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Jyoti Zingade
BDS, MDS, Private Practitioner
Visnagar, Gujarat, India

Pavan Gujjar
BDS, MDS, Reader, Department of Oral and Maxillofacial Pathology Narsinhbhai Patel Dental College and Hospital Visnagar, Gujarat, India

Arun Kumar
BDS, MDS, Private Practitioner
Hyderabad, Telangana, India

Correspondence
Pavan Gujjar
BDS, MDS, Reader, Department of Oral and Maxillofacial Pathology Narsinhbhai Patel Dental College and Hospital Visnagar, Gujarat, India

Etiopathogenesis and recent diagnostic modalities of pemphigus: A review

Jyoti Zingade, Pavan Gujjar and Arun Kumar

Abstract
Pemphigus is a group of potentially fatal dermatoses with both cutaneous and oral manifestations. Characterized by the appearance of vesicle or bullae, their manifestations in the oral cavity often precede those on the skin by many months or may remain as the only symptoms of the disease. Rupture of these lesions leads to erosions or ulcerations on the surface, hence making the diagnosis even more difficult. Hence, knowledge of the clinical presentation of these disorders with accurate diagnosis and the proper evaluation of the disease activity using recent diagnostic modalities are necessary for providing appropriate treatment and prognosis. The present article enlightens the recent diagnostic modalities that can be used for the diagnostic purpose of pemphigus.

Keywords: Auto-immune, Acantholysis, Desmoglein, immunoglobulin

Introduction
Pemphigus belongs to a group of potentially life threatening organ-specific autoimmune mucocutaneous blistering diseases. It is a term derived from the Greek Pemphix (bubble or blister) characterized by epithelial blistering affecting cutaneous and/or mucosal surfaces [1-6].
Pemphigus comprises a group of autoimmune skin diseases. The two major groups are pemphigus vulgaris (PV) and pemphigus foliaceus (PF). Their difference lies in the level of acantholysis, with the former in the suprabasilar level and the latter in the subcorneal level. Other forms of pemphigus have been described, namely pemphigus herpetiformis, IgA pemphigus and paraneoplastic pemphigus (PNP) [5, 7, 8].

Epidemiology
PV is an uncommon disease with an annual incidence of 1 to 5 per million population per year. Most commonly develops in the fourth to sixth decades of life with equal male and female predilection. There is a fairly strong genetic background to pemphigus with linkage to HLA class II alleles and ethnic groups such as Ashkenazi Jews and those of Mediterranean region Other predisposing factors include garlic, drugs (Captopril, Penicillamine, Rifampicin), radiation, surgery, stress, HHV8 virus, pregnant females [2, 3, 4, 7].

Etio-Pathogenesis
Various theories have proposed to understand the pathogenesis of Pemphigus. It is known that autoantibodies play an important role in the pathogenesis and development of PV.
The Desmoglein compensation theory, 'Multiple hits' hypothesis, Antibody-induced apoptosis theory, The basal-cell shrinkage hypothesis and The apoptolysis theory. Among all Desmoglein compensation theory clarified the basic pathophysiology of pemphigus and the classification with clinical features, and has been widely used in diagnosis and assessment of efficacy and prognosis. This theory is based on the distribution of Desmoglein 1 and 3 (Dsg 1 and 3) in the skin and mucosa. This landmark concept states that the existence of any one Dsg type is sufficient to maintain the integrity of the epidermis and mucosa. The clinical typing of pemphigus (PV and PF) are determined on the basis of both the differential antigenic distribution and generation of autoantibodies.[8]The disease is characterized histologically by acantholysis which means loss of cell-cell adhesion between keratinocytes, and immuno-pathologically by the presence of immunoglobulin directed towards the cell surface of keratinocytes or circulating autoantibodies are directed against Dsg1 and/or Dsg3 [1, 5, 9].

PV is the most common variant and is characterized by circulating immunoglobulin G (IgG) antibodies directed against Dsg3, with about half the patients also having Dsg1 autoantibodies [6]. whereas**,** in PF body's immune system produces IgG autoantibodies that target the intercellular adhesion glycoprotein Dsg-1. The binding of these autoantibodies to Dsg-1, which is principally expressed in the granular layer of the epidermis, results in the loss of intercellular connections between keratinocytes (acantholysis) and the formation of subcorneal blisters within the epidermis [10, 11].

Pemphigus lesions with associated antigen and antibody [12]

Type	Clinical Features	Associated Antibody	Target Antigens
Pemphigus vulgarus (PV) i. Mucosal PV ii. Cutaneous–mucosal PV iii. Pemphigus vegetans	Persistent, painful oral lesions; skinfolds are affected; vegetans-like; fetid, reddish plaques	 IgG IgG IgG	 Desmoglein 3 Desmogleins 1 and 3 Desmogleins 1 and 3
Paraneoplastic pemphigus	Characterized by proliferation of various types of tumours, particularly lymphoid hemopathies	IgG	Desmoplakin I/II, desmogleins 1 and 3, envoplakin, periplakin, antigen 170 and 230 kilodalton
IgA pemphigus	Exudative lesions with vesicopustules	IgA	Desmocollin 1 and another unidentified antigen
Herpetiform pemphigus	Rosette-like lesions	IgG	Desmogleins 1 and 3
Drug-induced pemphigus	Mainly cutaneous lesions	IgG	Heterogeneous

Clinical Features

PV affects the mucosa and the skin, resulting in superficial blisters and chronic ulceration. Various mucosal surfaces may be involved, including ocular, nasal, oral, pharyngeal, laryngeal, upper respiratory, and ano-genital mucous membranes. Because the clinical presentation is the first indicator for further investigations, it is critical that clinicians recognize the variety of lesions, which may be a sign of PV. Oral mucosal lesions are almost invariably present, underlining the decisive role of the dental professional in promptly diagnosing the pathology [2, 4, 5]. During active stage of the lesion, when lateral pressure is applied on the blister or perilesional skin or normal appearing skin, it results in removal of upper layer of epidermis this test is known as Nikolsky's sign, named after Pyotr Vasilyewich Nikolsky, who first described this sign in 1896 [13]. It may be used as a diagnostic tool in the assessment of patients presenting with oral ulcerations. There are two types of nikolsky's sign are described – wet nikolsky's sign in which after separation of epidermis the base of skin is moist, glistening, and exudative, and the dry nikolsky's sign, if the base of eroded skin is relatively dry. Active disease is readily appreciated by presence of a wet nikolsky's sign, while a dry nikolsky's sign indicates re-epithelization beneath a remnant blister top [14]. This test is not specific for PV, however, because it can be provoked in other diseases such as paraneoplastic pemphigus, oral lichen planus, mucous membrane pemphigoid, epidermolysis bullosa, linear IgA disease, lupus erythematosus, dermatomyositis, chronic erythema multiforme, or graft versus-host disease. Clinicians should therefore not hesitate to pursue a definitive diagnosis by using proper laboratory investigations [9, 13]

Laboratory Investigations [2]

1. Exfoliative cytology
2. Histopathology
3. Direct immunofluorescence microscopy (DIF) of perilesional skin
4. Serological detection of serum autoantibodies against epithelial cell surface by indirect immunofluorescence microscopy (IIF).
5. Enzyme-linked immunosorbent assay (ELISA).

Exfoliative cytology or Tzanck Test

A fresh lesion is scraped with the end of a spatula and tissue is spread over two slides then the slides are fixed by methyl alcohol and stained with Giemsa or Papanicolaou stain. On positive smear many separate acantholytic, round, epithelial cells will be seen with large deeply staining nuclei and prominent nucleoli called as tzanck cells. These are characterised by degenerative changes which include swelling of nuclei and hyperchromatic staining [2, 4]

Histopathology

Preferentially, a 4 mm- punch excision should be taken of a fresh (<24 h) small vesicle or 1/3
of the peripheral portion of a blister and 2/3 perilesional skin (placed in 4% formalin solution) for routine histopathological analysis: intraepidermal suprabasal acantholysis in PV and PNP, or acantholysis at the granular layer in PF [2, 3, 4]

Recent Diagnostic Modalities

Fluorescence

Fluorescence and phosphorescence are both types of luminescence. When molecules with Luminescent properties absorb light, they emit light of a different wavelength. With fluorescence the emission of light occurs extremely rapidly after the absorption of excitation light, whereas with phosphorescence emission continues for milliseconds to minutes after the energy source has been removed [15].

Immunofluorescence

Immunofluorescence studies are considered the gold standard for the diagnosis of autoimmune blistering diseases. It is a histochemical laboratory staining technique used for demonstrating the presence of antibodies bound to antigens in tissue or circulating body fluids. It is an antigen-antibody reaction where the antibodies are labelled with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope. Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescence. Commonly used fluorochromes are Acridine Orange, Rhodamine, Lissamine and Calcofluor white. The fluorochromes commonly used in immunofluorescence are fluorescein isothiocyanate (FITC) (green) and tetramethyl rhodamine isothiocyanate (red).

When fluorescein (FITC) is excited by a blue (wavelength 488nm) light, it will emit a green (520nm) colour [15, 16].

Types of Immunofluorescence [15, 17]

1. Direct immunofluorescence
2. Indirect immunofluorescence

Direct Immunofluorescence (DIF) (Patient's Biopsy)

It is a one-step procedure. Skin biopsy specimen For DIF, biopsy specimen may be taken with the help of a 3-4 mm punch from perilesional skin or mucosa, a few mm to 1 cm from the edge of lesion. An uninvolved site should be taken to maximize the chances of a positive finding. Mucosal biopsies may be positive, despite the absence of clinical involvement in bullous and cicatricial pemphigoid when the skin is negative. Skin biopsy specimen is either quick frozen or placed in transport medium.

If for any reasons, the tissue cannot be frozen immediately, it is best stored at 4 °C, wrapped in saline-moistened gauze for no longer than 24 hours.

Alternatively, acceptable results can be obtained by preserving the biopsy specimens in Michel's transport medium, containing ammonium sulfate that prevents the degradation of tissues. Specimens can be stored in this medium for as long as two weeks. After removal from the fixative, the specimen must be washed three times for ten minutes each time, in the buffer and then subsequently frozen and stored at -70 °C until used. For sectioning, the metal chunk bearing the frozen tissue specimen is placed in the cryostat. Sections of 4-6 μm thickness are cut in a cryostat and taken off the cryostat by gently touching with a glass slide. The slides are then allowed to air dry for 15 minutes. Incubation with FITC-conjugates: After rinsing in a phosphate buffered saline (PBS), slides are overlaid in a moist chamber with FITC-conjugate for 20-30 minutes at 37 °C.

As a routine, the following antisera are recommended: anti-IgG, anti-IgA, anti-IgM, anti-C3 and anti-fibrinogen. The unreacted anti-serum is washed off in phosphate buffer solution for 30 minutes. The slides are then allowed to drain and should now be cover-slipped using a drop of buffered glycerine and examined under the fluorescence microscope [18].

Advantages of DIF include shorter sample staining times and simpler dual and triple labeling procedures.

Disadvantages of DIF include lower signal, generally higher cost, less flexibility and difficulties with the labelling procedure when commercially labeled direct conjugates are unavailable [17, 18]

2. Indirect Immunofluorescence (Patient's Serum)

Indirect immunofluorescence (IIF) is a two-step procedure for demonstrating circulating autoantibodies in a patient's serum. It is 10 times more sensitive than DIF. Patient's blood (5-10 ml) is taken, centrifuged to extract serum after centrifugation. Serial dilutions (1:10, 1:20, 1:40…) are made.

First Step: This consists of contact between the serum and sections of appropriate substrate, which contains the corresponding antigen. A large number of tissue substrates including monkey esophagus, guinea-pig lip/esophagus, rabbit lip/esophagus, rodent urinary bladder and normal human skin are used. 8 Sections of substrate on glass slides are incubated with (primary unlabelled) patient's serum for 30 minutes. This is followed by three 10-minutes washes in PBS.

Second Step: The second step of IIF is identical to the staining procedure of the DIF technique [16-18]

Complement Indirect Immunofluorescence (Patient's serum)

This is a very sensitive, three-step serologic technique, even more sensitive than routine IIF, by which very small quantities of circulating antibodies are detected by means of their high affinity to fix complement. The first step of this technique is analogous to the first step of IIF, except for the fact that complement in the serum in question is inactivated before and by heating for 30 minutes at 56°C. If present, serum complement-fixing antibodies bind to the antigen, during incubation with the normal tissue substrate. In the second step, the tissue sections are overlaid with a source of complement, usually a fresh normal human or guinea pig serum. The complement-fixing antibody already bound to the antigen, activates the complement cascade and generates numerous molecules of C3 at the same time. The third step consists of revealing complement C3 by staining the sections with FITC-conjugates anti-human C3 antibody. Sections are washed in PBS after different incubations. Sections are examined under a fluorescence microscope.

Advantages of IIF include greater sensitivity than DIF. Commercially produced secondary antibodies are relatively inexpensive, available in an array of colors, and quality controlled.

Disadvantages of IIF include the potential for cross-reactivity and the need to find primary antibodies that are not raised in the same species or of different isotypes when performing multiple-labelling experiments [15, 16, 18]

Enzyme-Linked Immunosorbent Assay (Elisa)

ELISA studies have been found to be a sensitive and specific tool for pemphigus diagnosis. This method uses purified recombinant human dsg-1 to detect PF IgG autoantibodies in patient serum. ELISA also provides a quantitative way to measure the amount of circulating autoantibodies, which can be used to monitor a patient's response to treatment [10, 18].

Interpretation and Microscopic Appearance of Pemphigus

Pemphigus vulgaris	DIF of perilesional skin shows linear/granular deposition IgG and C3 in a characteristic "fish net or chicken wire" pattern. IIF shows a pattern similar to DIF in 70% of patients. ELISA detects the circulating autoantibodies directed against Dsg 1 and 3
Pemphigus foliaceus	antibodies are directed against Dsg 1 which is expressed strongly within the superficial layers of the epidermis DIF pattern in PF is similar to PV ELISA detects antibodies only against Dsg 1 in PF.
Pemphigus erythematosus	DIF in PE shows "dual" pattern of staining pemphigus like in the epidermis along with granular LE like staining with IgG. IgG is detected in nearly 100% of patients whereas C3 is present in 50%–100% of cases.
Linear IgA Disease	DIF of perilesional skin shows linear staining of BMZ with IgA. IIF on salt-split skin shows "roof pattern" in majority of patients

Conclusion

Ultimately, the diagnosis of PF is based on three criteria, the overall clinical picture, including patient history and physical examination; the histopathological findings of the biopsy; and the presence of autoantibodies as detected by direct and indirect immunofluorescent studies. Neither one of these alone is diagnostic of pemphigus.

References

1. Pan M, Liu X, Zheng J. The pathogenic role of autoantibodies in pemphigus vulgaris. Clin Exp Dermatol. 2011; 36(7):703-7. doi: 10.1111/j.1365-2230.2011.04092.x. Epub 2011 Sep 9.
2. Michael Glick. Burket's Oral Medicine. People's Medical publishing house; 12th edition, 2015, 104-110.
3. Rajendran R, Sivapathasundaram B. Shafer's Textbook of Oral Pathology.6th edition.
4. Ravikiran Ongole, Praveen BN. Textbook of Oral Medicine Oral Diagnosis and Oral Radiology. 2nd edition.
5. Crispian Scully Pemphigus Vulgaris: Update on Etiopathogenesis, Oral Manifestations, and Management. Crit Rev Oral Biol Med. 2002; 13(5):397-408
6. Black M, Mignogna MD, Scully C. Number II. Pemphigus vulgaris. Oral Dis. 2005; 11(3):119-30.
7. Shamimul Hasan, Nabeel Ishrat Khan, Osama Adeel Sherwani, Vasundhara Bhatt, Himangi Srivastava. Pemphigus Vulgaris: An insight on conventional and emerging treatment modalities. Int. Res J Pharm. 2013; 4(3):8-12.
8. Dr. Chan PT. Review on Pathogenesis of Pemphigus. 2002; 10(2):62-68
9. Fassmann A, Dvořákován N, Izakovicova Holla, Lydie, Vaněk J, Wotke J. Manifestation of pemphigus vulgaris in the orofacial region. Case report. Scripta Medica Facultatis Medicae Universitatis Brunensis Masarykianae. 2003; 76:55-62.
10. James KA, Culton DA, Diaz LA. Diagnosis & Clinical Features of Pemphigus Foliaceus. Dermatologic clinics. 2011; 29(3):405-412. doi:10.1016/j.det.2011.03.012.
11. Stanley JR, Amagai M. Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. N Engl J Med. 2006; 355(17):1800-10.
12. Ben Lagha N, Poulesquen V, Roujeau JC, Alantar A, Maman L. Pemphigus vulgaris: a case-based update. J Can Dent Assoc. 2005; 71(9):667-72.
13. Ettlin, Dominik A. Pemphigus. Dental Clinics. 49(1):107-125
14. Bharathi U, Naresh Lingaraju, Srisha Basappa, Mahesh MS. Oral Pemphigus Vulgaris: A Case Report and Review 4. IOSR Journal of Dental and Medical Sciences. 2014; 13(11):I, 24-29
15. Paul Robinson J. PhD, Jennifer Sturgis BS, George L. Kumar PhD. Immunofluorescence. IHC Staining Methods, Fifth Edition year, 61-65
16. Dr. Renuka Verma, Dr. Akhtar Riaz. Immunofluorescence: A Review. International Journal of Oral Health Research & Review. 4(3):8-14.
17. Aoki, Valéria, Sousa Jr, Joaquim X, Fukumori, Lígia MI *et al*. Direct and indirect immunofluorescence. Anais Brasileiros de Dermatologia. 2010; 85(4):490-500
18. Mohan KH, Pai S, Rao R, Sripathi H, Prabhu S. Techniques of immunofluorescence and their significance. Indian J Dermatol Venereol Leprol. 2008; 74(4):415-9.