Rubella post – vaccinational antibody response among rubella - serpositive individuals

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Abstract

Background: Rubella is an acute febrile illness that affects children and young adults. However, infection during early pregnancy may result in serious abnormalities of the fetus. Live attenuated vaccine controls rubella infection in industrialized countries.

Objective: To determine the antibody response to rubella virus in seropositive volunteers after vaccination with live attenuated rubella vaccine.

Methods: Fifty two rubella virus seropositive volunteers have been included in the study, their ages ranged between 15-45 years. 26 of them were vaccinated with rubella vaccine and the rest were injected with diluent supplied with the rubella virus vaccine (placebo). Antibodies against rubella virus were detected in volunteer's sera prior to, one and four weeks after vaccination, using ELISA method.

Results: There was elevation in the serum antibodies after vaccination. The Optical Density (OD) readings were 1.69 and 2.02 during first and fourth week respectively. Data analysis showed that there was a significant difference of OD value among seropositive vaccinee. And there was a significant elevation of serum antibody in the first week, but the fourth week had very high OD readings, which may reflect an increase in the concentration of antibodies.

Conclusion: Rubella vaccine was safe, and effective, and there was an elevation in serum antibody titer among vaccinee.

Key words: Rubella vaccination, IgG antibody, seroposive individuals.

IRAQI J MED SCI, 2008; VOL.6 (3):99-103

Introduction

Rubella is a febrile exanthematic disease that is benign in children and causes arthralgia and less frequently arthritis in adults. Although rare, complications such as thrombocytopenia, encephalitis, Guillain–Barré syndrome, myocarditis and pericarditis may appear in adults. The major complication of rubella is its teratogenic effects when pregnant women contract the disease, especially in the early weeks of gestation [(1)]. The virus can be transmitted to the fetus through the placenta and is capable of causing serious congenital defects, abortions, and stillbirths. Fortunately, because of the successful immunization program initiated in the United States in 1969, rubella infection and congenital rubella syndrome rarely are seen today [(2)].

Given the availability of a highly efficacious vaccine, in 1998, the European Regional Committee of the WHO, established the objective of reducing the incidence of CRS to <1/100 000 live births by 2010. The number of European countries that administer two doses of rubella vaccine has increased considerably in recent years, although coverage is still suboptimal in some countries [(3)]. Finland, which introduced a vaccination policy in 1982, eliminated CRS in 1986 and post-natal rubella in 1996[(4)]. So the aim of this study is to check the potency of the available rubella virus vaccine.

Materials and methods

In this study a total of 60 volunteers were chosen, one of them was seronegative (IgG specific for RV). Hence excluded from the study,

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Received: 6th July 2008, Accepted: 16th December 2008.

another 7 subjects were lost during follow up study for variable reasons.

The age and sex matched volunteers were subdivided into two groups.

Group 1

This group includes 26 individuals (Table 1) and was vaccinated with rubella vaccine. The mean age group was (30.2) years. 18 (69.2%) of the individuals were males and 8 (30.8%) were females

Group II (control)

This group includes 26 individuals and was injected with the diluent supplied with rubella vaccine (placebo). The mean age group was (27.8) years. The male constituted 69.2% of these individuals whereas 30.8% were females (Table 1).

Both groups were followed up for four weeks after vaccination.

Rubella Vaccination:

The reconstituted lyophilized live vaccine was given subcutaneously to group I in a volume of 0.5 ml which contained at least 1,000 Tissue culture infectious dose 50% (TCID50) of attenuated RV vaccine prepared from Schwarz strain according to the manufacturer instructions. The diluent supplied with the vaccine was given subcutaneously in a volume of 0.5 ml to the second group.

Serological analysis:

Blood samples were obtained from the volunteers prior to, one and fourth weeks after vaccination. The samples were left to coagulate at room temperature and serum was obtained by centrifugation. An aliquot of the serum obtained was frozen at -20°C until serological analysis. Rubella IgG antibodies were determined by ELISA (Diasorin, Saluggia-Vercelli, Italy).

Enzyme Immunoassay for the Determination of IgG Antibodies.

Antibodies against RV were detected in volunteer's sera (prior to, one and four weeks after vaccination) using ELISA-test, which done according to the manufacturer instruction and as follows:

Test procedure:

The sera were diluted 1/101 and mixed well, then 100ul of undiluted control sera and diluted samples pipetted in duplicate into respective wells of the microtiter strips (except the well for the blank), the plate was covered and incubated for one hour at room temperature. Wells then emptied by aspiration and unbound sera were removed by three cycles of washing, then 100ul of anti-IgG-HRP conjugate was added into each well, then plate was covered and incubated for 30 minutes at room temperature, then unreacted HRP-Abs were washed by 3 cycles of washing by ready to use washing solution. Then, 100ul of ready to use substrate (TMB) was added into each well, then plate was covered and incubated for 15 minutes at room temperature in the dark, then 100ul of 1M H_2So_4 (stopping reagent) to stop substrate reaction and after thoroughly mixing the color was stable for 30 minutes and the absorbance was measured at 450nm using an ELISA reader.

The low positive control served as the cut-off value and when the absorbance of the subject sample was more than 10% above the cut-off value, the result regarded as positive and the absorbance 10% below the cut-off value, the result regarded as negative, results in between these could not clearly be defined and they were regarded as questionable. The higher Optical Density (OD), the higher levels of anti- immunoglobulins are present. The mean cut off value was calculated with OD of (1.208), thus any OD reading higher than this OD reading by 10% was considered as positive and any OD reading below by 10% was considered as negative

(according to the manufacturer instruction).

Results

Rubella specific IgG antibody responses.

Serum IgG level against RV in selected subjects had been measured by using ELISA technique, which measure the optical density (OD) of readings.

One subject out of 60 gave negative results, and considered as seronegative subjects, and excluded from the study, the rest of them were seropositive for rubella specific IgG. So all the remaining subjects had antibody to rubella prior to inoculation of vaccine.

Raising serum antibody titer after rubella vaccination:

The mean OD reading of group 1 was 1.52 while the mean OD of group II was 1.56 before vaccination. During the first week of rubella vaccination the mean OD was 1.69, in the fourth week the mean OD reading was 2.02 (Figure 1)

Data analysis showed that there was a significant difference of OD value among seropositive vaccines. There was a significant elevation of serum antibody in the first week (*P* value ***0.004***); the rubella titer in fourth week had very high OD readings, which may reflect an increase in the concentration of antibodies (*P* value ***0.004***) (Table 2).

Table 1: Age and sex distribution of selected volunteers

Group	***No. Of individuals***	***Male***	***Female***	***Age/year (mean)***
		No. (%)	**No. (%)**	
Group I	26	18 (69.2)	8 (30.8)	30.2
Group II	26	18 (69.2)	8 (30.8)	27.8
Total	52	36	16	29

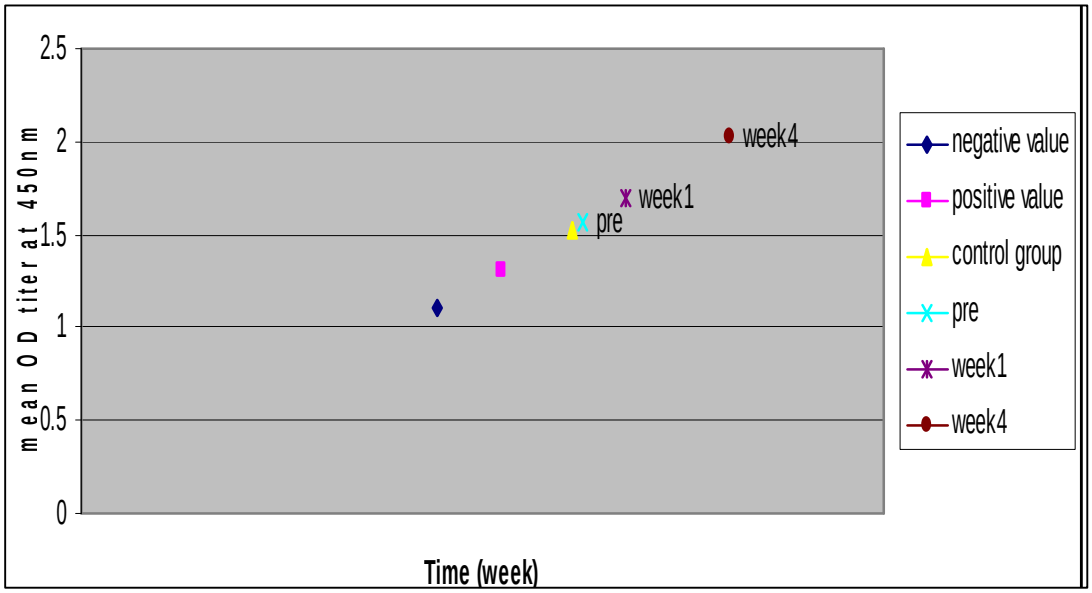

Figure 1: Mean rubella serum antibody titer (OD) between revaccination and postvaccination among seropositive individuals

Table 2: RUBELLA IgG levels [By ELISA (OD)] between prevaccination and postvaccination among seropositive individuals

Pre		***Week 1***		***P value***	***C.S.***
Mean	***Variance***	***Mean***	***Variance***		
1.56	**0.03**	**1.69**	**0.02**	***0.004***	***S.***
Pre		***Week 4***			
Mean	***Variance***	***Mean***	***Variance***		
1.56	**0.03**	**2.02**	**0.018**	***0.001***	***S.***
Week 1		***Week 4***			
Mean	***Variance***	***Mean***	***Variance***		
1.69	**0.02**	**2.02**	**0.018**	***0.004***	***S.***

C.S.: comparison significance.
N.S.: Not significant.
S.: Significant.
P value: probability of chance factor to be the origin of difference.

Discussion

All the selected individuals had preexisting antibody against Rubella virus, after vaccination of those individuals, a significant elevation of rubella specific antibody levels were observed during first week and forth week after inoculation of the vaccine while the was no significant differences of OD values during the follow up period for the control group.

Humoral immunity is not essential for recovery from rubella infection. Active B cell proliferation during the first week of infection may reflect expansion of virus specific clone since specific antibody appear and increase to peak levels 2-3 weeks later [(5)], and persist for life, which may be important in preventing reinfection [(6)]. Our results of an in vitro study in lymphocyte culture indicated that preexisting antibody play a very important role in prevention of infection [(7)].

The main objective of rubella vaccination programs is to prevent congenital rubella syndrome, i.e. to avoid infections during pregnancy. To reach this objective, two not incompatible strategies have been formulated: (i) to vaccinate all fertile women; (ii) to obtain vaccination coverage >95% in children. The first strategy is followed mainly by the United Kingdom, where screening of pregnant women in order to offer postpartum vaccination to susceptible women is widespread [(8)]. The second strategy has been implemented by countries such as Finland and Sweden, where, after selectively vaccinating girls from 1975, double universal vaccination with the Measles Mumps Rubella (MMR) was introduced in 1982[(4,9)].

It is probable that some vaccinated women did not remember having been vaccinated or did not have the correct documentation, as suggested by other

reports both on rubella [10, 11] and on other vaccine preventable diseases [12].

For successful rubella virus eradication vaccination campaign targeting till the age of 15 regardless of history of rubella infection or vaccination status should be done in addition to routine vaccination program.

In conclusion, the available rubella vaccine is safe and effective as there was elevation of serum antibody titer among vaccinated individuals.

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