

Diagnosis of Vesicullo Bullous Lesions- Simplified

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ABSTRACT

Pathological condition affecting the oral cavity is diagnosed accurately for their appropriate management. Knowledge of clinical presentation of these disorders is necessary because as such oral vesiculo bullous lesion gets ruptured and become erosions, ulcerations hence making the diagnosis of vesiculo bullous lesions more difficult. In this article various diagnostic procedures of vesiculo bullous lesions is explained.

Keywords: Pathological, Vesicullo bullous.

INTRODUCTION

Vesiculo bullous lesions are a distinct group of oral disorders characterized by the formation of vesicle or bullae. And it is uncommon to see vesicle and bullae intra orally because due to constant masticatory pressure vesicles and bullae get ruptured and it becomes ulcers and erosions.¹ The diagnosis can be made histopathologically, clinically, and immunological methods. And thorough clinical history should be asked from the patient which includes presence of vesicles and bullous anywhere else in the body like skin, genitalia, and eyes. Since many oral lesions can cause lesions in the dermatological regions. Vesicle is defined as a superficial blister, 5 mm or less in diameter usually filled with clear fluid. And bulla is defined as a circumscribed collection of free fluid greater than 5 mm. In this article various procedures have been explained to diagnose the condition of vesiculo bullous lesions.

There are three categories by which oral vesiculo bullous lesions can be diagnosed.

1. Clinical
2. Histological
3. Molecular

Diagnostic procedures for vesiculo bullous lesions

Nikolsky's test

It was first described by Piotr Vasiliyevich Nikolsky, a Russian dermatologist.² He related that after rubbing the skin of the patient who was affected by pemphigus, there was a blistering or denudation of the epidermis with a glistening moist surface underneath.³ this was later confirmed by Lyell who described Nikolsky's sign in patients with toxic epidermal necrolysis. It is classically seen in pemphigus vulgaris. However other lesions showing sign for this are pemphigus foliaceus, graft versus host disease, paraneoplastic pemphigus, epidermolysis bullosa, oral lichen planus, bullous

pemphigoid, mucous membrane pemphigoid, chronic erythema multiforme, dermatomyositis.⁴ this test cannot be performed in oral cavity because the blisters get ruptured. In oral cavity, after applying mucosal pressure, when ulceration or blisters appears, the test is said to be positive. Mucosal pressure can be by blowing air or using a blunt instrument or by finger.

METHOD

Done by applying lateral pressure with the index finger which gives shearing force to disrupt the intercellular adhesion⁵

Biopsy-^{6,7}

Factors to be considered

1. Ulcerated tissue site to be avoided (because roof will not be present and sometimes the site may be masked by secondary inflammation and necrosis)
2. To stop topical steroid (in order to prevent false negative results)
3. Two biopsy specimens from the affected site (one specimen to be kept in 10% neutral buffered formalin for H & E staining and other is submitted in Michel's medium for DIF)
4. Sample of the patient's serum or blood
5. Sample/ lesions collected to be fresh (less than 24 to 48 hrs old)

Tzanck test^{8,9}

Tzanck in 1947, used cytology for diagnosis of VB disorders particularly herpes simplex. It is a very simple and rapid technique. Samples taken should be fresh

Procedure for Tzanck test

1. Cleaned and dried area
2. At the base of the blister a sterile needle is inserted
3. Then smear is taken from the blister which contains acantholytic cells
4. Smear is then prepared on a clean glass slide and stained using Leishman stain
5. The smear shows the presence of Tzanck cells which are formed during detachment

Tzanck cell is a large round keratinocyte with a hyperchromatic nucleus and peripheral

condensation of the chromatin

Indication

- giant cells identification that accompany in viral infections
- Acantholysis, in case of pemphigus.

Le test or le cell inclusion phenomenon

Hargraves was the one who first explained this for SLE. typical LE cell will develop if the serum from the patient suffering from SLE is added to buffy coat of the normal blood. LE cell may be neutrophils or macrophages that has engulfed the denatured nucleus of an injured cell and contains LE body.

Immuno fluorescence

IF is an antigen antibody reaction where the antibodies are labeled with the dye (fluorescent dye) and then the antigen antibody complex can be seen using UV microscope.¹⁰ Coons developed IF. Used for detection of antigens in the tissues. Gold standard technique for detection of autoimmune blistering diseases is through DIF.

Two basic methods

1. Direct immunofluorescence
2. Indirect immunofluorescence

Principle of fluorescence

An atom or molecule absorbs a quantum of light, an electron jumps to a higher energy level, thus displaces an electron from its shell. When this displaced electron returns back to its original ground state, it emits a quantum of light. This phenomenon is called photoluminescence and is of two types: Fluorescence and phosphorescence.

Fluorescence is the property by which when illuminated by a light certain substances of certain wavelength, reemit the light to a longer wavelength. In phosphorescence, even after the exciting light is cut off, emission continues to persist.

Immunofluorescent Techniques

Direct Immunofluorescence

It is the one step procedure in which fluoresceinated antibodies are applied to the frozen section of the skin.

1. Apply fluorescein- conjugated anti- human IG antibodies in patients tissue specimen
2. Wash off excess
3. View with UV microscope

Indirect immunofluorescence

1. Apply patients serum, antibodies bind to homologous structures in the section of monkey oesophagus
2. Wash off excess
3. Apply fluorescein-conjugated anti-human Ig antibodies
4. Wash off excess
5. View with UV microscope

Salt split technique

The purpose of this technique is to differentiate between two similar immunologically mediated disease having similar clinical features (case of bullous pemphigoid and epidermolysis bullosa) ¹¹

It is of two types- direct and indirect ¹²

Direct technique is performed on freshly taken patient skin biopsy or by routine DIF, the one that has previously been investigated, whereas in indirect technique, substrate used is a normal human skin, cryocut sections are prepared after artificially inducing the junctional split, and then IDIF with patient's serum is carried out.

Elisa and western blot technique

For diagnosis of pemphigus vulgaris and foliaceus. This technique can detect antibodies to desmoglein 1 and 3

1. To the surface of plastic tubes, absorb antigen and then excess Ag is removed by washing
2. Antiserum of the patient is added and excess Antibody is removed
3. Enzyme linked alkaline phosphatase is added and excess removed and incubated at 37 c
4. Finally corresponding substrate p- nitro phenyl phosphate is added
5. Optical density of yellow product is measured by spectrophotometer

CONCLUSION

Diagnosing autoimmune VB disease still remains in a dilemma. Immunoprecipitation, Western blot analysis, and elisa are the newer techniques which have evolved and gradually being used in the domain of immunobullous diseases. However, these investigations are complex, expensive, and more time consuming. The gold standard in diagnosing VB lesions is IF as it simple, reproducible, and less time is consuming technique.

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