Research Article

Enteroviruses Incidence in Cases of Aseptic Meningitis with Special Reference to Enterovirus 71 in Sudan - 3

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ABSTRACT

Background: Aseptic meningitis epidemics may pose various health care challenges.

Aim: This study was carried out to determine the incidence of enteroviruses among aseptic meningitis patients with emphasis on Enterovirus 71 (EV71) in Khartoum, Sudan.

Objective: To generate preliminary information about of enteroviruses and Enterovirus 71 (EV71) in patients with aseptic meningitis in Khartoum State, Sudan.

Method: Cerebrospinal fluid specimens were collected from 89 aseptic meningitis patients from different Khartoum Hospitals (Mohammed Alamin Hamid Hospital, Soba Teaching Hospital, Omdurman Military Hospital, Alban Gadeed Teaching Hospital and Police Hospital) within February to May 2015. Among these 89 patients, 43 (48%) were males and 46 (52%) were females. The patient’s age ranged between 1 day and 30 years old. The collected specimens were assayed to detect enteroviruses and EV71 RNA using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique.

Result: Forty six (51.7%) specimens were found positive for enteroviruses. Out of these 46 specimens, 10 (22%) specimens were found positive for EV71.

Conclusion: In conclusion, incidence and the role of Enteroviruses especially EV71 in cases of aseptic meningitis in Khartoum State was documented. Further studies using various diagnostic methods should be considered to determine these viruses on a national level.

Keywords: Enterovirus 71; Meningitis; RT-PCR; Sudan

INTRODUCTION

Viral meningitis is the inflammation of meninges (the membrane that cover the brain and spinal cord) as a result of viral infection [1,2]. The annual incidence of viral meningitis is higher than meningitis caused by other etiological agents [1]. Meningitis disease cannot be reliably diagnosed on clinical features alone. The clinical picture may be difficult to distinguish from infections caused by bacteria such as Mycobacterium tuberculosis, Mycoplasma pneumoniae, Treponema pallidium, Borrelia burgdofi, Leptospira spp. and Brucella spp. [3-5]. However, viral meningitis is usually less serious than bacterial meningitis but, in some instances viral meningitis can lead to serious outcomes including death [1]. Meningitis may represent as headache, fever, neck stiffness, nausea, vomiting, photophobia, sleepiness, confusion, irritability, delirium and coma [1,2]. Infants may present with bulging fontanelle, paradoxic irritability, high pitched cry and hypotonia [2]. As there is no specific treatment, patients with viral meningitis usually receive palliative treatment. Viral meningitis is caused by many viruses such as enteroviruses (the most common), West Nile virus, herpes viruses’ types 1 & 2, measles virus, and mumps virus [1].

Enterovirus 71 (EV 71) belongs to Enteroviruses genus in Picornaviridae family. It is very small RNA non enveloped icosahedral virus [6-8]. It’s genome is composed of a positive sense non segmented linear single strand RNA with a molecular weight of 2-2.6x 10^6 dalt on and approximately 7.4 kb in length [7,9-11]. EV 71 has 6 genotypes, A to F with genotypes B and C being divided into sub-genotypes B0-B5 and C1-C5, respectively [7,8]. The linear genome is organized in a single long Open Reading Frame (ORF) flanked by Un-Translated Region (UTR) at 5’ and 3’ ends. The 3’ UTR is followed by a variable length of poly A tract. The ORF encodes poly proteins which can be divided into 3 different regions on the genome from P1 to P3 and encoded in a single poly protein of 2194 amino acid [5,12,13]. The P1 region encodes the structural proteins, while P2 and P3 genomic regions code for the non-structural proteins [12,13].

Several outbreaks of aseptic meningitis specially in Eastern Mediterranean region are caused by EV 71 [5]. Diagnosis of EV 71 is based on laboratory diagnosis including virus isolation, serological techniques (such as ELISA) or molecular technique (like PCR). Molecular techniques are the main methods that are currently used for detection of EV 71 during outbreaks [3-5].

MATERIALS AND METHODS

Ethical approval

The ethical approval to conduct this study was obtained from the Federal Ministry of Health, Khartoum, Sudan.

Study area

The study was conducted in Khartoum State; central Sudan during the period from February to May 2014. The samples were collected from five different hospitals in Khartoum State namely: Mohammed Alamin Hamid Hospital, Soba Teaching Hospital, Omdurman Military Hospital, Alban Gadeed Teaching Hospital and Police Hospital. Patients included in this study had negative results for bacterial meningitis and were suspected as having aseptic meningitis.

Data collection

The personal and clinical data were collected by reviewing the hospitals records. The collected data included gender, age, date of sample collection, place of collection and the result of bacterial meningitis examination.

Samples collection

A total of 89 Cerebrospinal Fluid (CSF) samples were collected from 89 (43 males and 46 females) in two age groups (1 day -15 years n =79, and 16 years -30 years n =10) patients. The samples were collected under aseptic conditions from the arachnoid space by experienced healthy workers. The samples collected were then transported on ice to the Department of virology, Central Laboratory (Ministry of High Education), where they were stored at -80°C until use.

RNA extraction

Viral RNA was extracted from CSF by using Viral Gene _Spin TM DNA/RNA Extraction Kit (Korea) according to manufacturer’s instructions.
REVERSE TRANSCRIPTION

Reverse transcription was performed on extracted RNA by using Maxime RT PreMix Kit (iNtRON BIOTECHNOLOGY, South Korea), cDNA synthesis was carried out at a temperature of 45°C for 60 minutes, followed by Reverse transcriptase inactivation at 95°C for 5 minutes.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The RT-PCR was performed by processing the cDNA with primers that are specific for Pan-Enteroviruses and EV71 [14]. The Pan-Enterovirus primers used consisted of forward primer 5’- CAAGCACTTCTGTTTCCCCGG-3’ and reverse primer 5’- ATTGTCACCATAAGCAGCCA-3’, which were predicted to amplify a fragment of 435bp. The primers specific for EV 71 were forward 5’-GTCCTTAATTCGCACAGCACAGCT-3’ and reverse 5’-CCGT CCGCACTGAGAATGTACCCAT-3’, which were predicted to amplify a fragment of 507bp (Figures 1 and 2). The reaction was performed in 25µl total volume using Maximum PCR PreMix tubes (i-Taq). The volume included 5ul master mix, 1ul forward primers, 1ul reverse primer, 5ul cDNA and 13ul distilled water. The PCR amplification of PAN Enteroviruses and EV 71 was performed in 40 cycles consisting of denaturation for 1 minute at 94°C, primer annealing for 1 minute at 50°C and elongation for 1 minute at 72°C by using Techno PCR Machine (Japan).

RESULTS

Out of 89 CSF samples tested for enteroviruses; 46 (51.7%) samples were positive for Pan-enteroviruses by RT-PCR. Out of these 46 samples, 10 (21.7%) representing 11.2% of the total samples were positive for EV 71 when tested using EV 71 RT-PCR (Table 1).

The incidence of Enteroviruses and E71 detection was higher in females than in males and in the age group 16-30 yrs old than in the younger groups (1 day -15 yrs old) age group (Tables 1 and 2) with significant difference (p 0.027) regarding the incidence of enterovirus infection between males and females (Table 1).

DISCUSSION

EV 71 is the predominant cause of hand, foot and mouth disease in children and the epidemics of severe neurological diseases (aseptic meningitis) which is the most common type of viral meningitis [3,8]. Little is known about the epidemiology of Enteroviruses in Sudan in particular and Africa in general [9].

The present study focused on determining the incidence using molecular detection (PCR) of Enteroviruses infection in cases of aseptic meningitis with emphasis on EV 71 in Khartoum State, Sudan. A total of 89 samples of CSF had been involved in this study. Forty six (51.7%) samples were positive for Enteroviruses of which 10 (11.2%) samples were positive for EV 71.

The result obtained in this study using RT-PCR, were in disagreement with published results from outbreaks in Brunei, that reported the incidence of EV 71 at 38%, but similar to that reported from South Korea(19%) and Thailand ( 9%) [3-5].

In this study the EV 71 infection occurred mainly in the age group 1 day -16 years but there was no significant statistical relation between EV 71 infection and patient’s age (p value 0.577). In Cyprus, Gaza, China and Taiwan outbreaks EV 71 mainly affects children more than adults which is in agreement with our study results, but in one outbreak in Romania the adults were more affected than children [1].

According to our results, females were more significantly afflicted with EV 71 infection than males (p value 0.027). In contrast, in India
it was reported that the aseptic meningitis was more common in males than in females [2].

These conflicting results from different countries indicate the need to determine the type of enteroviruses in meningitis patients occurring in particular geographical areas to serve as guide in choosing a suitable measures of disease prevention.

Laboratory diagnosis is also important in managing enteroviruses infection. Rapid and accurate enteroviruses diagnosis improve medical management by allowing timely implementation of appropriate infection control strategies for individual and public health response to meningitis, and limitation of unnecessary investigation or antibiotic therapy [2-4,15].

The present findings should highlight the need for the establishment in Sudan of rapid, sensitive and specific diagnostic techniques (such as the one used herin) for better diagnosis of enteroviruses infections especially in the high risk groups. It also represent an alarming indicator of the disease in the population in Sudan. In addition taking all of our findings into consideration, they may indicate complex epidemiology of enteroviruses in the country.

To best of our knowledge this is the first attempt to identify the causative viral agents of meningitis infections in Sudan using molecular techniques.

Finally, the results obtained should call for wider surveillance at the national level in order to fully elucidate the true status of EV 71, enteroviruses and enteroviruses virus spp. involved, as well as other causes of viral meningitis in Sudan.

CONCLUSION

Incidence and existence of enteroviruses and EV 71 in Sudan were documented through detection of enteroviruses and EV71 RNA in CSF samples indicating high infection among aseptic meningitis patients in Sudan. Moreover, the Enteroviruses and EV71 detection using RT-PCR was established locally. Generally, these findings are useful for further studies since there is little available information about enteroviruses and EV71 infection in Sudan.

AUTHORS’ CONTRIBUTION

Marwa Abdelmoneim Almakki the sample collection; Marwa Abdelmoneim Almakki and Mohamed O. Mustafa conducted the RNA extraction and RT-PCR assay; Khalid A. Enan revised the PCR assay reaction and validation and contribution to the concept and design the study.

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