Time-series analysis comparing the prevalence of antibodies against nine viral species found in umbilical cord blood in Japan


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Title page

Time-series analysis comparing the prevalence of antibodies against nine viral species found in umbilical cord blood in Japan


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Running title

Seroprevalence for nine viruses in women of childbearing age
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Summary

In this study, we investigated the prevalence of antibodies against nine viral species found in umbilical cord blood obtained from 561 neonates in 2013. Serum IgG antibodies against the following viruses were measured: herpes simplex virus (HSV), varicella–zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), measles virus (MV), rubella virus (RV), mumps virus (MuV), and human parvovirus B19 (HPV B19). A survey questionnaire for past history and maternal immunization status was simultaneously administered for the vaccine-preventable diseases of varicella, measles, rubella, and mumps. The results were compared with previous data collected in 2001–2002 from 378 umbilical cord blood samples. Virus seroprevalence data were as follows: HSV, 54%; VZV, 96%; EBV, 96%; CMV, 67%; HHV-6, 100%; MV, 95%; RV, 94%; MuV, 64%; and HPV B19, 55%. The seroprevalence of CMV, MV, and MuV was significantly lower in 2013 than that in 2001–2002 (CMV, 76%; MV, 98%; MuV, 93%). Compared with 2001–2002 data, the mean IgG antibody values of the four vaccine-preventable diseases were significantly lower and vaccination coverage for those diseases among mothers was significantly higher. Thus, focus should be turned to the future antibody levels in women of the childbearing age.
Introduction

Changes in the social environment regarding the widespread use of vaccines have had a dramatic effect on the epidemiology of viral infections in Japan. Some viral infections acquired in utero or during the birth process are significant causes of fetal and neonatal mortality and are important contributors to early and later childhood morbidity.

Congenital cytomegalovirus (CMV) infection is the leading non-genetic cause of sensorineural hearing loss, and CMV infection affects 0.2%–2.2% of live births in industrialized countries (1,2). Pre-existing maternal antibodies to CMV is the most important protective factor against congenital CMV infection, and primary CMV infection is more likely than non-primary infection to cause symptoms at birth and long-term disability (3). However, the seroprevalence among pregnant women ranges between 65% and 87% and has recently been decreasing in Japan (4,5).

Congenital rubella syndrome (CRS) is rare in developed countries as a result of established rubella immunization programs. However, in Japan, there was a large outbreak of rubella in 2012–2013. More than 16,000 cases of rubella and 45 infants with CRS were reported from 2012 to 2014 through the National Epidemiological Surveillance of Infectious Diseases (6).

Prevention of mother-to-child transmission is extremely important, and transplacental maternal antibodies are important in the prevention and alleviation of viral infections in the fetus and neonate. The aim of this study
was to investigate the prevalence of antibodies against nine viral species found in umbilical cord blood: herpes simplex virus (HSV), varicella–zoster virus (VZV), Epstein–Barr virus (EBV), CMV, human herpesvirus 6 (HHV-6), measles virus (MV), rubella virus (RV), mumps virus (MuV), and human parvovirus B19 (HPV B19).

**Materials and Methods**

Umbilical cord blood samples were obtained from 561 neonates born at ≥ 36 weeks of gestation between January and December 2013 at Konan Kosei Hospital, Aichi, Japan; in cases of twins, the elder infant was included in this study. Serum IgG antibodies against the following viruses were measured at SRL Limited (Tokyo): HSV, VZV, EBV, CMV, HHV-6, MV, RV, MuV, and HPV B19. Because of sample insufficiency, the number of samples screened for EBV, HHV-6, and HPV B19 decreased to 558, 550, and 558, respectively. Antibodies against HHV-6 were measured using a fluorescent antibody (FA) assay. Antibodies other than those against HHV-6 were measured by enzyme immunoassays (EIAs) using the Herpes IgG-EIA Kit for HSV, Varicella-Zoster IgG-EIA Kit for VZV, EB VCA-IgG-EIA Kit for EBV, Cytomegalovirus IgG-EIA Kit for CMV, Rubella IgG-EIA Kit for RV, Mumps IgG-EIA Kit for MuV, and Parvo IgG-EIA Kit for HPV B19 (all from Denka Seiken, Co., LTD., Tokyo, Japan). An anti-HHV-6 IgG FA titer ≥10 was considered positive. An IgG antibody value of ≥4.0 was considered positive for HSV, VZV, CMV, MV, RV, and MuV. For EBV and HPV B19, an IgG
antibody value of $\geq 1.0$ was considered positive.

A survey questionnaire of past history and maternal immunization status was used for the vaccine-preventable diseases varicella, measles, rubella, and mumps. In 2001–2002, we performed a similar study using 378 umbilical cord blood samples measured IgG antibodies by the same methods in this study at SRL Limited (Tokyo) with the exception that EBV antibody was measured by enzyme-linked immunosorbent assay (ELISA) using the EBV VCA IgG Kit (Dia Sorin, Italy) (7). The results of the present study were compared with those of the 2001–2002 study. Written informed consent was obtained from all participants. The study was approved by the Konan Kosei Hospital’s clinical research review committee. Statistical analyses were performed using the chi-square test, Mann–Whitney U test, or Tukey’s test. A probability ($P$) value $< 0.05$ was considered statistically significant. The SPSS software package version 19 (SPSS, Chicago, IL) was used to perform all statistical analyses.

Results

In both 2001–2002 and 2013, peak maternal age group was aged 30–34 years (Fig. 1). The mean maternal age was 32.7 years in 2013, which was significantly higher than that recorded in 2001–2002 (29.6 years; $P < 0.01$).

The virus seroprevalence in the umbilical cord blood samples was as follows: HSV, 54%; VZV, 96%; EBV, 96%; CMV, 67%; HHV-6, 100%; MV, 95%; RV, 94%; MuV, 64%; and HPV B19, 55% (Table 1). The seroprevalence of
CMV, MV, and MuV was significantly lower in 2013 than that in 2001–2002. Seroprevalence of nine viral species according to maternal age in 2013 is shown in Figure 2. Seroprevalence was significantly low in ≤24 year group for RV, in 25–29 year group for HSV, and in 30–34 year group for VZV, EBV, and MV, and was significantly high in ≤24 year group for HSV and in 30–34 year group for RV. The mean IgG antibody values of the four vaccine-preventable diseases among seropositive individuals were as follows: VZV, 26.7; MV, 24.6; RV, 28.1; and MuV, 9.4 EIA units (Table 2); these values were significantly lower than the EIA values obtained in 2001–2002.

Of the 561 mothers, 501 (89%) completed the questionnaire. Vaccination coverage among mothers who received at least one dose was as follows: varicella, 17%; measles, 53%; rubella, 38%; and mumps, 26% (Table 3). These rates were found to be significantly higher than those observed in 2001–2002. The previous infection rates of the mothers were as follows: varicella, 82%; measles, 33%; rubella, 37%; and mumps, 54% (Table 3). The rates for measles, rubella, and mumps were significantly lower in 2013 than those observed in 2001–2002. The mean IgG antibody values in the vaccinated group were significantly lower than those in the naturally-infected group for all the four vaccine-preventable diseases in 2013 (Table 4).

Discussion

In recent years, the mean age of the pregnant woman is increasing in Japan. According to the Population Survey Report from Ministry of Health,
Labour and Welfare, mean childbearing age had risen to 31.6 years in 2013 from 29.7 years in 2001. Consistent with this, our study also found an increase in the mean maternal age. In addition, we found that the seroprevalence of HSV, CMV, MuV, and HPV B19 in umbilical cord blood was relatively low in this time-series analysis investigating sero-status of nine viral species. Moreover, a significant decrease in seropositivity for CMV, MV, and MuV was seen over time. When examining the seroprevalence according to maternal age, some significant findings were observed in several viruses, but no consistent trends were observed with respect to the maternal age.

The seroprevalence of HSV was reduced considerably in Japan from the 1970s through the 1990s (8). In recent years, the Japanese seropositive rate for HSV-1 and HSV-2 in females was reported 63.3% and 9.3%, which is similar to that reported in other developed countries (9). This study revealed that the seroprevalence of HSV was 53%, comparable with a recent report, and has continued to be a relatively low value over the past twelve years. Neonatal HSV infection causes serious morbidity and mortality, leaving many survivors with permanent sequelae. The incidence of neonatal herpes infection varies widely from 1.6 to 33 per 100,000 live births, being 2.6 in Japan (10-12). First-time infection of the mother is the most important factor for transmission of genital herpes from the mother to the fetus or the newborn. The incidence of neonatal HSV infection in the UK was reported to increase 11-fold over the past two decades with a significant rise in first-episode genital herpes (13). In Japan, however, the number of reports of
female genital herpes infection has roughly unchanged in the last decade, and the incidence of neonatal herpes has declined compared with the previous national survey (12, 14).

CMV is the most common congenital viral infection, with a birth prevalence of about 0.2%–2.2% in developed countries and 0.31% in Japan (1,2,15). Congenital CMV infection causes significant clinical consequences not only at birth but also later, presenting as neurological sequelae, including sensorineural hearing loss and developmental delay. The seroprevalence of CMV increases with age depending on geographic area and socioeconomic status. CMV seroprevalence was reported to have been decreasing recently in Japan (4). In this study, the seropositive rate for CMV was significantly decreased from 76% in 2001–2002 to 67% in 2013, and this result is similar to previous reports (4,5). Though significant congenital morbidity occurs following both primary and non-primary maternal infection, the presence of maternal antibodies to CMV is the most important protective factor against congenital CMV infection (3). Because CMV seroprevalence has been decreasing in Japan, measures to prevent CMV infection in reproductive women has become extremely important.

Intrauterine infections with EBV and HHV-6 are regarded not to be related to congenital anomalies. In addition, the maternal seroprevalence of EBV and HHV-6 identified in this study has been maintained at more than 95% over the past 12 years. Hence, intrauterine infections with these viruses are considered rare.
HPV B19 infection during pregnancy is associated with fetal death, particularly in the first half of pregnancy. In addition to causing fetal loss, HPV B19 is cytotoxic to fetal red cell precursors and may cause anemia and hydrops fetalis. Antibodies to HPV B19 are found in 30%–80% of adults (16-18). This study found that the seroprevalence of HPV B19 was 54% and comparable with data from another report from Japan (19). The seropositivity was significantly increased in 2013 compared with 12 years previously. There has been a regional outbreak of HPV B19 every 5–6 years in Japan, and this probably affected the increase in seropositive rate.

Among vaccine-preventable diseases, the seroprevalences of VZV, MV, and RV were high, although the MV seropositivity rate significantly decreased in 2013 compared with that observed in 2001–2002. Despite RV seroprevalence being maintained at a high level in adult women, in 2012–2013, a large outbreak of rubella occurred in Japan involving over 16,000 cases and resulting in 45 cases of congenital rubella syndrome being reported from the second half of 2012 to 2014 (6). The majority of rubella cases occurred in males aged 20–49 years. Their seropositivity rates ranged from 73% to 90% and were lower than those observed in females (20). The seroprevalence discrepancy was considered to be the result of the past immunization policies for rubella in Japan. Since 2006, a measles-rubella vaccine has been administered to both boys and girls at the age of 1 year and in the preschool years. The vaccination rate in the first phase has recently maintained more than 95%, so it will be maintained at a high seropositivity rate without
difference between the sexes in the future. However, the vaccination rates of previous generations were low in males. Thus, in order to prevent future rubella outbreaks, there is a need for strategies to identify and vaccinate susceptible persons, both males and females.

The seroprevalence of MuV was 64% in 2013, dramatically decreasing from 93% in 2001–2002. More precisely, the rate of testing positive in the equivocal range was increased considerably from 6% in 2001–2002 to 27% in 2013, whereas the seronegative rate was 2% in 2001–2002 and 9% in 2013. The cause of these changes may be the natural declination of antibody titers, in addition to the lower antibody levels of MuV compared with other viruses. Fetal mumps infection is considered not to be related to congenital malformations but to be related to abortion and preterm birth. It is necessary to pay attention to the mumps infection in pregnant women.

For the vaccine-preventable diseases, the mean IgG antibody values of all the four diseases significantly decreased among seropositive individuals in 2013 compared with those observed in 2001–2002. For all four virus-related diseases, the vaccination coverage rates of the mothers who received at least one dose were found to be significantly higher in 2013 than those seen in 2001–2002. Past infection rates of the mothers were significantly lower in 2013 than those seen in 2001–2002 for measles, rubella, and mumps. The reduction in antibody levels may be due to the increasing number of mothers without natural infection. This decrease may also be the result of a reduction of the booster effect on wild-type strains to the acquired immunity via
vaccination. It is expected that the rate of natural infections caused by vaccine-preventable diseases will further decrease in future because infants have been immunized with the varicella vaccine on a routine basis since 2014, similar to the measles-rubella vaccine. In view of these changes, measures against mother-to-child transmission will be required, including the development of efficient vaccine policies.

In this study, we used the umbilical cord blood, because collecting umbilical cord blood samples is non-invasive to both mothers and neonates. The antibody levels of umbilical cord blood are similar or higher than those of the mother (21-23). In order to investigate the maternal seroprevalence, there are no issues related to using umbilical cord blood.

There have been several studies reporting the seroprevalence of various viruses in Japanese pregnant women; however, these studies investigated only a few viruses. The advantage of this study is that we investigated a wide variety of viruses, up to nine species. Furthermore, we also analyzed the development of seroprevalence over time. Thus, the findings of this study will be valuable in terms of informing policies related to mother-to-child infection control. The limitation of this study is that the data pertaining to past history and maternal immunization depended on a questionnaire. The rate of mothers who were able to confirm their immunization record was not even 30%. However, there were not many differences between the entire results and those of mothers who confirmed their immunization record. Hence, we consider our findings rather reliable.
In conclusion, the seroprevalence of HSV, CMV, MuV, and HPV B19 in umbilical cord blood was relatively low; therefore, it is necessary to pay attention to these viral infections in pregnant women in Japan. The mean antibody values for the four vaccine-preventable diseases were significantly lower at the time of the study than those observed 12 years previously. Thus, focus should be turned to the future decline of antibody levels in women of the childbearing age.

**Conflict of interest** None to declare.


Table 1. Seroprevalence of nine viral species in umbilical cord blood

<table>
<thead>
<tr>
<th></th>
<th>2001–2002</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N= 378)</td>
<td>(N= 561)</td>
</tr>
<tr>
<td>HSV</td>
<td>55.8</td>
<td>53.8</td>
</tr>
<tr>
<td>VZV</td>
<td>97.6</td>
<td>95.9</td>
</tr>
<tr>
<td>EBV</td>
<td>98.4</td>
<td>95.5</td>
</tr>
<tr>
<td>CMV</td>
<td>75.7</td>
<td>67.2 b</td>
</tr>
<tr>
<td>HHV-6</td>
<td>99.5</td>
<td>100.0</td>
</tr>
<tr>
<td>MV</td>
<td>98.4</td>
<td>94.8 a</td>
</tr>
<tr>
<td>RV</td>
<td>96.3</td>
<td>94.3</td>
</tr>
<tr>
<td>MuV</td>
<td>92.9</td>
<td>64.0 b</td>
</tr>
<tr>
<td>HPV B19</td>
<td>45.5</td>
<td>55.4 b</td>
</tr>
</tbody>
</table>

(%)  a: \( P < 0.05 \)  b: \( P < 0.01 \)

HSV; herpes simplex virus, VZV; varicella–zoster virus, EBV; Epstein–Barr virus, CMV; cytomegalovirus, HHV-6; human herpesvirus-6, MV; measles virus, RV; rubella virus, MuV; mumps virus, HPV B19; human parvovirus B19
Table 2. Mean IgG antibody values of four vaccine-preventable diseases among seropositive individuals

<table>
<thead>
<tr>
<th></th>
<th>2001–2002</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>VZV</td>
<td>33.2 ± 29.8</td>
<td>26.7 ± 31.7</td>
</tr>
<tr>
<td></td>
<td>(369)</td>
<td>(538)</td>
</tr>
<tr>
<td>MV</td>
<td>53.8 ± 36.3</td>
<td>24.6 ± 29.1</td>
</tr>
<tr>
<td></td>
<td>(372)</td>
<td>(532)</td>
</tr>
<tr>
<td>RV</td>
<td>41.9 ± 32.7</td>
<td>28.1 ± 26.8</td>
</tr>
<tr>
<td></td>
<td>(364)</td>
<td>(529)</td>
</tr>
<tr>
<td>MuV</td>
<td>19.9 ± 14.8</td>
<td>9.4 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>(351)</td>
<td>(359)</td>
</tr>
</tbody>
</table>

EIA unit, (N), a; $P < 0.01$

VZV: varicella–zoster virus, MV: measles virus, RV: rubella virus, MuV: mumps virus
Table 3. Rates (%) of vaccination and previous infection of the mothers

<table>
<thead>
<tr>
<th></th>
<th>Vaccination</th>
<th>Previous infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicella</td>
<td>4</td>
<td>17(^a)</td>
</tr>
<tr>
<td>Measles</td>
<td>19</td>
<td>53(^a)</td>
</tr>
<tr>
<td>Rubella</td>
<td>19</td>
<td>38(^a)</td>
</tr>
<tr>
<td>Mumps</td>
<td>9</td>
<td>26(^a)</td>
</tr>
</tbody>
</table>

\(^a\): \(P < 0.01\) \(^b\): \(P < 0.05\)
Table 4. Mean IgG antibody values of the naturally-infected group and the vaccinated group in 2013

<table>
<thead>
<tr>
<th></th>
<th>Naturally-infected group</th>
<th>Vaccinated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EIA unit, (N), a; ( P &lt; 0.01 ), b; ( P &lt; 0.05 )</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>28.0 ± 34.7 (358)</td>
<td>9.9 ± 6.1 a (25)</td>
</tr>
<tr>
<td>MV</td>
<td>40.1 ± 51.4 (103)</td>
<td>16.7 ± 16.6 a (191)</td>
</tr>
<tr>
<td>RV</td>
<td>30.6 ± 28.6 (134)</td>
<td>22.4 ± 23.0 b (137)</td>
</tr>
<tr>
<td>MuV</td>
<td>7.6 ± 6.3 (163)</td>
<td>5.5 ± 4.8 a (146)</td>
</tr>
</tbody>
</table>

VZV: varicella–zoster virus, MV: measles virus, RV: rubella virus, MuV: mumps virus
Figure 1. Maternal age observed at different time periods

- 2001–2002 (N = 378)
- 2013 (N = 561)
Figure 2. Seroprevalence of nine viral species according to maternal age in 2013

* $P < 0.05$