Changes in the prevalence of the measles, rubella, varicella-zoster, and mumps virus antibody titers in Japanese pregnant women

Masachi Hanaoka, Michi Hisano, Noriyoshi Watanabe, Kana Sugawara, Yukari Kambe, Eriko Kanda, Haruhiko Sago, Tatsuo Kato, Koushi Yamaguchi*

National Center for Child Health and Development, Tokyo, Japan

A R T I C L E   I N F O

Article history:
Received 23 July 2012
Received in revised form 4 March 2013
Accepted 11 March 2013
Available online 21 March 2013

Keywords:
Measles
Rubella
Varicella-zoster
Mumps
Antibody titer
Pregnant women

A B S T R A C T

In the present study, immunity against infectious diseases, which are capable of influencing both the mother and fetus during pregnancy and the infant in the postnatal period, were assessed in pregnant women to elucidate the necessity of vaccination during the childbearing age. It was determined that there was a trend of increases in the proportion of patients that had low antibody titers observed at a young age. Overall, after adjusting for age, low antibody titers of measles (≤4 via the neutralization test [NT]), rubella (≤16 via the hemagglutination inhibition [HI]), and varicella and mumps (plus minus or negative on the enzyme-linked immunosorbent assay [ELISA]) indicated that the rates of necessity for vaccination against measles, rubella, varicella, and mumps were 27.6%, 16.1%, 3.0%, and 23.8%, respectively. In Japan, acquired immunity for measles, rubella, and mumps was dependent on vaccination, whereas acquired immunity for varicella was dependent on natural infection. We recommend that women be vaccinated after delivery, as these vaccines are live, and thereby, are contraindicated during pregnancy.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Understanding the seroprevalence of viral antibodies is important not only for preventing viral infections in the local area, but also several infectious diseases that are capable of influencing the mother and fetus during pregnancy and the infant in the postnatal period. Viral infections during pregnancy can aggravate the mother, and lead to abortion, preterm delivery, growth retardation, malformation, and/or congenital infection of the fetus. Acquired immunity, transferred from the mother to the fetus though the placenta, continues to protect the baby from infections up to approximately 6 month after delivery [1,2]. Furthermore, acquired immunity in the mother protects against child-to-mother infections during nursing and provides protection and stability following pregnancy.

Antibody titers against viruses are affected by the patient's history of both natural infections and immunizations, and are country dependent. With respect to the history of immunizations in Japan (Table 1), the measles vaccine was initiated in 1966 and adopted into the regular national immunization program from 1978 onwards. In Japan, a single-antigen, live attenuated vaccine is inoculated only once in children aged 12–72 months, with an approximately 70% coverage rate. The measles vaccine has been incorporated into the measles, mumps, and rubella (MMR) vaccine since 1989; however, the MMR vaccination was terminated in 1993 due to the adverse effects of aseptic meningitis resulting from the mumps vaccine. In 1994, the vaccination policy was changed from being compulsory to becoming voluntary, and consequently, the coverage rate thereafter has been lower than that of other countries [3,4]. In 2006, the measles–rubella (MR) vaccine, a two-dose vaccination administered at 1 and 5–6 years of age, was introduced [5]. Moreover, the rubella vaccination program was initiated for women aged 12–<16 years (junior high school students) in 1977, and then the MMR vaccination for children was initiated in 1989 [6]. The MMR vaccination was terminated in 1993, as described above, and the rubella vaccination was then continued as a monovalent rubella vaccine until 1994. Thereafter, the rubella vaccination was no longer administered to junior high school students, but instead to children. Although the coverage rates of MMR and monovalent rubella vaccines are unclear, it appears that these rates have been sufficient in preventing outbreaks of rubella among children, and no significant epidemics have been observed since 1999. However, a local outbreak of rubella among young adults was observed in recent years, with 10 cases of congenital rubella syndrome reported in 2004 [6,7]. The varicella-zoster vaccine was started in 1987 as a voluntary vaccination, and currently has a coverage rate of...
approximately 30% [8]. The mumps vaccine was initiated in 1981, and incorporated into the MMR vaccine program between 1989 and 1993. After discontinuing the MMR vaccine program, the mumps vaccine became a monovalent vaccination [9,10].

In the present report, we analyzed the titers of antibodies against measles, rubella, varicella-zoster, and mumps viruses of pregnant women, who later delivered children at our institute located in Japan, and evaluated the changes in the prevalence of antibody titers according to the birth years of the mothers.

2. Materials and methods

2.1. Study design

We retrospectively surveyed the prevalence of measles, rubella, varicella-zoster, and mumps virus antibody titers of 13,924 Japanese pregnant women, who sought antenatal care at the National Center for Child Health and Development in Tokyo, Japan.

2.2. Neutralization test for determining the measles antibody titer

The measles antibody titer was determined via a neutralization test (NT). Virus suspensions containing the 100 median tissue culture infective dose (TCID<sub>50</sub>) were added in serially double-diluted serum, and the mixtures were incubated at 37 °C for 60 min while shaking. The virus-serum mixtures were inoculated onto monolayers of VERO cells in 96-wells plate (NUNC, Thermo Fisher Scientific Inc., Penfield, NY, USA), incubated at 35 °C for 3 days, and then the cytopathic effect (CPE) was assessed. The antibody titer was determined as the highest dilution of serum that produces a 50% or more inhibition in CPE. Measles NT antibody titers of 1:8 are approximately equivalent to >500 mIU/ml [11].

2.3. Hemagglutination inhibition (HI) test for measuring the rubella antibody titer

The rubella HI test was performed on U-bottom microtiter plates (Corning, Corning, NY, USA) with goose erythrocytes and the Baylor strain as the antigen. Serum were pretreated with 12.5% of kaolin in PBS at 15–25 °C for 20 min, and adsorbed into 25% of goose erythrocytes at 4 °C for 60 min with occasional shaking. Two-fold serial diluted sera were incubated in 4 U of hemagglutinin and 0.125% of goose erythrocytes at 15–25 °C for 60 min. The titers of the specific antibodies were determined at the final dilutions that completely inhibited hemagglutination. Rubella HI antibody titers of 1:16 are approximately equivalent to 30 IU/ml [12].

2.4. Enzyme-linked immunosorbent assay for measuring the varicella-zoster and mumps antibody titers

Varicella-zoster and mumps antibody titers were measured using an enzyme-linked immunosorbent assay (EIA). 96-Well
plates (Corning) were coated with viral antigens dissolved in PBS buffer with a pH 7.4. The plates were washed three times with PBS-Tween (0.05%) buffer. The serum was diluted and incubated in the coated plates at 15–25 °C for 1 h. After three washings with PBS-Tween buffer, the plates were incubated with goat anti-human-IgG-peroxidase (Denka Seiken Co. Ltd., Tokyo, Japan) at 15–25 °C for 1 h. The plates were washed three times, and then incubated with a substrate solution (tetramethylbenzidine) at 15–25 °C for 30 min. The color reaction was stopped with 0.6 N of sulfuric acid, and the plates were read at 450 nm using a Microplate Reader model 3550 (Bio-Rad, Richmond, CA, USA). The results were evaluated according to the manufacturer’s instructions (Denka Seiken Co. Ltd.).

3. Results

We focused on assessing the immunity of pregnant women in Japan against the above-mentioned infectious diseases during the childbearing age.

The vaccination programs for measles and rubella were initiated in the 1970s and have maintained a good coverage rate. However, the coverage rate of the MMR vaccine remains unclear since the vaccination program was started in 1989 as an option in parallel with each monovalent vaccine. The vaccine program was terminated in 1993 because of the adverse effect of aseptic meningitis due to the mumps vaccine. Instead of the MMR vaccine, the MR vaccine was introduced as a two-dose vaccine in 2006 [5]. Consequently, the current nationwide coverage is higher than 95%. Dramatic declines in susceptibility may be expected, albeit not for another decade or more. In spite of the current increase in vaccinations, there is still an increase in the ratio of pregnant women who have negative measles and rubella antibody titers at a young age (Fig. 1A and B).

The varicella-zoster virus vaccine was adopted in 1987. Despite being noncompulsory and having a low coverage rate, the ratio of pregnant women with a negative varicella-zoster virus antibody titer was low. Indeed, there was a small increase in the number of negative antibody titers observed in women born after 1985 (Fig. 1C).

The mumps vaccine was started in 1981 as a voluntary vaccination and then was incorporated into the MMR vaccine between 1989 and 1993. After termination of the MMR vaccine program, the mumps vaccine was reverted back to a monovalent vaccination with a coverage rate of approximately 30%. There was a tendency for titer decreases in women born after 1982 (Fig. 1D).

Overall, after adjusting for age, the necessity rate for vaccination against measles, rubella, varicella, and mumps were 27.6%, 16.1%, 3.9%, and 23.8%, respectively (Table 2).

4. Discussion

Viral infections during pregnancy are a risk not only for the mother, but also for the fetus. Measles during pregnancy is associated with an increased rate of preterm birth and, possibly, miscarriage [13,14]. Rubella is associated with an increased risk of miscarriage and fetal death. Additionally, congenital rubella syndrome (e.g., deafness, visual problems, cardiac defects, bone lesions, and neurologic abnormalities including intellectual disability) may occur in the first trimester [14]. Primary varicella infections during the first 20 weeks of gestation can induce

---

**Fig. 1.** Changes in the prevalence of antibody titers against measles (A), rubella (B), varicella (C), and mumps (D) viruses according to birth year.
Table 2
Antibody titers against measles, rubella, varicella and mumps viruses during the 1st trimester of pregnancy.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Antibody titer</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles (neutralization assay)</td>
<td>1:X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 4 (negative)</td>
<td>923</td>
<td>997</td>
<td>(10.2)</td>
</tr>
<tr>
<td>4</td>
<td>1579</td>
<td>1676</td>
<td>(17.4)</td>
</tr>
<tr>
<td>8</td>
<td>2427</td>
<td>2674</td>
<td>(26.8)</td>
</tr>
<tr>
<td>16</td>
<td>2081</td>
<td>2289</td>
<td>(23.0)</td>
</tr>
<tr>
<td>32</td>
<td>1239</td>
<td>1362</td>
<td>(13.7)</td>
</tr>
<tr>
<td>64</td>
<td>618</td>
<td>781</td>
<td>(6.8)</td>
</tr>
<tr>
<td>128</td>
<td>177</td>
<td>204</td>
<td>(2.0)</td>
</tr>
<tr>
<td>256</td>
<td>16</td>
<td>20</td>
<td>(0.2)</td>
</tr>
<tr>
<td>512</td>
<td>4</td>
<td>5</td>
<td>(0.04)</td>
</tr>
<tr>
<td>1024</td>
<td>2</td>
<td>3</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9066</td>
<td></td>
</tr>
<tr>
<td>Rubella (hemagglutination inhibition assay)</td>
<td>1:X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 8 (negative)</td>
<td>377</td>
<td>414</td>
<td>(3.6)</td>
</tr>
<tr>
<td>8</td>
<td>342</td>
<td>376</td>
<td>(3.2)</td>
</tr>
<tr>
<td>16</td>
<td>982</td>
<td>1080</td>
<td>(9.3)</td>
</tr>
<tr>
<td>32</td>
<td>1877</td>
<td>2065</td>
<td>(17.8)</td>
</tr>
<tr>
<td>64</td>
<td>2536</td>
<td>2812</td>
<td>(24.0)</td>
</tr>
<tr>
<td>128</td>
<td>2288</td>
<td>2516</td>
<td>(21.6)</td>
</tr>
<tr>
<td>256</td>
<td>1382</td>
<td>1518</td>
<td>(13.1)</td>
</tr>
<tr>
<td>512</td>
<td>627</td>
<td>695</td>
<td>(5.9)</td>
</tr>
<tr>
<td>1024</td>
<td>162</td>
<td>188</td>
<td>(1.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10,573</td>
<td></td>
</tr>
<tr>
<td>Varicella (enzyme-linked immunosorbent assay)</td>
<td>Units (IgG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 2.0 (negative)</td>
<td>108</td>
<td>121</td>
<td>(1.2)</td>
</tr>
<tr>
<td>2.0–4.0 (plus minus)</td>
<td>246</td>
<td>269</td>
<td>(2.7)</td>
</tr>
<tr>
<td>More than 4.0 (positive)</td>
<td>8698</td>
<td>9564</td>
<td>(96.1)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9052</td>
<td></td>
</tr>
<tr>
<td>Mumps (enzyme-linked immunosorbent assay)</td>
<td>Units (IgG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 2.0 (negative)</td>
<td>396</td>
<td>433</td>
<td>(6.5)</td>
</tr>
<tr>
<td>2.0–4.0 (plus minus)</td>
<td>1061</td>
<td>1208</td>
<td>(17.3)</td>
</tr>
<tr>
<td>More than 4.0 (positive)</td>
<td>4665</td>
<td>5130</td>
<td>(76.2)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6122</td>
<td></td>
</tr>
</tbody>
</table>

congenital varicella syndrome (e.g., limb hypoplasia, microcephaly, dermal scarring, ocular defects) [15]. Mumps in the first trimester may be associated with fetal death [16]. Accordingly, to fully prevent symptomatic infection during pregnancy, higher antibody titers of both measles and rubella are required in those of childbearing age than in the general population [11,17–19]. Vaccination has been recommended for those of childbearing age who have low antibody titers of measles (≤4 via the NT), rubella (≥16 via the HI test), or varicella and mumps (plus minus or negative on EIA) in Japan. The antibody titers at pregnancy are affected by the patient’s history of immunizations, infections, and maintenance of the titers after the onset of the initial immune response. These findings may be related to interventions such as voluntary vaccination programs and exposure to natural infections and national epidemics according to birth year.

Immunology of measles and rubella was mainly maintained by the high vaccination coverage rate. Albeit the yearly increases in coverage rate, there was a tendency for the ratio of pregnant women with negative measles and rubella antibody titers at a young age to increase. This may be a result of the successful prevention of natural infections and a decrease in the titer of the vaccine per se. However, conclusions should not be made based on our results with respect to the rubella antibody titers in women born in 1986 or later, as at that time, the administration of the rubella vaccine was changed from junior high school students to elementary school students. Thus, women born in 1986 were only 26 years old in 2012, and many have not yet been pregnant. Varicella and mumps vaccinations were performed as voluntary vaccinations and both had low coverage rates. Therefore, it was assumed that sufficient stimulation for the generation of adequate antibody titers against both viruses may have been achieved through natural infection; in particular, immunity to varicella was strongly influenced by natural infection [20–22]. The mumps vaccine was incorporated into the MMR vaccine program for 5 years, likely affecting patients who were immunized during that period, and the increase in negative mumps antibody titers after 1982 may be a result of the MMR vaccine program or a decrease in the incidence of epidemics. Another hypothesis is that mumps vaccination or natural infection may not generate robust B-cell memory [23]; this would explain why the seropositivity of mumps was lower than that of varicella even though both mumps and varicella vaccinations were voluntary and the vaccination rate of both vaccines was similar.

In Japan, the coverage rates of measles and rubella vaccines have been comparatively high, while those of varicella and mumps vaccines have been low. There are several possible reasons for these fluctuations in coverage rates. The most tangible reason is that the compulsory vaccination led to a high coverage rate. Reasons for the decrease in coverage rate include the adverse effects of the vaccine, which parents are concerned about, the frequent schedule changes, and the complicated recommendations, with vaccinations changing from being compulsory to voluntarily.

The present study revealed the changes in the prevalence of the maternal antibody titers against measles, rubella, varicella, and mumps viruses in Japan. It was observed that the tendency for increases in the ratio of negative antibody titers against these infections was on the rise in Japan. It is unlikely that non-pregnant women are examined for antibody titers of these viruses and receive the vaccinations when antibody titers are deemed
insufficient in adulthood. Thus, screening for immunity against several viruses during pregnancy is important for preventing viral infections, and examinations during pregnancy may be a good opportunity for assessing immunity and administering vaccinations; vaccinations should be recommended for pregnant women who have insufficient antibody titers after delivery.

5. Conclusion

The current status of the antibody titers suggests that immunity against these viral infections should be verified, and that patients who have low antibody titers during childbearing age should be vaccinated in Japan. From a sociological perspective on prevention, a vaccine schedule for young children should be established and vaccinations should be performed for fathers who have inadequate immunity in order to “cocoon” the baby from exposure to vaccine-preventable diseases. As potential immunization interventions are geared only for mothers during the perinatal period, we should disseminate information about immunization for young children and fathers through the mothers at this time.

Authors’ contribution

Koushi Yamaguchi and Tatsuo Kato designed and organized the study; Masachi Hanaoka, Michi Hisano, Noriyoshi Watanabe, Kana Sugawara, Yukari Kanbe, Eriko Kanda, and Haruhiko Sago performed the study and analyzed the data; Masachi Hanaoka and Koushi Yamaguchi wrote the paper. All authors have read and approved the final manuscript.

Acknowledgments

This work was supported in part by the Foundation of Vaccination Research Center (Japan), a Grant for Child Health and Development from the Ministry of Health, Labor and Welfare, and a Grant for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labor and Welfare.

Conflict of interest: The authors have no potential conflicts of interest to report.

References