1. HERPES SIMPLEX VIRUS (HSV)

"The name herpes comes from the Greek *'herpein'* - 'to creep'; these viruses cause chronic/latent/recurrent infections". Epidemiology of the common herpes virus infections puzzled clinicians for many years. The herpes viruses are a large family of viruses commonly infecting many animal species including humans. Approximately 100 herpes viruses have been isolated, at least one for most animal species which has been looked at *(Roizman and Pellett, 2001)*. In the latest report of the International Committee on Taxonomy of Viruses Herpesvirus Study Group *(Davison and Clements, 2005)*, the family Herpesviridae consists of three subfamilies: Alphaherpesvirinae (containing the Simplexvirus, Varicellovirus, Mardivirus and Iltovirus genera), Betaherpesvirinae (containing the Cytomegalovirus, Muromegalovirus and Roseolovirus genera) and Gammaherpesvirinae (containing the Lymphocryptovirus and Rhadinovirus genera). To date, there are eight known human herpes viruses *(Roizman and Pellett, 2001)*. Among them, herpes simplex virus (HSV) belongs to the family Alphaherpesvirinae and the genus Simplex virus *(Davison et al., 2007)*. The term herpes simplex was introduced in 1906. Convincing evidence that there were two types of HSV namely HSV-1 and HSV-2 was established in the 1960s *(Schneweis, 1962; Dowdle et al., 1967)*.

1.1. Virus classification *(Davison et al., 2007)*

Group:	Group I (dsDNA)
Family:	Herpesviridae
Subfamily:	Alphaherpesvirinae
Genus:	Simplexvirus
Species:	*Herpes simplex virus*

1.2. HSV nomenclature *(Davison et al., 2007)*

Formal name	*Abbrev.*	*Common name*	*Abbrev.*
Human herpesvirus 1	HHV-1	Herpes simplex virus [type] 1	HSV-1
Human herpesvirus 2	HHV-2	Herpes simplex virus [type] 2	HSV-2

The two viruses cross react serologically and differ in their mode of transmission. HSV-1 spread by direct contact, usually involving saliva or droplet spread from infected patients or carriers. HSV-2 is transmitted sexually or from a maternal genital infection to new born. This results in different clinical features of human infection *(Malkin, 2002).*

2. STRUCTURE OF HSV

HSV is an enveloped-virus with a large (150-250 nm in size) linear double-stranded DNA *(Mathewson Commission, 1929)*. The viral particle is wrapped from outermost by a lipid envelope with viral DNA enclosed within a capsid size of 85-110 nm in diameter. The structure between the envelope and capsid is called tegument *(Jerome and Ashley, 2003; Whitley, 2004; Davison and Clements, 2005)*. There are 25-30 structural proteins in these three layers which form the structure of the virion. There are glycoprotein protrusions on the surface of the envelope with antigenic properties (gB, gC, gE, gG, gI, gJ, gM). The capsid surrounding the viral genome consists of 162 capsomers and is icosahedral in shape. Icosahedral structure of capsid is supported by structural proteins, particularly VP21 and VP22a *(Jerome and Ashley, 2003; Whitley, 2004; Davison and Clements, 2005)*. Non-structural proteins play role in DNA replication and transcription regulation.

Herpesvirus

Virion

Nucleocapsid

Capsomeres

Protein

DNA

Tegument

Envelope

Glycoprotein spikes

Nucleocapsid
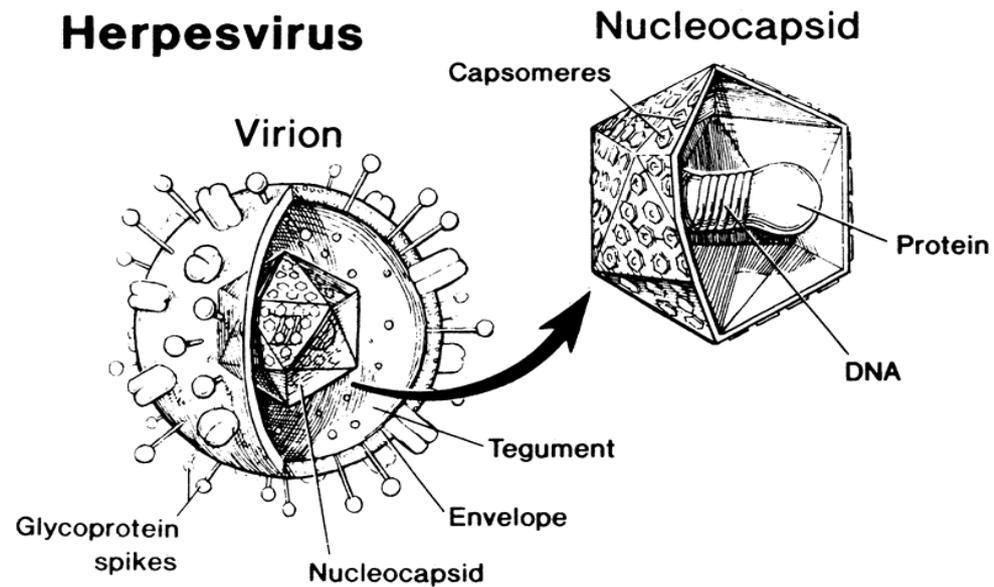
Schematic drawing of the virus particle (virion) of HSV-1 *(Liesegang, 1992)*.

These non-structural proteins act especially as enzymes. The most important of these enzymes are; DNA polymerase, deoxyribonuclease, ribonucleotide reductase, protein kinase and thymidine kinase. These enzymes are also involved in viral DNA replication *(Erturk, 1999; Whitley and Roizman, 2001)*.

3. ETIOLOGY OF HSV DISEASE

Most individuals having serologic evidence of infection with HSV-1 and/or HSV-2 are mostly asymptomatic or associated with non-specific signs and symptoms of the disease *(Whitley and Roizman, 2001)*. However, when symptoms do occur, they tend to be more severe in primary infections as compared with recurrent infections.

The most common sites of HSV infection include the skin and mucosal surfaces *(Cook and Stevens, 1973; Whitley et al., 1998)*. In general,

infections caused by HSV-1 occur above the waist and those caused by HSV-2 occur below the waist *(Lafferty et al., 1987)*. However, over the last several decades considerable overlap in site of infection has evolved and this pattern may no longer be appropriate in certain patient populations *(Lipson et al., 1988)*. The main clinical manifestations of HSV, herpes febrilis and herpes genitalis have been known for long. Herpes febrilis were described in Roman time and genital herpes was described by the French physician Jean Astruc in 1736 *(Hutfield, 1966)*.

HSV-1 and -2 cause infections manifesting as dermatologic, immunologic, and neurologic disorders *(Simmons, 2002)*. It has been shown that, in HSV infections, the sensory nervous system, rather than skin and mucous membranes, is the primary target *(Gosselin et al., 1992)*. After primary infection is established, the peripheral nervous system is the main target of the virus *(Corey et al., 1986; Whitley et al., 1998)*. The ability of HSV to lytically infect cells of the central nervous system (CNS) is illustrated by sporadic cases of potentially fatal encephalitis (*Davis et al., 1983; Schlitt et al., 1986; Koskiniemi et al., 1996; Dennett et al., 1997; Aberle et al., 2002; Widener and Whitley, 2014; Moon et al., 2014)*.

3.1. Infections of the Peripheral Nervous System

An association between HSV and the peripheral sensory nervous system has been recognized *(Simmons, 2002)*. During primary infection, virus is transported via sensory ganglia, most commonly in the trigeminal, cervical, or lumbosacral ganglia to establish a chronic latent infection *(Stevens et al.,*

1989). Depending upon the transport of virus to these ganglia, viral reactivation ensues in manifestation of spectrum of diseases ranging from gingevostomatitis to keratoconjuctivitis, genital diseases, encephalitis, and infections of new born.

Disease	Clinical Manifestation
Orolabial infection	HSV-1 commonly reactivates from the trigeminal ganglion to cause the cutaneous and mucocutaneous manifestations of recurrent facial herpes or cold sores and 20 to 40% of adults experience this disease *(Embil et al., 1975; Bader et al., 1978; Huff et al., 1981; Openshaw and Bennett, 1982; Lowhagen et al., 2002)*. A symptomatic primary HSV-1 infection, usually occurring in children, is gingivostomatitis characterized by lesions in and around the oral cavity *(Kuzushima et al., 1991)*.
Ocular HSV infections/ keratoconjunctivitis	Infection of the trigeminal nerve by HSV is a major cause of corneal scarring and visual loss *(Darougar et al., 1985; Simmons, 2002)*. Infection may be unilateral or bilateral followed by several complications *(Gallaso and Pavan-Langston, 1990)*.
Genital herpes	Genital herpes is a consequence of the lumbosacral ganglia infection with HSV. Primary infection, defined as the first encounter with HSV-1 or HSV-2, is clinically most severe, and most likely to be symptomatic *(Corey et al., 1983; Corey and Spear, 1986; Engelberg et al., 2003)*.
Cutaneous infections	*Herpetic whitlow.* This condition, caused by HSV-1 or -2 infections of the cervical and thoracic sensory nerves, is a painful infection of the digits seen predominantly in health care professionals *(Feder and Long, 1983; Klotz, 1990)*. *Eczema herpeticum.* Herpes infections also can result in severe cutaneous infection such as atopic eczema *(Bork and Bra¨uninger, 1988)* and frequently affects the head and neck if associated with autoinoculation from orolabial herpes *(Yoshida and Amatsu, 2000)*. *Erythema multiforme and Stevens-Johnson syndrome.* Erythema multiforme, an acute, usually self-limiting inflammatory syndrome, and Stevens-Johnson syndrome are often caused by recurrent HSV infections *(Huff et al., 1983; Schofield et al., 1993)*.
Neonatal herpes	Neonatal HSV infections are rare consequences of HSV Infection via three distinct routes: in utero infection, intrapartum contact, and postnatal acquisition with mother as the most common source of infection *(Sullivan-Bolyai et al., 1983; Tookey and Peckham, 1996; Mindel et al., 2000; Brown et al., 2003)*.
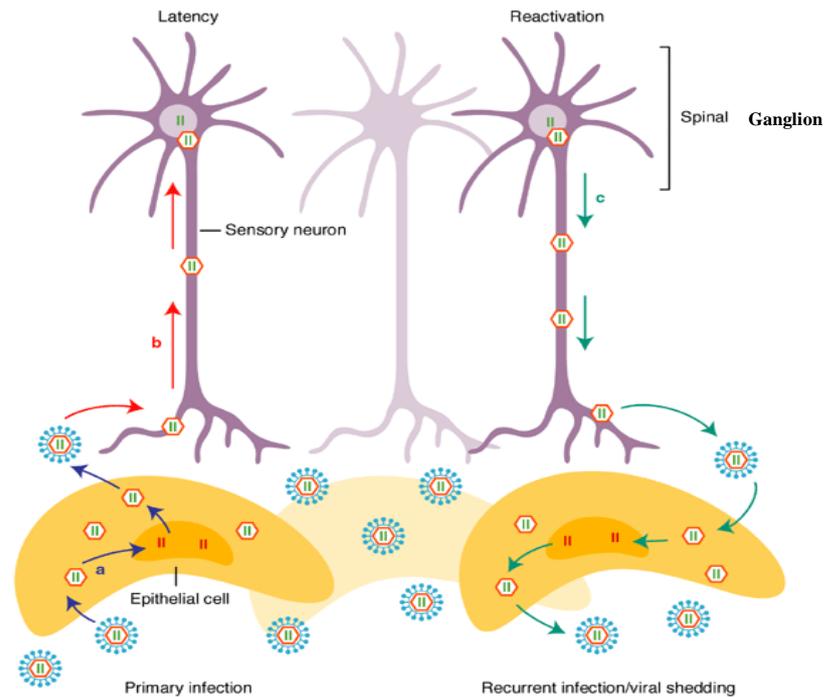
HSV infection, latency and reactivation *(Lachmann, 2003)*.

3.2. Infections of the Central Nervous System

HSV typically cause CNS illnesses of infectious and post-infectious nature which can result from a primary or, more commonly, a reactivated HSV infection *(Schmutzhard, 2001)*. HSV is the most common cause of sporadic fatal encephalitis *(Mathewson Commission, 1929; Smith et al., 1941; Meyer et al., 1960; Whitley and Alfrod 1980; Puchhammer-Stöckl E et al., 1990; Riancho et al., 2013; Sili et al., 2014; Jain et al., 2014)*. Other less common neurologic syndromes associated with HSV infection include recurrent aseptic meningitis (Mollaret's meningitis), brainstem encephalitis, ascending myelitis, post infectious encephalomyelitis, a variety of movement disorders and atypical pain syndromes, and temporal lobe epilepsy *(Schmutzhard, 2001; Simmons, 2002; Davison et al., 2007)*.

4. HERPES SIMPLEX ENCEPHALITIS (HSE)

The connection between HSV and infection of the CNS was suspected in the 1920s, when a vaccination trial was conducted *(Mathewson Commission, 1929)*. The initial cases with convincing evidence of herpes simplex encephalitis (HSE) included a child in 1941 and an adult in 1944 *(Smith et al. 1941; Zarafonetis and Smadel 1944)*. HSE has been considered to be caused almost exclusively by HSV-1 *(Whitley and Lakeman, 1995; Dennett et al., 1997; Kennedy and Chaudhuri, 2002; Hjalmarsson et al., 2007; Mailles and Stahl et al., 2009)*. HSV type-2 is rarely seen in healthy adults and usually causes benign CNS infection *(Berger et al., 2008)*. The typical CNS-manifestation of HSV-2 is meningitis, whereas severe meningoencephalitis is seen in immunosuppressed individuals *(Najioullah et al., 2000; Mommeja-Marin et al., 2003)*. Nevertheless, in 4-7% of the patients with non-neonatal focal herpetic encephalitis, HSV-2 infection has been implicated *(Nahmias et al. 1982; Aurelius et al. 1993; Sauerbrei et al., 2000; Mateen et al., 2014)*.

4.1. Epidemiology of HSE

HSV is involved in most ubiquitous of human infections and worldwide ~90% of people has been infected with one or both viruses *(Malkin et al., 2002)*. HSV cause approximately 10% of all acute encephalitides with a worldwide reported incidence of 2-4/1 million/year *(Johnson, 1998; Levitz, 1998; Stahl et al., 2011)*. Similar incidence rates have been reported in United States and European countries *(Longson, 1984; Hjalmarsson et al., 2007; Mailles et al., 2009; Child et al., 2012)*. Recently, studies have been done to determine

the etiology and epidemiology of viral encephalitis including HSE in the children and adults of Uttar Pradesh, India *(Beig et al., 2010; Jain et al., 2014)*. There is no seasonal variation of HSE throughout the year *(Whitley and Gnann, 2002)* and the disease does not show any gender difference *(Whitley et al., 1982)*. Approximately one-third of the cases are younger than 20 years and half of the patients are over the age of 50 *(Koskiniemi et al., 1996)*.

4.2. Clinical manifestations of HSE

There are no specific symptoms in HSE that differ from other encephalitides *(Whitley et al., 1982; Kohl, 1988; Rozenberg, 2013)*. About half of the patients experience a prodormal illness within a week before the onset of encephalitic symptoms which includes fever, headache and general malaise *(Riancho et al., 2013)*. Symptoms usually reach maximal level in 2-3 weeks *(Roos, 1999; Loon et al., 2004)*. During the acute onset, the symptoms include fever to almost 100%, headache, personality and behavioural changes, and focal neurological signs whereas, seizures occur in 33-67% cases and to a lesser extent, 36%, hemiparesis accompanies *(Whitley et al., 1986; Raschilas et al., 2002; Sili et al., 2014)*. HSE is often complicated by seizures *(Annegers et al., 1988; Sellner and Trinka, 2012)*. The incidence of post-HSE epilepsy has been estimated to approximately 24% *(Michael et al., 2012)*. Simple or complex partial seizures and secondary generalized seizures are also observed *(McGrath et al. 1997)*. In children, the incidence is higher (44%) *(Elbers et al., 2007; Ward et al., 2012)*.

Some patients after the initial acute HSE develop new neurological symptoms *(Steiner, 2011)*. There are at least three pathophysiologic mechanisms that may account for these complications: delayed symptoms induced by the initial infection, immunological mediated inflammatory disorders as in post infectious encephalitis, or recurrence of intracerebral viral replication *(Koenig et al., 1979; Kimura et al., 1992; De Tiege et al., 2003; Yamada et al., 2003; Landau et al., 2005)*. The incidence of these symptoms is reported to be between 4% and 26% and occurs more commonly in children *(Kimura et al., 1992)*.

A further sub classification of HSE cases, the "prolonged HSE group"has been suggested, to enable identification of patients with a more severe prognosis. *(Shoji, 2009; Taira et al., 2009)*. In human immunodeficiency virus (HIV) infected patients the typical clinical picture of HSE is rare however, a number of cases of adult HSV-2 meningoencephalitis have been reported in HIV patients *(Schiff et al., 1998; Mommeja-Marin et al., 2003)*.

4.3. Pathogenesis of HSE

HSV replication is a multi-step process. All herpes viruses induce long-term latent infections in the host. However, this process is not fully elucidated *(Griffin, 2000; Serter, 2002)*.

4.3.1. *Latent infection*

HSE development occurs with the entry of CNS disease causing virus to the brain. The source of virus resulting in brain infection has not been adequately

defined. The occurrence of infection in susceptible individual (namely one who is seronegative), requires contact of virus with mucosal surfaces or damaged skin *(Cook and Stevens, 1973)*. The viruses penetrate to mucosal receptors. HSV-1, due to its affinity to sensory and autonomic nerves, settles into the trigeminal nerve and olfactory tract ganglion neurons *(Barnett et al., 1994; Shivkumar et al., 2013)*.
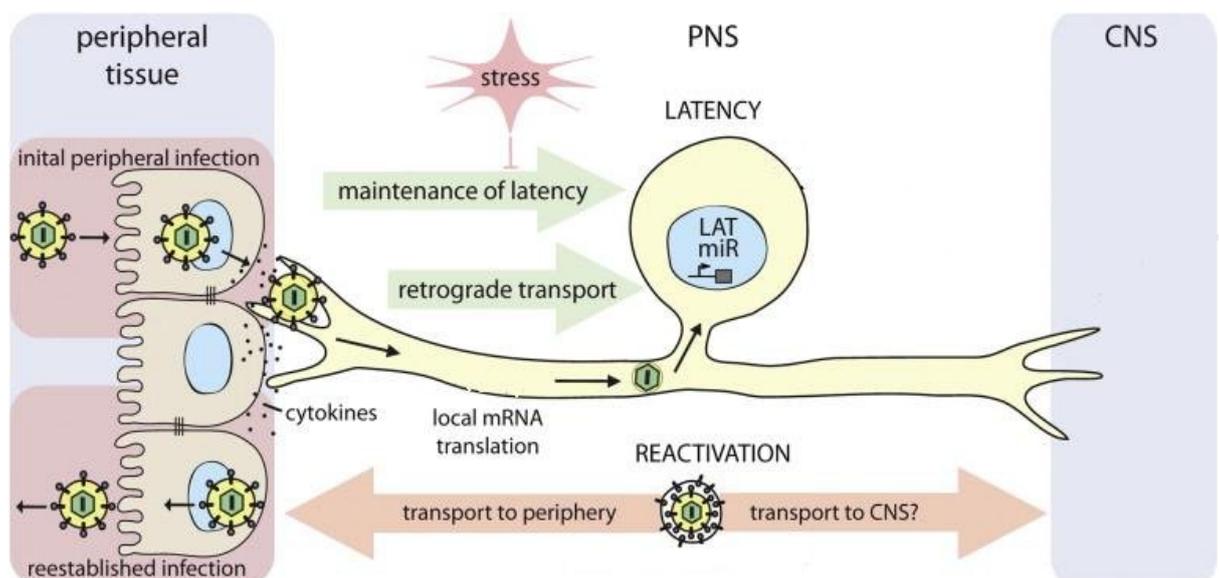
Establishment and Control of HSV latency in peripheral nervous system neurons. Virus enters the host organism through mucosal/epithelial surfaces. Entry to CNS is through retrograde axoplasmic transport *(Orkide et al., 2013)*.

Sacral ganglia have been shown to harbor HSV-2 *(Wilson and Mohr, 2012)*. HSV has the ability to establish the latency, persist in an apparent inactive state and can be reactivated by proactive stimulus. Within the day of infection, the naked virus (nucleocapsid) reaches the nerve cell nucleus, and injects its

genetic material. It then resides in nucleus of nerve cell in a dormant form. During latency HSV-1 viral activity is restricted, but a viral gene, called Latency Associated Transcript (LAT) is shown to be abundantly expressed and is responsible in establishing latency *(Perng et al., 2000; Held and Derfuss, 2011)*.

4.3.2. *Infection of the CNS and Reactivation of the virus*

After primary HSV infection, direct CNS invasion from nasal mucosa of the mouth and throat through trigeminal nerve and olfactory tract has also been suggested *(Whitley and Lakeman, 1995; Levitz, 1998; Schmutzhard, 2001)*. The disease has also been suggested to occur by virus reactivation in peripheral regions such as olfactory bulb or trigeminal ganglia and their transport to the CNS *(Griffith et al., 1967; Stroop and Schaefer, 1986; Steiner et al., 2007)*. After this primary reactivation, HSV-1 has been suggested to settle in certain areas of CNS such as the basal parts of the frontal lobes and limbic system parts of the temporal lobe via retrograde axoplasmic transport *(Cook and Stevens, 1973; Barnett et al., 1994; Schmutzhard, 2001)*. Certain animal studies have shown that virus can remain latent in the neuronal sub-populations of brain stem and cerebellum *(Rock and Frasher, 1983; Baringer and Pisani, 1994; Lewandowski et al., 2002)*. However, for humans, there is no evidence for such an access route.

A study showed that HSV reaches CNS through by both olfactory and trigeminal nerves *(Johnson et al., 1968)*. However, which of these nerve

tracts uniformly leads to HSV infection in the CNS of human is still not clear. In studies conducted on patients diagnosed with HSE, herpes virus particles along the olfactory tract were shown by electron microscopic examination of tissue samples in some patients *(Twomey et al., 1979; Dinn, 1980; Ojeda et al., 1983; Whitley et al., 1986)*. A model resembling the entry of HSV-1 in humans from the trigeminal ganglia to the CNS has been demonstrated *(Bereczky-Veress et al., 2008)*. HSV-2 infection after primary infection of the genital tract establishes latency in the sacral dorsal route ganglia. However, its entry in to the CNS via neuronal pathways has not yet been defined *(Whitley, 2004)*.

4. 4. Diagnosis of HSE

HSE is a severe viral infection of the human CNS and the most common cause of non-epidemic encephalitis contributing to 10–20% of viral encephalitis cases *(Banatvala, 2011)*. The clinical criteria (symptoms) are not reliable enough to differentiate between different causes of encephalitis, as numerous neurological syndromes may mimic HSE *(Whitley et al., 1982; Kohl, 1988)*. Therefore accurate and rapid diagnosis of HSV infections is utmost important as effective therapy is possible with antiviral drugs such as acyclovir. The therapy has to be initiated very early after the onset of disease to decrease lethality and to minimize the number of patients sustaining persistent neurological damage *(Erlich et al., 1989)*. The current clinical and laboratory analysis for diagnosis of HSE diagnostics is based on medical history and examination followed by analysis of cerebrospinal fluid (CSF) for

the identification of the infecting organism by viral cultivation, brain biopsy, polymerase chain reaction (PCR) and serology. However, due to inherent limitations of these tests the diagnostics of HSE is always challenging. The sensitivity is increased with the combination of these neurodiagnostic tests, but the specificity is still insufficient *(Dupuis, 1999; Griffin, 2000)*. The development of a HSE diagnostic test is to confirm, as rapidly as possible, a clinical impression that observed symptoms result from HSV infection.

4. 4. 1. Brain biopsy

In the past, the only method used to prove HSE was brain biopsy *(Maccallum et al., 1964; Morawetz et al., 1983)*. Brain tissue so obtained has to be examined by an experienced neuropathologist using histopathology, antigen and immunofluroscence staining. Before the advent of PCR analysis of the CSF, presence of virus particle in brain tissue obtained by biopsy was considered the gold standard for the diagnosis of HSE *(Lakeman and Whitley, 1995; Widener and Whitley, 2014)*. However, brain biopsy is an invasive procedure and is rarely performed.

4. 4. 2. Neurodiagnostic tests

The findings of computed tomography (CT) may be subtle or absent and therefore, specificity and sensitivity of brain CT for diagnosis of HSE is of little value *(Neils et al., 1987; Struffert and Reith, 2000)*. Typical changes found in CT that correlates with HSE are areas of low density causing localized mass effect which appear due to hemorrhagic brain lesions in temporal lobe

(Enzmann et al., 1978; Zimmerman et al., 1980, Marco de Lucas et al., 2006).

Magnetic resonance imaging (MRI) may suggest the findings of HSE earlier than CT; however, normal MRIs have been reported in approximately 10% of HSE patients confirmed by PCR *(Schlesinger et al., 1995; Sener, 2001, 2002)*. The characteristic MRI finding of HSE includes hyperintense areas in inferior lobes, frontal and parietal lobes and bilateral temporal lobe *(Aribas, 1996; Roos, 1999; Taber and Hurley, 2010)*. Abnormalities such as frontobasal and temporobasal hyperintense signal changes may also be seen on diffusion weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) when conventional; sequences are normal or show only minimal lesions *(Schroth et al., 1987; Maihofner et al., 2002; Djukic et al., 2003; Hatipoglu et al., 2008)*.

Electroencephalography (EEG) provides limited information for the diagnosis of HSE. Frontotemporobasal dysrhythmia and a slowdown in frequency are observed in the EEG *(Whitley et al., 1982; Bewermeyer et al., 1987; Al-Shekhlee et al., 2006)*. Periodic lateralized epileptiform discharges originating from the temporal lobe develops in some patients. However, their absence does not exclude or significantly reduce the likelihood of HSE *(Upton & Grumpert, 1970; Smith et al., 1975; Chien et al., 1977; Longson, 1984; Panagariya et al., 2001)*.

4. 4. 3. Examination of the CSF

With disease progression, CSF analysis demonstrates elevated mononuclear cells and protein. Lymphocytic pleocytosis in CSF, of between 100 and 500 cells/mm^3 occurs in most of the patients with HSE *(Whitley, 2004)*. Polymorphonuclear leukocyte domination is seen in CSF in the early periods of HSE *(Griffin, 2000; Hanson et al., 2007; Riera-Mestre et al., 2009)*. The elevated protein of approximately 60-700 mg/dl is obtained in 80% of patients, however, values higher than this is rarely obtained. CSF glucose level is almost always within the normal range and CSF examination can be normal in 5-10% of cases especially in children *(Griffin, 2000; Mook-Kanamori et al., 2009; Jakob et al., 2012)*.

4. 4. 4. CSF virus culture

Although viral culture is considered the "gold standard" for HSV detection, it has several inherent limitations, including a lack of 100% sensitivity and hence it is less preferred for diagnostic purpose *(Boerman et al., 1988; Calvario et al., 2002; Tyler, 2004)*. It is difficult to isolate viruses from the CSF using standard culture technique e.g. HSV CSF culture are positive in only about 4% of adult patient although the viruses may be isolated from 25-40% of CSF cultures obtained from infants with neonatal herpes *(Boivin, 2004)*. The main problem with cell culture is the long period (up to 4 weeks) required for a result to be available. Therefore CSF cultures are of limited value in the diagnosis of HSE. It is also not possible to culture virus in many

clinical laboratories because of non-availability of the essential infrastructure with respect to various biosafety requirements.

4. 4. 5. *PCR of HSV DNA in the CSF*

Molecular methods such as PCR for the diagnosis of viral CNS infections are now recognized as the standard laboratory method for the diagnosis of viral infections. Several studies using PCR protocols have been reviewed and have demonstrated the utility of the laboratory molecular diagnosis of HSE and other CNS infections *(Aurelius et al., 1993; Yerly et al., 1996; Read et al., 1997; Tang et al., 1999)*. PCR has been considered to revolutionize the HSE diagnosis by amplifying HSV nucleic acid from CSF *(Aurelius et al., 1991, Koskiniemi et al., 1996; Baringer, 2000)*.

PCR has been found to be a valuable tool for the early diagnosis of HSE. PCR followed by oligonucleotide hybridization has been done in CSF samples of HSE and its comparison has been done with culture to obtain 100% sensitivity and specificity for the detection of HSV DNA in CSF *(Puchhammer-Stöckl et al., 1990; Rowley et al., 1990)*. Lakeman and Whitley *(Lakeman and Whitley, 1995)* reported the sensitivity of PCR for laboratory diagnosis of HSV CNS disease where HSV DNA was amplified in the CSF of patients whose tissue failed to yield the virus by conventional techniques. Utility of nested PCR assay has also been studied in CSF samples of patients with HSE, in relation to HSV/viral antigen in a brain biopsy sample or at necropsy where almost 100% PCR result remained positive in

samples drawn up to 27 days after the onset of neurological symptoms *(Aurelius et al., 1991)*. Detection of HSV-1 and HSV-2 DNA by nested PCR in cases of immunocompetent post neonate patients with suspected HSE has also provided good results where intrathecal HSV-2 antibody response confirmed the findings *(Aurelius et al., 1993)*.

4. 4. 6. Real-time PCR

PCR analysis by real-time monitoring of DNA amplification reactions has been described earlier *(Higuchi et al., 1992)*. Real-time PCR has been introduced recently for the detection of HSV DNA in the CSF *(Kessler et al., 2000; Weidmann et al., 2003; Kawada et al., 2004)*.

Real-time PCR can be performed using different chemistries, such as SYBR Green dye *(Wittwer et al., 1997)* and TaqMan *(Livak et al., 1995)*. Both systems have proven useful in monitoring and quantification of pathogens in clinical specimens. However, the two assays demonstrate variable sensitivities and specificities. The use of individual TaqMan probes is superior to SYBR Green technology because TaqMan probe assays are more specific and lack false-positive results due to nonspecific amplification products, which are detected by SYBR Green technology *(Weidmann et al., 2003)*. The advantages of using SYBR Green over a 5'-nuclease assay with TaqMan probes are the relative simplicity and the reduced cost of SYBR Green compared to TaqMan probes *(Paudel et al., 2011)*. Real-time SYBR green PCR has been shown to be more sensitive than Taqman assay for the

detection of DNA of infectious organism *(Ponchel et al., 2003; Hanaki et al., 2014)*.

Real-time quantitative PCR is the reliable detection and measurement of products generated during each cycle of the PCR process which are directly proportional to the amount of template prior to the start of the PCR process. The measurement of PCR products as they accumulate, i.e., quantitative real-time PCR (qPCR) allows quantitation in the exponential phase of the reaction and therefore removes the variability associated with conventional PCR. The standard curve method has been used in circumstances when absolute quantitation is critical for the investigator, for example in quantitation of viral load *(Borg et al., 2003; Lin et al., 2004)*. SYBR Green based qPCR also provides a rapid, easy and accurate diagnosis of herpes virus CNS disease including viral load as supplementary information. A quantitative system using real-time PCR assay has recently been developed to measure relative quantitiy of HSV in patient's CSF samples *(Ryncarz et al., 1999; Kimura et al., 2002; Muñoz-Almagro et al., 2008; Murphy and Caliendo, 2009)*. Quantification of HSV DNA has also been used as a means of evaluating the antiviral effects of candidate drugs *(Schloss et al., 2009)*. It has been shown that quantitation of HSV viral load may be useful for assessing the prognosis, and may provide additional information for the management of HSV infection *(Domingues et al., 1998)*.

4. 4. 7. Antibody detection in the CSF

In earlier studies, detection of anti-HSV antibodies in CSF has been used as another method for diagnosis of HSE *(Cesario et al., 1969)*. It has been shown that detection of antibodies specific to HSV in CSF is useful for HSE diagnosis at periods of removal of virus from CSF (usually the first 1-2 weeks after infection) *(Lanciotti et al., 2000)*. Detection of HSV specific immunoglobulin M (IgM) and immunoglobulin G (IgG) in CSF have been widely used *(Petersen and Marfin, 2002; Holzmann, 2003)* using several immunological methods such as neutralization, complement incorporation reaction, haemagglutination, indirect immunofluorescence, "radioimmunoassay" and enzyme linked immunosorbant assay (ELISA) *(Griffin, 2000; Serter, 2002)*.

HSV-1 infections elicit strong antibody responses to the HSV-2 and vice versa due to high sequence homology between the two viruses *(McClung et al., 1976; Eberle and Courtney, 1981)*. Type-common as well as type-specific antibody responses have been determined using serological assays to detect CSF antibodies and discriminate between HSV-1 and HSV-2 infections *(Kahlon et al., 1987; Ashley, 1998; Görander et al., 2003)*.

The approach of peptide synthesis has advanced the use of synthetic peptides to study protein-protein interactions *(Merrifield, 1963)*. Several immunodiagnostic tests for the detection of antibodies have been developed for the detection of viral, bacterial and parasitic diseases based on synthetic

peptides derived from antigenic proteins *(Gómara and Haro, 2007)*. Studies have been done to detect antibodies using peptides designed with the aid of online software *(Morey et al., 2010)*.

Reports have shown that high titers of HSV specific antibodies in CSF are characteristic of HSE and measurement of these titers could be useful for early diagnosis of HSE *(Levine et al., 1978; Hanada et al., 1988)*. Several methods for evaluating ELISA have been reported for the determination antibody titer values in the CSF of patients infected with HSV *(Roberts-Thomson et al., 1976; Cremer et al., 1982)*. However, recent reports evaluating the role of different assays for the determination of antibody titer in CSF of HSE patients are lacking. Studies have been done to develop single dilution ELISAs for the determination of antibody titer against antigenic proteins of several infecting organisms *(Kumar et al., 2003; Dey et al., 2008)*. However, there is no report available for the determination of antibody titers in HSE using the method of single dilution ELISA.

Studies have been done determining whether there is a correlation between the magnitude of DNA load and specific seroresponsiveness. In this context, correlation between viral load and antigen specific antibody titers has been determined for several viruses *(Besson et al., 2006; Pastrana et al., 2012)*. However, no report is available determining the correlation between antibody titer and viral load in the CSF of HSE patients.

4. 4. 8. <u>Antigen detection in the CSF</u>

The detection of the viral antigen can be an alternative to isolation of the virus or the detection of viral nucleic acids, IgG, or IgM. A rapid antigen detection test that uses ELISA for the detection of HSV infection may be a more accurate diagnostic method for patients in the acute stage of infection.

ELISA systems are specific but lack sensitivity (sensitivities ranging from 46 to 76%) in detecting HSV antigen directly from clinical specimens *(Morgan and Smith, 1984; Warford et al., 1984)*. Additionally, immunologic analyses of CSF for the presence of specific antigens have not yielded sensitive tests for the laboratory diagnosis of HSV-related CNS disease *(Coleman et al., 1983; Ho and Hirsch, 1985)*. Studies have been reported, based on the evaluation of hyperimmune sera for antigen detection in clinical samples for the diagnosis of certain infections *(Kashyap et al., 2010)*.

HSV antigens could be effectively detected in the samples, but cross reactivity could not be avoided due the similar sequence of large sized protein. For instance in a study, ELISA was developed for the detection of circulating *Candida* mannan antigen based on the use of a monoclonal antibody, as the antigen cross-reacts with a wide range of different *Candida* species *(Rimek D et al., 2003)*. The use of a peptide that targets a specific region of the protein as an immunogen, may overcome these problems and hopefully generate antibodies with lesser cross reactivity *(Wang RW et al., 1996)*. Anti-peptide antibodies are excellent tools for biologic research and discovery *(Saravanan*

et al., 2004; Pattnaik et al., 2006). Synthetic peptides are used for the production of antipeptides by immunizing rabbits against the peptides conjugated to keyhole limpet hemocyanin (KLH). Studies have been reported, based on the evaluation of antipeptide antibodies for antigen detection in clinical samples for the diagnosis of some infections *(Pattnaik et al., 2006)*. However, there is no report about the diagnostic utility of antipeptide antibodies in HSE.

Antigen concentrations have been well correlated with virus load by several workers *(Kimura et al., 2002; Ganji et al., 2011; Marchetti et al., 2011)*. Correlation between quantitative antigen titers and viral load has been demonstrated for several diseases such as for hepatitis B and hepatitis C virus *(Thompson et al., 2010; Shen et al., 2011; Park et al., 2012)*. Some workers have shown that, viral load below the detection limit of the PCR was predictive of the loss of envelope antigen *(Zöllner et al., 2001)*. The findings of studies for determination of antigen titer may provide new insights into viral pathogenesis and have practical implications for the use of quantitative serology as a clinical biomarker in HSE.