High susceptibility to cytomegalovirus infection of pregnant women in Flanders, Belgium

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Abstract

Maternal antibodies against cytomegalovirus (CMV) infection offer, to some extent, protection against congenital CMV infection.
This study describes the seroprevalence of CMV-specific IgG in 220 parturient women during pregnancy, at delivery, at 3 months after delivery and in their cord blood (Flanders, Belgium, 2006-2008). ELISA was used to measure IgG. Of this population, 30% had positive IgG titres. Active transplacental transport was confirmed with a ratio 1.15/1. Elevated maternal IgG titre and increased parity, but not age, were significantly associated with higher seroprevalence of CMV-specific IgG in the cord blood.
These data indicate a high susceptibility to CMV among fertile women. Prenatal prevention and other strategies to prevent intra-uterine infection are of critical importance in a highly susceptible population.

Key words: Cord blood, cytomegalovirus, maternal antibodies, pregnancy, seroprevalence, transplacental transport.

Introduction

Congenital CMV infections are of considerable public health significance, as they are the leading non-genetic cause of hearing impairment in children and can be responsible for mental disabilities and other neurological handicaps. The disease burden of congenital CMV infection is similar to that of congenital rubella before the introduction of the universal rubella vaccination (Arvin et al., 2004).

One of the main determinants in the protection of foetuses from infection and the risk of primary or recurrent infection is the immune status of women at childbearing age. Transmission of virus during pregnancy occurs in 20-40% of primary CMV infections and in 0.2-2.2% of recurrent CMV infections (Revello et al., 2002). Improved hygiene in industrialised regions of the world delays the infection until adulthood, which often leaves young adults unpro-

tected (Stein et al., 1997; Arvin et al., 2004). Several factors are related to elevated CMV susceptibility at childbearing age, including lower age, lower parity, higher educational level, and a better economical situation (Gratacap-Cavallier et al., 1998; Fowler et al., 2004; Alanen et al., 2005).

The aim of the present study was to describe the seroprevalence of CMV-specific IgG during the puerperium period and in the cord blood of a cohort of women in Antwerp, Belgium. These data allow us to compare the seropositivity rate of this study's subjects with those of studies in other European countries

Table I. — General characteristics of the women.	
Number	220
Mean age in years (min-max)	29.8 (22.2-39.9)
Primipara	76.3%
Delivery through Caesarean section	20%
Educational level	82.5% bachelor's or master's degree

and to identify factors influencing CMV sero-positivity.

Material and Methods

A prospective multi-centre study was conducted in the province of Antwerp, Belgium. The study was performed in accordance with the Helsinki Declaration, the ICH-GCP and procedures established by the Belgian legal code. The protocol was approved by the Ethics Committee of the University Hospital of Antwerp. The detailed study design has been described in previous articles (Leuridan E., 2010; Leuridan et al., 2011). Healthy pregnant women aged 18 to 40 years were recruited beginning in April 2006 with follow-up lasting until November 2008. Exclusion criteria included an impaired immune system in either the mother or child and the use of immunoglobulins or blood products during the study period. Informed consent was obtained. A questionnaire was completed regarding demographics, vaccination history and medical and obstetrical history.

Venous whole blood (10 cc) was collected from the umbilical cord and from the women during pregnancy (week 36), at delivery (day 0-2) and at three months postpartum. Samples were centrifuged at 2000 rpm within 8 hours after sampling and were stored at -20°C.

CMV-specific IgG were assayed in all three blood samples from the subjects and cord blood samples using ETI-CYTOK-G Plus (Diasorin®, Saluggia, Italy) for CMV-specific IgG detection. The cut-off value used to determine IgG was 0.4 IU/mL according to the optical density (De Paschale et al., 2009). All samples were tested at the microbiology laboratory of the University Hospital of Antwerp.

Statistical analysis was performed with SPSS® (SPSS Inc., Chicago) version 16.0 software. Data were examined for normality and were not normally distributed. Therefore, non-parametric tests (Kruskal-Wallis, Wilcoxon signed-rank test) were used to compare the different geometric mean titres (GMT) of antibodies at different time points and to evaluate the potential influences of other variables on levels of IgG seropositivity.

Results

1. *Population*

A total of 221 women participated in the original study. One woman refused CMV testing. All participants resided in Belgium at the time of inclusion. Of all of the participating women, 76.3% were expecting a first child (regardless of previous missed abortions), 16.3% were expecting a second child (36/220) and 5.4% were expecting a third child (12/220). Two women were expecting a fourth child (1%) and 2 were expecting a fifth child (1%). The majority of women (94.6%) were of Belgian nationality.

In general, the women's education level was high, and 82.5% had a bachelor's or a master's degree. Five percent had a secondary school education level, and 9.5% had received a vocational education. For a small number of women (3.0%), the educational level was unknown (Table I).

2. *Serological results*

Thirty percent of the samples (64/212) collected at week 36 of pregnancy had positive, CMV-specific IgG antibody titres.

GMT were not significantly different between maternal values at different time points (Table II) but differed significantly between maternal IgG values at delivery and IgG values in the cord blood ($p < 0.0001$; Kruskal-Wallis). Note that the reported p-values should be considered with the appropriate significance level, while taking multiple tests into account.

Non-parametric tests were used to measure the possible influence of several variables on the $\log_{10}$ value of CMV-specific IgG in cord blood. The $\log_{10}$ value of CMV-specific IgG at week 36 of pregnancy significantly influenced the $\log_{10}$ value of CMV-specific IgG in the cord blood ($p < 0.0001$; Wilcoxon signed-rank test). Elevated parity (more than 1 previous child) was a predictive factor for higher maternal antibody titres in cord blood ($p < 0.0001$; Wilcoxon signed-rank test).

Table II. — GMT and percentage of CMV IgG- positive women at three different time points (week 36, at delivery and 3 months after delivery) and in cord blood samples.

Time point (available samples)	Week 36 (N = 212)	At delivery (N = 188)	In Cord (N = 182)	Month 3 postpartum (N = 212)
GMT IgG in IU/mL (95% CI) (% positive)	1.48 (1.34-1.63) (64/212 = 30.2%)	1.44 (1.32-1.58) (53/188 = 28.2%)	1.66 (1.48-1.86) (51/182 = 28%)	1.56 (1.42-1.72) (63/212 = 29.7%)

Educational level was split into 2 categories, including one large category for higher education (82%) and one small category for the secondary school level and for vocational education. A higher educational level was borderline predictive for lower seropositivity (p = 0.035). The GMT of CMV-specific IgG in the higher education category was 1.5 IU/mL (1.34-1.68) (N = 183), while the GMT was 14.5 IU/mL (2.9-70) (N = 32) in the secondary/vocational education category.

Age was categorised into 4 categories of 5 years each and was not associated with CMV-specific IgG titres (p = 0.37), nor was nationality (p = 0.078).

3. *Comparison with ESEN 2 data*

The CMV-specific IgG seroprevalence data derived from the present study were compared with the Belgian data from the European Seroepidemiology Network 2 (ESEN 2) database, which were collected in 2002 (Nardone et al., 2003) (CMV results not published).The prevalence of CMV-specific IgG in Belgian females in the age category of 18-40 years was 28.4% (N = 579), whereas, in the more narrow age category of 22-40 years (similar to that used in the present study), the prevalence of IgG was 27.5% (N = 400, CI 0.003).

Discussion

A CMV-specific IgG seroprevalence of 30% was found in women of childbearing age in Flanders, Belgium. This percentage is low compared with those observed in other countries (Table III) and with the 54% seroprevalence reported by Naessens et al. in pregnant women (N = 7140) in Brussels, Belgium (Naessens et al., 2005). Naessens et al. reported a median parity of 1.8 children in contrast to a median parity of 0.0 children in the present study. Parity is known as a predisposing factor for CMV seropositivity. However, comparing the present data with the Belgian ESEN2 data (2002, not published), a similarly high susceptibility (27.5%) was reported, as in our smaller cohort. The percentages of seropositive

pregnant women in a population vary considerably. Table III gives a limited overview of available European data.

Several factors are related to CMV seropositivity. Seroprevalence has been shown to increase with age (Gratacap-Cavallier et al., 1998; Fowler et al., 2003) and parity (Tookey et al., 1992; Gratacap-Cavallier et al., 1998; Alanen et al., 2005). Furthermore, socioeconomic background is inversely related to CMV seroprevalence (Gratacap-Cavallier et al., 1998). In different studies conducted in the United States, socioeconomic status, promiscuous sexual behaviour and an increased number of children were related to past exposure to CMV (Chandler et al., 1985; Marshall et al., 2005). Recently, Dowd et al. confirmed that significant racial and socioeconomic disparities influenced CMV seroprevalence in all age categories in the United States (Dowd et al., 2009). In a Finnish study, social environment was also found to be the most powerful influencing factor, predicting both IgG seroprevalence and recurrences of infections in pregnancy. Specifically, 60.9% of participants were seropositive in the upper socioeconomic strata, whereas 76.4% were seropositive in lower socioeconomic strata (Alanen et al., 2005). In contrast, in a study in the United States in 2003, Fowler et al. found no relation with socioeconomic conditions, but obtained strong evidence that pre-conception maternal seropositivity and a maternal age of greater than 25 years were highly protective against CMV infection of the foetus (Fowler et al., 2003). The strikingly low reported seroprevalence (30.4%) in pregnant Irish women (Table I) was related to elevated socioeconomic status and a lower number of children compared with immigrant women, who had significantly higher seropositivity rates (89.7%) (Knowles et al., 2005).

In the present study population, maternal IgG titre and parity, but not age, significantly influenced the titres of CMV-specific IgG in cord. Overall, the educational level in the study's subjects was relatively high. Differences in GMT were found between the two categories of educational level; however, the groups were small and the data was not normally distributed. Larger databases are needed to confirm

Table III. — Seroprevalence of CMV-specific IgG antibodies in pregnant women in Europe in the last decade.

Ref	Year	Country	Population	N	Age	% positive for CMV-specific IgG
Alanen A, et al.	2000	Finland	Pregnant women	558	16-45 years	56.3%
Knowles S, et al.	2005	Ireland	Pregnant women	670	Child-bearing age	30.4%
Eskild A, et al.	2005	Norway	Pregnant women	1238	Child-bearing age	70%
Naessens A, et al.	2005	Belgium	Pregnant women	7140	Child-bearing age	53.9%
Barbi M, et al.	2006	Italy	Prenatal screening	1200	Child-bearing age	79.9%
De Paschale M, et al.	2005-2007	Italy	Pregnant women	2817	Child-bearing age	68.3%
Gaytant M, et al.	2005	The Netherlands	Pregnant women	7524	16-47 y	41%

this low seroprevalence in childbearing women and to elucidate the possible influencing factors.

An active transport of IgG to the neonate was found with a woman/cord blood ratio of 1.15/1. Maternal CMV (IgG) antibodies are available to the foetus as early as in the sixth week of gestation (Jauniaux et al., 1995). Active transport across the placenta increases with advancing gestation, and the most efficient placental transfer occurs after 34 weeks of gestation with the FcRn receptor (Bergamini et al., 1977). Maidji et al. (2006) suggested that CMV virions use the IgG Fc receptor as a means to enter and propagate in the placenta. This finding could explain the efficacy of hyperimmune IgG for the treatment of primary CMV infection during gestation. A higher efficiency of placental transport of CMV IgG was shown in German children compared with children in Mali, possibly due to differences in nutrition and co-morbidity (Sarateanu et al., 1983).

Peckham et al. (1983) analysed samples from the routine screening of pregnant women to detect evidence for CMV infection, and reported that 3/1000 infants were congenitally infected. Of these, 67% were born to mothers with a primary CMV infection during pregnancy, and 17% were born to mothers with a recurrent infection (Peckham et al., 1983). Fowler et al. (Fowler et al., 1992) (1992) diagnosed symptomatic CMV infection at birth only in the group of women who experienced their primary infection during pregnancy. After 4.7 years of follow-up, however, one or more sequelae were perceived in 25% of the primary infected group (13% mental disabilities, 15% hearing loss, 8% bilateral hearing loss) and 8% (0% mental disabilities, 5% hearing loss) of the recurrent infected group. Re-infection with a different strain can lead to intrauterine transmission of CMV and symptomatic congenital disease (Boppana et al., 2001); therefore, immunity prior to conception yields only partial protection against intrauterine transmission.

Are maternal antibodies protective against CMV infection after delivery? Mothers are also the main source of perinatal CMV infection (Natali et al., 1997) through contaminated genital secretions and milk. Although higher titres of maternal antibodies are detected in cord blood than in the maternal circulation following the birth of a full-term child, maternal antibodies appear unable to prevent naturally-acquired primary CMV infections in newborns, as diagnosed by virus excretion in the urine (Leinikki et al., 1978; Reynolds et al., 1978; Gotlieb-Stematsky et al., 1983; Jauniaux et al., 1995). Maternal antibodies can be detected in children for 2-4 months (Leinikki et al., 1978) without significant protective effects against infections acquired at birth (Reynolds et al., 1973, 1978).

The reported high susceptibility to CMV in women at childbearing age and the potential exposure of woman and foetus to contaminated semen should be taken into consideration when drafting recommendations on screening of donor semen used in assisted reproductive technology (Barratt et al., 1998; Liesnard et al., 2001).

Conclusion

Because susceptibility to CMV infection during childbearing age was high in this particular population of pregnant women, preventing infection during

pregnancy is important. Because vaccination is not an option yet, the main actions that can be undertaken are prenatal prevention (Adler et al., 2004), prenatal screening and postnatal screening (Ludwig et al., 2009). No country in the world has yet implemented a specific universal program of prenatal or in-pregnancy screening for CMV susceptibility. Screening for CMV is recommended for all pregnant women at the beginning of pregnancy in Flanders by the Association of Obstetricians (VVOG, 2000). In Switzerland, screening is recommended for specific risk groups (Frischknecht et al.), and other countries (e.g., the United States) advise hand hygiene and avoiding contact with saliva and other bodily fluids from young children. Such hygiene counselling in France resulted in lower quarterly incidences of CMV infections during pregnancy (Vauloup-Fellous et al., 2009).

Some authors are not convinced of the power of screening for all women because no real therapy or vaccine is available, but public awareness should also be considered. Currently, public awareness is insufficient (Jeon et al., 2006, Ross et al., 2008), but adherence to preventive measures might be more effective if women are informed of their serological status (Vauloup-Fellous et al., 2009).

Vaccination may offer a resolution to the problem of CMV infection during pregnancy, provided that neutralising antibodies through immunisation would offer protection against vertical and perinatal CMV infection (Boppana et al., 2001). Currently, several trials are being conducted on the safety and efficacy of newly developed vaccines (Pass, 2009; Pass et al., 2009; Schleiss, 2009).

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