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LACK OF ASSOCIATION BETWEEN CYTOMEGALOVIRUS INFECTION AND HYPERTENSIVE DISORDERS IN PREGNANCY: A CASE-CONTROL STUDY IN DURANGO, MEXICO

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It is not clear whether infection with cytomegalovirus (CMV) is associated with hypertensive disorders in pregnant women. Through a case-control study design, 146 women suffering from hypertensive disorders in pregnancy (cases) and 146 age-matched normotensive pregnant women (controls) were examined for the presence of anti-CMV IgG and IgM antibodies with enzyme-linked immunoassays. IgM seropositive samples were further assayed by enzyme-linked fluorescent assay (ELFA).

Anti-CMV IgG antibodies were found in 138 (94.5%) controls and in 136 (93.2%) cases (odds ratio [OR] = 0.78; 95% confidence interval [CI]: 0.30–2.05; $P = 0.62$). High (>18 IU/ml) levels of anti-CMV IgG antibodies were found in 37.7% of the 138 seropositive controls and in 34.6% of the 136 seropositive cases (OR = 0.87; 95% CI: 0.53–1.43; $P = 0.59$). Anti-CMV IgM antibodies were found in 1 (0.7%) of the controls but in none of the cases using ELFA ($P = 1.0$). Seropositivity to CMV was not associated with a previous preeclampsia and was similar among cases regardless their mean systolic and diastolic blood pressures, and mean arterial blood pressure.

No serological evidence of an association between CMV infection and hypertensive disorders of pregnancy was found. Further research to elucidate the role of CMV in hypertensive disorders in pregnancy should be conducted.

Keywords: cytomegalovirus, seroprevalence, preeclampsia, HELLP syndrome, eclampsia, infection, epidemiology

Introduction

Cytomegalovirus (CMV) is a DNA virus of the Herpesviridae family and is widely distributed around the world [1]. Major routes of CMV infection are person-to-person contact [2] and blood transfusion [3]. Infections with CMV are persistent [4], and their reactivations contribute for shedding of infectious virus [2, 5]. Immunocompromised individuals infected with CMV may develop a severe disease including encephalitis, pneumonia, retinitis, and hepatitis [6]. Furthermore, CMV is an important pathogen leading to congenital infections [7–9]. The clinical spectrum of congenital infections varies from asymptomatic [10] to severe disease including mental retardation, cerebral palsy, hearing loss, and neurodevelopmental delay [9, 11]. Infections with CMV may occur in placenta [12–14]. In addition, inflammation and edema in placenta induced by CMV infections have been observed in cases of preeclampsia [13, 14]. Preeclampsia and other hypertensive disorders in pregnancy are major health problems leading to maternal and perinatal morbidity and mortality [15–17]. Worldwide estimates indicate that about 8.5 mil-

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lion pregnant women suffer from preeclampsia every year [18], and 4.6% and 1.4% of all deliveries are associated with preeclampsia and eclampsia, respectively [19]. The role of CMV in hypertension disorders in pregnancy is controversial. Several serological studies have failed to demonstrate a correlation of CMV infection and hypertensive disease in pregnancy [20–22]. In contrast, several serological studies have found an association of CMV infection with preeclampsia [23–25]. Therefore, we sought to determine the association of CMV infection and hypertensive disorders in pregnant women in Durango City, Mexico.

Materials and methods

Study design and study populations

We performed a case-control study to assess the association of CMV infection with hypertensive disorders in pregnancy using serum samples from a recent *Toxoplasma gondii* study in women in Durango City, Mexico [26]. Serum samples were obtained in a public hospital (General Hospital) from the Secretary of Health from November 2011 to September 2013. Cases and controls were matched by gender, age, attending hospital, and residence place. A 1:1 ratio for matching was used.

Women suffering from hypertensive disorders in pregnancy

Inclusion criteria for the group of cases were: 1) pregnant women at their 24–42 weeks of pregnancy suffering hypertensive disorders and proteinuria attended in the Department of Gynecology and Obstetrics of the General Hospital in Durango City, Mexico; and 2) who agreed voluntary participation in the study. Hypertensive disorders during pregnancy were mild preeclampsia, severe preeclampsia, eclampsia, and HELLP syndrome. Mild preeclampsia was defined as blood pressure ≥140/90 mmHg on 2 occasions, at least 6 h apart, and proteinuria of ≥300 mg/24 h. Severe preeclampsia was considered as blood pressure ≥160/110 mmHg on 2 occasions, at least 6 h apart, and proteinuria of ≥5 g/24 h. Eclampsia was diagnosed when hypertension, proteinuria, and seizures in a patient were found. HELLP syndrome was defined as hypertension, proteinuria and presence of hemolytic anemia, elevated liver enzymes, and low platelet count. All eligible women attended in the Department of Gynecology and Obstetrics of the General Hospital during the study period were invited to participate. In total, 146 patients suffering from hypertensive disorders in pregnancy were included in the study. All of them resided in Durango City. In total, 146 cases were enrolled in the study. Of them, 27 had mild preeclampsia, 95 severe preeclampsia, 16 eclampsia, and 8 HELLP syndrome. Mean age of the cases was 23.51 ± 6.41 years (range: 15–39 years).

Control pregnant women

Inclusion criteria for the control group were: 1) pregnant women without hypertensive disorders, diabetes, or nephropathy before or during pregnancy attended in the Department of Gynecology and Obstetrics of the General Hospital in Durango City; 2) to have a normal pregnancy with systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg; 3) patients without any underlying disease; and 4) who agreed voluntary participation in the study. Thus, 146 control women were included in this case-control study. Controls were 23.44 ± 6.17 (range: 15–39) years old, and their age was comparable to the one in cases ($P = 0.92$).

General clinical characteristics including age, number of pregnancies, cesarean sections, and deliveries, history of miscarriages and stillbirths, trimester of present pregnancy, history of preeclampsia, systolic and diastolic blood pressures, and mean arterial pressure from all participants were obtained.

Laboratory tests

Serum samples of the participants were kept frozen until analyzed. The presence of anti-CMV antibodies in serum samples was determined by commercially available enzyme immunoassays (EIA). Sera were analyzed for anti-CMV IgG antibodies by the "Cytomegalovirus IgG (CMV IgG)" kit (Diagnostic Automation Inc., Calabasas, CA, USA). In addition, all sera were tested for anti-CMV IgM antibodies by the "Cytomegalovirus IgM (CMV IgM" kit (Diagnostic Automation Inc., Calabasas, CA, USA). All tests were performed following the manufacturer's instructions. The cut-off values for IgG and IgM seropositivity were obtained by firstly multiplying the mean optical densities of IgG and IgM calibrators by the correction factor (0.50) of the calibrator to obtain the corrected mean cut-off value; secondly, the CMV G and M indexes were calculated by dividing the optical density of each sample by the corrected mean cut-off value. A serum sample was considered positive for IgG or IgM antibodies when a CMV G index or a CMV M index was greater than 1.1, respectively. Negative and positive controls were included in each run. Samples positive for IgM by EIA were further tested by a commercially available enzyme linked fluorescent assay (ELFA): "CMV IgM Vidas" (BioMériux, France).

Statistical analysis

We performed the statistical analysis with the aid of the software Epi Info version 7, and SPSS 15.0 (SPSS Inc., Chicago, Illinois). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, a reference seroprevalence of 65.6% [27] as the expected frequency of exposure in controls, and an odds ratio of 2.3. The result of the sample size

calculation was 134 cases and 134 controls. We compared the age values among the groups by the paired Student's *t* test. The Pearson's χ^2 test and the two-tailed Fisher's exact test (when values were small) were used to assess the association between CMV seropositivity and clinical data of the pregnant women. We calculated the odds ratio (OR) and 95% confidence interval (CI), and a *P* value of <0.05 was considered statistically significant.

Ethics statement

We used only archival serum samples and clinical data from a previous study [26]. The original study was approved by the Institutional Ethical Committee of the General Hospital of the Secretary of Health in Durango City, Mexico. A written informed consent was obtained from all participants.

Results

Anti-CMV IgG antibodies were found in 138 (94.5%) of the 146 controls and in 136 (93.2%) of the 146 cases (OR = 0.78; 95% CI: 0.30–2.05; *P* = 0.62). High (>18 IU/ml) levels of anti-CMV IgG antibodies were found in 52 (37.7%) of the 138 seropositive controls and in 47 (34.6%) of the 136 seropositive cases (OR = 0.87; 95% CI: 0.53–1.43; *P* = 0.59). Anti-CMV IgM antibodies were found in 22 (15.1%) of the 146 controls and in 15 (10.3%) of the 146 cases by using EIA. These EIA IgM positive samples were further tested by ELFA. Anti-CMV IgM antibodies were found in 1 (0.7%) of the 146 controls but in none of the 146 cases using ELFA (*P* = 1.0). Seroprevalence of CMV infection was similar among cases regardless the diagnosis: mild preeclampsia (23/27: 85.2%), severe preeclampsia (89/95: 93.7%), eclampsia (16/16: 100%), and HELLP syndrome (8/8: 100%) (*P* = 0.21).

Seropositivity to CMV did not vary with age (*P* = 0.58). In addition, seropositivity to CMV was not associated (*P* > 0.05) with number of pregnancies, history of deliveries, cesarean sections, miscarriages, stillbirths, or a previous preeclampsia (*Table 1*).

Systolic blood pressure (157.98 ± 17.93 mmHg) in CMV positive cases was similar to that (157.00 ± 21.10 mmHg) in CMV negative cases (*P* = 0.86). Diastolic blood pressure (103.42 ± 10.44 mmHg) in CMV positive cases was similar to that (104.00 ± 12.64 mmHg) in CMV negative cases (*P* = 0.86). There was no difference (*P* = 0.96) in the mean arterial blood pressure among seropositive and seronegative cases (121.64 ± 11.77 mmHg and 121.80 ± 14.82 mmHg, respectively).

Discussion

Infection with CMV has been associated with essential hypertension [28, 29]. In a meta-analysis of three studies, researchers found a significant association between CMV and essential hypertension [29]. However, studies on the association between CMV infection and hypertensive disorders in pregnancy have shown conflicting results [20–25]. Therefore, the present study aimed to determine whether CMV infection is associated with hypertensive disorders in a sample of pregnant women in Durango City, Mexico. Our results of tests including qualitative detection of anti-CMV IgG antibodies, quantitative measure of anti-IgG antibody levels, and qualitative detection of

Table 1. Bivariate analysis of clinical characteristics and rates of IgG seropositivity to CMV in cases

Characteristics	Women tested	Seroprevalence of CMV infection		*P* value
	No.	No.	%	
Pregnancies				
1	75	70	93.3	0.25
2	31	28	90.3	
3	18	17	94.4	
4	8	8	100.0	
5	11	11	100.0	
6	1	1	100.0	
7	2	1	50.0	
Trimester of pregnancy				
2	2	2	100.0	1.00
3	144	134	93.1	
Deliveries				
Yes	65	60	92.3	0.75
No	81	76	93.8	

Table 1. *(cont'd)*

Characteristics	Women tested	Seroprevalence of CMV infection		*P* value
	No.	No.	%	
Cesarean section				
Yes	102	97	95.1	0.16
No	44	39	88.6	
Miscarriages				
Yes	25	25	100.0	0.21
No	121	111	91.7	
Stillbirths				
Yes	3	2	66.7	0.19
No	143	134	93.7	
History of preeclampsia				
Yes	21	19	90.5	0.63
No	125	117	93.6	

anti-CMV IgM antibodies indicate that seropositivity to CMV was equally observed in patients suffering from hypertensive disorders of pregnancy and age-matched pregnant women without these disorders attended in the same hospital. Stratification by clinical types of hypertensive disorders did not show a link between CMV and mild preeclampsia, severe preeclampsia, eclampsia, and HELLP syndrome. Thus, our results do not support an association between CMV infection and hypertensive disorders of pregnancy. Our results conflict with those reported in three previous studies. In a Canadian study, researchers found a significant increase of CMV seropositivity and higher anti-CMV IgG antibody levels in women with preeclampsia than normal pregnancy controls [24]. In another study, Xie et al. found that women with early-onset preeclampsia with HELLP syndrome had a significantly higher CMV IgG seropositivity rate than matched normal and non-pregnancy controls [25]. In addition, women with early-onset preeclampsia had higher anti-CMV IgG antibodies than women with late-onset preeclampsia and normal pregnancy [23]. In contrast, our results agree with those reported by other researchers. Soydinc et al. found that anti-CMV IgG and IgM antibodies seropositivities were not significantly different between pregnant women with preeclampsia and healthy pregnant women [20]. In a Chinese study of 52 pregnant women with preeclampsia and 34 women with uncomplicated pregnancy in their third trimester, seroprevalence of recent and long-dated CMV infections was similar in women with preeclampsia and women with normal pregnancy [21]. In a Norwegian study, no evidence of an effect of CMV IgG seropositivity on the likelihood of developing preeclampsia was found [22].

We found a considerable number of serum samples with positive results of anti-CMV IgM antibodies using EIA. False positive results have been reported in anti-CMV IgM antibody tests [30]. Therefore, to increase the specificity of IgM seropositivity, we used two methods to test for anti-CMV IgM antibodies (EIA and ELFA). Results of ELFA yield only one positive sample for IgM antibodies. The sample was from a control women, and thus, no association of this infection marker with hypertensive disorder of pregnancy was found. This result is in line with the lack of association of CMV IgM seropositivity and preeclampsia reported in a Turkish study [20] and a Chinese study [21].

Our study has some limitations. We enrolled a relatively small cohort of pregnant women, and subgroups of hypertensive disorders were also small. Women from only one public hospital were included in the study. Most women attended in the participating hospital have a low socioeconomic status. Therefore, further studies with a larger sample size, including women from several hospitals, with a higher number of participants with several types of hypertensive disorder of pregnancy and with a variety of socioeconomic status, should be conducted.

Conclusions

No serological evidence of an association between CMV infection and hypertensive disorders of pregnancy in patients in a public hospital in Durango City, Mexico was found. Our results conflict with those reported in previous studies. Therefore, further research to elucidate the role of CMV in hypertensive disorders in pregnancy should be conducted.

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Competing interests

The authors declare that no competing interests exist.

References

1. Hanley PJ, Bollard CM: Controlling cytomegalovirus: helping the immune system take the lead. Viruses 6, 2242–2258 (2014)
2. de Jong MD, Galasso GJ, Gazzard B, Griffiths PD, Jabs DA, Kern ER, Spector SA: Summary of the II International Symposium on Cytomegalovirus. Antiviral Res 39, 141–162 (1998)
3. Lindholm PF, Annen K, Ramsey G: Approaches to minimize infection risk in blood banking and transfusion practice. Infect Disord Drug Targets 11, 45–56 (2011)
4. Goodrum F, Caviness K, Zagallo P: Human cytomegalovirus persistence. Cell Microbiol 14, 644–655 (2012)
5. Cannon MJ, Schmid DS, Hyde TB: Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol 20, 202–213 (2010)
6. Foti G, Hyeraci M, Kunkar A, Ieropoli G, Sofo D, De Lorenzo S, Carpentieri MS: Cytomegalovirus infection in the adult. Minerva Med 93, 109–117 (2002)
7. Hyde TB, Schmid DS, Cannon MJ: Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. Rev Med Virol 20, 311–326 (2010)
8. Kenneson A, Cannon MJ: Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 17, 253–276 (2007)
9. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK: The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev 26, 86–102 (2013)
10. Buonsenso D, Serranti D, Gargiullo L, Ceccarelli M, Ranno O, Valentini P: Congenital cytomegalovirus infection: current strategies and future perspectives. Eur Rev Med Pharmacol Sci 16, 919–935 (2012)
11. Johnson J, Anderson B, Pass RF: Prevention of maternal and congenital cytomegalovirus infection. Clin Obstet Gynecol 55, 521–530 (2012)
12. Laifer SA, Ehrlich GD, Huff DS, Balsan MJ, Scantlebury VP: Congenital cytomegalovirus infection in offspring of liver transplant recipients. Clin Infect Dis 20, 52–55 (1995)
13. Rana S, Venkatesha S, DePaepe M, Chien EK, Paglia M, Karumanchi SA: Cytomegalovirus-induced mirror syndrome associated with elevated levels of circulating antiangiogenic factors. Obstet Gynecol 109, 549–552 (2007)
14. Higgins L, Vause S, Tower C: Cytomegalovirus infection in association with early onset pre-eclampsia. BMJ Case Rep 2010, bcr0320102803 (2010)
15. Fajardo-Dolci G, Meljem-Moctezuma J, Vicente-González E, Venegas-Páez FV, Villalba-Espinoza I, Pérez-Cardoso AL, Barrón-Saldaña DA, Montesinos-Gómez G, Aguirre Güemez AV, Novoa-Boldo A, Mendoza-Larios LA, López-González R, Mazón-González B, Aguirre-Gas HG: Analysis of maternal deaths in Mexico occurred during 2009. Rev. Med. Inst. Mex. Seguro Soc 51, 486–495 (2013)
16. Jido TA, Yakasai IA: Preeclampsia: a review of the evidence. Ann. Afr. Med 12, 75–85 (2013)
17. Lo JO, Mission JF, Caughey AB: Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol, 25, 124–132 (2013)
18. Anderson UD, Olsson MG, Kristensen KH, Åkerström B, Hansson SR: Review: biochemical markers to predict preeclampsia. Placenta 33, S42–47 (2012)
19. Abalos E, Cuesta C, Grosso AL, Chou D, Say L: Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol 170, 1–7 (2013)
20. Soydinc HE, Kan I, Dal T, Evsen MS, Sak ME, Ozler A, Turgut A, Yildiz I: Evaluation of the relationship between preeclampsia and seropositivity of infectious disease in maternal plasma. Clin Ter 164, e199–202 (2013)
21. Zhang JF, Zhang WY: Relationship of cytomegalovirus, *Chlamydia pneumoniae* and herpes simplex virus type 2 infections with preeclampsia. Zhonghua Yi Xue Za Zhi 92, 1413–1415 (2012)
22. Strand KM, Odland ML, Iversen AC, Nordbø SA, Vik T, Austgulen R: Cytomegalovirus antibody status at 17–18 weeks of gestation and pre-eclampsia: a case-control study of pregnant women in Norway. BJOG 119, 1316–1323 (2012)
23. von Dadelszen P, Magee LA, Krajden M, Alasaly K, Popovska V, Devarakonda RM, Money DM, Patrick DM, Brunham RC: Levels of antibodies against cytomegalovirus and *Chlamydophila pneumoniae* are increased in early onset pre-eclampsia. BJOG 110, 725–730 (2003)
24. Xie F, Hu Y, Magee LA, Money DM, Patrick DM, Krajden M, Thomas E, von Dadelszen P; Toxemia Study Group: An association between cytomegalovirus infection and preeclampsia: a case-control study and data synthesis. Acta Obstet Gynecol Scand 89, 1162–1167 (2010)
25. Xie F, von Dadelszen P, Nadeau J: CMV infection, TLR-2 and -4 expression, and cytokine profiles in early-onset preeclampsia with HELLP syndrome. Am J Reprod Immunol 71, 379–386 (2014)
26. Alvarado-Esquivel C, Vázquez-Alaníz F, Sandoval-Carrillo AA, Salas-Pacheco JM, Hernández-Tinoco J, Sánchez-Anguiano LF, Liesenfeld O: Lack of association between *Toxoplasma gondii* infection and hypertensive disorders in pregnancy: a case-control study in a Northern Mexican population. Parasit Vectors 7, 167 (2014)
27. Alvarado-Esquivel C, Hernández-Tinoco J, Sánchez-Anguiano LF, Ramos-Nevárez A, Cerrillo-Soto SM, Estrada-Martínez S, Martínez-Ramírez L, Pérez-Álamos AR, Guido-Arreola CA: Seroepidemiology of cytomegalovirus infection in pregnant women in Durango City, Mexico. BMC Infect Dis 14, 484 (2014)
28. Tang N, Li JW, Liu YM, Zhong H, Wang LM, Deng FM, Qu YY, Hui J, Cheng J, Tang B, Huang G, Guo SX, Li XZ, Wei LL, He F: Human cytomegalovirus infection is associated with essential hypertension in Kazakh and Han Chinese populations. Med Sci Monit 20, 2508–2519 (2014)
29. Hui J, Qu YY, Tang N, Liu YM, Zhong H, Wang LM, Feng Q, Li Z, He F: Association of cytomegalovirus infection with hypertension risk: a meta-analysis. Wien Klin Wochenschr 128, 586–591 (2016)
30. Weber B, Berger A, Rabenau H. Human cytomegalovirus infection: diagnostic potential of recombinant antigens for cytomegalovirus antibody detection. J Virol Methods 96, 157–170 (2001)