

Research article

Isolation and Identification of Sabin poliovirus in middle and southern Iraqi provinces

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ABSTRACT

Three hundred and sixty feces samples were collected from children and infants who have diarrhea and other symptoms that associate with Acute Flaccid Paralysis (AFP). The samples were collected from middle and southern provinces of Iraq. The clinical cases were equal or less than four years old. Nineteen positive poliovirus positive cases were found post preliminary culture. Poliovirus type I was found in six cases, Poliovirus type II was found in three cases and Poliovirus type III was found in six cases. Four cases containing mix poliovirus types. All positive cases were screened in confirmation test and the results showed that 9 isolates of type I, 3 isolates of type II and 10 isolates of type III. All isolated viruses were sabin type and no wild type poliovirus was identification in current study. It can be concluded that the poliovirus sabin maybe associated with Acute Flaccid Paralysis.

Keywords: Acute Flaccid Paralysis, Poliovirus, Sabin, Wild type.

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INTRODUCTION

Poliovirus belong to picornavirus, despite the damage it caused in nerve tissue the poliovirus has been placed in the enteroviruses family of viruses that live in the gastrointestinal system [1]. It is formed of single strand of RNA enclosed in a protein coat that protects it from environment attack. Humans are thought to be Poliovirus `s only host, which is why WHO (World Health Organization) launched an eradication program [1]. Poliovirus associated paralysis in the US since 1974 have

been associated with the oral live virus vaccine [2, 3]. This virus gave certain symptoms cause Acute Flaccid Paralysis (AFP) and it is responsible for poliomyelitis the last came from two Greek words, Poli which means gray and myelitis means inflammation of spinal cord, poliomyelitis can cripple and kill vulnerable individuals specially children within days [4]. The European and Eastern Mediterranean regions of the WHO have made substantial progress toward the goal of eradicating



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poliomyelitis by 2000 [5]. As of June 1998 only two foci of known wild poliovirus transmission remained in the border areas of these two WHO regions: Southeastern Turkey/ northern Iraq and Tajikistan/Afghanistan [6,7]. Eradication has dependent on the use of the live oral polio vaccine (OPVs) originally developed by Sabin [8]. But the last associated with many problems, First the live oral polio vaccine is known to be able to cause poliomyelitis in rare cases in both vaccines and their contacts [3]. Second some hypogammaglobulinemic patients are known to excrete virus for long periods of time, giving opportunities for the virus to evolve to transmissible and virulent forms [9, 10]. The present study aimed to show the prevalence and presence of wild and sabin (vaccine of poliovirus) poliovirus in stools of many cases that collected from Iraqi provinces south and middle.

MATERIALS AND METHODS

Samples

The standard method of Eissa was followed [11]. Two to four grams of feces of 360 samples were collected from children and infants who have diarrhea and other symptoms that associate with Acute Flaccid Paralysis (AFP). All samples were collected from Al-Sadar learning hospitals in middle and south provinces of Iraq. The period of collection was from 1-1-2008 to 1-7-2010. All samples were collected from patients aged less than four years.

Preparation of fecal samples for virus isolation

The samples were mixed with 10 ml of Phosphate Buffer saline (PBS) pH 7.2, 1 gm of glass beads and 1 ml of chloroform. The tubes were closed and shaken vigorously for 20 minutes using a mechanical shaker. All tubes were spun for 20 minutes at 1500 g in a refrigerated centrifuge. Fecal suspensions were stored at -20 °C till using [11].

Virus isolation

Fecal suspensions were inoculated into tissue culture tubes of RD cells line (cells line derived from human rhabdomyosarcoma) culture. The supernatant of positive culture was re-cultured into tissue culture tubes of L20B cells line (mouse cell line expressing the gene for the human cellular receptor for poliovirus). The cells were culture in Eagle's minimum essential medium with Earle's salt solution (MEM) and fetal bovine serum (FBS), were purchased from Sigma, St. Louis, Mo. All cell culture

media contained HEPES buffer, L-glutamine, penicillin, streptomycin, gentamicin sulfate, and amphotericin B. Cell cultures were grown in CO2 incubators at 35.5 °C and 4.5% CO2. Stock cell cultures were grown in 75-or 162-cm2 plastic flasks (Costar, Corning, N.Y.) with 5 or 10% FBS-MEM. Cultures were read the day after inoculation to check for both Cytopathic effect (CPE) and contamination or toxicity problems. If neither of the above were noted, they were read routinely three times per week (Incubation period at least one week) [11, 12].

Identification of the virus (Poliovirus)

Neutralization method was used to identification of Poliovirus, this method was applied in flat micro titer plate, and specific anti-sera for this virus (WHO Lab.) were used for Neutralization method [11].

Intertype differentiation of Poliovirus.

ELISA method (RIVM) was used to identify the Sabin and wild types of Poliovirus and subtypes of virus I, II and III [13].

RESULTS AND DISCUSSION

All samples were taken from patients with AFP symptoms. They were aged four years old because this age is more sensitive to infect with Poliovirus [13, 14]. Virus has been isolated from patient's feces instead of other samples because the gastrointestinal tract is the primary site of replication for virus [11]. All samples were cultured on RD cells line at first step to isolate enteroviruses and the second step the positive samples (CPE on RD cells line) re-cultured on L20B cells line because the first cells line is more sensitive for enteroviruses and the second cells line are used to isolate poliovirus because it has specific receptors for poliovirus only [13]. When preliminary culture was happened the total samples that gave positive to poliovirus were 19 samples and number of samples that have poliovirus type I, II and III were 6, 3 and 6, respectively. In addition numbers of samples that carried mix types of poliovirus were 4 (**Table 1**).

The results of confirmation test were shown in **Table 2**. These results showed numbers of samples that contain type I, type II and type III were 9, 3 and 10, respectively. All isolated viruses were sabin type and there is no wild type of poliovirus was isolated in present study.

Table 1. Results of preliminary culture shows number of samples that have total, types and mix types of poliovirus.

	AFP cases specimens	Poliovirus types					Number of Negative cases
		Total	I	II	III	Polio mix	
Number of samples	360	19	6	3	6	4	341

AFP: Acute Flaccid Paralysis

Table 2. Poliovirus types post running in confirmation test that dependent on L20B cell line.

	Polio type I		Polio type II		Polio type III	
	Wild	Sabin	Wild	Sabin	Wild	Sabin
Number of samples	0	9	0	3	0	10

Fortunately, all positive cases were carrying sabin virus but the number of positive cases in Iraq was higher than in countries that surround Iraq such as Iran, Jordan, Kuwait, Syria and Saudi Arabia [15]. But the number of positive cases in Iraq was less than high risk countries such as Afghanistan and Somalia [15]. The factors than affect on transmission of the virus include extent of crowding, level of hygiene, water quality and Sewage handling facilities [13]. In Iraq there is much interaction among all transmission factors that mention above, that is why high rate of poliovirus separating (Sabin) was found. In Iraq the schedule of vaccination may be not regulated because the security situation that will result increasing in doses in certain area and decreasing in another area. Recently, sabin may be acting as a parent for new risk generation of Sabin calls cVDPV (clone of vaccine derived poliovirus). The last was responsible for 21 confirmed polio cases (including two fatal cases) on Caribbean island of Hispaniola in 2000-2001 [16, 17]. The presence of new generation of sabin in nature and in carriers is very risky. Thus, in Iraq the people may be infected with cVDPV. The immunological status of patients should be studies especially T lymphocytes as the role of this cells in regulating the immune system and developing autoimmune disease [18] That is why; we suggest many point should be raised in mind of researchers; first the immunological status of patients has to be investigated, second, the new method should be followed to prevent separation of sabin in Iraq such as study the ability of sabin poliovirus to develop infection in healthy host.

Conflict of interest

The author declares that he has no conflict of interests.

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