



Summary

Human herpesviruses cause various acute, sub-acute, and chronic disorders of the CNS and peripheral nervous systems in adults and children. Among herpesviruses, HSV-1 and -2 causes a wide spectrum of clinical manifestations like meningitis, encephalitis, myelitis etc. in the CNS of infants as well as adults. HSE represents one of the most severe infectious diseases of the CNS. However, clinical criteria (symptoms) are not reliable enough to differentiate between different causes of encephalitis, as numerous neurological syndromes may mimic HSE. The effective antiviral drugs are available, and therefore, early rapid and reliable diagnosis has become important. In India as of today, HSE appears to be under diagnosed, probably due to lack of awareness and sensitive diagnostic facility. Therefore, development of HSE diagnostic tests in this context are required to confirm, as rapidly as possible, a clinical impression that observed symptoms result from HSV infection.

The first aim of the study was to ascertain the usefulness of PCR methodology to routine diagnostic testing for HSV infections of the CNS in clinical specimens submitted for PCR analysis of the CSF at our Institute. The prevalence of HSV genotype (HSV-1 or -2) was also determined in the study population. Secondly, I developed real-time PCR methodologies including the qPCR assay. This protocol could estimate the extent of viral replication in CNS of HSE patients and hence was used for disease prognosis and for monitoring HSE treatment. PCR was negative in cases where CSF has been obtained at late times following onset of illness or because of low viral load in CSF. I also determined the type-common and type-specific antibody response

to HSV-1 or -2 against the synthetic peptides of the envelope glycoprotein of HSV in CSF of HSE patients. Immunologic analyses of CSF for the presence of specific antigens have not yielded sensitive tests for the laboratory diagnosis of HSV-related CNS disease. I developed better methods to improve sensitivity. HSE patient's hyperimmune sera and antipeptide antibodies were utilized to develop a more specific and cost effective antigen detection ELISA. I also correlated the status of antigen, antibodies and viral load in initial and follow up samples of patients so as to determine the stage at which any of the moiety will have diagnostic significance.

The aim of the study as discussed in **Chapter 1** was to ascertain the usefulness of PCR methodology for diagnosis of HSV infections of the CNS in clinical samples of patients admitted to a tertiary care centre, Central India Institute of Medical Sciences, Nagpur. The in-house PCR assays were developed by optimizing the conditions for the detection of HSV and the results were compared with the commercial kit. Two sets of gene targets were used for the development of in-house PCR assay, one targeting the UL30 gene (existing primers) for type-common detection of HSV and the other targeting the type-specific region of US3 gene (newly designed) of HSV-1 and HSV-2 to differentiate between the two on the basis of PCR. The results obtained in in-house PCR assays were similar to the results obtained by the commercial kit for detecting the presence of HSV DNA in CSF. Among 140 CSF samples of patients initially suspected for HSE out of a total of 319 cases, 35 CSF samples were found to be positive for the presence of HSV by PCR using commercial kit and in-house PCR protocol using existing primers,

whereas 105 cases were negative for both. Type-specific analysis using newly designed primers showed that the 35 samples were positive by HSV-1 PCR, whereas none of the samples were positive by HSV-2 PCR.

In **Chapter 2**, the sensitivity and specificity of the real-time PCR was assessed using the two fluorescence chemistries i.e., Taqman probe with reporter and quencher dye and SYBR-green dye as the sources of the fluorescence. A qPCR assay was then developed to measure viral load of HSV and evaluation of possible relationships was done between HSV DNA concentration in CSF with that of patients clinical and laboratory manifestations. The prevalence of the type of HSV in the study population was also determined using type-specific real-time PCR analysis. The SYBR-Green based real-time PCR assay for the detection of HSV was found to be more sensitive as compared to that of Taqman based detection system and therefore the former was further used for detection and quantification purposes. The qPCR results showed that in patients with higher viral loads in their CSF, greater number of cases showed the presence of lesions in the brain as revealed by computed tomography or magnetic resonance imaging scan. They required acyclovir therapy for longer duration and had poor clinical outcome than the patients with lower viral loads in their CSF. The real-time analysis using type-specific primers also showed the presence of predominantly HSV-1 genotype in the study population.

The next aim of my study was to identify an immunodominant epitope determining the antibody response to HSV in CSF of HSE patients (**Chapter**

3). The synthetic peptides that resembled type-common as well as type-specific domains of glycoproteins gB and gG of these viruses were evaluated by ELISA for binding with IgM and IgG antibodies in CSF samples from HSE and non-HSE patients. The QLHDLRF peptide, derived from gB of HSV was found to be an immunodominant epitope in the IgM and IgG antibody response. The total positivities of 86% and 75% for both IgM and IgG antibodies were obtained in the patients with confirmed and suspected HSE, respectively using this peptide. These results demonstrate that a synthetic peptide-based diagnosis of HSE can be an efficient and easily accessible alternative. This is the first report describing antibody detection using synthetic peptides derived from HSVs in diagnosis of HSE using patients' CSF samples. After the development of antibody detection ELISA using synthetic peptide, I then determined antibody titer against the identified immunodominant epitope in CSF of HSE patients. The method of single CSF dilution ELISA was adopted to determine the IgM and IgG antibody titer values in CSF of HSE patients. The antibody titers obtained in ELISA using the synthetic peptide were also correlated with HSV DNA levels obtained from the qPCR assay. A weak negative correlation of IgM antibody titers and a positive correlation of IgG antibody titers were obtained with viral load in the CSF of HSE patients. This assay gave reproducible results and helped to save sample, time and cost of reagents. As per my knowledge, this is the first report describing the use of synthetic peptide of HSV for determining antibody titer in CSF of HSE patients.

In **Chapter 4**, development of a sensitive ELISA protocol for HSV antigen detection is discussed. An in-house ELISA protocol was developed for HSV antigen detection by assessing the usefulness of hyperimmune sera isolated from HSV seropositive patients. To improve the specificity of the method, protein A was incorporated in the protocol for ELISA. The modification in the ELISA method utilizing hyperimmune sera along with protein A for HSV antigen detection yielded good sensitivity and specificity for antigen detection in both CSF and sera and hence can be useful for the diagnosis of HSE.

Another method for detection of specific antigen of HSV was developed as discussed in **Chapter 5**. The ELISA protocol was developed for HSV antigen detection and quantitation by assessing the usefulness of anti-peptide antibodies obtained against potential peptides of HSV. CSF samples of HSE and non-HSE patients were analyzed using a panel of anti-peptide antibodies against synthetic peptides of HSV glycoprotein gB. The cases of confirmed and suspected HSE showed good positivity for the detection of HSV antigen in CSF using anti-peptides against synthetic peptides QLHDLRF and MKALYPLTT. The concentration of HSV antigen was also determined and was found to be higher in confirmed HSE as compared to suspected HSE group. The viral load as determined by the qPCR assay correlated well with antigen concentration obtained using the two anti-peptides in CSF of confirmed HSE group. This is the first report describing the use of antibodies obtained against synthetic peptides derived from HSV in diagnostics of HSE using patients' CSF samples.

The developed techniques presented in the thesis can be used in the identification of HSV infections of the CNS. The conventional PCR assay can be used for detecting the presence/absence, whereas the real-time PCR assay can help to determine the load of the organism in clinical samples. The synthetic peptide-based approach can be an efficient and easily accessible alternative for detection as well as titer estimation for IgM and IgG antibodies against HSV using patients' CSF samples for the diagnosis of HSE. The developed antigen detection protocols using hyperimmune sera and antipeptide antibodies demonstrates a unique approach for identifying and estimating whole and specific antigens of HSV. Thus, the developed methods can be useful for the diagnosis as well as prognosis of HSE.