Original Article

Cytomegalovirus Specific Cell-Mediated Immunity Status in Women with Preeclampsia: A Case-Control Study

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Abstract

Background: Preeclampsia, a pregnancy-specific complication, has been associated with cytomegalovirus (CMV) infection in observational studies. CMV-specific T cell response plays a major role in viremia clearance. We explored whether CMV-specific cell-mediated immunity (CMI) status is associated with preeclampsia in pregnant women.

Materials and Methods: CMV-specific CMI was assessed using CMV-QuantiFERON (QF-CMV) assay in plasma serum of 35 women with preeclampsia as well as 35 normal pregnant controls, retrospectively. Participants were matched for gestational age in a 1:1 ratio. The proportion of reactive results, the mean value of interferon-gamma (IFN-γ) level produced in mitogen and antigen tubes were compared between the cases and controls through Chi-square and Wilcoxon rank-sum tests, respectively. The odds ratio and confidence interval were calculated as well.

Results: No significant differences observed between demographic characteristics of the case and control groups. The QF-CMV assay turned reactive (QF-CMV [+]) Women with preeclampsia had lower mean IFN-γ levels in antigen tube compared with normal pregnant controls. There were no statistically significant differences in the value of mitogen tube between case and controls women with suppressed CMV-CMI were 6.3 times more likely to have preeclampsia. This result even strengthened after adjustment for age, gestational age, and gravidity.

Conclusions: Our findings support an association between suppressed CMV-specific CMI and preeclampsia.

Keywords: Cytomegalovirus, immunity, Iran, preeclampsia, pregnancy, QuantiFERON-cytomegalovirus

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Introduction

Preeclampsia, one of the pregnancy-induced complications, is manifested by hypertension and proteinuria in the second half of pregnancy and is associated with maternal and fetal morbidity and mortalities. According to the World Health Organization, 10% of all maternal death in Asia was due to preeclampsia.[1,2] The worldwide incidence of preeclampsia has been reported to be approximately 4.6% of all deliveries. In Iran, this disorder involved 5% of all pregnancies.[1]

The pathogenesis of this complication has remained unclear. However, multiple pathological processes including inflammation, impaired placentation, and endothelial dysfunction have been suggested in its pathogenesis.[3] The initial step in the establishment of this disorder is impaired implantation and shallow trophoblastic invasion.[4] Interestingly, some studies have revealed that cytomegalovirus (CMV) infection can result in abnormal placental function and implantation.[4,5,6,7] Other study proposed that it is plausible

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that CMV infection causes preeclampsia by an increase in angiogenic factors.[8] Previous studies evaluating the association of anti-CMV antibodies in pregnancy and the development of preeclampsia had controversial conclusions.[9,10,11] For instance, in one study in Canada, CMV antibody seropositivity and anti-CMV IgG titers were more prevalent and higher, respectively in women with preeclampsia versus normal pregnant controls.[9] In contrast, another research has not found any association between serological evidence of CMV infection and preeclampsia.[11] It is notable that the seroprevalence of CMV antibody is common in developing countries.[12]

It is well known that host immune response to CMV infection mostly mediated by cell-mediated immunity (CMI) and a CMI would restrict the risk of CMV infection reactivation. Whether the immune deficiency to CMV or primary or secondary infection with this organism is responsible for the development or reactivation of CMV infection in preeclampsia, it is still unclear. QuantiFERON-CMV (QF-CMV) test is an *in vitro* method to identify patients with CMV-specific CD8+ T-cells. In this assay, the specific interferon-gamma (IFN-γ), produced primarily by CD8+ T-cell stimulated by CMV antigens, is assessed.[13,14] It is notable that the accuracy and efficacy of this method for CMI monitoring have been previously confirmed.[15,16] To our knowledge, this investigation is the first attempt to assess the association between CMV-related CMI and preeclampsia. Nevertheless, surveys investigating the relationship between CMV infection and preeclampsia have resulted in conflicting data.

This study aimed to compare the host CMI response to CMV in a sample of Iranian pregnant women with and without preeclampsia.

Materials and Methods

Study design and study population

This case-control study was conducted on pregnant women referring to the Obstetrics and Gynecology Department of Al Zahra Hospital, affiliated to Isfahan University of Medical Sciences, Iran in 2017. After employing the Cochran's formula, we included 35 individuals in each group. The probability of outcome was estimated at 81% and 96% in the case and control groups, respectively. We applied 0.05 for type 1 error and 80 for the power of the study.

Participants considered the case group if they meet the criteria for preeclampsia and consent to participate in the research. Preeclampsia is defined as (1) blood pressure equal to or above 140/90 mmHg at two separate occasions, at least 6 h apart and proteinuria in the second half of pregnancy (2) hypertension and proteinuria (3) eclampsia and or toxemia. Urine dipstick of ≥1+ or urine protein of ≥0.3 g/24 h used as criteria for diagnosing proteinuria. To be eligible for control, pregnant women should (1) be a suitable match for an individual in the case group regarding her gestational age and have no underlying disease including autoimmune or immunodeficiency disorders and no prior or current history of infective disorder (2) assent to participate in the study. To reduce the effect of potential confounding factors, we matched the two groups with frequency matching for gestational age.

Demographic features of the participants including age, number of pregnancies, history of miscarriage and stillbirth, and gestational age were obtained.

Laboratory test

QF-CMV (Qiagen, Germantown, MD) assay performed according to the manufacturer's guide.[17] In brief, 5 mm^3 of whole blood was taken from each participant. Each sample then collected into each of the QF-CMV collection tubes including a CMV antigen tube, a nil control tube, and a mitogen tube. The mitogen tube used as a positive control, particularly when there is a suspicion on the immune status of the individuals. Then, the tubes were incubated at 37°C for 16–24 h, centrifuged, and the amount of IFN-γ measured in the supernatant by QuantiFERON ELISA (QuantiFERON-CMV, Cellestis, a QIAGEN Company, Australia). The third person blinded to the clinical status of the participants performed all the assays. According to the test instruction, a test rated as a reactive result, when the IFN-γ value was ≥0.2 IU/mL (after subtraction of nil tube). The test recognized as a nonreactive result when the value was <0.2 IU/mL and the mitogen was ≥0.5 IU/mL. If the mitogen tube was <0.5 IU/mL, the test noted as an intermediate result. For the purpose of analysis, intermediate tests were regarded as nonreactive.

Statistical analysis

Our data were analyzed by means of StatsCrop (version 11) software version 18. To describe data, we used mean, standard deviation, and percentage. Considering the nonnormal distribution of data as well as the small sample size, the Wilcoxon rank-sum test was used to compare the continuous variables between cases and controls. The Chi-square test was employed to analyze the differences in proportions. Crude and adjusted odds ratio (OR) and 95% confidence interval (CI) were also calculated using logistic regression. *P* considered statically significant when $P < 0.05$.

Ethics

The Ethics Committee of the Isfahan University of Medical Sciences approved the study design under approval no. 194307. Each participant provided written consent after having been fully explained about the method and goal of the study.

Results

Participant's characteristics are shown in Table 1.

There are no significant differences between the demographic characteristics of the case and control groups.

Our data revealed that the mean value of antigen was significantly higher in the control group ($P = 0.028$), but no significant differences was observed between cases and controls in the mean value of mitogen tube ($P = 0.209$). Our

Table 1: General and clinical characteristic of the cases and controls

Variables	Group		*P*
	Women with preeclampsia (cases)	Women with normal pregnancy (controls)	
Age (year), mean±SD (minimum-maximum)	29.88±5.60 (19-41)	27.68±4.14 (19-34)	0.101*
GA (day), mean±SD (minimum-maximum)	257.5±23.7 (198-282)	261.1±21.1 (188-289)	0.617*
Pregnancy history			
Gravid, mean±SD (minimum-maximum)	2.00±1.26 (1-7)	2.23±1.19 (1-6)	0.285*
Number of abortions (%)			
One	20	9	0.121#
Two	6	0	0.121#
Antigen (mean±SD)	1.57±1.79	2.40±2.21	0.028*
Mitogen (mean±SD)	3.53±1.67	3.53±1.67	0.209*
CMV reactivity, *n* (%)			
Nonreactive	10 (28.5)	3 (8.6)	0.004#
Intermediate	3 (8.5)	0	
Reactive	22 (63.0)	32 (91.4)	

*Wilcoxon rank-sum test, #Chi-square test. GA: Gestational age, CMV: Cytomegalovirus, SD: Standard deviation

results also showed that the percentage of nonreactive results were 28.6% higher in the cases than the controls ($P = 0.004$). Figure 1 compares the IFN-γ levels of the CMV tube and the mitogen tube in the two groups of cases and controls.

There was a trend toward higher IFN-γ level in normal pregnant controls in both CMV and mitogen tubes. For the CMV tube, the mean ± SD IFN-γ level was 1.57 ± 1.80 in women with preeclampsia and 2.40 ± 2.27 in women with normal pregnancy ($P = 0.028$). In mitogen tube, nevertheless, the mean ± SD IFN-γ level was 3.53 ± 1.67 in the cases and 4.23 ± 1.52 in controls ($P = 0.209$).

Crude and adjusted OR and 95% CI were also calculated and visualized in Table 2.

Women with nonreactive results were 6.3 times more likely to manifest preeclampsia ($P = 0.008$). This result even strengthened and reached 12.7 times after adjustment for age, gestational age, and gravidity ($P = 001$).

Our data failed to find any statically significant relationship between preeclampsia and other variables (age and gravidity) in the crude model ($P > 0.05$). However, adjusting for the effect of other variables, we observed that preeclampsia is associated with increased age and nulliparity. In other words, with each year increase in age of mother, preeclampsia risk would increase 1.2 times ($P = 0.005$). In addition, women with multiple gestations were less likely to manifest preeclampsia during their pregnancy ($P = 0.030$).

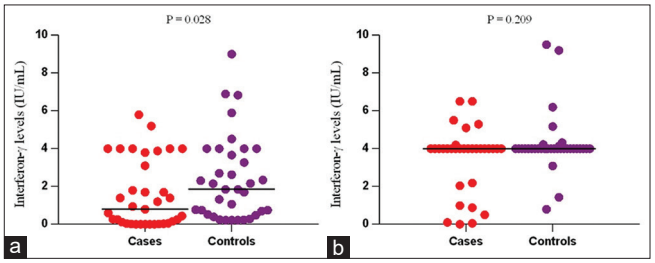

Figure 1: IFN-γ levels in the cases and controls. Black lines are median levels. *P* are calculated using Wilcoxon rank-sum test. (a) IFN-γ levels from the CMV tube. (b) IFN-γ levels from the mitogen tube. IFN-γ: Interferon-gamma, CMV: Cytomegalovirus

Discussion

Herein, we showed a statically significant association between preeclampsia and suppressed CMI to CMV. This relationship even gets stronger after modifying for demographic characteristics of individuals. In other words, women with preeclampsia were more likely to show suppressed CMI to CMV compared with the women in the control group. It is of particular note that our participants showed inadequate CMI response only to CMV antigens. The cellular response to phytohemagglutinin evaluating the overall CMI observed to be intact in all of the participants indicating that they were not immunodeficient. In agreement with this result, Kumar *et al*. suggested that CMV infection in solid-organ-transplant patients reoccur more depending on the pathogen-specific immune suppression rather than the overall degree of immunodeficiency.[17]

To our knowledge, this investigation is the first attempt to assess the association between CMV-related CMI and preeclampsia. Other studies have estimated the association between CMV-related CMI and its reoccurrence in solid-organ-transplant patients.[18-21] For instance, a study has shown that CMV-specific T cell reactivity before transplantation inversely correlates to the risk of CMV viremia and disease after transplant.[22] Conversely, a reactive CMV-specific immune response has linked to spontaneous clearance of CMV viremia even without treatment.[17] Taken these data, we can hypothesize that a negative CMI response to CMV is associated with CMV reactivation or reoccurrence during pregnancy.

Studies observed an association between CMV infection and essential hypertension.[23] There is evidence that considered

Table 2: Multivariable-adjusted odds ratio and 95% confidence interval for preeclampsia

Variables	COR (95% CI)	*P*	AOR (95% CI)	*P*
Nonreactivity	6.30 (1.60-24.7)	0.008	12.86 (2.68-61.6)	0.001
Age (year)	1.09 (0.99-1.22)	0.070	1.23 (1.06-1.42)	0.005
Gravidity (*n*)	0.85 (0.57-1.26)	0.435	0.52 (0.29-0.94)	0.030

OR: Odds ratio, COR: Crude OR, AOR: Adjusted OR, CI: Confidence interval

a role for chronic CMV infection in initiating atherogenesis. Interestingly, CMV has been identified within athermanous plaques. These associations have further approved by epidemiological studies finding a relationship between atherosclerotic cardiovascular disease and CMV infection. Mechanisms trying to explain this observation are the direct local effect of the infectious agent on the vascular components (smooth muscle cells or macrophages within the atherosclerotic plaques) and stimulation of systemic inflammatory response. Notably, CMV-related villitis has been associated with preeclampsia.[10] Moreover, CMV has been observed to result in the generation of reactive oxygen species and cause vasoconstriction.[18] Nevertheless, surveys investigating the relationship between CMV infection and preeclampsia have resulted in conflicting data.[9,10] von Dadelszen *et al*. have shown that anti-CMV antibody titers were higher in preeclamptic women compared with normal pregnancy controls.[10] In contrast, in our unpublished project evaluating a quantitative measure of anti-CMV IgG titer and avidity, as well as qualitative detection of anti-CMV IgG and IgM, we confirmed that CMV antibody seroprevalence was similar among preeclamptic women and normal controls attending in the same hospital. Nevertheless, preeclamptic women had lower mean anti-CMV IgG titers compared to healthy pregnant. Another research suggested that women with repeated miscarriages, which are likely part of the spectrum of diseases that includes preeclampsia, have an impaired lymphoproliferative response to CMV.[10,24] This possible lower CMV-specific humoral immunity in preeclamptic women might relate to the reactivation of chronic CMV infection in the course of pregnancy and the later placental vasculopathy causing preeclampsia. Since the cell-mediated immune response plays an essential role in limiting virus reactivation and replication,[17] we determined to assess whether there is a change in CMV-specific cell-mediated immune response in preeclampsia.

There are hypotheses trying to explain the association between CMV and preeclampsia. CMV infection affects arterial walls directly causing endothelial-platelet dysfunction and acute atherosis, which may explain the uteroplacental ischemia seen in preeclampsia. This infection also affects innate immune response indirectly inducing pro-inflammatory cytokines through activation of TLR-2 and TLR-4 and CD 14 and resultant placental injury manifesting as preeclampsia.[3,25] Furthermore, another research has suggested that CMV infection at the uteroplacental junction, in the absence of maternal or fetal features, may act as a contributing factor in placental damage and dysregulated trophoblast invasion seen in preeclampsia.[26] These findings may suggest that weak cell-mediated immune response to CMV is responsible for the development of CMV viremia and recurrence as well as possible complications in placenta and resultant preeclampsia. Thus, we may hypothesize that strong CMI can lower the CMV viremia level or decrease the risk of recurrence and prevent further complications. However, future studies are needed to prove this finding.

Our study has some limitations. First, we failed to match the participants individually; however, no significant differences observed in the demographic features of the cases versus the controls. Second, our sample size was small, but to our knowledge, the design of the study was very unique and this study was the first attempt assessing the correlation between CMV-specific cell-mediated immune response and preeclampsia. Still, this study had some other strength. Using the QuantiFERON assay is among the strength of the study due to its feasibility and minimal processing and clinical expertise. However, the determination of IFN-γ release by CD8+ T-cells alone and not including CD4+ T cells may be a limiting factor. In addition, only 21 HLA-associated epitopes are used in this assay, therefore some cases of rare HLA haplotype might not be covered, eventuating in falsely nonreactive results in the presence of immunity such that may happen in our study among normal pregnancy controls. However, it has been claimed that the QuantiFERON assay covered more than 98% of the population by recent epidemiologic study.[27] Another strength is that a technician blinded to the clinical status of the participants did all the evaluations.

Conclusion

In this study, we confirmed our hypothesis that preeclampsia is associated with a change in CMV-specific CMI. Our findings need to be approved in a larger, more representative scale that followed longitudinally.

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Conflicts of interest

There are no conflicts of interest.

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