Comparing Seroprotective Levels of IgG Antibody Against Single and Two Shots of Measles-Rubella-Mumps (MMR) Vaccine in Children Aged 12 Months to 15 Years Old in Southern Iran, Ahvaz in 2018

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Abstract: *Background and Objective*: Despite inclusive measles and rubella vaccination programs, the epidemics are still present in some areas. The present study aimed to evaluate the efficacy of these two vaccines simultaneously by testing the levels of IgG antibodies in children aged 12 months to 15 years old in Southern Iran.

Methods: This was a cross-sectional study on serum blood samples of 400 children aged 12 months to 15 years old in Ahvaz, Southern Iran in 2018. The children were divided into the following age groups: age group of 12 months to 17 months and 29 days who received a single dose of Measles, Rubella, Mumps (MMR) vaccine; age group of 18 months to 10 years old who received two doses of MMR vaccine at the ages of 12 months and 18 months; and age group of 11 to 15 years old who received two doses of MMR vaccine at the age of 12 months and 4-6 years old. In the assessments, the protective levels were defined as per the guidelines of the ELISA kit (VIRCELL Co., Spain) as follows: The serum IgG antibody levels above 11 IU/ml were defined as positive protective level, 9 to 11 IU/ml as an equivalent state, and the serum antibody level below 9 IU/ml as a negative state. Accordingly, the equivalent and negative cases were reported as negative and cases above 11 IU/ml were positive.

Results: The male to female ratio was 1.02. Most of the patients (69%) were in the age group 18 months to 10 years old. In all age groups, the number of people with a high serum anti-rubella and anti-measles IgG antibody level was higher than the protective level. There was no significant difference between the mean serum anti-rubella and anti-measles IgG antibody level between the age groups (P= 0.515, P= 0.176, respectively). For rubella, 86.66% of females and 83.16% of males, and for measles, 86.36% of females and 83.66% of males were seropositive, and there was no significant difference by two sexes (P= 0.486 and P= 0.412, respectively).

Conclusions: The frequency of seropositive anti-measles and anti-rubella vaccine was not significantly different between the subjects with a single dose and those with two doses of MMR vaccine. Further studies are needed to evaluate the adequacy of the MMR vaccine for Iranian children. Moreover, due to the lower average serum IgG antibody level measles and rubella in males, further investigation is needed to find out the causal relationship between the lack of appropriate response to vaccine injection and male gender.

Keywords: Rubella, Measles, IgG, Vaccination, Serum Level.

INTRODUCTION

Measles and rubella are common infectious diseases caused by viruses from the Paramyxoviridae and Togaviridae family that induce classic childhood exanthem and respiratory infections [1-3]. Most deaths due to acute measles are due to secondary infections resulting from the suppression of immune responses [4-6]. After the early days of birth, effective vaccination coverage is a major contributor to the prevention of these infectious diseases. In different countries, age recommendations for the first dose of Measles, Rubella, Mumps (MMR) vaccination are 9 to 18 months old based on respective national programs. The first MMR vaccination should be done when the child's immune system is mature enough to effectively respond to the vaccine, and when maternal antibodies decrease and don't interfere with the vaccine response [7-9]. The World Health Organization recommends the first dose of measles, rubella, and mumps vaccine (MMR) in measles endemic countries for 9 months old children, in countries with less measles transmission for 12-month-olds [5, 10, 11]. Although the measles vaccine provides high levels of protection, the outbreak has continued to be reported in vaccinated children, and it continues to lead to cases of infection and mortality, especially in developing countries [12, 13]. These outbreaks are due to several factors such as

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E-ISSN: 2292-2598/19

improper storage of the vaccine and prolonged exposure to light, as well as host factors such as the inactivation of the vaccine virus by high levels of maternal antibodies [14]. Based on studies conducted in different parts of the world and different epidemiological conditions, immunity to measles varies, and on average, the community's safety level against this disease is reported to be 66% to 100% [15]. Due to the small epidemics of measles, rubella and mumps in vaccinated individuals, and to ensure the protection of children and adults against measles, the continuous monitoring of vaccinated individuals, especially a few years after receiving the last dose of vaccine is necessary [4]. Limited studies have been carried out to determine the antibody after Measles and Rubella vaccinations simultaneously and most studies on measles immunization have been performed in the early months after vaccination. Therefore, the present study was aimed to evaluate the efficacy of the vaccine by testing the level of IgG antibodies in children aged between 12 months and 15 years old in Ahvaz. This age group was chosen because there is enough time after vaccination to create immunity against the vaccine by the immune system.

METHODS

All procedures of this study were approved by the local Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethics code: IR.AJUMS.REC.1397.795), which were in complete accordance with the ethical standards and regulations of human studies of the Helsinki declaration (2014). This study was conducted as a cross-sectional study on serum blood samples of children aged between 12 months and 15 years old who were referred to the laboratories in the city of Ahvaz, Khuzestan, Iran during 2018.

After enrolment of the subjects and before the start of the study, researchers clearly explained the experimental procedures, objectives of the study and possible benefits and risks of participating in the study to the parents of children and then the written consent forms were collected from the parents or guardians of all the subjects participated in this study. Total of 400 children, according to a record obtained from their parents were selected and evaluated as a study group, who were vaccinated according to the national protocol, have not had measles and rubella or an immunocompromised disease so far, and did not receive blood or blood products or immunosuppressive drugs. Patients' demographic information including age and gender was recorded in each patient's information form. As Routinely, for the probability of repeating the test cause of laboratory errors, about 2 cc of the blood sample of all patients is going to be stored for a maximum of 72 hours in the laboratory refrigerators at -70 cc temperature.

We examined the collected 2 cc blood sample of the subjects using an ELISA kit (VIRCELL Company, Spain) to determine the level of anti-rubella and anti-measles IgG serum according to the age groups. According to the predefined range of the kit, individuals who had anti-rubella IgG level above 11 IU/ml were defined as positive, those with serum antibody level between 9 to 11 IU/ml as an equivalent state, and those with serum antibody level below 9 IU/ml were considered as a negative state. Since the children in this age range do not receive the same level of the vaccine dose at the first and second intervals, the children were divided into the following age groups: 12 months to 17 months and 29 days who received a single dose of the vaccine, those aged 18 months to 10 years who were given two doses of MMR vaccine at the age of 12 months and 18 months; age group of 11 to 15 years old who received two doses of MMR vaccine at the age of 12 months and 4-6 years old. Those children who received the MMR vaccine in other protocols different than the defined scheme were excluded from the study. The inclusion criteria of the study included children aged 12 months to 15 years and oral parenting consent for participation in the study. Exclusion criteria included a history of measles or rubella or both, the presence of diseases and immune disorders, and receiving blood and blood products, or immunosuppressive drugs or not following national vaccination protocol.

After collecting data, were analyzed using SPSS software version 23. In this study, the results were reported using descriptive statistics including mean, standard deviation, frequency, and frequency percentage. To compare the mean of the variables studied in the three age groups, ANOVA was used and independent T-test was used to compare the independent variables. The significance level was less than 0.05.

RESULTS

The number of females (49.5%) and males (50.5%) were almost equal in this study. The highest number of patients was in the age group of 18 months to 10 years (69%), 11 to 15 years (25.5%), and 12 to 17 months (5.5%), respectively (Table **1**).

Table 1: Patient's Demographic Information

Variables		N	%
Gender	Female	198	49.5
	Male	202	50. 5
Age Range	12 – 17 (months)	22	5.5
	18 (months)–10 (years)	276	69
	11 – 15 (years)	102	25.5

The highest percentage of seropositivity for Rubella vaccine was for the age group of 11 -15 years (92.16%) (receiving two doses of the vaccine with a 6-year interval) and for Measles was for the age group 18(month)-10(year) (85.51%). Also, in all age groups, the number of people with serum IgG antibodies above the protective level was significantly higher than those with serum levels below the protective level, and the highest percentage of subjects with serum levels above the protective level was related to the age group 18 months to 10 years (85.51%) (receiving two doses of the vaccine with a 6-month interval) (Table **2**).

The highest mean serum anti-rubella IgG was in the age group of 11-15 years old (22.9%) (receiving two doses of the vaccine with a 6-year interval) and the age group was 18 months to 10 years (22.88) (receiving two doses of the vaccine with a 6-month interval). The highest mean serum anti-measles IgG was in the age group 12-17 months (22.05) (receiving a single dose of vaccine).

Statistical analysis of different age groups using ANOVA showed that there was no significant difference between the mean level of serum anti-rubella and anti-measles IgG antibodies between the age groups (P = 0.515, P = 0.176, respectively) (Table **3**).

In both sexes, the highest percentages of children with serum anti-rubella IgG antibody level above the protective level were in the age group of 11-15 years old (receiving two doses of the vaccine with a 6-year interval), and the lowest frequency was related to the age group of 12-17 months (receiving a single dose of vaccine). Totally, 86.36% of females and 83.16% of males were seropositive for rubella, and there was no significant difference between the two sexes (P = 0.486).

Among females, the percentage of serum anti-measles IgG levels above the protective level in two age groups of 18 months to 10 years and 11-15 years was approximately equal to and greater than that of the

Table 2: Percentage of People with Higher and Below the Protective Level

Age Range	Serum IgG antibody level (N, %)			
	Rubella		Measles	
	Positive (n=339)	Negative (n=61)	Positive (n=340)	Negative (n=60)
12 – 17 (month)	17 (77.27)	5 (22.73)	18 (81.82)	4 (18.18)
18 (month) – 10 (year)	228 (82.61)	48 (17.39)	236 (85.51)	40 (14.49)
11 – 15 (year)	94 (92.16)	8 (7.84)	86 (84.31)	16 (15.69)

Table 3: Comparison of Mean Serum Anti-Rubella and Anti-Measles IgG Antibodies by Age Group

Age Range	Serum IgG antibody level (Mean ± SD)	
	Rubella	Measles
12 – 17 (month)	20.35 ± 9.89	22.05 ± 10.87
18 (month) – 10 (year)	22.88 ± 10.39	19.06 ± 8.24
11 – 15 (year)	22.90 ± 9.07	18.42 ± 7.73
P-Value	0.515	0.176

Table 4: Comparison of Percentage of Serum Anti-Rubella and Anti-Measles IgG Antibodies Level and Above Protective Level Based on Gender

Gender	Age Range	Serum IgG antibody level (N, %)			
		Rubella		Measles	
		Positive	Negative	Positive	Negative
Female	12 – 17 (month) (n= 12)	10 (83.33)	2 (16.67)	10 (83.33)	2 (16.67)
	18 (month) – 10 (year) (n= 126)	106 (84.13)	20 (15.87)	109 (86.51)	17 (13.49)
	11 – 15 (year) (n= 60)	55 (91.67)	5 (8.33)	52 (86.67)	8 (13.33)
	Total	171 (86.36)	27 (13.63)	171(86.36)	27 (13.63)
Male	12 – 17 (month) (n= 10)	7 (70)	3 (30)	8 (80)	2 (4)
	18 (month) – 10 (year) (n= 150)	122 (81.33)	28 (18.67)	127 (84.67)	23 (15.33)
	11 – 15 (year) (n= 42)	39 (92.86)	3 (7.14)	34 (80.95)	8 (19.05)
	Total	168 (83.16)	34 (16.83)	169 (83.66)	33 (16.33)

age group of 12-17 months. However, among males, the highest percentage of serum anti-measles IgG above the protective level was in the age group of 18 months to 10 years, and the two age groups of 12-17 months and the 11-15 years old had a nearly equal frequency. Also, 86.36% of the females and 83.66% of the males were seropositive against measles, and there was no significant difference between the two groups (P = 0.412) (Table **4**).

The ANOVA showed no significant difference between the mean level of anti-rubella IgG antibody between different age groups (P >0.05). In male patients, although the mean serum anti-rubella IgG antibody in the age group of 12-17 months (receiving a vaccine) was lower than that of the other age groups, there was no significant difference between the mean of different age groups (P >0.05).

Although the mean level of serum anti-measles IgG antibody in the 12-17 months of age group (receiving a single dose of vaccine) in both sexes was higher than that of other age groups, this difference was not significant in the different age groups of male and female patients (P >0.05) (Table **5**).

DISCUSSION

In the present study, 84.8% of children had anti-rubella IgG antibody and 85% had anti-measles IgG antibody levels above the standard positive level defined by the Kit.

In our study, the percentage of seropositivity in children aged 12-17 months receiving a single dose of MMR vaccine was 77.27% for rubella and 81.82% for measles, while in Esteghamati *et al*., who conducted a cohort study on 240 Iranian children aged 12-15 months in 2015, the percentage of seropositive individuals for IgG after receiving a single dose of MMR vaccine was 73.8% for rubella and 75.8% for measles [13]. They concluded that since at least one-quarter of children do not require a protective level after a single dose of the vaccine, there is a need for a second dose of the vaccine, while in our study, the proportion of seronegative people was less than one-quarter of the population studied [13]. In other words, the efficacy of a single vaccination in our study was more likely to be observed, perhaps because of the smaller sample size of our study (22 children) in comparison with 240 children, or it could reflect more attention of Iranian

Table 5: Comparison of Mean Serum Anti-Rubella and Anti-Measles IgG Antibodies by Age Group and Gender

Gender	Age Range	Serum IgG antibody level (Rubella) (Mean ± SD)	P-value	Serum IgG antibody level (4) (Mean ± SD)	P-Value
Female	12 – 17 (month) (n= 12)	23.67 ± 10.91	0.947	22.34 ± 10.52	0.396
	18 (month) – 10 (year) (n= 127)	22.76 ± 9.93		19.01 ± 8.04	
	11 – 15 (year) (n= 60)	23.04 ± 9.63		19.39 ± 7.69	
Male	12 – 17 (month) (n= 10)	16.36 ± 7.09	0.141	21.71 ± 11.85	0.204
	18 (month) – 10 (year) (n= 149)	22.98 ± 10.80		19.10 ± 8.42	
	11 – 15 (year) (n= 42)	22.70 ± 8.30		17.03 ± 7.65	

public health care system on the vaccines' cold chain and proper injection of vaccine and better education of health care providers. Moreover, in recent years, due to the progress of the laboratory and the increasing attention of families and medical centres and paediatricians, the genetic and immunological diseases of children have been diagnosed at an earlier age. Therefore, children with immunodeficiency, who were not likely to respond adequately to vaccination, were more carefully excluded from the study and a more realistic outcome was shown for the effectiveness of receiving a single dose of MMR vaccination.

Our findings showed that there was no significant difference between the mean serum level and the percentage of vaccinated people with an appropriate level of serum anti-rubella and anti-measles IgG antibodies in different age groups who had been vaccinated one or two times. In the study of Tsuji *et al*. (2015) in Japan, 163 children were examined and the seropositivity for rubella (88.2% after single vaccination and 96.6% after a double vaccination) and for measles (5.5% 86% after a single vaccination and 91% after a double vaccination) was reported [16]. They concluded that despite the protective coverage of 95.5% after a single vaccination and 93% after double vaccinations in Japan in 2013, there are still measles and rubella outbreaks in the country. Therefore, they concluded to prevent outbreaks in Japan, an over 95% coverage is needed, and in this population, it is necessary to inject two doses of vaccine, which reduces the seronegative function appropriately [16]. In Iranian society, due to different genetics, administration of one or two doses or more of the vaccine may not have different efficacy, and based on our results patients who did not respond with a single dose vaccination seems that they will not respond in the second turn. However, to prove it, further studies needed.

On the other hand, Parizi *et al.,* in their study of 200 children younger than 3 years in Kerman Iran, observed a significant relationship between a number of vaccines injected and better coverage of measles and rubella vaccination [17]. As the antibody level decreases over time, it seems that the result is more valid in our study due to the wider population and more age dispersion, which results in a longer time interval from the second vaccine.

In this study, there was no significant difference between the mean serum IgG antibodies for females in different age groups. Among the males, although the mean serum anti-rubella IgG in the age group 12-17 months who received a single dose of vaccine was significantly lower than the two other groups receiving vaccine in two times, there was no statistically significant difference between them, which was consistent with the study of Parizi *et al*. This significant difference in the comparison of the mean serum antibodies among children's age groups may be related to genetic issues because genetic diseases are more prevalent in Khuzestan province due to the high prevalence of familial and ethnic marriages. Therefore, the male children, due to having a chromosome X and a chromosome Y, are at greater risk of developing and expressing genetic mutations that are not necessarily categorized as genetic diseases but affect the immune response to the vaccine. Of course, no significant difference statistically has been showed likely due to the small volume of the population studied, which requires further studies.

In the study of Kontio *et al*. (2016) in Finland, 187 children who were 3 years old and received one dose of MMR vaccine at different ages during their lifetime were studied, they observed the serum antibody following the vaccination was lower in males compared with females and also they concluded with increasing age in males, the immune system response to MMR vaccine would improve [9].

In the reported outbreak of measles in Southern Iran in 2012 seven cases of measles were confirmed [18]. Except for a case of 35 years old, whose vaccination status was unknown, all cases have had adherence to vaccination protocol. This study concluded that more immunologic and vaccine quality investigations are necessary to figure out the main cause of the outbreak. Nonetheless, due to the warm weather, probable bad temperature management of cold chain and technical problem in vaccination are the main suspicious reasons for the outbreak. based on results of our study, although in all age ranges, the mean serum anti-measles IgG antibody in males was lower than that of females, there was no significant difference between serum anti-measles IgG antibody levels between different age groups of females and males.

According to these findings, along with the results of the study by Kontio *et al*. (2016) in Finland, which suggested that in males, the response of the antibody after the MMR vaccination would improve with ageing, the need for more examinations on relation of male gender and immune system respond to MMR vaccination is obvious which is going to help to find a

solution to increase the immunity level of this group of people and improve coverage of MMR seropositivity in society. Considering the lack of significant relationship between the number of vaccines and the level of serum antibody after MMR vaccination this question has been mentioned that people who did not respond appropriately after a single dose of the vaccine, may not respond at all, no matter how many times MMR vaccine they have been injected. This is important because decreasing unnecessary doses of vaccination will reduce the economic burden of vaccination on public health care systems, however, to prove this, more studies with higher sample sizes are required. On the other hand, the lower level of serum antibodies anti-measles and anti-rubella after MMR vaccination in males and the last measles epidemic episode of Fars, Southern Ian, in 2012, 4 out of 7 confirmed cases were male, makes it necessary to investigate further studies to find the causality and a solution to improve this reduction in male immune responses [18]. A new approach to reach this objective is increasing the frequency of administering a vaccine for male gender or changing the age of MMR vaccination for male children.

This study had several limitations including small sample size as we selected study group from patients who were referred to the lab and we had no information about the reason of tests their doctors prescribed for them which is maybe interfere with the immune system respond to vaccination, also limited study time and the high price of kits. Finally, considering the mortality of measles and rubella in communities and importance of ensuring the high coverage of MMR vaccination in societies, we suggest to think and design much more studies on this subject and there may be needed to change our approach to MMR vaccination respond just like what is recommended for HBV as checking antibodies after MMR vaccination and revaccination who does not have good respond at routine vaccination protocols.

CONCLUSION

This study was aimed to comparatively assess the seroprotective levels of IgG antibody against single and two shots of MMR vaccine in children aged 12 months to 15 years old in Southern Iran, Ahvaz in 2018. Our findings showed that the seroprevalence of anti-measles and anti-rubella antibody in patients who received two shots of MMR vaccine was not significantly different from those with a single-dose vaccine. This suggests the probability that those who did not respond appropriately after a single dose would probably not respond with further doses. Therefore, further studies are needed to recommend whether a single dose MMR vaccine is sufficient for the Iranian children's population. In the present study, in all age ranges and different vaccination cycles, the mean level of serum anti-measles and anti-rubella IgG antibody in males was lower than that in females. Also, the percentage of male seropositive in the age group of 12-17 months and 18 months to 10 years was lower than that of females of the same age group. The recent epidemic in Fars, Southern Iran in 2012, has raised the necessity of conducting further investigation on the causal relationship between the inappropriate immune system response to MMR vaccine and gender [18]. Since the immune population against measles and rubella virus in this study was 84.75% and 85.25%, respectively, and both were less than 95%, with this level of coverage, there is a risk of epidemic occurrence every few years, and a more detailed plan for vaccination against these diseases and evaluating the effect of vaccination on changing the serum level of antibody in the community seems necessary. Further studies should be conducted to evaluate the adequacy of the MMR vaccine for Iranian children. Moreover, due to the lower average serum IgG antibody level measles and rubella in males, further investigation is needed to find out the causal relationship between the lack of appropriate response to vaccine injection and male gender.

ACKNOWLEDGEMENT

This study was financially supported by the Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Grant No.: OG-9738).

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Received on 05-08-2019 Accepted on 07-09-2019 Published on 12-09-2019

DOI: https://doi.org/10.6000/2292-2598.2019.07.03.12