

Role of Non-Polioviruses in Acute Flaccid Paralysis (AFP)

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ABSTRACT

Acute Flaccid Paralysis (AFP) is a complex syndrome caused by different infectious and non-infectious agents, mostly by Enteroviruses. Mass vaccination against Polioviruses with Oral Poliovirus Vaccine (OPV), under the supervision of World Health Organization (WHO) has endeavored to eradicate AFP and Poliomyelitis caused by Poliovirus. Although Poliovirus has been eradicated in most countries, the societies have not released of AFP outbreaks, yet. Most researches emphasis on Non-Polio Enteroviruses (NPEV) as main isolates in AFP affected patients. Because the traditional methods of cell culture and neutralization used in corresponding laboratories cannot detect some of the agents, it is recommended to use more sensitive diagnostic methods, such as Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), for increasing the rate of isolation.

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Key words: Acute flaccid paralysis; Non-Polio Enteroviruses; RT-PCR; Cell Culture

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INTRODUCTION

Acute Flaccid Paralysis (AFP) is a rapid and sudden onset of weakness of muscles in different parts of the body, including foot, hand and

rarely muscles of respiration tracts and swallowing system, that begins anytime between after birth and under 15 years of age and does not progress further after its increase through 4-5 days of symptoms^[1,2,3,4]. Non-immune individuals usually (72%) show an unapparent infection, and paralytic disease is exclusive for only 1/1000 to 1/100^[5]. The symptom starts with fever, vomiting, constipation or sometimes diarrhea, headache, fatigue, stiffness in the neck as well as pain in the limbs. The acute paralysis is seen mainly in young children under 15 years of age^[5,6].

The term "flaccid" emphasizes on a lack of spasticity and other signs including hyperreflexia, extensor plantar responses and clonus in the Central Nervous System (CNS)^[3,4,5]. "Paralysis" or Plegia in muscles is a severe loss of contraction in the muscle cell as a result of an interruption in the signaling from CNS to the muscles (in contrast to Paresis that is a slight defect in motor strength)^[3,7]. In case of AFP caused by Enteroviruses, the neuron cells expressing enteroviral receptor (N-terminal V-type immunoglobulin-like domain of hPVR or CD155)^[8] (especially anterior horn cells, are affected, and their destruction is responsible for inflammation and cell loss in the spinal cord^[5,7]. Sometimes, AFP can persist forever (Residue Paralysis), and can even lead to death because of a defect in the respiratory muscles^[3]. The exact pathological mechanism of AFP has not been discovered^[8]. Based on the symptom manifestations, poliomyelitis is classified as spinal poliomyelitis (acute flaccid paralysis, bulbar poliomyelitis (paralysis of respiratory muscles) and bulbospinal poliomyelitis^[6].

AFP is a complex disease caused by different microbial, chemical and physiological agents. Viruses that can cause AFP include some viruses such as Enteroviruses, mumps virus, Rabies virus^[3], some Arboviruses such as Togavirus, Japanese encephalitis virus^[9], Human Immunodeficiency Virus (HIV)^[3,8], West Nile virus^[10], herpes simplex virus^[8], cytomegalovirus^[3], the varicella-zoster virus^[3] and Epstein-Barr Virus^[3]. Certain bacterial infections and toxins can also cause AFP. These include *Campylobacter jejuni*^[5], *Clostridium botulinum*^[5,11], Diphtheria^[5,11], *Mycobacterium tuberculosis*^[5,8,12], *Treponema pallidum*^[8], tetanus^[5,13,14] and *Moraxella*^[8] and Mycotoxins (*Penicillium islandicum* and *Penicillium citrinum*)^[15]. Of the parasites that can cause AFP, one can name some *Giardia lamblia*^[5,8], *Toxoplasma gondii*^[5,7,8]. Other agents include shellfish's saxitoxin^[5] and ichthyotoxism^[8], insects (Cockroach, beetle, Tick paralysis, Lepidoptera larvae, wasp venom, spider venom)^[5], Reptiles-snake venom (Cobra, krait,

mamba, Australian elapid and sea snake)^[5], plants and plant toxins [daisy, monkhood, sweet pea, bean (Cassia), parsley, berries, blossoms (Gelsemium) Evergreen (Cycas), bush tea shrub (Heliotropium), Melochia species, parsnips (Oenanthe), Phenolic pigment in the cottonseed, Trecresyl phosphate (in Jamaican ginger tonic)]^[5, 6], Heroin^[6], Antibiotics (Aminoglycosides, Polymyxin B, Tetracyclines)^[5, 17, 18], Pesticides [Trichlorfon, leptophos (Phosel), Dipterex, dichlorvos (DDVP), Isofenphos (Oftanol)]^[5], Metals (lead and Barium)^[5], Volatile hydrocarbons (Methyl Butyl Ketone, Hexane and Carbon disulfide)^[3, 16], mouse poisons (eg, diethyltoluamide, triethyl-dodecyl-ammonium bromide and dithiobiuret)^[11, 16], Cantharidin, and some physiological situations such as Hypokalemic (eg, hyperthyroidism, hyperaldosteronism^[5] and Familial Sjogren's Syndrome)^[5, 11, 13], Normo/Hyperkalemic Hypophosphatemia^[3, 5, 16] (Familial Adynamia Episodia Hereditaria of Gamstorp)^[5], Asthma (Polio-like Hopkins' syndrome)^[16], Werdnig-Hoffmann disease^[5], porphyric polyneuropathy^[5], Wohlfart-Kugelberg-Welander disease^[5], and also, China paralytic syndrome Guillain-Barre syndrome (GBS)^[16, 19, 20], transverse myelitis and Bell's palsy^[5, 18].

There are no unique clinical symptoms to differentiate between different cases of AFP^[17]. AFP is distinguished from GBS based on their clinical symptoms; in addition to the absence of fever, headache, vomiting, nausea and pleocytosis in GBS, the syndrome causes a symmetrically paralysis along with sensory changes^[19].

ENTEROVIRUSES

Enteroviruses, one of the main factors of AFP in children, are a group of viruses belonging to the Picornaviridae family^[17, 21] that contain a positive stranded RNA covered by an icosahedral capsid^[16]. Although Enteroviruses were previously classified based on their pathogenicity and serology into Polioviruses, Echoviruses and Coxsackie A and B, the viruses have extensive similarity in biological aspects, and therefore this type of classification is usually time consuming and labor intensive, and can lead to complication in research and laboratory diagnosis. A new classification based on genotyping (VP1 gene)^[22-26] classifies the human Enteroviruses into 4 groups (HEV A, B, C and D), and all of the serotypes are placed in the 4 subdivisions (Table 1)^[23, 26]. VP1 is one of the Capsomers on the surface of the viruses, which plays a significant role in their antigenicity^[25]. Because the new molecular method is

based on amplifying the viruses' RNA to DNA (reverse transcription-polymerase chain reaction)^[22], it is more sensitive and user friendly for detecting the isolates' genotype^[23, 26]. Also, as Enteroviruses are divided into 4 groups only, the classification can resolve the complexity of serological tests for detecting more than 80 Enteroviruses^[26].

Enteroviruses are transferred via fecal-oral route and respiratory droplets, and can replicate in gastrointestinal tracts and nasopharynx^[17, 32]. They can then shed to the blood stream and cause viremia, which seed different organ systems, including lung, liver, heart and CNS. Up to now, different clinical manifestations ranging from asymptomatic infection and mild febrile sickness, (acute gastroenteritis, respiratory tract infection, hand-foot and mouth disease and aseptic meningitis) to severe and lethal illnesses (such as pulmonary, Sepsis, hemorrhagic conjunctivitis, myocarditis, encephalitis and AFP) are attributed to these viruses^[5, 26]. A variety of factors, including age, gender, health conditions, the immune system, host cell membrane receptors and dietary) can affect the severity of disease. It has been shown that there is a close association between the molecular type of Enteroviruses and the clinical manifestations. For example, aseptic meningitis and gastrointestinal symptoms are mostly caused by HEV-B, while most Hand-foot-and-mouth diseases are caused by HEV-A^[26].

ENTEROVIRAL ACUTE FLACCID PARALYSIS

Historically, Polioviruses I, II and III (Enteroviruses C in new classification) were the most significant member of Enteroviruses. Approximately 1% of polio infections lead to viremia and infection of motor neurons and cause irreversible weakness of lower limb and respiratory (5-10%) muscles, AFP and residual paralysis^[46].

Mass vaccination against Poliovirus with the Sabin vaccine or the Oral Poliovirus Vaccine (OPV)^[29, 30], started in 1989^[7] and has decreased the number of poliomyelitis affected persons from 350 000 (1998) to 1500 (2008)^[47], and from 125 countries in 1998 to four nations in 2008^[14]. A country can be declared as Poliovirus-free if it meets three criteria: (1) the mass vaccination against poliovirus covers more than 80% of the targets; (2) No wild-type poliovirus has been isolated for the past three years; and (3) a sensitive investigation on suspected persons and proper surveillance of Acute Flaccid Paralysis^[29, 30, 48]. AFP surveillance endeavors to report all flaccid paralysis in less than 15 years old children, including GBS and excluding Trauma. AFP

Table 1 Serotypes of Enteroviruses. The serotypes that have been recognized as etiology of AFP are underlined ^[4, 11, 12, 17, 18, 19, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45]

	Coxsackievirus A	Coxsackievirus B	Enteroviruses	Echovirus	Polioviruses
Enteroviruses A	CV-A2 ^[27] , CV-A3 ^[28] , CVA4 ^[18] , CV-A5 ^[27] , CV-A6 ^[32] , CV-A7 ^[29] , CV-A8, CV-A10 ^[28] , CV-A12 ^[27] , CV-A14 ^[18] , CV-A16 ^[18]		EV-71 ^[18] , EV-76 ^[33] , EV-89, EV-90 ^[27] , EV-91, EV-92		
Enteroviruses B	CV-A9 ^[19] , CV-A ^[23]	CV-B1 ^[29] , CV-B2 ^[29] , CV-B3 ^[32] , CV-B4 ^[29] , CV-B5 ^[12] , CV-B6 ^[29]	EV-69, EV-73 ^[34] , EV-74 ^[34] , EV-75 ^[34] , EV-77 ^[17] , EV-78 ^[34] , EV-79, EV-80 ^[35] , EV-81 ^[33] , EV-82 ^[27] , EV-83 ^[33] , EV-84, EV-85, EV-86 ^[37] , EV-87, EV-88, EV-93 ^[33] , EV-97 ^[37] , EV-98, EV-100, EV-101, EV-106, EV-107	E-1 ^[4] , E-2 ^[29] , E-3 ^[12] , E-4 ^[32] , E-5 ^[12] , E-6 ^[12] , E-7 ^[12] , E-8, E-9 ^[32] , E-11 ^[36] , E-12 ^[12] , E13 ^[36] , E-14 ^[29] , E-15, E-16 ^[32] , E-17, E-18 ^[18] , E-19 ^[18] , E20 ^[29] , E-21 ^[29] , E-24 ^[4] , E25 ^[29] , E-26 ^[4] , E-27 ^[33] , E-29, E-30 ^[36] , E-31, E-32, E-33 ^[11]	
Enteroviruses C	CV-A29, CV-A11 ^[32] , CV-A13 ^[33] , CV-A17 ^[33] , CV-A19 ^[18] , CV-A20 ^[28] , CV-A21 ^[32] , CV-A22 ^[18] , CV-A24 ^[19]		EV-95, EV-96 ^[28] , EV-99, EV-102, EV-104, EV-105, EV-109		PV-1 ^[31] , PV-2 ^[31] , PV-3 ^[31]
Enteroviruses D			EV-68, EV-70 ^[33] , EV-94 ^[33]		

surveillance, indeed, is considered as the most sufficient method for evaluating the Poliomyelitis eradication program (PEP); at least one case of AFP per 100 000 children below 15 years of age annually is considered an effective AFP surveillance. In addition, it is compulsory to report all AFP cases within 14 days, and two stool samples must be collected at least every other day, and be sent to AFP laboratory center via a cold chain transferring system (80% of AFP cases need to have adequate specimens). All cases need to be followed up for 60 days in order to determine probable residual paralysis cases^[2,16,39,48,49,50]. Cell culture (on RD, L20b and Hep-2) and neutralization are the routine and conventional methods for identification of Enteroviruses from stool. However, because some of Enteroviruses cannot replicate in mentioned cell lines, identification based on molecular methods, such as RT-PCR, is recommended^[12,18,21].

Although the rate of poliomyelitis has been declined through the mass vaccination program, AFP and residual paralysis cases are reported continuously. The cases occur all around the world, even in the Poliovirus eradicated countries^[12,18,30,48,52]. Iran, for example, is one of the countries in which poliovirus has been eradicated since 2001, but the rate of reported AFP increased gradually from about 400 cases in 2004 to 600 cases in 2007^[15]. The situation has had scientists to study on different agents of AFP in children. Because Non-Polio Enteroviruses are capable of replication in motor neurons, they could be considered as a probable cause of this huge prevalence of AFP^[12,48]. Researches on Vaccine-Derived Polioviruses (VDPV) showed that the attenuated vaccines are one of the potential reasons of AFP and sometimes even residual paralysis^[12,18,30,51,48,52]. Although OPV is an effective vaccine for preventing polio dependent paralysis, it has some disadvantages^[53]. It is worth mentioning that the vaccine can lead to Vaccine Associated Paralytic Poliomyelitis (VAPP) in very rare cases. In addition, by passing through different human hosts, the attenuated virus can recover its genetic materials and return to its virulent wild type^[54]. An OPV is defined as a VDPV when it shows more than 1% genetic divergence in VP1 genes versus the Sabin strain^[55,56]. Numerous outbreaks worldwide, such as Cambodia^[57], China^[58], the Philippines^[58], Madagascar^[59], Indonesia^[60], Iran, Israel^[60] and other countries indicate the high probability of genetic conversion, and is affected by different factors such as population density, sanitation level, socio-economic status, level of immunity in the population, dietary habits and so on.

ROLE OF NON-POLIO ENTEROVIRUSES IN AFP

In addition to VDPV that has been isolated only from a small group of AFP affected patients, Non-polio Enteroviruses (NPEVs) are considered as main suspected agents. Different types of Enteroviruses have been associated with Acute Flaccid Paralysis (Table 1)^[12,18,30,48,52]. However, because NPEVs circulate easily through the world, their isolations are not absolutely associated with their etiology in paralysis^[32]. Therefore, assuming that Non-Polio Enteroviruses are causal agents of AFP needs further investigation. According to the guidebook issued by the WHO, during 1967-1970, just less than 1% of the patients who were infected with Non-Polio Enteroviruses showed paralysis. The report emphasizes on Non-Polio Enteroviruses, including Coxsackie virus, Echovirus and new discovered Enteroviruses as major factors of AFP^[18]. The WHO reported a same percent of non-polio enteroviral AFP during 2000-2003^[5]. Nowadays, such reports are being issued all around the world. Scottish researchers reported isolation of Coxsackie virus

from 12 cases out of 77 patients with RP during 1956-1973^[5]. Australia showed that 15% of AFPs in 1963, three years after eradicating indigenous Poliomyelitis in 1960, caused by Non-Polio Enteroviruses. Nigeria has also reported that 14.6% of AFP during 2002-2003 was caused by Non-Polio Enteroviruses, and 24 cases of them with isolates of Echovirus serotypes showed residual paralysis^[18]. After that, a dramatic increase of Non-Polio Enteroviral AFP from 20% to 54% was reported in India^[36]. Furthermore, there are a big number of sporadic cases of AFP in which Non-Polio Enteroviruses were isolated^[18,20,15,19,31]. EV71 is one of the examples which numerous sporadic and outbreaks of its infection with neuron motor disorders, such as AFP, has been reported^[11,15], and is considered as the most common NPEV associated with AFP and Poliomyelitis like paralysis^[20]. Coxsackievirus A9 (CVA9), Coxsackievirus B1 (CVB1) and Echoviruses 6, 9 and 18 constitute 54% of isolations from AFP cases during 2006-2008^[61]. It is important to mention that although it is rare, NPEV induced AFPs can lead to Residual paralysis^[5]. However, most patients show a complete recovery^[20].

Since these viruses exist worldwide, and numerous outbreaks of them are recorded, and because the clinical signs of NPEV induced AFP are similar to the AFP induced by Poliovirus, Non-Polio Enteroviruses are considered as one of the most important indicators in AFP surveillance. Therefore, the differential diagnosis of AFP based on laboratory tests is one of the important factors in order to evaluate the role of Non-Polio Enteroviruses in such disorders^[32].

As mentioned above, the evidences indicate a new pattern of AFP disease in which NPEV and VDPV are considered as critical factors. Therefore, poliomyelitis centers should improve their diagnostic methods in order to detect the viruses. Enteroviruses are conventionally identified by inoculation of the chloroform-treated stool samples in RD and L20b cell culture, and then detecting their serotypes by neutralization test^[18,22]. However, some of the viruses such as Coxsackie viruses, cannot grow in routine cell cultures, and need to be cultured in additional cell cultures such as Hep-2. Furthermore, applying a combination of two or three different cell lines makes the method costly and time consuming^[22,60]. Last but not least, contamination of the cell cultures with different bacteria and fungi, especially *Mycoplasma Sp.*, is one of the most common problems in such laboratories^[18,22]. RT-PCR (Reverse Transcriptase Polymerase chain reaction) is a sensitive method using a primer prepared from VP1 gene that enables laboratories to identify the viruses directly from stool samples. Such molecular techniques not only improve laboratories' sensitivity for detection of the virus, but are also more convenient and user friendly, and of course, more economic^[12,18,21,22,32,60]. Conceivably, adequate financial resources should be provided to equip the laboratories and to improve the probability of causality.

CONCLUSION

Nowadays, Non-Polio Enteroviruses (NPEV) and Vaccine Derived Polioviruses (VDPV) are the main suspects of poliomyelitis and AFP, excluding wild type Poliovirus in Afghanistan, India and Nigeria. It appears NPEV and VDPV are becoming new concerns of the WHO for eradication of the AFP. As current laboratory approaches, cell culture and neutralization, are not able to detect all of the serotypes, new strategies such as RT-PCR are recommended for a better surveillance of AFP.

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