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**RESEARCH ARTICLE**

# Non-polio Enteroviruses Implicated in Residual paralysis among cases of acute flaccid paralysis affecting Iraqi children under 15 years

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## *Abstract*

Acute flaccid paralysis (AFP) is a clinical syndrome known to be manifested in humans by infectious (bacterial or viral) or noninfectious (metabolic disorders or trauma or metal toxicity) causes or post-infectious autoimmune condition. Enteroviruses (EVs)[Poliovirus (PV) and Non-Polio Enterovirus (NPEV)] are among the most common viruses infecting humans worldwide.In Iraq, the isolation and identification of NPEV serotypes from AFP cases has been reported rarely, although the incidence has been reported often. Thus, this study was performed to prove the NPEV role in residual paralysis occurrence among Iraqi children with AFP infection. Results showed 36.33% of AFP cases produced byEVs that distributed to 33.67% as NPEVs and 2.67% as Sabin-like poliovirus.After investigation at 60 day Follow-up to all AFP positive cases, a persistent muscular weakness residual paralysis was found in 44 (40%) of AFP cases. All those cases produced by NPEVs that typing as: echoviruses and coxsackie viruses were 36(81.4%) and 8(18.6%), respectively.The current study concluded that high ability of NPEV (especially echovirus) to produce residual paralysis in children with AFP. Thus, we recommend using molecular typing method to increase the rate of isolation.



## Introduction

Acute flaccid paralysis (AFP) is a clinical syndrome known to be manifested in humans by infectious (bacterial or viral) or noninfectious (metabolic disorders or trauma or metal toxicity) causes or post-infectious autoimmune condition (eg: GuillianBarre syndrome [GBS]) (Marx et al., 2000; Israeli et al., 2012).

Enteroviruses (genus Enterovirus, family Picornaviridae) are among the most common viruses infecting humans worldwide. Enteroviruses are associated with diverse clinical syndromes ranging from minor febrile illness to severe, potentially fatal conditions (e.g.,AFP) (Pallansch and Roos, 2001; Khetsuriani and Parashar, 2003).Based on the pathogenicity in humans andexperimental animals, enteroviruses(EVs) were classified into four groups,polioviruses (PVs), coxsackieviruses A (CVA), coxsackieviruses B(CVB), echoviruses (E), and the numbered enteroviruses (EV) (Minor et al., 1995; Apostol et al., 2012).Since polioviruses and some non-polio enteroviruses can cause acute flaccid paralysis, all suspect cases must undergo thorough virological investigation.

For instance, Echovirus has been implicated in multiple human disease syndromes, including paralysis (Hughes et al., 2003). Most of the AFP cases in United States America were found to be caused by NPEV in the post vaccination era (Dietz et al., 1995). Several cases of paralysis were reported in association with enteroviruses, especially caxsackie virus in Scotland (Gris and Bell, 1984). Paralysis has also been reported in association with coxsackie viruses B2-B6, enterovirus 71 and echovirus types 3,4,6,9,11,19 and 22 (Saeed et al., 2007).

The presence of enterovirus in a clinical sample can be readily determined by applying the specimen to a

proper cell culture system, or directly by molecular detection of the 5'-NTR conserved region of the viral genome. For serotyping, a neutralization test has traditionally been used (WHO, 2004).

In Iraq, the isolation and identification of NPEV serotypes from AFP cases has been reported rarely, although the incidence has been reported often. Thus, this study was performed to prove the NPEV role in residual paralysis occurrence among Iraqi children with AFP infection.

## Materials and Methods

### 1- Sample collection

A total of 600 stool samples were collected from 300 children under 15 years with acute flaccid paralysis during the period October 2010 to March 2011 from all Iraqi provinces. Two stool specimens per AFP case, 24 hours apart and within 14 days of the onset of paralysis were sent in cool boxes with ice packs to maintain the cold atmosphere for the national polio laboratory. A clinical diagnosis of AFP was 60 days follow-up and analyzed for HEV per AFP case.

### 2- Virus Isolation

The samples were prepared to stool suspension according to WHO manual (2004). All samples were treated with chloroform and antibiotics in PBS to remove bacteria and fungi and to prevent virus aggregates. Each stool suspension was inoculated in healthy monolayer of RD cells and then it providing with maintenance medium supplemented with 2% fetal bovine serum. The inoculated tubes were placed to incubator at 36$^{o}$C for 7 days and examined daily for the specific enteroviruscytopathic effects (CPE) of rounded, refractile cells detaching from the surface of tubes. The cells with CPE up to 75% were harvested and stored at -20$^{o}$C, whereas those negative results were repassaged on to another RD cells. Positive isolates on RD cells were passaged on L20B cells to identify polioviruses (Soji et al., 2007; Odoom et al., 2012). Samples that showed CPE on L20B were confirmed with international laboratory for WHO to differentiate between wild and vaccine polioviruses.

### 3- Microneutralization test

The typing sera kit was provided by the National Instituteof Public Health and the Environment, Bilthoven, theNetherlands (RIVM). The kit contained reference-typingsera against 21 of the 64 known human NPEV serotypescombined as nine antiserum pools. 50 µl of antisera wasadded to the appropriate wells of microtiter plate. Eachisolate was tested in duplicate against all the NPEV antiserumpools using 10-3 and 10-4dilutions. 50 µl of both the dilutions were dropped in respective wells and incubatedat 37°C for one hour. After incubation, 100 µl of RD cellsuspension was distributed into these wells and the plateswere incubated at 37°C after covering with non-toxicsealer. Virus controls and cell controls were run along forcomparison. The plates were examined daily, till the viruscontrol showed 4+ CPE. The identification was made byanalyzing the pattern of inhibition of CPE by the antiserumpools (Saeed et al., 2007).

### 4- Statistical Analysis

The data and graphs were carried out using spss program version 20 IBM. The proportion and their frequencies were checked by applying chi-square test. The P-values < 0.05 considered statistically significant.

## Results

**Identification of isolates:**

Results of RD cell cultured were showed 36.33% of AFP cases produced by HEVs that distributed to 33.67% as NPEVs which grow on RD only, and 2.67% as PVs grow on both L20B and RD. As shown in figure 1, AFP cases distribution accordance to cell culture selection. On the other hand, no case associated with wild poliovirus was found, but all these cases were characterized as Sabin-like poliovirus.

The distribution of positive cases among patients with AFP in fourteen Iraqi provinces was summarized in table 1. Baghdad province the most predominant of infection followed by Basra province (mid and south provinces) were found.

Fig. 2 shows the manifestation HEVs among Iraqi province during six months in this study. The appearance of PVs between provinces was not statistical significant ($X^2$ =0.059, P=0.808), whereas the NPEV positively in AFP cases was found to be significantly in mid and south than north provinces ($X^2$ =228.4, P<0.0000001).

**Serotyping:**

Table 2 shows the serotyping of NPEVs that growth on RD cell only. However, majority of isolates 72.48% were echovirus by antisera pools. Echovirus appearance was significantly high in an implicated with AFP cases as compared to other types of HEVs ($X^2$ =134.85, P<0.0000001).Afterinvestigation at 60 day Follow-up to all AFP positive cases, a persistent muscular weakness residual paralysis was found in

44 (40%) of AFP cases. Meanwhile 65(60%) of cases in our observations did get complete recovery from paralysis caused by non-polio enteroviruses. Residual paralysis of AFP cases was significantly as compared to recovered cases ($X^2$ =4.05, P<0.05). All those cases produced by NPEVs that typing as: echoviruses and coxsackie viruses were 36(81.4%) and 8(18.6%), respectively. Significant presence of echovirus in residual paralysis was found ($X^2$=17.818, P<0.0000001).

**Table 1 Frequencies of Enterovirus positive cases in patients among different Iraqi provinces**

| provinces | Patient number with AFP | Positive cases of HEVs | |
|---|---|---|---|
| | | number | % of total positive cases |
| Baghdad | 60 | 44 | 40.37 |
| Basrah | 40 | 26 | 23.85 |
| Bablyon | 10 | 3 | 302.75 |
| Anbar | 10 | 1 | 0.92 |
| Diyala | 10 | 4 | 3.67 |
| Duhok | 10 | 0 | 0 |
| Erbil | 10 | 0 | 0 |
| Karbala | 10 | 2 | 1.83 |
| Maysan | 10 | 5 | 4.59 |
| Muthanna | 10 | 1 | 0.92 |
| Najaf | 10 | 3 | 2.75 |
| Ninawa | 20 | 1 | 0.92 |
| Qadisiyyah | 20 | 5 | 4.59 |
| Sulaymaniyyah | 10 | 0 | 0 |
| Salah ad-Din | 10 | 1 | 0.92 |
| Kirkuk | 10 | 0 | 0 |
| Thi-Qar | 20 | 6 | 5.5 |
| Wasit | 20 | 7 | 6.42 |
| Total | 300 | 109 | 36.33 |

**Table 2 Observations of Vaccine polioviruses, Coxs, Echo, and untypeable entero viruses in positive cases that collected from patients suffering from AFP in Iraq.**

| Serotyping of viruses | No. (%) Isolated of viruses | No. (%) in Residua Paralysis |
|---|---|---|
| Vaccine polioviruses | 8 (7.34) | 0 |
| Coxsackie virus | 18 (16.51) | 8 (18.6) |
| ECHO virus | 79 (72.48) | 36 (81.4) |
| untype able | 4 (3.67) | 0 |
| Total | 109 | 44 |

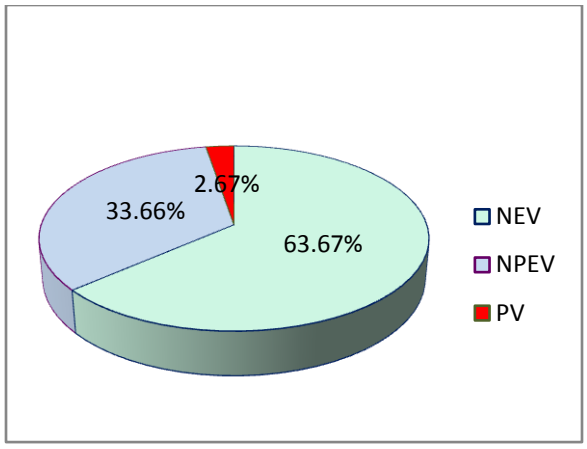


Fig. 1 AFP cases distribution according to cell culture results. Non-enteroviruses (NEV) represented more than half of these cases, which no grow on specific cell lines.

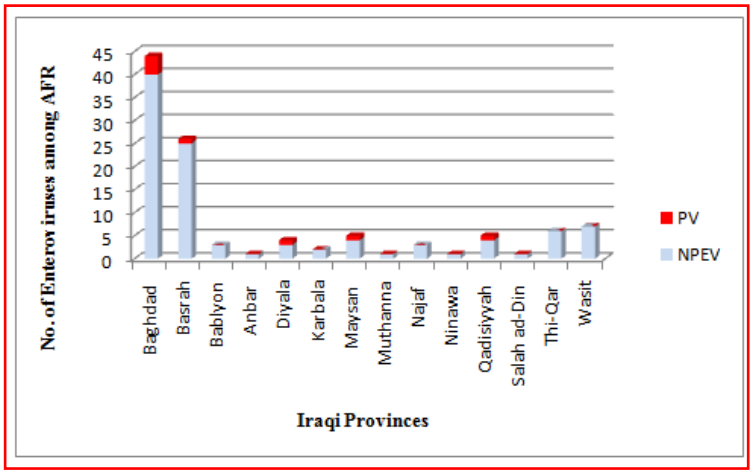


Fig. 2. Enteroviruses appearance among Iraqi provinces during the period October 2010 to March 2011. Significant occurrence of NPEV in mid and south from north provinces, while there is no significant difference in PV present.

## Discussion

The frequency of NPEVs in test group may be explaining the main role of echovirus to cause paralysis (AFP) in Iraqi children. Meanwhile, absence or reduce of wild or vaccine poliovirus due to good WHO strategy on poliomyelitis eradication in Iraq.

The highest percentage of EVs was observed in Iraqi provinces that happened because the highest population is concentrating in the middle and south of Iraq. High temperature of south Iraqi provinces as compare to north Iraqi provinces lead to high incidence of NPEVs that considered one of important etiology of AFP cases. The other reasons are the contamination of these provinces water was higher as comparing to north provinces. That is normally happening and expected because in everywhere the down flow of rivers is highly biologically and chemically contaminated (Lenna et al., 2005).The boarder normally connected with Iranby many small rivers. We suspect that these rivers are contaminated with NPEVs and basically are known that NPEVs are one of water borne infectious diseases agent that is why these rivers may play an important role in incidence of polioviruses in Iraq.

The present results are partially matching with the results that reported by other investigators, in Egypt, from 1000 stool samples isolated NPEV (176) (17.6%) by tissue culture (Salwa et al., 2009). In Saudi Arabia study of 72 AFP in children with paralytic polio were investigation only 19% (Ramia et al., 2010).The same with that study done in Latin America Between years 1989 and 1991. Total of (3112) AFP cases, representing 52% of all non-polio were studied, representing an overall annual incidence rate of(one cases) 1/100,000 children < 15 years old Follow-up investigations showed a persistent muscular weakness at 60 day (Olive et al., 1997). Only in Nigeria reflect the study of non-Polio enteroviruses (NPEV) by State was not in agreement with our results and during the years 2002-2003 implicated with suspected residual paralysis is presented of only 307 NPEVs induced AFP which count for only 24 (7.8%) patients had residual paralysis(Soji et al., 2007).

Currently, there are no vaccines available for NPEVs, but as efforts is intensified on poliovirus eradication, there is need to assess the impact of the disease burden caused by this group of viruses with a view to finding permanent solution where possible. We therefore speculate that NPEVs may predominate in its toll to cause paralysis after Polio must have been eradicated. Meanwhile, improved sanitation and general hygiene, provision of portable drinking water and discouraging overcrowding will to a great extent reduce the incidence of NPEVs infections (Soji et al., 2007).

## Conclusion

The current study concluded that high ability of NPEV (especially echovirus) to produce residual paralysis in children with AFP. Thus,we need to continue and stress the importance of careful clinical evaluation for cases of AFP caused by NPEVs among the differential diagnosis and expert review of cases with residual paralysis.On the other hand, we

recommend using molecular typing method to increase the rate of isolation.

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