Immune response to 1 and 2 dose regimens of Measles vaccine in Pakistani children

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Abbreviations: EPI, Expanded Program on Immunization; GMC, Geometric mean antibody concentrations; HELP, Health Education and Literacy Program; MMR, Measles Mumps and Rubella; NGO, Non-governmental organization; OD, Optical density; WHO, World Health Organization

Measles is a significant problem in Pakistan despite vaccine coverage rates reported at 80%. The purpose of this study was to determine the serologic response in children after one dose of measles vaccine at 9 mo versus two doses at 9 and 15 mo of age. From March through December 2006, children were enrolled from immunization clinics and squatter settlements in Karachi. Blood samples were taken from children in Group A at 9–10 mo of age prior to measles vaccine and 8 to 11 weeks later; from children in Group B at 16–17 mo of age after receiving 2 doses of measles vaccine; and from children in Group C who had received at least one dose of measles vaccine by 5 y of age. After the first dose of measles vaccine, 107/147 (73%) of children in Group A were seropositive, 157/180 (87%) of children in Group B were seropositive after two doses and 126/200 (63%) of children in Group C were seropositive at 5 y of age. The post-vaccination geometric mean antibody concentrations were higher in females than males in groups A (irrespective of pre-vaccination antibody levels) and B. The serologic response to one and two doses of measles vaccine was lower in children in Karachi than has been reported in many other countries. Two doses of vaccine were significantly better than one dose. An in-depth investigation is needed to determine the reason for the lower-than-expected protection rates. Differences in immunogenicity between genders need to be further studied. Recent introduction of supplemental measles vaccine doses should help control measles in Pakistan.

Introduction

Measles is an important cause of morbidity and mortality in young children in some countries despite the availability of an effective vaccine.¹ High immunization coverage is needed to prevent outbreaks and two doses of measles vaccine are more effective than one dose.^{2–5} Seroconversion rates are lower when the vaccine is given before one year of age,⁶ and malnutrition, gender and socioeconomic factors could be associated with the differences in immune responses.^{7–9}

Pakistan is one of 47 countries identified by the World Health Organization (WHO) with a high measles burden. Of the 19.1 million infants worldwide that WHO estimates did not receive measles immunization in 2010, 600 000 were in Pakistan. Measles occurs primarily among children younger than five years of age in Pakistan with a peak incidence in the second year of life; only 50% of affected children had a history of receiving measles vaccine.^{10,11} Reported immunization coverage rates for measles vaccine in Pakistan at one year of age is 80%.¹²

At the time of our study, measles vaccine was recommended at 9 mo of age; children could also receive a dose of measles, mumps

and rubella (MMR) vaccine at 15 mo through the private sector. The crude vaccine efficacy of one-dose measles vaccine has been reported to be 75% in Pakistan.¹³ In 2008, Pakistan instituted a two-dose measles vaccine schedule for all children at 9 and 15 mo of age in the Expanded Program on Immunization (EPI) program. The objective of this study was to determine the serologic response in children who have received one dose of measles vaccine at 9 mo or two doses at 9 and 15 mo of age. We also tested children at 5 y of age to evaluate seropositivity rate from a single dose of measles vaccine at the start of school.

Results

A total of 547 infants were enrolled, 158 in Group A, 186 in Group B and 203 in Group C. Twenty subjects were excluded due to missing laboratory results and the final analysis was conducted on 527 infants (147 in Group A, 180 in Group B, and 200 in Group C). The mean age of Group A infants was 9.8 mo (sd 1.4), Group B 15 mo (sd 2.6), and Group C 64 mo (sd 4.8).

Of the children in Group A, 35/147 (24%) were seropositive at enrollment, but the geometric mean antibody concentrations

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Table 1. Pre- and post-vaccine serologic response and Geometric mean antibody concentrations

	*Group A						**Group B Post vaccination			***Group C Post vaccination		
	Pre vaccination n = 147 (%)			Post vaccination n = 147 (%)			n = 180 (%)			n = 200 (%)		
	Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total
Gender	67	80		67	80		81	99	180	108	92	200
Age at enrollment months (Mean and SD)	9.8 ± 1.5	9.8 ± 1.4	9.8 ± 1.4	NA	NA	NA	14.6 ± 2.4	15.5 ± 2.7	15.1 ± 2.6	63.8 ± 4.8	64.4 ± 4.8	64.1 ± 4.8
Seropositive 95% CI	13 (19) [10–29]	22 (28) [18–38]	35 (24) [17–31]	54 (81) [71–90]	53 (66) [56–77]	107 (73) [66–80]	70 (86) [79–94]	87 (88) [81–94]	157 (87) [82–92]	59 (545) [45–64]	67 (723) [64–82]	126 (63.0) [56–70]
Seronegative 95% CI	52 (78) [67–88]	57 (71) [61–81]	109 (74) [67–81]	5 (8) [1–14]	20 (25) [15–35]	25 (17) [11–23]	9 (11) [4–18]	10 (10) [4–16]	19 (11) [6–15]	36 (33) [24–42]	21 (23) [14–32]	57 (28) [22–35]
Indeterminate 95% CI	2 (3) [–1–7]	1 (1) [–1–4]	3 (2) [–0.3–4]	8 (11) [4–20]	7 (9) [2–15]	15 (10) [5–15]	2 (2) [0–6]	2 (2) [0–5]	4 (2) [0–5]	13 (12) [6–18]	4 (4) [0–9]	17 (9) [5–12]
GMC (mIU/mL) 95% CI	19.1 [9.2–39.6]	34.9 [16.3–74.5]	26.5 [15.7–44.9]	1476.6 [924.3–2358.8]	446.9 [254.1–785.8]	770.5 [525.6–1129.4]	1422.6 [935.4–2163.7]	1316.3 [898.9–1927.7]	1363.1 [1030.7–1802.7]	518.6 [322.5–833.9]	1030.3 [673.4–1576.5]	711.2 [514.6–982.7]
Female/male GMC (mIU/mL) ratio <i>P</i> value	0.55 0.346			3.30 0.001			1.08 0.665			0.50 0.0374		

Note: *A = Children 9–10 mo of age prior to measles vaccine and 8–11 weeks post measles vaccine. **B = Children 16–17 mo of age who have received 2 doses of measles vaccine. ***C = Children 5 y of age who have received one dose of measles vaccine. *P* value for difference in total post-vaccination seropositivity rates between group A and B is 0.003, Group A and C is 0.11, and Group B and C is < 0.001. *P* value for difference in total post-vaccination GMC between Group A and B is 0.055, Group A and C is 0.40, and Group B and C is 0.001. *P* value for difference between male and female seropositive rates in Group A pre-vaccine is 0.25, Group A post-vaccine is 0.051, Group B is 0.77 and Group C is < 0.02.

(GMC) was low. Post-vaccination, 107/147 (73%) were seropositive (Table 1) and the geometric mean antibody concentration following measles vaccines increased 29-fold (95% CI: 15.3–55.1). For children in Group B who had received 2 doses of measles vaccine, the seropositivity rate was 157/180 (87%). Mean fold-increase cannot be estimated, since only post-vaccination data were collected for these children. For the 5-y-old children in Group C who had received only 1 dose of measles vaccine, the seropositivity rate was (63%). The seropositivity rate for measles IgG antibodies was higher in children in Group B as compared with Group A (87% vs. 73%; *P* value = 0.003) and Group C (87% vs. 63%; *P* value: < 0.001). The gender differences in seropositivity rates were marginally significant in Group A (81% for females vs 66% for males; *P* value 0.051), as compared with Group C where females were less likely to be seropositive than males (55% vs. 73%; *P* value < 0.02). In Group A, the post-vaccination GMC was significantly higher in females as compared with males (1477 vs. 447; *P* value < 0.002) while in Group C females had lower GMC as compared with males (519 vs. 1030; *P* value < 0.037). There were no significant gender differences in seropositivity rates or GMC for Group B.

Table 2 compares seropositivity in pre- and post-vaccination sera for children in Group A. Seropositivity rates post-vaccination were similar for children who were seronegative and seropositive at enrolment.

Discussion

At the time of this study, measles vaccine was provided at 9 mo of age as part of the EPI program and the second dose was available only through the private sector. This study found a low serologic response of 72% after the first dose of measles vaccine and 87% after two doses of vaccine at 9 and 15 mo. Another study in Karachi found that only 55% of children 12–59 mo of age had evidence of immunity in oral fluid.¹⁴ Similar low rates of 64–71% seroconversion and 44–76% seroprotection have been reported after one dose of measles vaccine from other developing countries.^{15–18}

The low serologic response after 1 or 2 doses of measles vaccine could be due, in part, to the persistence of maternal antibodies. In our study, 24% of children had residual maternal antibodies. Ceyhan et al. obtained similar results when children were vaccinated before 12 mo of age in Turkey.² Some earlier studies have suggested that giving vaccine prior to 12 mo of age alters the immune response to revaccination conferring low levels of protection.¹⁹ However, other studies could not duplicate these results and postulated that the low rates could be due to the serological tests used.²⁰ Our study used the standardized and commercially available ELISA method used by WHO reference laboratories.^{21,22} A study comparing this commercial ELISA with plaque reduction neutralization test showed 92% agreement, with a sensitivity

Table 2. Post- vaccination serologic response by prevaccination status for children vaccinated at 9–10 mo of age

Group A post-vaccine	Pre-vaccination Sero-positive n = 35 (%)	Pre-vaccination Sero-negative n = 109 (%)	Pre-vaccination Indeterminate n = 3 (%)
Seropositive	26 (74.3)	80 (73.4)	1 (33.3)
Seronegative	5 (14.3)	18 (16.6)	2 (66.7)
Indeterminate	4 (11.4)	11 (10.0)	-

Note: *The differences in post-vaccine seropositivity, seronegative and intermediate rates between previously seropositive and seronegative infants were all insignificant (P value > 0.7).

of 90% and specificity of 100%.²³ Although plaque reduction neutralization titer ≥ 120 is the most widely accepted correlate of protection for measles, use of the WHO international standard can allow for the comparison of commercially available ELISA results with immune responses that are considered protective.^{24,25} The serologic response rates obtained in this study are consistent with the results from an earlier study in Karachi, which estimated the protective efficacy from one dose of vaccine to be 70%.¹⁰

Low serologic responses in our study could also be due to improper vaccine storage.²⁶ We reviewed temperature charts going back 2 y at the central EPI vaccine storage facilities and interviewed the EPI personnel and no evidence of breaks in the cold chain were found. Similar visits were conducted at the EPI vaccine administration centers where all processes seemed to have been appropriately followed.

Female children had a higher serologic response and higher GMC than male children in Group A. Similar sex differences have been reported in responses to measles and hepatitis B vaccines.^{27,28} Females have been noted to exhibit stronger humoral immune responses to several vaccines.²⁹ However, in our study male children in Group C had higher responses GMC than females. We cannot explain these results. It is possible that the male children who did not respond to vaccine had higher disease rates that resulted in higher immune responses from natural infection in Group C. Further research is needed to address this issue.

There are some limitations to our study. Our study was confined a geographic location in Karachi where vaccines were given by both the EPI and a non-governmental organization (NGO). However, all children enrolled in our study had immunization records available. We did not measure the nutritional status of the children enrolled in the study. However, another study from Karachi reported no differences in the serologic response rate for children based on nutritional status.¹⁴ Similar results on nutritional status have been reported by other studies.³⁰

WHO recommends that every child should receive two doses of measles vaccine. There is overwhelming evidence that overall progress in reducing measles-related morbidity and mortality is linked to increased vaccination coverage and the opportunity for children to receive a second dose of measles vaccine.³¹ Our data support the need for a routine second dose of measles vaccine, which has now been instituted in Pakistan. Further studies need to be conducted to assess the antibody titers for children 5 y of age at the start of school following two doses of measles vaccine in Pakistan.

Methods

The study was conducted in Karachi, Pakistan. Children were enrolled between March and December 2006 from the government immunization clinics in the squatter settlements of Khuda ki Basti, Neelam colony and Lower Ghizri. Health care and other services in this area were provided by government EPI centers as well as a non-governmental organization, Health Education and Literacy Program (HELP). Experienced Lady Health Visitors who were also trained vaccinators conducted enrollment and interviews. They were further trained by the study investigators to administer standardized data collection tool. Group A children were enrolled and blood samples were taken at the time of measles vaccination at 9–10 mo of age and again at home 8–11 weeks later. Blood samples from Group B children were taken at 16–17 mo of age after they received 2 doses of measles vaccine and samples were taken from Group C children, who had received one dose, by 5 y of age. Children in Group A were identified at the EPI clinics at the time of immunization and enrolled serially. Children in Groups B and C were identified through household visits of children who had received immunization at the HELP clinics. As the children were provided EPI services through HELP, the EPI records were maintained and confirmed the immunization status of these children.

Children with known immune deficiency, immediate hypersensitivity to measles vaccine and those with history of having had measles or a household contact who had measles were excluded from the study. After obtaining informed consent, parents or guardians were interviewed to obtain child's age, immunization history and demographic data and 1–3 ml of blood was drawn using venipuncture or fingerstick.

Monovalent measles vaccine and diluents (manufactured by Sanofi Pasteur) were provided by the EPI (Lot numbers: Z5172-1, Z5410-1, Z6425-1, Z6323-1, Z6453-1, Z6350-1) with expiration dates from March to October 2007. Merck and Co manufactured the MMR vaccine available through private sector. A 0.5 mL dose of vaccine was injected subcutaneously in the outer mid-thigh or upper arm, depending on the age of the child, using a 1/2–5/8 inch needle. The vaccine was reconstituted as per manufacturer's guidelines and stored at 2–8°C. Any leftover vaccine was discarded at the end of the immunization session.

A commercial enzyme-linked immunosorbent assay (Enzygnost® Anti-Measles Virus/IgG, Dade Behring, Germany) was used for detecting measles IgG antibodies at Kharadar General Hospital, Karachi. ELISA results were read as optical

density (OD) and the antibody concentration was determined in mIU/ml calculated by an equation using the OD and constants specific for the reagent's batch according to manufacturer's instructions. Sera with OD value less than 0.100 nm were considered negative while OD value higher than 0.200 nm was classified as positive. Sera with OD value 0.100–0.200 were considered equivocal or inconclusive. The protective threshold for ELISA-based antibody level has not been established. Based on the OD measurement, we estimated the cut-off value to positivity as greater than 0.200 nm or 300 mIU/mL or neutralization titer of 120.^{25,32} A serologic response was defined as seroconversion (in previously seronegative children) or 4-fold rise in post-vaccine antibody concentration as compared with pre-vaccine status in children.

The data were double-entered in Microsoft Access (Microsoft Corporation) and analyzed using Stata (Stata Corp LP). The

seroconversion rates for each group were calculated along with 95% confidence intervals. Chi square test was used to compare proportions and student t test or Mann-Whitney U test for comparing means between groups. Two-sided *P* values are reported and values 0.05 or less are considered statistically significant. GMCs were computed as arithmetic means of logarithmically transformed values along with 95% confidence intervals. All children were included in GMC analyses irrespective of their seropositivity status. Bridge Research Ethical Committee, Karachi and the Committee on Human Research at the Johns Hopkins Bloomberg School of Public Health, Baltimore Maryland, approved the protocol.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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