement for the award of M.D. Degree **Branch XII A, M.D., (Dermato Venerology)** and is a bonafide research work carried out by him under direct supervision and guidance.

Dr. S. Krishnan, M.D., D.D
Additional Professor,
Department of Dermatology,
Govt. Rajaji Hospital,
Madurai Medical College,
Madurai.

Dr. H.Syed Maroof Saheb, M.D., D.D
Professor and Head,
Department of Dermatology,
Govt. Rajaji Hospital,
Madurai Medical College,
Madurai.

ACKNOWLEDGEMENT

Gratitude cannot be expressed through words. True, but unexpressed gratefulness weighs heavily on one's heart. I may be permitted here to record valuable guidance, help, co-operation and encouragement from my teachers, colleagues and various other persons directly or indirectly involved in the preparation of this dissertation.

First of all I would like to acknowledge my thanks and sincere gratitude to our beloved Prof. **Dr.H.Syed Maroof Saheb,** Professor and Head of the department of dermatology, GRH & MMC, Madurai for his valuable advice and encouragement.

I profoundly thank **Dr.S.Krishnan,** Addl. Professor, dept. of dermatology, MMC & GRH, for his valuable guidance.

I express my deep sense of gratitude and thanks to my teachers **Dr.A.S.Krishnaram, Dr.G.Geetharani,** and **Dr.A.K.P.Vijayakumar** Assistant Professors, for their valuable guidance, timely advice, constant encouragement and easy approachability in the preparation of this dissertation. Their profound knowledge in dermatology gave me immense confidence throughout the study.

I would also like to acknowledge my thanks to my teachers **Dr.S.Ragunath** and **Dr.K.Senthilkumar,** Assistant Professors, Department of Dermatology, GRH & MMC, Madurai, for their constant support and encouragement.

I would like to express my thanks to Prof. **Dr.Gomathinayagam,** Professor and head of the department of pathology, MMC, Madurai.

I owe thanks to the Dean, Principal and Medical Superintendent for allowing me to conduct this study.

I owe thanks to my fellow postgraduate colleagues for their constant help and constructive criticism.

I am thankful to the technicians of the pathology department. I also would like to thank the staff nurses for their help in biopsy procedures.

I owe a lot to my family, which has always stood by me my career.

I owe a lot to all my patients without whom this study would not have taken off, and the authors, who have worked on this subject, from whose wisdom and experience. I have been benefited immensely.

CONTENTS

INTRODUCTION

Vesicles and bullae are reaction patterns of the skin to injury, and thus can be caused by a wide variety of conditions. Many skin diseases present with blisters, but only in some of them does blistering occur as a primary event. This group of disorders has been traditionally termed ‘the vesiculobullous disorders’. Most primary vesiculobullous diseases are either genetic or immunologic. The latter group, also called the ‘autoimmune vesiculobullous diseases’ is the focus of this study.

The autoimmune vesiculobullous diseases are a heterogeneous group of diseases. They are classified on the basis of their clinical, histopathological, and immunopathological features. Thus, they can be broadly classified histopathologically into epidermal and subepidermal blistering dermatoses. These can be further categorized, based on their immunopathological features, into several individual diseases.

Though these disorders are rare in the general population, for a given patient, the impact of the diseases on the quality of life can be devastating. The severity is often variable and the course is unpredictable, and may even be fatal. Moreover, some auto immune vesiculobullous diseases may be markers for internal malignancy.

Since the days of Walter lever who differentiated pemphigus from pemphigiod, dramatic progress has been made in the understanding of these diseases, with the advent of investigative techniques such as electron microscopy, immunoprecipitation, immunoblotting and molecular genetic analysis. In the therapeutic front, dexamethasone cyclophosphamide pulse therapy has improved the prognosis, and emerging therapies like biologicals hold much promise for the future.

But despite the explosion of knowledge regarding the etiopathogenesis, the exact mechanism that triggers autoimmunity in these patients remains largely unclear.

HISTORICAL ASPECTS

Historical aspects of diseases

1884 - Louis Duhring described Dermatitis Herpetiformis.

1894 - Piotr Vasiliyevich Nikolsky described Nikolsky sign

1943 - Acantholysis first demonstrated as a characteristic feature of pemphigus bullae[1].

1947 - Cytology was introduced in skin disorders by Tzanck.

1949 - Civatte separated cicatricial pemphigoid from pemphigus.

1953 - Walter lever distinguished Pemphigus from pemphigoid[2].

1964 - Beutner and Jordan discovered circulating antibodies against cell surface of keratinocytes from the sera of pemphigus patients[3].

1967 - Beutner et al demonstrated anti basement membrane zone antibodies in bullous pemphigoid[6].

1973 - Roenigk et al proposed the clinical criteria for EBA.

1975 - Chorzelski and Jablonska recognised LABD as a separate entity based on immuno pathologic findings.

1979 - Pemphigoid vegetans was first reported by Winkelmann R.K. and Su. W.P.

1980 - Nieboer et al separated EBA from BP by immunoelection microscopy[7].

1982 - Stanley et al characterized the target antigens of pemphigus by immunoprecipitation and immunoblotting[4].

1990 - Paraneoplastic pemphigus was first recognised as a distinct entity by Anhalt et al.

1991 - Amagai et al demonstrated that pemphigus is an anti-cadherin autoimmune disease by isolation of the cDNA of pemphigus antigens[5].

Epidemiology

The epidemiological aspects of autoimmune vesiculobullous diseases are covered in the table below.

Disease	Incidence	Geographic pattern	Age	M:F	HLA association
Pemphigus vulgaris & vegetans	About 1.3 per million per year	Higher in Jews and people of Mediterranean origin	Middle age	M=F	DR4 (subtype) DQ1, Subtype DQB1, 0503 DRB1(0402)
Pemphigus foliaceus	.3 cases per million per year	Higher in Brazil, Finland and Tunisia	Middle age	M=F	
Fogo selvagum	3.4% on endemic areas of Brazil[8]	Endemic around the rivers of Brazil	Children and young adults		DR1
Bullous pemphigoid	7 per million[9] per year	Worldwide	Old age (few infants & children)	M=F	DQB1*0301 DQ7 (males)
Cicatricial pemphigoid	1 per million per year	No geographic predilection	Middle old age	F:M=2:1	DQB1*0301
Pemphigoid Gestationis	1:1700[10] 1:10,000[11] deliveries	-	-	Pregnant woman	DRB1*0301, DRB1*0401, C4 null allele in 90%
Linear IgA Disease 1. Childhood 2. Adults	About 1 in 2.5 million per year[12] ”	Childhood variant more common in developing countries	<5 yrs >40 yrs	Slight female preponderance ”	HLS –B8, TNF2 allele (76%) HLA-B8 (some) TNF-2 allele

Disease	Incidence	Geographic pattern	Age	M:F	HLA association
EB acquisita	0.25 per million per year[13]	More common among Asians and African – Americans	Adults (few children)		HLA –DR2
Bullous SLE	0.2 per million[14]	More common or African Hispanic descent	Young adults	F>M	HLA DR2
Dermatitis herpetiformis	1.3 per[15] million per year in Finland	Common in North-European descents. Rare in far east	Middle age	M:F =1.5:1	DQ1, DQB8, DQW2, DQ(α1*501) DQ(α1*03)

In an Indian study by Arya et al[58], pemphigus vulgaris was the commonest vesiculobullous disease, comprising 61.4% of the cases studied,

followed by pemphigus foliaceus.

In the study by K.K.Das et al[57], pemphigus vulgaris was the commonest 40.8%, followed by dermatitis herpetiformis 36.2%, bullous pemphigoid 16%, CBDC 5.3% and Hailey-Hailey disease 1.4%.

Etiopathogenesis

The etiopathogenisis of various autoimmune vesiculobullous diseases are summarised in the table given below:

Disease	**Antibody isotype**	**Target antigen**	**Antigen KDa**	**Epitope**	**Location**
Pemphigus vulgaris/ vegetans	IgG (few IgM, IgA)	Desmoglein3 Desmoglein1	130KDa 160KDa	Amino terminal of extracellular domain	Desmosome
Pemphigus foliaceus	IgG	Desmoglein1	160KDa	”	”
IgA pemphigus	IgA1	Desmocollin1		-	Desmosome
Fogo selvagum	IgG	Demoglein1	160KDa	Aminoterminal of extracellular domain	Desmosome
Bullous pemphigord	IgG (few IgA)	BPAG1 BPAG2	230KDa 180KDa	α-helix terminal regions Non collagenous region just	Hemidesmosome

				outside the membrane	
Cicatrical pemphigoid	IgG, IgA	BPAG1 BPAG2 Laminin 5 β4 integrin	20KDa 180KDa 600KDa 205KDa	? Distal extra cellular domain α-submit -	Hemidesmosome anchoring filament ” ”
Pemphigoid vegetan	IgG_4	BPAG1	230 KDa	-	Hemidesmosome
Pemphigoid gestationis	IgG	BPAG1 BPAG2	230KDa 180KDa	? Transmembrane	Hemidesmosome
Linear IgA dermatosis (both childhood & adult)	IgA		97KDa 120KDa 285KDa	Soluble ectodomain of BPAG2 ” ?	Anchoring filament ” ?
EB acquisita	IgG	Type VII collagen	290KDa	Fibronectin like region of NC1 domain	Anchoring fibril
Bullous SLE	IgG, IgA	”	”	” (some)	Anchoring fibril
Dermatitis herpetiformis	IgA	Unknown	-	-	Upper dermis

In a recent Indian study by Bhushan Kumar et al[64], it has been reported

that the titre of antibodies against desmoglein-1 correlated with the severity of the skin disease, and anti desmoglein-3 antibody titre correlated well with oral lesions of pemphigus vulagris. However, anti demoglein 3 antibodies were found in detectable levels in all patients with pemphigus foliaceus, apart from anti desmoglein-1 antibody.

Clinical features

The clinical features of the various autoimmune vesiculobullous diseases are summarised in the tabular column given below:

Disease	**Cutaneous distribution**	**Mucosal involvement**	**Pattern of skin lesions**	**Scarring**	**Disease association**
1. Pemphigus vulgaris	Scalp, face flexures may be generalised	Common orpharynx, conjunctiva genitalia	Flaccid blisters, erosions show no tendency to heal, nikolsky sign & bulla spread sign +ve, flexural vegetations	-	Other auto immune disease, thymoma. Rarely BP[45], may evolve into P.foliaceus & vice vasa
2. Pemphigus vegetans	Flexural	Oral	Vesicles, pustules, erosions, vegetating plaques	-	-

3. Pemphigus foliaceus	Scalp, face, chest, upper back (seborrheic) may be generalised	-	Scaly papules, crusted erosions, erythroderma	-	-
4. Epidemic pemphigus foliaceus	Head, neck, generalised	Uncommon	Flaccid blisters, erosions, verrucous lesions, erythroderma	-	-
5. Bullous pemphigoid	Trunk, limbs flexures	Common, minor	Urticated plaques, tense blisters, milia	-	-
6. Cicatrical pemphigoid	Infrequent (30%)	Major, severe desquamative gingivitis conjunctivitis scarring, symble pheron	Tense blisters, erosions milia	+++	Autoimmune diseases
7.Pemphigoid gestationis	Umbilicus, generalised	Minor	Urticated plaques, tense blisters	-	Autoimmune thyroid disease
8. Linear IgA dermatosis 1. CDBC 2. Adult LAD	Perineum, face trunk, limbs Trunk, limbs	Majority (few severe) ” Majority (few severe)	Urticated plaques, annular lesions, Urticated plaques, tense blisters milia	 + +	Ulcerative colitis lymphoma

9. EB acquisita	Variable generalised	Some (few severe)		++	Inflammatory bowel disease
10. Bullous SLE	Variable, generalised	Minor	Urticated plaques tense blisters	-	SLE
11. Dermatitis herpetiformis	Elbows, knees, buttocks symmetrical	Minor	Papulovescicles excoriations	-	gluten sensitive exteropathy, lymphoma

CYTODIAGNOSIS OF AUTOIMMUNE VESICULOBULLOUS DISORDERS

Tzanck smear is a rapid preliminary test used in the diagnosis of blistering diseases. A smear is made from the floor of a freshly opened vesicle. It is allowed to dry and flooded with equal quantity of water and Giemsa or Leishman's stain. After 30-40 seconds, the slide is rinsed, air-dried, and examined for acantholytic cells.

Acantholytic cells

An acantholytic cell is rounded, with an enlarged nucleus with peripheral condensation of chromatin and prominent nucleoli. There is a perinuclear halo, with the peripheral parts of the cell staining more darkly. In older cells the nucleus may be pyknotic.

In pemphigus vulgaris and vegetans, typical, rounded acantholytic cells

are seen. In pemphigus foliaceus and pemphigus erythematosus, cells tend to be cuboidal, with a small nucleus and more prominent cytoplasm. Keratohyaline granules and evidence of keratinisation may be seen. Occasional multinucleated cells may be seen[69].

Eosinophils are commonly seen in bullous pemphigoid, but may be seen in dermatitis herpetiformis also.

Histopathology in autoimmune vesiculobullous diseases

1) Pemphigus vulgaris

A small, early vesicle is preferable. Scalpel biopsy of the intact blister should be done. If punch biopsy is to be done, the lesion should be frozen by a refrigerant spray. If no new blister is seen, an old one may be moved to the neighbouring skin by finger. The new cleavage reveals early specific changes.

Early lesions show eosinophilic spongiosis in the lower epidermis. This is a manifestation of acantholysis, rather than true spongiosis. Developed lesions show clefts and suprabasal blisters. Acantholysis extends to the adnexal structures.

The basal keratinocytes are detached from each other, but remain attached to the basement membrane, because the hemidesmosomes are intact. This gives an appearance of ‘row of tombstones’.

As the blister ages, a mixed inflammatory infiltrate appears in the dermis. There may be epidermal downgrowth or villi.

In patients with only oral erosions, DIF from the perilesional oral mucosa is more sensitive than lesional biopsies for HPE.

2) Pemphigus Vegetans

a) Neumann type : Early lesions have the same histopathology as pemphigus vulgaris. Later, there is formation of villi and verrucous epidermal hyperplasia. Eosinophilic spongiosis and eosinophilic pustules are present. Acantholysis may be absent in older lesions.

b) Hallopeau type : Early lesions have suprabasal clefts with plenty of eosinophils. There are more eosinophilic abscesses than Neumann type[20].

3) Pemphigus foliaceus

There is acantholysis in the granular layer, leading to subcorneal cleft and detachment of str.corneum. The number of acantholytic cells is small. There may be eosinophilic spongiosis. Dyskeratotic granular keratinocytes are diagnostic.

4) Pemphigus erythematosus

Histology similar to pemphigus foliaceus. Interface dermatitis may be

seen in rare cases[21].

5) Ig A pemphigus

a) SPD type : There are subcorneal vesiculopustules with minimal acantholysis

b) IEN type : Has intraepidermal vesiculo pustules with neutrophils

6) Paraneoplastic pemphigus

Variable. EMF-like, LP-like, pemphigus – like and Bullous pemphigoid- like features may be seen. PNP may present with lichenoid interface dermatitis without acantholysis[22].

7) Bullous pemphigoid

The blister arises at the dermo epidermal junction[23]. Two patterns are seen.

Blisters arising on as erythematous base show a cell-rich pattern. Eosinophilic papillary abscesses may develop, along with lymphocytes and neutrophils in superficial and deep demis. Eosinophilic spongiosis may occur[24].

Blisters on a clinically normal skin show cell poor pattern, with a scant perivascular lymphocytic infiltrate, with some eosinophils[24].

8) Pemphigoid gestationis

There is eosinophilic spongiosis, papillary dermal edema and a perivascular infiltrate of lymphocytes and eosinophils[25]. Focal necrosis of basal keratinocytes leads to subepidermal blister[26].

9) Cicatricial pemphigoid

The subepidermal bulla extends down the adnexa. Neutrophils, lymphocytes and histiocytes predominate in the infiltrate. Eosinophils may be present. Lamellar fibrosis beneath the epidermis is the hallmark.

10) Linear IgA dermatosis

Features are similar to dermatitis herpetiformis. A subepidermal blister with neutrophils and fibrin in the lumen is seen. Early lesions show neutrophils concentrated in the dermal papillae and lined along the dermoepidermal junction.

11) Epidermolysis bullosa acquisita

In the commonest type (bullous pemphigoid-like), sub epidermal blisters with lymphocytes and neutrophils are seen. Eosinophils are variable. In the classical form, non-inflammatory subepidermal blisters with fibrosis and milia

formation are seen.

12) Bullous SLE

Three patterns may be seen

a) Most common is the DH-like pattern

b) Second pattern (25%) is subepidermal blister with a neutrophil – rich leukocytoclastic vasculitis[27]

c) Third pattern is severe basilar vacuolation with subsequent blister formation.

13) Dermatitis herpetiformis

Typical features are seen in perilesional erythematous skin. Neutrophils accumulate in the tips of the dermal papillae[28]. Early blisters are multilocular, which later become unilocular. Apoptotic keratinocytes may be seen above the miroabscesses.

DIRECT IMMUNOFLUORESCENCE IN VESICULOBULLOUS DERMATOSES

Procedures

- A 3-4mm punch biopsy from the inflamed, but unblistered perilesional skin is preferred.

- If there is a delay of more than 24 hrs before processing, the specimen should be kept in Michel's medium, which contains.

  5% Ammonium sulphate

  Magnesium sulphate

  N – ethyl maleimide (K^+ inhibitor)

  Citrate buffer (pH 7.25)

- Specimens may be kept in Michel's medium for 2 weeks at room temperature and for several weeks in refrigerator.

- While processing, the specimen is washed, embedded in OCT (optimized cutting temperature) compound, and snap frozen. 6μ sections are made, and incubated with antihuman IgG, IgM, IgA and C3, which are tagged with fluorescein isothiocyanate. Sections are visualised in fluorescent microscope[29].

Salt Split Skin

- The human skin is split at the level of lamina lucida when incubated for 48-72 hrs 1M Nacl. If it is used as substrate DIF and IIF studies become more sensitive[30] and differentiation of subepidermal bullous diseases is possible.

DIF Patterns

1) Pemphigus Vulgaris

- Squamous intercellular IgG in upto 100%[31], in a chicken wire pattern
- DIF remains positive for many years after the clinical disease has subsided[32].
- False positive tests may be seen in spongiotic dermatitis, psoriasis and insulation of serum.

2) Pemphigus vegetans

Squamous intercellular IgG present in all reported cases[33] .

3) Pemphigus foliaceus

Two patterns have been described commonly, full thickness squamous inter cellular IgG is seen. Rarely IgG may be localised to the upper layers[34].

4) Pemphigus erythematosus

Squamous intercellular IgG seen in >75% of cases, along with deposits of IgM and IgG (positive lupis band) in the DEJ.

5) Ig A Pemphigus

Reveals squamous intercelleular IgA throughout the epidermis. Complement and other Igs are usually absent[35]. Some cases show both IgA and IgG[36].

6) Paraneoplastic Pemphigus

Squamous intercellular IgG is seen along with granular deposits of C_3 in the dermoepidermal junction[37]. Linear deposits of C3, IgG, IgM or granular deposits of IgG and C3 may also be seen along the BMZ[38].

7) Bullous pemphigoid

Linear BMZ deposits of C3 seen in upto 100% of cases, IgG is seen in 65-95% of cases[39]. IgA and IgM are seen in 25% of cases.

8) Pemphigoid gestationis

Linear BMZ deposits of C3 is seen in 100% of cases[40]. IgG is seen in 30-40% of cases.

9) Cicatricial pemphigoid

Linear BMZ IgG and C3 are seen in 80% of cases. Mucous membrane is the preferred site for DIF studies[41]. Salt splitting increases the sensitivity [42].

10) Linear IgA dermatosis

Linear BMZ IgA, deposits seen in 100% of cases[43]. In the lamina lucida type, IgA is seen on the epidermal side of the salt split skin. In the sublamina dense type, IgA, localizes to the dermal side. This type has now been classified as IgA mediated EBA.

11) Epidermolysis bullosa acquisita

Linear BMZ deposits of C3, IgG and less commonly IgM and IgA are seen. Increasing number of immunoreactants along the BMZ favours a diagnosis of EBA over bullous pemphigoid. In salt split skin, IgG is seen on the floor of the split[44].

12) Bullous SLE

IgG and C3 are seen in 100% of cases in the BMZ. In 50% of cases the pattern is linear and in 25% it is granular. IgM and IgA are seen in 50% and 60% of cases, respectively.

13) Dermatitis herpetiformis

Granular deposits of IgA can be seen within the dermal papillae[45]. False negative results may occur if inflamed lesions are biopsied[46]. DIF results are not altered by dapsone therapy.

INDIRECT IMMUNOFLUORESCENCE

IIF is a semiquantitative procedure in which double immunolabelling is done to evaluate the presence and titre of circulating antibodies, or to specifically localise an antigen in the skin.

Procedure

The serum is serially diluted. The substrates most commonly used are

a. Monkey esophagus – pemphigus vulgaris[46]
b. Guinea pig esophagus – pemphigus foliaceus[47]
c. Human salt split skin – subepidermal blistering diseases
d. Murine bladder epithelium – paraneoplastic pemphigus

The serially diluted serum is incubated with the substrate for 30 mins at room temperature and washed. Antibodies bound to the substrate are detected by incubation with FITC-labelled goat antihuman IgA or IgG.

IIF PATTERNS

1. Pemphigus vulgaris

>80% have circulating anti cell surface IgG[48]. There is a positive, but imperfect correlation between the antibody titre and disease activity[49] in pemphigus vulgaris and pemphigus foliaceus.

2. Pemphigus foliaceus

Squamous intercellular IgG deposits seen in 80-90% of cases.

3. Pemphigus erythematosus

Using monkey esophagus, IIF reveals ICS deposits of IgG in 80% of cases ANA is positive in 30-80% of cases.

4. IgA pemphigus

Positive in less than 50%[50] of cases.

5. Paraneoplastic pemphigus

Circulating antibodies that bind to rat bladder epithelium is seen in all cases. Immunoblotting and immunoprecipitation are more sensitive and specific, and at a minimum, antibodies to envoplakin and periplakin should be demonstrated[51].

6. Bullous pemphigoid

Positive in 70-80% of cases. Titres have poor correlation with disease activity. In salt split skin, antibodies bind to roof alone (80%) or roof and floor (20%).

7. Pemphigoid gestationis

Using in vitro complement fixation, anti BMZ IgG is seen in virtually all patients.

8. Cicatricial pemphigoid

In salt split skin, IgG and / or IgA binds usually to the epidermal side, although combined pattern and dermal binding can also occur.

9. Linear IgA dermatosis

Using salt split skin, low titre IgA is demonstrated in most cases of CDBC and LAD[52].

10. EB acquisita

50% of cases show anti BMZ zone antibodies, localised to the floor of salt split skin[53].

11. Bullous SLE

Circulating anti BMZ antibodies are rare. However, salt split studies increase the sensitivity and antibodies localize to the floor of the split[54].

12. Dermatitis herpetiformis

Using monkey or pig gut as substrate, circulating anti-endomysial IgA can be seen in 50-100% of cases. Anti reticulin, anti gluten, anti bovine serum albumin and anti lactoglobulin antibodies may also be present.

OTHER DIAGNOSTIC METHODS

ELISA

In pemphigus, antigen specific ELISA has been shown to be more sensitive, specific and to correlate with disease activity better than IIF[55].

Immunoperoxidase methods have roughly the same sensitivity as immunofluorescence studies[56].

Immunoprecipitation and immunoblotting

They detect antigens as protein bands of different molecular weights separated by electrophoresis. Immunoblotting requires denaturation of substrates, whereas immunoprecipitation does not. The former recognizes antibodies against linear epitopes while the latter recognizes antibodies against conformational epitopes. Immunoblot is easier because immunoprecipitation requires radiolabelling.

AIMS OF THE STUDY

This study of autoimmune vesiculobullous diseases was undertaken.

1. To find out the presence of different autoimmune vesiculobullous diseases and their incidence encountered among patients attending skin OPD.

2. To find out the various morphological patterns, symptoms, and systemic association if any.

3. To correlate the clinical, cytological, histopathological and immunofluorescence features of various autoimmune vesiculobullous diseases.

MATERIALS AND METHODS

The material for this study was from the patients attending the skin OPD, Government Rajaji Hospital, Madurai Medical College, Madurai, with autoimmune vesiculobullous diseases of the skin during the period of May 2004 – May 2006.

In total 40 patients with vesiculobullous lesions were subjected to a detailed clinical evaluation, followed by cytological, hispathological and direct immunofluorescence studies. Out of this 40 cases, 32 were confirmed as cases of autoimmune vesiculobullous diseases, and were included in the study after getting informed consent. The remaining 8 cases whose cytology, histopathology and immunofluorescence were found to be otherwise, were excluded from the study.

A detailed history was elicited with particular reference to the age of onset, duration, site, morphology of lesions, progression, symptoms, occupation, and family history.

Thorough general, systemic and dermatological examinations were done.

Routine laboratory investigations like blood for Hb%, TC, DC and ESR, urine examination for albumin, sugar and microscopy, and special investigations like x-ray chest, ultrasonogram, and CT of abdomen, were performed wherever necessary.

Tzanck smear was done in every patient. The smear was air dried stained with Leishman's stain and studied.

Skin biopsies were taken after informed consent. After thorough cleaning of the selected part with spirit or povidone –iodine solution, the area was infiltrated with 2% lignocaine and a bit of skin involving the whole thickness was removed by elliptical incision method. The specimens were preserved in 10% formalin and submitted to histological section of pathology department, Madurai Medical College. Specimens were studied with routine H&E stain. For direct immunofluorescence studies, after careful selection of the part to be biopsied, biopsy was done as described above and the specimen was preserved in Michel's medium and sent to the Department of Skin & STD, Kasturba Hospital, Manipal.

Indirect immunofluorescence test was done wherever necessary, to arrive at the correct diagnosis.

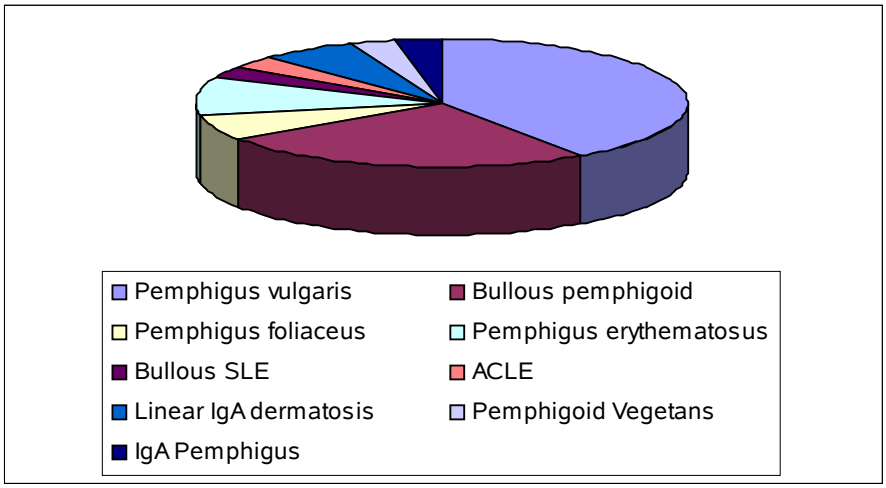
OBSERVATIONS & RESULTS

In this study, 32 cases of autoimmune vesiculobullous disease were studied from the outpatient department of dermatology, Govt. Rajaji hospital, Madurai medical college, Madurai from May 2004 to May 2006. The following observations were made.

The various autoimmune vesiculobullous diseases encountered were

Disease	**No. of Patients**	**Percentage (%)**
Pemphigus vulgaris	13	42%
Bullous pemphigoid	8	25%
Pemphigus foliaceus	2	6%
Pemphigus erythematosus	3	9%
Bullous SLE	1	3%
ACLE	1	3%
Linear IgA dermatosis	2	6%
Pemphigoid Vegetans	1	3%
IgA pemphigus	1	3%
Total	**32**	**100%**

Pemphigus vulgaris was the most common vesiculobullous disease (42%) followed by bullous pemphigoid (25%) pemphigus foliaceus (6.25%), pemphigus erythematosus (9.3%), bullous SLE(3%), acute cutaneous lupus erythematogus (3%) linear IgA dermatosis (6.25%), pemphigoid vegetans (3%) and IgA pemphigus (3%) were the other diseases seen.



AGE DISTRIBUTION

The age of onset of various diseases were

Age of onset	PV	BP	PF	PE	LAD	BSLE	ACLE	PgV	IgAP	TOTAL
0-10	-	-	-	-	-	-	-	-	-	-
11-20	-	-	1	-	1	1	-	-	-	3
21-30	1	2	-	1	-	-	-	-	-	3
31-40	5	1	-	-	-	-	-	-	-	6
41-50	4	2	-	1	1	-	-	-	-	6
51-60	3	1	1	-	-	-	1	1	-	7
61-70	-	2	-	1	-	-	-	-	-	3
>71	-	-	-	-	-	-	-	-	1	1
Total	**13**	**8**	**2**	**3**	**2**	**1**	**1**	**1**	**1**	**-**

PV = Pemphigus vulgaris

BP = Bullous pemphigoid

PF = Pemphigus foliaceus

PE = Pemphigus erythematosus

LAD = Linear IgA dermatosis

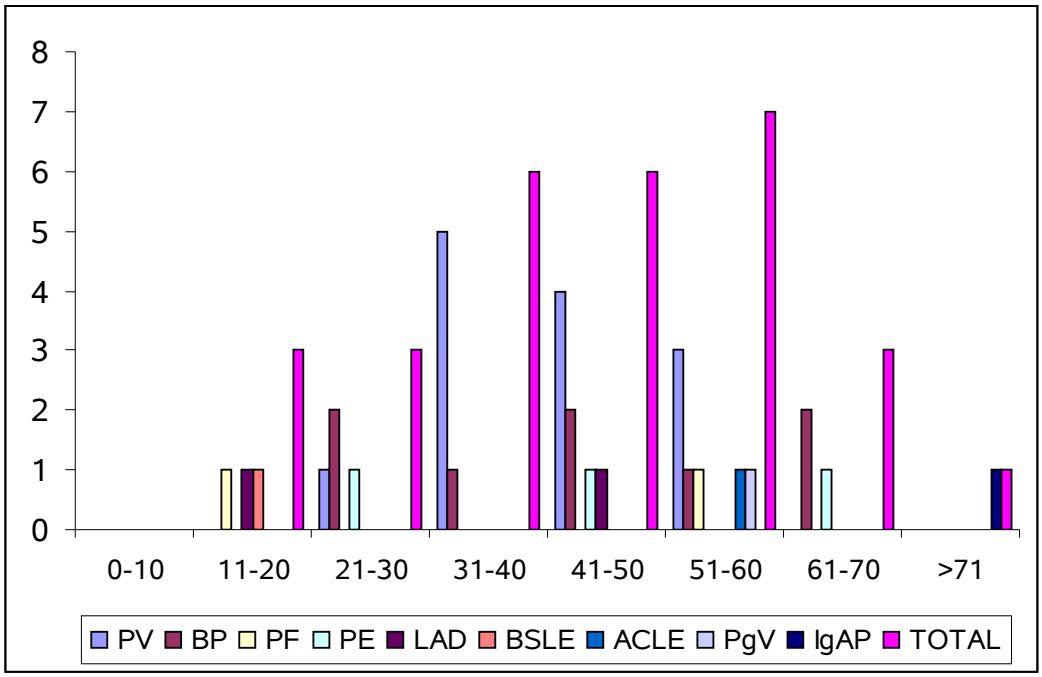
BSLE = Bullous SLE

ACLE = Acute cutanoues LE

PgV = Pemphigoid vegetans

IgAP = IgA pemphigus

According to our study, the mean age of onset for pemphigus vulgaris was 42, and that for other diseases were bullous pemphigoid 51 yrs, pemphigus foliaceus 36 yrs, pemphigus erythematosus 46 yrs, LAD 35 yrs, ACLE 52 yrs, bullous SLE 18 yrs, pemphigoid vegetans 60 yrs and IgA pemphigus 75 yrs.



SEX DISTRIBUTION

In our study, the male: female ratio for individual diseases were as follows:

Disease	Total	Male	Female	M:F
Pemphigus vulgaris	13	4	9	1:2
Pemphigus foliaceus	2	1	1	1:1
Pemphigus erythematosus	3	2	1	2:1
Bullous pemphigoid	8	3	5	3:4
LAD	2	2	-	-
Bullous SLE	1	-	1	-
ACLE	1	-	1	-
Pemphigoid vegetans	1	1	-	-
IgA pemphigus	1	-	1	-
Total	**32**	**13**	**19**	**2:3**

Duration

The mean duration of disease was 1.7 years, with a maximum of 15 yrs, and a minimum of 1 month.

Distribution

The distribution of lesion in the various diseases were as follows

Region	PV	PF	PE	BP	LAD	ACLE	BSLE	PgV	IgAP
Scalp	10	2	3	1	1	-	-	1	-
Face	10	2	3	3	2	1	1	1	-
Trunk	13	2	3	8	2	1	1	1	1
Limbs	13	2	-	8	1	-	1	1	-
Flexures	7	-	-	8	-	-	-	1	1
Seborrheic distribution	-	-	-	-	-	-	-		

PV = Pemphigus vulgaris BSLE = Bullous SLE

BP = Bullous pemphigoid
PF = Pemphigus foliaceus
PE = Pemphigus erythematosus
LAD = Linear IgA dermatosis
ACLE = Acute cutanoues LE
PgV = Pemphigoid vegetans
IgAP = IgA pemphigus

Mucosal involvement

The pattern of mucosal involvement was as follows

Mucosa	PV	PF	PE	BP	LAD	ACLE	BSLE	PgV	IgAP
Oral	13	-	-	4	2	1	1	-	-
Conjuctival	2	-	-	-	1	-	-	-	-
Genital	4	-	-	-	-	-	-	-	-

PV = Pemphigus vulgaris
BP = Bullous pemphigoid
PF = Pemphigus foliaceus
PE = Pemphigus erythematosus
LAD = Linear IgA dermatosis
BSLE = Bullous SLE
ACLE = Acute cutanoues LE
PgV = Pemphigoid vegetans
IgAP = IgA pemphigus

The mucosal involvement was severe in all patients with pemphigus vulgaris, and preceded the skin lesions by a mean duration of 2 months.

Mild to moderate involvement of oral mucosa was seen in 4 of the 8 patients with Bullous pemphigoid (50%).

Morphology of the lesions

The various morphological patterns observed and their incidence in the diseases encountered are tabulated against the clinical diagnosis.

Lesion	PV	PF	PE	BP	LAD	ACLE	BSLE	PgV	IgAP
Flaceid blister	13	2	3	-	-	-	-	-	1
Flaccid blister with scaling	-	2	3	-	-	-	-	-	-
Tense blister	-	-	-	8	2	1	1	1	-
Targetoid lesion	-	-	-	5	-	-	-	-	-
Annular lesion	-	-	-	-	1	-	-	-	-
Hypopyon	-	-	-	-	-	-	-	-	1
Scarring	-	-	-	-	1	-	-	-	-
Milia	-	-	-	-	-	-	-	-	-

PV = Pemphigus vulgaris

BP = Bullous pemphigoid

PF = Pemphigus foliaceus

PE = Pemphigus erythematosus

LAD = Linear IgA dermatosis

BSLE = Bullous SLE

ACLE = Acute cutanoues LE

PgV = Pemphigoid vegetans

IgAP = IgA pemphigus

Signs

Nikolsky sign and Asboe-Hansen sign were elicited, and the results are given below, against the respective clinical diagnosis.

Sign	PV	PF	PE	BP	LAD	BSLE	BSLE	PgV	IgAP
Nikolsky sign	11	2	1	1	-	-	-	-	1
Asboe-Hansen sign	11	2	1	1	-	-	-	-	1

PV = Pemphigus vulgaris

BP = Bullous pemphigoid

PF = Pemphigus foliaceus

BSLE = Bullous SLE

ACLE = Acute cutanoues LE

PgV = Pemphigoid vegetans

PE = Pemphigus erythematosus IgAP = IgA pemphigus

LAD = Linear IgA dermatosis

Cytology

The findings of tzanck smear examination of the different patients is summarised below, under their respective clinical diagnosis.

Finding	PV	PF	PE	BP	LAD	BSLE	ACLE	PgV	IgAP
Acantholytic cell	13	2	3	-	-	-	-	-	-
MNGC (few)	3	-	-	-	-	-	-	-	-
Eosinophils	-	-	-	6	-	-	-	1	-
Neutrophils	-	-	-	-	2	1	-	-	1

PV = Pemphigus vulgaris BSLE = Bullous SLE

BP = Bullous pemphigoid ACLE = Acute cutanoues LE

PF = Pemphigus foliaceus PgV = Pemphigoid vegetans

PE = Pemphigus erythematosus IgAP = IgA pemphigus

LAD = Linear IgA dermatosis MNGC = Multinucleated giant cell

The multinucleated giant cells were few in number and were smaller, and had fewer nuclei than those seen in viral infections.

Histopathology

The histopathology of the different patients with their respective clinical diagnosis in shown below:

Region	Feature	PV	PF	PE	PV/BP	BP	LAD	ACLE	BSLE	PgV	IgAP	CP
Epidermis	Subcorneal bulla	-	2	3	-	-	-	-	-	-	1	-
	Suprabasal bulla	13	-	-	-	-	-	-	-	-	-	-
	Dyskeratotic cells	-	1	0	-	-	-	-	-	-	-	-
	Eosinophils	4	-	-	-	2	-	-	-	-	-	-
	Neutrophils	2	-	-	-	-	-	-	-	-	1	-
	Basal vacuolopathy	-	-	3	-	-	-	1	-	-	-	-
	Others	-	-	-	-	-	-	Spongiosis (1)	-	Epidermal hyperplasia (1)	-	-
Dermis	Subepidermal bulla	-	-	-	1	8	1	1	1	1	-	1
	Hemorrhage	-	-	-	1	2	-	-	-	-	-	-
	Eosinophils	-	-	-	-	8	-	-	-	1	-	-
	Neutrophils	-	-	-	-	1	1	-	1	-	-	1
	Lamellar fibrosis	-	-	-	-	-	-	-	-	-	-	1
	Vasculitis	-	-	-	-	-	-	-	1	-	-	-

Part	Immunoreactant		PV	PF	PE	PV/BP		BP	LAD	ACLE	BSLE	PgV	IgAP
						1	2						
ICS	IgG	Strong	11	2	3	1	-	-	-	-	-	-	-
		Weak	1	-	-	-	-	-	-	-	-	-	-
	C3	Strong	4	-	2	1	-	-	-	-	-	-	-
		Weak	2	2	1	-	-	-	-	-	-	-	-
	IgA		-	-	-	-	-	-	-	-	-	-	1
	IgM		-	-	-	-	-	-	-	-	-	-	-
BMZ	C3	Linear	-	-	2	-	1	8	1	-	-	1	-
		Granular	-	-	-	-	-	-	1	1	1	-	-
	IgG	Continuous	-	-	-	-	-	6	1	-	1	-	-
		Discontinuous	-	-	-	-	-	2	1	1	-	-	-
	IgA		-	-	-	-	-	1	2	-	1	-	-
	Fibrinogen	Continuous		-	2	-	-	2	1	-	-	-	-
		Discontinuous	1	-	-	-	-	-	1	-	-	-	-
	IgM	Continuous	-	-	2	-	-	-	1	-	1	-	-
		Discontinuous	-	-	-	-	-	-	-	1	-	-	-
Vessel wall					IgM fibrogen	-	-	-	-	-	C3 IgM	-	-

PV = Pemphigus vulgaris BSLE = Bullous SLE BP = Bullous pemphigoid

ACLE = Acute cutanoues LE PF = Pemphigus foliaceus PgV = Pemphigoid vegetans

PE = Pemphigus erythematosus IgAP = IgA pemphigus LAD = Linear IgA dermatosis

DISCUSSION

The study was conducted during the period May 2004 – May 2006 at the Department of Dermatology, Govt. Rajaji Hospital, Madurai Medical College, Madurai.

Incidence

32 cases of autoimmune bullous diseases were diagnosed in about 1,92,750 patients who attended the skin OPD during the period of study.

In our study, pemphigus vulgaris was the commonest disease (42%) followed by Bullous pemphigoid (25%), pemphigus erythematosus (9%), pemphigus foliaceus (6%) and linear IgA dermatosis (6%). K.K.Das (IJDVL – 2003, vol-69, issue-1)[57], in his study, also reported that pemphigus vulgaris (40.8%) was the commonest. However dermatitis herpetiformis (36%) which was the next common in their study, was not encountered in our study. This may be due to geographic variation in the incidence. However, larger studies are required to settle the issue.

Age distribution

Majority (78.1%) of the patients were between 21-60 yrs of age. This is in concordance with the study by Arya et al[58], who recorded 80% in that age group.

Sex distribution

In our study, the male:female is approximately 2:3. In the study by Arya et al, the ratio is 1.4:1. Thus, our study shows an increased incidence in women, especially in pemphigus vulgris, where the male:female ratio is 1:2. In the world literature, the incidence is accepted to be equal to both the sexes for pemphigus, and slightly higher in females for bullous pemphigoid.

Extraordinarily high prevalence of pemphigus in females has been reported in certain countries. For example, in the study by Shafi M et al[65], in libya, published in IJDVL, the female:male ratio for pemphigus vulgaris was 90:19, and 100% of the pemphigus foliaceus patients were female.

Morphology and distribution

The morphology of lesions and their distribution in our series followed the pattern seen in earlier studies[59,60,61]. However, involvement of oral mucosa was seen in 100% of the pemphigus vulgaris patients, as against 72% reported by Arya et al. Neither of our patients with pemphigus foliaceus had oral

mucosal involvement (0%), as against 12% reported by Arya et al. All 3 cases (100%) of pemphigus erythematosis showed involvement of the malar area. Flexural involvement was noted in 6 of the 8 cases (75%) of bullous pemphigoid, as described in earlier studies.

Clinical signs

Nikolsky sign and Asboe-Hansen sign were positive in all the cases of pemphigus, (100%) and one patient with paraneoplastic Bullous pemphigoid. In their series, Arya et al reported positive Nikolsky sign in 97.2% cases of pemphigus vulgaris and 94.7% cases of pemphigus foliaceus. The presence of positive Nikolsky sign in bullous pemphigoid is well documented, in the literature.

Tzanck smear

Acantholytic cells were seen in 100% of the cases of pemphigus vulgaris, pemphigus foliaceus and pemphigus erythematosus. Arya et al reported acantholytic cells in 96% of cases of pemphigus vulgaris and foliaceus. Multinucleated cells were seen in 3 of the 13 patients (23%) with pemphigus vulgaris, in concordance with the study by Barr et al[69]. Eosinophils were seen in 75% of Bullous pemphigoid cases. The correlation between the presence of multinucleated giant cells and coexistant herpes simplex infection, and its implication in the prognosis, needs further study.

Histopathology

Suprabasal bulla was seen in 100% cases of pemphigus vulgaris. Arya et al, in their study, reported 81.4% suprabasal bulla and 18.6% mid dermal bulla. The later was seen in older bullae due to regeneration. Since early blisters were carefully chosen for biopsy in our study, mid dermal bulla was not seen.

An inflammatory infiltrate was seen in 46.1% of pemphigus patients, against 53.5% reported by Arya et al. Eosinophils were seen in 30.7% and neutrophils in 15.3% in our study, against 25.6% and 20.9% respectively, in the study by Arya et al.

In pemphigus foliaceus, subcorneal bulla was seen in 100% of cases, as against 60% of cases in the study by Arya et al. Dyskeratotic cells were seen in one of the two patients (50%), whereas, only 8% of the cases showed the same in the study by Arya et al.

Subepidermal bulla (100%) and eosinophils (100%), were the most consistent findings in Bullous pemphigoid, in our study, which is concordant with earlier studies[23].

Lamellar fibrosis of the dermis was seen in a patient of LAD, presenting as cicatricial pemphigoid.

Direct immunofluorescence

The direct immunofluorescence findings in our series were in concordance with those described in earlier studies. Intercellular cement substance deposits (100%) and linear C_3 BMZ deposits (100%) were the most consistent findings seen in pemphigus vulgaris and Bullous pemphigoid respectively.

In a 50 yrs old male patient who had been diagnosed as a case of cicatricial pemphigoid based on clinical features and histopathology, the diagnosis was revised after the DIF showed a strong linear IgA band in the basement membrane zone, along with weak C_3 deposits. The patient showed good response to dapsone therapy. This emphasises the importance of DIF, in the diagnosis, and thereby, its value in determining the therapy and hence, prognosis of autoimmune bullous disorders.

Indirect immunofluorescence

A 36 yr old female presented initially with flaccid blisters positive tzanck smear, and positive Nikolsky sign. Direct immunofluorescence showed features of pemphigus vulgaris. A few weeks later, she presented with clinical

features suggestive of bullous pemphigoid. Tzanck smear was negative and histopathology showed subepidermal bulla. An indirect immunofluorescence study found IgG against intercellular cement substance in 1:80 dilutions. Thus, a final diagnosis of pemphigus vulgaris was made.

Naveed Sami et al[66], have reported a series of 13 patients who initially presented with clinical and immunofluorescence features of bullous pemphigoid, who subsequently demonstrated co-existent DIF and IIF features of bullous pemphigoid and pemphigus vulgaris. Cholzelski et al[67], and Leibovici et al have described similar patients. However, our patient initially presented with pemphigus vulgaris, and then progressed to manifest a bullous pemphigoid – like presentation. The patient is on dexamethasone – cyclophosphamide pulse therapy, and is being followed up.

Associations

In our study, the commonest coexisting disease was non-insulin dependent diabetes mellitus in 6 patients (18.75%), and hypertension in 2 patients (6.25%). Ischaemic heart disease was associated in one patient.

A 27 years old female patient who presented bullous pemphigoid of 1 month duration, was diagnosed to have multiple liver secondaries in ultrasonography. However, the patient died before the primary malignancy

could be established. Association of bullous pemphigoid with internal malignancies has been described in earlier studies by Ogawa et al[62].

A history of diclofenac ingestion prior to the onset of linear IgA dermatosis was elicited in a 16 yr old male. Similar findings have been reported in earlier studies.

In contrast to earlier studies by Callen JP et al[63], and et al, no association with other autoimmune diseases could be established in our study.

Rare and interesting disease

A 60 yrs old male who presented tense bullae initially, and vegetative lesions subsequently, was diagnosed as a case of pemphigoid vegetans, based on the following findings.

1. Vegetative lesions
2. Marked acanthosis with subepidermal blister and eosinophils
3. Linear BMZ deposits of C_3

Winkelman et al have reported a case of pemphigoid vegetans with findings similar to our case.

SUMMARY

32 patients, diagnosed with autoimmune vesiculobullous diseases, based on history, clinical features, cytology, histopathology, and immunofluorescence were included in the study.

Incidence

The incidence was 1.7:10,000, among 1,92,750 patients who attended the skin OPD during the period of study.

Sex

13 patients were male (40.6%) and 19 patients were female (59.4%). The male:female ratio was approximately 2:3.

Age

78.1% of the patients were between 21-60 years of age.

Morphology and distribution

The morphology of lesions and their distribution were concordant with those described in the literature. Oral mucosal involvement and flaccid bullae was noted in 100% of the cases of pemphigus vulgaris. Tense bullae (100%) and targetoid lesions (62.5%) were the commonest lesions seen in bullous pemphigoid.

Nikolsky sign and Asboe-Hansen sign

These signs were positive in 100% of the pemphigus patients, and one patient with paraneoplstic bullous pemphigoid.

Tzanck smear

Acantholytic cells were demonstrated in 100% cases of pemphigus. Eosinophils could be seen in 75% of bullous pemphigoid cases multinucleated cells were seen in 23% of pemphigus vulgaris patients.

Histopathology

Histopathological features consistent with pemphigus vulgaris were seen in 13 patients, pemphigus foliaceus in 2 patients, pemphigus erythematosus in 3 patients, IgA pemphigus in one patient, bullous pemphigoid in 8 patients, linear IgA dermatosis in one patient, and bullous SLE, acute LE and pemphigoid vegetans in one patient each. One patient who was diagnosed as cicatricial pemphigoid based on histopathology, was later diagnosed as linear IgA dermatosis based on DIF.

Immunofluorescence

Based on direct immunofluorescence findings, a diagnosis of pemphigus vulgaris was made in 13 patients, pemphigus foliaceus in 2 patients, pemphigus erythematosus in 1 patient, bullous pemphigoid in 8

patients, linear IgA dermatosis in 2 patients acute cutaneous LE in one, bullous SLE in one patient and pemphigoid vegetans in one patient.

One patient who had DIF findings of pemphigus vulgaris initially and bullous pemphigoid 2 months later, was subjected to indirect immunofluorescence and was finally diagnosed as pemphigus vulgaris.

Association

Non insulin dependent diabetes mellitus was the commonest associated disease, seen in 18.75% cases, followed by hypertension (6.25%), ischaemic heart disease (3%), internal malignancy (3%) and diclofenac ingestion (3%).

CONCLUSION

1. Autoimmune vesiculobullous diseases were rare skin diseases comprising only a very small percentage of patients attending skin OPD.
2. Pemphigus vulgaris was the most common autoimmune vesiculobullous disease, followed by bullous pemphigoid.
3. Pemphigus vulgaris occurred more commonly in females.
4. Majority of the patients were between 21-60 yrs of age.
5. Tzanck smear is a useful bedside tool which points towards the final diagnosis.
6. Histopathological examination is of paramount importance in arriving at the diagnosis.
7. Direct immunofluorescence technique is a useful supplement to routine histopathology, and may prove to be of diagnostic value and therapeutic significance in selected cases.
8. Indirect immunofluorescence is helpful in arriving at the correct diagnosis in selected cases.

BIBLIOGRAPHY

1. Arndt IcA, Feingold Ds. Nengl. J.med 1970; 282 1154-5
2. Lever WF, pemphigus. Medical 32:1, 1953
3. Beutner EH, Jordan RE, proc. soc. Exp. Biol. med 1964:117, 505-10
4. Stanley JR, Yaar M, Hawley NP, J. clin. Invest 1982; 70:281-8
5. Amagai M, Klaus V, Stanley JR, cell .1991; 67, 869-77
6. Beutner EH, Jordan RE, et al. JAMA, 1967 ; 200 :751-6
7. Nie boer C et al ; Br. J. Dermatol, 102 :383,1980.
8. Warren SJ, et al, New enj J. dermatol 343: 23, 2000
9. Bermard P et al, Arch Dermatol 131 :48, 1995
10. Roger D et al Arch Dermatol 130:734, 1994
11. Kolodny RG et al Am J obstret gynecol 104:39, 1969
12. Wojnarowska F. et al, J am acad dermatol, 1988;19:792-805
13. Bernard P et al, Arch damatol 1995; 131:48-52
14. Hall RP et al, Ann int med 1982,77:165-70
15. Reunala T et al DH in Finland, Acta dam venerol 1978 :58, 505-10
16. Barr RJ, cutaneous cytology, JAAD, 1984, 10 :163-80
17. Asboe-Hansen-J invest Dermatol 1960, 34:5
18. Lever WF, pemphigus and pemphigold, Springfield IL, Charles C.

Thomas, 1965

19. Arndt .K, Harrist TJ, N.eng.J. med 1980, 303:35
20. Ahmed AR, Blose DA, pemphigus vegetans, int,j, dermatol 1984, 23:135
21. Lever WF, J am acad dermatol, 1979, 1:2
22. Stevens SR, Anhalt GJ, arch dermatol 1993; 129:866
23. Lever WF, pemphigus, med. Baltimore, 1953, 32:1
24. Bushkell LL, Jordan RE, J am acad dermatol 8:648, 1983
25. Pierard, thiery.M.et al, Arch belg Dermatol syphilig, 1969, 25:31
26. Prorost TT, Tomasi TB, J chh invest 1973; 52:1779
27. Hall RP, Lawley TJ, Ann intern med 1979, 97:165
28. Pierad J, Ann Dermatol syphiligr (parij) 1963: 90: 121
29. Nousari HC, An halt GJ, Mamrae of clinical laboratory technology, ASM press, 2002:1032
30. Gammon WR et al, J invest dermatol, 82:139,1984
31. Korman NJ, perphigus, J am acad dermatol 1988 :18,1219
32. Judd KP, lever WF, Arch dermatol, 1979, 115:428
33. Lever WF, J am acad dermatol 1979, 1:2
34. Brystyn JC, Abel E, Arch dermatol 1974; 110:857
35. Hodak E, David M, clin enp dermatol 1990; 15:443
36. OHNO H, miyagawa S, atypical pemphigus, 1994:189:115

37. An halt GJ, kim SC, N,eng J med, 1990:323, 1729

38. Mutasim DF, Anhalt GJ, Dermatol clin, 1993:11:473

39. Harrist TJ, cutaneous immunopathology 1979:10:625

40. prorost TT, Tomasi TB, J. clin invest dermatol 1973;52:1779

41. Fine JD, et al J invest dermatol 82:39,1984

42. Gammon WR et al, j amacad dermatol 22:664,1990

43. Kelly S, Wojnarowska F et al, Br.J. dermatol 118:31,1988

44. Gammon WR, fine JD et al, Bullous diseases Igaku shoin, 1993:75

45. Cormane R, pathol tutr, 1967:2:170

46. Cholzerski TP, Bentner EH, J. invest dermatol, 1971: 56: 373

47. Harman, KE, et al Br. J. dermatol, 142 142: 1135, 2000

48. Harman, KE, et al Br J dermatol, 142: 1135, 2000

49. Krasny, SA et al, immunopatholgoy of skin, wiley, 1987 P.207

50. Hashimoto T, inamoto N et al, Arch, dermatol 1987 ; 123 :1062

51. Kiyokawa C et al, J invest dermatol, 11 : 1236, 1998

52. Wojnarawska et al, Br J dermatol, 132 : 150,1995

53. Woodley DT et al, immunol sec 1989, 46-547

54. Barton DD, fine JD, J am acad dermatol, 1985, 84:472

55. Amagi Mm et al, J. immunol 159: 2010, 1997

56. Cerio R, macdonald DM, J am acad dermatol 1988: 19:747

57. K.K. Das – IJDVL 2003, vol-69, issue-1-16-18

58. Arya SR et al, IJDVL 1999:65:168:171

59. Singh R, pandhi RK, IJDVL, 1973 ;39 :126-132

60. Hands F, Agarwal RR, IJDVL 1975; 39:100-171

61. Kandhari KC, pasricha JS, IJDVL, 1965;31:62-71

62. Ogawa H et al, J Dermato Sci 9: 136, 1995.

63. Callen JP et al, J Am Acad Dermatol, 3: 107, 1980.

64. Kumar B, Arora S, Kumaran MS, IJDVL, 2006; 72: 203-206.

65. Shafi M et al, IJDVl, 1994; 60:140-143.

66. Naveed Sami, Kailash Bohl, Beutner EH Dermatology 2002; 202: 108-117.

67. Chorzelski et al. Arch Dermatol 109; 849, 1974.

68. Leibovici et al. Int J Dermatol, 28; 259, 1989.

69. Bart RJ, cutaneous cytology, J. am.acad dermatol 1984, 10: 163-80.

PROFORMA

Name : Age / Sex: Occupation: Income: S/E status:	Address:

General history

d) HT / DM :

e) PT / Heart Disease :

f) Malignancy :

g) Smoking / Alcohol :

h) Precipitating factors :

   i. Drugs :

   j. Infection :

   k. Diet :

   l. Trauma :

   m. Sweating :

   n. Sun exposure :

o) Oral / Genital herpes:

Family history

p) Marital status :

q) Children :

r) Obstetric history :

s) P/S Done or not :

t) Menstrual history :

u) H/o other auto immune Disease in the patient :

v) H/O auto immune diseases in relatives :

H/O vesiculobullous disease

w) Date of onset :
x) Site of first lesion (mucosal / skin) :
y) Morphology of first Skin lesion and it's Evolution :
z) Treatment received :

aa) Date of registration at GRH :
bb) Number of acute exacerbations:

Onset	Duration	PPt. factors	Treatment

Condition on First Visit

4. General Condition

cc) wt :
dd) CVS :
ee) RS :
ff) Abd :
gg) Others :

❖ **Oral Lesion**

❖ **Active Skin lesions**

hh) Number of bullae :
ii) Distribution – groups / discrete / along lines of trauma:
jj) Tense/flaccid :
kk) Contents – clear / pustular / hemorrhagic:
ll) Nikolsky sign & bulla spread sign:
mm) Are of erosions :
nn) Symptoms :

4. Healing skin lesions

oo) Hyper / hypo pigmentation: • Scarring :

pp) Milla : • Peripheral extension :

Investigations
LFT
RFT
Sr. Electrolytes
Hemogram
ECG
Tzanck
Biopsy
DIF
USG abdomen
Others

NAME:													
Date	**Com plaints**	**New lesions skin / mucosa**	**Persisten t lesion oral/ scalp/ skin**	**Treatment DCP / others**	**Wt.**	**BP**	**Urine ASD**	**TC**	**DC**	**Hb**	**Blood USC**	**LFT / Tzanck / Others**	**Remarks / Blister free period / menstrual history**

KEY TO MASTER CHART

ul = upper limb

ll = lower limb

tghs = thighs

ax = axilla

sc = scalp

trnk = trunk

gen = genital

conj = conjunctival

P = present / positive

A = absent

N = negative

PV = pemphigus vulgaris

BP = bullous pemphigoid

PF = pemphigus foliaceus

PE = pemphigus erythematosus

LAD = Linear IgA dermatosis

PgV = Pemphigoid vegetans

EBA = epidermolysis bullosa acquisita

DLE = Discoid lupus erythematosus

BSLE = bullous SLE

PNP = paraneoplastic pemphigus

NIDDM = non – insulin dependant diabetes mellitus

HT = hypertension

ACA = acantholytic call

Neu = neutrophil

EOS = eosinophil

Sub epi = sub epidermal