ACUTE FLACCID PARALYSIS SURVEILLANCE: LOOKING BEYOND THE GLOBAL POLIOMYELITIS ERADICATION INITIATIVE

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Abstract. In 1992 surveillance of acute flaccid paralysis (AFP) cases was introduced in Malaysia along with the establishment of a national referral laboratory at the Institute for Medical Research. The objective of this study was to determine the incidence, viral etiology and clinical picture of AFP cases below 15 years of age, reported from 2002 to 2007. Six hundred seventy-eight of 688 reported cases were confirmed as AFP by expert review. The clinical presentation of acute flaccid paralysis in these cases was diverse, the most commonly reported being Guillian-Barre syndrome (32.3%). Sixty-nine viruses were isolated in this study. They were Sabin poliovirus (25), Echovirus (22), Cocksackie B (11), EV71 (5), Cocksackie A (1), and untypable (5). Malaysia has been confirmed as free from wild polio since the surveillance was established.

INTRODUCTION

Acute flaccid paralysis (AFP) is a clinical syndrome characterized by rapid onset weakness of limbs, often with weakness of respiratory muscles and difficulty in swallowing. If untreated, AFP may lead to death due to failure of respiratory muscles.

Surveillance for AFP is an important activity of public health programs in many countries because it is the key strategy used in the Global Polio Eradication Initiative by the World Health Organization (WHO, 1988). WHO's commitment to the Global Polio Eradication Initiative, has achieved impressive progress with only four countries remaining endemic for polio. These countries are India, Pakistan, Nigeria and Afghanistan (WHO, 2008). WHO's success has been attributed largely to a sensitive surveillance strategy, which requires countries to conduct surveillance of all AFP cases using a standard case definition. Active AFP surveillance in children below 15 years of age is ongoing until global eradication is achieved, to monitor risk of importation of wild polioviruses into non-polio endemic countries. With near eradication of polio, AFP surveillance has directed the focus on other causes of this alarming clinical syndrome.

AFP is caused by many conditions, including viral infections, notably, poliomyelitis, Guillain Barre syndrome (GBS), transverse myelitis, metabolic neuropathies and trauma. In poliomyelitis, poliovirus invasion of the anterior horn cells of the spinal cord can manifest with asymmetrical weakness of limbs with no sensory symptoms or signs. Non-polio enteroviruses (NPEV) may also cause paralytic disease, but it is usually less severe than poliomyelitis. Most cases are due to enterovirus

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70, enterovirus 71, coxsackie A7 and echoviruses (Solomon and Wilson, 2003). Many studies have found GBS to be a leading cause of AFP (Marx *et al,* 2000). Also known as Acute Inflammatory Demyelinating Polyneuropathy, GBS is an immunologically mediated para-infectious or post-infectious process causing damage to the lower motor neurons in the peripheral nerves or nerve roots. The underlying pathology, precise cellular basis or pathophysiologic mechanisms for some causes of AFP are still not clearly understood. Accurate diagnosis of the cause for AFP has important implications for therapy and prognosis of cases.

In Malaysia, the Virology Unit at the Institute for Medical Research in Kuala Lumpur was designated as the National Reference Laboratory for Poliomyelitis Eradication (NRLPE) by the WHO in 1992. Under the AFP surveillance program, clinicians are required to notify every case of AFP, in children under the age of 15 years to the State Health Office using a standard AFP surveillance form, wherein the relevant clinical and epidemiological data of the patient is recorded. Two fresh stool specimens collected 24 hours apart and within 14 days of onset of paralysis are required to be sent in appropriate transport conditions to the NRLPE. Each year a significant number of samples are received by our laboratory from hospitals throughout Malaysia for laboratory investigation of AFP cases. In this study, we analyzed AFP surveillance data from January 2002 to December 2007, to determine the incidence, viral etiology and clinical picture of AFP, in children below 15 years.

MATERIALS AND METHODS

Samples

Between January 2002 and December 2007 the NRLPE received 1,437 stool specimens from 688 reported AFP cases sent from hospitals throughout Malaysia. Another 138 specimens, comprised of throat swabs and cerebrospinal fluid, were also received from these cases. The specimens were accompanied by an AFP notification form with details of patient personal and clinical history.

Cell cultures

All stool specimens were processed with chloroform before inoculation into RD and L20B cell lines from our laboratory stock held in liquid nitrogen at low passage. The cultures were grown in monolayers in 75 cm^2 tissue culture flasks in Earles basal medium supplemented with L-glutamine, Hepes buffer, pH 7.2, sodium bicarbonate, 10% fetal calf serum, 100 IU /ml penicillin G and 100 µg/ml streptomycin sulfate. Cells from confluent cultures were resuspended in maintenance medium and the concentration was adjusted to 6 X 10^5 cells/ml for use in the assay. Inoculated cell cultures were examined daily for cytopathological effects (CPE). The WHO standard protocols for pre-treatment of stool specimens and virus isolation by cell culture were followed (WHO, 2004).

Microneutralization test procedure

Positive cell cultures were confirmed by microneutralization assay using standard WHO antisera. WHO standard enterovirus antiserum pools (0.05 ml) were distributed into wells of a microtiter plate followed by 0.05 ml of virus dilutions from 10^{-1} to 10^{-7}. The plates were covered and incubated at 36ºC in a carbon dioxide incubator for 1 hour. After incubation 0.01 ml of cell culture suspension, containing approximately 1.5 x 10^5 were added to all wells. The antiserum pools that prevent the development of CPE indicated the identity of the virus isolated. Poliovirus isolates were sent to the Victorian Infectious Disease Reference Laboratory (VIDRL) in Melbourne, Australia for further identification and intratypic differentiation.

Controls

The controls incorporated in the assay

included cell control to check for normal cell morphology and virus control to detect virus infectivity with complete CPE. Back titration was included to confirm that the amount of virus used in the assay was within an acceptable range.

AFP clinical picture

All AFP reported cases were followed up for 60 days to ascertain residual paralysis. This activity was monitored by the Surveillance Unit, Disease Control Division of the Ministry of Health by communication with clinicians attending to the AFP cases and from monthly notification data obtained from State Health Departments. Clinical assessment of all reported AFP cases were also reviewed at the Expert Polio Review Meetings.

RESULTS

During the period of this study, 688 AFP cases below age 15 years were reported to our laboratory. Following review by the Polio Expert Group, 10 cases were classified as non-AFP by reassessment of clinical findings and only 678 were confirmed as AFP. The annual incidence of reported AFP cases and specimens received are shown in Table 1. This may not indicate the actual incidence of AFP, as many cases may not have been reported to our laboratory for viral investigation. Since AFP notification is part of the poliomyelitis eradication exercise some cases may have been omitted when a definite diagnosis by history, for example trauma, did not indicate the need for laboratory investigation at the time of clinical assessment. Enteroviruses were found in 69 cases (4.9%) out of 1,416 stool specimens received from these AFP cases. Polioviruses was found in 25 cases (36.2%). All these polioviruses were confirmed as vaccine related Sabin-like strains by the VIDRL, the WHO Reference Laboratory in Melbourne, Australia. The remaining 44 virus isolates were non-polio enteroviruses. The identification and distribution of these viruses are shown in Figs 1 and 2. Echoviruses accounted for the majority of non-enterovirus isolated from the AFP cases in this study (50%), followed by coxsackie B viruses (25%)

Table 1
Annual reported AFP cases in children below 15 years of age and specimens received for laboratory investigation.

Year	Reported AFP cases	Confirmed AFP cases	Incidence rate (Per 100,000)	Stool specimens	Enteroviruses isolated
2002	107	107	1.3	222	10 PVSL
					11 NPEV
2003	100	98	1.18	191	6 PVSL
					10 NPEV
2004	120	114	1.35	243	8 NPEV
2005	138	137	1.61	292	5 PVSL
					5 NPEV
2006	116	116	1.34	239	4 PVSL
					4 NPEV
2007	107	106	1.21	229	6 NPEV
Total	688	678	Range1.2~1.6	1,416	69 Enteroviruses

PVSL = Vaccine related Sabin-like polioviruses; NPEV = Non-polio enteroviruses

and enterovirus 71 (11.4%). Table 2 shows the clinical diagnosis of the 678 confirmed AFP cases included in the study. For convenience of presentation, some of less frequent clinical presentations have been grouped using a neuro-anatomical approach. Fourteen of the 678 AFP cases did not have a specific clinical diagnosis other than a report of AFP. When the available clinical data was inadequate, the AFP cases were followed up with the attending pediatrician in charge to get a full case report. These cases either had adequate

Table 2
Clinical diagnosis of confirmed AFP cases from 2002 to 2007.

Clinical diagnosis	*N*	%
Condition		
Guillain-Barre syndrome (Including acute and chronic inflammatory demyelinating polyneuropathy, post-infection demyelination)	219	32.3
Transverse myelitis	36	5.3
Meningitis / meningoencephalitis	85	12.5
Viral encephalitis	26	3.8
Vaccine associated poliomyelitis	3	0.4
Enteroviral parainfectious flaccid paralysis	2	0.3
Critical illness (acute excerbation of bronchial asthma due to pneumonia, upper respiratory tract infection with hyperemesis, bronchopneumonia and diarrhea)	4	0.6
Site / Factor (Contition)		
Brain (encephalomyelitis / myelitis , cervical cord tumor, cerebral tumor, cerebral atropy acute lymphoblastic leukemia, rheumatic chorea, recurrent transient ischemic attack, cerebral ataxia, right sided thalamic infarct, extrapyramidal cerebral palsy, partial seizures, Moya-moya disease with intracranial hemorrhage, cerebral edema due to fall, acute demyelinating encephalomyelitis (ADEM, multiple sclerosis, cerebrovascular accident, mitochondrial myopathy)	114	16.8
Musculoskeletal (viral myositis, septic arthritis, transient synovitis, musculodystrophy, viral myalgia)	62	9.1
Nerve (peripheral neuropathy, traumatic neuritis, post-infective neuritis, neuropraxia secondary to infection, vasculitis, Miller-Fisher Syndrome)	23	3.4
Neuropathies associated with systemic / metabolic disorders (hypokalemic periodic paralysis, nephrotic syndrome, porphyric crisis, renal tubular acidosis, acute urinary retention) hypokalemia due to diarrhea and malnutrition, Bartter's Syndrome, Inborn error of metabolism	63	9.3
Spinal cord (spinal cord tumor, spinal cord injury, extradural compression, syringomyelia, spinal dural AV fistula	16	2.4
Neuromuscular junction (myasthenia gravis, botulism, jelly fish sting)	6	0.9
Nerve root (brachial plexitis)	5	0.7
Not specified	14	2.1
Total	678	100

ADEM- acute demyelinating encephalomyelitis

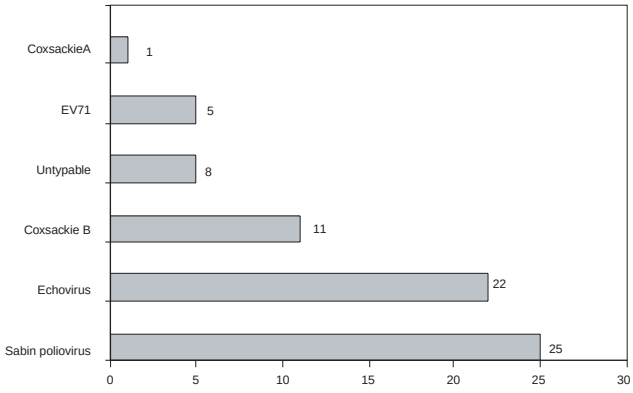

Fig 1–Distribution of Enteroviruses isolated from AFP cases.

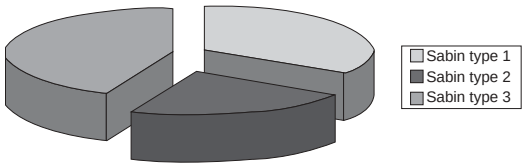

Fig 2–Distribution of Sabin-like poliovirus isolated from AFP cases: Sabin type 3 (43.8%) > Sabin type 1 (34.3%) > Sabin type 2 (21.9%).

stools which were confirmed as non-polio by laboratory investigations or fully recovered motor function. Since this AFP reporting system was based on the national polio eradication program, the Expert Review Group determined these cases as non-polio AFP.

DISCUSSION

The AFP surveillance system provides a sensitive tool for investigating AFP cases in children, with careful clinical evaluation of the differential diagnosis and expert review of cases. Accurate diagnosis requires a precise knowledge of the etiology and underlying pathophysiology. Analysis of the clinical findings and diagnosis reported for the 678 cases in this study showed that the underlying causes were diverse.

No wild poliovirus was isolated during this period. Malaysia has been free of wild poliovirus circulation since the mid 1980s and is one of the countries listed in the Western Pacific Region as nonendemic for poliovirus. The last major outbreak of poliomyelitis in Malaysia occurred in 1977 with 121 cases, including 4 deaths (IMR, 1977). The number of poliomyelitis cases decreased dramatically from 1978 following an effective National Oral Polio Vaccine Immunization Program introduced in 1972. However, three cases of poliomyelitis were reported in 1992 probably due to importation of wild poliovirus (IMR, 1992/1993). Since 1993, no wild poliovirus has been identified; our success being attributed to good immunization coverage and an effective AFP surveillance system. Three of the cases in the study were identified as possible vaccine associated paralytic poliomyelitis (VAPP). VAPP is diagnosed when a vaccine strain of poliovirus is isolated from the stool of an AFP case, where oral polio vaccine was given in the last 30 days preceeding onset of paralysis. Sabin-like poliovirus type 1 and type 3 were isolated from these cases.

With near eradication of poliomyelitis, viruses other than polioviruses have been reported to cause AFP (Solomon and Wilson, 2003). Among these viruses are echoviruses, coxsackie viruses and enterovirus 71, which are also members of the enterovirus genus. Japanese encephalitis virus and West Nile virus have also been reported to cause this syndrome (Solomon *et al*, 1998; Glass *et al*, 2002). In our study, 44 non-polio enteroviruses (NPEV) were isolated; the most predominant, as seen from Fig 1, were the echoviruses. Further investigation by immunofluorescent method using specific antisera pools showed that these were echovirus types 3, 6 and 11. The clinical presentations in these cases included aseptic meningitis, meningoencephalitis, viral encephalitis and GBS. Five out of 44 NPEVs were enterovirus 71 and the clini-

cal presentations in these cases included acute encephalopathy and enteroviral monoplegia. EV 71 has been reported in countries of the Asia Pacific region to cause large outbreaks of hand foot and mouth disease (HFMD) (Cardosa *et al*, 2003). In Malaysia HFMD outbreaks occurred in 1997 and in 1999, where EV71 was reported to cause the neurological complications in some children which included acute flaccid paralysis and brain stem encephalitis (Chan *et al*, 2000).

Gullain Barre Syndrome (GBS) accounted for 32.3% of AFP cases in this study with an overall annual incidence rate of 0.42. In the absence of wild poliovirus induced poliomyelitis, GBS is the most common cause of AFP reported in many parts of the world, accounting for over 50% in many industrialized and developing countries (Marx *et al,* 2000). It is interesting to note that in a previous study of AFP surveillance in Malaysia, data from 1997 to 2001 showed that GBS was found in 30.2% of AFP cases, with an annual incidence of 0.36% (Hussain *et al*, 2004). The majority of GBS cases in the AFP surveillance were diagnosed based on typical clinical features, such as progressive ascending and symmetrical paralysis of the limbs with or without cerebrospinal fluid abnormalities. Not all cases were confirmed by nerve conduction studies, which can only be carried out at regional centers where these facilities and pediatric neurologists are available. Hussain *et al* (2004) found that central nervous system infections were more common in Malaysia than other countries.

Among other significant causes of AFP reported during this period were transverse myelitis (5.3%) and neuropathies occurring from metabolic disorders (9.3%). Metabolic disorders included acute hypokalemic periodic paralysis, hypokalemia from malnutrition and acute gastroenteritis as well as porphyria and renal tubular acidosis. As seen in Table 2, the other conditions and causes attributed to AFP during this surveillance period were broad. These conditions included central nervous system disorders, infantile botulism, jelly fish stings, and weakness associated with critical illnesses, such as acute excerbation of bronchial asthma secondary to pneumonia.

In conclusion, despite the great reduction in the number of poliomyelitis cases following the Global WHO Poliomyelitis Campaign, AFP continues to be an important neurological presentation in children. The list of underlying causes of AFP is broad and complex. Clinicians need to have a detailed knowledge of the differential diagnosis to ensure effective and timely management. The current WHO AFP surveillance provides a sensitive tool for investigating AFP cases with careful clinical evaluation of the differential diagnosis and expert review of cases. It is therefore crucial that AFP surveillance be conducted even in the absence of wild poliovirus transmission.

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