

Original Research Article

Determination of ELISA reactive mumps IgG antibodies in MMR vaccine recipients in comparison with MMR vaccine naïve children: a cross sectional study

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ABSTRACT

Background: Mumps is a vaccine preventable infectious disease characterized by parotitis. In India mumps vaccines are not currently used under NIP. Waning of vaccine-induced immunity is considered to play a central role in the reemergence of mumps. The comprehensive data on the seroepidemiology of measles, mumps, and rubella (MMR) as well as studies which compare the antibody titer among mumps vaccine naïve with mumps vaccinated children are lacking. The aim of our study is to estimate and compare Mumps specific antibody titer in children with and without MMR vaccine. Current study is a cross sectional observational study.

Methods: In 2019/2020, blood samples were collected from 100 healthy children attending immunization clinic in government medical college Kota and associated J. K. Lon maternal and child care hospital Kota. The samples were investigated for MMR IgG antibodies using ELISA.

Results: Out of total 100 children included in the study 32.27% vaccinated and 4.83% Non-vaccinated were positive for Mumps IgG antibody in the age group of 6m to 6 year. While in the age group of 6year to 12 year there was 31.57% and 26.57% positivity respectively. The seroprevalence of measles, mumps and rubella antibodies among 50 MMR vaccinated children was 94%, 64%, and 96% respectively. A high measles and rubella seroprevalence was observed among all children age groups, suggesting an effective control program, while the mumps seroprevalence decreased significantly with age.

Conclusions: The maximum vaccine effectiveness against mumps for 2 doses of MMR vaccine is $\approx 96\%$ therefore the herd immunity threshold to block mumps virus transmission is $\geq 86\%$. As per our study only 64% of the vaccinated children were found to have IgG Mumps Ab. In view of morbidity following mumps infection there is a need to incorporate mumps vaccine along with measles and rubella vaccine in the NIP instead of MR.

Keywords: Antibody, Measles, Mumps, Vaccination

INTRODUCTION

Mumps is a contagious disease characterized by parotitis. Although perceived as a benign childhood disease, mumps was the leading cause of viral meningitis and encephalitis, as well as permanent deafness, in children before the widespread vaccination.¹ It is a vaccine-

preventable disease that is endemic in most parts of the world.² The burden of mumps remains high (100-1000 cases/100000 population) in countries that don't supply routine mumps vaccination, with epidemic peaks each 2-5 years.^{3,4} However, mumps disease outbreak have occurred even in countries using mumps vaccination in their national immunization programs (NIPs).⁵⁻⁷

According to WHO, Southeast Asia region (SEAR) reported 36,352 cases of Mumps in 2013, however there's no information on the cases reported from India.⁸

Mumps, despite being a widely prevalent disease everywhere in the country, is taken into account as associate degree insignificant public ill health in India, primarily due to poor documentation of clinical cases and lack of published studies. There's no across the nation representative knowledge on incidence of the illness.⁹ Although the illness is sometimes mild but its burden shouldn't be underestimated. Up to 10% of Mumps patients developed aseptic meningitis; a less common however more serious complication is encephalitis, which might end in death or disability; and permanent deafness, orchitis and pancreatitis are other untoward effects that may be prevented by vaccination.¹⁰ There's no specific treatment and vaccination is the solely effective way to forestall the disease.¹¹

Measles is the most common cause of vaccine-preventable death in children, and is that the eighth leading reason of death worldwide. Nationwide Measles-Rubella vaccination was introduced in 2017.¹² Measles cases in India have dropped considerably by banging 43% between 2015 and 2016 for the first time as India builds on its acute anterior poliomyelitis obliteration campaign experience to eliminate measles by 2020. By 2018, India planned to achieve 40 crore children with the measles vaccine, that is predicted to significantly scale back the illness burden and facilitate India meet its 2020 elimination target.¹³ Prior to introduction of vaccination program, Rubella was endemic worldwide. Epidemic still occur in developing and tropical countries each four to seven years, however the lack of monitoring programs, along with the absence of severe clinical symptoms, has created them troublesome to assess.¹⁴

Vaccination

The Mumps vaccine was first licensed in the U.S. in 1967. Since the initiation of the Mumps vaccine, reported cases of Mumps have decreased by 99% from 185,691 cases in 1968 to only 906 cases in 1995.¹¹

Mumps vaccination has been incorporated into the regular vaccination schedule of the many countries, usually with measles and rubella vaccines in triple formulation (MMR) at nine months, fifteen months, and four to six years, or as 2 doses at twelve to fifteen months with the second dose between four to six years of age.¹⁵⁻¹⁷ These vaccines have enabled the WHO to ascertain worldwide strategies for the advanced management of measles and rubella resulting in an elimination target in some regions. However, in contrast to rubella and measles, secondary vaccine failure happens oftentimes within the case of Mumps and circulation of Mumps virus inside highly immunized populations has been frequently reported.¹⁶

There are lower clinical attack rates among immunized populations; however epidemics occur much within the second and third decade usually with severe complications.¹⁸ The monovalent measles vaccine is not offered for normal use. Measles-containing immunizing agent (MMR/MR) ought to be administered once nine months getting on (270 days) under NIP of India.

Rubella vaccine is usually given together with measles and Mumps as MMR vaccine between twelve and fifteen months getting on, with a succeeding booster shot before school entry or in adolescence.^{19,20} Rubella vaccination ought to be given to all women of child bearing age found to possess low or undetectable antibody titer, preferably one month before conception or postnatal. Immunization during pregnancy should be avoided because of risk of CRS.²¹

Government of India has recently introduced some modifications within the NIP supporting the recommendation of National technical advisory group on immunization (NTAGI) where four new immunizing agents are introduced and recommended conversion of MMR vaccine to MR vaccine (measles-rubella).²² As a result of it, it is felt that the disease burden of Mumps isn't much significant in India. Only a few studies are conducted to search out the seroprevalence of antibodies against mumps in Indian children. Indian academy of pediatrics (IAP), however, recommends the continuation of this vaccine since incidence of mumps remains high in Indian children. During this study we shall try to verify the antibody level against mumps in 2 groups of children attending immunization clinic at Government medical college Kota and associated groups of hospitals, J. K. Lon maternal and child care hospital, a tertiary care hospital in Kota.

Aims and objectives

Aim of current study was comparison of mumps specific antibody titer in children with and without MMR vaccine. Objectives of current study were; estimation of mumps specific IgG antibody in children, MMR naïve and MMR vaccinated and comparison of mumps specific IgG antibody level in above children after quantitative estimation.

METHODS

The study was conducted in the department of microbiology, Government medical college, Kota and department of pediatrics of associated J K Lon maternal and child care hospital, Kota, Rajasthan. It was a Cross sectional observational study lasted from May 2019 to May 2020 for a period of 1 year in which 100 children between age group of six months to twelve years were investigated. They were divided into two groups. Fifty MMR vaccine naïve and fifty MMR vaccinated children between six months to twelve years of age. Immuno-compromised children, children presenting with acute

febrile illness, children who have received blood products or immunoglobulins 3-11 months back, children above 12 years of age were excluded from study. IgG antibodies against measles, mumps, and rubella were measured in the sera by enzyme-linked immunosorbent assay (ELISA) using specific commercially available kits (Diesse Enzywell, Italy). For each test run, control and standard sera were included independent of the number of microtest strips used. The standard sera were set up in duplicate. All tests were performed and interpreted as per kit manufacturer's instructions.

The assay utilizes the microtiter plate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter well. The purified and inactivated mumps/measles virus antigen is bound to the solid phase (8-well strip). The specific immunoglobulins are bound to the antigen through incubation with diluted human serum. After washing, incubation is performed with the conjugate, composed of human IgG monoclonal antibodies labelled with peroxidase enzyme which is detected colorimetrically by adding an enzyme substrate. The optical density of the resulting dye complex was read spectrophotometrically at single wavelength of 450 nm. The colour which develops is proportional to the concentration of specific antibodies present in the serum sample. The average absorbance for each pair of duplicate test results was calculated.

Statistical analysis

The continuous data was described as mean and standard deviation, while discrete data was described as proportions. The association between qualitative variables was measured chi-squared test. The level of significance was considered at 5% ($\alpha=0.05$). The Matlab 2016 and JASP 0.11.1.0 statistical package were used for statistical analysis.

Table 2: Gender wise positivity of IgG Mumps antibody in MMR vaccinated and non-vaccinated children.

MMR vaccination	Male		Total	Female		Total
	Positive N (%)	Negative N (%)		Positive N (%)	Negative N (%)	
Vaccinated	17 (62.96)	10 (37.04)	27	15 (65.22)	8 (34.78)	23
Non-vaccinated	9 (30)	21 (70)	30	4 (20)	16 (80)	20
Total	26	31	57	19	24	43

This data showed that there was significant association between MMR vaccination and Mumps IgG antibody status ($p<0.001$). There was 32.27% and 4.83% positivity in vaccinated and Non-vaccinated children respectively in the age group of 6M to 6years. While in the age group of 6 years to 12 years there was 31.57% and 26.57% positivity respectively. This data showed that there was significant association between MMR vaccination and antibody status in 6 months to 6 years age group ($p<0.001$) but in 6 year to 12 year age group ($p = 0.05$) was not significant as shown in (Figure 1).

RESULTS

Among the 100 children included in the study mean age was 6.7 years (SD=3.7 years) with male to female ratio of 57: 43. Most of the children were from urban area (86%) and literate families (85%). Among all the children percentage of children vaccinated with MMR and MR vaccine was 50% and 78% respectively.

Out of 50 vaccinated children 68% (N=34) were from 6m-6year and 32% (N=16) were from 6 year-12 year age group. While among Non-vaccinated children 56% (N=28) and 44% (N=22) were from 6 months to 6 years and 6 years to 12 years age group respectively as shown in (Table 1). Age wise distribution showed significant association between MMR vaccination and antibody status in 6 months to 6 years age group ($p< 0.001$) but association in 6 year to 12 year age group ($p = 0.05$) was less significant.

In this study 62.96% (N=17) males and 65.22% (N=15) females were positive for Mumps IgG antibody among vaccinated children. While among Non-vaccinated Children only 30% (N=9) males and 20% (N=4) females were positive for IgG mumps antibody shown in (Table 2).

Table 1: Age wise distribution of MMR vaccinated and naïve children.

MMR vaccination	6 m to 6 yr N (%)	6 yr to 12 yr N (%)	Total
Vaccinated	34 (68)	16 (32)	50
Non-vaccinated	28 (56)	22 (44)	50
Total	62	38	100

In MMR non-vaccinated children total positivity for IgG Mumps antibody was 26% (N=13) while 74% (N=37) were negative for all the age groups and gender. This data showed insignificant seropositivity among non-vaccinated group. Out of 50 vaccinated Children included in the study 100% (N=19) males and 93.33% (N=14) females were positive for Measles IgG antibody in the age group of 6 months to 6 years. While among 6 years to 12 years age group 100% (N=8) males and 75% (N=6) females were positive for IgG. Measles antibody, data showed there was significant association between MMR

vaccination and measles antibody status in all the age group irrespective of gender.

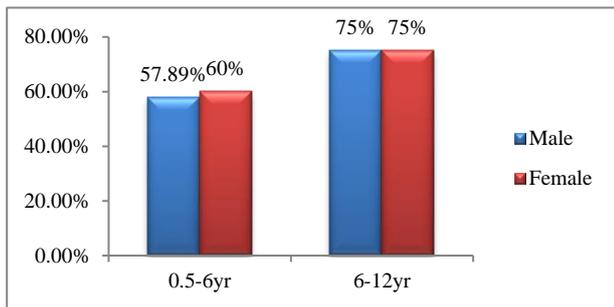


Figure 1: Age wise positivity of IgG mumps antibody in MMR vaccinated and non-vaccinated children.

Out of 50 vaccinated Children included in the study 89.47% (N=17) males and 80% (N=12) females were positive for Rubella IgG antibody in the age group of 6m to 6 year. While among 6year to 12 year age group 100% (N=8) males and 75% (N=6) females were positive for IgG rubella antibody showing significant association between MMR vaccination and rubella antibody status in all the age group irrespective of gender. Total 78% children were MR vaccinated among all the children. Among 50 MMR vaccinated children 90% (N=45) also received MR vaccination. While among Non-vaccinated children only 66% (N=33) received MR vaccination as shown in (Table 3). This represents that rate of MR vaccination is high in country to that of MMR vaccination. As in the current schedule of NIP, MMR vaccination is not recommended while private institutions give only MMR vaccines.

Table 3: Percentage distribution of MR vaccination among MMR vaccinated and MMR non-vaccinated children.

MMR vaccination	MR vaccinated N (%)	MR non-vaccinated N (%)	Total
Vaccinated	45 (90)	5 (10)	50
Non-vaccinated	33 (66)	17 (34)	50
Total	78	22	100

DISCUSSION

The use of vaccine in developed countries successfully reduced Mumps virus infection though outbreaks are often reported. But in developing countries, due to absence of effective vaccination due to various reasons such as illiteracy, poor reach to health care services, poor economy, Mumps is still a major health issue. The burden of disease in India has still not been clearly defined because of very few studies over it. The present study represents the current scenario of MMR vaccination in our country.

As per WHO, MMR vaccine has been included in National Immunization Program of 121 countries.²² In India it is not a part of NIP. Recently measles and rubella elimination program has been launched by WHO in India which targets 95% coverage of measles immunization in community with 2 doses of MR vaccine given 1 dose at 9-12 months and 2nd dose at 16-24 months of age.²³ Mumps is not a part of this schedule as it is believed that Mumps is a disease of moderate morbidity and mortality in Indian setup.⁹ Numerous studies both from India and other countries have reported many outbreaks of Mumps in children and young adults both unvaccinated and vaccinated mostly occurring in age group of 5-10 years.²⁴ In a prospective study conducted at Finland, where Mumps is almost eradicated, antibodytiter of measles, mumps and rubella vanished within 20 years but antibody titers of mumps decreased the most, suggesting poor immunogenicity of the vaccine.²⁵

In the present study among the 100 children 50% were MMR vaccinated and 50% were not. Out of 50 vaccinated children 68% (N=34) were from 6 months to 6 years and 32% (N=16) were from 6 years to 12 years age group. While among Non-vaccinated children 56% (N=28) and 44% (N=22) were from 6 months to 6 years and 6 years to 12 years age group respectively. Similarly in a study done by Wang et al 42.25% children were from <6 years and 57.75% were from 6-12 years age group.²⁶ In contrast to a prospective study done by Rout et al 33.33% <6 years were included in the study in compare to 61.11% from 6-12years age group.²⁷

64% (N=32) of the 50 vaccinated children were found to have IgG Mumps antibody. Out of these positive maximum children 62.5% (N=20) belong to age group of 6m-6year. This denotes that antibody response declines with increasing age and booster vaccination is required to maintain immunity against Mumps. Also it can be due to low seroconversion rate of Mumps antibody leading to low immunity. Exact time of vaccination cannot be estimated in India as in India most people belong to rural population and does not maintain records. For prevention of a disease outbreak of Mumps at least 90% of vaccine coverage of the population should be present to maintain and effective herd immunity as supported by previous studies.²⁸ In our study only 64% of vaccinated population had immunity against Mumps which is inadequate to prevent an outbreak. Similarly in a study done by Wang et al among all positive children 70.39% belong to <6year Age group for IgG Mumps antibody. In contrast to study done by Adam et al 63.6% were positive for IgG mumps antibody, 34.01% of them belong to <6 years age group and 65.99% belong to >6 years age group.^{26,29} In their study the mumps and rubella seroprevalence increased significantly with age documenting active wild type circulation.

Among the 50 nonvaccinated children, 26% (N=13) of children had presence of IgG mumps antibodies. It was seen that children aged 6-12 years had higher IgG mumps

seroconversion as compared to <6 years. This could be possibly due to prior exposure to a case of mumps or boosting of natural immunity with age. These results may be an overstatement of the current scenario within the community since the study was conducted on a limited number of participants due to various constraints; also the distribution of participants according to their age and gender was not uniform in each age group. Also it can be due to more chances of accounting an infection in later ages so mumps vaccination is necessary for these children.

Studies from other countries where two doses of MMR vaccination are recommended have reported seroconversion rate of 63-95% after 2nd dose.³⁰ Low seroconversion rate are also expected in communities reporting mumps outbreaks. Among the 50 MMR vaccinated children 94% (N=47) were positive for IgG Measles antibodies. This shows high seroconversion rate of measles among the vaccinated individuals. Similarly, in study done by Adam et al and Wang et al among the MMR vaccinated children 93.5% and 95% respectively were positive for IgG Measles antibodies.^{26,29}

In the present study 96% (N=48) out of 50 MMR vaccinated children were positive for IgG rubella antibody, 60.42% (N=29) of which are from 6 months to 6 years of age while rest 39.58% (N=11) are from >6 years of age group. Similarly a study done by Poethko-Muller et al 75% were from 6m-6year of age. In contrast a study done by Adam et al only 17% were from 6m-6year of age. This data showed that the seroconversion for Rubella is also high among MMR vaccinated children.

Out of 50 MMR vaccinated children only 64% have IgG Mumps Ab in compare to 94% IgG Measles and 96% IgG positivity of rubella. Overall Mumps seroconversion is poor due to 1st dose of vaccine, so another booster of MMR is required to complete coverage of Mumps and prevent infection. In present study, total 78% children were MR vaccinated among all the children. Among 50 MMR vaccinated children 90% (N=45) also received MR vaccination. While among Non-vaccinated children only 66% (N=33) received MR vaccination. This represents that rate of MR vaccination is high in country to that of MMR vaccination as MR (measles-rubella) vaccine is currently not available in the private sector in India. Hence in view of morbidity following mumps infection, it has been recommended by IAP that MMR is administered instead of MR. Irrespective of previous vaccination status, additional dose of MR vaccine during MR campaign for children 9 months to 15 years, is to be administered, keeping in mind the need to support national programs.³²

In view of these results the decision of government of India and NTAGI on replacing MMR with MR vaccine may require reconsideration since mumps rivals measles and rubella in its ability to cause outbreak and possible congenital malformations in children. Removing mumps

vaccine from states with <70% of MMR vaccine coverage can also lead to potential outbreak in future once MR vaccine completely replaces MMR vaccine. The results of this study were in concordance with previous reports that a single dose is not sufficient to prevent clinical mumps and natural immunity in Indian children is not sufficient enough to offer protection upon exposure to the agent. Also poor efficacy in vaccinated children causes a rightward shift in the epidemiology and affects older age group, children age young adults. This widens the clinical severity of disease. Blanket withdrawal of mumps component of MMR cannot be a final decision without a strong backup of long term epidemiological data.

CONCLUSION

The maximum vaccine effectiveness against mumps for 2 doses of MMR vaccine is ≈96% therefore the herd immunity threshold to block mumps virus transmission is ≥86%. As per our study only 64% of the vaccinated children were found to have IgG mumps Ab. This concludes firstly low seroconversion rate of mumps antibody leading to low immunity and secondly the antibody response declines with increasing age and booster vaccination is required to maintain immunity against Mumps. We concluded high seroconversion rate of measles and rubella among vaccinated group. Hence in view of morbidity following mumps infection, the results highlight the need to incorporate mumps vaccine along with measles and rubella vaccine in the NIP instead of MR. Irrespective of previous vaccination status, additional dose of MR vaccine during MR campaign for children 9 months to 15 years, is to be administered, keeping in mind the need to support national programs. By preventing measles, rubella and mumps together we produce significant savings for our country and communities.

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