Case report

First detection of enterovirus 71 from an acute flaccid paralysis case with residual paralysis in Iran

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Abstract

In an attempt to determine the types of non-polio enteroviruses (NPEVs) in acute flaccid paralysis (AFP) cases in Iran, we detected enterovirus 71 (EV71) in an AFP case with residual paralysis for the first time. Cell culture detected no enteroviruses, while RT-PCR and subsequent sequencing revealed that the specimen was positive for EV71. EV71 is the causative agent of a variety of diseases from hand, foot and mouth disease to severe neurological complications and is now considered as an important cause of childhood acute flaccid paralysis. © 2008 Elsevier B.V. All rights reserved.

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1. Introduction

EV71, a member of genus Enterovirus, Picornaviridae family, is the causative agent of a variety of diseases ranging from hand, foot and mouth disease to severe neurological complications like meningitis, encephalitis and polio-like paralysis (Melnick, 1984). Since its discovery in 1969 (Schmidt et al., 1974) there have been reports of sporadic cases and also large outbreaks of EV71 infection with severe neurological complications in which fatal cases have occurred (AbuBakar et al., 1999; Alexander et al., 1994; Gilbert et al., 1988; Ishimaru et al., 1980; Nagy et al., 1982; Tagaya et al., 1981).

Like poliovirus, EV71 infection may display an affinity for anterior horn cells (Chumakov et al., 1979), and it is the most common NPEV associated with poliomyelitis-like paralysis (Melnick, 1984).

EV71 is genetically most related to Coxsackievirus A16 and comparison of complete genomic sequences of EV71 with poliovirus failed to detect any correlate for the neurovirulence of the virus. Phylogenetic analysis of complete VP1 sequences of EV71 isolates has led to identification of three genotypes: A–C (Brown and Pallansch, 1995). Genotype A is represented by only one isolate (BrCr-CA-70). Two other genetic lineages (B1, B2 and C1, C2) showed worldwide distribution. Another report from India has shown that there would be another genotype designated as genotype D (Deshpande et al., 2003).

Epidemiology of EV71 is unknown in Iran and outbreaks of hand, foot and mouth disease have not been reported from our country. Also, cases of encephalitis, meningitis and polio-like paralysis due to EV71 had not been recognized prior to this study.

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In an attempt to determine the types of non-polio enteroviruses in AFP cases in Iran, we detected EV71 in an AFP case with residual paralysis for the first time. Cell culture detected no enteroviruses, while direct extraction of RNA from stool specimen, RT-seminested PCR (RT-snPCR) and subsequent sequencing revealed that the specimen was positive for EV71.

2. Case report

A 17-month-old girl, who was originally from Ilam province in west border of Iran, was admitted to a pediatric hospital in Tehran on 20 August 2005 due to persisting acute flaccid paralysis. Date of paralysis onset was 21 June 2005, so the patient had been being paralyzed for almost 2 months at the time of admission. The patient had presented with symmetrical paralysis in lower limbs that had reached maximum deficit in 4 days. There was neither fever nor sensory loss at onset of paralysis. The preliminary diagnosis was Guillain-Barre syndrome but the committee for classification of AFP cases eventually categorized the case as Myopathy.

Two stool specimens of the patient were collected at the time of admission to the hospital and the day after. Both specimens were sent to the National Polio Laboratory for poliovirus surveillance.

Suspensions of the stool specimens were inoculated in L20B and Rhabdomyosarcoma (RD) cells based on World Health Organization manual for polio laboratories network (Polio Laboratory Manual, 2004). Neither of the specimens produced cytopathic effect in cells and the case was reported as negative on 9 September 2005.

With the objective to determine NPEV types of epidemiological significance, a retrospective study was carried out to detect and identify NPEVs in AFP cases, especially the ones who had residual paralysis after 60 days of paralysis onset. Based on our previous study (Shoja et al., 2007), to maximize the NPEV detection, RNA was extracted directly from the processed stool specimens and RT-PCR using PanEV primers, which amplifies a 114 bp region in 5’UTR of all enteroviruses, was performed.

The specimens which were positive with PanEV primers were subjected to RT-snPCR (Nix et al., 2006, with slight modifications). This RT-snPCR amplifies a 350–400 bp sequence from 3’ end of VP1 region of the genome which can be used for sequencing and type identification. Briefly, cDNA was produced by random hexamer primer, and RT-snPCR was performed using AN88/224 primer set as outer primers and AN88/AN89 as seminested inner primers. The amplified segments were subjected to direct sequencing and BLAST analysis was carried out for the partial VP1 sequences to search for closely similar sequences in the database. Blast analysis revealed EV71 in one of the specimens, which belonged to the above-mentioned patient. The sequence showed 96% similarity to strain HEV71 804/NO/03 which belonged to genotype C1 and had been detected in Norway (Witsø et al., 2007).

3. Discussion

As a result of the successful initiative to globally eradicate poliomyelitis, Iran has been free of wild polioviruses since 2001. In spite of this fact, the number of AFP cases not only has not decreased, but also is gradually increasing in Iran so that in 2007 there were almost 600 AFP cases (compared to ~400 AFP cases in 2004) detected by our AFP surveillance system, from none of which wild poliovirus was isolated.

The case presented in this report, was one of the AFP cases detected by our AFP surveillance system in 2005. Unfortunately, the stool specimens had been collected 2 months after paralysis onset due to the patient’s parents’ ignorance of submitting her to the hospital in time, and obviously the virus titer had decreased drastically so that it could not be isolated in RD cells with the routine two passage numbers.

Cell culture is a time consuming procedure and sometimes it does not have adequate sensitivity for EV71 isolation. EV71 seems to be fastidious and growth of this virus is often slow compared to the other enteroviruses, and multiple blind passages are often required for EV71 to be isolated. A variety of human and primate cell lines, including primary Rhesus monkey kidney cells, diploid human lung fibroblast cells, RD cells, and vero cells can be used to isolate EV71 (Hanson et al., 1988).

During the recent years, there have been reports of emerging PCR techniques and different primer sets which have been used for EV71 detection (Oberste et al., 1999; Oberste et al., 2000). The technique used in this study was a very sensitive method so that 10 RNA copies per reaction could be detected (Nix et al., 2006).

The EV71 presented in this report was initially reported as negative and the case was classified as AFP due to Myopathy. With the sensitive method we used, EV71 detection could be feasible. This finding opened a new window to a probable cause for increasing rate of AFP cases in Iran. There are untypable NPEVs in the archive of Iran National Polio Laboratory, which have had no reaction with the routine antisera used for NPEV typing and so they have been reported as “untypable NPEV”. Now with the detection of the first EV71 in Iran, another investigation should be scheduled to trace probable existence of EV71 in these specimens.

Despite some understanding of the variability in clinical manifestations and epidemiologic pattern, little is known about the contribution of EV71 to overall AFP in different countries. Public health personnel and pediatricians should be alerted to the possible role of NPEV infections in the differential diagnosis of AFP, particularly in the areas where the circulation of wild poliovirus has been ceased. The laboratory diagnosis of all AFP cases should routinely include tests capable of detecting EV71 as well as other NPEVs.
once the primary objective of poliovirus eradication has been achieved.

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