SEROLOGY OF RUBELLA AND SURVEILLANCE OF CONGENITAL RUBELLA SYNDROME IN HANOI WHERE AN OUTBREAK HAS OCCURRED

Bang Nguyen Van [1], Anh Nguyen Thi Van [2], Van Vu Thi Tuong [3], Thai Trieu Thi Hong [4], Thuong Nguyen Van [5], Gulam Khandaker[7,8] and Elizabeth Elliott[8,9]

1. Department of Pediatrics, Hanoi Medical University, Hanoi, Vietnam

2. Department of Medical Education and Skill Laboratory, Hanoi Medical University, Hanoi, Vietnam

3. Department of Microbiology, Bach Mai University Hospital, Hanoi, Vietnam

4. National Hospital of Pediatrics, Hanoi, Vietnam

5. Department of Pediatrics, Saint Paul Hospital, Hanoi, Vietnam

7. National Centre for Immunization Research and Surveillance (NCIRS), The Children's Hospital at Westmead, NSW, Australia

8. Discipline of Pediatrics and Child Health, the Children's Hospital at Westmead Clinical School, Sydney Medical School, the University of Sydney, NSW, Australia

9. Australian Pediatric Surveillance Unit (APSU), Sydney, NSW, Australia

ABSTRACT

Congenital rubella syndrome (CRS) is preventable illness but stays frequent in developing countries where rubella vaccination is not incorporated in national program of vaccination.

Objectives

This study aimed at obtaining baseline information on acquired immunization status of school girls as well as the rate of susceptibility and rubella infection in pregnant women around a rubella outbreak in Hanoi.

Methods

During and after the rubella outbreak in 2011, sera from 136 school girls were tested for rubella-specific IgG (Rs-IgG) and 140 pregnant women were tested for rubella-specific IgG and for rubella-specific IgM (Rs-IgM) using electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostic) with cut-off value ≥10 IU/mL for IgG and ≥0.3 IU/mL for IgM. During the surveillance period, 113 infants were identified with confirmed CRS and clinically analyzed.

Results

Rs-IgG antibodies was positive in 125/136 (91.9%) school girls and 119/140 (85%) pregnant women, with strong-to-extreme concentration in 86/136 (63.2%) school girls versus 19/140 (13.6%) in pregnant women (p<0.002). Rs-IgG was negative in 21/140 (15%) and Rs-IgM antibodies was positive in 27.9% (39/140) pregnant women, of whom 38 (27.1%) a co-existence

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Corresponding Author: Bang Nguyen Van
Address: Email: hongbang52@yahoo.com

of Rs-IgG and Rs-IgM were documented. During the surveillance period 113 infants were identified with confirmed CRS. Clinical manifestations of CRS included low birth weight <2,500g (86%), congenital heart disease (63.7%), hearing impairment (63.7%), ophthalmological involvement (46.9%). Other clinical features included: thrombocytopenia (85%), neonatal purpura (74.3%), splenomegaly (63.7%), hepatomegaly (62.8%) and blueberry muffin rash (61.1%). During the surveillance period the estimated annual incidence of CRS was 1.13/1,000 live birth (95% CI 0.92-1.34).

Conclusion

This preliminary, baseline per/post epidemic information showed a high rate of acquired immunization in female adolescents but also high rate of susceptibility and very high rate of rubella infection in pregnant women, as well as a very high burden of CRS in Vietnam.

***Key-words:** Acquired immunization, rubella infection, school girls, pregnant women, congenital rubella syndrome (CRS).*

1. BACKGROUND

Rubella usually is a mild, febrile rash illness in children and adults. However, infection early in pregnancy, particularly during the first 16 weeks of gestation can result in miscarriage, stillbirth or an infant born with congenital rubella syndrome (CRS) [1, 2]. The World Health Organization (WHO) estimates that around 238,000 children are born with CRS every year worldwide, the majority live in developing countries [2-4]. The frequency of CRS varies in different parts of the world, depending on levels of naturally acquired immunity, overcrowding and immunization policies and health practices [3-6]. During epidemics of rubella, CRS has been reported in 0.6-2.2/1,000 live births [2-3]. The WHO and the Children's Vaccine Initiative have produced guidelines for surveillance of CRS and rubella [5]. As of 2013, targets for accelerated rubella control and CRS prevention have been established by the Western Pacific Region (WPR) [4]. WHO guidelines also recommend that, for countries in Stage 1 (planning for rubella vaccination), the surveillance should focus not only on establishing case-based CRS detection but also on serological status of rubella in childbearing age and pregnant women [3,4]. Currently, 130 WHO member countries have intruded rubella vaccine in their national immunization program. However, 59 of the remaining WHO member states, representing 60% of the world's child population still do not have rubella in their routine immunization schedule; Vietnam being one of those countries. In 2004-2005, at least 2 rubella epidemics were suspected in Vietnam, and then in late 2010 and early 2011, an epidemic of rubella had been confirmed [5,6]. However, there is a paucity of data on the burden of CRS in Vietnam apart from few sero-epidemiological studies 10]. During 2012, the WHO passive surveillance reported 7,259 cases of rubella and 189 cases of CRS in Vietnam; however, this is a gross underestimate of the actual burden. In view of efforts to incorporate rubella vaccine into the national immunization program in Vietnam, it is prerequisite to establish the national baseline data of disease burden of CRS and determine rates of acquired immunization in young women as well as to identify the rate of rubella susceptibility in child-bearing women. Therefore, this prospective study, conducted around the outbreak of rubella epidemic in late 2010 and early 2011, aimed at (1) assessing the status of acquired immunization against rubella in school girls, (2) investigating the risk of congenital rubella syndrome by exploring the susceptibility (seronegative rate) as well as the recent rubella infection rate in pregnant women and (3) surveying the burden of CRS in infants born during a rubella epidemic in Hanoi, Vietnam.

2. METHODS

In order to assess the status of acquired immunization in potential childbirth-age subpopulation, after obtaining written consent from their family, blood sample of female students aged from 14 to 17 years from Hanoi-Amsterdam high school in Hanoi town were taken to determine former rubella infection rate (dosage of IgG in serum). To assess the risk of congenital rubella syndrome, pregnant women presenting in Vietnam-France hospital and accepting to participate to the study had been enrolled. Information of the study was explained to participants and signed consent form was taken as the evidence of agreement.

Burden of CRS was assessed by clinical examination in all infants with suspected or clinically-confirmed CRS used WHO's criteria [5]. Serum samples were collected in all suspected or clinically-confirmed CRS infants admitted to departments of neonatology, cardiology and pediatrics of Bach Mai tertiary teaching hospital and national hospital of pediatrics in Hanoi. Informed consent procedure was also applied to all patients' parents or guardians.

Whole blood samples were taken using plain bottles, preserved in vaccine thermos, then transported within one hour to the study laboratory (Virology Section of Microbiology Department of Bach Mai hospital) where the serum was separated and stored at -70°C. Sera from teenagers were qualitatively tested for rubella-specific IgG by using technique of electro-chemiluminescence immunoassay (ECLIA) (Roche). Sera from young pregnant women and from infants with suspected or clinically-confirmed CRS were measured for Rs-IgMby the same method as for IgG. The threshold value of IgG was ≥10IU/mL and the positive cutoff value for IgM was ≥0.3 IU/mL. The software was used for the data handling and analysis was IBM SPSS Statistics version 20.

3. RESULTS

There were 136 female teenagers from Hanoi-Amsterdam high school participating in the study, and 140 pregnant women recruited from French hospital in Hanoi (79.5% living in Hanoi). Demographic characteristics, rubella contact and vaccination history of the 2 study subpopulations were summarized in table 1.

Table 1. Demographic characteristics, rubella contact and vaccination history

Study subpopulation characteristics		Frequency	Percentage
	In school girls		
Age groups (years)	14-15	129	94.9
	16-17	7	5.1
Mother's age at birth	20-29	93	68.4
	30-35	32	23.5
	≥36	11	8.1
	In pregnant women		
Age groups (years)	≤25	40	28.6
	26-29	42	29.3
	≥30	58	42.1
Order of present pregnancy	First	73	52.1
	Second	46	32.9
	≥Third	21	15.0

Number of miscarriage	None	120	85.7
	One	17	12.1
	≥Two	3	2.2
Rubella contact history during present pregnancy	No	120	85.7
	Yes	20	14.3
Stage of pregnancy when rubella contact occurred	Unknown	124	88.5
	1st trimester	8	5.7
	2nd trimester	5	3.7
	3rd trimester	3	2.1
Rubella vaccination in childhood	No	132	94.3
	Yes	8	5.7
Rubella vaccination prior to present pregnancy	No	129	92.1
	Yes	11	7.9

A history of contact with persons suspected or confirmed rubella reported in 20 (14.3%) of pregnant women, of them 8 (5.7%) in the first trimester. Vaccination against rubella in childhood (5.7%) and prior to present pregnancy (7.9%) was verbally declared. Results of rubella serology in school girls and pregnant women wered presented in table 2.

Table 2. Rubella serology in school girls and pregnant women around the outbreak

Rubella-specific antibodies			N	%
In school girls (N = 136)	IgG	(-)	125	91.9
		(+)	11	8.1
In pregnant women (N = 140)	IgG	(-)	21	15.0
		(+)	119	85.0
	IgM	(-)	101	72.1
		(+)	39	27.9

Rs-IgG was positive in the vast majority of school girls (91.9%) and in 85% of pregnant women. Rs-IgM was positive in 27.9% of pregnant women (39/140). There was a coexistence of IgG and IgM positivity in 38/39 (27.2%) among IgM positive pregnant women. The concentration of Rs-IgG in school girls and pregnant women was depicted in table 3.

Table 3. Distribution of Rs-IgG concentration in study subpopulations

Rubella-specific IgG	School girls, n (%)	Pregnant women, n (%)	p
<10 IU (negative)	11 (8.1)	21 (15.0)	<0.02
10 - <50 IU (weak)	10 (7.4)	35 (25.7)	<0.01
50 - <200 IU (moderate)	29 (21.3)	63 (45.7)	<0.02
200 - <500 IU (strong)	40 (29.4)	14 (10.0)	<0.01
≥500 IU (extreme)	46 (33.8)	5 (3.6)	<0.001
Total	136 (100)	140 (100)	

A strong and extreme titer of Rs-IgG was observed in 86/136 (63.2%) school girls vs. 19/140 (13.6%) in pregnant women (p<0.002); reversely, these were 98/140 (71.4%) pregnant women vs. 39/136 (28.7%) school girls in the group with low-to-moderate antibody titers (p<0.02).

During the surveillance period we identified 113 infants with confirmed CRS (all laboratory-confirmed). Among the CRS cases, 107 were recruited from NHP (15 from infectious disease unit, 51 from neonatology and 41 from cardiology) and 6 from pediatric department of Bach Mai hospital. Mean age of the infants at CRS diagnosis was 38.4 days (range: 1 hour to 152 days) and 61% were girls. 86% (97/113) were born with birth weight less than 2,500g and 42% were born pre-term (gestational age under 37 weeks). Clinical features of CRS in this subpopulation are presented in Table 4.

Table 4. Clinical signs and symptoms of CRS

Clinical manifestations	Frequency (N=113)	Percentage
Thrombocytopenia[a] (<100.000/mm^3)	96	85.0
Neonatal hemorrhage (purpura)	84	74.3
Congenital heart malformation	72	63.7
Splenomegaly	72	63.7
Hepatomegaly	71	62.8
Anemia	69	61.1
Blueberry muffin rash	69	61.1
Hepatitis (liver enzymes > 2 UNL)	54	47.8
Microcephaly[b]	41	36.3
Hearing loss at 1st month	37	32.7
Deafness at birth	35	31.0
Cataract	29	25.7
Other eye diseases	24	21.2
Developmental delay	3	2.6

[a] Thrombocytopenia lasted for more than 15 days in 71 cases, [b] Head circumference at birth <2 SD for fetal age.

Clinical manifestations of CRS included low birth weight <2500 g (86%), congenital heart disease (63.7%), hearing impairment (63.7%), ophthalmological involvement (46.9%). Other clinical disorders included hepatomegaly (62.8%), hepatitis (47.8%), splenomegaly (63.7%), thrombocytopenia (85%), neonatal purpura (74.3%), anemia (61.1%), blueberry muffin rash (61.1%), microcephaly (36.3%) and mental retardation (2.6%). There are 44 CRS cases with multi-organ abnormality, of them 16 cases with 3 major CRS defects, 28 cases with 2 major CRS defects (14 cases with deafness and heart defects, 14 cases with eye and heart defects). Most of congenital heart defects in children with CRS could be detected by ultrasound (Figure 1).

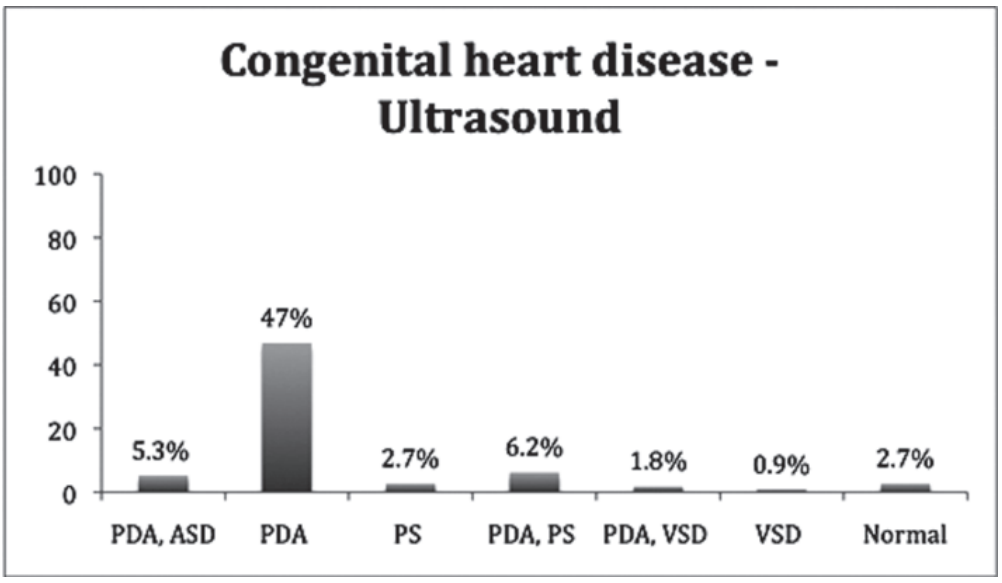

Figure 1. Distribution of heart defects detected by ultrasound in children with CRS

PDA: persistent ductus arteriosus, ASD: Atrial septal defect, VSD: ventricular septal defect, PS: pulmonary stenosis, "Normal": absence of congenital heart abnormality but lethal acute heart failure.

4. DISCUSSION

This was the first study in Vietnam, in close collaboration with Vietnam-Australia "Hoc mai" (Learning Forever) Foundation, allowing to assess in the same time the rates of acquired immunization in school girls, susceptibility to rubella in pregnant women, and congenital rubella syndrome (CRS) in infants during and just after the outbreak of rubella epidemic in late 2010 and early 2011.

In recent years, rubella and its consequence (CRS) have been occupied an increased importance in public health. In 45 developing countries, where serosurveys in women of childbearing age have enrolled more than 100 individuals, the proportion of women who remained susceptible to rubella (i.e. seronegative) was 10% in 13 countries, 10-24% in 20 countries and 25% or higher in 12 countries [4]. In the present study, we found seropositivity in 91.9% of school girls aged from 14 to 17 years old. This rate is clearly higher than 67.7% from Vietnamese young women community living in Taiwan [8]. Our results showed that the proportion of young women susceptible to rubella situated in the lowest group among above-cited 45 developing countries. Nevertheless, our serosurvey was carried out just after rubella outbreak, during which inevitably a certain proportion of young girls acquired natural immunization, then lowered proportion of young women susceptible to rubella in the community.

Our study carried out during rubella epidemic showed 27.9% (39/140) of young pregnant women (aged 18 to 35 years) got primary infection, i.e. at very high risk of giving infants with CRS. This unacceptable high rate of rubella infection in pregnant women was production of multi-facet factors issuing from data of our study, including nearly null vaccination, negative or low-to-moderate titers of protective IgG antibodies (15%, 27.5% and 45.7%, respectively), total absence of prenuptial rubella serology for screening susceptibility status and recommending vaccination. In the convergence of these favorable conditions for rubella infection, with current outbreak of rubella prevailing in the community, it is not surprising to get so high

percentage of primary infection of rubella in this group of pregnant women.

It is well known that primary rubella infection occurring during pregnancy will induce fetal infection and high rate of CRS in infants, depending on the term of gestation. The infection occurring in the first 12 weeks of pregnancy causes congenital rubella infection in 90% with almost a 100% risk for congenital defects. From 13 to 17 weeks, the risk of infection is about 60% with about 50% risk for congenital defects. From 18 o 24 weeks, the risk of infection is about 25% with hardly any risk of congenital defects [1-4]. Our data showed that in the present conditions, it is very hard to obtain exact detail on history of rubella contact even during outbreak. We could documented only 20/140 (14.3%) cases in whom a suspected history of contact with persons suffering from disease with rashes; half of the cases happened in the first trimester of gestation.

In our study, among 21 pregnant women staying susceptible to rubella (IgG negativity), only one case infected, the rest of 20 cases fortunately stayed intact through the current outbreak of rubella. Adding to 39 infected cases, we could count 59/140 (42.1%) pregnant women susceptible to rubella before the current outbreak of rubella. This figure suggests a terrible perspective for this subpopulation being subject to contracting rubella at any time, including pregnancy time if an outbreak of rubella happens.

Among 39 pregnant women acutely infected by rubella (IgM positive), except the above-mentioned case with IgG negativity, in the rest of 38 cases, a co-existence of rubella-specific IgG and IgM was noted with low to moderate titers of IgG in a majority of cases (25.7 and 45.7%, respectively) and only small percentage of strong or extreme titers of IgG (10 and 3.6%, respectively). This distribution is total inverse to those in school girls investigated just after the outbreak (table 3). It is hardly to know if these cases with low protective titers of IgG antibodies who contracted acute rubella from were among those who had been totally susceptible to rubella (IgG negative) then infected by rubella for a rather long duration that allows the body to produce IgG plus residual IgM as previously reported. However, it is really impossible to classify them among those who recently infected by rubella with phenomenon of "persistent IgM response" as 6 original cases reported in United Kingdom [9]. The fact that all of 5 cases with extreme titers of IgG (≥500 IU/mL) possessed very low titers of IgM (virtually 0.34; 0.30; 0.36; 0.31 and 0.38 IU/mL) suggests an acute infection of rubella lasted for a certain time and then IgM was on the way of waning. In daily practice, coexistence of IgG and IgM and lack of previous serum stored for retesting of paired samples always cause anxiety in couples and create stressful dilemma for obstetricians because it is impossible in such a circumstance to diagnose or to determine exact or approximate moment of the infection in relation to gestational age of fetus, consequently impossible to shape the appropriate advice (continuing or stopping the pregnancy) to couples during counseling. In such a scenario, most of the time one should require referring obstetricians in referral fetal medicine center with availability of modern techniques (test for avidity of antibodies, viral diagnostic techniques and procedures such as PCR, amniocentesis), specialists and experts. Rubella immunization is yet to be implemented in the national immunization program in Vietnam. As suggested by the WHO/CDC recommendation [3,4,10], in low-income countries without national rubella immunization program, it is important to have pre-pregnancy or preferably prenuptial screen for rubella to all pretending brides to obtain a baseline and useful immunological status which enables vaccination at least 3 months prior to pregnancy planning in seronegative young women of child bearing age.

Our study is one of the first prospective surveillance on CRS in Vietnam allowing identifying 113 laboratory confirmed cases of

CRS during a 10 month surveillance period. Our estimated incidence of CRS in Vietnam was high (1.13/1,000 live birth, 95% CI 0.92-1.34), although is likely still an underestimate. The previous study reporting CRS incidence in Vietnam is based on rubella seroprevalence among pregnant women and their estimated CRS incidence was higher than our (234 per 100,000 live births) [6]. Another study conducted at the National Obstetric Hospital in Hanoi reported an incidence of 210 per 100,000 live births [11]. These differences could be due to several factors including under-reporting, lack of diagnostic facilities and referral services or failure to recognize infants with less severe clinical manifestations. Moreover, we were unable to include CRS cases that resulted in early pregnancy loss (e.g. miscarriage and abortion). After the 2011 rubella outbreak in Vietnam, 281 CRS cases have been identified and reported through the WHO passive surveillance system (189 in 2011 and 92 in 2012). However, our surveillance shows that the number of CRS cases identified by passive surveillance only represents the tip of the iceberg. The actual burden of CRS in Vietnam is yet to be determined.

Beside transient clinical manifestations of CRS (e.g. thrombocytopenia, blueberry muffin rash), serious and persistent defects of CRS were highly prevalent in our cohort. Almost two-third (63.7%) children had sensoroneural hearing loss and nearly half had eye defects (e.g. cataract, retinopathy). Multiorgan involvements were common and majority of children would suffer from life-long disability. The number of children with hearing loss in our cohort was comparable to published literature [1,13]. However, the frequency of other impairments was higher than reports from other parts of the world [12,13].

In limited conditions and resources, we could neither be able to increase sample size nor design a representative study with several study sites or settings, nor carry out serial tests for rubella serology in pregnant women as recommended by WHO and CDC experts [2-4]. The fact that the study was carried out during and just after an outbreak of rubella influences in some extent on the serology results as well as on the interpretation of baseline immunological status of school girls and pregnant women. Despite our best effort there are several limitations to our study. Our reported incidence of CRS is likely to be an underestimate as our surveillance was not designed to capture any abortion or stillbirth due to congenital rubella infection. Moreover, CRS cases with subtle defects (e.g. mild hearing impairment) might have been missed as newborn hearing screening is not routine in Vietnam. However, our study generated valuable information on the current burden of CRS in Vietnam and also provided future directions for a larger national study.

5. CONCLUSION

This preliminary, baseline per/post epidemic information showed a high rate of acquired immunization in female adolescents but also high rate of susceptibility and very high rate of rubella infection in pregnant women, as well as a very high burden of CRS in Vietnam, warranting further comprehensive studies to determine robust, nationwide epidemiological data prior to establishing the national program of immunization against rubella and identify appropriate methods for CRS surveillance in Vietnam.

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