IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 4 Ser.2 (April. 2023), PP 09-13
www.iosrjournals.org

Oral Viral Infections: Laboratory Diagnosis - A Review

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ABSTRACT:
Viral pathogen-induced illnesses frequently affect the oral cavity. Viruses isolated from the oral cavity may act as primary or secondary pathogens, direct causal agents, or adjuncts in the disease process. The mucosal processes and evolutionary stages of viruses vary, and many viruses have comparable clinical manifestations. The investigator must frequently consider the oral lesions in light of the overall clinical picture and rely on laboratory tests to make the correct diagnosis. Oral mucosal diseases have been linked to both DNA and RNA viral families. The laboratory diagnosis of a few common viral diseases of the oral cavity is discussed in this article.

KEY WORDS: *Oral Cavity, Viral Infections and Laboratory Diagnosis.*

Date of Submission: 22-03-2023 Date of Acceptance: 05-04-2023

I. INTRODUCTION

The infectious pathology that affects oral tissues is a viral disease of the oral cavity. Either cellular degeneration or an immunological response to viral proteins can result in viral diseases.Typically, viral infections develop suddenly and are accompanied by a single, multiple blisters, or ulcerations. Viral infections are commonly associated with periodontal diseases. Due to the correlation between the development of tumors and specific viral infections, it is crucial in dental practice to report oral disease early and refer patients for treatment.Health care professionals are urged to keep up-to-date on virally driven infections that may affect the health and clinical care of their relevant specialty as a result of the evolving epidemiology of viral infections.[1]

In most instances, relying solely on clinical data to make an accurate virus diagnosis is unreliable. Known epidemic settings, where a large number of cases may present with a similar clinical picture, are examples of exceptions. However, accurate virus diagnosis nearly always requires laboratory testing.The operation of virus diagnostic laboratories has undergone a significant revolution in recent decades, and they now play a crucial part in the clinical management of patients.

The most common viruses that cause infection in oral cavity are listed below:

1. Herpes simplex virus (HSV)
2. Human papilloma virus
3. Mumps
4. HIV
5. Epstein Barr Virus (EBV)
6. Varicella Zoster
7. Cytomegalovirus (CMV)

II. Herpes simplex virus (HSV)

A significant pathogenic virus known to cause mucocutaneous conditions in the oral cavity and genital region is the herpes simplex virus (HSV). It belongs to the family of herpes viruses. The two main herpes virus types that can be distinguished by distinct antibodies are HSV-1 and HSV-2. Herpetic whitlow is the term used to describe herpetic infections that arise after salivary contamination over the finger.[2]

The skin, lips, oral cavity, eyes, genital tract, and central nervous system are among the anatomical sites where the herpes simplex virus (HSV) infects. Generalized or widespread HSV infection can develop in people whose immune systems have been damaged by cancer, organ transplantation, inherited immune deficiency disease, or AIDS. It can also happen in newborns who contract the infection after the virus is transmitted through the birth canal. Most diseases that spread widely are fatal.

Diagnosis: Over a 25-year period in our practice, HSV has accounted for more than 40% of the viruses that we find in cell cultures. The "gold standard" for HSV detection, with the exception of cerebrospinal fluid (CSF)

samples taken from patients with central nervous system diseases, is the virus' optimal replication in human diploid fibroblast cell cultures, which results in detection rates that typically exceed 30%.

Modifications to the HSV cell culture detection method, the shell vial assay, and genetically modified host cells shorten the diagnostic window to 24 to 48 h after immunization, but conventional tube cell cultures must be used in addition to these methods to reach the highest level of diagnostic sensitivity. Similar to this, when low titers of HSV are present, attempts to directly detect HSV from clinical specimens using the enzyme-linked immunosorbent assay (ELISA), latex agglutination, nucleic acid probe, and fluorescent antibody methods typically fail.[3]Recent studies have suggested that PCR may be more effective at detecting HSV infections than antigen detection or cell culture techniques.

III. Human papilloma virus

Human papilloma viruses (HPV) comprise agroup of DNA viruses of approximately 200 different sub-types. They can also be classified as oncogenic and non-oncogenic. An infection with the human papilloma virus can result in a variety of typically benign skin lesions or squamous mucosal lesions. These include plantar warts, flat warts, and oral papillomas. Human papilloma virus is typically transmitted by direct contact with an infected lesion, transmission via oral fluids or food utensils may account for the acquisition of multifocal epithelial hyperplasia

Squamous Papilloma: Squamous papilloma is the most typical oral HPV lesion. (SP). These appear as tiny projections that resemble fingers, resulting in lesions with a rough or cauliflower-like surface.

Diagnosis: The diagnosis of HPV (Squamous papilloma) is confirmed by Histopathology of lesional tissue with the viral proteins being identified by immunohistochemical studies. Genotyping of the virus is possible,but it is not a routine diagnostic investigation[4]

Multifocal epithelial hyperplasia: Heck's disease, also known as multifocal epithelial hyperplasia (MEH), is most likely the clinically significant HPV oral disorder and results in multiple soft, flat, or rounded elevated nodules.

Diagnosis: The clinical picture and occasionally a review of the patient's genetic history can be used to make the diagnosis of MEH. Although types 13 and 32 are the most frequently associated types, accounting for 75–100% of the isolated HPV, many other HPV genotypes have been found in MEH lesions. Histopathology will confirm the presence of epithelial proliferation typical of HPV, and immunohistochemistry of lesional tissue can identify HPV-derived proteins. Although genotyping is not a common investigation, it is not likely to help with disease management.[5]

IV. Mumps

The mumps virus, an RNA paramyxovirus belonging to the Rubula virus genus, causes the acute, self-limiting disease known as epidemic parotitis. The virus spreads through droplets, and patients are contagious for an additional 9–10 days after the salivary gland swelling begins, which occurs 48–72 hours later. The infection takes 15 to 21 days to become contagious.

Diagnosis: The clinical picture is almost always used to make the diagnosis of mumps. While viral culture from saliva, urine, or cerebrospinal fluid (CSF) is technically possible but rarely performed, it can provide confirmatory evidence of recent infection. The detection of mumps-specific IgM or IgA class antibodies can do this. The most widely used and confirmatory test for mumps is RT-PCR (Reverse transcription – polymerase chain reaction) RT-PCR on buccal swabs is the ideal specimen for diagnosis. Acute mumps infection can be detected by the presence of serum mumps IgM. IgM response could be Transient, slow, or undetectable. This could be as a result of prior exposure to the mumps virus, whether through vaccination or unintentional infection. A person with clinically compatible mumps symptoms who tests negative for IgM does not necessarily have the mumps. Results should be interpreted carefully because false negatives are frequently encountered. The ability to identify IgM is enhanced by serum collection three to ten days after the onset of parotitis. IgG seroconversion, or a significant increase in IgG antibody titer between serum specimens from the acute and convalescent phases, is another way to identify an acute mumps infection.[6]

V. HIV

A wide range of oral disorders can arise in HIV disease, due to the failure of cellmediated immunity. In fact, it was likely that everyone with HIV would eventually develop oral disease before ART was available.

The HIV salivary gland disease itself, bacterial sialadenitis, intraparotid lymphadenopathy, primary or metastatic non-Hodgkin's lymphoma, or Kaposi's sarcoma are just a few of the lesions that can cause the salivary gland disease associated with HIV infection.

Diagnosis:

Enzyme immunoassays: In 1985, the first enzyme immunoassay (EIA) to identify HIV antibodies was released. Using viral lysate as the antigen, these first-generation immunoassays identified immunoglobulin G (IgG) antibodies to HIV type 1 but were unable to identify antibody response to various HIV-1 clades. They tested positive about 6 to 8 weeks after the infection. The sensitivity and specificity of currently used tests are not present in first-generation assays.

By creating viral antigens from recombinant proteins or peptides, the second-generation assays improved specificity.About a week earlier than the first-generation assays, they were able to identify infection.

The creation of third-generation assays was a significant step forward because they were able to identify both HIV-1/2 immunoglobulin M(IgM) and IgG and did so as early as 3 weeks after infection. Additionally, they are thought to be more sensitive than previous generations.

Although similar combination assays have been used in other nations, fourth-generation assays started to be made available in the US in 2010. P24 antigen and HIV-1/2 IgG and IgM antibodies are both detected at the same time. Fourth-generation assays have succeeded in greatly reducing the time to diagnosis, reaching as early as 2 weeks after infection thanks to their ability to detect p24 roughly 5-7 days after the appearance of nucleic acid.[7]

Confirmatory test: Due to their higher specificity, Western blot or indirect immunofluorescence assay (IFA) have historically been used as confirmatory tests. Using a substrate to create a pattern that can be interpreted as positive, negative, or indeterminate, the Western blot assay looks for antibodies that bind to fixed HIV proteins.

To test for the presence of antibodies, the IFA, a less popular alternative, combines serum or plasma samples with T cells that express HIV antigens. A fluorescent molecule-coated antihuman antibody is then used to identify the bound antibodies.

Once seroconversion takes place, the EIA/Western blot combination has shown to have high sensitivity (99.3%–99.7%) and specificity (99.7%). Confirmatory tests, on the other hand, can lag behind a third or fourth generation assay by as much as 3 weeks due to their ability to only detect IgG antibodies, which can result in false-negative results if the test is carried out prior to seroconversion.

Rapid HIV test: Rapid HIV antibody tests typically use flow-through cassettes or lateral-flow devices with porous membranes to find anti-HIV IgG and IgM in oral fluid, whole blood, plasma, or serum samples.

They have the benefit of having a turnaround time of 30 minutes or less, which is crucial for populations with poor follow-up and for women who are in labour when they present. the majority of HIV-1 and HIV-2 detections. Since lateral flow tests do not need a laboratory, they can be carried out anywhere.

They have specificities between 99.7% and 99.9% and sensitivities between 99.3% and 100%.

Rapid tests based on oral fluid are especially desirable for use in environments with scarce resources and for self-testing at home. They have been discovered to have accuracy that is comparable to blood tests and high accuracy in higher-risk populations, such as clients of STI clinics.[8]

VI. Epstein Barr Virus (EBV)

The herpes virus family includes the significant pathogen Epstein-Barr virus (EBV), which is known to infect B cells in the oropharyngeal epithelium. Infants and young children who contract EBV do not show any symptoms, but adolescents and adults who contract the virus develop infectious mononucleosis (kissings disease). Pharyngitis, lymphadenopathy, and fever make up the classic EBV triad. Fever, lymphadenopathy, pharyngitis, hepatosplenomegaly, oral ulcerations, rhinitis, or cough are additional signs of EBV infection. Children under the age of 4 are less likely to experience hepatomegaly, rhinitis, or cough. Myocarditis, hepatitis, hemolytic anemia, thrombocytopenia, aplastic anemia, splenic rupture, encephalitis, and seizures are among the side effects of EBV.[9]

Diagnosis: Different lab procedures have been used to identify EBV infection. Both non-specific heterophile antibodies and specific anti-EBV antibodies can be detected through tests, and EBV DNA can be detected using molecular biology techniques.

Heterophile Antibodies: Heterophile antibodies, which mainly accompany mononucleosis brought on by the EBV, are antibodies that agglutinate cells from other animal species. They reach their peak 2–5 weeks after the onset of the symptoms and then rapidly decline, though they may sporadically last for 6–12 months.

Due to the ease of the tests, finding heterophile antibodies can be beneficial in cases of primary infection..

Specific EBV Antibodies: The specific tests for anti-EBV antibodies use various substrates or antigens and technologies, with immunofluorescence assays (IFAs) or enzyme immunoassays (EIAs) with enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassay (CLIA) versions or more recent multiplex flow immunoassays being the most frequently used for the routine screening of EA IgG, EBNA-1 IgG, V (MFI).

IFAs typically utilise Burkitt's lymphoma patients' EBV-transformed B cell lines, whereas EIAs utilise purified native or recombinant proteins, synthetic peptides, or fusion proteins. (complete proteins or fragments of the

proteins encoded by the EBV genes). The differences in the sensitivity and specificity of the various assays are likely caused by the type and preparation of the antigens used.

Immunoblotting: To validate the screening assays, numerous immunoblotting tests have been created using viral lysates of EBV-transformed cells and recombinant antigens. Antibodies against the cell material present in patients with mononucleosis are thought to have no effect on the latter.

However, anti-p72 IgM cannot be detected by immunoblots for IgM, which may be useful for verifying the specificity of the VCA IgM detected by screening assays.VCA IgM can also be found using immunoblots for IgM in patients with acute infections.

Molecular Biology: The presence of EBV DNA has been detected and the viral load has been measured using a variety of different approaches, techniques, and protocols. Although different materials have been subjected to dot blotting, Southern blotting, PCR, and in situ hybridization, their differences in sensitivity and specificity have produced results that should be carefully considered. Real-time PCR is particularly sensitive and very helpful for defining infection status, especially in immuno-compromised patients, according to more recent studies.[10]

VII. Varicella Zoster

The Herpesviridae family of big, dsDNA viruses includes the varicella zoster virus (VZV). They cause diseases with a variety of clinical symptoms, such as mucocutaneous, genital, and neurological disorders, the most common of which are mucocutaneous lesions.

The cause of varicella, also known as chickenpox, or VZV, is an infectious, acute illness characterised by an itchy, vesicular rash on the face, chest, back, and stomach. Neonatals and people with compromised immune systems are regarded as high-risk groups for infection because the virus is most commonly spread through droplets, aerosols, or direct contact.[11]

Diagnosis:

Tzanck smear assay: Long used for the diagnosis of different skin and mucocutaneous infections with HSV and VZV, the Tzanck smear assay is a cytological, morphological examination technique.Clinical smear samples were routinely taken and transferred to glass slides, where they were stained with different dyes like Giemsa-Wright, hematoxylin, eosin, or the Papanicolaou stain and examined under a light microscope to determine the presence of the cytopathic effects. These samples could also have been taken directly from herpes or varicella lesions without any specific pretreatment. The Tzanck smear assay's poor sensitivity—only 40–50% compared to culture—limits its effectiveness, though.

Direct fluorescent antibody test: The direct fluorescent antibody (DFA) test is an assay that uses pathogen-specific fluorescein-tagged antibodies to directly identify the presence of VZV antigen in specimens without the need for a secondary antibody reaction. In particular for the detection of VZV, the clinical sensitivity of this diagnostic technique is significantly higher than that of the Tzanck smear test.

Viral culture: It is frequently used to assess the sensitivity and specificity of other diagnostic methods because VZV isolation in viral culture has long been regarded as the gold standard diagnostic modality for detecting active infection. Viral culture's capacity to isolate VZV relies on carrying out proper sample collection, transportation, and storage procedures as well as cultivating with the right permissive cell line.

Serological tests: Serology is frequently used for epidemiological research, including obstetrical risk evaluations for VZV infections, and for determining VZV immune status. 10.6% of pregnant women in Italy were found to be VZV susceptible, according to a cross-sectional study, indicating that vaccination is necessary to lower the risk of foetal complications. However, diagnosing an acute VZV illness using serology is rare.[12]

VIII. Cytomegalovirus (CMV)

The Herpesviridae subgroup Betaherpesvirinae contains the genus Cytomegalovirus (CMV). The human herpes virus type 5 (CMV), which only infects people, is one of the eight species that make up the genus. The endemic CMV virus typically effects people during infancy and is latent for a lifetime. It does not exhibit seasonal variations. Breast milk, saliva, and urine all have the potential to spread CMV to young children and babies. Usually silent, primary CMV infection can occasionally produce symptoms similar to a cold. However, in individuals who have undergone a transplant, as well as those who have HIV infection or other immune deficiencies, CMV infection can result in pneumonia, mononucleosis, and liver dysfunction.

Diagnosis: The diagnostic gold standard for congenitally CMV-infected infants is CMV detected in the urine or saliva shortly after delivery by culture or polymerase chain reaction because congenitally CMV-infected infants shed CMV in both urine and saliva. (PCR). To distinguish between congenital and acquired infections in the urine, the time frame is restricted to just 3 weeks following delivery. If a newborn becomes infected after delivery, CMV spreads and establishes latency, so it may take a few weeks before a test yields a positive result. Due to its lower cost and immunity to the effects of sample preservation and transportation, real-time PCR

technology has significantly improved the diagnostic possibilities. As a result, real-time PCR assay has lately been employed to verify the diagnosis of CMV-DNA found in urine or saliva.[13]

IX. CONCLUSION

The oral cavity is impacted by a large number of DNA and RNA viruses. Careful examination of the oral mucosa and oropharynx assists the clinician in making a diagnosis when combined with cutaneous features. Regular oral cavity examinations are essential for maintaining good oral health and determining the best course of action for any necessary treatments. Physicians will need to account for individual circumstances and special situations because there isn't an algorithm that can fit every situation. Physicians can adapt the suggested algorithm to each patient they encounter, though, if they are fully aware of the traits and restrictions of the diagnostic testing that is currently available. By knowing about the available test modalities it is easier to carry out the treatment procedure.

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Dr. Yuvaraja G B (CRI), et. al. "Oral Viral Infections: Laboratory Diagnosis - A Review." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 22(4), 2023, pp. 09-13.