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Detection of Sabin Poliovirus Serotypes among Vaccinated Iraqi Children with AFP Syndrom

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Abstract

Poliomyelitis is a viral disease caused by an enterovirus known as poliovirus and is well known for its role in causing paralysis in children, the virus is only infectious in humans and does pass into the central nervous system and cause various degrees of paralysis, poliovirus passes newcomer disabuse of suppliant to alms-man thumb the fecal-oral route infected persons still shed the virus in their stool allowing the virus to infect others. The main aim of this study was isolating and differentiation of poliovirus strains (Sabin virus) from the stool samples of children received polio vaccine $_{T}$OPV and suffering from acute flaccid paralysis.

In this study use the cell culture system as the gold-standard method for Culture and isolation of Sabin polioviruses newcomer disabuse of the clinical moderate samples of sharp-witted flaccid paralysis AFP cases.

Total of two hundred and fifty (250) stool test was taken from vaccinated children with $_{T}$OPV who suffering from acute paralysis in Iraq.The samples were collected during the period of the study from (March to November in the year 2017) Were transferred under cold situation the children were stratified according to provinces .Stool test taken within 48 hours were each patient before the fourteenth days after the onset of AFP .

The study that 35 (14%) samples were positive for Sabin for poliowere isolated from AFP cases. Showed CPE effect on L20B cells only all the samples were from patient vaccinated with the type of OPV in varying number of orally $_{T}$OPV dose.

The current study which was done in different Iraqi provinces shows that (Sabin for polio of type 3) were isolated from AFP cases had the highest prevalence affecting 18 cases (51. 42 %) out of 35 Sabin of polioviruses AFP. The present study showed that the maximum frequency of (Sabin for polioviruses typo 3) virus isolate was reported in Baghdad 7 (38.8%) and both provinces Anbar and Salahdeen shows 3 (16.6%) followed by Wasit and Dyiala 2 case (11.11%), then Duhok 1 case (5.5%).

Ten 10 cases (28.57%) of the Sabin AFP polio were mixing isolated (Sabin of polioviruses typo 3 with type 1) these positive cases found in Anbar province in high prevalence 4 cases (40%) followed by Baghdad, Erbil 2 case (20 %) for each then , Duhok and Tamim 1 case (10 %) .

Seven 7 (20 %) stool samples were positive for Sabin for polio type 1 serotype were isolated from AFP cases the large number of the diagnosis was obtained from a patient in the Baghdad isolate 3 cases (42.85 %)) followed by Sulymania 2positive cases (28.57 %) then Qadysia and Basrah 1 case (14.28% .

Keywords: AFP , OPV, CPE , ITD, $_{T}$OPV

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الكشف عن الأنماط المصلية لفيروس شلل الأطفال في الأطفال العراقيين الذين تم تطعيمهم مع متلازمة AFP

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الخلاصة

التهاب سنجابية النخاع الشوكي هو مرض فايروسي ناتج عن احد الفايروسات المعويه يعرف باسم فيروس شلل الاطفال (polio) والذي يلعب الدور الرئيس في احداث الشلل في الاطفال . يكون هذا الفيروس معديا للانسان فقط حيث يمر الى الجهاز العصبي المركزي ويسبب درجات متفاوتة من الشللوينتقل الفيروس من شخص إلى اخر عبر الطريق البرازي الفموي و لا يزال الأشخاص المصابون بالفيروس يراقون الفيروس في البراز مما يسمح للفيروس بإصابة آخرين. في هذه الدراسة تم استخدم نظام الخلايا الزرعيه كطريقة معيارية– ذهبيه في تشخيص وعزل عدد من فيروسات شلل الاطفال من عينات البراز السريرية لحالات الشلل الرخو الحاد.تهدف الدراسة الى عزل وتفريق فايروسات شلل الاطفال نوع سابين (polio Sabin (Vaccine virus من السامبلات البراز من لاطفال اللذين قد تلقوا لقاح الشلل الفموي ومن اللذين اصيبوا بالشلل الرخوي. تم جمع ما مجموعه مائتان وخمسون (250) عينة من البراز من الأطفال الذين لقحوا بلقاح شلل الاطفال الفوي OPVوقد عانوا من اصابات مرض للشلل الرخو الحاد في العراق. تم جمع العينات خلال فترة الدراسة من مارس إلى نوفمبر2017 حيث نقلت من مصادر جمعها تحت ظروف مبردة وتم توزيعها استنادا الى موقع المحافظات التي اخذت في الدراسة واخذت عينات البراز في غضون 48 ساعة من بعضها البعض في موعد لا يتجاوز اسبوعان من بداية تاريخ ظهور اعراض الاصابه بمرض الشلل.

بينت الدراسة ان مايقارب من 35 عينة (14٪) نتائج الفحص إيجابية لفيروسات شلل الأطفال سابين التي تم عزلها من حالات AFP .وأظهرت جميعها تأثير فايروسي خلوي على خلايا نوع LB فقط حيث كانت جميع عينات المرضى من الأطفال الذين لقحوا بلقاح شلل الفوي (OPV VACCINE)وبعدد من الجرعات المتفاوتة من OPV Vaccine . تبين الدراسة الحالية التي أجريت في مختلف المحافظات العراقية بان فايروس شلل الاطفال سابين النمط الثالث3 Sabin polioviruses serotypo قد تم عزله من حالات AFP. حيث كان أعلى معدل انتشار و تم عزل 18 حالة (51. 42٪) من أصل 35 عزله من فيروس شلل الأطفال سابين AFP. وأوضحت الدراسة الحالية أن الحد الأقصى لمعدل تكرار لفيروس Sabin) polioviruses sero typo 3) قد تم تسجيله في بغداد هو 7 حالات (38.8%)ومحافظات الأنبار وصلاح الدين لكل منهما ثلاث حالات 3 (16.6٪) يليهما حالتان لكل من محافظة واسط ومحافظة ديالى 2 (11.11٪)ثم حالة واحدة في محافظة دهوك 1 (5.5 ٪) .

تم عزل 10 حالات (28.57%) من فيروسات شلل الاطفال سابين AFP وهي مزيج من العزلات للنمط 1 مع النمط 3 (Sabin polioviruses sero typo 3 with type 1) وجدت هذه الحالات الإيجابية في محافظة الأنبار في معدل انتشار مرتفع 4 حالات (40٪) تليها محافظة بغداد ومحافظة أربيل حالتان لكل منهما 2 (20٪) وثم محافظة دهوك محافظة التاميم حالة واحدة لكل محافظة 1(10٪).

سبعة عينات (20٪) للبراز كانت موجبة لفيروسات شلل الاطفال سابين AFP من النمط الاول Sabin polioviruses type 1 حيث عزلت من حالات AFP وتم الحصول على أكبر عدد من العزلات

من الأطفال في محافظة بغداد بعدد 3 حالات (42.85٪) يليها حالتان 2(28.57٪) في محافظة السليمانيه ثم حالة واحدة 1 (14.28٪) في كل من محافظة البصرة والقادسية .

Introduction

The enteroviruses are a sort of the family picornaviridae, scientific differentiation of polio virus contained Species ; Enterovirus and Subtype ; Polio virus a grouping that includes rhino and hepatitis type A virus. Enter virus, the genus that includes poliovirus [1].Picornaviruses are having icosahedral capsid shape that lacks a viral core and carries the single-strand of RNA genome[2].Acute flaccid paralysis or (AFP) is a clinical disease that known to be manifested in humans by infectious (bacterial or viral) or noninfectious case (metabolic disorders or trauma or metal toxicity) causes or post-infectious autoimmune situation. The danger of infection is low socioeconomic status, poor sanitation and living in overcrowded residential areas .Poliomyelitis is an acute communicable transmitted viral disease suffering the humans that are considering the only natural hosts for viruses mainly children in age under 5 years. The disease is caused by 3 serotypes of Poliovirus (Poliovirus types1, 2, and 3), [3]. The virus has been reclassified as Enterovirus spp. in the entero virus genus. The virus can transmit through too by contaminated food and water and multiplies in the GI, the incubation period is sometimes seven - fourteen days vary three - thirty five days,[4]. It can invade the nervous system.The polio has capsid contains sixteen copies each of the 4 polypeptides of viral VP1, VP2, VP3, and VP4. The arrangement of these proteins in the capsid creates as icosahedral symmetry shape [5].The surface of virion is covered by star-shaped pig mesas at its fivefold axes covered by deep canyons and three-bladed propellers. These are situated at threefold axes divide by saddle depressions straddling twofold axes these proteins are VP1, VP2, and VP3 all have an eight-stranded β-barrel fold, but they have many different shaped loops on their N- and C-terminal extensions. [6]. Viruses grow only in living cells a virus cannot replicate outside of live a cell, therefore totally dependent on a host cell material in order to grow and spreads.[2]. Thus the host cell must give the energy and synthetic machinery to the virus and the low molecular-weight precursors for the made of all both viral proteins and nucleic acids.[7]. The SS (+) sense RNA play acts as mRNA. The 5′ end of RNA that has a long genome that can fold into several stem-loops. The VPg of protein and the stem-loops mimic the cap-binding complex, and these permits to the binding of the mRNA to the ribosome of the host cell. RNA codes of the virus for all the proteins into one large these Protein molecule called poly-protein.

The most common form of paralytic poliomyelitis (79%) attacks motor neurons in the spinal cord ,(Figure-5) which are responsible for movement of the muscles, including those paralyses in the arms, trunk, and legs and breathing problems [8]. The virus may break the muscles on both sides of the body, but the mainly of paralysis occurred asymmetrically. Any limb may be destroyed such as one leg or more than limb, one arm, or both legs and all both arms. Paralysis is usually more acute nearly where the limb joins the body than distally the fingertips with toes. [9].

No specific of treatment is available except that supportive measures in paralytic poliomyelitis these measures contained use antibiotics to prevent infections in weakened muscles, analgesics for pain, moderate exercise and a nutritious diet [10].Treatment of virus often needs long-term rehabilitation, such as physical therapy, braces, with corrective shoes and, in some states , orthopedic surgery, However there is a possible to prevent the infection with viruses through active immunization Campaigns[11]. There are two kinds of vaccines for Polio available worldwide. In activated virus Vaccine IPV and Sabin Oral Polio Vaccine OPV, [12]. The first Vaccine was dropped contain the attenuated virus. The attenuated viral strains it has the ability to revert to a form that can cause neuron virulence and paralysis this serotype called Vaccine derived poliovirus (VDPV) [13]. have transmissibility from human to others patient.

Materials and Methods

1- Stool sample collection

A total of two hundred and fifty (250) samples were included in this study patient children vaccinated with OPV give clinical stool specimen were collected from them,who infected with AFP After having been examined them by a pediatric neurologist doctor, these children were under 5 years old, from different Iraq provinces. All samples were collected during March to November 2017. All samples received within 48 hours of each other not later than 14 days after the onset of disease. Stool samples were selected from those gets by Surveillance system for polio in Iraq and sent to the Iraqi National polio laboratory (INPL) under good Status.

Processing of stool specimens

All facal samples must be mixed with the chloroform to removing bacteria ,fungi, contaminated [14] and cytotoxic lipids to separated virus aggregates. Manipulation of stool material was inside a functional BSC Class II . For Preparations, or processing of stool samples tests for virus diagnostics. The fecal specimen was processed according to protocols for virus isolation and typing[14].these methods identified different poliovirus isolates into types 1, 2 and 3 all stool specimens were inoculated , 0.2 ml of the 10 ml of stool with chloroform-extracted in two cells RD and two types of L20 cell monolayer's in 50 ml monolayer of cell culture flasks. Negative samples at first isolation underwent two serial blind cultures in both cell lines, and positive samples on RD cells were reculturing on L20B for specific amplification of poliovirus[15].

2- **polio Virus Isolation and identification**

Samples were inoculated on cell line L20B (Figure-1).The test adopted for AFP follow up and Laboratory tests,these methods used for isolation and micro neutralization for identification of positive isolates were virus as described and recommended by WHO PolioLaboratory Manual 2004 and, from 2008 onwards, the supplemental manual of the New Algorithm Technique (Figure-1) [13].

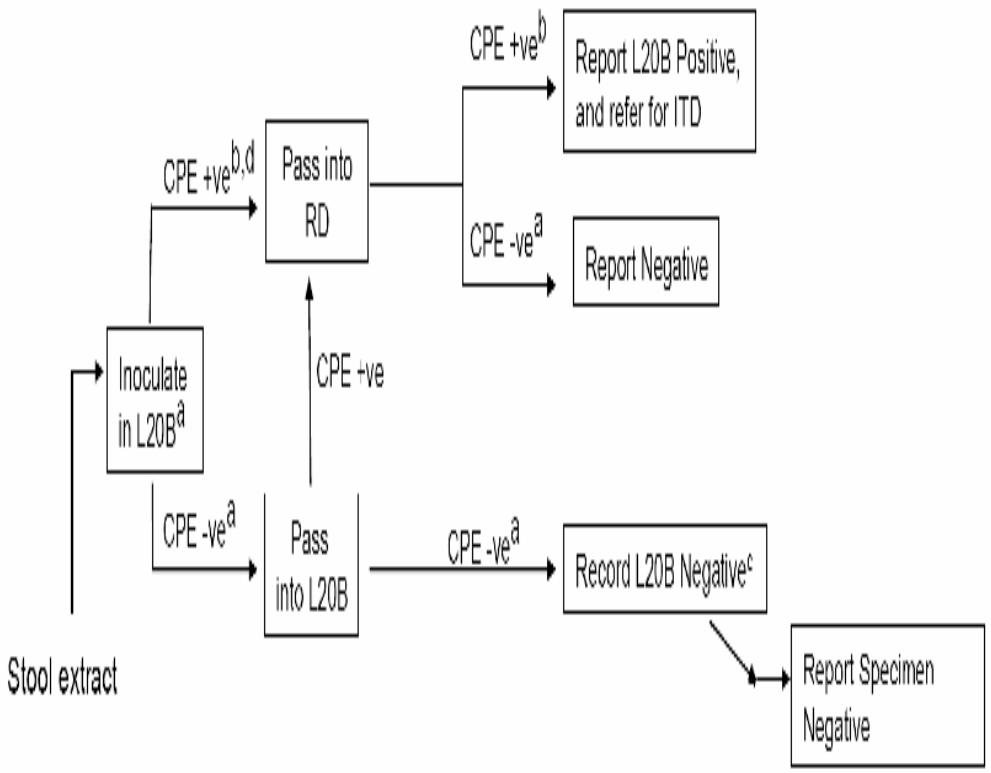

Figure 1-L20B Passage flowchart

According to the methods in protocols that recommended by the WHO [4], PV is stereotyping was carried out via platting assays that have 96-well of tissue culture plates using polyclonal antiserum against all types of PV such as 1, 2 and 3 . The antisera-virus mixtures were incubated for one hour at 36°C. Suspensions of cultures were put to the micro-plate which was examined daily to observe the CPE. The antiserum that stopped the grow of CPE indicated the identity of the virus.

Table 1-Determination the type of virus in the poliovirus serotyping test accordingto the CPE in micro-titration wells .[16].

PP (PV1+PV2+PV3)	Polio 3 (PV1+PV2+)	Polio 2 PV1+PV3	Polio 1 (PV2+PV3)	Virus Serotyping
NO CPE	**NO CPE**	**NO CPE**	CPE	PVs serotype 1
NO CPE	**NO CPE**	CPE	**NO CPE**	PVs serotype 2
NO CPE	CPE	**NO CPE**	**NO CPE**	PVs serotype 3
NO CPE	**NO CPE**	CPE	CPE	Mixture of PVs serotype 1 & 2
NO CPE	CPE	**NO CPE**	CPE	Mixture of PVs serotype 1 & 3
NO CPE	CPE	CPE	**NO CPE**	Mixture PVs serotype 2 & 3
NO CPE	CPE	CPE	CPE	Mixture (PV1+PV2+PV3)
CPE	CPE	CPE	CPE	No PV S or mixture of PVswith other NPEVs

for PV identification use the unknown viruses isolate with 100 TCID50 and a cell suspension of isolating from a tube culture, showing 3+ to 4+ CPE in L20B healthy cell cultures is expected to include about 105–106 TCID50/ 50 µl. (Figure-2)

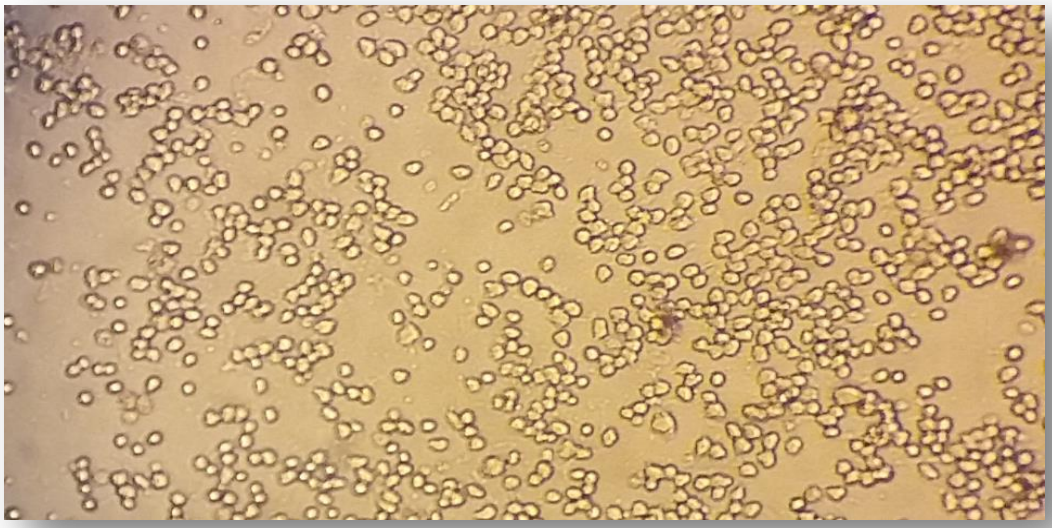

Figure 2 -CPE observed in L20B cells affected by the poliovirus.

Only Dilutions of 10-3 and 10-4 have been selected for use in this study [17]. A gradual decreasing dilution of the virus is included in each plate to allow counting of the titer of virus actually present in that test. Polio typing was performed at the (INPL) by utilizing monospecific anti-PV pooled anti-sera RIVM/WHO tools. Neutralization assay steps done as W.H.O manual [14].

3- Statistical Analysis

The data and graphs were done by using spss program version 20 IBM. The ratio and their frequencies were checked by stratifying of the chi-square test. The P-values < 0.05 take into account statistically significant.

Results

Identification of viral isolates:

The Polio typing method was tested for neutralization of virus used for identification and differentiation of polio isolates to different types. In this study Polio Virus identification was

performed by using a culture of tissue system with monospecific polyclonal for polio virus anti-serum to types 1, 2 and 3 complexes of antiserum pools [18].

High-titer of polyclonal anti-sera were used and mixed with about CCID50 of the of unknown stool isolate This study confirms the presence of different strains of Sabin polio that cause AFP among children in the test group that shown in (Table-2), from 35/250 (14%) positive Sabin poliovirus AFP isolated in the test group, the study showed 7 (20%) Sabin poliovirus AFP type 1, 18 (51.42%) Sabin poliovirus AFP type 3, and 10 (28.57%) Sabin polioAFP type 1with 3 together (mix). The present study showed the highest number of Sabin polioviruses was in the case of type 3 followed by a mix and the lowest in type 1 while we did not find any sero typing of type 2 in this study. (Table-2).

Table 2- Distribution the different types of poiloviruses AFP cases among suspected AFP patients

Sabin Poliovirus AFP serotyping	Patient group NO.250	
	Number positive case	Percentage%
PV1	7	20
PV2	0	0
PV3	18	51.42
MIX PV1+, PV3	10	28.57
Total	35 (14%)	100

The study shows the largest number of the Sabin poliovirus AFP dignosis were taken from children in Baghdad province having 12/35 (34.28%), followed by Anbar province having 7/35 (20%),

Salahdeen province 3 /35 (8.57%) and (Sulymania, Erbil, Duhok, Diala and Wasit) having 2/35 for each of them.While the remaining 3 (8.57%) isolates were obtained from children in the three provinces, 1 (2.85%) from Basrah province, 1(2.85%) from Qadysia province and 1 (2.85%) from Tamim. (Table-3).

Table 3-Distribution of Sabin Polio virus AFP isolates by location of children

Provinces.	No. of Sabin polio virus AFP with the following type				
	Patient number	Mix type Type 1+Type 3	Sabin polio virus AFP Type 1	Sabin polio virus AFP Type 3	Sabin polio virus AFP Type 2
BG	40	2	3	7	0
BS	20	0	1	0	0
BA	18	0	0	0	0
AN	20	4	0	3	0
DY	20	0	0	2	0
DU	20	1	0	1	0
ER	15	2	0	0	0
KR	4	0	0	0	0
MS	3	0	0	0	0
MU	3	0	0	0	0
NJ	4	0	0	0	0
NI	0	0	0	0	0
QA	10	0	1	0	0
SU	15	0	2	0	0
SL	10	0	0	3	0
TA	15	1	0	0	0
TH	15	0	0	0	0
WA	18	0	0	2	0
Total	250	10	7	18	0

Discussion

In Nigeria study by Baba *et al* [19] focused on isolation and identification three Poliovirus Sabin AFP types 1+2 and the twenty three Poliovirus PV3 , this result is consistent with the report of Adu *et*

al [20], that found three strains of Sabin virus serotype 1 with 2 were isolate in children who had complete vaccination by receive 3 doses of Polio Vaccine.

The recurrence of AFP Sabin PV-positive feces tests that acquired in this examination by cells of tissue (Table-2) was contrasted with those outcomes in other clinical investigations analyzing.[21]. revealed secludes of numerous PV1, PV2, and PV3 in successive 30, 45, and 20% of stool examples, separately while our L 20B culture strategy test yielded a lot higher recurrence of sabin PV3 18 (51.42%) disengagement, however, bring down the recurrence of sort 1 Sabin poliovirus 7 cases (20%) and no detached of Sabin AFP PV2 (0%) . These outcomes vary from our examination, on the grounds that the distinctive cell lines utilized for PV developing in cell , the diverse infection development conditions, and the diverse antiserum utilized for composing of infection may represent the show up a the difference.

A study in the UK by Ramsay et al [22] give the idea that 49, 48, and 12% of stool examples gathered after post immunization were certain for both PV1, PV2, and PV3, separately the make of this investigation can't help contradicting aftereffects of our investigation and as indicated by[23].Twenty cases 20 disconnected Sabin Polio infection among them were ten analysis of sort 2 Sabin AFP from the stool of youngsters with intense loss of motion in Nigeria were recognized and affirmed by CDC while in our examination need secluded sort 2 Sabin AFP 0 (0%). Another examination by[24]. certain the nearness of 13 S AFP of polioviruses that reason sickness, off these around seven Sabin Poliovirus type 1 this is predictable with our outcomes in regards to the event of Sabin Polio infection AFP type one.

The increasing frequency of Sabine Polio viruses AFP infection was found in Baghdad 12 (34.28 %) of these cases include all types Polio isolates, these are Sabine Polio viruses AFP type 1, Sabine Polio viruses AFP type 3, and Sabine Polio viruses AFP mixture type one 1 with type three 3 (Table- 3).This is signal to clusters or out peak occurred in Baghdad [25].during the time of our study suggests that in spite of highly frequency of supplemental immunizations and many children were adequately received of vaccinated against poliovirus during vaccination coverage during NIDs and additional campaigns from house -to house all these factors seem to have contributed and supported our conclusion that OPV must be phased out to secure a lasting Polio-free world.

Conclusions

1-Transmission of Sabin poliovirus AFP type 1 among children with complete vaccinated by the oral vaccine for polio poses a serious threat to this virus eradication program in Iraq.

2-The ratio frequency of infection with Sabin polio virus AFP strain 3 (51.42%) found more than infection with sabin polio virus AFP strain 1 (20%) or mix isolated Sabin polioviruses typo 3 with type 1 (28.57%).

3-The incidence of a different type of sabin polio virus AFP in Iraqi provinces was shown with the highest frequency in Baghdad province as compared with other provinces.

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