Cytomegalovirus antibody status at 17-18 weeks of gestations and pre-eclampsia: A case-control study of pregnant women in Norway

KM Strand,[a] ML Odland,[a] A-C Iversen,[a] SA Nordbø,[b,c] T Vik,[b] R Austgulen[a]

[a] Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway [b] Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway [c] Department of Medical Microbiology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Correspondence: KM Strand, NTNU, Faculty of Medicine, Department of Cancer Research and Molecular Medicine, PO Box 8905, MTFS, N-7491 Trondheim, Norway. Email: krist@stud.ntnu.no.

Running title: Cytomegalovirus infection and pre-eclampsia

Objective To assess the association between maternal cytomegalovirus (CMV) antibodies in mid-pregnancy and pre-eclampsia.

Design Nested case-control study.

Setting Pregnancies registered in the Norwegian Mother and Child Cohort Study (MoBa), a large population-based pregnancy cohort (1999-2006).

Sample 1500 pre-eclamptic (cases) and 1000 non-pre-eclamptic (controls) pregnant women.

Methods Plasma samples and pregnancy-related information were provided by the MoBa. Antibody status (CMV IgG and CMV IgM) and levels (CMV IgG) at 17-18 weeks of gestation was determined by enzyme-linked immunosorbent assay (ELISA).

Main outcome measures Pre-eclampsia, as defined in the Medical Birth Registry of Norway.

Results There was no evidence of an effect of CMV IgG seropositivity on likelihood of developing pre-eclampsia, and CMV IgG antibody levels among seropositive women did not differ between groups. Adjusted for maternal age, parity and smoking, the odds ratio for pre-eclampsia in CMV IgG seropositive women was 0.88 (95% confidence interval 0.74-1.04; $P = 0.17$). The proportions of IgM seropositive women did not differ between cases and controls ($P = 0.98$). Among nulliparous women, the proportion seropositive for CMV IgG was slightly lower among cases (53.5%) than controls (59.8%) ($P = 0.03$). Subgroup analyses were performed for cases with early/late onset manifestations, with preterm delivery and/or neonates with small for gestational age, but antibody status did not differ between pre-eclampsia subtypes and controls.

Conclusions Maternal antibodies to CMV was not associated with pre-eclampsia in our study. The results suggest that CMV infection unlikely is a major cause of pre-eclampsia.

Keywords Cytomegalovirus, infection, pre-eclampsia, pregnancy, epidemiology.

Introduction

Pre-eclampsia is a complication of pregnancy, characterised by the presence of elevated blood pressure and proteinuria occurring in a pregnant woman after 20 weeks of gestation.[1] Pre-eclampsia occurs in 2-7% of pregnant women and is a major cause of maternal and perinatal morbidity and mortality.[1] However, the underlying cause of pre-eclampsia is poorly understood.

Cytomegalovirus (CMV) infection is a frequent viral infection in human pregnancy, with severe consequences for neonatal morbidity and long-term neurological development.[2] Following a primary infection, lifelong CMV latency is established in the host. Incidents of recurrent CMV infection in pregnancy are more common than primary infections, and most often caused by reactivation of latent virus, although reinfection with a different strain of CMV is also possible.[2] In seronegative women, primary CMV infection occurs in 1-4 % of pregnancies and transmits frequently to the fetus (30-40%).[3] However, the majority of all congenital infections are estimated to be due to recurrent maternal infection, although the transmission rate is low (1%).[4] Infection by CMV in a pregnant woman may evoke mononucleosis-like symptoms, but most women are asymptomatic.[5]

In early pregnancy, CMV infection can cause fetal death and miscarriage.[6-7] It has also been hypothesized that early CMV infections may adversely affect placentation, with subsequent pregnancy complications, such as pre-eclampsia.[8-9] Proper placentation depends on successful spiral artery remodeling ensured by optimal fetal trophoblasts invasion and angiogenic function of maternal Natural Killer (NK) cells in deciduas.[10] An established initial step of pre-eclampsia pathogenesis, is insufficient trophoblast invasion and NK cell interaction, leading to unconverted narrow spiral arteries and shallow placentation.[11] CMV infection induces impaired invasiveness in trophoblasts, direct activation of NK cells, and

may interfere with trophoblast interaction with NK cells by regulating trophoblast HLA expression.[8, 12 13] Hence, it is plausible that CMV infection in early pregnancy may underlie the inadequate spiral artery remodeling and shallow placentation of pre-eclampsia.

A small case-control study by Carreiras et al. reported an increased risk for developing pre-eclampsia in women with specific HLA-G alleles combined with CMV infection.[14] Previous studies investigating the potential association between CMV antibodies in pregnancy and development of pre-eclampsia are inconsistent.[15-17] Risk for developing pre-eclampsia has been shown associated with both 1) increased CMV immunoglobulin (Ig) G seroprevalence;[15] 2) increased CMV IgG antibody levels;[15-16] and 3) decreased CMV IgG seroprevalence.[17] Accordingly, the relation between CMV infection and pre-eclampsia remains uncertain.

Most previous studies have had limited power to make reliable estimates of association. The Norwegian Mother and Child Cohort Study (MoBa) is a prospective pregnancy cohort, with biological specimens and background data from more than 100 000 pregnancies, providing a unique opportunity with sufficient statistical power to investigate the association between CMV infection and pre-eclampsia. Using these prospectively collected data from the MoBa we, in the present study, wanted to explore the hypothesis that women who develop pre-eclampsia are more likely to have positive CMV IgG or IgM antibodies or higher CMV IgG antibody levels at 17-18 weeks of gestation than mothers who do not develop pre-eclampsia.

Methods

Subjects

The MoBa is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health.[18-19] Participants were recruited from all over Norway from 1999-2008 and 38.5% of invited women consented to participate. By 2006, the cohort included 74 000 pregnancies and 75 000 children. Blood samples were obtained from the mothers during pregnancy and from mothers and children (umbilical cord) at birth. Follow-up is conducted by questionnaires at regular intervals and by linkage to national health registries. Several sub-studies are conducting additional collections of data and biological materials.

The current study is based on version 4 of the quality-assured data files of participants recruited during the period 1999-2006. The data collected from the MoBa questionnaires were combined, using personal identification numbers, with data from the Medical Birth Registry of Norway (MBRN),[20] to obtain information on pre-eclampsia in pregnancy and additional data concerning the mothers, their pregnancy and the newborns. Since 1967, the MBRN has used standardised forms, completed by midwifes and physicians, to record information on all births in Norway.[21] The study was approved by the Regional Committee for Medical Research Ethics in South-Eastern Norway. Informed consent was obtained from each MoBa participant upon recruitment.

Variables

The primary outcome variable in this study was pre-eclampsia, as registered in the MBRN. Diagnostic criteria for pre-eclampsia used by the MBRN are a) hypertension (i.e. systolic blood pressure (BP) ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg) and proteinuria occurring after 20 weeks of gestation, or b) hypertension, proteinuria and oedema or c) eclampsia and/or toxaemia.[22] Proteinuria was defined as protein concentration ≥ 0.3 g/24 h urine or ≥ +1 dipstick.

Pre-eclampsia is a heterogeneous syndrome, and the mechanisms implied in the pathology may differ.[23-25] Pre-eclampsia was therefore divided into subgroups according to clinical phenotypes of disease, and these subtypes were studied as secondary outcome variables. Subgroups explored included a) early onset pre-eclampsia; b) late onset pre-eclampsia (i.e. clinical manifestations before or after the start of the 34th week of gestation); c) pre-eclampsia combined with the birth of a small for gestational age (SGA) infant (i.e. birth weight for gestational age less than the tenth percentile adjusted for sex);[26] d) pre-eclampsia restricted to preterm birth (i.e. delivery before 37 weeks of gestation) and e) pre-eclampsia in combination with SGA and preterm birth.

The inclusion criteria to our study were 1) present information about pre-eclampsia in the MBRN and 2) available plasma samples in the MoBa biobank (1999-2006). With consideration of economical expenses, 1500 pre-eclamptic and 1000 non-pre-eclamptic pregnancies were randomly selected as cases and controls, respectively. Fourteen case pregnancies were found not to fulfil the diagnostic criteria for pre-eclampsia, and these were excluded. Four control women were withdrawn by the MBRN because they discovered an error in the variable eclampsia for the year 2006. Twenty-one women had participated with two pregnancies in our sample (16 case women and five controls), and their latest registered pregnancy were excluded. The final study sample therefore included 1470 women with pre-eclamptic pregnancies and 991 women with non-pre-eclamptic pregnancies.

To assess exposure to CMV, plasma samples collected from the mothers at 17-18 weeks of gestation were examined. The 2461 plasma samples collected from the pregnant women were stored at -80°C. Levels of CMV IgM and IgG antibodies were determined by enzyme-linked immunosorbent assay (ELISA) (Medac, Hamburg, Germany). Seropositivity was defined according to the guidelines given by the manufacturer. The CMV IgM test used was an anti-IgM capture (μ-capture) ELISA, while the CMV IgG test was an indirect ELISA.

The CMV IgG levels were reported as Arbitrary Units per ml (AU/ml). Women with equivocal results (18 women for IgM antibodies (11 cases and seven controls), 57 women for IgG antibodies (22 cases and 35 controls)) were excluded from analysis. Determination of antibody status was conducted blinded; without knowledge of pre-eclampsia status.

We obtained information on the pre-eclampsia diagnosis (early/late onset), the mothers (maternal age at delivery, diabetes, chronic hypertension and civil status), the indexed pregnancies (parity, gestational age at birth, twin pregnancy) and the neonates (infant sex, birth weight) from the MBRN. Women's parity was dichotomised as nulliparous (no previous births) or parous (at least one previous birth). In the MBRN, gestational age at birth is calculated from routine ultrasonographic (US) measurements of biparietal diameter at 17-18 weeks of gestation according to Norwegian standard curves, and if US measurement is missing, from the date of the last menstrual period. Information on the mother's level of education, native language and smoking in pregnancy was obtained from the MoBa questionnaire completed at 15 weeks of gestation. Mothers reported their education on a seven level scale. We recoded this information into four levels of education (Elementary School (level I), Secondary School (level II), maximum four years of Higher education (level III) and more than four years of Higher education (level IV)). Participants were asked to state their native language if other than Norwegian. We dichotomized native language as Norwegian or other. Women were asked "Do you smoke now (after you became pregnant)?". We defined women as smokers ("sometimes" or "daily") or non-smokers ("no").

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 17) (SPSS Inc., Chicago, IL). We excluded the latest registered pregnancy of all women who participated with two pregnancies during the inclusion period. Student *t* test was used to compare differences in means between pre-eclamptic and non-pre-eclamptic

pregnancies for continuous variables. Mann-Whitney U-test was applied for comparison of continuous variables with a non-normal distribution. Differences in proportions were analyzed using Chi square statistics. Logistic regression was used to calculate crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) for pre-eclamptic pregnancies in mothers who were seropositive for CMV, compared to CMV seronegative women. In these analyses, a variable was considered a confounder if it could theoretically be associated with both exposure (CMV) and outcome (pre-eclampsia). Thus, maternal age, parity and smoking in pregnancy were studied as possible confounding variables. In addition, confounding by parity (i.e. nulliparous vs. parous) was also studied in stratified analyses.

The study group comprised 90 twin pregnancies, 80 among cases and 10 among controls. Since the risk of pre-eclampsia triples in multiple pregnancies,[27] we explored antibody differences between cases and controls by first including twin pregnancies, then excluding them.

Two-sided *P* values lower than 0.05 was considered statistically significant. The number of cases and controls were based upon power calculations assuming a first-trimester CMV IgG seroprevalence of 70% among pregnant women in Norway.[28] Based upon these calculations, by including 1500 cases and 1000 controls, our study had 80% power to detect a 5 % higher proportion of CMV IgG antibody positive women in the case than in the control group ($\alpha = 0.05$).

Results

Antenatal maternal data and their infants' status at birth are shown in Table 1. The proportion of women with positive CMV IgG antibody status did not differ between cases (56.6%) and controls (60.5%) (*P* = 0.06) (Table 2). After adjustment for maternal age at delivery, parity and smoking in pregnancy the OR of pre-eclampsia for women with IgG antibodies was 0.89

(95% CI 0.74-1.05; $P = 0.17$). When only CMV IgG seropositive women were considered, the level of CMV IgG was equal between cases and controls ($P = 0.8$) (Table 2). The proportion of women seropositive for CMV IgM in the two groups was identical (1.2%) ($P = 0.98$). The adjusted OR for pre-eclampsia was 1.07 (95% CI 0.48-2.36; $P = 0.87$) for women seropositive for CMV IgM.

When twin pregnancies were excluded from the above analyses, the results were essentially unchanged (data not shown).

Among nulliparous women, the proportion of CMV IgG seropositive women was lower among cases (53.5%) than controls (59.8%) ($P = 0.03$). After controlling for maternal age and smoking, the OR for developing pre-eclampsia was 0.76 (95% CI 0.59-0.97; $P = 0.03$) for CMV IgG seropositive women compared with seronegative women. Regarding CMV IgM among nulliparous women, the proportions of seropositive women did not differ between the case and control groups ($P > 0.6$) (data not shown). Among parous women, there was no evidence to suggest a difference in CMV seropositivity between cases and controls ($P > 0.5$) (data not shown).

Table 3 shows the number of women in each of the five subgroups of pre-eclampsia and some of their characteristics. Among women with late onset pre-eclampsia, 732 (56.5%) women were seropositive for CMV IgG compared to 578 (60.5%) of women without pre-eclampsia ($P = 0.06$) (Table 4). When these analyses were stratified according to parity, nulliparous case women had a lower prevalence of CMV IgG seropositives (53.1%) than control women (59.8%) ($P = 0.03$). After adjustment for maternal age and smoking, nulliparous CMV IgG seropositive women had reduced OR for late onset pre-eclampsia (OR 0.75, 95% CI 0.58-0.96; $P = 0.02$). There were no differences in the proportions of CMV IgG seropositive women between any of the four other case subgroups and controls (Tables 4 and

5). When CMV IgG seropositive women were considered, there was no evidence to suggest a difference in levels of CMV IgG between subgroups of preeclampsia and controls ($P > 0.09$) (Table 4, Table 5). There were no differences in CMV IgM seropositive women between any of the five pre-eclampsia subgroups and controls ($P > 0.15$) (data not shown).

Discussion

In this large population-based study, we were not able to confirm our hypothesis that women who develop pre-eclampsia are more likely to have detectable CMV IgM antibodies at 17-18 weeks of gestation or to have higher prevalence or higher CMV IgG antibody levels than the control group. Thus, our results suggest that CMV infection in early pregnancy unlikely plays a role in development of pre-eclampsia.

The strengths of our study include the prospective recoding of perinatal data in the MBRN, the prospective, population based design of the MoBa, the large sample size and that all testing was blinded. Also, the proportion of CMV IgM seropositive women (1.2%) was within the expected range.[29] Misclassification in the MBRN, registering normal pregnancies as pre-eclampsia and vice versa, could potentially dilute the differences between cases and controls, and lead to an underestimation of a potential association between CMV infection and pre-eclampsia. However, we recently evaluated the validity of the pre-eclampsia diagnosis in the MBRN for the period 1967- 2002 and found that pre-eclampsia could be confirmed in 88.3% of the pre-eclamptic pregnancies recorded in the MBRN (Liv Cecilie Vestrheim Thomsen, manuscript *in prep.*). The accuracy of the diagnosis is probably even higher in the latest decades, due to improved diagnostic criteria, and we therefore consider such misclassification to be of less significance in our study. Economic constraints limited the

total number of women available for analyses, and due to the aim of the study, the number of cases was prioritised. Importantly, this did not alter the statistical power of the analyses.

Multivariable analyses did not indicate confounding by maternal age, smoking and parity, although in analysis restricted to nulliparous women, the proportion of CMV IgG seropositive women was slightly lower in the pre-eclampsia group. This finding was also observed among the subgroup of women with late onset pre-eclampsia. Both findings are in the same direction, and coherent with the borderline non-significant difference in proportions of seropositive women between the pre-eclampsia and the control group (Table 2). We are not aware of obvious biological explanations for these small differences. However, we emphasise that in this relatively large study population, *P*-values between 0.01 and 0.05 should be interpreted with caution. Even if the results could be due to multiple comparisons, we did not adjust for multiple comparisons, since the results were coherent, and since several authors have advised against such adjustment.[30-32] On the other hand, lack of statistical significant findings in some of the other subgroup analyses including early onset pre-eclampsia and those included in Table 5 should also be interpreted with caution due to a more limited statistical power in these analyses.

A limitation of our study is that antibodies to CMV were measured only at 17-18 weeks of gestation. Women seronegative at this time might have become infected later in pregnancy (seroconversion), and we cannot exclude that CMV infection may play a role later in pregnancy. However, the hypothesized impact of CMV infection on pre-eclampsia is that it may disturb the trophoblast invasion in first trimester.[14] If CMV infection played a major role in the pathogenesis of pre-eclampsia, it would be reasonable to expect a higher level of antibodies or a higher proportion of seropositive mothers in the pre-eclamptic group, even at 17-18 weeks of gestation.

Another potential limitation to our study is that a higher proportion of control women had equivocal results for IgG compared with cases, which could suggest selection bias. However, since the largest proportion was in the control group, it seems unlikely that this could explain our results.

Several investigators have reported an association between maternal CMV infection and pre-eclampsia.[14-17] Most of these studies had a limited number of case women. In the largest study to date, Xie et al. compared 78 women with pre-eclampsia to 109 normal pregnancy controls, and found higher levels of CMV IgG and that CMV IgG seropositivity was more common in the pregnancies complicated by pre-eclampsia (53% versus 27%, chi-squared $P < 0.01$).[15] However, in contrast to our study population, their participants included several ethnic groups in which the seroprevalence of CMV is known to vary,[2] and the women were recruited from a single tertiary referral centre.

Although von Dadelszen and co-authors reported elevated levels of CMV IgG in a small group of women with early onset pre-eclampsia (n=9), their results agreed with ours in that mid-trimester women demonstrated no difference in the proportion of CMV IgG seropositive mothers between women who later developed pre-eclampsia and women with normal pregnancy outcomes.[16] A recent systematic review was also in agreement with our observations.[33] However, Trogstad et al. reported that women seronegative to CMV IgG in early pregnancy were at increased risk of developing pre-eclampsia, although not statistically significant (OR 1.6, 95% CI 0.8-3.2).[17] The authors discussed whether this finding might suggest susceptibility to acquire a primary CMV infection during pregnancy. Somewhat in consistence with their results, we found that nulliparous, CMV IgG seropositive women had a marginally lower OR of developing pre-eclampsia. The same finding was observed among women with late onset pre-eclampsia. However, the differences between the groups were

minor, and we therefore consider it unlikely that late CMV infection plays a central role in the pathogenesis, even of late onset pre-eclampsia.

The impact of recurrent CMV infection on pre-eclampsia could not be accurately assessed in our study. CMV IgM serology at 17-18 weeks points out some, but not all, active infections (primary and recurrent), and CMV IgG serology does discriminate between women who have a latent infection and those with an active infection. Determination of CMV IgG levels in CMV IgG positive women is only indicative of active infections when only one blood sample is tested, but may, when interpret with caution, be an additional indicator to CMV IgM serology. Since no differences in CMV IgM and IgG prevalence or CMV IgG levels were detected between women with or without pre-eclampsia the occurrence of active CMV infections does not seem to differ between the two groups. Moreover, if recurrent infection with CMV was a major cause of pre-eclampsia, women seropositive for CMV IgG would more often develop the disease. Despite limitations in serology interpretations, primary and recurrent CMV infections are from these data unlikely contributors to the development of pre-eclampsia.

Conclusion

In our large cohort study, we found neither increased levels of CMV IgG nor higher proportions of CMV seropositives (CMV IgG/IgM) among pregnant women who later developed pre-eclampsia. Thus, our findings do not suggest that CMV infection is an important cause of pre-eclampsia.

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Disclosure of interests

There are no conflicts of interest.

Contribution to Authorship

KMS analysed the data and wrote the manuscript. MLO participated in data analyses and writing of the manuscript. ACI contributed to research hypothesis, study design and revision of the manuscript. SAN contributed to the study design and data interpretation and was responsible for serological testing. TV supervised the statistical analyses and data interpretation and revised the manuscript. RA was responsible for research hypothesis, study design and revision of the manuscript. All authors approved the final version of the submitted manuscript.

Details of ethics approval

The study was approved by the Regional Committee for Medical Research Ethics in South-Eastern Norway, date: 27 February 2006, reference number: S-06072.

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Reference list

1 Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005;365:785-99.

2 Stagno S. Cytomegalovirus. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 5th edn. Philadelphia (PA): W. B. Saunders Co; 2001. p. 389-424.

3 Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Walton PD, et al. Primary Cytomegalovirus Infection in Pregnancy. *JAMA: The Journal of the American Medical Association* 1986;256:1904-8.

4 Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. *Clin Infect Dis* 2011;52:e11-3.

5 Mocarski ES, Shenk T, Pass RF. Cytomegaloviruses. In: Knipe DM, Howley PM, editors. *Fields Virology*. 5th edn. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 2701-72.

6 Griffiths PD, Baboonian C. A prospective study of primary cytomegalovirus infection during pregnancy: final report. *Br J Obstet Gynaecol* 1984;91:307-15.

7 Tanaka K, Yamada H, Minami M, Kataoka S, Numazaki K, Minakami H, et al. Screening for vaginal shedding of cytomegalovirus in healthy pregnant women using real-time PCR: correlation of CMV in the vagina and adverse outcome of pregnancy. *J Med Virol* 2006;78:757-9.

8 Fisher S, Genbacev O, Maidji E, Pereira L. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. *J Virol* 2000;74:6808-20.

9 von Dadelszen P, Magee LA. Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? *Acta Obstet Gynecol Scand* 2002;81:642-8.

10 Eastabrook G, Hu Y, von Dadelszen P. The role of decidual natural killer cells in normal placentation and in the pathogenesis of preeclampsia. *J Obstet Gynaecol Can* 2008;30:467-76.

11 Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005;308:1592-4.

12 Yamamoto-Tabata T, McDonagh S, Chang HT, Fisher S, Pereira L. Human cytomegalovirus interleukin-10 downregulates metalloproteinase activity and impairs endothelial cell migration and placental cytotrophoblast invasiveness in vitro. *J Virol* 2004;78:2831-40.

13 Le Bouteiller P, Pizzato N, Barakonyi A, Solier C. HLA-G, pre-eclampsia, immunity and vascular events. *J Reprod Immunol* 2003;59:219-34.

14 Carreiras M, Montagnani S, Layrisse Z. Preeclampsia: a multifactorial disease resulting from the interaction of the feto-maternal HLA genotype and HCMV infection. *Am J Reprod Immunol* 2002;48:176-83.

15 Xie F, Hu Y, Magee LA, Money DM, Patrick DM, Krajden M, et al. An association between cytomegalovirus infection and pre-eclampsia: a case-control study and data synthesis. *Acta Obstet Gynecol Scand* 2010;89:1162-7.

16 von Dadelszen P, Magee LA, Krajden M, Alasaly K, Popovska V, Devarakonda RM, et al. Levels of antibodies against cytomegalovirus and Chlamydophila pneumoniae are increased in early onset pre-eclampsia. *BJOG* 2003;110:725-30.

17 Trogstad LI, Eskild A, Bruu AL, Jeansson S, Jenum PA. Is preeclampsia an infectious disease? *Acta Obstet Gynecol Scand* 2001;80:1036-8.

18 Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C. Cohort profile: The Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol* 2006;35:1146-50.

19 Ronningen KS, Paltiel L, Meltzer HM, Nordhagen R, Lie KK, Hovengen R, et al. The biobank of the Norwegian Mother and Child Cohort Study: a resource for the next 100 years. *Eur J Epidemiol* 2006;21:619-25.

20 The Medical Birth Registry of Norway. General information [cited 2012 1. February]; Available from: http://www.fhi.no/eway/default.aspx?pid=238&trg=MainArea_5811&MainArea_5811=5895:0:15,3320:1:0:0:::0:0

21 Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstet Gynecol Scand* 2000;79:435-9.

22 Trogstad L, Magnus P, Moffett A, Stoltenberg C. The effect of recurrent miscarriage and infertility on the risk of pre-eclampsia. *BJOG* 2009;116:108-13.

23 Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am J Obstet Gynecol* 1996;175:1365-70.

24 Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet* 1993;341:1447-51.

25 Vatten LJ, Skjaerven R. Is pre-eclampsia more than one disease? *BJOG* 2004;111:298-302.

26 Skjaerven R, Gjessing HK, Bakketeig LS. Birthweight by gestational age in Norway. *Acta Obstet Gynecol Scand* 2000;79:440-9.

27 Coonrod DV, Hickok DE, Zhu K, Easterling TR, Daling JR. Risk factors for preeclampsia in twin pregnancies: a population-based cohort study. *Obstet Gynecol* 1995;85:645-50.

28 Eskild A, Jenum PA, Bruu AL. Maternal antibodies against cytomegalovirus in pregnancy and the risk of fetal death and low birth weight. *Acta Obstet Gynecol Scand* 2005;84:1035-41.

29 Hyde TB, Schmid DS, Cannon MJ. Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. *Rev Med Virol* 2010;20:311-26.

30 Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43-6.

31 Bacchetti P. Peer review of statistics in medical research: the other problem. *BMJ* 2002;324:1271-3.

32 Altman D. Statistics in medical journals: development in the 1980s. *Stat Med* 1991;10:1897-913.

33 Conde-Agudelo A, Villar J, Lindheimer M. Maternal infection and risk of preeclampsia: systematic review and metaanalysis. *Am J Obstet Gynecol* 2008;198:7-22.

Table 1. Antenatal maternal data and infant status at birth in cases with and controls without pre-eclampsia in pregnancy. Data are number of women/infants (%) unless otherwise specified

	Pre-eclampsia	
	Yes	**No**
	(*n* = 1470*)	**(*n* = 991*)**
Maternal data		
Age at delivery (years) (mean ± 1SD)	29.3 ± 4.7	29.9 ± 4.5
Nulliparas	921 (62.7)	427 (43.2)
Smoking in pregnancy	101 (7.2)	102 (11.4)
Chronic hypertension	46 (3.1)	6 (0.6)
Diabetes	25 (1.7)	4 (0.4)
Civil status		
Living with partner	1413 (96.2)	951 (96.3)
Education		
Elementary	41 (3.0)	36 (4.0)
Secondary	553 (40.7)	319 (35.0)
Higher ≤ 4 years	535 (39.4)	367 (40.3)
Higher > 4 years	230 (16.9)	189 (20.7)
Native language		
Norwegian	1414 (96.3)	949 (95.8)
Other	55 (3.7)	42 (4.2)
Infant data		
Gestational age in weeks (mean ± 1SD)	37.7 ± 3.2	39.5 ± 1.7
Gestational age < 37 weeks	383 (26.3)	40 (4.1)
Small for gestational age	264 (18.1)	59 (6.0)

* Numbers for some covariates do not total because of missing data.

Table 2. Cytomegalovirus (CMV) IgG and CMV IgM antibodies at 17-18 weeks of gestation and odds ratios (OR) with 95% confidence intervals (CI) for development of pre-eclampsia in women seropositive for CMV IgG and CMV IgM

	Pre-eclampsia		**Crude OR (CI)**	***P* value**	**Adjusted* OR (CI)**	***P* value**
	Yes	**No**				
	N (%)	**N (%)**				
IgG level, median (IQR) among seropositive women**						
	10.8 (7.4-14.2)	10.5 (7.6-14.3)		0.8		
IgG antibodies						
Yes	819 (56.6)	578 (60.5)	0.85 (0.72-1.01)	0.06	0.89 (0.74- 1.05)	0.17
No	629 (43.4)	378 (39.5)				
IgM antibodies						
Yes	18 (1.2)	12 (1.2)	1.01 (0.49-2.11)	0.98	1.07 (0.48- 2.36)	0.87
No	1441 (98.8)	972 (98.8)				

IQR, Interquartile range.

*Adjusted for maternal age, parity and smoking in pregnancy.

** Levels of CMV IgG are measured in Arbitrary Units per millilitre.

Table 3. Maternal data and infant status in clinical subgroups of pre-eclampsia

	Early onset pre-eclampsia (*n* = 155)	Late onset pre-eclampsia (*n* = 1314)	Pre-eclampsia and SGA (*n* = 264)	Pre-eclampsia and premature (*n* = 383)	Pre-eclampsia, SGA and premature (*n* = 117)	Non-pre-eclamptic pregnancy (n= 991)
Maternal data						
Age at delivery (years)	29.9 ± 4.5	29.3 ± 4.8	29.4 ± 4.8	29.8 ± 4.5	29.9± 4.9	29.3 ± 4.5
Nulliparous	99 (63.9)	822 (62.6)	178 (67.4)	253 (66.1)	75 (64.1)	427 (43.2)
Smoking in pregnancy	12 (7.8)	89 (7.1)	20 (7.9)	26 (7.0)	12 (10.4)	102 (11.4)
Infant data						
Gestational age (weeks)	32.9 ± 3.8	38.3 ± 2.6	36.1 ± 3.8	33.4 ± 2.9	32.7 ± 3.2	39.5 ± 1.7
Gestational age < 37 weeks	128 (83.7)	255 (19.5)	117 (44.3)	383 (100)	117 (100)	40 (4.1)
SGA infant	58 (37.9)	206 (15.8)	264 (100)	117 (30.5)	117 (100)	59 (6.0)

Data are n (%) or mean ± standard deviation; SGA, small for gestational age; Early onset pre-eclampsia, onset of pre-eclampsia before 34 weeks of gestation.

Table 4. Cytomegalovirus (CMV) IgG antibody levels (AU/ml) and status in pre-eclampsia subgroups

	Early onset pre-eclampsia (n = 155)	Late onset pre-eclampsia (n = 1314)	Non-pre-eclamptic pregnancy (n= 991)
IgG level, median (IQR) among seropositive women			
	11.5 (8.9-15.2)	10.6 (7.2-14.1)	10.5 (7.6-14.3)
IgG antibodies			
Yes	86 (56.6)	732 (56.5)	578 (60.5)
No	66 (43.4)	563 (43.5)	378 (39.5)

IQR, Interquartile range; AU, Arbitrary units.

Table 5. Cytomegalovirus (CMV) IgG antibody levels (AU/ml) and status in pre-eclampsia subgroups

	Pre-eclampsia and SGA (n = 264)	Pre-eclampsia and premature (n = 383)	Pre-eclampsia, SGA and premature (n = 117)	Non-pre-eclamptic pregnancy (n= 991)
IgG level, median (IQR) among seropositive women				
	10.3 (6.4-14.2)	11.5 (7.6-14.4)	10.8 (5.9-14.1)	10.5 (7.6-14.3)
IgG antibodies				
Yes	145 (55.8)	213 (56.6)	70 (60.9)	578 (60.5)
No	115 (44.2)	163 (43.4)	45 (39.1)	378 (39.5)

IQR, Interquartile range; AU, Arbitrary units.