

POLYMERASE CHAIN REACTION ASSAY OF CEREBROSPINAL FLUID FOR DETECTION AND QUANTIFICATION OF HERPES SIMPLEX VIRUS DNA FOR DIAGNOSTIC AND PROGNOSTIC EVALUATION OF HERPETIC ENCEPHALITIS. (ABSTRACT)*. THESIS. SÃO PAULO, 1997.

RENAN BARROS DOMINGUES **

Herpes simplex virus (HSV) DNA detection in cerebrospinal fluid (CSF) samples by polymerase chain reaction (PCR) has been recently evaluated as a rapid non-invasive diagnostic assay for HSV encephalitis (HSE) diagnosis. It has been demonstrated that PCR is a sensitive and specific assay for HSE diagnosis when comparing to viral isolation from brain tissue obtained by biopsy. However, further studies are necessary to clarify the precise role of HSE diagnosis by PCR in the clinical practice. Also, because of the invasiveness of brain biopsy, it has been suggested that CSF PCR may reveal a wider spectrum of the disease than previously recognized by brain biopsy studies.

In this study, two different PCR protocols for HSV DNA amplification from CSF samples were described; one that amplifies a 179 bp region of DNA polymerase gene and other that yields 148 bp products from glycoprotein B gene. Both PCR products were applied to CSF samples obtained from 29 patients with focal, 12 patients with mild and 8 patients with diffuse encephalitis.

PCR was positive in 15 of 29 (51.7%) patients with focal encephalitis, and in 3 of 12 (25%) patients with mild encephalitis. As demonstrated by enzymatic digestion of DNA polymerase products, 17 (94.4%) PCR positive patients had Herpes simplex virus type 1 (HSV-1) infection and one (5.6%) had Herpes simplex virus type 2 (HSV-2) encephalitis. The presence of temporal abnormalities on electroencephalography, computed tomography of the brain, or cranial magnetic resonance imaging showed a high correlation with a positive PCR. The use of PCR has allowed the diagnosis of HSE in atypical and less severe forms of encephalitis, demonstrating a wider spectrum of clinical presentations of HSE than previously known.

A competitive PCR (QC-PCR) assay was developed to quantify HSV-1 DNA in the CSF of 16 patients with HSV-1 encephalitis. The viral quantification aimed to correlate the amount of HSV-1 DNA with severity of disease and outcome, and assess the effect of acyclovir treatment on viral DNA levels. An internal standard (IS) with an internal 25 bp deletion that uses the same primers as the target HSV-1 DNA was generated. A standard curve was constructed for each experiment by coamplification of known amounts of HSV-1 DNA and the IS. Quantification of the samples was obtained by coamplification with the samples with the IS and by plotting the ratio sample/IS onto the standard curve. Using this assay, higher amounts of HSV-1 DNA in CSF correlated with increased severity of disease, higher age, and poor outcome. Also, a decline in HSV-1 DNA was observed only in patients with good clinical response to acyclovir treatment. The application of this assay proved to be helpful in the prognostic evaluation and for monitoring the antiviral treatment of patients with HSE.

The use of PCR techniques by experienced personnel is an important tool for the management of encephalitis, favoring a better outcome of the HSE patients.

KEY WORDS: polymerase chain reaction, cerebrospinal fluid, Herpes simplex encephalitis.

* Reação em cadeia por polimerase para detecção e quantificação do genoma do vírus Herpes simples em amostras de líquido céfalo-raquidiano na avaliação diagnóstica e prognóstica da encefalite herpética (Resumo). Tese de Doutorado, Faculdade de Medicina da Universidade de São Paulo (Área: Patologia). Orientadora: Ana Maria Crous Tsanaclis.

** Rua Maestro Cardim 808, 01323-001 São Paulo SP, Brasil.

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