Original Article

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Cerebrospinal Fluid (CSF) Analysis for Herpes Simplex Virus (HSV) Detection by Real-Time Polymerase Chain Reaction

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Abstract

The current work demonstrated about the clinical relevance of the molecular characterization of Herpes Simplex Virus in CSF specimens for the detection of Neurotrophic virus i.e HSV genotypes 1 & 2 in meningitis/ encephalitis cases.

Keywords: Herpes; Real Time PCR; Amplification Plot; Genotypes.

Introduction

Infection with the herpes simplex virus, commonly known as herpes, can be due to either herpes simplex virus 1 (HSV-1) or herpes simplex virus 2 (HSV-2). HSV-1 is mainly transmitted by oral to oral contact. HSV-2 is a sexually transmitted infection that can cause painful genital ulcer disease [1,2].

Herpes simplex virus - type 1 (HSV-1), Herpes simplex virus - type 2 (HSV-2), HSV-1 is a highly contagious infection, which is common and endemic throughout the world. The virus causes lifelong infection, and there is no cure, although treatment can reduce symptoms. It is mainly transmitted through oral-oral contact and causes or labial herpes / herpes labialis, or "cold sores". HSV-1 can also be transmitted to the genitals through oral-genital contact, leading to genital herpes. In addition, mothers with HSV-1 genital infection can transmit the virus to the neonate during labour, which can cause neonatal herpes, a rare but fatal condition [4-7].

Materials & Methods

Cerebrospinal fluid was collected from various Departments of Shri Mahant Indiresh Hospital, Patel Nagar, Dehradun, and Uttarakhand. 20 cerebrospinal fluids (CSF) were collected from the patients with Neurological disorders which mainly include seizures, epilepsy, meningitis, hydrocephalus, altered sensonium etc. Further the CSF was preprocessed for DNA isolation and DNA was extracted by silica column method [8-10].

Results

The amplification of glycoprotein gene in HSV was done by Qiagen Rotor-gene Q Real Time PCR. Pre PCR mix was prepared as per the manufacturer's protocol (artus HSV-1/2 PCR Kit CE-QIAGEN). All the 20 cases were processed for the molecular genotyping of HSV-1 and HSV-2 along with exogenous internal control which is used to check for any artifacts in processing and in PCR protocol. Direct detection of the florescence can be detected as specific amplification plots which can be visualized in channel cycling Green (470nm) and cycling Orange (detector 610nm) and Internal Control fluorescence channel cycling Yellow (530nm- 555nm) for HSV-1, HSV-2 and internal control respectively. Out of 20 cases, 02 came positive (as tabulated in Table 1).

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Table 1: Molecular profiling of HSV 1 &2 in CSF specimens (10 cases only)

Sr. No.	Type of specimen	HSV-1/2 RESULT						NTC^	RESULT
		HSV-1	CT#	HSV-2	CT	IC*	CT#		
1	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
2	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
3	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
4	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
5	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
6	CSF	Positive	26.17	Negative	-	positive	26.86	28.07	Positive
7	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
8	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
9	CSF	Negative	-	Negative	-	Positive	26.86	28.07	Negative
10	CSF	Positive	27.95	Negative	26.65	Positive	26.86	28.07	Positive

#CT- Cycle Threshold
***IC- Internal control**
^NTC- No template control

Samples	Sequence Channel
HSV1	Green
HSV2	Yellow
Internal control	Orange

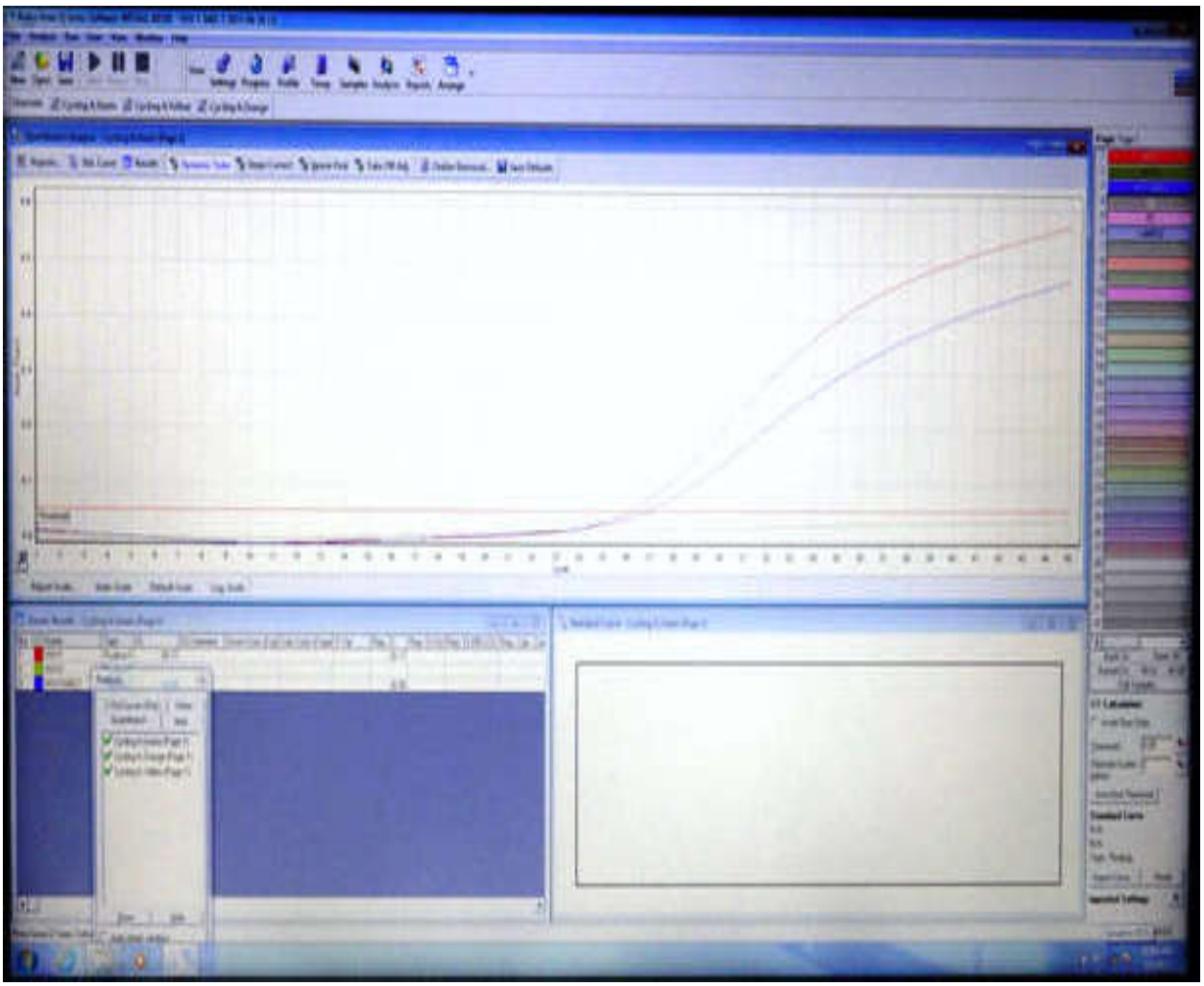

Fig. 1: Real time PCR amplification plot for HSV-1 positive control (HSV-1 RG PC) in fluorescence Channel Cycling Green. No signal detected in Cycling Green & Yellow channels

Discussion and Conclusion

HSV encephalitis is a serious infection but diagnosis previously required brain biopsy in certain cases due to low sensitivity of CSF culture and serology [11,12]. PCR now allow the detection of HSV DNA from CSF with 95% sensitivity thus avoiding invasive brain biopsy. Viral meningitis, commonly caused by either enteroviruses or HSV is more reliably detected by PCR when compared to culture and in a shorter time (one verses up to 5 days). HSV PCR can be multiplexed with other pathogens responsible for meningitis. Genital ulceration due to HSV, usually due to HSV type II infection is now routinely detected by PCR in many clinical microbiology laboratories due to its increased sensitivity over viral culture. Genital herpes is the most common causes of genital ulcer disease in the developed world [13-15]. HSV 1 classically presents as herpes gingivostomatitis an infection of the oral mucosa. It can also cause conjunctivitis, keratitis, and herpetic whitlow. HSV 2 is most common cause of genital ulcer in the United States. More than 95% of recurrent disease is due to HSV 2. The main application for HSV sub typing is with regard to the clinical issue of recurrent infection. Most painful and annoying recurrent genital herpes is due to HSV 2, and almost all recurrent cold sores or fever blisters are due to HSV 1. However, genital herpes also can be caused by HSV 1. This type of genital herpes is much less frequently recurrent and each recurrence usually last only a few days. It has been documented that as many as one third of herpes infections are due to HSV 1, particularly in adolescent and young adult. In this result we have concluded that out of the ten clinical samples of patients suspected for HSV infection, one was found to be infected by HSV-1 and one was infected with HSV-2 [16, 17]. These results were interpreted by the green signals of HSV-1 sample, yellow signals of HSV-2 sample and orange signals of the internal control.

Conflict of Interest: None

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