REVIEW

A Comprehensive Review and the Diagnostic Procedures in Autoimmune Vesiculobullous Lesions

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Abstract

Introduction: Vesiculobullous lesions are distinct skin and oral disorders with diverse clinical presentations. Diagnosing these lesions in the oral cavity is challenging due to masticatory forces, external factors, and fragile mucosa, often leading to ulceration. Various causes of vesiculobullous diseases include viral infections, allergies, autoimmune diseases, and genetic factors. Accurate diagnosis relies on physical examination, mucosal scrutiny, histological analysis, and immunological assessment. Therefore,general practitioners and dentists need a thorough understanding of these disorders and diagnostic procedures.

Objective: To review the various diagnostic procedures for autoimmune vesiculobullous diseases of skin and oral mucus membrane.

Materials and Methods: Review articles ,Case reports, , and Original research papers published in various electronic databases such as Google Scholar, , Crossref, PubMed,and e-Books are included in this review article.

Result and Conclusion: This review underscores basic histology and molecular components of the adhesive junctions, along with etiopathogenesis of autoimmune vesiculobullous lesions, and importance of patient history, clinical examination, histological tests, and molecular techniques in diagnosing these lesions.

Keywords: Autoimmune, Vesiculobullous Lesions, Desmosomes, Hemidesmosomes, Basal Lamina, Immunofluorescence

Introduction

Vesiculobullous lesions represent a distinctive category of skin and oral disorders, manifesting with diverse clinical presentations. However, inspecting oral vesiculobullous lesions poses challenges due to the influence of masticatory forces, external factors, and the friable nature of mucosa, often leading to ulcerations of vesicles and bullae. The etiology of vesiculo-bullous lesions encompasses viral infections, allergies, autoimmune diseases, and, in certain cases, genetic factors. These lesions exhibit complex features, emphasizing the pivotal role of general physical examination, mucosal scrutiny, histological analysis, and immunological assessment in achieving accurate diagnoses. For healthcare professionals, particularly general practitioners and dentists, a comprehensive understanding of the clinical presentation of these disorders and familiarity with the associated diagnostic procedures are imperative. This comprehensive review highlights the importance of thorough patient history, clinical examinations, histological tests, and molecular techniques in diagnosing oral and skin vesiculobullous lesions.

An insight on the Oral Mucus Membrane and Skin

The oral mucosa, a moist tissue membrane, lines the structures of the oral cavity. It spans from the vermilion border of the lips and labial mucosa to the palatopharyngeal folds. Histologically, it consists of three layers namely surface squamous stratified epithelium or oral epithelium (varies in thickness and keratinization based on location and function), lamina propria (a connective tissue layer beneath the epithelium), and submucosa (a dense irregular connective tissue found deepest, but absent in areas where the lamina propria directly attaches to bone or muscle)[1]. At the junction of the epithelium and connective tissue, two structures, the basal lamina and the basement membrane, can be found. The basal lamina which is epithelial in origin, is visible under an electron microscope, while the basement membrane can be seen under a light microscope. The oral mucosa is similar to skin, composed of epithelium and connective tissue, akin to the epidermis and dermis. Skin epithelium is always orthokeratinized, whereas oral epithelium can be nonkeratinized or keratinized (ortho or parakeratinized),

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lacking a stratum lucidum. Lamina propria of skin consists of hair follicles, sebaceous, and sweat whereas oral mucosa contains salivary glands.[2]

Specialized regions of the plasma membrane of epithelial cells create junctions between cells and the extracellular matrix. Cell-cell adhesive junctions primarily involve cadherins, calcium-dependent proteins that bind homotypically with cadherins on adjacent cells. Catenins, cytoplasmic adapter proteins, connect cadherins to the cytoskeleton. In the oral mucosa, desmosomes are the intercellular junctions. Desmosomal cadherins, desmoglein, and desmocollin, interact with adjacent cell proteins, forming a dense line in the intercellular space. Catenins in desmosomes include desmoplakin, plakoglobin, and plakophilin, creating an electron-dense plaque on the cytoplasmic side, anchoring intermediate filaments.[3]

Hemidesmosomes anchor cells to the basal lamina, consisting of the electron-lucent lamina lucida and the electron-dense lamina densa, and connect to the extracellular matrix. Key transmembrane adhesive molecules in hemidesmosomes include integrin α6β4, which binds to laminin, and collagen XVII (BP180). Similar to desmosomes, hemidesmosomes have cytoplasmic adapter proteins like BP230 and plectin, forming a dense plaque that attaches intermediate filaments.[3]

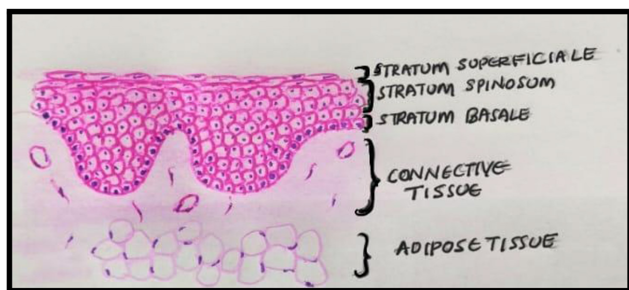

Fig. 1: Histology of the buccal mucosa

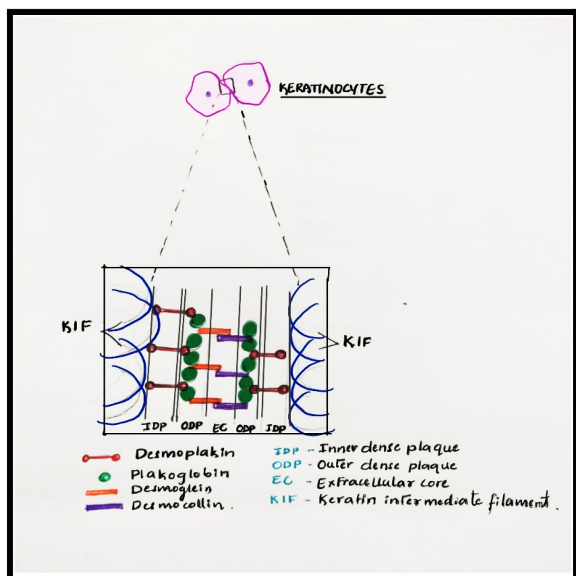

Fig 2: Structure of a desmosome

Desmosomal and hemidesmosomal integrity is important in maintaining tissue cohesion and diverse mechanisms that can disrupt their function are genetic mutations, infections and autoimmune diseases.[3]

Vesiculobullous Lesions

A vesiculobullous lesion is a mucocutaneous disease characterized by fluid-filled blisters of varying sizes. Vesicles are small blisters measuring 0.5 cm or less in diameter, while bullae are larger blisters exceeding 0.5 cm in diameter.[4]

In this review, 2 types of classifications are enumerated based on etiology and based on plane of separation.

I. Classification based on etiology

Table I : Classification based on etiology.

S. NO.	ETIOLOGY	VESICULOBULLOUS LESION
1.	Autoimmune	Pemphigus, Pemphigoid, Dermatitis Herpetiformis, Pemphigoid Gestationis, Bullous SLE, Linear Iga Dermatosis, Epidermolysis Bullosa Acquisita, Paraneoplastic Pemphigus
2.	Familial	Hailey Hailey Disease, Epidermolysis Bullosa
3.	Infectious	Varicella, Herpes Zoster, Herpes Simplex, Bullous Impetigo, Bullous Scabies.
4.	Others	Burns, Diabetic Blister, Toxic Epidermolysis Bullosa, Fixed Drug Eruption, Porphyrias, Bullous Erythema Multiforme

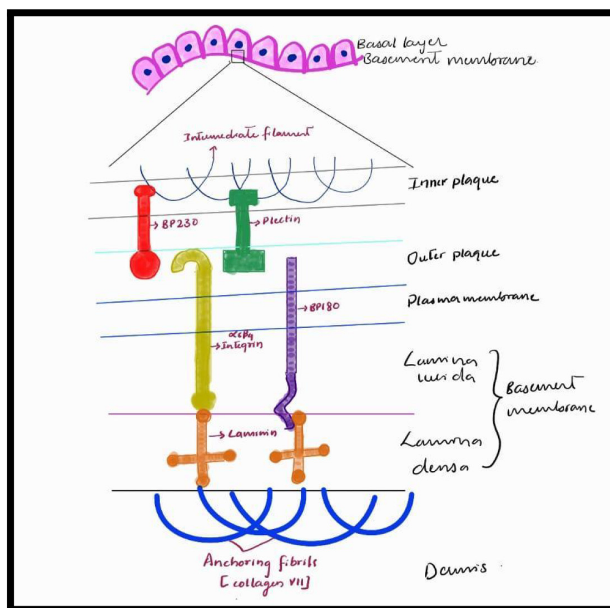

Fig 3 : Structure of a hemidesmosome.

II. Based on the specific plane of separation classification is given by Fitzpatrick, [4]

Table II: Classification based on plane of seperation.

1	INTRAEPITHELIAL SPLIT	1. GRANULAR LAYER Pemphigus Foliaceous Pemphigus Erythematosus Bullous Impetigo Frictional Blisters
		2. SPINOUS LAYER Benign Familial Pemphigus Herpes Simplex Virus Herpes Zoster And Varicella Eczematous Dermatitis
		3. SUPRABASAL LAYER Pemphigus Vulgaris Pemphigus Vegetans Darier's Diseases
		4.BASAL LAYER Erythema Multiforme Toxic Epidermal Necrolysis (TEN) Lichen Planus Lupus Erythematous Epidermolysis Bullosa Simplex
2	SEPARATION AT DERMO-EPIDERMAL JUNCTION	1. SPLIT AT LAMINA LUCIDA Bullous Pemphigoid Cicatricial Pemphigoid Epidermolysis Bullosa Junctional Dermatitis Herpetiformis 2. SPLIT BELOW BASAL LAMINA (SUBLAMINA DENSA) Epidermolysis Bullosa Acquisita Epidermolysis Bullosa Dystrophica Linear Iga Dermatosis Bullous SLE

A common etiopathogenesis is autoantibodies binding to adhesion molecules in desmosomes, hemidesmosomes, and sometimes the basement membrane, causing inflammation and loss of adhesion. This leads to the formation of intra- or subepidermal blisters.[5] The antigens are as given in the table

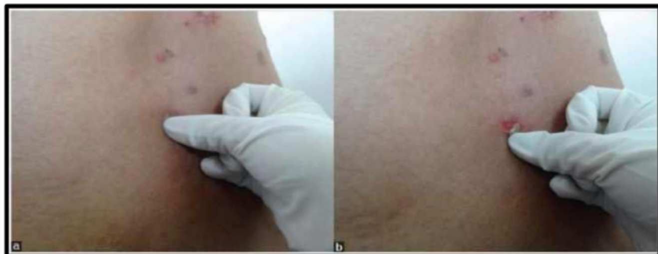

Fig. 4 (a) Eliciting Nikolsky's sign on perilesional skin. Note the tangential pressure, (b) Eliciting Nikolsky's sign, peeling of skin revealing moist erosion[12]

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Table III

Autoimmune VB Lesions	**Antigens**
Pemphigus vulgaris	Desmoglein 1 & 3
Paraneoplastic pemphigus	Desmoglein 1 & 3, plakins
Pemphigus foliaceous	Desmoglein 1
IgA pemphigus	Desmoglein 3, Desmocolin 1 & 2
Pemphigus herpetiformis	Desmoglein 1
Cicatricial pemphigoid	BP180,Laminin 5
Bullous pemphigoid	BP180 &230
Epidermolysis bullosa aquisita	Type VII collagen
Epidermolysis bullosa simplex	Keratin 5 & 14
Epidermolysis bullosa junctional	Laminin 5,Type XVII collagen
Epidermolysis bullosa dystrophica	Type XVII collagen
Erythema multiformae	Desmoplakin
Dermatitis herpetiformis	Tissue transglutamase
Bechets disease	HLA B51

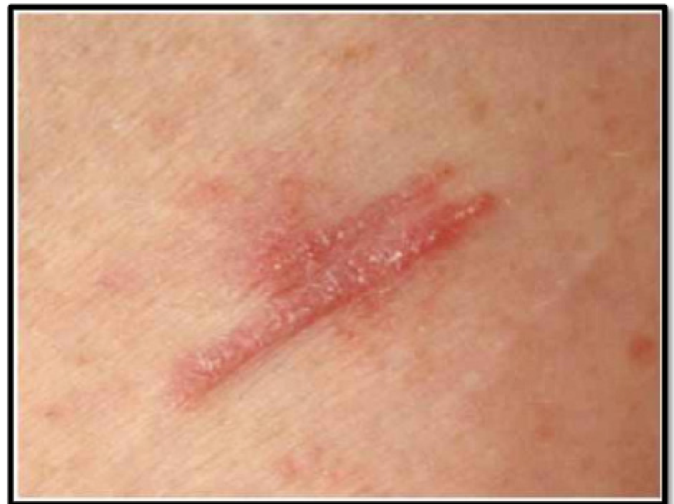

Fig. 5: linear koebner phenomenon in Lichen planus[23]

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Vesiculobullous disorders are a diverse group of skin conditions with varying symptoms. They significantly impact patients and their families, leading to substantial economic costs. These diseases have been extensively studied. Identifying intact vesicles and bullae is challenging due to the fragile nature of oral mucosa, often resulting in erosions or ulcerations. Diagnosing vesiculobullous lesions is further complicated by the need to differentiate them from other ulcerative, immune-mediated, and neoplastic diseases. Therefore, understanding the clinical presentations and diagnostic procedures for these disorders is crucial.[6]

Daignostic Procedures

The pathological assessment of blisters requires a detailed analysis, focusing on the separation plane, the mechanism behind blister formation, and the nature of the inflammatory infiltrate, noting its presence or absence. Advances in investigative dermatology have expanded the scope of diagnosis. Key techniques for evaluating patients with vesiculobullous diseases include traditional histopathology and confirmatory tests such as direct and indirect immunofluorescence[7]

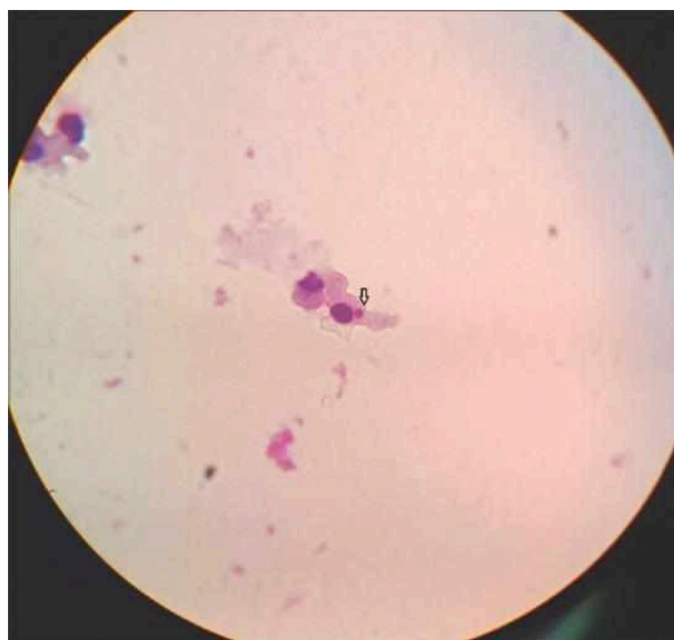

Fig. 6: Giemsa stain reveals the LE cell showing the characteristic Haematoxylin body (arrow) [28]

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Diagnostics procedures that aid in the diagnosis of vesiculobullous lesions can be classified as follows

Table IV: Classification of diagnostic procedures in vesiculobullous diseases.

CLINICAL TESTS	HISTOLOGICAL TESTS	MOLECULAR TECHNIQUES
Nikolsky's test	Tzanck test	Immunofluorescence
Pathergy test	LE cell phenomenon	ELISA
Kobner phenomenon	Biopsy	Immunoblotting and immunoprecipitation
Brocques phenomenon		

❖ **Nikolsky's Test**

Nikolsky's sign, first described by Russian dermatologist Piotr Vasiliyevich Nikolsky (1858-1940)[8], he stated that rubbing the skin of patients with pemphigus foliaceous, lead to blistering or denudation of the epidermis with a moist, glistening surface underneath. This indicated weak adhesion between the corneal and granular cell layers, even on lesion-free skin.[9] Lyell confirmed these observations in 1956, identifying Nikolsky's sign in toxic epidermal necrolysis patients.[10] Applying lateral pressure with the index finger disrupts intercellular adhesion, showing Nikolsky's sign clinically. If there is less disruption of intercellular adhesion, may only be visible microscopically, requiring serial sections to detect. In the oral cavity, Nikolsky's sign is often indicated by tissue ulceration or blistering after pressure from a finger, air, or blunt instrument.[11]

When pressure is exerted for Nikolsky's sign if it leaves moist, glistening, and eroded base it is 'Wet Nikolsky's sign whereas in 'Dry Nikolsky's sign' dry base is noticed after erosion of skin.[13,14]

The marginal Nikolsky's sign is when the erosion extends on surrounding normal skin by rubbing the skin in perilesional area. The direct Nikolsky's sign is when the erosion is induced on the normal skin that is far from the lesions.[13,14]

"Microscopic Nikolsky's sign", induced by tangential pressure, reveals subclinical changes in pemphigus vulgaris and foliaceus, confirmed by biopsy. It may be more sensitive for quick diagnosis and histopathological assessment.[13,14]

"False Nikolsky's sign (Sheklakov's sign)" is elicited by pulling the edge of a ruptured blister, extending the erosion into surrounding normal skin. It indicates subepidermal blistering disorders like cicatricial pemphigoid, bullous pemphigoid, epidermolysis bullosa, dermatitis herpetiformis, porphyrias, and bullous systemic lupus erythematosus (SLE).[13,14]

The "pseudo Nikolsky's sign," or "epidermal peeling sign." is positive as a result of necrosis and not acantholysis. It is seen in Stevens–Johnson Syndrome, toxic epidermal necrolysis, some burn cases.[13,14]

The phenomenon associated with this sign called "Nikolsky's phenomenon" where the superficial epidermis is felt to slide over the deeper layers, resulting in delayed blister formation instead of an immediate reaction to form erosion as in Nikolsky's sign.[13,14]

Asboe-Hansen sign, named after Danish physician Dr. Gustav Asboe-Hansen, also known as the bulla spread sign, involves applying pressure to the roof of an intact blister. The blister extends with a sharp angle in Pemphigus Vulgaris and a rounded border in bullous pemphigoid.[8,9]

❖ **Pathergy Test**

The pathergy phenomenon, first described by Blobner in 1937, involves altered tissue reactivity to minor trauma.[15] The pathergy test, an unspecific hypersensitivity response to a needle prick, results in erythematous induration, papules, or sterile pustules at the trauma site. This reaction is seen in Behcet's disease and neutrophilic dermatoses like pyoderma gangrenosum and Sweet syndrome.[15]

There is no standardized method for the pathergy test. Common techniques include intradermal, subcutaneous, and intravenous approaches, with the intradermal needle prick being most common. This test uses substances like normal saline, monosodium urate crystals, or streptococcal antigens. There are two pathergy tests: the oral pathergy test (OPT) and the skin pathergy test (SPT). The OPT involves pricking the lower lip mucous membrane with a 20-gauge blunt needle. Although less sensitive than the SPT, the OPT is easier to perform. Any resulting ulcer or pustule is considered a positive result, regardless of size.[16]

For the skin SPT, a 20-gauge needle is typically inserted bevel up at a 45-degree angle to a depth of 3-5 mm.[17] The site is evaluated 24 to 48 hours later. Erythema without induration around the needle prick site is considered a negative result. A papule with an erythematous halo is viewed as a positive result, and if the papule transforms into a pustule, it indicates a strongly positive reaction.[16]

❖ **Kobners Phenomenon**

The Koebner phenomenon, first described by Heinrich Koebner in 1876, involves the appearance of new skin lesions on previously unaffected skin following trauma. Also known as the isomorphic response, these new lesions mimic the patient's existing skin condition.[18,19] This phenomenon can vary over time, with individuals shifting between Koebner-negative and Koebner-positive states. It is commonly observed in conditions such as psoriasis (11-75% incidence), vitiligo (21-62%), and lichen planus.[18,20,21] The new lesions resemble the underlying skin disease both clinically and histologically.[19]

Boyd and Nelder classified clinical entities with reported Koebnerization into four categories:[20]

Table V: Boyd and Nelder classification of koebnerization.

S. NO.	CATEGORY	DISEASES
1.	**Category I (true koebnerization)** Koebner phenomenon is seen in all patients by any form of insults	Psoriasis, Lichen Planus, And Vitiligo
2.	**Category II (pseudo-koebnerization)** Koebner phenomenon gets produced by the seeding of infectious agents along with sites subjected to trauma, usually from scratching.	Molluscum Contagiosum,Verruca Vulgaris
3.	**Category III (occasional lesions)** Koebner phenomenon is seen in some but not all	Darier disease,Erythema multiforme
4.	**Category IV (questionable trauma-induced process)** all disorders with a dubious association with trauma	Pemphigus Vulgaris And Lupus Erythematosus

Since this publication in 1990, there are other conditions where Koebner phenomenon is demonstrated (leukocytoclastic vasculitis, eruptive xanthomas, and mycosis fungoides.)[22]

❖ **Brocq's Phenomenon**

Brocq's phenomenon is characterized by subepidermal hemorrhage upon scraping classical lesions of lichen planus. Notably, this contrasts with psoriasis, where scratching leads to pinpoint bleeding on the lesion surface[24].

❖ **Tzanck Test**

The Tzanck smear, was pioneered by French physician Tzanck in 1947, and this test was primarily employed in viral infections such as herpes simplex, varicella, and herpes zoster. It aids in identifying viral giant cells and acantholysis in pemphigus.[25]

Fresh samples are essential for the test. The suspected area is dried and cleaned, followed by inserting a sterile needle at the blister base.[24] Obtained contents are then smeared onto a clean glass slide, left to air dry, and stained with Leishman stain.

Tzanck cells, or acantholytic cells, appear during microscopic examination. These large, rounded keratinocytes exhibit a hypertrophic nucleus, hazy or absent nucleoli, and abundant basophilic cytoplasm. Their morphology shifts from polyhedral to rounded as they detach from other epithelial cells.[26]

Besides Leishman stain, alternative stains for Tzanck smear include giemsa, haematoxylin and eosin, wright, papanicolaou,

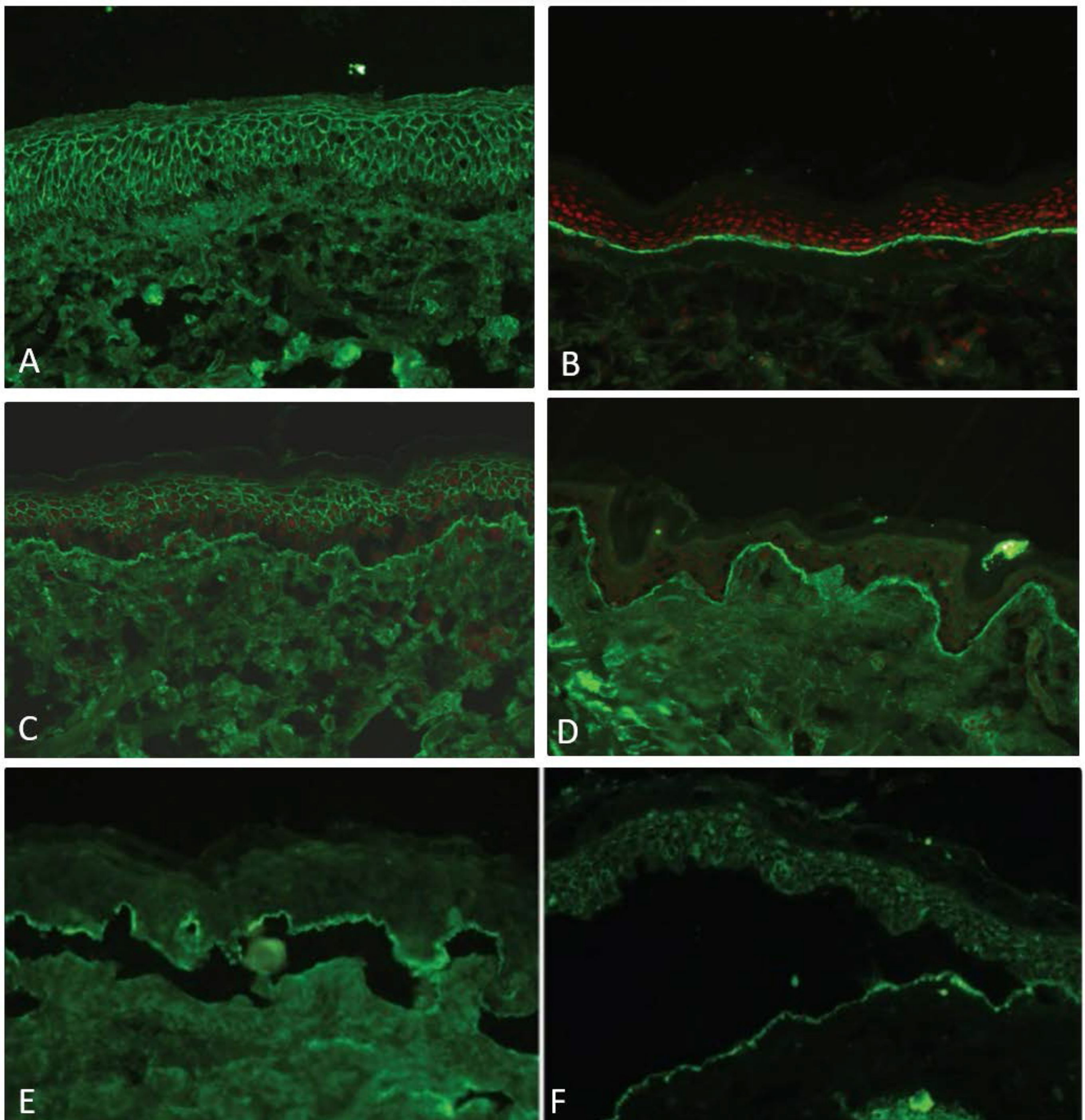

Fig. 7: A-Intercellular staining of the epidermis with IgG in pemphigus (fluorescein isothiocyanate, ×200)

B- Linear basement membrane zone staining with C3 in bullous pemphigoid (fluorescein isothiocyanate, ×200)

C- Both intercellular and basement membrane zone staining with IgG in a patient with paraneoplastic pemphigus (fluorescein isothiocyanate, ×200)

D- Linear basement membrane zone staining with IgA in linear IgA disease (fluorescein isothiocyanate, ×200)

E&F- Salt-spit technique showing IgG staining on the epidermal side of the split skin in bullous pemphigoid (E) and staining with IgG on the dermal side in epidermolysis bullosa acquisita (F) (fluorescein isothiocyanate, ×200) [40]

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methylene blue, and toluidine blue.[27]

LE cell phenomenon

The Lupus Erythematosus (LE) cell test, initially described by Hargraves for systemic lupus erythematosus (SLE) confirmation, involves identifying specific cellular inclusions indicative of SLE. In tissues, the presence of reddish-purple amorphous inclusions, known as haemotoxylin or LE bodies, signifies the degraded nuclear material of an injured cell phagocytized by intact phagocytes, a pathognomic sign of SLE.[24]

LE cells are not naturally present in peripheral blood, necessitating the use of defibrinated blood to promote their formation. This involves vigorously shaking venous blood with glass beads or forcing clotted blood through a sieve, rupturing leukocytes and exposing nuclear material. The sample is then incubated at 37°C, leading to the formation of LE cells, followed by centrifugation. Buffy coat extraction, smearing, staining, and examination complete the process. During incubation, antinuclear antibodies in the serum bind to exposed nuclear material, opsonized by phagocytes, resulting in LE cell formation.[28]

Historically, LE cell testing in buffy coat was utilized for SLE diagnosis but has been replaced by antinuclear antibody (ANA) testing. Although less sensitive and labor-intensive than ANA testing, awareness of LE cells is crucial in interpreting cytology specimens, facilitating a rapid SLE diagnosis.[28,29]

❖ **Biopsy**

When obtaining a biopsy for patients with Vesiculobullous (VB) eruptions, careful consideration is needed to elicit intra and subepithelial split histologically. Ulcerated tissues should be avoided as they may not reveal the vesicle's roof and could be obscured by secondary inflammation and necrosis.[30]

To prevent false negative results, patients are advised to discontinue topical steroids at least a month before the biopsy procedure.[31]

A 3-4 mm punch biopsy of uninvolved skin and an unblistered perilesional skin, obtained from an elliptical biopsy, are generally considered adequate specimens.

Number of Biopsy Specimens: It is ideal to obtain two biopsy specimens from the representative site, or a single biopsy specimen can be divided into two equal specimens.

One specimen is preserved in 10% neutral buffered formalin for hematoxylin and eosin staining, while the other is submitted in Michel's medium for direct immunofluorescence (DIF) studies.

A sample of the patient's serum or blood is required for both indirect immunofluorescence (IDIF) and direct immunofluorescence (DIF) studies.

In VB disease, the choice of lesions for sampling is crucial. The ideal lesions are fresh (less than 24-48 hours old), intact, non-excoriated vesiculobullae, with normal or erythematous perilesional skin included in the biopsy field.[32]

❖ **Immunofluorescence**

In 1940, Coons introduced Immunofluorescence (IF) using the blue fluorescing compound beta anthracene.[33] IF involves an antigen-antibody reaction where antibodies are labeled with a fluorescent dye. The resulting antigen-antibody complex is visualized using an ultraviolet (fluorescent) microscope.[34]

Substances exhibiting fluorescence in IF are termed fluorochromes. Notable examples include:

Fluorescein isothiocyanate (FITC) - apple-green color

Tetramethylrhodamine isothiocyanate (TRITC) - red color

Phycoerythrin - displaying red fluorescence.[34,35]

In IF, five panels are utilized, each targeting specific components:Anti-IgG,Anti-IgM,Anti-IgA,Anti-C3,Anti-fibrinogen.

These panels play a crucial role in immunofluorescence for the detection of various antibodies and antigens.

Direct Immunofluorescence

DIF is a one-step procedure that involves application of fluoresceinated antibodies to a frozen section of the skin[32]

Indirect immunofluoroscence

IDIF is a two-step procedure in which patient smear is layered on the substrate followed by the application of fluoresceinated antibodies[32]

Complement Indirect Immunofluorescence

Antigens and antibodies bind to one another to generate many molecules of complement 3 (C3). In some situations, few IgG or IgM antibodies bind to tissue antigens that they cannot be detected by IIF, complement IIF is helpful to detect antigen(s) in these conditions.[36]

Interpretetion of DIF

1. IgG staining in intercellular space staining [“chicken-wire” or “fish-net” appearance]

This pattern is seen in all types of pemphigus except IgA pemphigus but may be localized to or more intense along the upper layers of epithelium in PF.[37] C3 deposition follows the same pattern as IgG, but it is less intensely stained compared to IgG and usually detected in patients with active disease.37

2. IgA staining in intercellular space staining

This is a distinctive feature observed in IgA pemphigus and involves two recognized types: subcorneal pustular dermatoses (SPD) and intraepidermal neutrophilic (IEN) types[40]. In SPD type, IgA deposition predominantly occurs in the upper epidermal layers, while in IEN type, it is observed throughout the epidermis or constrained to the lower epidermis.[38].

3. Intercellular and basement membrane zone staining

The dual staining of epidermal intercellular substance (ICS) and basement membrane zone (BMZ) is observed in two conditions: Pemphigus erythematosus (PE) and Paraneoplastic pemphigus (PNP).[37]

A. Pemphigus erythematosus (PE) is a variant of Pemphigus Foliaceus (PF) characterized by the immunopathological coexistence of PF and lupus erythematosus (LE).[39] Direct Immunofluorescence (DIF) reveals ICS in a "fish-net" pattern along with granular BMZ staining with IgG, resembling a "lupus band."

B. Paraneoplastic pemphigus (PNP) exhibits autoantibodies against desmosomal proteins (Dsg 1 and 3, desmoplakin, envoplakin, and periplakin) and BMZ protein (bullous pemphigoid [BP] 180). PNP shows weak, diffused DIF positivity in ICS and linear BMZ staining, similar to Bullous pemphigoid with IgG and C3.[37]

4. Linear basement membrane zone staining

The linear deposition of IgG, C3, or both along the Basement Membrane Zone (BMZ) is a characteristic feature seen in various conditions, including Bullous Pemphigoid (BP), Mucous Membrane Pemphigoid (MMP), Pemphigoid Gestationis (PG), Epidermolysis Bullosa Acquisita (EBA), Bullous Systemic Lupus Erythematosus (SLE), and Anti-p200 Pemphigoid.[40] The intensity of BMZ staining with C3, compared to IgG, aids in distinguishing pemphigoid group disorders (BP, MMP, PG) from EBA. However, this pattern may not always be discernable under microscopy.[41] PG is specifically characterized by exclusive BMZ staining with C3. In the absence of clinical history, differentiation between EBA and Bullous SLE might be challenging, as Direct Immunofluorescence (DIF) features can be similar in these conditions.

Linear IgA Disease (LAD) exhibits an exclusive linear deposition of IgA along the BMZ, representing a pathognomonic feature. Occasionally, C3 deposition may also be observed.[42]

5. Granular basement membrane zone staining

Systemic Lupus Erythematosus (SLE) showcases the presence of immunoreactants deposited in a granular pattern along the Basement Membrane Zone (BMZ). This phenomenon is known as the "lupus band test." The granular deposition is evident in both lesional and sun-protected, nonlesional skin. It is typically associated with IgM, this pattern may also involve other immunoreactants or three or more immunoreactants. In contrast, Discoid Lupus Erythematosus (DLE) exhibits BMZ staining with these immunoreactants, characterized by a more homogeneous and thick appearance.[40]

6. Ragged or shaggy BMZ staining

The distinctive staining with fibrinogen is a characteristic feature observed in Lichen Planus (LP). While Direct Immunofluorescence (DIF) is not commonly recommended for LP, it proves beneficial in specific scenarios like LP–Lupus Erythematosus (LE) overlap and mucosal LP. In these situations, DIF aids in distinguishing Lichen Planus from other mucosal erosions such as Pemphigus Vulgaris (PV) and Mucous Membrane Pemphigoid (MMP).[37]

7. Papillary dermal staining

In the diagnosis of Dermatitis Herpetiformis (DH), granular deposits of IgA in the papillary dermis are a distinctive feature. There are less frequent occurrences of a similar pattern with other immunoreactants such as C3 and fibrinogen.[43]

Other types of immunofluorescence techniques available are Salt split technique, Antigenic Mapping Method, Double Staining Method, Sandwich Technique, Calcium Enhancement Indirect Technique.[13]

ELISA

When enzymes (such as peroxidase) react with an appropriate substrate, a color change occurs, which is used as a signal to detect the presence of antibody or antigen, therefore it was essential that enzyme has to be linked to an appropriate antibody.[32]

Furthermore, for the diagnosis of pemphigus vulgaris and foliaceous, there are commercially available ELISA tests that are both sensitive and specific. These tests facilitate the detection of circulating antibodies targeting desmoglein 1 and 3. The titers obtained through this technique directly correlate with disease activity. In cases of paraneoplastic pemphigus, reactivity to envoplakin and/or periplakin can be identified through immunoblotting using extracts from cultured human keratinocytes. Alternatively, a recently developed ELISA utilizes a recombinant envoplakin N-terminal fragment for detection.[43]

❖ **Western Blotting**

Immunoblotting and immunoprecipitation techniques involve the use of recombinant proteins or extracts derived from various sources, including dermis, epidermis, bovine gingiva, amnion membrane, or cultured keratinocytes.[44] These methods are instrumental in detecting a range of antibodies, such as anti-p200 autoantibodies, anti-laminin γ1 autoantibodies, antibodies targeting C-terminal stretches of BP180, and the soluble ectodomain of BP180. Additionally, these techniques are valuable for identifying autoantibodies against cell-derived forms of envoplakin, periplakin, desmoplakin, BP180, BP230, α4β6-integrin, laminin 332, and type VII collagen.[45]

Conclusion

Although numerous clinical diagnostic methods and histological examinations of biopsies are available, it is crucial to confirm biopsy specimens through Direct Immunofluorescence (DIF) microscopy. For patients positive on DIF microscopy, obtaining sera is essential for further Indirect Immunofluorescence (IIF) studies. While molecular diagnostic techniques like ELISA and immunoblotting are valuable for confirming diagnoses and monitoring disease prognosis, they come with drawbacks such as high costs, time consumption, and the need for technical expertise. Despite these alternatives, Immunofluorescence (IF) remains the gold standard for diagnosing VB lesions due to its simplicity, reproducibility, and time efficiency.

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