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A cross-sectional sero epidemiological prevalence of polio virus antibodies post-immunization with three doses of TOPV

SA Tswana, C Berejena, SR Moyo, O Mudyarabikwa

SUMMARY

A cross-sectional sero epidemiological study to detect the presence of antibodies to polio virus types 1, 2 and 3 was undertaken. A total of 437 infants with an average of 40 subjects per province was enrolled in this study. All the subjects had completed the three doses of TOPV.

Blood samples were aseptically collected by heel pricking on calibrated filter papers and immediately transported to the laboratory for processing. In the laboratory, standardized techniques were used to detect neutralizing antibodies to polio virus. Antibodies with a titre of >1:32 were detected in all studied subjects. Some infants developed titres as high as 1:1024. However, polio virus type 3 showed a higher antibody titre than the other two types. Differences in titres were observed from province to province.

INTRODUCTION

In Zimbabwe the schedule of immunization of infants is a three dose programme of triple oral poliomyelitis vaccine (TOPV), at 12, 16 and 20 weeks of age. Recent epidemiological data from many developing countries suggest that poliomyelitis is a far more serious public health problem than actually suspected, whereas the problem of the same disease is far reduced or in some cases, eradicated in several of the developed countries.

It has been reported that the three doses of TOPV as currently recommended may not be adequate for effective protection of infants in tropical countries due to poor conversion rates or due to other enteroviruses. Consequently paralytic poliomyelitis occurs in vaccinated populations, and thus may justify the recommended fourth dose.

More recently studies performed in Harare at Mbare clinic showed a good sero conversion to all three types of polio virus while cases of poliomyelitis within the Harare area are now a rare occurrence. There are however, a number of cases still being reported, but these cases are not confirmed by laboratory tests.

This study was designed in order to determined the efficacy of the TOPV in infants who had already received three doses.

MATERIALS AND METHODS

Study population: The study population consisted of 437 infants less than nine months old who had completed TOPV and were brought to the clinics to receive the measles vaccine. Both male and female candidates were included in the study. Only African infants were sampled since these were the only infants who were brought to the randomly selected clinics.

Clinics were randomly selected from all of the 10 provinces, however Chitungwiza was taken as the 11th province. Results were analyzed separately for each province.

Collection of specimens: Samples were collected from volunteers only. Written consent was obtained from the mothers before any samples were collected. All relevant information was recorded.

Blood samples were aseptically collected by heel pricking on calibrated filter papers. These filter papers were calibrated to absorb 0.05ml of blood. A total of 0.10ml (2 x 0.05ml) of blood was collected from each infant. Immediately after collection samples were placed in an ice box and were transported to the laboratory for processing. If not examined on the same day, samples were stored at -70°C, until examined.

Determining of neutralizing antibodies: The neutralizing antibodies were detected from the samples using the micro-neutralizing test which consists of two steps namely, virus titration and the actual neutralization test.
The virus titration: This was done to determine the right concentration of virus to be used in the actual test. The virus titre was determined as the highest dilution giving cytopathic effect (CPE) in 50 pc of inoculated cultures (TCID50).

The test procedure was performed by the micro technique which was done by preparing serial ten fold dilutions of the polio viruses, types 1,2 and 3 from 10^4 to 10^5. Fifty micro litres of each virus dilution were added to a row of four wells in a micro-titre plate already containing 50ml of maintenance media. The plates were than incubated for three hours at 36°C. A concentration of 1 x 10 Hep2 cells/0,1ml was added to all the wells containing the virus plus the cell controls. The plates were sealed with adhesive tape and incubated at 36°C for 10 days. They were examined daily using an inverted microscope for appearance of CPE. The titre of the virus was determined by the Karber Formula.

The actual micro-neutralizing test: 25ml of maintenance media was added to each well to be used for assay of serum. Serial dilutions of the eluted blood samples were done starting from 1:8 to 1:2 048.

This was followed by dilution of 25ul standardized polio virus types 1,2 and 3 containing 100 TCID50 to every well of poliovirus types 1,2 and 3 except the serum control wells, which received 25ml of the maintenance media. The plates were then incubated at 36°C for three hours followed by overnight incubation of 4°C. Then a concentration of 1 x 10 Hep2 cells/0,1ml was added to all the wells.

Appropriate controls were also included in the test. The plates were sealed with adhesive tapes and incubated at 36°C. The plates were examined daily for 10 days using an inverted microscope to confirm that the cells were monolayering, the sera were not toxic and that the challenge viruses were showing CPE.

RESULTS

Our of 437 infants immunized against polio virus types 1,2 and 3 it was noted that all had developed circulating neutralizing antibodies at nine months of age, with titres of >1:32. However, distribution of antibodies for each type varied from province to province (Table 1). Although all immunized infants seroconverted and developed high titres (up to 1:1 024), waning of antibodies was shown to increase. Our findings show that these infants showed a higher antibody titre to polio virus type 3 than the other two types (Figure I). On the other hand type 1 had a better seroconversion than type 2. It was also observed that several of the infants had developed antibody titres of between 1:32 and 1:1 024. Surprisingly infants from both Mashonaland Central and Mashonaland East had titres >1:512 only for type 2 which was not compatible with other provinces nor with other poliovirus types. Further, a high antibody titre for type 3 in infants from both Mashonaland West and Manicaland ranged from 1:32 to 1:64 with a decreasing scattered distribution in higher titres, whereas the opposite in titres was observed for type 2 in Midlands and Manicaland.

Table I: Post immunization distribution of polio virus neutralizing antibodies (Har=Harare, Bul=Bulawayo, Chi=Chitungwiza, MC=Mashonaland Central, ME=Mashonaland East, MW=Mashonaland West, MN=Matabeleland North, MS=Matabeleland South, Mid=Midlands, Man=Manicaland, Mas=Masvingo, Zimb=Zimbabwe).

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DISCUSSION

The introduction of polio vaccine has drastically reduced both the incidence and prevalence of poliomyelitis both in developed and developing countries. Very rare sporadic cases if any are reported from few developing countries. In Zimbabwe occurrence of poliomyelitis is now uncommon. The reduction in the prevalence of the wild virus could probably be due to a high vaccination coverage. Even though cases of poliomyelitis are becoming scarce, developing countries should not be relaxed, but continue with the Extended Programme of Immunization against the disease.

Our study shows a 100 pc seroconversion to OPV after three doses. However, there were variations in seroconversion as per province. It should however, be noted that all study infants had protection to polio virus. In this study, a titre of 1:16 was considered protective. Sero-responses of infants and children to three doses of OPV had been described elsewhere. Obviously in our study there was a progressive increase in the titre in the sero-response to OPV after an additional dose. This was in agreement with our previous study and studies by John, Paesa and Simoes, et al which showed a drastic increase in titre with subsequent doses. Although all our study subjects sero-converted, there was somehow a very low rate of seroresponse to poliovirus type 2 from the study of infants in both Mashonaland Central and East.

We could not explain this. In this study we observe a very high rate of sero-response to poliovirus type 3, which is in contrast to our previous study which showed a low rate of seroresponse to this type.

In conclusion, we recommend continuation with vaccination and a close monitoring of seroconversion, especially in provinces where decreased antibody titres were observed.

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