Antibody Response of Children Receiving Multiple Doses of Oral Polio Vaccine in Disease Free Area of India

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Abstract: Maintenance of high levels of immunity to poliomyelitis is priority in India as transmission of wild polioviruses in its two states has never been interrupted. Determination of serological profile of vaccines in disease endemic as well as disease free areas is important for evaluation of efficacy to the extended polio vaccination program in the country. A hospital based study to determine antibody levels against three poliovirus serotypes in selected children was performed in Pune city which has been disease free since, last 11 years. Levels of neutralizing antibodies against poliovirus types 1, 2 and 3 in the sera of group of 90 children below 2 years of age and have participated in Pulse Polio Immunization (PPI), were determined. The geometric mean antibody titers were 1.811, 1.659 and 1.226 for poliovirus types 1, 2 and 3, respectively. Significantly high antibody titers for poliovirus types 1 and 2 were found after 6 polio doses. However, about three to five fold decrease in the antibody titers for poliovirus type 3 was noted. About 17.7% of serum samples showed poliovirus type 3 antibody titer less than 1:52. In view of the current type 3 epidemic in India, the study suggest that perhaps, single round of PPI and a booster of monovalent type 3 following routine immunization schedule may be required to create adequate immunity in children in a disease free area such as Pune. Targeting of all the children below 5 years of age for each PPI may not be necessary. This study also suggests that protective antibody titer in India should be redefined.

Key words: Poliomyelitis, neutralizing antibody, seroprevalence, pulse polio immunization, poliovirus

INTRODUCTION

The World Health Assembly resolved to global eradication of polio in 1988. About 67 countries in the world had already achieved the interruption of wild polioviruses by then. The regions of Americas, Western Pacific and Europe eliminated polio thereafter. Countries such as Taiwan, Oman, China, Bangladesh, Philippines, Sri Lanka also succeeded in eradicating polio by implementing large scale supplementary immunizations.

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(Aylward and Maher, 2006). Because India was still unable to control polio, a pulse polio immunization program was launched in India in 1995. It is being implemented every year since, then which includes administration of two doses of Oral Polio Vaccine (OPV) at an interval of 1 month, simultaneously to all the children below the age of 5 years (Shah et al., 2006). Transmission of indigenous wild polioviruses in 26 states and 7 union territories was successfully interrupted following multiple supplementary immunization activities. Poliovirus transmission has however, still persisted in two Northern States Bihar and Uttar Pradesh (UP) (Anonymous, 2009). These two areas experienced epidemics caused due to poliovirus type 1 in the years 2002 and 2006 infecting 1560 and 660 children, respectively (Thacker, 2007). About 866 and 559 cases of paralytic poliomyelitis were reported in the country in the years 2007 and 2008. Of these, 96.5% were from the affected reservoirs (Anonymous, 2009). The genetic studies show that viral spread from endemic reservoir areas in UP and Bihar has been responsible for all cases of polio in India and several polio free countries including Bulgaria, Angola and Democratic Republic of Congo (Anonymous, 2009).

The ongoing wild poliovirus transmission despite high vaccination coverage has been a cause of great concern. More alarming is the record of vaccination status of reported paralytic poliomyelitis cases, which shows that nearly 85% of children contracting poliomyelitis had received four or more doses of OPV (Paul, 2008). Failure of effect of vaccine in these children may not be related to lactation in vaccination coverage or loss of vaccine potency, as vaccine potency in the country is being monitored since 1988 and high coverage through supplemental immunization, PPI has also been assured throughout the country (Shah et al., 2006). An in-depth analysis of the failure to terminate wild poliovirus transmission has recently been carried out by reviewing nearly 100,000 cases of acute flaccid paralysis from 1997 in India. The study has pointed out extremely low, about 9-18% vaccine efficacy in the two high risk states (UP and Bihar) and about 21% in rest of India. High population densities and poor sanitation conditions leading to high incidences of diarrheal diseases could have affected vaccine efficacy (Grassly et al., 2006).

As long as wild poliovirus transmission continues in the country, the remaining polio free states are at risk of re-emergence. The parameter indicative of protection accepted by WHO and Polio Eradication Committee of India appears to be based on failure to record any case of acute flaccid paralysis caused due to wild polioviruses in vaccinated area. Emphasis is laid on type of resurgences, wild poliovirus and genetic characterization of wild and/or vaccine strains of virus isolated from stool samples (Anonymous, 2009). There is no doubt that these data are important. However, based on these studies it may not be possible to find out the safe level of immunity needed to be achieved in healthy vaccinated population to categorize them as risk free. Surveys for evaluation of immune status of the target population would thus, be crucial to identify the risk areas. Antibody titers achieved by children from disease free states and endemic states might be useful as a guide line for designing appropriate immunization strategies. The estimation of antibody titers against vaccine, vaccine derived and/or wild strains in vaccinated children in affected areas might prove to be important research effort towards development of modified vaccines. Unfortunately, not much effort has been directed to this issue in India with the result that protective titers are not well defined.

We have conducted a modest hospital based study to determine antibody levels against the three poliovirus types in vaccinated children from Pune city. Ninety children in the age group of 8-24 months, which is most susceptible age group as 80% of polio infections are being contracted in this age, were the study subjects. Information on socioeconomic status
of the family, dates of OPV vaccination and medical history especially with reference to respiratory and Gastro Intestinal (GI) tract infections of these children was noted. This study, in particular, was done to estimate the antibody response of children residing in relatively poliomyelitis free area. Estimation of antibody levels has further importance for Pune city as there is a constant influx of migrants from affected areas to the city in search of work and for education.

MATERIALS AND METHODS

Selection Criteria for the Study Population

Ninety children in the age group of 8 to 24 months, visiting the OPD of Pediatric Department of Bharati Hospital, Pune have been included in the present study. Studies were conducted during the year 2008-2009. Children visiting Bharati Hospital mostly reside in the nearby area of Katraj, Dhankawadi (a suburb in Pune). They belong mostly to lower socioeconomic class. All the 90 children had taken about 4 doses of OPV through routine immunization. Fifth dose was received at an age of 8-9 months during their regular visit to hospital. All the children had taken two additional doses in Jan and Feb Pulse Polio Immunization (PPI) rounds of 2008. Children born before December 2006 had taken 2 more doses during 2007 FPI program round. Records of birth weight of children, weight at every visit and the details of their health especially, infections were noted. All children included in this study had thus, received minimum 6 doses of OPV. About 2 mL of blood was collected with prior consent of parents, from all the children as they visited the hospital. Serum was separated and stored at -20°C.

Poliovirus Neutralizing Antibody Assay

The titers of neutralizing antibodies against poliovirus types 1, 2 and 3 were determined by microneutralization assay (WHO, 1997). Sera were diluted two-fold beginning from 1:2 to 1:8196. 50 μL of each of the serum dilution was mixed in 8 wells of microtiter plate with 50 μL of 100 TCD₃₀ of poliovirus Sabin vaccine strains (Enterovirus Research Center, India). Plates were incubated at 37°C for 3.0 h. After incubation, 50 μL of Vero cell suspension containing 1×10⁴ cells were plated to each well. Plates were further incubated for 7 days. Microscopic examination was performed on day 7 for presence or absence of cytopathic effect. Appropriate controls of cells, cells+ virus and cells+ serum were kept in each plate. The cytopathic effect was assessed under phase contrast microscope. Titers were calculated as reciprocal of the highest dilution of the serum that protected cells from 50% of replicate wells against cytopathic effect of a given virus type. A titer of 1:10 was defined as indicative of protective immunity.

RESULTS

Data were uploaded in an Access database. Geometric mean titers against each poliovirus serotypes were determined with respective 95% confidence intervals using Minitab 14 software. Testing of hypothesis for comparison of antibody titers to three poliovirus serotypes and to different doses of oral polio vaccine was done by applying nonparametric test. The antibody titers were also graphically determined.

Antibody titers of children who have taken over 6 doses of OPV are shown in Table 1. Antibody prevalence was found to be 100% for the three poliovirus serotypes. The geometric mean antibody titers were 1.811, 1.659 and 1.226 for poliovirus types 1, 2 and 3,
Table 1: Geometric mean antibody titers of vaccinated children in various age groups

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>No. of children</th>
<th>No. of total OPV doses taken</th>
<th>*GMAT for poliovirus type</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-12 months (subgroup 1)</td>
<td>21</td>
<td>6</td>
<td>1:236, 1:381, 1:117</td>
</tr>
<tr>
<td>12-18 months (subgroup 2)</td>
<td>40</td>
<td>7</td>
<td>1:1070, 1:682, 1:235</td>
</tr>
<tr>
<td>19-24 months (subgroup 3)</td>
<td>29</td>
<td>&gt;8</td>
<td>1:1353, 1:934, 1:344</td>
</tr>
<tr>
<td>6-24 months (total)</td>
<td>90</td>
<td>6-9</td>
<td>1:811, 1:659, 1:226</td>
</tr>
</tbody>
</table>

* Geometric mean antibody titers

Fig. 1: Comparison of antibody response to poliovirus type 1 (PV1), type 2 (PV2) and type 3 (PV3). Datasets of antibody response to three poliovirus serotypes are compared using box plot. The box denotes 2nd quartile, i.e., 50% of the data. The upper hinge (edge) of the box indicates the 3rd quartile or 75th percentile and the lower hinge indicates the 1st quartile or 25th percentile of the dataset. The line in the box indicates the median value of the data (1:912, 1:707 and 1:240 for PV1, 2 and 3, respectively). The whiskers (ends of the vertical lines) indicate the minimum and maximum data values. The points outside the ends are outliers.

respectively. Antibody titers were highest for poliovirus type 1 (Kruskal Wallis test; p<0.001) which ranged from 1:44 to 1:5128. Antibody titers for poliovirus type 2 ranged from 1:32 to 1:5623. However, the titers of antibodies to poliovirus type 3 were 3 to 5 fold lower or sometimes even 10 fold lower than the corresponding titer against type 1. They ranged from 1:18 to 1:2096. Antibody titers for three poliovirus serotypes and their comparison are graphically shown in Fig. 1. The box denotes the distribution of antibody titers, the range of which is quite high for poliovirus type 1 and 2 whereas it is much lower for poliovirus type 3. About 17.7% of serum samples showed antibody titer less than 1:52 for poliovirus type 3. Of these children 6 had taken 6 doses, 8 had taken 7 doses and 1 child had taken 8 doses of TTOPV.

Total number of children in the study group could be divided into 3 subgroups on the basis of their age and number of OPV doses taken. Those in the age group of 8-12 months and have taken 6 doses; 13-18 months and have taken 7 doses and 19-24 months and have taken 8 or more doses of OPV could be grouped in subgroups 1, 2 and 3, respectively. Figure 2-4 show the comparison of dose wise antibody response to poliovirus type 1, 2 and 3, respectively. The geometric mean titers after administration of 6, 7 and 8 doses for poliovirus type 1, 2 and 3 are also given in Table 1. Increments in geometric mean antibody titer after 6, 7 and 8 doses of OPV were observed. We did not attempt to estimate per dose increment as no significance could be determined, in particular, for increments after 7 and
Fig. 2: Antibody response of subgroup 1 (Sg1), subgroup 2 (Sg2) and subgroup 3 (Sg3) to poliovirus type 1. The description of box plot is same as given in Fig. 1. Sg1 (Subgroup 1) denotes children who are in the age group 8-12 M and have taken 6 doses of TOPV (No. 21), Sg2 denotes children in the age group 13 - 18 M and have taken 7 doses of TOPV (No. 40) and Sg 3 denotes children in the age 19-24 M (No. 29). The median values were 1.251, 1.912 and 1.1188 for Sg1, Sg2 and Sg3 respectively for PV1. The description of box plot, Sg1, Sg2 and Sg3 remains same for Fig. 3 and 4.

Fig. 3: Antibody response of subgroup 1 (Sg1), subgroup 2 (Sg2) and subgroup 3 (Sg3) to poliovirus type 2. Median values for Sg1, Sg2 and Sg3 for PV2 were 1.512, 1.732 and 1.831, respectively (p = 0.051).

8 doses for poliovirus type 1 and for type 3 (p>0.05) from our sample size. For poliovirus type 2, no increments could be demonstrated (Kruskal Wallis test; p<0.001) due to much variation in the antibody titers.

An attempt was also made to correlate the antibody titers with number of respiratory and gastrointestinal tract infections suffered by the children. The results are graphically plotted in Fig. 5a-c. In these graphs, red squares represent the respiratory infections and black circles denote the diarrheal infections. Some of these children have reported as high as 8-12 episodes of each infection. No correlation could be determined for number of infections and antibody titers to poliovirus type 1 and 2 as high titers could be demonstrated to children who have suffered from as many as 8 episodes of infection. Lower antibody titers of poliovirus type 3 however, correlated with lower birth weight (data not shown) and more than 6 episodes of intestinal infections. The lowest antibody titer in this study was demonstrated by a 9 month old baby who had not been breastfed at all.
**Fig. 4:** Antibody response of subgroup 1 (Sg1), subgroup 2 (Sg2) and subgroup 3 (Sg3) to poliovirus type 3. Median values for Sg1, Sg2 and Sg3 for Pv3 were 1:125, 1:218 and 1:354, respectively (p = 0.013)

**Fig. 5:** (a-c) Correlation of respiratory tract (denoted in red) and gastrointestinal tract (denoted in black) infections suffered by the children and the antibody response to PV1, PV2 and PV3, respectively. In the graph the red squares represents children who suffered from 2-12 episodes of respiratory tract infections and black circles represents the GI tract infections

**DISCUSSION**

Battle against polio is far from over in India. It has remained as one of the 4 countries that have never interrupted transmission of indigenous poliovirus. The 32 new importation events reported during the year 2008, from polio endemic countries to 15 polio free nations and resulting in 96 polio cases signifies the threat posed by polio endemic reservoirs (Anonymous, 2009). All polio free countries are pressurized to maintain sufficient levels of immunity and efficient AFP surveillance as long as circulation of wild polioviruses continues. In spite of very high performing eradication program, challenges are faced in interrupting the remaining chains of wild poliovirus transmission in India. These include high population
density, large birth cohorts, poor sanitation and high burden of enteric disease that compromises OPV efficacy. New vaccination strategies based on scientific data would be required to overcome the current challenges. Serological surveys comprise an important part of surveillance studies needed to generate scientific data required to guide eradication and post eradication risk management.

A hospital based seroprevalence study to evaluate the antibody response to poliovirus type 1, 2 and 3 strains was conducted in Pune city, a disease free area of India. Ninety children in the age group of 8 months to 2 years were selected randomly as they visited pediatric department of one of the city based general hospital. The vaccination status, health status, number of episodes of respiratory tract and gastrointestinal tract infections suffered by the child, economic status of parents and other relevant information was noted. All the selected children had taken about 4 to 5 doses of OPV in Routine Immunization (RI) and about 2 to 4 doses in Pulse Polio Immunization (PPI) rounds.

The neutralizing antibody titers of these children were significantly high against poliovirus type 1 and 2. The geometric mean antibody titers were found to be 1: 811 and 1: 659 for poliovirus type 1 and 2, respectively. About 74% of the serum samples showed antibody titer greater than 1:512. Significantly high titer indicates that children who have taken more than 6 doses of OPV have been well protected against poliovirus type 1 and 2. This is particularly important in the view of wild poliovirus (WPV) importations detected in last few years. It has been observed that importation events involving WPV1 and Vaccine Derived PV2 (VDPV2) in 14 polio free countries in the last two years, resulted in 70 polio cases and circulation of wild polioviruses in the community for more than one year (Anonymous, 2009). The higher antibody values for PV 1 and 2 suggest protection of children from such importations in the area of study.

Much lower, about 3 to 5 fold lower antibody titer were observed against poliovirus type 3. The geometric mean antibody titer was found to be 1:226. Only 17% of the serum samples showed antibody titer greater than 1: 512. Lower antibody titers to poliovirus type 3 have also been noted in earlier seroprevalence studies conducted in India and in other countries including Greece, Portugal, Netherlands and Germany (Frantzidou et al., 2005; Starka et al., 1999; Conyn van et al., 2001; Miranda et al., 2007). However, lower antibody titer, below even 1:52 for 17.7% of serum samples were noted in the present study even after administration of 6 to 9 doses of OPV. Of the 21 children who showed lower antibody titers, 6 had taken 6 doses of OPV, 8 had taken 7 doses and 1 child had taken 8 doses of OPV.

Children in the study group could be divided into those who had taken 6, 7 and 8 (or more than 8) doses of OPV. The geometric mean antibody titer values of each of these groups show per dose increment in the antibody titer. However, when the antibody titers of each of these groups were plotted separately no significance could be determined for increments of 7 and 8 doses of OPV from our sample size (p>0.05).

Concurrent intestinal infections have been identified as one of the important cause of low vaccine efficacy in India (Patriareca et al., 1991). We therefore, attempted to correlate the number of infections suffered by the child with the antibody titers. Almost all the children included in the study, as is very common in India, have suffered from about 4-5 episodes of respiratory infections and about 2-4 episodes of GI tract infections. Some of the children have suffered from as many as 14-16 episodes of infection in all. The correlation scatter plot showed that number of infections suffered by the child did not interfere with the antibody titers for polio virus type 1 and polio virus type 2. Children who demonstrated antibody titers less than 1:52 for PV1 also demonstrated lower titer for PV2 and 3 irrespective of number of infections suffered by the child. Lower PV3 titer to some extent correlated with the lower birth weight, more than 6 episodes of respiratory and/or intestinal tract infection and no breast feeding.
Although, lower antibody titers to poliovirus type 3 as compared to poliovirus type 1 and 2 are observed, overall our results show 100% seroprevalence in children who had taken more than 6 doses of OPV. Hundred percent seroprevalence after administration of 7 doses were noted by Jaiswal et al. in earlier study performed in India (Jaiswal et al., 2000). Thus, apparently, in city like Pune, where no WPV infected poliomyelitis case has been detected for last decade, it can be said that all children after receiving 6 doses of OPV seem to be protected from WPV infection. This indeed was observed in most of the countries which relied on OPV for eradication of poliomyelitis including Oman, China, Brazil and Mexico (Sutter and Maher, 2006).

Analysis of 1433 paralytic poliomyelitis cases caused due to wild poliovirus in the years 2007 and 2008 in India has shown that about 77% of cases had received more than 7 doses of OPV (Anonymous, 2009). It thus, suggests that multiple doses of vaccine failed to provide protection against wild polioviruses. Similarly, in a study conducted on Vaccine Associated Paralytic Poliomyelitis (VAPP) cases in 1999, in India, Kohler et al. (2002) have noted that about 53.3% of VAPP cases developed paralysis after taking fourth or higher dose of OPV (Kohler et al., 2002). These observations differ from our laboratory findings utilizing neutralizing antibody assays using vaccine strains. It suggests that although titer of 1:10 is considered as adequate protective antibody titer, it may not actually be a protective titer in India. In an earlier study performed by Deshpande and Dave (1992), it was observed that when an antibody titer was 1:143 against vaccine strain it was as low as 1:31 against the locally prevalent wild poliovirus strains circulating in India. These results suggest that 1:10 antibody titer generated against vaccine strains may not be sufficient to provide protection from the so called stubborn (genetic variants) strains of WPV currently circulating in polio endemic reservoirs. This emphasizes the need to redefine the protective antibody titer in India. Wild poliovirus strains were not available to attempt such studies in our laboratory; however, we strongly believe that it will be extremely important to determine protective antibody levels for both OPV and Inactivated Polio Vaccine (IPV) to plan for the strategies to govern polio immunization policies in India.

CONCLUSION

Overall, this study shows that administration of 6-9 doses of TOPV renders the children well protected for poliovirus type 1 and 2 whereas about 17.7% of children still possess antibody response lower than 1:52 to poliovirus type 3. Since, this response is against vaccine strain of poliovirus type 3, it could well be a borderline response to wild type strain. The routine immunization schedule followed by one round of pulse polio immunization is enough to provide immunity against poliovirus type 1 and 2. To increase the rather low immunity of poliovirus type 3, a third booster dose of monovalent poliovirus type 3 after PPI round may be required to create complete immunity. The present policy of targeting all children below 5 years of age in the entire country for repeated PPI, which requires huge financial and human resources, perhaps may need to be reconsidered.

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REFERENCES


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