

Congenital Rubella Syndrome: A Case Report on Changes in Viral Load and Rubella Antibody Titers

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To our knowledge, this is the first report of the use of real-time reverse transcription–polymerase chain reaction to assess changes in viral load in a patient with congenital rubella syndrome (CRS). Rubella-specific antibody titers were also determined. The patient was a male neonate born to a primipara with rubella infection at 10 weeks of gestation. He had no symptoms at birth, but rubella virus was detected in his pharynx, blood, and urine. His mental and physical development was normal for 1 year; however, he was diagnosed with deafness at 13 months of age. Thus, the patient was diagnosed with CRS. Rubella infection in the pharynx was almost constant until 5 months of age; however, it increased dramatically at 6 months of age. No infection was detected at 13 months. Rubella-specific immunoglobulin M titer was consistently low until 9 months of age and then decreased gradually until it became negative at 20 months of age. Rubella-specific immunoglobulin G titer was high at birth. However, it decreased at 3 months and increased again at 4 months. This titer peaked at ~9 months and then decreased again at 13 months. This case shows that the period after the decline in maternal antibody titers, not the neonatal period, may be the most contagious period in patients with CRS.

Rubella belongs to the family *Togaviridae* and is a causative virus of congenital rubella infection (CRI).¹ CRI refers to all outcomes associated with intrauterine rubella infection, including miscarriage, stillbirth, a combination of birth defects, and asymptomatic infection.² Congenital rubella syndrome (CRS) refers to a CRI that occurs in various congenital defects (eg, deafness, cataract, and heart abnormalities).^{2,3} Deafness is usually congenital, but a few cases of deafness after birth have been reported.^{3,4} Papania et al⁵ reported in 2014 that since 2004 the incidence of rubella in the United States was <1 per 10 000 000 individuals and that the incidence of CRS was <1 per 5 000 000 births. Thus, on the basis

of this information, we can say that rubella has generally been eliminated from the United States. On the other hand, there are many individuals with rubella worldwide because the rubella vaccine has not been introduced in several countries. In fact, in Asia, there are many patients with rubella and CRS/CRI.^{6,7} In Japan, rubella epidemics have been suppressed by public measles/rubella vaccination of children. However, from 2012 to 2013, a rubella outbreak occurred, and 45 patients with were reported from week 42 in 2012 to week 40 in 2014.^{8,9}

Patients with CRS and some patients with CRI continue to produce the rubella virus for a long time,¹⁰ and these patients spread the infection.^{11–13}

abstract

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Dr Nagasawa examined the patient, collected samples, and drafted the initial manuscript; Dr Ishiwada examined the patient and reviewed and revised the manuscript; Mr Ogura performed real-time reverse transcription–polymerase chain reaction and reviewed and revised the manuscript; Dr Ogawa performed virus isolation and reviewed and revised the manuscript; Drs Takeuchi and Shimojo examined the patient and reviewed the manuscript; Dr Hishiki examined the patient, collected samples, and reviewed the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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However, to our knowledge, there are no data on how rubella viral load and titers of rubella antibody change in patients with CRS/CRI. To clarify these changes in rubella viral load, proper infection control measures need to be applied. This article reports the case of a patient diagnosed with CRI at birth, and later with CRS after the development of hearing loss. Changes in rubella viral load were also assessed by performing real-time reverse transcription–polymerase chain reaction (RT-PCR) assays and measuring the titer of antibodies to rubella.

PATIENT PRESENTATION

The patient was a male neonate born to a 19-year-old primipara who did not have rubella infection and had not been vaccinated before pregnancy. She developed rubella on the third day of 10 weeks of gestation. On the fifth day of 20 weeks of gestation, the amniocentesis fluid was positive for rubella, as determined by RT-PCR. However, no abnormality was found on ultrasonography and the growth of the fetus was good during the gestational period. Finally, the patient was born via vaginal delivery on the third day of 39 weeks of gestation.

At birth, the patient's general condition was good, with Apgar scores of 8 (at 1 minute) and 9 (at 5 minutes). His birth weight was 2770 g. Physical examination revealed no microcephalus and no hepatosplenomegaly. There were no ophthalmologic complications. Laboratory values were as follows: white blood cell count of 10 600 per μL , platelet count of 94 000 per μL , an aspartate aminotransferase level of 60 U/L, an alanine aminotransferase level of 17 U/L, a total immunoglobulin (Ig) M level of 101 mg/dL, and a rubella-specific IgM titer of 10.7 (normal value: <0.8). The automatic auditory brainstem response was normal. No congenital cardiac anomaly was

detected on echocardiography. Rubella RT-PCR assays of whole blood, urine, stomach fluid, and pharyngeal sampling were positive. From these findings, CRI was diagnosed. The patient had been admitted to our hospital, but because he had no symptoms he was discharged from the hospital with his mother.

At the 1-month medical check-up, the patient's eyes tracked light normally and he reacted appropriately to sound. His body weight was 4092 g, and the platelet count was 166 000 per μL . The patient met normal physical and mental developmental milestones. However, at ~13 months of age, his parents reported that he always held toys to his left ear. Otitis media with effusion was diagnosed and was treated, and the patient underwent an audiology assessment. Bilateral sensory deafness was found, and CRS was diagnosed.

During almost all monthly check-up sessions after birth up to 20 months of age, samples were obtained to assess the following: rubella virus in whole blood, urine, and pharynx by real-time RT-PCR (Fig 1) and RT-PCR; isolation of the rubella virus from a pharyngeal swab sample; and rubella-specific IgM and IgG serum levels (Fig 2). Real-time RT-PCR, RT-PCR, and virus isolation were performed at the Division of Virology, Chiba Prefectural Institute of Public Health, as previously described.^{14,15} The cutoff for our assay was 1.0×10^3 copies per mL of the real-time RT-PCR reaction sample. When a sample was positive by RT-PCR, the sample was assessed as positive even if the viral load was lower than the cutoff value in the current study. In addition, rubella-specific serum antibodies were assayed at an outside commercial clinical laboratory by using the enzyme immunoassay (EIA) kit Virus Antibody EIA "Seiken" Rubella IgG and IgM (Dennka, Tokyo, Japan). Standard values for the assay are <0.8 (IgM) and <2.0 (IgG),

respectively, and the maximum value detected by this test was 128. These assays were used to determine the EIA score. The IU (IU/mL) of rubella-specific IgG is 2.3 times the EIA score for rubella-specific IgG, but there are no data about converting the EIA score of rubella-specific IgM into IU of rubella-specific IgM.

The rubella virus titer in the patient's blood had decreased markedly at the age of 4 months and was undetectable at 9 months of age (Fig 1). The rubella virus titer in the pharynx had been almost constant from 1 to 5 months and drastically increased at age 6 months ($\sim 1.7 \times 10^6$ copies per mL). Subsequently, the titer decreased after 7 months of age and was not detected at 13 months. Rubella titer in the urine peaked at 7 months ($\sim 2.0 \times 10^4$ copies per mL) and was not detected at 10 months. The pharyngeal viral load was the highest, followed by that in blood and urine, which had the lowest titer load.

Isolation of the rubella virus from pharyngeal swab samples was consistently positive until the age of 9 months. Cultures remained negative at 10 and 11 months, even though RT-PCR of the pharynx swab was positive at 11 months.

Rubella-specific serum IgM and IgG levels are shown in Fig 2. The IgM level was consistently low until age 9 months, and then decreased gradually until 20 months at which time it was negative. The high serum IgG level decreased at 3 months, increased at 4 months, peaked at ~9 months, and then decreased again at 13 months. Serum IgG increased again after the measles/rubella vaccine was administered.

DISCUSSION

Our patient was asymptomatic at birth but was diagnosed with CRS at 13 months of age because of bilateral sensory deafness. The patient's viral load and the antibody specific to

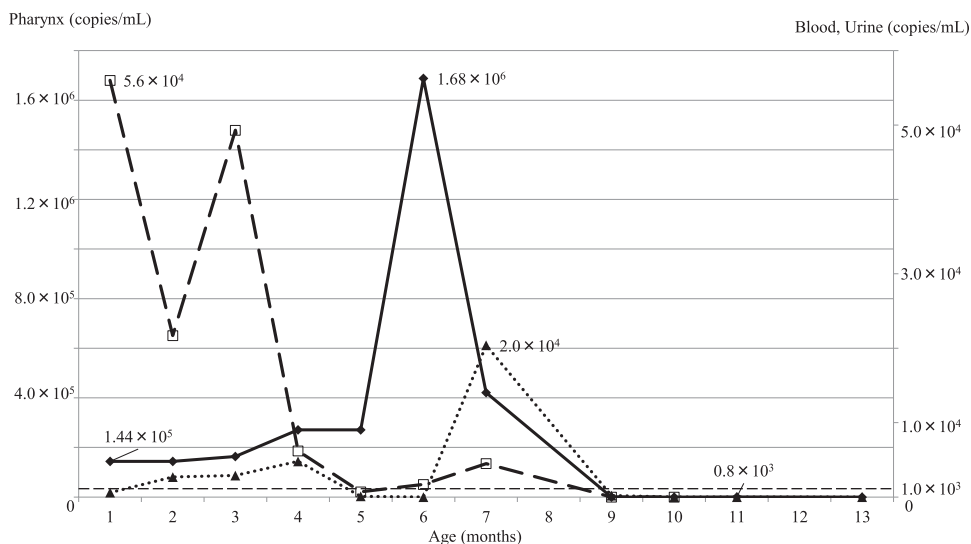


FIGURE 1

Real-time RT-PCR results showing changes in rubella viral load. Open squares: rubella viral load in blood; solid diamonds: pharynx; solid triangles: urine. Numerals in the graph indicate the copy number of rubella virus. Dashed horizontal line: cutoff value determined from real-time RT-PCR for this assay.

rubella during his clinical course were examined. To our knowledge, this is the first report of changes in viral load and antibody titers in a patient with CRS.

According to a previous report, the positive rate of rubella-specific IgM is ~20% in patients with CRS from 12 to 18 months of age and is ~5% from 18 to 24 months of age.¹⁶ In our patient, rubella-specific IgM decreased gradually from ~9 months of age and was negative at 20 months of age. These results were not contradictory to those in a previous report.

The positive rate of maternal rubella-specific IgG decreases with time.^{16,17}

In addition, the antibody titer of rubella-specific IgG in patients with CRS tends to be higher several months after birth than in neonates.¹⁸

In the current case, rubella-specific IgG decreased until 3 months of age and increased at 4 months of age. The decrease in IgG titers by 3 months of age was related to changes in maternal IgG levels. The subsequent increase could be caused by a patient's increase in antibody levels.

In the current patient, the rubella viral load in the pharynx was more than that in blood and urine, and virus shedding continued from this site for a long time. Furthermore, the

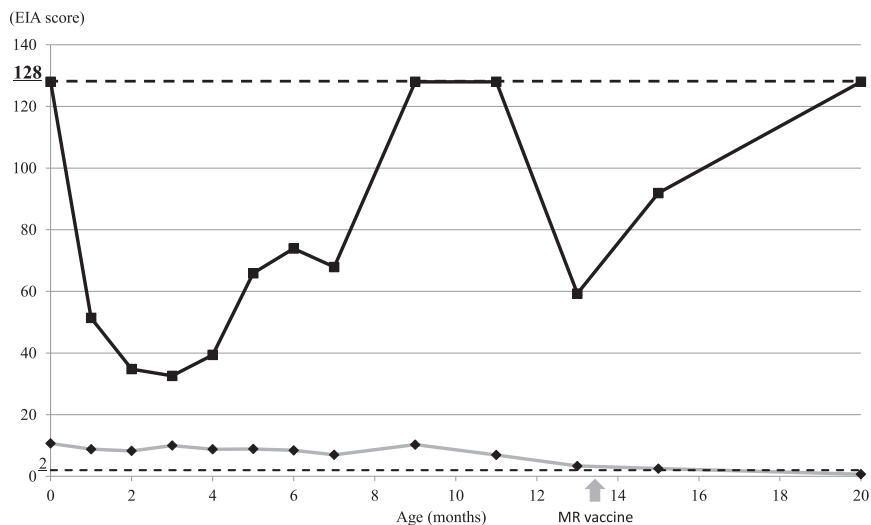


FIGURE 2

Changes in rubella-specific serum antibody isotype titers. Solid squares: rubella-specific IgG; solid diamonds: rubella-specific IgM. Transverse dashed line at 128: upper limit of this assay. Transverse dashed line at 2: cutoff value of IgG of this assay.

rubella viral load in the pharynx was highest at 6 months, suggesting that infection is mostly transmitted in patients with CRS at about 6 months of age. Schiff and Dine¹³ reported that newborns with congenital rubella were an important source of infection, but to our knowledge, there are no previously published data regarding the precise time of infection transmission. In the current patient, the rubella viral load in the

pharynx at 6 months of age was >10 times that at 1 month of age, which is the age reported in a previous study in which CRS was contagious.¹³

In our case, the rubella viral load in the pharynx and that in the urine was almost constant at ~5 months of age, increased at 6 months of age, and then decreased at 7 months of age. On the other hand, the rubella viral load in blood increased at 3 months of age

and then decreased at 4 months of age. These changes may be related to changes in rubella-specific IgG titers. The increase at ~6 months of age might be caused by the decrease in maternal IgG levels, and the increase in rubella-specific IgG levels in the patient led to another decrease. The rubella virus in blood may react with serum IgG more quickly, and therefore the virus load in blood decreased earlier than the viral load in the pharynx and urine. Certainly some other immune cells, such as cytotoxic T cells, natural killer cells, and monocytes, are involved in eliminating rubella-infected cells.¹⁹ Therefore, changes in viral load in this patient may have been caused by the activation of these immune cells with age. Unfortunately, the activity of these cells was not examined in the current case.

Persistent viral replication after birth may cause continuous damage of the

involved tissues.³ In fact, the rubella virus may have been detected in the lens of patients with congenital cataract a few years after birth.^{20,21} In the current case, hearing loss developed after birth. The viral load of the inner ears was not checked, but the virus load at this area might be high. Long-term follow-up is needed in patients with CRS/CRI, even if they have no symptoms at birth because there is no way to predict the deafness and deafness in children is difficult to notice.²²

This is a single case report, and therefore whether changes in viral load and specific rubella antibody titers seen in this case would apply to other congenital rubella cases is unknown. The information in this report, in combination with that in other reports, will provide in-depth knowledge regarding changes in viral load and specific rubella antibody

titers in patients with congenital rubella.

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ABBREVIATIONS

CRI: congenital rubella infection
 CRS: congenital rubella syndrome
 EIA: enzyme immunoassay
 Ig: immunoglobulin
 RT-PCR: reverse transcription-polymerase chain reaction

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