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 immunosorbent assays. 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
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. 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” (BioMérieux, 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 χ² 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). Sero-prevalence 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

| 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    |        |

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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.

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