

The possible role of cytomegalovirus infection and pro-inflammatory IL_2 cytokine in preeclampsia

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Abstract

Background and Objective: Fragmentary evidence suggests that trophoblast viral infection may play a role in placental dysfunction leading to complications like Preeclampsia. Among these, placental exposure to CMV induces an inflammatory response that precedes invasive trophoblast cell death. This study aimed to assess the frequency of anti-CMV IgG seropositivity and IL2 level in serum in patients with preeclampsia compared to normotensive control pregnant women.

Methods: A total of 90 women were enrolled, of which 60 had preeclampsia and 30 normotensive pregnant women as the control. A serum sample was collected from each subject and was investigated for anti-CMV IgG serostatus and IL-2 concentration using Enzyme-Linked Immunosorbent Assay (ELISA) based kits.

Results: In preeclamptic and normotensive pregnant women, 33.3% and 16.6% of the tested sera were seropositive for anti CMV IgG antibodies respectively ($P = 0.136$). Early onset preeclamptic women revealed a high frequency of anti-CMV seropositivity (80%) when compared with late onset Preeclampsia (20%). Cytokine assessment revealed a higher IL-2 level in preeclamptic women seropositive for anti-CMV IgG although statistically was not significant ($P = 0.14$), but in normotensive women, the IL-2 level was significantly higher in sera seropositive for anti-CMV IgG ($P = 0.02$).

Conclusion: This study delineates a high frequency of anti-CMV IgG antibodies particularly those with early onset preeclampsia; with no significant difference for the IL-2 level.

Keywords: Preeclampsia; CMV; IL-2 cytokine.

Introduction

The origin of preeclampsia, a hypertensive disorder unique to pregnancy is still a matter of debate and numerous theories have suggested. The pathophysiology of the disease involves impaired trophoblast invasive, abnormal genetic polymorphism, vascular endothelial cell activation, immune tolerance by the maternal immune system and an exaggeration of a systematic inflammatory process.¹ Perturbation of the immune system is a central feature in the maternal generalized inflammatory response of preeclampsia;² among player microbial factors like *Chlamydia pneumonia*,³ *Chlamydia*

trachomatis,⁴ *Helicobacter pylori*,⁵ *Human papillomavirus*,⁶ and cytokines like TNF- α ,⁷ IL-1,⁸ IL-6,⁹ IL-12,¹⁰ IL-18,¹¹ and INF- γ ¹² are thought to be involved in the pathogenesis of preeclampsia for a long time.^{13,14} Data strongly focused on the alteration of the equilibration between TH1 and TH2 lymphocytes in preeclamptic trophoblast,¹⁵ in favor of an increase of TH1 profile with the consequence of defect in the expression of IL-2.^{16,17} During steady-state conditions IL-2 produced by CD4⁺ T helper cell in secondary lymphoid organs and to lesser extent, by CD8⁺ T cell, Natural killer cell (NK),¹⁸ can be synthesized in small amount by activated Dendritic cell (DC)

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and mast cell.¹⁹ Interleukin-2 (IL-2) is a pleiotropic cytokine produced after antigen activation that plays pivotal roles in the immune response. Discovered as a T cell growth factor, IL-2 additionally promotes CD8⁺ T cell and natural killer cell cytolytic activity and modulates T cell differentiation programs in response to antigen, promoting native CD4⁺ T cell differentiation into T helper 1 (Th1) and T helper 2 (Th2) cells while inhibiting T helper 17 (Th17) and T follicular helper (Tfh) cell differentiation. Moreover, IL-2 is essential for the development and maintenance of T regulatory cells and activation-induced cell death, thereby mediating tolerance and limiting inappropriate immune reactions.²⁰ IL-2, TNF- α , and IFN- γ are characteristic of T helper 1 (Th1)-type immunity, induce several cell-mediated cytotoxic and inflammatory reactions.²¹ The association between CMV placental infection and adverse obstetric outcomes revealed that CMV can inhibit placental cytotrophoblast cell invasion through extracellular matrices; that consequently associated with placental dysfunction.²²⁻²⁴ CMV can also damage trophoblast cell via induction of TNF- α and the consequences of adverse obstetric outcomes.²⁵ There are several potential mechanisms by which CMV infection might contribute to the pathogenesis of preeclampsia. It has been reported that preeclampsia is associated with Toll-like receptor (TLR) up-regulation, CMV infection can activate inflammatory cytokines response via CD⁺4 and Toll-like receptor 2. This binding may contribute to the stimulation of immune pro-inflammatory cytokines observed in preeclampsia. Second, CMV can augment the expression of macrophage-colony stimulate factor and result in pro-coagulant activation through altering the coagulate balance of endothelial, all of which could trigger the maternal syndrome of preeclampsia. Third, CMV infection, as well as other infectious agents may be involved in lowering the threshold level at which the clinical phenotype of preeclampsia is triggered.²⁶

Therefore, this study aimed to compare the frequency of seropositivity of anti-CMV IgG and IL-2 levels between preeclamptic and normotensive control pregnant women and to determine whether the level of IL-2 correlated with anti-CMV IgG seropositivity in both groups.

Methods

This study is a case-control prospective approach conducted at Maternity and Obstetrics hospital in Erbil city, Kurdistan, Iraq. The women enrolled were 60 preeclamptic and 30 normotensive control pregnant women. This number of controls seems to be statistically representative to the size of test samples. Though it is the minimum quantity that can be taken as control. Nevertheless, this study was intended to undertake test samples rather than controls considering all the limitations during sample collection and challenges in performing the experiments. The preeclamptic cases were at gestational age 26-39 weeks. The preeclamptic women were further classified as early onset preeclampsia (<34 weeks gestation) and late onset preeclampsia (>34 weeks gestation). Preeclampsia was diagnosed according to the criteria recommended by the American college of obstetrician and gynecologists in 2002.²⁷ Thus systolic blood pressure of 140 mmHg or higher and diastolic blood pressure of 90 mmHg or higher on two occasions occurring after 20 weeks gestation in pregnant women with previous history of normotensive blood pressure and detectable protein in the urine (\geq 1+1 by dipstick or \geq 0.3g/24 hrs) were diagnosed as preeclampsia cases. Blood samples were collected from both groups and the sera were separated and stored at -40°C until performance of the ELISA assay; the procedure as written in the leaflet of kit was: Prepare 1:40 dilutions by adding 5 μ l of the test samples, negative control, positive control, and calibrators to 200 μ l of sample diluents. Mix well; dispense 100 μ l of diluted sera, calibrator, and controls into the appropriate

wells. For the reagent blank, dispense 100 µl sample diluents in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature, Remove liquid from all wells. Repeat washing three times with washing buffer. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature; Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer; dispense 100 µl of (3,3',5,5'-tetramethylbenzidine) TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature. Add 100 µl of Stop Solution to stop the reaction, make sure there are no air bubbles in each well before reading, Read optical density at 450 nm immediately for detectable of anti-CMV IgG antibody and estimation of the concentration of IL-2 cytokine in both groups. Serum IL-2 level was measured with ELISA kit using Human Interleukin (IL-2) provided by (BioVision – USA, Cat: K4798-100) according to manufacturer instruction. The standard range: 82-102% and Sensitivity: 94.6%). Also, Anti-CMV IgG was assessed by ELISA Kit (AccuDiag™ – USA, Cat: 1201-11 Sensitivity: 97.0% and Range: 64-92%).

Ethical consideration:

The study proposal was approved by the Research Ethics Committee of the College of Health Sciences, Hawler Medical University.

Statistical analysis:

The statistical package for the social sciences (version 21.0) was used to attain the descriptive and inferential analysis of data. Thus, the results of the studied groups were analyzed statistically using the student t-test for numeric data, Chi-square test for non-parametric data, and Spearman correlation to determine the collective effect of parameters. The association between groups was assigned as statistically significant at *P* value less than 0.05.

Results

Table 1 shows that there was no statistically significant difference in frequency of anti-CMV IgG seropositivity (33.3%) in sera of preeclamptic patients, when compared with its frequency (16.6%) in sera of normotensive control pregnant women (*P* >0.05). According to the onset of disease, 80 % the seropositive cases were confined to early onset disease and 20% among late onset preeclampsia (Table 2).

Table 1: Seroprevalence of anti-CMV IgG in preeclamptic and normotensive pregnant women.

Test	Cases No. %	Control No. %	Total No. %	<i>P</i> value
Anti-CMV IgG Positive	20(33.3%)	5(16.6%)	25(27.8%)	0.136
Negative	40(66.6%)	25(83.4%)	65(72.2%)	
Total	60(100%)	30(100%)	95(100%)	

Table 2: Distribution of anti-CMV IgG seropositivity according to the onset of preeclampsia .

Test	Early onset preeclampsia		Late onset preeclampsia	
		No. %		No. %
Anti-CMV IgG Positive		16(88%)		4(20%)
Negative		4(20%)		16(80%)

(Tables 3) clarify the mean concentration of IL-2 cytokine in preeclamptic women seropositive and negative for anti-CMV IgG. (36.1 ± 10.8 VS 27.1 ± 16.24); statistically no significant differences was seen ($P = 0.14$). But in normotensive pregnant women, a significant difference was observed between anti-CMV IgG seropositive and negative. (15.1 ± 10.7 VS 1.17 ± 2.4) $P = 0.02$. Figure 1 and 2 delineates the correlation between IL-2 concentration and anti-CMV IgG level

in both preeclamptic and normotensive pregnant women; both might have a causal factor in the pathophysiology of the preeclampsia. The correlation coefficient value of the tested parameters namely anti-CMV IgG and IL-2 concentration in preeclamptic cases were ($r = 0.43$), ($P = 0.14$) which refer to positive correlation, meanwhile was negative in normotensive women ($r = 0.39$), ($P = 0.02$) as shown in Figure 1 and 2, respectively.

Table 3: Mean IL-2 serum concentration among Preeclamptic and normotensive Seropositive and negative for anti-CMV IgG.

Studied group	No.	Concentration of IL-2 (pg/ml) (Mean±SD)	CMV serostatus		t. value	P value
			Seropositive in CMV IgG	Seronegative in CMV IgG		
Preeclamptic women	60	IL-2 (Mean±SD)	36.1 ± 10.8	27.1 ± 16.24	0.14	>0.05
Normotensive pregnant women	30	IL-2 (Mean±SD)	15.1 ± 10.7	1.17 ± 2.4	0.02	<0.05*

IL-2: interleukine-2; SD Standard Deviation;
 *paired sample t-test; $p < 0.05$ statistically significant.

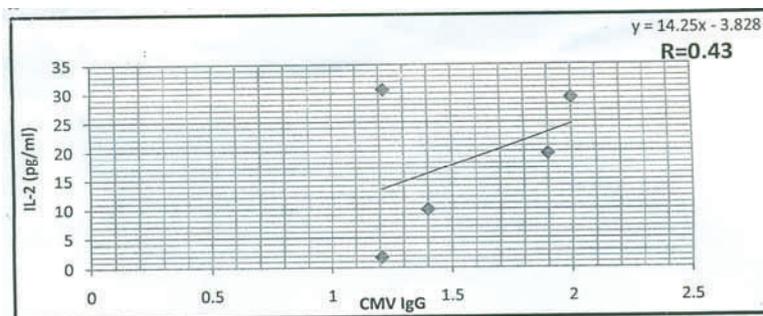


Figure 1: Correlation between IL2 level and anti-CMV IgG in preeclamptic women. r- Spearman rank correlation Coefficient.

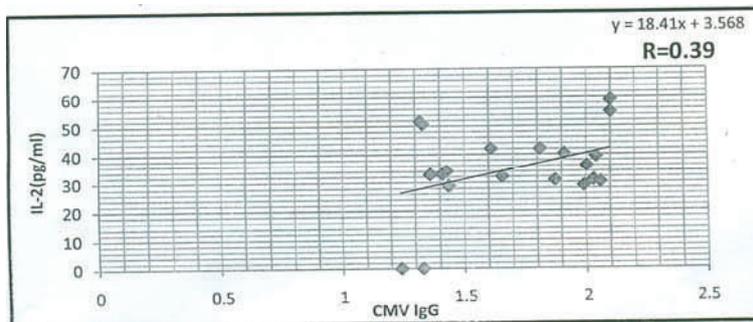


Figure 2: Correlation between IL-2 level and anti-CMV IgG in normotensive women. r- Spearman rank correlation Coefficient.

Discussion

Preeclampsia is a common disease in pregnant women and a leading cause of entire vascular endothelial cell dysfunction.²⁸ Epidemiologic studies have shown that maternal infection and preeclampsia are connected and the link could be via Toll-like Receptor activation.²⁹⁻³¹ In early pregnancy, CMV infection can cause fetal death and miscarriage,³² adversely affect placentation with subsequent pregnancy complication such as preeclampsia.³³ The data from the present study showed that seropositivity for anti-CMV IgG was higher in preeclamptic patients as compared to normotensive control pregnant women, but statistically non-significant ($P > 0.05$). Regarding the relation between anti-CMV IgG seroprevalence and the onset of disease, the seroprevalence was higher among the patients with early onset preeclampsia cases. Association between CMV antibodies in pregnancy and development of preeclampsia is inconsistent.³⁴⁻³⁶ It has been confirmed the elevated CMV antibodies level³⁵ and genomic DNA load in women with early onset preeclampsia was reported or confirmed.^{37,38} However, Strand *et al.*³⁹ reported lower anti CMV IgG seropositivity in preeclamptic women than healthy. The risk for developing preeclampsia was confirmed by others to be associated with increased prevalence of CMV IgG together with increased CMV IgG antibodies level.^{34,35} This may be explained by the fact that the increased risk for developing preeclampsia in women with specific HLA-G alleles may be combined with CMV infection.⁴ It has been stated that CMV infection impairs trophoblast differentiation and invasion. This leads in turn to down-regulate matrix metalloproteinase (MMP) activity in uterine micro vascular endothelial cells and differentiating invading cytotrophoblast.²⁵ In addition to reduced MMP activity early in placental development could impair cytotrophoblast. Remodeling of the uterine vessels and eventually restrict fetal growth in affected

pregnancy.²⁵ Also, Fisher *et al.*⁴¹ demonstrated that endothelial cells are targets of infection by CMV. Thus, remodeling of uterine spiral arterioles suggests that CMV can spread from maternal vascular to endovascular cytotrophoblast in the uterus. Our results agree with other who reported that women with early-onset PE (<34 weeks) have higher IgG HCMV titers than women with late-onset.³⁵ Moreover, in the placenta, the increased cytokine production especially TH1 profile may be an important component of the immune inflammatory response that impairs trophoblast invasion and results in trophoblast death.⁴² The impact, are associated with adverse outcomes that include fetal growth restriction preeclampsia and spontaneous preterm delivery. Of those TH1 cytokine IL-2 plays a key role in regulating the balance between immunity and tolerance.^{35,43,44} In this study, preeclamptic women seropositive for CMV-IgG, the IL-2 concentration was elevated but statistically not significant in comparison with seronegative CMV-IgG level ($P > 0.05$). Results obtained in this study indicated that the mean concentration of IL-2 was significantly higher in normotensive women seropositive for CMV-IgG as compared to seronegative group ($P < 0.05$). Of interest in this study finding a positive correlation between IL-2 and CMV-IgG seropositivity in preeclamptic women. A study done by Gallersen *et al.*²⁸ demonstrated that during pregnancy, HLA-G is expressed on trophoblastic cells at the maternal fetal interface and may be involved in the local immune response to viral infection. The reduced HLA-G expression is a feature of the preeclamptic placenta.²⁸ In this regard, CMV-infected cytotrophoblast down-regulate HLA-G which may play a role in maternal immune tolerance⁴¹ and consequently alter the critical materno-fetal immune interface leading to preeclampsia.²⁸ The possible role of IL-2 elevation in the pathophysiology of PE especially during the first trimester of

pregnancy may be linked to the IL-2 reduced angiogenesis in placenta.^{45,46} In addition, IL-2 elevation in PE can lead to IL-2 induced proliferation and natural killer activation.^{46,47} Consequently, NK-cell of decidual origin acquired a broad cytolytic potential in response to IL-2 cytokine that may have a role in the control of unduly invasive trophoblast.⁴⁷

Conclusion

This study concluded that a high frequency of IgG anti-CMV in preeclamptic women especially in early onset PE that reflects the important role of CMV in the pathoetiology of preeclampsia. IL-2 as TH1 marker in preeclamptic women seropositive for IgG anti-CMV revealed a non-significant elevation with surprisingly a positive moderate correlation. In future, this study needs to be extended to evaluate the role of other cytokines and microbial factors like IFN- γ , TNF- α , *Helicobacter pylori* and *Chlamydia pneumonia*.

Competing interests

The authors declare that they have no competing interests.

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