REVIEW ARTICLE

Laboratory Methods for Diagnosis of Herpes Simplex Infection: A Review

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Introduction:

Genital herpes simplex virus infection is extremely common throughout the world and epidemiological surveys demonstrated a rising infection rates in most countries[1,2]. In our country, the seroprevalence of HSV-2 was found 63% and 38.2% in different studies among STD clinic attendees[3,4]. Herpes simplex infection is usually identifiable by its characteristic lesion: a thin walled blister on an inflamed base of skin. However, other condition can resemble herpes and so the diagnosis based on visual inspection is inaccurate[5]. In addition, majority of HSV infections are asymptomatic or unrecognized and most transmission to partners, or less commonly to the neonate occurs during asymptomatic condition which has been identified as a major public health concern[6,7,8]. Infection with HSV has also been shown to increase the risk of acquisition or transmission of HIV infection[9].

HSV-2 is the most common cause of genital herpes, whereas HSV-1 is the most common cause of facial herpes or cold sores, although cross infection can frequently occur[10]. However, genital HSV-2 infection is twice as likely to reactivate and recurs 8-10 times more frequently than genital HSV-1 infection[11]. So, it is important for doctors to determine whether the genital herpes infection is caused by HSV-1 or HSV-2, as the type of herpes infection influences prognosis and treatment recommendations[5]. As such, definitive diagnosis of HSV infection by laboratory testing is essential which can help patients to receive appropriate treatment, encourage them to contribute to the control of their disease and, with proper education, reduce the risk of HIV transmission.

The purpose of this paper is to review the microbiology, epidemiology and laboratory diagnosis of herpes simplex infection.

Microbiology:

HSV-1 and HSV-2 are the common names for the species formally named human herpes virus 1 and human herpes virus 2, respectively. They are classified in the genus simplex virus in the subfamily Alphaherpesvirinae in the family Herpes viridae by the International Committee on the Taxonomy of Viruses (ICTV)[12]. The diameter of the virion varies from 150 – 200 nm, and consists of a relatively large double stranded, linear DNA genome, surrounded by a tegument, capsid and envelope in a lipid bilayer carrying a virus encoded 11 different glycoproteins. The viral capsid (100-110 in diameter) is a closed shell in the form of an icosadeltahedron (T=16) with 162 capsomers arranged as 12 pentamers (vertices) and 150 hexamers (face and edges)[13].

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Epidemiology:

There is no conclusive data regarding the exact extent of HSV infection in the human population. World wide, it was estimated to be approximately 86 million people in 1999[9]. The prevalence of HSV-1 infection is higher than HSV-2 in most geographical areas of the world. HSV-1 prevalence increases in a linear fashion with increasing age, with females having a higher seroprevalence than males[14]. However, significant variation in prevalence is observed in different regions. In central Africa, HSV-1 seroprevalence is high (>90%) in population above 15 years of age. Comparatively, lower prevalence of HSV-1 is reported from India (33%) and Japan (50-60%)[15]. In Bangladesh, in Dhaka city, the prevalence was 46% in prepubescent children of 1-12 years of age[16].

Seroprevalence of HSV-2 is virtually non-existent in persons younger than 12 years of age, and high rates of infection are prevalent between the ages of 15-40 years, correlating with sexually active population[17]. HSV-2 seroprevalence is generally highest in Africa and in parts of America, and modest in western and southern Europe, usually lower in northern Europe and North America, and in Asia it is lowest. HSV-2 seroprevalence in sub- Saharan Africa is among the highest in the world, reaching approximately 80% in individuals of above 35 years[15]. In Asia, seroprevalence of HSV-2 was 63% and 23% among STD clinic attendees from Bangladesh and Japan respectively.[3, 18] In a recent data from Bangladesh, seroprevalence of HSV-2 in STD clinic attendees was found 38.2%[4].

Transmission:

Herpes simplex virus (HSV) may be transmitted from both symptomatic and asymptomatic individuals and may occur with either primary or recurrent infections. Infection with HSV-1 usually results from direct contact with infected oral secretions or lesions. Infection with HSV-2 usually results from direct contact with infected genital secretions during sexual activity, via contact with genital lesion[19]. In addition, vertical transmission of HSV may occur between mother and child during childbirth. However, the risk of infection is reduced in absence of active blisters[20].

Pathogenesis:

Primary infection begins when HSV is exposed at mucosal or cutaneous surfaces, generally the oral cavity or genital tract. At the site of infection HSV produces a short lived viraemia and then travels along the innervating neuronal axon to the neuronal cell body. Once within the neuron, the virus enters a latent state in which the usual cascade of gene expression and cytopathic result of productive infection does not occur. During latency, the HSV genome remains in the nucleus of the neuron as circular, extra-chromosomal DNA[21]. The site of viral latency is related to site of primary infection; for HSV-1 the trigeminal ganglia, and for HSV-2 the sacral ganglia, are the most common sites. Other dorsal root ganglia, including the superior cervical, vagal and geniculate ganglia, may also harbor viruses[13]. The virus remains in this state for the life time of the host, or until the proper signals reactivate the virus. After the reactivation of latent virus by a variety of inducers, eg. sunlight, hormonal changes, trauma, stress, or fever, new progeny

are generated. The progeny virus then travel through the neuronal axis to the site of primary infection, reinitiate a lytic replication cycle and produces recurrent infection[21].

Laboratory diagnosis:

Direct methods:

The direct methods are the most relevant in patient presenting with active lesions. The sensitivity of these methods depend on the stage of lesion (sensitivity is higher in vesicular than in ulcerative lesions), or whether the patient has a primary or recurrent episode of the disease (higher in primary infections), whether the sample has taken from early lesion and whether the sample is from an immunosuppressed or an immunocompetent patient (more virus is found in immunosuppressed patients)[22].

Cell culture: Isolation of HSV in culture is the preferred virological test and still considered the 'gold standard' for patients who seek medical treatment for genital ulcers or other mucocutaneous lesions[23]. Culture isolates can be typed and tested for antiviral susceptibility. Both primary cell cultures and established cell lines have been used for the diagnosis of herpes infections[22]. The specificity of cell culture is virtually 100%. However, in cell culture system, cell line is potentially hazardous, and contamination by bacteria and fungi is an inherent problem. Morover, it should be done in specialized laboratories and which is relatively expensive[24].

Embryonated chorioallantoic membrane inoculation: The chick embryo is a versatile host system in diagnostic virology, especially for isolation of some viruses[25]. It requires less preparation and upkeep than cell culture[26]. The size and appearance of pocks on the chorioallantoic membrane afford a quick diagnostic criterion for herpes simplex virus.[24] Chick embryos are used almost exclusively because of their easy availability, low cost, convenient size and relative freedom from latent infection and extraneous contamination[25].

Inoculation into animals: Corneal inoculation into rabbits is the classical method for isolating herpes simplex virus. Intracerebral inoculation into mice is more sensitive than intraperitoneal route but has no advantage in comparison to the egg method[27].

Antigen detection: Antigen detection tests are simple, rapid to perform and may be used instead of or in addition to viral culture. Common methods used for the detection of HSV antigen are direct fluorescence antibody test and enzyme immunoassay.

Immunoflurescence test: The sensitivity of antigen detection by DFA method is same as or greater than that of culture[28]. But due to specificity of the monoclonal antibodies and antigenic complexcity of the two HSV types, some monoclonal antibodies may only react with certain strains of HSV isolates, some may cross react with HSV-1 and HSV-2, and some may fail to react with some strain due to lack of specific antigenic determinants[29].

Enzyme immunoassay: A number of ELISA procedures are available for the rapid identification of HSV antigen in clinical specimen. This immunoassay has been shown to be twice as sensitive as culture for detecting HSV in late stage genital lesion and equivalent to culture for the detection of HSV in early stage genital lesion because they are not relient upon the detection of infectious virus[13].

Cytological detection by Tzank's smear: HSV infection causes typical cytopathic changes in genital epithelial cells. Scraping of lesions followed by Wright-Giemsa staining identifies the presence of multinucleated giant cells and eosinophilic intranuclear inclusions typical of HSV[19]. The test can be performed when an urgent result is needed. However, the method has low sensitivity and does not distinguish between HSV-1 and HSV-2 or between HSV and Varicella - Zoster virus[30].

Electron microscopy: Electron microscopic examination of negatively stained vesicle fluid presents one of the most rapid methods for the detection of HSV. This procedure, although rapid, is relatively insensitive and a specimen must contain at least 106 or more particles per milliliter to allow detection of virus[13].

Virus DNA detection: Virus DNA detection by polymerase chain reaction (PCR) is increasing in use. The test relies on the amplification of HSV DNA by multiple sequential temperature cycles. It can detect HSV DNA from later stages of lesions more readily than virus culture.[30] PCR is more expensive than culture, as such, Centre for Disease Control (CDC) recommended PCR test as the test of choice for detecting HSV in spinal fluied for the diagnosis of the central nervous system. It is not cleared by the USA Food and Drug Administration for testing of genital lesions[31]. However, many laboratory use PCR for identification of herpes from a lesion area, because it has a higher sensitivity and specificity.

Indirect method (serological study):

Serological testing is useful in symptomatic patients when direct method yield negative results and in asymptomatic patients to determine past or present infection. However, it does not identify the site of infection, for example, whether around the genital or facial area. This test may give false-negative results in the early stage of infection in which antibodies may not reach detectable levels and in immunocompromised patients due to delayed antibody response. As such, the test results must be interpreted in the light of the clinical situation and performed by an expert who knows how to interpret the result[10].

Accurate type specific test are based on only glycoprotein G1 (gG-1) for HSV-1 and glycoprotein G2 (gG-2) for HSV-2 that allows differentiation between established infection with HSV-1, and HSV-2 respectively[23]. Test that combine many HSV proteins without signaling out g^G are still being used and have a high chance of missing HSV-2 antibodies in people who are infected with HSV-2 or giving a positive test for HSV-2 antibodies when the person has been infected by only HSV-1. Therefore, any one seeking an accurate diagnosis of genital herpes must be sure to get a g^G- based type –specific serological test. Recommended tests include:

Here Select: This include ELISA (Enzyme-linked immunosorbent assay) or Immunoblot.

Biokit HSV-2: This test detects HSV-2 only. Its major advantages are that it requires only a finger prick and result are provided in less than 10 minutes.

Western blot test: It is highly accurate in differentiating HSV-1 and HSV-2 antibodies when used with a step to cross- adsorb antibodies to HSV-1 and HSV-2 antigens. However, it is expensive to perform, takes several days, and is not commercially available[31].

Conclusion:

The chance of missing herpes in a lesion by culture or flurescent antibody is much higher than with PCR. As such, if PCR is not affordable or available, and then a type specific serology blood test is good alternative for diagnosis of herpes infection, because both PCR and type specific serology have high level of sensitivity and specificity. However, these test are not 100% specific and therefore, there is potential for false positive results. False positive results have serious implications. Therefore, results should be interpreted in the context of clinical presentation and patient history.

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