ISSN:0975-542X

Comparative diagnostic method of herpes encephalitis by LAMP (Loop-Mediated Isothermal Amplification) and MRI (Magnetic Resonance Image) method in Tertiary Care Center

Rahman W[1], Dhole TN[1], Nag VL[1], Maurya AK[1], and Pradhan S[2]
[1]Department of Microbiology & [2]Neurology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow UP – 226104, India

Abstract:
Herpes Simplex Encephalitis (HSE) is the most common cause of fatal sporadic acute encephalitis occurring worldwide, contributing to 10-20% cases of viral encephalitis. The present study was aimed to which method cheap, rapid and cost-effective method in the diagnosis of herpes simplex encephalitis (HSE). Out of fifty cases of suspected HSV encephalitis, seven cases were positive magnetic resonance image (MRI) that shows T2 hyperintence lesion in temporal lobe. Five by loop-mediated isothermal amplification (LAMP) method. Maximum number of cases was seen in 13-20 years of age group. As compared to MRI, LAMP method is 71.4% sensitive, 100% specific with positive prediction value of 100% and negative prediction value of 93.48%. MRI findings are common in all confirmed cases of HSV encephalitis i.e. hyper intense signals on T2W1 in median temporal areas. We concluded that MRI is best investigation in diagnosis of HSE but is costly and not available in rural and semi urban area of developing countries like India. Although LAMP method is cheap, rapid and cost-effective method in the diagnosis of HSE.

Key words: *Herpes Simplex virus, Herpes Simplex Encephalitis (HSE), LAMP method.*

Introduction:

Herpes Simplex Encephalitis (HSE) is the most common cause of fatal sporadic acute encephalitis occurring worldwide, contributing to 10-20% cases of viral encephalitis[1]. More than 90% of the cases are caused by herpes simplex virus type I and 7% are caused by herpes simplex virus type 2 (HSV-2) [2]. More than two thirds of cases of encephalitis due to HSV-I appear to result from reactivation of endogenous latent HSV-I in individuals previously exposed to the virus[3]. There is bimodal age of distribution of HSE, with one third of cases occurring in those less than 20 years old and one half in those aged 50 years or more [4]. Globally 2-4 individuals per million populations per year get affected by this disease [5]. In the United States, prevalence equals one case per 250 000 population/year while in Sweden 2.5 per million population/year get affected [5]. There are no documented studies from India, however in older study HSV-1 encephalitis constituted a very low proportion (1.1%) of acute viral encephalitis cases seen in Eastern Uttar Pradesh (India) [6]. In India as of today, HSE appears to be under diagnosed, probably due to lack of awareness and sensitive diagnostic facility[1]. Serological and clear epidemiological studies for virus are not available in many part of the country, due to lack of virology research laboratory [1]. Thus percentage of cases of viral encephalitis and proportion of HSE are difficult to estimate. HSV can be detected in the laboratory using a variety of methods. These range from standard and rapid culture (shell vial) to a molecular amplification method[7]. CSF culture is of extremely low sensitivity for diagnosis of HSV encephalitis. Positive culture has been reported in less than 5% of cases[3]. Recent studies have suggested that detection of HSV DNA by PCR increases the sensitivity of viral infection detection compared to antigenic detection or cell culture methods[8]. Improvements in diagnosis and antiviral drug treatment have dramatically reduced the morbidity and mortality of HSV-1 encephalitis (HSE). In an effort to improve HSE by radiological and other molecular methods [9]. To develop an effective assay for rapid measurement of HSV DNA content, we evaluated LAMP based HSV specific DNA amplification method in our setup and examines its reliability for the detection of HSV DNA from CSF sample obtained from suspected

Table 1 : Primer targeting the HSV-1 and HSV-2 Gg Genes

Virus	Name of Primer	Sequence of Primer
HSV-1	HS1F3	5'-GCCGTTGTTCCCATTATCCC-3' (F3)
	HS1B3	5'-TACTTGGCATGGGGGGTG-3' (B3c)
	HS1F1P	5'-GTTGGGTGGTGGAGGAGACGTCCTTTTGGTTCTTGTCGGT-3' (F1c-F2)
	HS1B1P	5'-GGTCGTCCCTCGCATGAAGCGGCGTGGTAAGGCGTATG-3' (B1-B2c)
	HS1LPF	5'-TTGGTGGGAACCCCCGATAC-3' (LPFc)
	HS1LPB	5'- AACATGACCCAGACCGGCAC-3' (LPB)
HSV-2	HS2F3	5'-GGCCTTGACCGAGGACAC-3' (F3)
	HS2B3	5'-CGACTCCACGGATGCAGT-3' (B3c)
	HS2F1P	5'-TCGACTGAGGGTGCCATGGCGTCCTCCGATTCGCCTACG-3'(F1c-F2)
	HS2B1P	5'-TCAACCACTACTCCCCCCGACGCTTTCCTCCGGCGTAA-3'(B1-B2c)
	HS2LPF	5'-GCCGACACAGGGAGGGGCGT-3' (LPFc)
	HS2LPB	5'-GATGGCCACACAAGCCGCAA-3' (LPB)

Table 2: Relationship between LAMP and MRI finding in Positive HSE cases

No	Age (Yrs)	Sex	Diagnosis	LAMP Method	MRI Finding
1	19	F	SLE, Encephalopathy	Positive	T2 hyperintence lesion in Temporal Lobe
2	60	M	Viral Encephalitis	Positive	T2 hyperintence lesion in Temporal Lobe
3	69	F	Encephalopathy	Positive	T2 hyperintence lesion in Temporal Lobe
4	16	M	Herpes Encephalitis	Positive	T2 hyperintence lesion in Temporal Lobe
5	23	F	Herpes Encephalopathy	Positive	T2 hyperintence lesion in Temporal Lobe
6	20	M	Viral Encephalitis	Positive	T2 hyperintence lesion in Temporal Lobe
7	18	M	Viral Encephalitis	Positive	T2 hyperintence lesion in Temporal Lobe

herpes viral encephalitis cases and we compared these data with MRI (magnetic resonance imaging) findings.

Materials and Methods:

Sample

This study has been conducted at Sanjay Gandhi Post graduate Institute of medical

Sciences, situated at Lucknow, India. A total of 50 cases of >10 years of age with or without treatment history of suspected Herpes viral encephalitis admitted between 1st Nov 2006 to 15th Oct 2008.All the patients were diagnosed by clinical presentations, biochemical findings according to the universal standard for the diagnosis of viral encephalitis. Cerebrospinal fluid samples (CSF) were collected from these patients and further testing was performed on them. CSF samples from fifty cases of non-viral meningitis (withy laboratory confirmed of other etiology) were taken as negative controls.

Lamp method:

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method that amplifies DNA with high specificity, efficiency, and speed under isothermal conditions[10]. . The LAMP method requires a set of four primers (B3, F3, BIP, FIP) that recognize a total of six distinct sequences within the target DNA [11]. Primers for the HSV-1(Herpes simplex virus-1) and HSV-2(Herpes simplex virus-2) specific LAMP assays were designed based on the HSV-1 gG (Glycoprotein) and HSV-2 gG gene sequences, respectively (Table 1) . Primer BIP(Backward inner primer) for the gG genes of HSV-1 and HSV-2 contained the B1 direct sequence and B2 complementary sequence, each specific for the respective strains. Primer FIP(Forward inner primer) for the gG genes of HSV-1 and HSV-2 contained the F1 complementary sequence and the F2 direct sequence. Primers B3 and F3 for the gG genes of HSV-1 and HSV-2 were located outside the F2-B2 regions. As additional loop primers increase the amplification efficiency [12], loop primers specific for the HSV-1 gG and HSV-2 gG genes were also used. The primer sequences and binding locations have been described elsewhere 10]. The LAMP reaction was performed using a Loop-amp DNA amplification kit (Eiken Chemical, Tochigi, Japan). The 25 µl reaction mixtures contained 1.6 µM each of the FIP and BIP primers, 0.8 µM of each outer primer (F3 and B3), and 0.8 µM of each loop primer, 12.5 µl of 2X reaction mix, 1 µl of Bst DNA polymerase, and 5 µl of the extracted DNA solution. The mixture was incubated at 63^0C for 45 min in water bath.

The turbidity derived from magnesium pyrophosphate formation, due to release of pyrophosphate during polymerization, was detected. To avoid contamination between samples, different rooms were used for DNA extraction and LAMP set up, using filter containing pipette tips for aerosol protection. Positive reaction shown by appearance of turbid product or after addition of 1 µl SYBR green they appear green fluoresces in uv illumination and negative one seen as pink color.(Fig-2)

They can detected by gel electrophoresis (2.5% agrose). MRI done in our tertiary care hospital.

Results:

Out of fifty cases of suspected HSV encephalitis cases, seven cases were positive by MRI and five by LAMP method. None of the negative control sample was found to be positive by MRI and LAMP method. The maximum number of cases were seen in 13-20 years age group (Table-2).Table-1 shows that the incidence of confirmed HSV Encephalitis cases in the age group 13-20 years was significantly higher as compared to other age group (p=0.019).Considering MRI as gold standard, LAMP method showed a sensitivity of 71.4% and specificity of 100% with positive prediction value of 100% and negative prediction value of 93.48%(Table-2).All two cases LAMP method negative sample which were positive by MRI,had low HSV DNA copy number as we also study with Real time PCR.

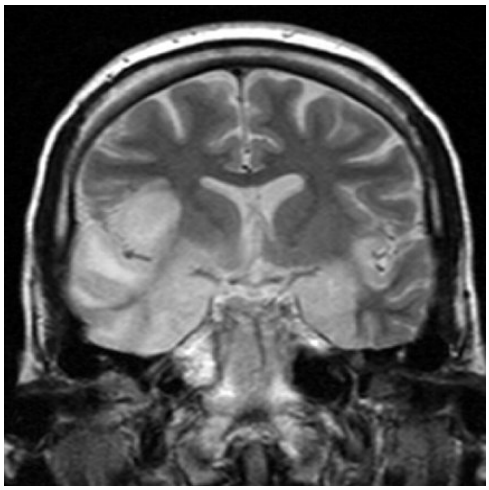

Fig 1 (MRI) Arrow shows T2 hyperintence and lesion in Temporal Lobe

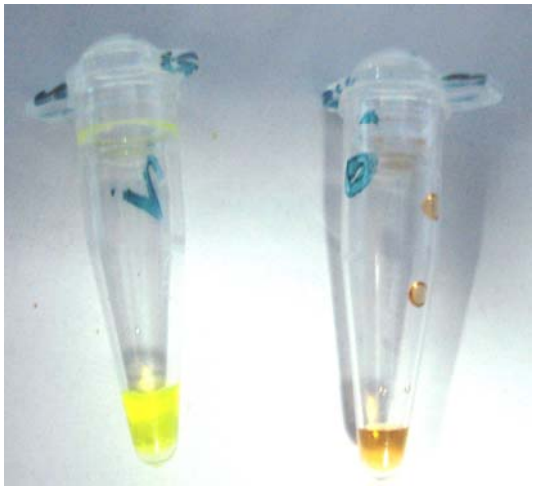

Fig 2 . (LAMP Reaction) Green is positive Pink colour is negative

Discussion:

LAMP is a novel technique for amplification of specific DNA sequences, and has several advantages over PCR [11, 12] .First; the specificity of LAMP is high because the LAMP method uses multiple primers, recognizing six distinct sequences in the target DNA. Second, the method is both rapid and simple; only 45 min are needed to amplify the target sequences. Third, the cost of the equipment is inexpensive compared with PCR; equipment cost remains one of the major reasons why PCR diagnostics have not been more widely utilized. With these advantages, LAMP has the potential to become adopted for widespread use in hospital laboratories.(13).

There have been no documented studies, which purely compare the sensitivity and specificity of LAMP method and MRI (as gold standard).In the our study Considering MRI as gold standard, LAMP method showed a sensitivity of 71.4% and specificity of 100% with positive prediction value of 100% and negative prediction value of 93.48%(Table-2).All 2 cases LAMP method negative sample which were positive by MRI, had low HSV DNA copy number.

Although real-time PCR offers more information than other tests currently available. However, this procedure should be used in conjugation with patient history and other laboratory data to support a clinical diagnosis. MRI appears to be the most sensitive and specific neuroimaging method for HSE. It shows hyperintense signals on T2W1 in median temporal areas bilaterally14.(Fig-1) Our study also shows same findings in confirmed case of HSE encephalitis. Brain biopsy was not performed in this study. Thus, real-time PCR with LAMP method and MRI may prove to be practical in initiating the acyclovir treatment in most centers in India.

References:

[1] Panagariya A, Jain RS, Gupta S, Garg A, Sureka RK, Mathur V, Herpes simplex encephalitis in North West India. Neurol India. 2001 Dec; 49:360-5.

[2] Aurelius E, Johansson B, Sköldenberg B, Forsgren M., Encephalitis in immunocompetent patients due to herpes simplex virus type 1 or 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. J Med Virol. 1993 Mar;39(3):179-86

[3] Nahmias AJ, Whitley RJ, Visintine AN, Takei Y, Alford CA Jr., Herpes simplex virus encephalitis: laboratory evaluations and their

diagnostic significance. J Infect Dis. 1982 ; 145:829-36.

[4] Sköldenberg B, Forsgren M, Alestig K, Bergström T, Burman L, Dahlqvist E, Forkman A, Frydén A, Lövgren K, Norlin K, et al. Acyclovir versus vidarabine in herpes simplex encephalitis. Randomised multicentre study in consecutive Swedish patients. Lancet. 1984; 2 :707-11.

[5] Whitley RJ,Alford CA,Hirsh MS et al .Vidarabine verses acyclovir therapy in herpes simplex encephalitis :NIAID collaborative antiviral study group.N Eng J Med 1986;314:144-49

[6] Gambhir IS, Singh NN, Singh DS, Gulati AK, Herpes simplex virus-1 encephalitis in eastern Uttar Pradesh. Comment in: J Assoc Physicians India.2001 Mar; 49:392.

[7] Boivin G, Diagnosis of herpesvirus infections of the central nervous system. Herpes. 2004; 11 Suppl 2:48A-56A.

[8] Espy MJ, Ross TK, Teo R, Svien KA, Wold AD, Uhl JR, Smith TF Evaluation of LightCycler PCR for implementation of laboratory diagnosis of herpes simplex virus infections. J Clin Microbiol. 2000 Aug;38(8):3116-8

[9] Klein D.Quantification using real-time PCR technology:application and limitation.Trends Mol Med.2002;8:257-60

[10] Enomoto Y, Yoshikawa T, Ihira M, Akimoto S, Miyake F, Usui C, Suga S, Suzuki K, Kawana T, Nishiyama Y, Asano Y (2005) Rapid diagnosis of herpes simplex virus infection by loop-mediated isothermal amplification method. J Clin Microbiol 43:951–955

[11] Nagamine K, Hase T, Notomi T (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probes 16:223–229.

[12] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:E63.

[13] Hiroshi Kimura Æ Masaru Ihira Æ Yoshihiro Enomoto Jun-ichi Kawada Æ Yoshinori Ito Æ Tsuneo Morishima Tetsushi Yoshikawa Æ Yoshizo Asano Rapid detection of herpes simplex virus DNA in cerebrospinal fluid: comparison between loop-mediated isothermal amplification and real-time PCR Med Microbiol Immunol (2005) 194: 181–185

[14] Soo MS,Tien RD,Gray L et al. Mesenrhombencephalitis: MR findings in nine patients. Am J Roentgenol. 1993 ;160:1089-93