

Testing of IgG and IgM against Rubella Virus in School Girls and Pregnant Women in Hanoi where an Outbreak had occurred

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Abstract

Background: Congenital Rubella Syndrome (CRS) is preventable illness but stays frequent in developing countries where rubella vaccination is not incorporated in national program of vaccination like in Vietnam actually.

Aims: 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, Vietnam.

Population and methods: During and after the rubella outbreak in 2011, sera from 136 school girls (aged from 14 to 17 years) and 140 pregnant women in Hanoi French hospital were tested for rubella-specific IgG and for rubella-specific IgM (only in pregnant women) by the technique of Electro-Chemiluminescence Immunoassay (ECLIA) (Roche Diagnostics) with cut-off value ≥ 10 IU/mL for IgG and ≥ 0.3 IU/mL for IgM.

Results: Rubella-specific 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 ($p < 0.002$). Rubella-specific IgG was negative (susceptible to rubella infection) in 21/140 (15%) and rubella-specific IgM antibodies was positive in 27.9% (39/140) pregnant women, of whom 38 (27.1%) a co-existence of rubella-specific IgG and IgM were documented.

Conclusions: 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, 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.

Keywords: Acquired immunization; Rubella infection; School girls; Pregnant women; Congenital Rubella Syndrome (CRS)

Introduction

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) [1-4]. The World Health Organization (WHO) estimates that around 238,000 children are born with CRS every year, the majority of whom live in developing countries [4-9]. The frequency of CRS varies in different parts of the world, depending on levels of naturally acquired immunity, overcrowding and immunization policies and practices [10-13]. During epidemics of rubella, CRS has been reported in 0.6-2.2/1,000 live births [6-7]. The WHO and the Children's Vaccine Initiative have produced guidelines for surveillance of CRS and rubella [5-13], and

targets for accelerated rubella control and CRS prevention have been established by the Western Pacific Region (WPR) [11-13]. 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 [6,10-13] but also on serological status of rubella in childbearing age and pregnant women [6,7,14-20].

In Vietnam, where rubella virus of genotype 2B circulates [21], the rubella vaccine, well-known for its effectiveness [5,10-13], has not been incorporated yet into the national immunization schedule. In late 2010 and early 2011, an epidemic of rubella had been confirmed [21-23]. Consequently, at least 7,259 cases of rubella had been identified resulting in 189 CRS cases in 2012 rubella outbreak in Vietnam, according to National Health Authority Reports to WHO [11]. However, few studies conducted antenatal serosurveys to assess the proportion of women at risk for rubella infection in pregnancy [22]. 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 due to CRS and determine rates of acquired immunization in young women as well as to identify susceptible rate in women of child-bearing age. Our study, conducted around the outbreak of rubella in late 2010 and early 2011, aimed at assessing (1) the status of acquired immunization against rubella in school girls, and (2) the susceptibility and the recent rubella infection rate in pregnant women. Data from this pilot study will be used for planning a larger multi-center study in view of controlling rubella and CRS in Vietnam.

Population and Methods

This prospective study was carried out between May 2011 and March 2012 in Hanoi, where an outbreak of rubella had occurred. After obtaining parental consent, blood samples of female students of Hanoi-Amsterdam high school were taken to determine acquired rubella immunization rate. Meanwhile, pregnant women who presented in Vietnam-France hospital for prenatal care were enrolled to investigate history of rubella contact and immunization and determine the rates of susceptibility as well as recent rubella infection. Whole blood samples were taken using plain bottles, preserved in vaccine thermos, then transported within 1 hour to the laboratory of Virology Section of Microbiology Department of Bach Mai hospital (WHO's reference laboratory of microbiology for Pacific Asian Region) 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 Diagnostics). Sera from young pregnant women were qualitatively tested for rubella-specific IgM and rubella-specific IgG by the same method. The threshold value of IgG at ≥ 10 IU/mL was considered as protective and the positive cutoff value for IgM was ≥ 0.3 IU/mL. The software used for the data handling and analysis was IBM SPSS Statistics version 20.

Results

There were 136 female teenagers from Hanoi-Amsterdam high school participating in the study; all of them were from 14 to 17 years old (average 14.8 ± 2.1 years old). The study also recruited 140 pregnant women from French hospital in Hanoi; their average age was 28.7 ± 4.7 (from 18 to 42 years old); most of them are from Hanoi (79.53%). Demographic characteristics, rubella contact and vaccination history of study subpopulations were summarized in the Table 1.

| Study population characteristics | Frequency | Percentage |
|--|-----------|------------|
| <i>In school girl subpopulation</i> | | |
| Age groups (years) | 14 | 22.1 |
| | 15 | 72.8 |
| | 16-17 | 5.1 |
| Mother's age at birth | 20-25 | 32.4 |
| | 26-29 | 36.0 |
| | 30-35 | 23.5 |
| | ≥ 36 | 8.1 |
| <i>In pregnant women subpopulation</i> | | |

| | | | |
|--|---------------|-----|------|
| Age groups (years) | ≤ 25 | 40 | 28.6 |
| | 26-29 | 42 | 29.3 |
| | 30-35 | 50 | 35.7 |
| | >35 | 8 | 6.4 |
| Rubella contact history during 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 pregnancy | No | 129 | 92.1 |
| | Yes | 11 | 7.9 |

Table 1: Demographic characteristics, rubella contact and vaccination history

Most of school girls were 14 and 15 years old (94.9%) and 68.4% of them were born when their mothers were 20-30 years old. A history of contact with persons suspected or confirmed rubella 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 declared verbally (not firmly documented). Results in serology of rubella in school girls and pregnant women were presented in the Table 2.

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

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

Rubella-specific IgG antibody was positive in the vast majority of school girls (91.9%) and in 85% of pregnant women. Rubella-specific IgM antibody 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 levels of rubella-specific IgG antibody in school girls and pregnant women were depicted in the Table 3.

| 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) | |

Table 3: Distribution of rubella-specific IgG levels in study subpopulations

Accordingly, 86/136 (63.2%) school girls vs. 19/140 (13.6%) pregnant women belonged to groups of strong to extreme titers of rubella-specific IgG antibody ($p < 0.002$); reversely, 98/140 (71.4%) pregnant women vs. 39/136 (28.7%) school girls belonged to groups of low-to-moderate antibody titers ($p < 0.02$).

Following-up the pregnancy outcome from 39 women with IgM positivity showed that 2 cases dropped out (probably hidden stopping pregnancy); 27/37 stopped pregnancy (18 in the first trimester, 9 in the early second trimester of gestation); and 10 finally completed their pregnancy. Among 10 newborns, 1 suffered from CRS, including PDA plus low birth weight (2.560 grams) and thrombocytopenic purpura; 2 with severe but transient thrombocytopenic purpura recovered without consequence; 2 others with low birth weight (2.500 and 2.700 grams). All these 5 newborns were IgM positive. The 5 other newborns stayed normal at birth and during neonatal period but impossible to follow up (neither clinical nor laboratory data possibly documented) due to lack of parental consent after birth.

Discussion

This was the first study in Vietnam, in close collaboration with Vietnam-Australia "Hoc Mai" (Learning Forever) Foundation, allowing assessing 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. Data on CRS will be published in another article.

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 [6,7,14-20]. 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 documented by Tseng et al. [18]. However, it is very similar to 92.5% in Turkish school girls reported by Karakoc et al. [19]. The data from different parts of the world were in accordance with our results. The proportion of young women susceptible to rubella in our study is 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 the natural immunization, and then lowered proportion of adolescents susceptible to rubella in the community.

Large proportion (63.2%) of school girls in our study belonging to the group of strong-to-extreme concentration of rubella-specific IgG antibodies in comparison to that in pregnant women (13.6%) might be an evidence backing-up for this argument. This important notion will be clarified by the results of an ongoing study on the same topic but with larger study population, sponsored by Hanoi municipality.

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, including very low rate of vaccination (5.7% in childhood and 7.9% prior to present pregnancy), negative or low-to-moderate titers of protective IgG antibodies (15%, 27.5% and 45.7%, respectively), total absence of prenatal rubella serology for screening susceptibility status. In the convergence of these conditions favorable for rubella infection, with current outbreak of rubella, it is not surprising to see so high rate of primary rubella infection in these pregnant women, while. This rate in others developing countries varied only between 1-2%.

It is well known that primary rubella 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; and from 18 to 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 details 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. It is not surprised that 27 (or even 29) among 39 pregnant women had chosen a termination of pregnancy. For the rest of 10 cases, whose pregnancy were completed, at least 1 cases of CRS identified and 4 others with some clinical signs of congenital rubella infection.

In our study, among 21 pregnant women stay susceptible to rubella (IgG negative), only one case infected, and 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 [24]. This figure suggests a terrible perspective for this population being subject to getting rubella infection at any time. 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 belonged to group with low

to moderate titers of IgG in 25.7 and 45.7% of cases, and only small percentage (10 and 3.6%, respectively) belonged to group with strong or extreme titers of IgG. This distribution is total inverse to those in school girls investigated just after the outbreak (Table 3). It is impossible to know if these cases with coexistence of low titers of IgG and IgM antibodies belong to newly produced IgG plus residual IgM as reported by Kremer et al. [25], or to phenomenon of "persistent IgM response" as 6 original cases reported by Best et al. [26]. 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) might suggest an acute infection of rubella but 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 for couples and create dilemma for obstetricians because it is impossible in such a circumstance to determine exact or approximate moment of the infection in relation to gestational age of fetus, consequently impossible to shape the appropriate advice to couples. In such a scenario, most of the time one should require referring obstetricians in referral fetal medicine centers with availability of specialists and experts and particularly modern techniques (test for avidity of antibodies, viral diagnostic techniques and procedures such as PCR, amniocentesis).

In the point of view of preventive medicine and public health, while waiting mass vaccination set up and become effective to eradicate rubella, it is crucial for low-income nations to apply prepregnant or preferably prenuptial screening for rubella to all pretending brides to obtain a baseline and immunological status which enables prescription of rubella vaccination in seronegative young women in term of controlling CRS.

In limited conditions and resources, we could neither be able to increase sample size nor design a representative study with several study sites, nor carry out serial tests for rubella serology in pregnant women as recommended by WHO experts [27]. 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. It warranted further well-designed studies for national baseline of rubella immunization and susceptibility that could endorse national policies and appropriate techniques for vaccination against rubella in perspective of attaining targets for accelerated rubella control and CRS prevention set up by WHO in Western Pacific Region [11-13].

Conclusion

In this preliminary research carrying during and just after an outbreak of rubella epidemic in Vietnam, we found a high rate of protective levels of rubella-specific IgG in school girls, and also an unacceptably high rate of acute infection of rubella in young pregnant women with high risk of CRS burden in the country. It warrants further robust studies by free-of-epidemic periods of time to obtain more credible baseline on immunological status of rubella in childbearing age women prior to setting up strategies for rubella control and CRS prevention.

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