Original Article

Time-Series Analysis Comparing the Prevalence of Antibodies against Nine Viral Species Found in Umbilical Cord Blood in Japan


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SUMMARY: In this study, we investigated the prevalence of antibodies against 9 viral species found in umbilical cord blood 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), mumps virus (MuV), and human parvovirus B19 (HPV B19). A survey questionnaire regarding past medical history and maternal immunization status was used to inquire about the vaccine-preventable diseases varicella, measles, rubella, and mumps was simultaneously administered. The results were compared with previous data collected in 2001–2002 from 378 umbilical cord blood samples. Viral seroprevalence data were: 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 were significantly lower in 2013 than in 2001–2002 (CMV, 76%; MV, 98%; MuV, 93%). Compared with the 2001–2002 data, the mean IgG antibody values of the 4 vaccine-preventable diseases were significantly lower, and vaccination coverage for those diseases among mothers was significantly higher. Thus, attention should be paid to antibody levels in women of childbearing age in the future.

INTRODUCTION

Changes in the social environment regarding widespread vaccination have dramatically affected the epidemiology of viral infections in Japan. Some viral infections acquired in utero or during delivery 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 are 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 been decreasing recently in Japan (4,5).

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

Prevention of mother-to-child transmission is extremely important, and transplacental transfer of maternal antibodies is important in the prevention and alleviation of viral infections in the fetus and neonate.

The aim of this study was to investigate the prevalence in umbilical cord blood of antibodies against 9 viruses: herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), measles virus (MV), 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 in 2013 at Konan Kosei Hospital, Aichi, Japan; for twins, only the elder infant was included in this study. Serum IgG antibodies against the following viruses were measured at SRL Limited (Tokyo, Japan): 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 against the remaining viruses were measured by IgG enzyme immunoassay (EIA) kits specific for each virus (all from Denka Seiken, Co., LTD., Tokyo, Japan). An anti-HHV-6 IgG FA value ≥ 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 ≥ 1.0 was considered positive.

A survey questionnaire regarding past medical history and maternal immunization status was used to inquire about the vaccine-preventable diseases varicella, measles, rubella, and mumps. In 2001–2002, we performed a similar study, using 378 umbilical cord blood samples...

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to measure IgG antibodies by the same methods used in this study, except that the EBV antibody value was measured by enzyme-linked immunosorbent assay using the EBV VCA IgG Kit (Diasorin, Saluggia, 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, USA) was used to perform all statistical analyses.

RESULTS

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

The viral 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 measured in 2001–2002. The seroprevalence of 9 viruses according to maternal age in 2013 is shown in Fig. 2. In the comparison of age groups, seroprevalence was significantly lower in the ≤24 year old group for RV, in the 25–29 year old group for HSV, and in the 30–34 year old group for VZV, EBV, and MV, and was significantly higher in the ≤24 year old group for HSV and in the 30–34 year old group for RV. The mean IgG antibody values of the 4 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 sig-

![Graph](image_url)

**Fig. 1.** Maternal age observed at different time periods.

**Table 1.** Seroprevalence (%) of 9 viral species in umbilical cord blood

<table>
<thead>
<tr>
<th>Species</th>
<th>2001–2002 (N = 378)</th>
<th>2013 (N = 561)</th>
</tr>
</thead>
<tbody>
<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</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</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</td>
</tr>
<tr>
<td>HPV B19</td>
<td>45.5</td>
<td>55.4</td>
</tr>
</tbody>
</table>

**Table 2.** Mean IgG antibody values of 4 vaccine-preventable diseases among seropositive individuals

<table>
<thead>
<tr>
<th>Species</th>
<th>2001–2002 (N = 378)</th>
<th>2013 (N = 561)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VZV</td>
<td>33.2 ± 29.8 (369)</td>
<td>26.7 ± 31.3(358)</td>
</tr>
<tr>
<td>MV</td>
<td>53.8 ± 36.3 (372)</td>
<td>24.6 ± 29.1(352)</td>
</tr>
<tr>
<td>RV</td>
<td>41.9 ± 32.7 (364)</td>
<td>28.1 ± 26.8(359)</td>
</tr>
<tr>
<td>MuV</td>
<td>19.9 ± 14.8 (351)</td>
<td>9.4 ± 6.6(359)</td>
</tr>
</tbody>
</table>

1) P < 0.05.  
2) 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.

![Graph](image_url)

**Fig. 2.** Seroprevalence of 9 viral species according to maternal age in 2013.
for all 4 vaccine-preventable diseases in 2013 (Table 4). The mean IgG antibody values in the vaccinated group were significantly lower than those in the naturally-infected group. Antigen titers in 2013 were significantly lower than those observed in 2001–2002. The mean IgG antibody titers were 30.6 ± 28.6 (134) in 2001–2002. The antigen titers were significantly lower than the EIA values obtained in 2001–2002 (1,2,15). Congenital CMV infection causes significant and harmful 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 has been decreasing recently in Japan (4). In this study, the seroprevalence rate for CMV decreased significantly, from 76% in 2001–2002 to 67% in 2013, similar to previous reports (4,5). Though significant congenital morbidity is observed 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 pregnant women have become extremely important.

Intrauterine EBV and HHV-6 infections do not cause congenital anomalies. In addition, the maternal seroprevalence of EBV and HHV-6 identified in this study remained above 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%, similar to another report from Japan (19). Seropositivity increased significantly in 2013 compared with 12 years earlier. There has been a regional outbreak of HPV B19 every 5–6 years in Japan, and these infections probably affected the increase in seropositivity.

Among vaccine-preventable diseases, the seroprevalences of VZV, MV, and RV were high, although the MV seropositivity rate decreased significantly in 2013 compared with that observed in 2001–2002. Despite RV

<table>
<thead>
<tr>
<th>Naturally-infected group</th>
<th>Vaccinated group</th>
</tr>
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<tbody>
<tr>
<td>VZV 28.0 ± 34.7 (358)</td>
<td>9.9 ± 6.1 (25)</td>
</tr>
<tr>
<td>MV 40.1 ± 51.4 (103)</td>
<td>16.7 ± 16.6 (191)</td>
</tr>
<tr>
<td>RV 30.6 ± 28.6 (134)</td>
<td>22.4 ± 23.0 (137)</td>
</tr>
<tr>
<td>MuV 7.6 ± 6.3 (163)</td>
<td>5.5 ± 4.8 (146)</td>
</tr>
</tbody>
</table>

EIA unit (N)

\( \text{P} < 0.01 \), \( \text{P} < 0.05 \).

VZV, varicella-zoster virus; MV, measles virus; RV, rubella virus; MuV, mumps virus.
Seroprevalence for Nine Viruses in Women of Childbearing Age

Seroprevalence remaining at a high level in adult women, a large outbreak of rubella occurred in Japan in 2012–2013. Over 16,000 infections, including 45 cases of congenital rubella syndrome, were reported from the second half of 2012 to 2014 (6). The majority of rubella cases occurred in men aged 20–49 years. Their seropositivity rates ranged from 73% to 90% and were lower than those observed in women (20). The seroprevalence discrepancy was considered to be the result of earlier rubella immunization policies 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 recently exceeded 95%, so a high seropositivity rate without a difference between the sexes is expected in the future. However, the vaccination rates of previous generations were low in boys. Thus, to prevent future rubella outbreaks, strategies are needed to identify and vaccinate susceptible persons, both men and women.

The seroprevalence of MuV was 64% in 2013, dramatically decreased from 93% in 2001–2002. More precisely, the rate of equivocal test results 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 decline of antibody titers, in addition to the lower antibody levels of MuV compared with other viruses. Fetal mumps infection does not cause congenital malformations, but is related to abortion and preterm birth. Mumps infections in pregnant women require attention.

For the vaccine-preventable diseases, the mean IgG antibody values of all 4 diseases significantly decreased among seropositive individuals in 2013 compared with those observed in 2001–2002. For all 4 virus-related diseases, the percentage of mothers who received at least 1 vaccination dose was significantly higher in 2013 than in 2001–2002. Past infection rates of the mothers were significantly lower in 2001–2002 than 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 from wild-type strains on the immunity acquired via vaccination. The rate of natural infections caused by vaccine-preventable diseases is expected to further decrease in the 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 umbilical cord blood, because collecting umbilical cord blood samples is non-invasive for both mothers and neonates. The antibody levels of umbilical cord blood are similar or higher than those of the mother (21–23). To investigate 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, including 9 viral species. Furthermore, we 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 less than 30%. However, there were not many differences between the total results and those of mothers who confirmed their immunization record. Hence, we consider our findings to be 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 4 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 childbearing age.

Conflict of interest None to declare.

REFERENCES


