CONGENITAL RUBELLA SYNDROME: A REVIEW OF LABORATORY DATA FROM 2002 TO 2011

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Abstract. Rubella infection in pregnant women during the first trimester of pregnancy can lead to fetal anomalies, commonly known as congenital rubella syndrome (CRS). The objective of our study was to analyze the serological test results among infants suspected of having CRS aged ≤12 months compared with their clinical status. Between January 2002 and December 2011, 3,279 serum samples from infants aged ≤12 months from government hospitals in Malaysia were examined for rubella specific IgM and IgG antibodies using a Axsym, automated analyzer (Abbott Laboratories). Forty-eight samples were positive for rubella specific IgM antibodies and 494 samples were positive for rubella specific IgG antibodies. These were then age stratified and their clinical history reviewed for any CRS symptoms. Fifteen of 38 rubella IgM positive infants (39.5%) aged <3 months, had a clinical appearance compatible with CRS. However, only 1 IgM positive infant aged 3 to 6 months and one infant aged 7 to 11 months had clinical appearance compatible with CRS. The most common abnormal findings in these cases were congenital heart defects and cataracts. Forty-eight point eight percent of IgM positive cases and 53.1% of IgG positive cases, had inadequate information in the chart to determine the presence of CRS. Clinical findings and timely laboratory diagnosis to determine the presence of CRS are important in infants born with congenital defects. Physicians should also be aware of the appropriate interpretation of these findings.

Keywords: rubella, CRS, diagnosis, specific IgM, IgG

INTRODUCTION

Rubella is a self-limiting viral infection caused by the rubella virus, a member of the Togaviridue family, *Rubivirus* genus. Rubella infection among pregnant women during the first trimester of pregnancy can lead to fetal anomalies and death of the fetus. Important consequences of congenital rubella infection (CRI) are miscarriages, stillbirths and congenital anomalies known as congenital rubella syndrome (CRS) (Robertson *et al*, 2003). As a result of these disabilities, some patients need expensive medical attention, lifelong support and social support.

Birth defects most commonly associated with CRS include auditory (*eg*, sensorineural deafness), ophthalmic (*eg*, cataracts, microphthalmia, glaucoma, chorioretinitis), cardiac (*eg*, patent ductus arteriosus, peripheral pulmonary artery stenosis, atrial or ventricular septal defects), and neurologic abnormalities (*eg*,

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microcephaly, meningoencephalitis, mental retardation). Infants with CRS often exhibit intrauterine growth retardation and postnatal growth delay (Singla *et al*, 2003). Other conditions associated with CRS include radiolucent bone defects, hepatosplenomegaly, thrombocytopenia, and purpuric skin lesions (CDC, 2001).

Immunization against rubella is the only effective way of preventing fetal infection during pregnancy and CRS. The World Health Organization (WHO, 2003) reported in 2002, 123 countries/territories were using the rubella vaccine routinely. In Malaysia, the monovalent rubella vaccine was introduced in 1987 for immunization among female schoolchildren 12 years old and in reproductive age women (Narimah, 2005). Although high vaccine coverage rates were reported, CRS cases were still encountered in hospitals and serological surveys indicated about 40% of the female population of child bearing age were susceptible (Cheong and Khoo, 2008). Since 2002, the rubella vaccine has been given in combination with mumps and measles (MMR) in Malaysia targeting both boys and girls at ages 1 and 7 years. MMR coverage rates in Malaysia have been above 85% (Saraswathy *et al*, 2009).

High immunization rates among children can reduce rubella risk for seronegative women but they do not eliminate the risk. Circulation of the rubella virus in the community, in the family or in other settings where susceptible women are present may result in CRS. The WHO has recommended all countries include the rubella vaccine in their immunization services and conduct surveillance for CRS and rubella. Besides monitoring the effectiveness of rubella vaccination programs surveillance can detect infants more rapidly and potentially reduce the consequences of the disease to the infants and their families through early provision of appropriate medical care.

The clinical diagnosis of CRI and CRS is difficult and may not always be possible. Infants moderately or severely affected by CRS are readily recognizable at birth, having more than one sign or symptom consistent with CRS. However, infants may present with a single defect; hearing impairment being the most common single defect (CDC, 2012). Deafness and other mild CRS deficits, such as some cardiac obnormalities may only be detected months or years after birth, or not at all (Lorraine *et al*, 2008). Clinical signs may be transient or confused with measles or parvovirus B19 infection (Thomas *et al*, 1999). Infants with CRS may shed the virus for a prolonged period in the urine, feces or skin and may be infectious until they are at least 1 year old (CDC, 2012).

Early diagnosis of infants with CRS requires a high index of suspicion to facilitate early intervention for specific disabilities. Infants with CRS and CRI may shed rubella virus for long periods (60% for the first 4 months of life) and appropriate infection control measures should be applied (WHO, 2006). Acquired rubella virus infection is transmitted via airborne droplets from the upper respiratory tract of active cases. Early diagnosis is important to prevent further spread of the virus. It is particularly important that pregnant women who are not rubella-immune not be exposed to infants with CRI. Laboratory confirmation is essential to confirm suspicion of CRI. Detection of virus specific IgM and IgG in serum is the most commonly used method to confirm the infection because it is simple to perform and does not require sophisticated equipment or training to conduct.

In this study, we reviewed laboratory data from suspected CRS cases between

2002 and 2011 among children aged less than 12 months. The objective of our study was to determine the predictive role of rubella antibodies in the diagnosis of CRS.

MATERIALS AND METHODS

Patient samples

The samples used in this study were obtained from theVirology Unit, Institute for Medical Research (IMR), Malaysia, from January 2002 to December 2011. The samples belonged to children aged less than 1 year. The tests were ordered by attending physicians for suspected cases of CRI and CRS. The samples were also accompanied by patient details and clinical history.

Clinical spectrum of congenital rubella infection

The clinical history and symptoms used to diagnose CRI/CRS, according to the CDC case definition were obtained from the forms (CDC, 2012). The symptoms were divided into categories. Category was based on clinical conditions described for suspected and probeble cases.

Category A: the presence of congenital cataracts, glaucoma, heart disease (most commonly patent ductus arteriosus or peripheral pulmonary artery stenosis), hearing impairment or pigmentary retinopathy.

Category B: the presence of purpura, hepatosplenomegaly, jaundice, microcephaly, hydrocephaly, intrauterine growth retardation, developmental delay, meningoencephalitis or radiolucent bone disease.

No clinical history or history non-specific for CRI: the presence of clinical symptoms non-specific for CRI and none of the problems found in Category A or Category B.

Rubella serological assay protocol

Serum samples were tested for rubella specific IgM and IgG using the Axsym Automated Analyzer (Abbott Laboratories, Abbott Park, IL) untill January 2011. Kit controls as well as in-house positive and negative controls from our laboratory were tested. Beginning in February 2011, serum samples were tested for rubella specific IgM and IgG using the Enzygnost Anti-Rubella-Virus IgM and IgG enzyme immunoassays (Siemens), respectively. The test procedure, interpretation and validation of test results were conducted following the manufacturer's instructions.

RESULTS

Between January 2002 and December 2011, a total of 3,279 serum samples were received from infants aged ≤12 months from government hospitals to test for rubella specific IgM and IgG. Of those 661 samples were performed among children with fever and rash. The serum samples were also tested for measles IgM and IgG using an Enzygnost ELISA kit (Dade Behring, Marburg, Germany). One hundred sixteen samples were positive for measles IgM; 48 were positive for rubella specific IgM and 494 were positive for rubella specific IgG (Table 1).

Cases positive for rubella serology were age stratified and their clinical histories were obtained from the laboratory investigation forms and reviewed to determine the presence of CRS symptoms (Table 2). Twenty-one out of the 48 cases (43.8%) positive for rubella IgM had no recorded clinical history. Seventeen cases were aged '0-3 month'. In 287 of the 494 cases (58.1%) positive for rubella IgG no history was recorded or the symptoms were non-specific for CRI.

Table 1
Specimens tested for rubella specific IgM and IgG during January 2002-December 2011 at the Institute for Medical Research, Malaysia.

Year	Number of specimens	Number of specimens positive for rubella IgM (ages in months)				Number of specimens negative for rubella IgG (ages in months)			
		0-3	4-6	7-11	12	0-3	4-6	7-11	12
2002	389	2	0	0	0	0	0	4	2
2003	444	4	1	0	3	2	0	0	4
2004	353	1	0	1	0	1	0	0	0
2005	452	6	0	0	0	6	0	2	0
2006	317	2	1	0	0	1	0	2	2
2007	302	2	1	0	2	1	1	0	4
2008	317	4	0	0	1	81	4	7	8
2009	370	9	1	0	2	217	8	4	7
2010	105	0	0	1	1	37	8	1	4
2011	230	2	0	0	1	68	2	1	5
Total	3,279	32	4	2	10	414	23	21	36

Table 2
Categorization of clinical findings among rubella IgM and IgG positive infants, 2002-2011.

Clinical category	Number of specimens positive for rebella IgM (ages in months) (*n*=47)				Number of specimens positive for rubella IgG (*n*=490)	Total
	0-3	4-6	7-11	12		
Category A: presence of congenital cataracts, glaucoma, heart disease (most commonly patent ductus arteriosus or peripheral pulmonary artery stenosis), hearing impairment or pigmentary retinopathy.	2	1	0	0	33	36
Category B: presence of purpura, hepatosplenomegaly, jaundice, microcephaly, developmental delay, meningoencephalitis or radiolucent bone disease.	13	0	1	4	174	192
No clinical history or history non-specific for CRI (*eg*, fever, rash only, sepsis or bronchopneumonia)	17	3	1	6	287	314
Total	32	4	2	10	494	542

DISCUSSION

Diagnosis of CRS among newborn children usually focuses on virus isolation from nasal, throat and urine samples or the detection of rubella-specific IgM in cord or child serum samples or virus detection by RT PCR (CDC, 2001). The demonstration of virus specific IgM has been the method of choice to diagnose CRS in many developing countries since the ELISA test is cost effective and easier to set up in hospital pathology laboratories. Rubella specific IgM can be found in nearly 100% of infected infants aged less than 3 months; however, IgM has been reported to gradually decrease to less than 50% by 12 to 18 months of age (Abraham *et al*, 1999). In our study, 15 out of 38 infants (39.5%) aged less than 3 months, with rubella specific IgM antibodies had findings categorizing them into either Category A or B, defining them as having CRS. The most common clinical problem reported in children aged less than 3 monghs with positive rubella IgM antibodies were congenital heart defects and cataracts. One out of 38 children (2.6%) aged 3-11 months with positive rubella IgM antibodies had congenital heart defects or cataracts. Although rubella specific IgM has a high predictive value for CRS among children aged less than 3 months, many infected children are not screened for rubella specific antibodies. Only 32 children were tested for rubella specific IgM between 2002 and 2011. One explanation for this is that some symptoms may be subclinical or difficult to detect in newborns, such as hearing defects. This requires a high index of suspicion. Rural hospitals in developing countries may be able to test for rubella antidodies and in rural areas women may deliver at home and may not present to a hosptial until months later.

The serological diagnosis of CRI/CRS is not without challenges (Best *et al*, 2002; Craig and Hardie, 2005). The sensitivities and specificities of assays differ from eachother (Tipples *et al*, 2004). Assays may not detect low circulating antibody levels. The results may depend on how much time has elapsed between infection and sample collection. Some infected newborns may not have detectable IgM antibodies at birth. This could be due to the presence of high titers of rubella specific IgG from the mother and child, interfering with IgM antibody binding. False positive rubella IgM results have been reported with other viral infections, such as with acute Epstein-Barr virus infection (infectious mononucleosis), cytomegalovirus infection and parvovirus B19 infection (Thomas *et al*, 1999). The limitations of the assays need to be understood by laboratory personnel in order to avoid misinterpretating the results (Jin and Thomas, 2007).

Where there is clinical suspicion of CRS in an infant but the IgM test result is negative or equivocal, rubella specific IgG should be examined. However, infants aged less than 6 months may have passive transfer of maternal IgG antibodies. In infants with Category A or B findings and a negative test for rubella specific IgM but a positive test for rubella specific IgG, laboratory follow-up is necessary to determine the persistence of IgG levels. The laboratory may request a second sample but this is rarely done. Each case must be assessed individually, taking into account factors such as age, vaccination history, maternal history and presence of clinical findings. A complete clinical and contact history of the patient should be obtained. IgG detected after 12 months may be indicative of rubella vaccination. In Malaysia the first dose of the MMR vaccine is given

to infants aged 12 months. Although vaccination history was not reported on the laboratory request form, fever and rash could have been post-vaccination side effects. Forty children in our study had positive rubella IgG antibodies and positive measles IgG antibodies, indicating MMR vaccination.

The clinical description of fetal anomalies is useful to support the laboratory diagnosis of CRS. However laboratory investigation forms may not have an adequate clinical history to correlate with the laboratory data. In our study 48.8% of IgM positive cases and 53.1% of IgG positive cases, either did not have any information or the information was inadequate or non-specific for CRI. It was difficult to determine the predictive value of the results for CRS without complete clinical information on the laboratory forms.

The laboratory findings are important for evaluating infants born with congenital defects. The WHO has recommended disease surveillance should focus on detecting cases of CRS. It is important for physicians to understand the importance of laboratory testing for CRS, the best timing for collection of laboratory specimens and the interpretation of the results and its limitations. Clinicians should never solely rely on the serologic detection of rubella specific IgM or IgG antibodies but must consider the clinical presentation.

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