Immune Response as a Result of OPV Vaccination Between Children and Young Adults in Jeddah, KSA

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Abstract: In the present study immune response against oral poliovirus vaccine was measured. Samples were collected from children and young adults within age from 1 month to 15 years. All samples were screened for the presence of antibodies against polioviruses by EIA. The samples were obtained from vaccinated individuals except those under two months of age. It was noticed that the children of 1-2 months accept a protective immunity against polioviruses from their mothers (maternal immunity). The responsiveness against poliovirus vaccine was varied much between children at different ages. It was noticed that some of the studied cases have not a protective level of antibodies which represents a high risk factor if they exposed to clinical doses of wild polioviruses. Accordingly, it is recommended to measure the protective antibodies against polioviruses at different intervals to check the validity of the used vaccine.

Key words: Immune response, OPV vaccination, children, young adults, Jeddah, KSA

INTRODUCTION

Polioviruses are classified into three distinct serotypes (type 1, type 2, and type 3) based on their reaction with reference panels of neutralizing antisera[2]. They belong to the genus enterovirus in the family picornaviridae. Polioviruses are stable at acid pH and can survive for weeks at room temperature and for many months at 0°C to 8°C. As with other enteroviruses, polioviruses are resistant to ether, 70% alcohol and other laboratory disinfectants. Treatment with 0.3% formaldehyde, 0.1 N HCl, or free residual chlorine at a level of 0.3 to 0.45 parts per million rapidly inactivates polioviruses, as does exposure to a temperature of 50°C or higher or to ultraviolet light [8]. Early work identified two distinct types of antigens in harvests from virus infected cells, which were designated as D antigen and C antigen. D antigen is largely but not exclusively associated with infectious virus and C antigen with empty capsids[7]. The poliovirus virion is small, with a diameter of 27 to 30 nm, and contains a single stranded molecule of RNA. A thin 20-sided shell composed of four virion proteins (VP1, VP2, VP3, and VP4) surrounds the RNA[4]. Several sites involved in virus neutralization have been identified on the surface of the poliovirus. For example, a site composed of amino acids 89 to 100 of VP1 is a major immunogenic site for type 2 and type 3 polioviruses, as judged by monoclonal antibodies induced in mice[9].

MATERIALS AND METHODS

Samples: Serum samples were collected from 200 children and young adults between one month and 15 years of age. They were selected randomly from King Abdulaziz Teaching Hospital, and Delivery and Children's Hospital, El Mosaaedeen, Jeddah, between May 2006 and January 2007. All samples were collected from OPV vaccinated children and young adults. Some of the children were taken one dose of the OPV and others were taken two doses but most of them were taken the three doses and the booster one.

EIA for Measurement of Immune Response Against OPV: EIA detection of polioviruses-specific antibodies was performed. Briefly, the EIA plate was coated (100 µl/well) with OPV trivalent vaccine diluted 1:20 in coating buffer (1 M Na2CO3; 1 M NaHCO3, pH 9.6) then incubated over night at 4°C. Plates were washed 3 times with PBS-0.05% Tween20 (PBST). Nonspecific binding was blocked by incubating the plates with PBST-4% bovine serum albumin (PBST-BSA; 200 µl/well).
μl/well) at 37°C for 2 hours. After 3 washes with PBST, wells were loaded with diluted sample sera in PBST-FCS (1:16; 100 μl/well), and plates were incubated at 37 °C for 2 hours. Plates were washed 3 times and then incubated with 1:10,000 (100 μl/well) Goat anti-human IgG to horseradish peroxidase (Koma Biotech Inc.; Korea) at 37°C for 1 hour. Plates were again subjected to 3 washes with PBST. For color development, TMB substrate was added (100 μl/well) and incubated at room temperature in dark place for color development through 15-30 min. The enzymatic reaction was stopped with 50 μl/well 2 M HCl, and the change in optical density (OD) was recorded at $\lambda_{max} = 450$ nm using a multiwell plate reader (BioTech., USA). Blank well (A1) was filled with washing solution, positive control was rabbit anti polioviruses 1, 2 & 3 and negative control was wells without samples. All control wells were treated identically as the rest of the plates.

RESULTS AND DISCUSSION

Evaluation of Immune Response Against OPV at Different Age Groups: Sera samples which collected from children and young adults were subjected to EIA to evaluate the protection level of antibodies for polioviruses. The evaluation assay included age group from 5 to 10 years,10 to 15 years, then 1 month to 12 months and finally 2, 3 & 4 years. The obtained results are represented in the following figures.

Discussion: During the twentieth century, many significant scientific advances were made which furthered the understanding of poliovirus. In 1949, poliovirus was grown by Enders on tissue culture, which paved the way for detailed research on the molecular biology of poliovirus. That same year, the virus was grouped into three immunological types, or serotypes. Subsequent to the discoveries of Avery et al and Hershey and Chase on DNA-mediated transformation of bacteria, RNA was identified as the infectious agent of poliovirus in 1957. Shortly thereafter, the basic steps of replication cycle of poliovirus were established, and the interaction of poliovirus with its antibody was analyzed. X-ray crystallographic and electron microscopic analysis of poliovirus contributed to its further characterization.

These advances all contributed to the development of the two vaccines which are currently used in the global campaigns to control and to eliminate poliomyelitis: Oral Polio Vaccine and Inactivated Polio Vaccine. IPV, a killed polio vaccine, is administered subcutaneously via injection while OPV, a live polio vaccine, is taken orally and more resembles the fecal-oral route of transmission of the virus.

The Oral Polio Vaccine (OPV) was developed in 1958 by Dr. Albert Sabin. Sabin attenuated the wild type poliovirus by passaging the virus in monkey kidney epithelial cells. The commonly used form of the oral polio vaccine is trivalent, which means that it contains live attenuated strains of the three serotypes of poliovirus. Trivalent OPV is characterized in vivo by efficient growth properties in the intestinal tract, unaltered immunogenic properties with respect to wild type progenitors, and attenuated neurovirulence after experimental intraspinal injection into primates. This means that an individual immunized with trivalent OPV induces long-lasting (frequently life-long) protective immunity of the gastrointestinal tract to all known forms of poliovirus.

In 1988, polio still ravaged about 350,000 people each year, causing damage that will last for the rest of their lives. The World Health Organization, UNICEF, the CDC, and Rotary International saw that polio could be entirely eradicated from the world. By the turn of the millennium, they had been 99 percent successful (only 2881 new cases in 2000).

But enormous challenges remained in bringing immunizations to 16 million children in war-torn Central and Western Africa. Using bicycles, boats, and canoes (and an amazing variety of methods to keep the vaccine cold and fresh), courageous vaccinators began visiting children in the Democratic Republic of the Congo, Congo-Brazzaville, Gabon, and in Angola - one of the most war-torn and landmine-infested areas in the world and each child had to be visited 3 times. Volunteers have been arrested and killed trying to protect children from this disease.

In the present study, different cell lines were used for OPV propagation. The cells which used represent different body organs in both human and animal trying to find new infection target for oral attenuated polioviruses. It was noticed that the only factor for attenuated poliovirus attachment and infection of a certain host cell is the presence of the specific receptors. These receptors are present in the Vero and RD cells.

One of the most important subjects related to poliovirus vaccination is the protection level which obtained as a result of polio vaccination. It was found that the antibodies level between children of age group 1 to 3 months is high enough and this is referred to the maternal antibodies where during the first few months of life, most infants have circulating IgG antibodies acquired from the mother before birth. There are no practical techniques to distinguish these
passively acquired antibodies from antibodies that the infant has made in response to immunization. Therefore, most investigators compare the antibody titers in cord blood or venous blood obtained prior to immunization with titers observed after immunization. Based on an estimated half-life of approximately 30 days (range 21 to 45 days), the expected level of passively acquired antibody is determined\cite{3}. If the titer obtained after immunization is fourfold greater than the expected titer of passive antibody, it is concluded that the infant has responded to the vaccine. At 6 month age till 12 month, it was noticed that the circulating antibodies were not enough to save protection of the children from infection with polioviruses. It was noticed also that the immune response of the female children is slightly higher than that of males (Fig. 4, 5 & 6).
The age groups of 2, 3 and 4 years didn't show any significant increase in antibody titer for both males and females which is not normal for attenuated poliovirus vaccine (Fig. 7). A slightly increase in antibody titers was observed between members of the age group 5 to 10 (Fig. 8, 9 & 10) and age group 11 to 13 years (Fig. 11, 12& 13). The immune response in this study was represented by IgG where IgM levels peak at about 2 weeks after exposure and disappear from the serum within about 60 days. IgG levels increase steadily and persisting serum antibody belongs to this class[1].

Tests for serum neutralizing antibodies are considered to be the most specific for determining the
Ivanov et al.\textsuperscript{[5]} describe several enzyme-linked immunosorbent assay (ELISA) techniques proposed to replace the neutralization test for detecting neutralization-relevant antibodies to polioviruses in recipients of inactivated poliovirus vaccine and oral poliovirus vaccine, and for seroepidemiologic studies. Comparisons of results from ELISA and the neutralization test suggest that ELISA variants, based on the principle of blocking or binding inhibition that emulate the neutralization test, might offer an alternative to the neutralization test. However, to replace the neutralization test with ELISA would first require extensive studies with very large numbers of serum samples, including sera having low titers of neutralizing antibodies, in order to obtain reliable and statistically sound validation.

Más Lago et al.\textsuperscript{[6]} collected samples of feces and sera obtained from 3-year-old children were studied to increase the knowledge about the circulations of virus protective antibody response to poliovirus infections\textsuperscript{[10]}.
Fig. 13: Immune response between females of age group 11-15 years.

vaccines during the massive campaigns. The use of the oral polio vaccine with schemes of massive campaigns allows the circulation of the virus vaccine 2 months after their completion. The use of continual vaccination schemes makes possible the circulation of the virus vaccine for longer periods of time. Even in populations with a low immunity coverage, epidemic outbreaks of the vaccine-derived virus may appear. The total of poliovirus vaccine isolated in 2-year-old children (11 cases, 11.0 %) and the boosts of neutralizing antibodies (51 cases, 51.0 %), show a contradiction between the verification of the infections caused by isolations of the viruses and the results of boosts. The low percentage of isolations of virus vaccine and the highly significant percentages of seroconversions or boosts to polio virus, allow inferring the occurrence of silent circulation. The silent circulation self limited to 2 months after concluding the campaign is due, among other causes, to the homologous or not induced response by the primary infection with the first dose of oral polio vaccine and by the secondary infections. The self limitation of the circulation of the polio viruses in massive campaigns constitutes an excellent prevention of the risks represented by the vaccine-derived viruses appearing in vaccinations with continual schemes.

**Recommendations:** As a result of this preliminary study, many observations were recorded:

- The ELISA is an alternative technique to the neutralization assay for measuring the immune response associated with poliovaccination.
- The observed low level of antibodies against OPV at different age groups turns our mind to necessity to repeating vaccination campaigns for vaccinated and unvaccinated individuals.
- The protective antibodies against polioviruses must be measured at different intervals to check the validity of the used vaccine.

**REFERENCES**

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