

UPDATE

# Cytomegalovirus Infection and Pre-Eclampsia

Rădulescu Carmen<sup>1\*</sup>, Huțanu Adina<sup>2</sup>, Gabor Rozalia<sup>3</sup>, Șincu Nina<sup>4</sup><sup>1</sup> Department of Obstetrics and Gynecology II, University of Medicine and Pharmacy of Tirgu.Mureș, Romania<sup>2</sup> Department of Central Laboratory, Emergency University Hospital, Tirgu Mureș, Romania<sup>3</sup> Department of Management and Economy, University Petru Maior of Tirgu Mureș, Romania<sup>4</sup> Department of Infectious Diseases, University of Medicine and Pharmacy of Tirgu Mureș, Romania

**Introduction:** Pre-eclampsia is a pregnancy-specific disease characterized by hypertension after 20 weeks of gestation and proteinuria. It is a major cause of maternal and perinatal morbidity and mortality. The pathogenesis of pre-eclampsia is not completely understood. In our study we investigated if there is a potential link between cytomegalovirus infection and pre-eclampsia and if cytomegalovirus infection is the triggering factor of pre-eclampsia. **Material and methods:** This study was carried out in a secondary care hospital between January 2014 and July 2015. We included two groups of pregnant women: 66 with pre-eclampsia and 62 without pre-eclampsia. Enzyme linked immunosorbent assay (ELISA) technique was performed to detect cytomegalovirus Immunoglobulin G levels in maternal serum. **Results:** The p-value between median values of positive cytomegalovirus IgG in pre-eclamptic women and in controls was not significant (p:0.867). Odds ratio for cytomegalovirus IgG in pre-eclamptic group (OR:0.967; 95%CI:0.535-1.748) do not differ greatly from the value recorded in the control group (OR:1.036; 95%CI:0.571-1.880). The risk for preterm birth in pre-eclamptic women with cytomegalovirus IgG positive values (OR:1.009; 95%CI:0.329-3.090) was greater than those which had term delivery (OR:0.994; 95%CI:0.472-2.095) but the two values are not very high. We found a positive correlation coefficient (0.217) at a "p" value of 0.08 between white blood cells, but a negative correlation coefficient between the percentage of neutrophils and cytomegalovirus infection. **Conclusion:** Cytomegalovirus infection may be associated with pre-eclampsia but it is unlikely to be the triggering factor of pre-eclampsia.

**Keywords:** Cytomegalovirus infection (CMV), pre-eclampsia (PE), Enzyme linked immunosorbent assay (ELISA), Immunoglobulin G (IgG), Interleukine (IL)

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## Introduction

Pre-eclampsia (PE) is an important cause of maternal and perinatal morbidity and mortality. It is a pregnancy-specific disease characterized by high blood pressure: 140/90 mmHg or more taken twice a day more than 4 hours apart after 20 weeks of gestation, except molar pregnancies [1], and proteinuria: 0.3 g or greater after a 24-hour urine sample [2].

The pathogenesis of PE is not completely understood. It is thought to involve: defective placentation, diminished perfusion of placenta, hypoxia, increased oxidative stress, production of lipid-laden foam cells as seen in atherosclerosis, release of antiangiogenic proteins into the maternal blood, excessive maternal inflammatory response, endothelial injury, generalized endothelial dysfunction, hypertension, activation of the coagulation cascade, multiorganic manifestations [3-13].

Pre-eclampsia was classified in early-onset (clinical onset of PE <34 weeks of gestation) and late-onset (PE at ≥34 weeks of gestation) [6,12]. There is also a hereditary predisposition [14]. The familial susceptibility to the development of pre-eclampsia may be mediated by single nucleotide polymorphisms in the Toll-like receptors gene sequences and can be associated with early-onset PE [15]. There are studies that indicate an association between pre-eclampsia and chronic infectious diseases [8,16,17].

Chronic infection-associated inflammation may trigger maternal response like pre-eclampsia [8,9,16].

Cytomegalovirus (CMV) is a Deoxyribonucleic acid (DNA) herpesvirus. It can be transmitted by contact with infected nasopharyngeal secretions, urine, saliva, semen, cervical secretions, blood. There may be intrauterine, intrapartum or neonatal infection from breast feeding. Up to 85 percent of women from lower socioeconomic backgrounds are seropositive by the time of pregnancy whereas only half of women in higher income groups are immune. Maternal antibodies do not prevent fetal infection [18]. Some seropositive women can be reinfected with a different strain of virus [18,19]. Infection with cytomegalovirus impairs extravillous cytotrophoblasts migration and efficient invasion of uterine wall and maternal spiral arteries induced by chemokine CXCL12 [10].

In our study we investigated if there is a potential link between cytomegalovirus infection and pre-eclampsia. It was estimated about 50,000 maternal deaths per year worldwide because of pre-eclampsia [20] and it is important an intensive surveillance to avoid adverse outcomes in PE and fetal or maternal demise.

## Material and methods

This study was carried out between January 2014 to July 2015 at the Obstetrics and Gynecology Department in Târgu Mureș University Hospital, Romania. Our Hospital and University Ethics Committee approved the study. The purpose of our study was explained to our patients

\* Correspondence to: Carmen Rădulescu  
E-mail: carmen.c.radulescu@yahoo.com

and a written informed consent was obtained from each participating woman. We included two women groups: 66 with pre-eclampsia and 62 without pre-eclampsia (controls, normotensive pregnant women). The blood pressure was taken in a semi-recumbent position, with a supported arm and appropriately-sized cuff using a manual sphygmomanometer, with Korotkoff V used to determine diastolic pressure.

Maternal venous blood samples (5 ml) were collected into vacutainer tubes without anticoagulants. After sample collection, immediately serum was separated by centrifugation and stored at -80°C until assayed at the Central Laboratory of Mureş County Emergency Hospital. Enzyme-linked immunosorbent assay (ELISA) was performed to detect cytomegalovirus Immunoglobulin G (Dia. Pro CMV IgG kit, ELISA, Italy) in maternal serum samples according to the manufacturers' instructions. A calibration curve, calibrated against the 1<sup>st</sup> World Health Organisation (WHO) international standard, made possible a quantitative determination of the antibody in the patient. The cut-off value for cytomegalovirus Immunoglobulin G (IgG) was of 0.5 IU/ml. Samples with a concentration lower than 0.5 WHO IU/ml were considered negative for anti Cytomegalovirus IgG antibody. Samples with a concentration higher than 0.5 WHO IU/ml were considered positive for anti Cytomegalovirus IgG antibody.

Statistical data were analyzed using SPSS 17.0 for Windows program.

To characterize the two groups of patients we used mean, standard deviation, the minimum and maximum values recorded in the study. We used one sample Kolmogorov-Smirnov test for evaluation the significance of average values of continuous variables, the socio-demographic variables and the levels of cytomegalovirus IgG.

Chi-squared test was used for categorical variables. Data were reported as mean± standard deviation and percentage for continuous demographic characteristics, median and range for the levels of cytomegalovirus IgG. We used Mann-Whitney U tests to compare the cytomegalovirus IgG levels of preeclamptic group and for controls. A *p* value < 0.05 was considered significant for all tests.

We also estimated the risk and 95% confidence interval (CI) of cytomegalovirus IgG in pre-eclamptic women and controls using SPSS 17.0 soft.

## Results

In table I are shown the maternal clinical characteristics and p-value of the 66 pre-eclamptic women and of the 62 healthy pregnancy control: age (years), the percentage of nulliparous women, the gestational age at delivery (in weeks), the mean systolic blood pressure (mmHg), the mean diastolic blood pressure (mmHg), the percentage of proteinuria, the birth weight (g), the percentage of smokers, body mass index (BMI) before pregnancy (kg/m<sup>2</sup>), the current BMI. Pre-eclamptic women had a higher mean blood pressure and a current BMI higher than controls.

The mean triglycerides values were of 266 mg/dl in PE group and of 209 mg/dl in the controls. In PE group, 77% had a family history of hypertension and 62% in the control group. In preeclamptic group 32% had a family history of diabetes and 19% in the control group. In PE group 23% had a family history of obesity and 19% in the control group.

As shown in table II, the median value of cytomegalovirus IgG in pre-eclamptic women is increased: 4.573 IU/ml with a minimum value of 0.00 and a maximum value of 71.3 IU/ml compared with the median value in the control group: 4.066 IU/ml with a minimum value of 0.00 and a maximum value of 304 IU/ml, but *p*-value: 0.867 (not significant) in Mann-Whitney U test.

The odds ratio (OR) for qualitative cytomegalovirus IgG (table III) was of 0.933 (95%CI:0.284-3.064). The OR for CMV IgG in pre-eclamptic group was of 0.967 (95% CI: 0.535-1.748) which do not differ greatly from the value recorded in the control group OR: .036 (95%CI: 0.571-1.880)

The pathophysiology of pre-eclampsia is believed to involve impaired placentation and an associated increase in systemic inflammation. In our study we have determined the white blood cells and the neutrophils percentage in pre-eclamptic women and in healthy pregnant women in order to confirm or infirm the hypothesis that cytomegalovirus infection produces a generalized inflammation and endothelial injury, pathogenic features of pre-eclampsia (Table IV).

Table IV shows that in PE group the median value of white blood cells (WBC) was of 11.645x10<sup>3</sup>/µL with a minimum value of 5x10<sup>3</sup> and a maximum value of 21.1x10<sup>3</sup>, higher than in the control group: 9.42x10<sup>3</sup>/µL

**Table I. Maternal and perinatal clinical characteristics in women with pre-eclampsia and in normal pregnancy control group**

Characteristic	Pre-eclampsia (n=66)	Normal pregnancy control (n=62)	P-value
Maternal age (years)	28 ±6.3 (14-44)	26 ± 7 (14-42)	0.120*
Nulliparous (%)	75 (I, multi)	79.4 (I, multi)	0.100*
Gestational age at delivery (weeks)	37.7 ± 2.3 (31-41)	38 ± 5.7 (38.5-41)	0.173*
Mean systolic blood pressure (mmHg)	152.9±17.9	114±7.4	0.000*
Mean diastolic blood pressure (mmHg)	97.15±11.1	70.6±5.5	0.000*
Proteinuria (%)	100	0	0.000*
Birth weight (g)	3076 ± 714 (1570-4730)	3218 ± 485 (1730-4280)	0.093**
Smoker (%)	73.5	79.4	0.500*
BMI before pregnancy	27.3 ±6.8 (15.6-48.1)	21.1 ± 2.8 (18-29)	0.019**
Current BMI	31.04 ± 6.6 (20.7-52.5)	25.7 ± 4 (20-41.5)	0.004**

\*Chi-square test; \*\*Kolmogorov-Smirnov test

**Table II. Median and range for CMV IgG in PE and in the control pregnant women**

Characteristic	Pre-eclampsia (n=66)	Normal pregnancy control (n=62)	P-value (Mann-Whitney U test)
CMV IgG IU/ml (median)	4.573 (0.00-71.3)	4.066 (0.00 – 304)	0.867

CMV: Cytomegalovirus; IgG: Immunoglobulin G; PE: Preeclamptic

**Table III. The estimated risk of CMV IgG in PE women and controls**

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for qualitative CMV IgG (negative < 0.5 IU/ml /positive over 0.5 IU/ml)	0.933	0.284	3.064
For PE cohort	0.967	0.535	1.748
For control cohort	1.036	0.571	1.880
Number of Valid Cases	128		

**Table IV. Median and range of white blood cells and neutrophils in PE and in control group**

Characteristic	Pre-eclampsia (n=66)	Normal pregnancy control (n=62)	P-value (Kolmogorov-Smirnov test)
White blood cells (/ $\mu$ L) (median)	11.645x10 <sup>3</sup> (5x10 <sup>3</sup> -21.1x10 <sup>3</sup> )	9.42x10 <sup>3</sup> (4.67x10 <sup>3</sup> -18.4x10 <sup>3</sup> )	0.000
Neutrophils (%) (median)	77.7% (57.5%-93.7%)	69.2% (52.38%-87%)	0.000

with a minimum value of 4.67x10<sup>3</sup> and a maximum value of 18.4x10<sup>3</sup> and p-value was significant (p=0.000).

In pre-eclamptic group we have found a positive directly correlation (Pearson correlation coefficient: 0.217) with a significant “p” value of 0.08 between cytomegalovirus IgG values and the number of white blood cells in 92% of women. In pre-eclamptic group Pearson correlation coefficient was negative between cytomegalovirus IgG value and the neutrophils percentage.

In normotensive pregnant women (controls) we did not find a statistically significant correlation between cytomegalovirus IgG and WBC count and also between cytomegalovirus IgG and the percentage of neutrophils.

We had determined pro-inflammatory cytokines: interleukine (IL) 6 and 16 between 35 and 40 weeks of gestation for a subgroup of 68 pregnant women (47 PE and 21 controls) but we did not find significant correlation between IL-6, IL-16, cytomegalovirus IgG infection in pre-eclamptic and in the control group; Pearson correlation coefficient was negative: -0.097 and a p-value of 0.524 between IL-6 and cytomegalovirus IgG and -0.63 and a p-value of 0.679 between IL-16 and cytomegalovirus IgG.

We had also calculated (Table V and table VI) if there is a greater risk in pregnant women with pre-eclampsia and positive cytomegalovirus IgG for premature birth and those at term delivery. So we have recorded 23 of 26 PE patients

with values of cytomegalovirus IgG over 0.5 IU/ml which delivered premature and 35 of 40 PE patients with positive cytomegalovirus IgG which delivered at term. According to table VI data, odds ratio for cytomegalovirus IgG in pre-eclamptic women which delivered premature and in those which had at term delivery was of 1.014 (OR:1.014; 95%CI:0.157-6.549). The risk in pre-eclamptic women with CMV IgG values of over 0.5 IU/ml which delivered premature was greater (OR:1.009; 95%CI:0.329-3.090) than those which delivered at term (OR:0.994; 95%CI:0.472-2.095) but the differences between the two values were not very high.

## Discussion

It is important to avoid adverse outcomes in pre-eclampsia such as maternal and fetal demise. A major goal of our study was to identify women at high risk of PE, to search if cytomegalovirus infection may influence the onset of pre-eclampsia.

Cytomegalovirus infection can increase in the host immune response and release chemokines and cytokine responses: Interferon gamma (IFN- $\gamma$ ), Tumour necrosis factor alpha (TNF- $\alpha$ ), Interleukines 4,8,18 (IL-4, IL-8, IL-18), modifies adhesion molecules: Intercellular adhesion molecule-1 (ICAM-1), Vascular cell adhesion molecule-1 (VCAM-1), E-selectin, increases matrix metallopro-

**Table V. CMV IgG, gestational age, premature, at term delivery crosstabulation**

		Gestational age, premature, at term		Total
		premature <38 weeks	at term 38-41 weeks	
CMV IgG	negative<0.5 IU/ml	3	5	8
	positive>0.5 IU/ml	23	35	58
	Total	26	40	66

**Table VI. Risk estimate for premature vs. at term delivery in CMV IgG seropositive women**

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for CMV IgG(negative<0.5 IU/ml/ positive over 0.5 IU/ml)	1.014	0.157	6.549
For cohort premature < 38 weeks	1.009	0.329	3.090
For cohort at term, 38-41 weeks	0.994	0.472	2.095
N of valid cases	66		

teinase (MMP-2) activity, resulting an alteration in vessel remodeling, enhance the adherence and infiltration of inflammatory cells that drive vascular disease. These pathophysiological mechanisms were found in atherosclerosis, transplant vascular sclerosis, vessel narrowing and acute organ transplant rejection [21]. In our study the mean triglycerides values were higher in PE group (266 mg/dl) than in the controls (209 mg/dl). Women who had pre-eclampsia more frequently develop cardiovascular disease later in life [22,23]. We had determined pro-inflammatory cytokines, interleukine (IL) 6 and 16 between 35 and 40 weeks of gestation for a subgroup of 68 pregnant women (47 pre-eclamptic and 21 controls) but we did not find a statistically significant correlation between IL-6, IL-16, cytomegalovirus IgG infection in pre-eclamptic and control group; may be the cause was our sample size relatively small.

Xie F *et al.* [9] studied cytomegalovirus Immunoglobulin G, M and A (IgG, IgM, IgA) seropositivity in pre-eclamptic and normal pregnancy controls. They found that cytomegalovirus IgG antibody level was higher in PE than in normal controls ( $p < 0.0001$ ) but no difference was observed in cytomegalovirus IgM or IgA among study groups. IgA is associated with primary, chronic, or recurrent infections and IgM suggests acute or primary infection. The authors concluded that cytomegalovirus infection may influence the occurrence of pre-eclampsia because cytomegalovirus infection can activate inflammatory cytokine responses via CD14 and Toll-like receptor 2. No infants had clinical evidence of congenital cytomegalovirus.

Rustveld LO *et al.* [16] collected data of published studies since 1964 on relationship between maternal infection and pre-eclampsia. The authors selected sixteen of 32 studies. They calculated OR (odds ratio), 95% CI and found in their analysis that any infection (bacterial or viral) was associated with a two-fold higher risk of pre-eclampsia. The association may provide a potential explanation for preeclampsia-related inflammation.

Conde Agudelo A *et al.* [8] examined the relationship between maternal infection and pre-eclampsia from forty-nine studies. They ascertained that there were no association between pre-eclampsia and presence of antibodies to Chlamydia pneumoniae, Helicobacter pylori, Cytomegalovirus, Human Immunodeficiency Virus (HIV) infection, malaria, herpes simplex virus type 2, bacterial vaginosis and Mycoplasma hominis. The authors found that the risk of pre-eclampsia was increased in pregnant women with urinary tract infection (pooled OR:1.57; 95%CI:1.45-1.70) and periodontal disease (pooled OR:1.76; 95%CI:1.43-2.18).

Gibson CS *et al.* [17] had investigated the role of fetal exposure to herpesvirus infection and pregnancy-induced hypertensive disorders, antepartum haemorrhage, birth weight <10th percentile, preterm birth, using polymerase chain reaction. They concluded that fetal exposure to herpesvirus infection was associated with pregnancy-induced

hypertensive disorders for term and preterm births. Cytomegalovirus belongs to herpesvirus family.

Strand KM *et al.* [24] studied cytomegalovirus IgG and IgM in plasma sample of a cohort of 1500 women with pre-eclampsia and 1500 healthy pregnant women. They concluded that maternal antibodies to cytomegalovirus was not associated with pre-eclampsia; their result suggested that cytomegalovirus infection was unlikely be a major cause of PE.

The results of our study suggest that the median value cytomegalovirus IgG seropositivity in pre-eclamptic women was increased compared with the median value in the control group but "p" value was not significant. IgG cytomegalovirus indicates previous infection. When we had calculated the odds ratio for cytomegalovirus IgG in pre-eclamptic group we evaluated that it did not differ greatly from the value recorded in the control group. When we studied the risk for preterm birth in pre-eclamptic women CMV IgG positive compared to those who delivered at term, the differences between the two values were not very high. We did not find a correlation coefficient between the percentage of neutrophils and cytomegalovirus IgG infection in pre-eclamptic women.

There are some limitations of this study. Our sample size was relatively small and we did not studied cytomegalovirus IgA which is associated with primary, chronic, or recurrent infection nor IgM which indicate acute or primary cytomegalovirus infection neither CMV-DNA plasma viral load, PP65 antigen; however Xie F *et al.* [9] did not observed any difference between IgM or IgA levels in their study.

It is necessary a larger number of patients to conclude that cytomegalovirus infection can influence the onset of pre-eclampsia.

In our study, no infants exhibited clinical evidence of congenital CMV infection.

## Conclusions

Preeclampsia is an important cause of maternal and neonatal morbidity and mortality. The pathogenesis of PE is not clear; some studies found an association between chronic infection-associated inflammation and pre-eclampsia. In our study, we concluded that cytomegalovirus infection is unlikely to be the triggering factor of pre-eclampsia. More studies are required to verify on a greater number of pregnant women if there is any relationship between cytomegalovirus infection and pre-eclampsia.

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## Conflicts of interest

The authors report no conflicts of interest.

## Abbreviation

Interferon gamma (IFN- $\gamma$ ), Tumour necrosis factor alpha (TNF $\alpha$ ), Intercellular adhesion molecule-1 (ICAM-1), Vascular cell adhesion molecule1 (VCAM-1), Interleukin 8 (IL-8), Body mass index (BMI)

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