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Detection of Herpes Simplex Viruses I andII in Cerebrospinal Fluid Specimens of Iraqi Children Presenting With Aseptic Meningitisby Using Real Time PCR Assay

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Abstract:
Introduction: *Acute meningitis is a frequent syndrome encountered in emergency rooms. Viral meningitis is a common infectious disease of the central nervous system (CNS) that occurs worldwide. It can occur at any age but is most common in young children. Viral meningitis caused by herpes simplex virus (HSV) is a self-limited illness of 2–5 days' duration characterized by CSF findings of pleocytosis and an elevated protein level with a normal glucose level10. Although Herpes simplex I and II account for only 31% to 4%1of all cases of aseptic meningitis, and/or meningoencephalitis caused by HSV I and II are more important due to their potentialclinical severity in the case of meningoencephalit*
Materials and Methods: *CSF samples were collected from 68 patients suspected of having aseptic meningitis and/or encephalitis at Medical City Hospital, Baghdad, Iraq, during the period between April 2013 and June 2014. Viral DNA was isolated from 400 µl of a CSF specimen by using the high pure viral nucleic acid kit (Roche, USA) according to the manufacturer's instructions. DNA amplification was carried out on a Rotorgene 6000 (Corbett Life Sciences, Sydney, Australia) using Applied Biosystems (Roche, Branchburg, New Jersey) TaqMan Universal PCR Master Mix R.*
Results: *Twelve/68 (17.6%) CSF samples were positive for HSV-1whereas 2/68 (2.9%) samples were positive for HSV-2. There was no mixed infection. Herpesviruses were observed during the winter months.The positive samples were within age range 1-4 years. Of the 55 HSV DNA–positive cases, 5/12 (42%) occurred in male patients and 7/12 (58%) occurred in female patients. The same pattern was observed for negative samples, 23/56 (41%) male and 33/56 (59%) female.*
Conclusion: *HSV- 1 and HSV-2 should be screened in cases of suspected aseptic meningitis. Further analysis of other agents and the stan¬dardization of biology molecular methodologies are future proposition to improve the laboratorial diagnosis of viral meningitis in Iraq.*
Keywords:*Aseptic meningitis, HSV-1, HSV-2, children, Iraq , Real time PCR*

I. Introduction

Acute meningitis is a frequent syndrome encountered in emergency rooms. Viruses and bacteria are the most common pathogens associated with this clinical picture[1].Viral meningitis is a common infectious disease of the central nervous system (CNS) that occursworldwide.It can occur at any age but is most common in young children[2].While the clinical course of viral meningitis and encephalitis may overlap, viral meningitis is usually self-limiting[3], whereas the mortality from viral encephalitis ranges from 4.6% to 29%[4-9].Viral meningitis caused by herpes simplex virus (HSV) is a self-limited illness of 2–5 days' duration characterized by CSF findings of pleocytosis and an elevated protein level with a normal glucose level[10]. Although Herpes simplex I and II account for only 31% to 4%[1]of all cases of aseptic meningitis, and/or meningoencephalitis caused by HSV I and II are more important due to their potential clinical severity in the case of meningoencephalitis[11].

Rapid etiologic diagnosis is very important to differentiate viral and bacterial meningitis so as to guide the introduction of antibiotic therapy early or avoid its unnecessary use for viral diseases.Conventional laboratory diagnostic methods, such as growth of a microorganism in culture and detection of specific antibody, are generally poor in the diagnosis of CNS infection. They are generally too slow, have low sensitivity and consequently they are of little use in diagnosing and treating the patient during the acute phase of the disease[12,13]. Molecular techniques are now considered the gold standard for the detection in cerebrospinal fluid (CSF) samples of the viruses responsible for CNS infections[14, 15]. Several studies have employed polymerase

chain reaction (PCR) for diagnosing CNS infections, especially for HSV, studies have demonstrated that PCR is highly sensitive and specific, in addition to being fast and less invasive[16, 17].

There are few confirmed diagnoses of viral CNS infections in Iraq. Consequently, the true incidence and etiology of viral meningitis are not well described.The purpose of the present study was to screen for herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) in febrile hospitalized patients, utilizing molecular diagnostics to improve the laboratory diagnostic assessment of these patients.

II. Materials And Methods

Specimens

CSF samples were collected from 68 patients suspected of having aseptic meningitis and/or encephalitis at Medical City Hospital, Baghdad, Iraq, during the period between April 2013 and June 2014. CSF samples were included in the study based on one or more signs of meningitis and/or encephalitis (severe headache, photophobia, nausea/vomiting, meningeal signs, petechial/purpural rash, altered mental status, seizures, and lethargy) and clinical indication for lumbar puncture, as determined by the attending physician.

CSF biochemical and cytological pattern of viral meningitis are: white blood cells>5 cells/mm^3 with predominance of lymphocytes, CSF glucose normal (>45 mg/dL), CSF lactate <3.5 mmol/L,as well as the negative cultures for bacteria[1, 18].Patients' ages ranged from 1-4 years, 40 cases were male and 28cases were female. Upon receipt in the diagnostic laboratory, the specimens were processed or stored at - 20°C until the study.

Viral DNA extraction

Viral DNA was isolated from 400 µl of a CSF specimen by using the high pure viral nucleic acid kit (Roche, USA) according to the manufacturer's instructions. DNA was stored at -20°C until PCR performed.

Real time PCR

PCR primers and probes were previously described[10] (Table 1)

Table 1. Polymerase Chain Reaction Primers and Probes

Virus (Target Gene)	Sense Primer 5 □ 3 □	Antisense Primer 5 □3 □	Probe 5 □3 □
HSV-1 (gD)	CGGCCGTGTGAC ACTATCG	CTCGTAAAATGGCCCCT CC	CCATACCGACCAC ACCGACGAACC
HSV-2 (gG)	CGCTCTCGTAAAT GCTTCCCT	TCTACCCACAACAGAC CCACG	CGCGGAGACATTC GAGTACCAGATCG

DNA amplification was carried out on a Rotorgene 6000 (Corbett Life Sciences, Sydney, Australia) using Applied Biosystems (Roche, Branchburg, New Jersey) TaqMan Universal PCR Master Mix R. The PCR thermocycler conditions were 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, then 60°C for 1 minute and 60°C for 7 minutes[10].

III. Results

A total of 68 CSF samples from patients with suspected aseptic meningitis were tested using the real time PCR protocol.Twelve/68 (17.6%) CSF samples were positive for HSV-1whereas 2/68 (2.9%) samples were positive for HSV-2 (figure 1). There was no mixed infection.

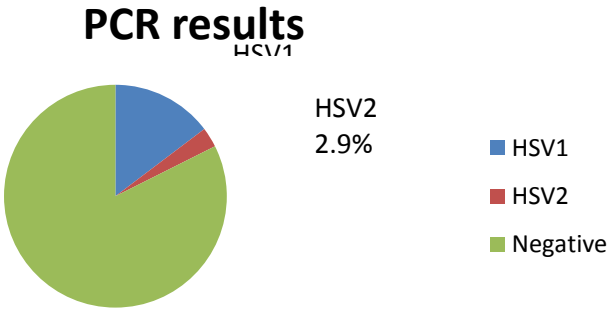

Figure 1 Percentage for positive samples in the period of the study for herpes simplex virus1 (HSV1) human herpes virus 2 (HHV2)

Herpesviruses were observed during the winter months.The positive samples were within age range 1-4 years. Of the 55 HSV DNA–positive cases, 5/12 (42%) occurred in male patients and 7/12 (58%) occurred in female patients. The same pattern was observed for negative samples, 23/56 (41%) male and 33/56 (59%) female.

IV. Discussion

Out of a total of 12/68 (17.6%) positive samples, 10 CSF samples were positive for HSV-1 and 2 CSF samples for HSV-2. Because of their neurotropic nature, HSV has been recognized as a relatively common CNS pathogen andthe involvement of herpesviruses as etiologic agents of meningitis or encephalitis has been extensively described[20-22].When compared to other studies the percentage of herpes simplex virus positive specimens of our study is similar to that reported by other investigators such as 15% in Iran[23]while other investigators reported results differ from our results either lower such as 1.3% in Brazil[24]or higher such as 33% in Middle East region[25]these differences may be due different in sample criteria: Middle Eastern study is done on CSF specimens from encephalitis patients only and excluded meningitis patient and vice versa in Brazilian study while our study is done on CSF specimens from meningitis and/or encephalitis patients as well as our study included childhood only while Middle Easternand Brazilian studies are done on children and adults. Many studies also reported the positivity of HSV1 is higher than HSV2. The high number of CSF samples negative for HSV that suggests other viruses cause of aseptic meningitis such as enteroviruses, varicella zoster virus, adenovirus, measles, rubella and mumpsornon-infectious conditions including autoimmune diseases and carcinomatous meningitis must be considered[26]. As well as different factors, including the time of sampling of the CSF specimens may be critical. Many studies observed that HSV DNA may be more readily detectable in the later phases after the onset of symptoms and not in the very acute phases[27, 28].

The difficulty in making a laboratory diagnosis using traditional techniques in cases of aseptic meningitis and encephalitis has stimulated the development of studies using real time PCR assays.HSV- 1 and HSV-2 should be screened in cases of suspected aseptic meningitis. Further analysis of other agents and the standardization of biology molecular methodologies are future proposition to improve the laboratorial diagnosis of viral meningitis in Iraq.

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