

## THE ASSOCIATION OF AGE AND NUTRITIONAL STATUS WITH SEROCOVERSION TO ORAL POLIOVIRUS VACCINE (OPV) IN BASRAH, IRAQ

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### ABSTRACT

*A seroepidemiological study was carried out in Basrah, southern Iraq from November 1997 until the end of April 1998. Blood samples were collected from 3 sources; Basrah Maternity and Child Hospital, the primary health care centers in Al-Zubair and Abu-Al-Khasib. The relevant information's were obtained from mothers through interview and/or vaccination cards. An Enzyme linked Immunosorbent assay (ELISA) was used for the detection of poliovirus IgG antibodies. Poliovirus antibodies were found in 92.85% with almost full seroconversion among children above 2 years of age, while seronegative cases were present among infant's children below the age of two years and those with old vaccination history and not boosted with additional booster doses. Malnourished children have relatively lower levels of antibody titer than well nourished children. Number of vaccine doses given to the child have a significant effect on antibody prevalence where high levels of immunity were found in children who received 5 doses or more while seronegative cases were detected among children who had been given 4 doses or less, There were relatively higher seropositivity among children who received an extra doses during the National Immunization Days (NID) than among those who did not receive extra dose(s). The antibody levels in the blood is partially affected by age and the number of vaccine doses given to the child where the proportion of samples with low antibody levels decreased as the age and number of vaccine doses increases. There was clear evidence for the overtime antibody losses among the unboosted children.*

### INTRODUCTION

The persistence of an infectious agent in human population is mainly determined by the interaction between contagiousness of the agent and the number of susceptible individuals in the community.<sup>[1]</sup> Since the contagiousness of poliovirus falls somewhat below that of other high transmissible agents. The control now depends on immunization practices of the country and the degree to which effective and long lasting immunity has been induced in all segments of the population without leaving unimmunized or incompletely immunized pockets of susceptibles who remain at risk.<sup>[2]</sup> It is known that in some developing countries, the live polio-vaccine has been repeatedly and fully administered to a large proportion of childhood population and nonetheless has not provide a 100% protection.<sup>[3]</sup> However, practical experience in Brazil and Mexico showed high levels of population immunity and significant reduction in the incidence can be achieved even in developing countries by the use of repeated doses of standard live poliovirus vaccine alone if given on regular basis to infants and young children.<sup>[2]</sup> The priorities of WHO was to achieve poliomyelitis eradication by the year 2000 include: insuring high level National Immunization Days (NIDs) in

countries with persistent wild poliovirus circulation and low vaccination coverage, establishing and strengthening sensitive and effective surveillance system of acute flacid paralysis (AFP).<sup>[4,5]</sup> Malnutrition due to protein restriction associated with poverty although does not affect the antibody levels but it has been found that it affects the affinity of antibodies produced<sup>[6]</sup>, so it enhances the susceptibility to infections in general.<sup>[7]</sup> In Iraq, as in many other countries where mass vaccination through NIDs with OPV has been introduced, the number of paralytic cases of poliomyelitis has continuously decreased.<sup>[8]</sup> In 1997, Iraq achieved the expected target rate in reduction of AFP cases. These are good indicators of the effectiveness of poliomyelitis control programme, nevertheless serological surveys can provide useful information to assess the immune status against poliovirus in our community.

***This study aimed at studying:*** the prevalence of poliovirus antibody among children in Basrah in relation to selected variables looking into the effect of age and number of vaccine doses received by the child on the seroconversion to OPV and the association of nutritional status with seroconversion.

## MATERIALS AND METHODS

**Study population:** A total of 308 infants and children with an age range of 1 day-13 years were randomly selected from three sources; Basrah child and maternity hospital, Abu-Alkhasib and Al-Zubair primary health centers. In addition, 3 blood samples from children who had paralytic poliomyelitis during childhood were used as positive control sera.<sup>[9]</sup> The study was carried out during the period from November 1997 through April 1998. All required informations were obtained on special questionnaire form designed for this study through mother's interview and/or from the child vaccination card. Child body weight was taken as an index for the nutritional status of the child.<sup>[10]</sup> Body weight for age of the child was categorized into normal and low weight according to WHO criteria.<sup>[11,12]</sup>

**Sampling:** Blood was taken either from veins or via finger or heel prick.<sup>[13]</sup> Serum was separated and kept frozen at -20C<sup>[14]</sup> until use.

**Elisa procedure:** ELISA procedure principally based on a standard method.<sup>[15]</sup> The optimum dilutions of the reactants were selected through a checkerboard titration ELISA (CB-ELISA). Poliovirus antigen (routinely used trivalent oral vaccine) was diluted in coating buffer (pH 9.6) and incubated overnight at room temperature to allow antigen binding to solid phase (polystyrene microtiter plates). Then unbound antigen was washed away before the addition of serum under investigation at the optimal dilution to these sensitized wells. After 2 hours incubation at room temperature, excess serum was removed by four additional washes, and wells were loaded with horse reddish peroxidase labelled anti-human conjugate. The conjugate will become attached to the antigen-antibody complex on the solid phase if present. After 4 additional washes, addition of substrate produced colour change at the reaction site. The intensity of the colour is proportionally related to the amount of specific antibody present in the sample.

The absorbance value of the wells was read at 492 nm using Dynetech microplate reader (MR600). Cut-off value was estimated as the mean of true negatives plus 3 SD, accordingly serum samples with OD values of less than 0.3 over the cut-off value were considered negative for poliovirus antibody.

## RESULTS

The overall antipoliovirus antibody positivity in this study was 92.8% (286/308). The vaccination coverage with at least one dose of OPV as obtained from the questionnaire form analysis was 99% (305/308). The ratio of seropositive to seronegative vaccines was 13.5/1 (284/21). There were 8 children who passed their seventh month of age still not completed the primary series of OPV, only one of them was seronegative. There were 84.4% of seropositivity among infants below one year of age which was increased to 92% among children by 1-2 years of age where all of this age group covered with primary vaccine doses and boosted with at least one dose and/or NIDs (Tables 1,4). However, the number of vaccinees with low levels of antibody was less than those with moderate or high antibody levels in this group. Children at age groups 3-5 and 6-13 years of age who had completed their primary doses and boosted with at least one booster dose of OPV showed almost comparable numbers of low levels of antibody (41.3% and 37%) and moderate or high levels of antibody (58.7% and 63%) for both age groups respectively. However, participants from the same age groups not boosted after the primary doses showed higher percentages of poliovirus seronegativity especially at age group 3-5 years (31.8%) compared to boosted individuals (1.3%). However, the number of participants with low antibody levels increased considerably as the time from the primary doses increased which indicate clear antibody loss overtime in the absence of booster doses at a suitable time following the primary vaccine doses.

**Table 1. Prevalence of poliovirus antibody in various age groups in relation to vaccination status.**

Age (years) (No. Tested)	No. (%) Positive	Antibody levels*		No. (%) Negative
		Low + N (%)	Mod/high+ N (%)	
Less than 1 (109)*	92(84.4)	50(54.3)	42(45.7)	17(15.6)
1-2 (50)	46(92)	21(45.7)	25(54.3)	4(8)
3-5: Boosted (76) Not boosted (44)	75(99) 30(68.2)	31(41.3) 21(47.7)	44(58.7) 9(20.4)	1(1.3) 14(31.8)
6-13: Boosted (74) Not boosted (87)	73(98.6) 74(85.1)	27(37) 58(66.7)	46(63) 16(18.2)	1(1.3) 13(14.9)

$\chi^2: 21.3, P<0.01, df: 3$

*Low antibody level: serum sample with O.D. value greater than 0.1 and less than 0.499, moderate or high: serum samples with an O.D. value of 0.5 or more, above the cut-off value. Some of individuals in the primary doses also considered in boosted group.*

The effect of nutritional status which is related to the index of child body weight corresponding to age of each child on the distribution of poliovirus IgG antibody is presented in (Table-2). Among 278 children with normal body weight, 260(93.5%) positive samples were found; while of 30 under weight children 26(86.7%) were positive. Although there was a slight difference between normal and the under weight children, it did not attain statistical significance ( $\chi^2: 1.02, P>0.05$ ).

**Table 2. The distribution of poliovirus IgG antibody in relation to child nutritional status.**

Weight for age	Antibody levels		Antibody levels*	
	Low Ab No. (%)	Moderate or High No. (%)	No. +ve No. (%)	No. -ve No. (%)
Normal	117(45)	143(55)	260(93.5)	18(6.5)
Under Wt.	21(80.8)	5(19.2)	26(86.7)	4(13.3)
Total	138(48.3)	148(51.7)	286(92.85)	22(7.15)

$\chi^2=1.17, P>0.05, df: 2.$

(Table-3) shows, the association of seroconversion to OPV with nutritional status of children where a high number (13.3%) of seronegative to OPV was found among the under weight children compared to a lower

number (6.5%) of seronegatives among the normal body weight children.

**Table 3. The association of poliovirus IgG with child body weight for age.**

Weight Age	No. Positive (%)	No. Negative (%)	Total No. (%)
Normal	260(93.5)	18(6.5)	278(100)
Under weight	26(86.7)	4(13.3)	30(100)
Total	286(92.9)	22(7.1)	308(100)

(Table-4), shows the seroconversion according to the number of vaccine doses taken by each child. There were samples with no detectable poliovirus antibodies even among children who had taken 4 doses of OPV, while among 90 children given 5 doses, only single seronegative case was found. Children who received 6 or more doses of OPV almost showed full seropositivity. The difference in antibody distribution in relation to the number of OPV doses given to each child was statistically significant ( $\chi^2: 29.68, P< 0.01$ ).The effect of participation in NIDs on antibody prevalence is shown in (Table-4). However, 179 participants were fully covered with primary doses and booster/or included in the NIDs by the 3<sup>rd</sup> year of their age, leaving 131 participants of study population neither boosted nor included in NIDs although were covered by the primary vaccine doses.

**Table 4. Prevalence of poliovirus antibody in relation to number of OPV doses and participation in NIDs.**

OPV doses	No. positive (%)	No. negative (%)	Ab levels of positives	
			Low n(%)	Mod / high n(%)
Primary (1-3)	110 (36.5)	12 (4)	56(51)	54(49)
Boosters* (4-6)	169 (66)	10 (3.3)	71(42)	98(58)
NIDs				
Yes	49(96)	2(4)	24(49)	25(51)
No.	89(82.4)	19(17.6)	58(65)	31(35)
Not vaccination	2(67)	1(33)	-	-

Most of them boosted before the conduction of NIDs.

7 Positive samples not included of inadequate vaccination history (Total of 8 unvaccinated or incompletely vaccinated, all positive except one).

## DISCUSSION

Survey studies on poliovirus antibodies in Arab countries are very rare and according to the available knowledge so far no similar study in Iraq dealing with the prevalence of poliovirus antibody among children post vaccination were carried out. Clinical data alone are of very limited value because the disease is sub-clinical in 90 to 95% of the cases<sup>[7,15]</sup>, and the cases which show the cardinal feature of the disease which is acute flacid paralysis without sensory loss represent only 0.1-2% of the cases.<sup>[16]</sup> So the study of seroepidemiology of poliovirus antibodies makes this of value to the country. The proportion of children who were immune to poliovirus was 92.8% leaving 7.2% seronegative to all the three types of poliovirus. These susceptible nonimmune children can be considered at risk of acquiring the disease if exposed to wild poliovirus<sup>[17]</sup>, in addition to their probable role in sustaining the transmission of wild virus.<sup>[18]</sup> However, they cannot be regarded as a serious immunity gap because they represent an isolated cases of vaccine failure or cases with incomplete vaccination living in a community with herd immunity against poliovirus. The prevalence of poliovirus antibody in this study was higher than that in some developing countries such as 78% in Nigeria<sup>[19]</sup>, 72% in Gambia<sup>[20]</sup> and comparable to that of 90% from northern Malaysia.<sup>[21]</sup> While it is lower than that from developed countries such as Germany where only 1-2% not have detectable antibody<sup>[22]</sup> and Italy where only 1% were seronegative among children up to 10 years old children.<sup>[23]</sup> The vaccination coverage with at least single dose among the study children was 99%, however, not all of them had received their doses of OPV at it's proper time where there were 7.3% of infants had passed the 7<sup>th</sup> month but they did not complete the primary doses of OPV. Age plays a significant role on the prevalence of poliovirus antibody among children. Negative samples were restricted largely to infants and children below two years of age and those not taken the booster doses at any time post the primary doses. Seronegative infants were either not vaccinated or not completed their primary doses yet, but unfortunately six of them had completed their primary doses. This indicates

that the primary doses are not adequate to give full protection to infants and this may be attributed to the limited efficacy of OPV in inducing high seroconversion rate among early age group in our community, as the case in developing countries.<sup>[3,24]</sup> This risky period of age seems to extend to the second year of life and ends when the child receives the booster dose(s) of vaccine. This is supported by that all the four negative cases found in the group of two years old had received an early single booster dose and no further boosting to reach the adequate protective level of antibody. So this study suggests that in general, age below 18 months may be regarded as a risk group for poliomyelitis in Basrah because the booster doses are not given before this age and we expect that the risky age will extend more if the child does not take the booster doses at its proper time. The decrease in seronegativity as the age increased might be due to the acquisition of immunity after taking the booster doses of OPV or probably from natural exposure to wild poliovirus in the environment, especially at the older age group since the virus is endemic in Iraq.<sup>[4]</sup> However, seroconversion among 8 unvaccinated cases indicates the wide distribution of vaccine virus shedding or wild virus in the surrounding. The low levels of anti-poliovirus antibody and the presence of seronegative among vaccinated children may be due to inadequate vaccination or due to overtime loss of antibody (waning immunity) especially the low levels of antibody among unboosted children which did not attain the protective levels, that may be overcome through the application of poliovirus vaccination schedule that suggested in 1997 by the USA advisory committee on vaccination practices where they recommended a two doses of inactivated poliovirus vaccine in infancy (2 and 4 months of age) for priming followed by two doses of OPV (12 and 18 months and at 4-6 years). It is expected to enhance the early immune responses and to give the needed immunity against poliovirus.<sup>[7]</sup> There was relatively higher seroconversion among children with normal body weight than among the under weight children. This finding may reflect the partial role of the nutritional status as presented by weight for the corresponding age on the immune response to OPV. These results are in

agreement with Adeigu et al<sup>[25]</sup> where they found that the state of nutrition affect the immune response to OPV to some extent. However, it was found that nutritional status does not only affecting the immunoglobulin levels but also affect their affinity<sup>[6]</sup> where malnutrition due to protein restriction associated with poverty imposed by the effect of sanction on Iraq for more than 10 years which in turn enhances the susceptibility to infectious diseases in general.<sup>[7]</sup> The number of OPV doses taken by the child have significant role on antibody prevalence among vaccinees. There were considerable proportions of children without detectable antibody or low levels of antibody even after 4 doses of OPV vaccine. However, from the fifth dose and more a convenient rate of seropositivity was found. The presence of interfering agents such as enteroviruses in the gut of the vaccinees may have a role of interference.<sup>[2]</sup> This finding is in agreement with others<sup>[26,27]</sup> in that seroprevalence is correlated with the number of OPV doses taken by the child. Children who had received an extra OPV doses during NIDs have relatively higher seropositivity which stress the need for these campaigns to continue further especially for children below 2 years of age and those with waning immunity. These finding are in agreement with Shin 1995<sup>[27]</sup> and Xu 1992.<sup>[28]</sup>

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