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RESEARCH ARTICLE

PREVALENCE OF HERPES SIMPLEX VIRUS IN CSF SPECIMENS OF NEONATES WITH ENCEPHALITIS BY USING REAL TIME PCR

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ABSTRACT

Background: *Herpes simplex* virus (HSV) causes a spectrum of clinical manifestations in the central nervous system (CNS). Neonatal HSV infection following exposure to the virus at delivery produces severe disseminated infection and can lead to death if not effectively treated.

Objective: The aim of this study is to detect the prevalence of HSV type I, and II in encephalitic neonates.

Materials and methods: A total of 100 CSF samples were collected from encephalitic neonates from Khartoum Hospitals. HSV type I and II were detected in patients' samples using real time PCR.

Results: Among the 100 encephalitis patients, 70 (70%) were males, and 30 (30%) females. HSV DNA was detected in Two patients (2%) using Real-time PCR. Positive sample Ct value was 19.45 were as positive control sample Ct value was 22.24. All negative samples Ct values were appear later after 36 Ct value.

Conclusion: HSV detection by real-time PCR in the present study indicated a low prevalence among encephalitic neonates included in the study poulation. Real time PCR procedure should be used in conjugation with clinical diagnosis to support a perfect sensitive, specific and quantities diagnosis.

Keywords: *HSV, Encephalitis, Real-time PCR.*

INTRODUCTION:

Herpes simplex virus (HSV) caused a wide spectrum of clinical manifestation in the central nervous system (CNS). Neonatal HSV infection following exposure to the virus at delivery produce severe disseminated infection and can lead to death if not effective therapeutic management is possible (1). There for rapid laboratory diagnosis is very essential for decreasing the lethality of HSV infection.

Today; real time PCR is recognized as reference standard method for sensitive, specific and quantitative diagnosis for CNS infection caused by HSV (2.3).

Studies on detection of HSV are usually based on home brew protocols. These assays commonly involved toxic and/or radioactive substances and high number of manipulations (4.5). to replace these short comings, molecular assays including rapid extraction protocol with non toxic substance and detection protocol based on non-radioactive and largely automated methods been developed.

In the present study qualitative and quantitative molecular assay based on rapid DNA extraction protocols and R.T PCR was evaluated.

Neonatal herpes simplex encephalitis is one of sporadic focal encephalitis (6.7). Estimates of the incidence of neonatal herpes encephalitis varied from 1 in 3000 to 1 in 20.000 live birth (8).

Women who acquired first episode of genital herpes during pregnancy are greater risk of transmitting the virus to their newborn than women with genital reactivation of latent infection (9).

HSV disease of newborn is acquired during one of three distinct time intervals, Intra Uterine (Inutero),per partum (perinatal) and post-partum (post natal).

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The improvement of mortality and morbidity achieved by using acyclovir at 60 mg /1kg / day ,delivered intravenously in three daily doses for 21days (10,11). Prevention can be achieved by using cesarean section delivery in women with active genital lesion which can reduce the risk of acquiring HSV to infants (12).

Ethical Construction:

Approval was taken from Al Neelain Ethical Committee.

Study design:

This is a descriptive cross sectional study done on patients attended to Jafer-Ibnoof hospital, Omdurman teaching hospital and Omdurman military hospital.

Patient:

AQ hundred patients known with clinical diagnosis including CT scan of encephalitis and suspected *herpes simplex* encephalitis were included in this study.

A total of 60% of the patient ware from Jafer-Ibnoof hospital, 20% from Omdurman military hospital and the rest 20%was from Omdurman teaching hospital.

Clinical specimens:

Sample:

CSF samples had been collected from infant with encephalitis (Age ranged from 1 day of life to 2years) from divert sex group in Khartoum state-Sudan prior to the start of therapy, all samples were the second CSF collected samples.

Samples were collected under aseptic condition by an experience healthy worker from the arachnoid space using a serial wide born needle inserted between fourth and fifth lumber vertebrae, then CSF was allowed to drip into dry serial container and then the samples kept frozen in –80c˚ tell examined.

DNA extraction and Rt. PCR:

DNA extraction was done from patient material using commercial QIA gene amp Blood kit cat No (51104) according to manufacture instruction as follow:

A20µl of QIA gen protease was pipetted in to the bottom of 1.5ml micro centrifuge tube then 200µl of CSF sample was added then 200µl of buffer AL was added to the sample and mixed by pulse vortexing for 15second then incubated at 56 c˚ for 10min"in water path" then briefly centrifuged the 1.5 ml micro centrifuge tube to remove the drops from inside of lid then 200µlethanol (96-100%) was added and mixed by pulse vortexing for 15 second ,after mixing the 1.5 micro centrifuge tube was centrifuged to remove the drops from the lid ,the mixture in the previous step was added carefully to the QIAamp mini spin column and the cap of the column was closed and then centrifuged at (8000rpm)for l min and then the QIAamp mini spin column was placed in a clean 2 ml collection tube and discard the tube conation filtrate,then the QIAamp mini spin column was carefully opened and a 500 µl Of buffer AW2 was added without wetting the rim and then centrifuged at full speed (14,000rpm)for 3min ,the old collection tube with filtrate was discharged and the QIA amp mini spin column was placed in anew 2ml collection tube and centrifuged at full speed for 1min .the QIA amp mini spin column was placed in a clean 1.5 ml micro centrifuge tube, and the collection tube containing the filtrate was discarded, then the QIAamp mini spin column was opened carefully and 200µl of buffer AE or distilled water was added and incubated at room temperature for 1min and then centrifuged for l min at 8000rpm, the DNA was kept frozen at -20c̊ till used.

The Real time PCR was performed by processing the extracted DNA from CSF samples with HSVI and HSVII Real time PCR Kit that manufactured by shanghiZj Bio-tech.co, Ltd.

The reaction was performed in 0.2 RT PCR plate.
To prepare 20µl reaction mix, 4 µl of PCR master mix , 1 µl from internal control (ic), and 13µl of water was added, the mixture was then dispended in RT-PCR Plate and then 2 µl from extracted DNA was added, positive and negative controls were included in different reactions.

The plate was sealed and amplified in TechneRT-PCR machine as following Protocol: 37˚ C for 2 min 1cycle, 94˚ C for 2 min 1cycle, 93˚ C for 19 sec, 60˚C for 60 sec then fluorescence was measured, and FAM was chosen.

Result was compared automatically by the RT.PCR instrument with a positive control HSV DNA sample as shown on figure (1&2).

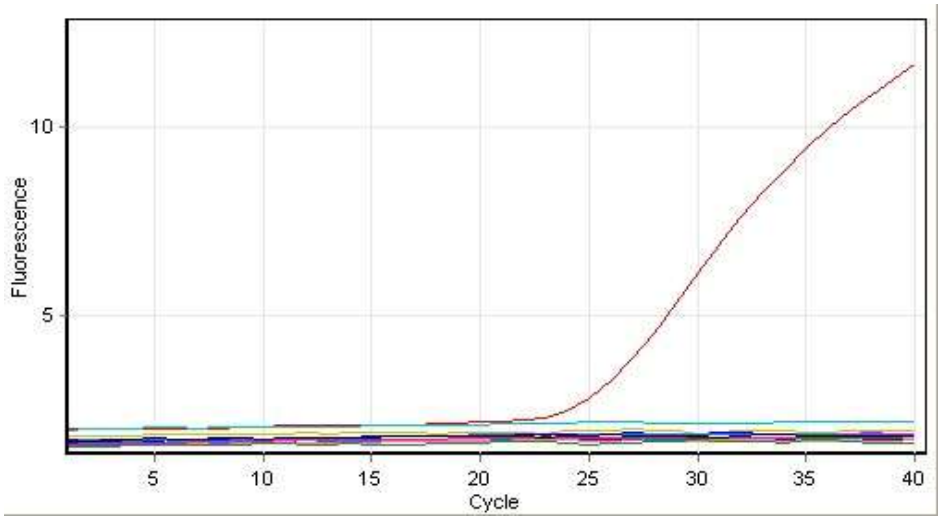

Figure 1: Amplification curve showed Ct value of the positive control(Ct: 22.24)

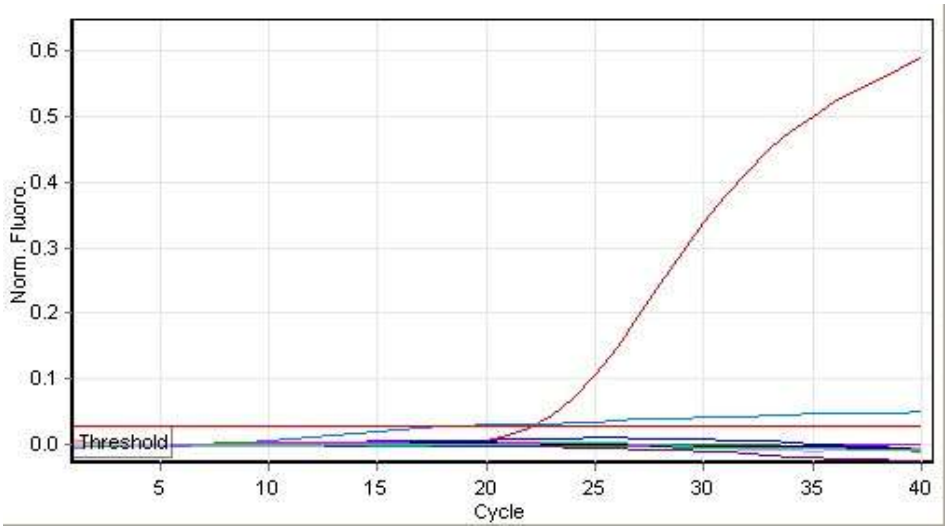

Figure 2: Amplification curve showed Ct value of the unknown sample (Ct: 19.45)

RESULTS:

Out of hundred cases of clinical diagnosis of encephalitis; 70 was female (70%) and 30 was male (30%) ,age ranged from 1 day of life to 2 years; HSV DNA was detected in two cases out of 100 (2%) table (1).

Table 1: frequency of positive and negative samples among study population.

	Positive samples	Negative samples
Sample	2	98
frequency	2%	98%

DISCUSSION:

The RT.PCR is a highly specific and sensitive method for detecting DNA.The DNA primers used in our assay were specific to detect HSV I and HSV 2.

As far as we know there were no false negative result in RT.PCR assays in the patient with clinical diagnosis of possible HSV encephalitis, so the risks of a false negative result was excluded specially when we use primers that identified only HSV I and HSV II in addition to that these types of herpes virus are genetically stable virus.

Our results indicated that the detection of HSV DNA in the CSF with a RT.PCR assay is a highly specific test for herpes

simplex encephalitis the assay was detected small quantities of HSV DNA but a precise estimate of its sensitivity cannot be provided because of that our patient did not have a brain biopsy and the clinical diagnosis of them was depend only on CT scan.

In this study two cases out of hundred was positive by Real time PCR for HSV, that indicated low prevalence of this virus among the target population, this finding was supported by another study done by (Pinninti , and Kimberlin 2013) (14), they mentioned that neonatal herpes simplex virus infections are uncommon, but because of the morbidity and mortality associated with the infection they are often considered in the differential diagnosis of ill neonates. The use of polymerase chain reaction for diagnosis of central nervous system infections and the development of safe and effective antiviral therapy has revolutionized the diagnosis and management of these infants.

Lastly the RT-PCR offers more information than other tests currently available; however this procedure should be used in conjugation with clinical diagnosis to support a perfect sensitive, specific and quantities diagnosis.

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