Original Article

Detection of Serum Antibodies against Measles, Mumps and Rubella after Primary Measles, Mumps and Rubella (MMR) Vaccination in Children

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Abstract

Background: In Iran, the measles, mumps and rubella vaccine (MMR) is administered in a two-dose protocol where the first dose is scheduled at 12 months of age. This study aims to determine the efficacy of the MMR vaccine by testing IgM and IgG antibody levels 4 – 7 weeks after primary vaccination.

Methods: A single group cohort study was performed on healthy children, 12 – 15 months of age, who were vaccinated at health centers affiliated with Shahid Beheshti University of Medical Sciences in Tehran, from January to April 2009. Children with negative vaccination and/or clinical history for measles, mumps or rubella were administered the first dose of the MMR live attenuated vaccine. IgG and IgM antibodies were checked by enzyme linked immunoassay (ELISA) in serum samples 4 – 7 weeks after vaccination. A child was considered seropositive if antibody levels were higher than the assay cut-off level set by the ELISA kit.

Results: Samples from 240 children were checked for antibodies against measles and rubella. Measles serum IgM level was positive in 71.7% of samples and IgG in 75.8%. The rubella serum IgM level was positive in 71.7% of children and IgG in 73.8%. From 190 blood samples that were checked for mumps antibodies, serum IgM was positive in 68.9% and IgG in 95.3%. No significant relationship was found between seropositivity and age or gender.

Conclusion: IgG and IgM antibody levels were below the assay cut-off levels against measles and rubella in approximately one-fourth of the children following primary MMR vaccination. A second dose was necessary to raise the level of protection against measles and rubella.

Keywords: Immunogenicity, measles, mumps, MMR vaccine, rubella

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Introduction

Measles remains a serious, fatal disease in the developing world with estimated case fatality rates that range between 5% – 30% of children in different areas of the world.[1,2]

According to figures published by the WHO, there were approximately 30 million cases of measles that have resulted in 745000 deaths in children younger than 15 years of age in 2001; most from countries with suboptimal immunization programs.[3] Measles vaccine, on its own or in combination with mumps and rubella, is highly effective and has resulted in a 78% worldwide decline in deaths from measles between 2000 and 2008.[1–3]

It has been estimated that even in countries where vaccination coverage approaches > 80%, a single dose of the vaccine leaves many children susceptible to measles. To eradicate measles and thus prevent mortality and morbidity associated with this highly infectious disease, the WHO recommends universal immunization of all children with two doses of the measles vaccine.[1–5] Since, with the successful implementation of the recommended vaccination schedule, natural infection with measles will gradually decrease and eventually disappear; the two-dose strategy aims to confer life-long immunity against measles in addition to improving vaccination coverage and providing herd immunity. Regional elimination of measles depends on the fulfillment of specific conditions, including vaccination coverage of at least 95% for the primary dose and at least 80% for the second dose.[6]

The WHO recommends that in order to eliminate congenital rubella syndrome and to prevent the complications associated with mumps, countries should use the measles, mumps and rubella (MMR) vaccine in a two-dose schedule for routine childhood immunization programs.[6,7]

Since 2004 the MMR vaccine, a live attenuated virus vaccine, has been included in the routine immunization program for children in Iran. All children are vaccinated with MMR at 12 months of age and receive a second dose at 18 months.

Although the measles vaccine provides a high degree of protection, outbreaks have been reported in vaccinated children. These outbreaks have been attributed to multiple factors which may include faulty vaccine storage and prolonged exposure to light, in addition to host factors such as inactivation of the vaccine virus by high levels of maternal antibodies.[8,9]

In view of the above reasons and in order to ensure that children

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and adults are protected against measles, ongoing surveillance in the vaccinated population is necessary. Detection of serological status by measuring antibody levels after receiving the vaccine provides objective data about vaccine efficacy and can define the need and timing for revaccination.

We performed this study to determine the efficacy of the MMR vaccine by testing IgM and IgG antibody levels at 4 – 7 weeks after first vaccine dose was administered to children who visited health centers affiliated with Shahid Beheshti University of Medical Sciences in Tehran.

Materials and Methods

Toddlers between the ages of 12 – 15 months who visited four health centers affiliated with Shahid Beheshti University of Medical Sciences, Tehran, Iran for routine immunization were selected for this study over a four month period from January until April 2009. The study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences.

Healthy children with no past history of MMR, epilepsy or other central nervous system disorder were included in the study. Children with acute febrile illness at the time of vaccination, immunocompromised children and those who had received blood products or immunoglobulins during the three to eleven months befor recruitment depending on the kinds and the doses of products were excluded.

After obtaining informed written consent from their parents, the children were vaccinated with the live attenuated MMR vaccine (Razi Institute, Tehran, Iran) which contained the AIK- HDC strain for measles, rubella vaccine Takahashi strain, and Hochino mumps strain. Vaccines were dispensed in multi-dose vials, (10 doses/vial), stored at 2 – 8ºC and reconstituted before vaccination. Reconstituted, unused vaccines were discarded within six hours.

Between 4 – 7 weeks post-immunization, blood samples were collected from the vaccinated children and antibody titers against MMR were checked by enzyme linked immunosorbent assay (ELISA) in the Pediatric Infections Research Center of Mofid Children's Hospital, Tehran. An Elisa kit manufactured by Dade Behring (Germany) was used for determination of measles and rubella antibody titers, and a mumps virus antibody ELISA kit (IBL, Hamburg; Lots MUM 181 and MUG 171) was utilized for evaluation of mumps antibody levels. All demographic and laboratory data were meticulously documented by trained members of the study team. Sample size was calculated assuming 80% seropositivity with precision of 6% under the 95% confidence interval (CI) according to the following formula**:**

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$$

Where $Z_{1-\alpha/2}$ is the upper (α/2)th quintile of the standard normal distribution, P is pre-assumed prevalence rate and d denoted maximum error. A sample size of 171 recruits was calculated. To compensate for a 10% dropout rate, we needed a total of 190 specimens. Samples were collected from 240 infants. Consecutive infants who reported for vaccinations were included in the study until the required sample size was attained.

Statistical analysis

Seropositivity was defined as the level of antibody higher than the assay cutoff, as specified by the ELISA kits. Equivocal titers were assigned to negative or positive groups by logistic regression. Geometric mean titers (GMTs) were calculated along with the 95% CI by calculating the anti-log of the conventional mean and 95% CI of the log-transformed data.

Results

We included 240 children between the ages of 12 and 15.7 months (mean 13.27 ± 0.43 months) in the study, of which 105 (55.3%) were females. All 240 samples were checked for measles and rubella antibodies, while 190 samples were checked for mumps antibody levels. After 4 – 7 weeks the seropositivity rates were as follows.

As measured by the Dade Behring Kit, the measles serum IgM level was higher than the assay cut-off in 71.7% of samples and IgG was higher in 75.8%. Also according to this kit, the levels of rubella serum IgM was higher in 71.7% and IgG in 73.8% of children. Using the IBL Kit, the mumps serum IgM level was positive in 68.9% and IgG level was positive in 95.3% of our vaccine recipients. No significant relationship was found between seropositivity and age or gender. With regards to geometric mean titers, all titers were within the detectable range. There was no need to substitute or omit any data to calculate the geometric mean (Table 1).

Discussion

In our study between 71% – 75% of children became seropositive for measles and rubella antibody, and more than 95% for mumps within 4 – 7 weeks post-immunization.

In the study performed by Redd et al. on children vaccinated with MMR the seroconversion rates for measles and rubella were approximately 87% for children vaccinated at 9 months of age, 95% for those vaccinated at 12 months, and 98% for those who received the vaccination at 15 months of age. However the response to mumps did not vary among different age groups.[10] In another study from Saudi Arabia, seropositivity rates for measles IgG antibody measured by ELISA before and one month after primary vaccination with MMR changed from 3.5% to100%.[11] In a sample of Singaporean children who received the MMR vaccination between 12 – 18 months of age, seroconversion rates for measles and rubella were reported to be 100% and 98% for mumps at 42 days post-vaccination.[6] Saffar et al. found the seroconversion rate for measles to be 90.5% but only 53% for rubella 4 – 8 weeks after primary vaccination with MMR in 12-month-old sero-negative infants.[12] In another report from Iran, an IgG assay was used to test for immunity against rubella before and after a mass campaign for measles-rubella vaccination in which 33 million doses of the measles-rubella vaccine were administered to individuals 5 – 25 years of age, nationwide. Results indicated that about 62% of 1940 vaccinees tested for the presence of antibodies against rubella were immune before vaccination and out of the 38% non-immune individuals, 98% developed antibodies after vaccination.[7] A similar study carried out in Northern Iran to check the immune response to the measles vaccine one year after the mass vaccination program found a rise in the seropositivity rate from 53% to 72.3% following the campaign (Table 2).[13]

Although all our subjects had detectable antibodies against MMR,, levels of IgG and IgM antibodies against measles and rubella were lower compared to some of the above mentioned stud-

Table 1. Geometric mean titers and 95% CI of IgM and IgG antibody levels 4-7 weeks after primary MMR vaccination in healthy children.

	Weeks 4-5 (n=131)	Weeks 5–6 (n=82)	Weeks 6-7 (n=24)
Measles			
IgM	0.14 (0.02–1.24)	0.16 (0.02–1.24)	0.22 (0.08–0.6)
IgG	0.15 (0.02–1.22)	0.17 (0.03–1.05)	0.18 (0.03–1.04)
Rubella			
IgM	0.13 (0.02–1.16)	0.17 (0.02–1.29)	0.20 (0.05–0.87)
IgG	0.20 (0.03–1.31)	0.21 (0.02–2.08)	0.19 (0.03–1.33)
Mumps			
IgM	24.99 (0.78–803.29)	16.09 (0.35–746.48)	8.25 (0.15–441.7)
IgG	88.77 (13.35–590.1)	81.51 (8.31–799.75)	109.35 (35.53–336.59)

Table 2. Comparison of IgM and IgG titers in the serum of subjects after measles, mumps and rubella (MMR) vaccination.

NO	Author name	Year of study	Sample size	Age	Method	Measles (%)		Mumps (%)		Rubella (%)	
						IgG	IgM	IgG	IgM	IgG	IgM
1	Redd et al.	2004	990	9 months 12 months 15 months	Indirect EIA*	87 95 98	---	92 89 93	---	91 94 96	---
2	Khalil et al.	2008	57	12 months	ELISA**	100	---	_	---	---	---
3	Lim et al.	2007	150	18 months–12	Immunoassay	100	---	98	---	100	---
4	Saffar et al.	2009	112	12 months	ELISA	90	---	---	---	53	---
5	Hamkar et al.	2006	1940	25 years–5	IgG avidity assay	98	---	---	---	---	---
6	Yekta et al.	2009	625	25 years–5	ELISA	72	---	---	---	---	---
7	Helfund et al.	1997	536	15 months	IgM capture EIA IgG indirect EIA	99.4	73	---	---	---	---
8	Helfund et al.	1998	209	9 months	EIA	85	79	---	---	---	---
9	Lumbiganon et al.	1998	223	9 months	HIA***	100–94	---	---	---	---	---
10	Kanbour et al.	2005	440	16 years–9	ELISA	74	---	---	---	---	---
11	Jaber et al.	2006	527	11 years–4	ELISA	71	---	65	_	90	---
12	Lee et al.	2006	Group I (n=116) Group II (n=127) Group III (n= 277)	15 years–0	EIA	Group I: ND Group II: 87 Group III: 82	---	---	---	---	---
13	Isik et al.	2003	116	9 and15 months	---	87	---	---	---	---	---
14	Tabatabaei et al.	2009	240	12 and 15 months	ELISA	75	71	95	69	73	**71**

* Enzyme immunosorbent assay; ** Enzyme linked immunosorbent assay; *** Hemagglutination inhibition antibody; ****Not determined

ies; this could be due to the fact that we measured both IgM and IgG antibody levels between 4 – 7 weeks post-vaccination. This finding was similar to a study by Helfand et al. where it was observed that the rate of IgM positivity (about 73%) at 4 weeks after measles vaccination was lower than expected.[14,15] In their study only 2% of those vaccinated had measles-specific IgM during the first week after receiving the first dose of the measles vaccine; the levels reached a peak by 3 weeks and began declining by 4 weeks, thus there was a narrow window of time for detectable IgM antibody. The authors have surmised that possibly IgM begins to decline before 4 weeks post-vaccination. In their study, rate of IgG antibody positivity was 14% at 2 weeks, 81% at 3 weeks and 85% at 4 weeks. As we measured IgG between 4 – 7 weeks, and the largest numbers of our cases were tested at 4 weeks, we do not know how many of our vaccinees would have later developed IgG antibody. If we had measured IgM levels between 3 – 4 weeks and IgG levels between 6 – 8 weeks after vaccination, the seropositivity rates might have been higher than our present figures. As such, our rates for seropositivity against measles and rubella are consistent with the expected seroconversion rates in infants from developing countries who are vaccinated before one year of age.[14]

Another reason for the low levels in our study could be that all individuals between 5 – 25 years of age were given the measles/rubella vaccination in a mass national campaign about four years before our study; there was a high likelihood that high circulating levels of maternal antibody interfered with the efficacy of the measles/rubella vaccine. Furthermore, we used multi-dose vials in which the vaccines were reconstituted when the first recipient was vaccinated. As a result, the reconstituted vaccine was kept for a few hours until all ten doses were administered, which might have resulted in a breach of the cold chain and loss of potency.

Some studies have claimed that timing of the first MMR vaccination if administered at 9 months of age or after the first birthday (between 12 – 15 months) does not affect the efficacy of the vaccine. Most authorities including the CDC and WHO recommend that in countries with widespread vaccine coverage and where

measles is not seen in the first year of life, the first dose should be deferred until 12 months of age in order to achieve a robust antibody response.[1–7,16–18]

The MMR vaccine induces long-term immunity in a majority of vaccinees; however, some vaccine recipients remain seronegative after a single dose, the rates of seropositivity may decline with age and outbreaks have been reported after a single dose, particularly in older children.[19,20]

The updated immunization schedule for MMR in Iran recommends that the first dose be administered at 12 months and the second dose at 18 months of age. The CDC schedule for routine childhood immunization states that the second dose should routinely be given before school at 4 – 6 years of age; however the center stipulates that the second MMR dose can be given before age 4 provided at least 28 days have elapsed since the first dose.[4] In Australia, the second dose of MMR has been changed from 4 years to 18 months according to the updated immunization schedule.[21] A study in Turkey has determinedthat seroconversion rates in healthy infants after the first dose of measles at 9 months of age rose from 77.6% to 81.9% after the second dose. The authors concluded that the two dose schedule caused a significant increase in seropositivity against measles.[22]

In our findings almost 25% of 12-month-old children had IgM and IgG antibody levels below the protective levels for measles and rubella at 4 – 7 weeks after primary vaccination with MMR. Thus, we have concluded that it is necessary to give the second MMR dose soon after the first dose and preferably not later than 18 months of age. Furthermore, to ensure seroconversion, it is important to conduct large scale studies to check antibody levels before and after the second vaccine dose. Further studies are required to determine if a third dose would be needed at school entry or later. Since vaccines should be reconstituted just before administration to the recipient and keeping reconstituted vaccine under suboptimal conditions results in variable loss of potency, it is essential to provide an ample supply of single-dose vials for health care clinics.

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