SEROPREVALENCE TO RUBELLA VIRUS POST MMR VACCINATION IN BASRAH, SOUTHERN IRAQ

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ABSTRACT

A seroepidemiological study was carried out in Basrah from October 2003 to October 2004. The study aimed on determination of the prevalence of rubella IgG antibody among children under 15 years of age and females at childbearing age, rates of vaccination and provide information on immunity gap in certain age groups post vaccination and to estimate the duration of vaccine induced immunity and the rate of overtime loss of rubella antibody post vaccination. A total of 1309 blood samples were collected beside 80 pairs of blood samples from children and 77 samples from females at childbearing age. Specimens were collected from primary health care centers and primary schools. An Enzyme-linked Immunosorbent Assay (ELISA) was used for detection of rubella IgG antibody. The overall vaccination coverage was 78.9% leaving 21.1% unvaccinated. There were a variation in vaccination coverage in different age groups, 66.9% among 2-5 years old children, 85% of 6-8 years old and 83.2% of 10-14 years old. The prevalence of rubella antibody and vaccination coverage in rural areas (37.6%) is significantly (P<0.05) lower than in urban areas (62.3%) among children received 2 doses of MMR. There is a significant decline in the levels of rubella antibody overtime post vaccination and losses of protective levels of antibody was quite evident by 3-4 years after the primary doses of rubella vaccine which stress the need for booster doses. The prevalence of rubella IgG antibody among females at childbearing age was 68.8% leaving 27.2% of those female were susceptible to rubella with increased risk of congenital rubella syndrome (CRS) if these females catch the infection during early pregnancy.

INTRODUCTION

The current rubella vaccine when administered to children is effective in induction of rubella immunity and prevention of rubella infections and CRS[1-3]. Although acquired rubella is a benign illness, its public health importance came from the ability of the virus to cross the placenta barrier causing infections which result in CRS and fetal death[4] . Following the introduction of rubella vaccine in 1969, the number of rubella cases and CRS in US has decreased by 99% from 12.5 million cases and 20000 new born with CRS to 271 cases of rubella and 5 cases of CRS in 1999 then to lower annual record of 81 cases in 2002[5-7]. Despite the marked drop in the incidence of rubella, it was estimated that up to 15% of adolescent and young adults remain susceptible]8]. Generally, vaccine induced immunity is assumed to be lifelong, although rubella antibody may fall below the protective level which is defined as haemagglutination inhibition (HI) antibody titer > 1:8 or > 15 IU/ml by ELISA[1,9]. However, many studies have documented that there may be waning of rubella antibody in adolescent who were vaccinated with rubella vaccine[7]. Therefore serological surveillance are needed to monitor the impact of immunization program and guide the future program activities[10], these surveillance permit statement on the immunity gap in certain age groups post vaccination, the level of mean antibody titer, the duration of immunity and on the course of passive immunity[1,11]. However, to interrupt rubella transmission uniformly, high level of vaccine coverage in children must be achieved and sustained; in fact when vaccination coverage is inadequate, the disease continue to circulate in the community with shift of the infections toward higher age group[1,7]. This is of great concern as it may lead to increased number of infection during pregnancy and increases the number of CRS cases[10]. The objectives of this study are to determine rubella vaccination coverage in Basrah, estimate the prevalence of rubella antibody among children under 15 years old and females at childbearing age, evaluate the age at initial rubella vaccination considering the pre-existing maternal antibody and its effect on vaccination program, and to estimate the duration of vaccine induced immunity, immunity gap in certain age group and the rate of overtime rubella antibody losses.

MATERIALS AND METHODS

Study population and sampling:

A total of 1309 blood samples as well as 80 pairs of blood specimens were collected from

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children below 15 years (1232 samples) and from females at childbearing age (77 samples) in Basrah governorate (estimated population size 1 760 037 inhabitants during 2000 census) from october2003 to October 2004. Rubella vaccine is administered as MMR at 15 months and at school entry to children and at 12 years to girls as well as the mass vaccination campaign targeted 6-12 years of age regardless of vaccination history during 2004. Community survey in stratified by urban area (683 samples/921047 population) and rural area (549 samples/838 990 population) from primary schools and primary health care centers from a variety of geographical locations within Basrah governorate to provide reasonable representative estimate of general population immunity. Special questionnaire form was prepared for data collection which was filled according to the informations obtained from the parents interview or from the child vaccination card/or the school health card. All blood samples were collected after taking the permission from the member of the family who brought the child to the medical centers. Most blood samples were collected on Wattman No.3 filter papers for serological testing according to a standard method[12] and for comparison other blood sample was obtained by vein-puncture to pool serum and all stored at -20C until use. Rubella IgG enzyme immunoassay kit (biocheck-BC-1080,) for the detection and quantitation of antibodies against rubella virus in human sera was used. All ELISA procedure was carried out according to the manufacturer's instructions. A modified ELISA procedure for detection of anti-rubella IgG antibody was developed and compared to the bioCheck commercial kit using routinely available rubella vaccine as antigen for coating ELISA plate wells in coating buffer at pH 9.6. The optimum dilutions for the reaction were selected through a chequerboard titration ELISA[13] and all tested samples in both methods gave comparable results. Rubella antibody concentration was estimated in international unit/ ml (IU/ml) through a standard curve to convert optical density(OD) values into IU/ml. Rubella IgG index less than 0.9 (< 15 IU/ml) are considered negative for IgG antibody and if it was 1.0 or greater (> 15 IU/ml) as seropositive (protective). Sera with OD values of 0.91-0.99 were considered equivocal after repeated test.

RESULTS

The number and proportion of serum samples classified as positive (posses the protective level of antibody), equivocal and negative in relation to age is presented in (Table-1). Seropositivity was 68% among infants in the age group 1-9 months which represent the residual of maternally derived antibodies. Seropositivity was increased to 76.6% in the age group 10-23 months, then decreased to 65.7% among age group 2-5 years and increased again to 74.3% and 69.4% in age groups of 6-9 and 10-14 years respectively. The highest proportion of negative sera (susceptible individuals) was found among those aged 2-5 and 10-14 years which was 17.3% and 16.8% respectively. These differences were statistically significant ($P<0.01$). Estimation of antirubella IgG antibody in blood samples of females at childbearing age showed that 27.2% of this group were seronegative (susceptible to rubella and 68.8% were seropositive, although most of them were vaccinated with rubella vaccine.

Table 1. ***The prevalence of rubella IgG antibodies according to age.***

		Serological results		
Age	Total No. (%)	Positive* No. (%)	Equivocal** No. (%)	Negative*** No. (%)
1-9 months	150(12.1)	102(68)	36(23.8)	12(7.9)
10-23 months	94(7.6)	72(76.6)	13 (13.8)	9 (9.6)
2 – 5 years	248(20.1)	163(65.7)	42(16.9)	43(17.3)
6 – 9 Years	400(32.4)	297(74.3)	46(11.5)	57(16.8)
10–14 years	340(27.5)	236(69.4)	47(13.8)	57(16.8)
Females at childbearing	77	53(68.4)	3(3.8)	21(27.2)
Total	1309(100)	923(70.6)	187(14.9)	199(14.4)

$X^2 = 16.2$ d.f. = 8 $P < 0.01$

* Positive (+): protective level of rubella IgG equivalent to ≥ 15 IU/ml

**Equivocal (+): Rubella IgG 10-14 IU/ml

*** Negative (-): Rubella IgG < 10 IU/ml.

The overall vaccination coverage among the study population was 78.9% leaving a significant proportion of unvaccinated children (21.1%). An equal rate of vaccination coverage

was recorded among children taken 3 and 2 doses of rubella vaccine (32.4%) and 14% of children covered with one dose only (Table-2). There was a slight difference in the percentage of children taken 3 doses in urban areas (52.7%) and in rural areas (47.2%). Also there were significant differences in the percentage of unvaccinated children in urban areas (45.9%) and rural areas (54%) (P<0.05). Also (Table-2) shows the relation of vaccination coverage to serological profile. The highest rate of seropositivity (84.3%) was observed among children covered with 3 doses while the lowest seropositivity was obtained among the unvaccinated group of children (35.2%) which is attributed to the natural exposure to wild virus. The number of vaccine doses taken by children showed significant differences (P<0.01) in the seropositivity between those immunized with 3 doses (84.3%) and children immunized with 2 doses (77.6%), and single dose (77.3%).

Table 2. ***The pattern of vaccination coverage and the number of vaccine doses in relation to residence and serological profile.***

		Urban No. (%)	Rural No. (%)	Positive No. (%)	Equivoc No. (%)	Negative No. (%)
3 doses	345 (32.4)	182 (52.7)*	163 (47.2)*	291 (84.3)**!	35 (10.1)	19 (5.5)
2 doses	345 (32.4)	215 (62.3)	130 (37.6)	268 (77.7)!	44 (12.7)	33 (9.5)
Single dose	150 (14)	92 (61.3)	58 (38.6)	116 (77.3)!	16 (10.6)	18 (12.1)
Unvaccinated	224 (21)	103 (45.9)	121 (54)	79 (35.2)**	50 (22.3)	95 (42.4)
Total	1064	592 (55.6)	472 (44.4)	754 (70.8)	145 (13.6)	165 (15.5)

**P < 0.05*
***X^2 = 17.5 P < 0.01*
! X^2 = 16.9 P < 0.01
The overall vaccination coverage 78.9% and 21.1% unvaccinated.

Table-3, shows the losses in the levels of antibody at different time intervals between sampling and rubella vaccination. The highest proportion of seropositivity was observed among children received the vaccine 1-2 years before sampling (71.2%), then declined to 68.1% and 58.5% by 3-4, 5-6 years post vaccination respectively, to reach it's lowest level (48.8%) after 7-8 years, but raised again to 59.2% in those who received the vaccine 9-13 years ago. These differences was statistically significant (P <0.01).

Table 3. ***Levels of rubella virus antibodies at different periods post vaccination***

Intervals between vaccination & sampling	Total No. (%)	Serological results		
		Positive No. (%)	Equivocal No. (%)	Negative No. (%)
1 – 2 years	52 (21.7)	37 (71.2)	9 (17.3)	6 (11.5)
3 – 4 years	66 (27.6)	45 (68.1)	12 (18.1)	9 (13.6)
5 – 6 years	53 (22.1)	31 (58.5)	13 (24.5)	9 17.0)
7 – 8 years	41 (17.1)	20 (48.8)	10 (24.4)	11 (26.8)
9 –13 years	27 (11.2)	16 (59.2)	6 (22.2)	5 (18.5)
Total	239	145 (60.6)	50 (20.9)	44 (18.4)

X^2 = 8.21 d.f. = 8 P < 0.01

DISCUSSION

Due to limited disease surveillance and reporting system, data on rubella and CRS in Middle East are scanty[4]. According to the available information, there are no rubella vaccine efficacy studies and no serological evaluation as yet available in Iraq. Clinical data by itself are of limited value because the clinical symptoms of the disease are mild in addition to the presence of many subclinical cases[14], so study of seroepidemiology of rubella makes this of value to the country. This study is the first attempt to present the results of population based serological survey in Basrah governorate, southern Iraq. The overall seropositivity was 70.8% *(with protective levels of antibody)* of vaccinated children, the majority of them below 10 years of age or recently vaccinated leaving the older age groups still susceptible due to interruption in the vaccination program. However, serological surveillance is an important tool for the evaluation of vaccination program as it monitors immunity in the population, thus providing information with which to identify further control measures and provides age specific profiles that enable the identification of susceptible cohort that can emerge following the implementation of vaccination program, in addition to that, the impact of rubella immunization program has demonstrated that if vaccination coverage fall below threshold of 80%, then there is an increase in CRS due to accumulation of susceptible adult females[15]. Knowledge of the distribution of infectious agent immunity in relation to age in a country is useful to determine the target population for an effective mass immunization program. Our study showed that the proportion of children with protective levels of antibody (> 15 IU/ ml) was lowest in age group 2-5 years old; this may indicate a rapid antibody loss or poor immune responses to virus vaccine. However, 74.3% of seropositivity was obtained among children of 6-9 years of age which reflect the effect of booster doses of MMR given at school entery age. In a study done in Africa[16] reported 74% seropositivity among children 5-9 years of age and 71% among 10-14 years old which is in consistence with our findings. The proportion of vaccinated children was higher in urban than the rural areas and there was a significant differences in the vaccination coverage with 3, 2, single doses and the unvaccinated children between the two areas, hence lower number of children with protective levels of antibody. The most common causes for this finding are attributed to the low vaccination coverage in rural areas, the unavailability of the vaccine, distant PHC, ignorance or neglect of the parents and low educational levels of the parents in rural areas; and probably better cool chain provided to the vaccine given to the children in the urban areas, all these causes may have role in sharing these differences[17,18]. These observations are in agreement with other studies[17,19,20]. Active transplacental transfer of viral IgG begins at about 6 months of gestation and IgG concentration in fetal serum reported to exceed maternal level by a ratio of 1:1.2 to 1:1.8 [21]. At birth till the age of 6 months 90.5% of infants posses the protective levels of antibodies in their serum, although it fade to 45.7% by 12 months of age[17,21,22]. Accordingly, only after antibody levels are low enough, the vaccine virus can induce immune responses because the pre-existing antibody limits the replication of the virus. However, the achievement of the goal of eliminating rubella is facilitated by vaccination at earliest possible time after the clearance of maternal antibody in order to keep the number of susceptible subjects in the population as low as possible[21]. Paired sera were collected from school children showed that the best seroconversion was observed in seronegative children and those with low levels of pre-existing antribody. Many studies showed that the pre-existing antibodies limits the replication of the virus in the majority of the low titer antibodies on revaccination[23], so infants vaccinated before their first birthday should not considered be vaccinated and preferably vaccinated by 15 months of age[1,3,5]. Rubella immunization has been a subject of great interest and controversy since the introduction of rubella vaccine in 1969. Of particular concern to many experts had been the possibility that antibodies and consequently protection would not be long lasting, although rubella vaccine induce immune responses that qualitatively similar to that induced by naturally acquired infections[24,25].

In this study we found that there is a significant decline in the levels of rubella immunity about

3-4 years post vaccination which indicates an overtime waning immunity. This may be attributed to the absence of natural booster effect, a direct result of vaccination which interrupted the circulation of wild virus[26]. This observation is in consistence with that reported by other studies[27,28]. However, in our community, after 1-2 years from vaccination 71.2% were seropositive, then the level decreased gradually to 62.1% and 48.8% by 3-4 and 7-8 years intervals respectively. However, the levels of antibodies increased among 9-13 years which reflect the impact of mass MMR vaccination campaign implemented in 2004 regardless of previous vaccination history. These differences in the results may be due to interrupted introduction of MMR vaccine, inadequate vaccination coverage or unavailability of the vaccine, so that the virus continues to circulate in the community with shift of infection (susceptibility) toward higher age groups including women of childbearing age and thereby increased the risk of CRS[7,10]. From the results of this study, we conclude that MMR vaccination coverage should be kept at least at a level above 80% taking in consideration the pre-existing maternal antibodies that interfere with vaccine efficacy. So the result stress on continues monitoring for the levels of antibodies post vaccination to ensure the maintenance of adequately protective levels since these levels could be lost overtime, hence achievement of this goal can be obtained through annual surveillance to the levels of antibodies post vaccination to recommend the implementation of booster doses required.

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