SEROPREVALENCE OF RUBELLA IMMUNITY AMONG WOMEN OF CHILDBEARING AGE IN BANGKOK, THAILAND

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Abstract. Rubella infection during the first trimester of pregnancy can cause congenital rubella syndrome in the fetus. This can be prevented with the live attenuated rubella vaccine given prior to pregnancy. This study aimed to determine rubella seroprevalence among non-pregnant women of childbearing age in Bangkok, Thailand, in order to inform congenital rubella preventive measures by measuring the prevalence of protective antibody levels and measure the levels of rubella-specific immunoglobulin G (IgG) antibody titers using an indirect enzyme-linked immunosorbent assay (ELISA). Serum samples were collected from 289 non-pregnant Thai women aged 28-40 years who presented to Rangsit University Healthcare medical center, Bangkok, Thailand for a check-up during 2014. A protective rubella IgG antibody level was determined to be ≥10 IU/ml. Eighty-seven point two percent [95% Confidence Interval (CI): 83.4-91.0] of study subjects (252/289) were found to have protective antibody levels. The mean ± standard deviation of antibody level was 41.4 ± 37.3 IU/ml (95% CI: 37.0-45.7 IU/ml). There were no significant differences in protective rubella IgG antibody levels by age group. Further population-based surveys are needed to determine if the levels found in our study are consistent throughout the Thai population.

Keywords: rubella, seroprevalence, seropositivity, IgG, immunity, childbearing age women

INTRODUCTION

Rubella infection, also known as German measles, is an important public health problem, especially in developing countries (Cutts and Vynnycky, 1999). It is a contagious disease caused by rubella virus in the family Togaviridae, genus *Rubivirus* (Collier and Oxford, 2000). The infection is often mild and self-limited, causing 2-3 days of fever and rash (Collier and Oxford, 2000). However, congenital rubella infection during the first trimester of pregnancy can cause congenital rubella syndrome (CRS) in the fetus (Miller *et al*, 1982). The complications of CRS include deafness, cataracts, heart defects, mental retardation, liver and spleen damage and can lead to abortion (Mellinger *et al*, 1995).

The Global Vaccine Action Plan (GVAP) endorsed by the World Health Organization (WHO) introduces the global goal to control rubella and CRS by 2020 (The Measles and Rubella Initiative, 2013). To achieve this goal, one of the strategies is maintenance of high levels of immunity in

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the population through vaccination with two doses of rubella-containing vaccine (The Measles and Rubella Initiative, 2013). In Thailand, rubella vaccination has been included in the Expanded Program of Immunization (EPI) by the Ministry of Public Health since 1986 (WHO SEARO, 2015). The first introduction of the rubella vaccine was in the form of a rubella-containing vaccine (RCV). It was recommended to be given during sixth grade to all girls aged 12 years (WHO SEARO, 2015). Following this, the Measles, Mumps, Rubella (MMR) vaccine was introduced in 1997; this was given to all girls upon completing primary school (WHO SEARO, 2015). Currently in Thailand, the MMR vaccine is recommended to be given in 2 doses, the first at age 9-12 months and the second at age 7 years (WHO SEARO, 2015). The incidence of reported cases of rubella in Thailand has decreased from 3.07 per 100,000 population in 1986 to 0.24 per 100,000 population in 2014 (Bureau of Epidemiology, 1986; 2014). Central Thailand has the highest prevalence of rubella with an incidence of 0.48 cases per 100,000 population in 2014 (Bureau of Epidemiology, 2014).

There have been few rubella immunity seroprevalence studies conducted within the last decade among Thai population (Boonruang and Buppasiri, 2005; Tharmaphornpilas *et al*, 2009). In 2004, Boonruang and Buppasiri (2005) determined the rubella immune status of 150 pregnant women aged 15-40 years, using the ELISA method; the seropositivity rate was determined to be 75%.

Supplementary rubella vaccination should be administered to susceptible women who are not pregnant since it contains a live attenuated virus (WHO, 2011). It is important to monitor changes in the prevalence of rubella immunity in this population to inform rubella and CRS control programs. There are no recent studies from Thailand regarding the prevalence of rubella immunity among women of childbearing age. We conducted this study to determine the seroprevalence of rubella immunity among non-pregnant women of childbearing age who presented to the Rangsit University (RSU) Healthcare institution in Bangkok, Thailand for a regular check-up. The results of this study will inform rubella and CRS control programs for the study population.

MATERIALS AND METHODS

Subject selection

This was a retrospective cross sectional study. Subjects were non-pregnant healthy women aged 28-40 years who presented to the out-patient department of the RSU Healthcare institution for a physical check-up during April-June 2014. Women with a history of immunodeficiency disorders were excluded from the study. The sample size was calculated based on assumed seroprevalence of rubella immunity of 75 (Boonruang and Buppasiri, 2005), with a 95 confidence level giving a required sample size of 289 participants. The participants were randomly selected from women of the study age presenting during the study period.

Serological testing

A serum sample was obtained from each subject and stored at -20°C until used. Each sample was examined for the presence of rubella IgG antibodies using a commercial enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions. Briefly, 100 µl of diluted serum (1:101) was added to wells of a microtiter plate coated with rubella virus antigen and incubated at room tem-

perature for 30 minutes. The wells were then washed three times with buffer and then 100 µl of peroxidase-labeled rabbit anti-human IgG was added to each well. The wells were then incubated at room temperature for 30 minutes protected from light. The wells were then washed again with buffer and then 100 µl TMB/H_2O_2 chromogen substrate was added to each well. The wells were again incubated at room temperature for 15 minutes. The reaction was then stopped by adding 100 µl 0.5 M sulfuric acid to each well and then gently mixing. The optical density at 450 nm was read using an ELISA reader (TECAN infinite F50; Tecan Trading AG, Männedorf, Switzerland). The experiment was conducted in duplicate; positive and negative control sera were also included. The results were recorded as rubella IgG concentration in international units per milliliter (IU/ml). Rubella immunity was defined as a rubella IgG level ≥10 IU/ml (Skendzel, 1996), an equivocal result was defined as a rubella IgG level 8-9.9 IU/ml and no rubella immunity was defined as a rubella IgG level <8 IU/ml.

Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for Social Services (SPSS), version 21.0 (IBM, Armonk, NY). The Kruskal-Wallis and chi-square tests were used to compare rubella IgG antibody levels and seropositivity rates by age groups for normally and non-normally distributed data, respectively. A *p*-value <0.05 was considered statistically significant.

Ethical considerations

Ethical approval for this study was obtained from the Ethics Committee, Research Institute of Rangsit University, Thailand (RSEC number 05-2013). The informed consent was exempted because the study is retrospective. Women without immunity were recommended to have a rubella vaccine.

RESULTS

Seroprevalence of rubella immunity

A total of 289 women were included in this study. The mean (±standard deviation (SD) age of subjects was 33.4 years (± 3.8; 95% Confidence Interval (CI): 33.0-33.9).

The seroprevalences of rubella IgG antibody results that were seropositive, equivocal and seronegative were: 87.2 (95% CI: 83.4-91.0), 3.1 (95% CI: 1.0-5.2), and 9.7 (95% CI: 5.9-13.5), respectively. The age group with the highest seropositivity rate was women aged 37 years (100; 95% CI: 100-100) and the lowest seropositivity rate was women aged 35 years (75; 95% CI: 50.0-93.8) (Table 1). The seropositivity rates did not differ significantly by age group ($p > 0.05$).

Rubella IgG antibody levels

The mean (±SD) rubella IgG antibody level was 41.4 (± 37.3; 95% CI: 37.0-45.7) IU/ml (data not shown). Among seropositive women, the mean (±SD) rubella IgG antibody level was 46.9 (± 36.8; 95% CI: 42.3-51.4) IU/ml. The highest rubella IgG antibody level among seropositive women was found among those aged 39 years (69.1± 62.1 IU/ml; 95% CI: 39.2-99.1) and the lowest rubella IgG antibody among seropositive women was found among those aged 40 years (36.9 ± 19.5 IU/ml; 95% CI: 26.9-46.9). There were no significant differences in rubella IgG antibody levels among seropositive subjects by age group ($p > 0.05$) (Table 2).

DISCUSSION

We studied the seroprevalence of

Table 1
Seroprevalence of rubella IgG antibodies among study subjects by age group.

Age (years)	Seropositive		Equivocal		Seronegative		Total		*p*-value[a]
	No.	% (95 CI)	No.	% (95 CI)	No.	% (95 CI)	No.	% (95 CI)	
28	26	86.7 (73.3-96.7)	1	3.3 (0-10.0)	3	10 (0-20.0)	30	10.4 (7.0-13.8)	
29	24	96 (88.0-100)	0	0 (0-0)	1	4 (0-12.0)	25	8.7 (5.6-12.1)	
30	19	86.4 (69.4-100)	1	4.5 (0-13.6)	2	9.1 (0-22.7)	22	7.6 (4.8-10.7)	
31	25	86.2 (72.4-96.6)	1	3.5 (0-12.9)	3	10.3 (0-24.1)	29	10 (6.6-13.1)	
32	19	76 (56.0-92.0)	2	8 (0-23.0)	4	16 (4.0-32.0)	25	8.7 (5.3-11.8)	
33	26	89.7 (76.8-100)	2	6.9 (0-17.2)	1	3.4 (0-10.3)	29	10 (6.6-14.2)	
34	22	91.7 (76.1-100)	0	0 (0-0)	2	8.3 (0-23.9)	24	8.3 (5.5-12.1)	0.699
35	12	75 (50.0-93.8)	2	12.5 (0-25.0)	2	12.5 (0-31.3)	16	5.5 (3.5-8.3)	
36	14	87.5 (68.8-100)	0	0 (0-0)	2	12.5 (0-31.3)	16	5.5 (2.8-8.6)	
37	12	100 (100-100)	0	0 (0-0)	0	0 (0-0)	12	4.1 (1.7-6.6)	
38	17	89.5 (73.7-100)	0	0 (0-0)	2	10.5 (0-26.3)	19	6.6 (4.2-9.3)	
39	19	90.5 (76.2-100)	0	0 (0-0)	2	9.5 (0-23.8)	21	7.3 (4.5-10.4)	
40	17	81 (61.9-95.2)	0	0 (0-0)	4	19 (4.8-38.1)	21	7.3 (4.5-10.7)	
Total	252	87.2 (83.4-91.0)	9	3.1 (1.0-5.2)	28	9.7 (5.9-13.5)	289	100	

Rubella immune status was defined as a rubella IgG antibody levels: ≥10 IU/ml is seropositive, 8- 9.9 IU/ml is equivocal, and <8 IU/ml is seronegative.
[a]The chi-square test was used to compare differences among age groups when $p<0.05$ was considered significant; CI, Confidence Interval.

rubella IgG immunity and antibody levels among Thai women of childbearing age. This study was conducted 28 years after the rubella-containing vaccine (RCV) campaign of 1986. Women in our study were born between 1974 and 1986; as the RCV program was begun in Thailand among girls aged 12 years. In our study, seropositivity was found in 87.2% of study participants. This is the first study to investigate rubella immunity among non-pregnant women, while previous studies mainly performed in pregnant women. A study conducted in 2004 in northeastern Thailand among pregnant women aged 15-40 years reported seropositivity rate of 75% (Boonruang and Buppasiri, 2005). In other countries, rubella seropositivity rates among pregnant women of childbearing age were reported to be 71.1% from Vietnam (Miyakawa *et al*, 2014), 84.3% from Bangladesh (Jubaida *et al*, 2011), 92% from Italy (De Paschale *et al*, 2012), 92.6% from Tanzania (Mwambe *et al*, 2014), 94.3% from Turkey (Uyar *et al*, 2008), and 97.1% from Mexico (Alvarado-Esquivel *et al*, 2016). The differences by country in rubella immunity may reflect immunization programs, the percentage of vaccine coverage, waning immunity after vaccination and incidences of rubella infection. In addition, a comparison of rubella seroprevalence to vaccine coverage might imply the vaccine effectiveness and waning immunity after vaccination in a population. However, this comparison could not be performed in our study since the data on RCV coverage in Thailand was limited in the early years of rubella immunization (UNICEF and WHO, 2013).

Table 2
Rubella IgG antibody levels among seropositive subjects by age group.

Age (years)	Seropositive[a] No. (%)	Mean rubella IgG antibody levels (95% CI) (IU/ml)
28	26/30 (86.7)	41.0 ± 29.6 (29.0-53.0)
29	24/25 (96.0)	47.8 ± 34.9 (33.1-62.5)
30	19/22 (86.4)	54.3 ± 39.4 (35.4-73.3)
31	25/29 (86.2)	43.8 ± 31.1 (31.0-56.6)
32	19/25 (76.0)	42.6 ± 38.4 (24.1-61.1)
33	26/29 (89.7)	39.8 ± 39.2 (24.0-55.6)
34	22/24 (91.7)	50.1 ± 30.7 (36.4-63.7)
35	12/16 (75.0)	40.4 ± 22.1 (26.4-54.5)
36	14/16 (87.5)	42.9 ± 26.0 (27.9-58.0)
37	12/12 (100)	39.9 ± 20.0 (27.2-52.6)
38	17/19 (89.5)	59.8 ± 50.5 (33.9-85.8)
39	19/21 (90.5)	69.1 ± 62.1 (39.2-99.1)
40	17/21 (81.0)	36.9 ± 19.5 (26.9-46.9)
Total	252/289 (87.2)	46.9 ± 36.8 (42.3-51.4)
p-value[b]	0.683	

Rubella IgG antibody levels are expressed as mean ± standard deviation (95% CI, Confidence Interval). [a]The seropositive was defined as a rubella IgG antibody level ≥10 IU/ml. [b]The Kruskal-Wallis test was used to compare rubella IgG antibody levels among age groups. A p-value <0.05 was considered significant.

In summary, 87.2 of our study subjects had immunity to rubella. This prevalence did not differ significantly by age groups. Further studies among other populations in Thailand are needed in order to guide rubella control programs to prevent CRS.

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