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Original Article

Chronic *Chlamydia pneumonia* Infection and Risk of Early-Onset Versus Late-Onset Preeclampsia

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Abstract

Preeclampsia is one of the challenges associated with mother and baby health. Preeclampsia and atherosclerosis share certain similarities. Atherosclerosis has been previously linked to chronic *Chlamydia pneumoniae* infection. This investigation aimed to establish the role of chronic *Chlamydia pneumoniae* in the development and onset of preeclampsia. The research was conducted from October 1, 2018 to September 30, 2019 in the department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital in Baghdad, Iraq. The study included 140 pregnant women divided into two groups: 70 women with early and late-onset preeclampsia and 70 women with late-onset preeclampsia. Group I (early-onset preeclampsia) included 35 singleton pregnant women who developed preeclampsia after 34 weeks with blood pressure>140/90 and proteinuria>1. Late-onset preeclampsia (group II) consisted of 35 singleton pregnant women who developed clinical preeclampsia after 34 weeks of pregnancy, with blood pressure>140/90 and proteinuria +1, and 70 healthy term pregnant women without complications who acted as the control group (Group III). Enzyme-Linked Immunosorbent Assay (ELISA) analyzers were utilized to measure serum *Chlamydia pneumoniae* IgG levels in all study groups. Women with early-onset preeclampsia had the highest median Chlamydia pneumonia IgG level, 0.3 U/ml, compared to 0.09 U/ml for women with late-onset preeclampsia and 0.19U/ml for healthy term pregnant women without complications; these differences were statistically significant (P=0.001). This study found that the IgG titer for *Chlamydia pneumoniae* was higher in early-onset preeclampsia than in late-onset preeclampsia and in healthy term pregnancy without complications. This substantial increase was a direct result of the onset of preeclampsia. This provided evidence for the pathophysiological connection between preeclampsia and the reactivation of a chronic or latent infection.

Keywords: Chlamydia pneumonia, Preeclampsia, IgG titer, Pregnancy

1. Introduction

Preeclampsia (PE) is a pregnancy disorder marked by the emergence of hypertension and proteinuria after 20 weeks of pregnancy in a woman who previously had normal blood pressure (BP). It is marked by an aberrant vascular response to pregnancy, linked to increased systemic vascular resistance, platelet aggregation, coagulation system activation, and endothelial cell dysfunction (1).

As described previously (2), the initiation time for PE is defined as:

• EO-PE (early-onset preeclampsia): (34 weeks of pregnancy) fetal/maternal morbidity/mortality is high.

• LO-PE (late-onset preeclampsia): (34 weeks gestation) fetal/maternal morbidity/mortality is reduced.

• The beginning of symptoms after delivery

All across the world, preeclampsia is a leading cause of maternal and newborn morbidity and death (3). Because the placenta of women with PE is abnormal and characterized by insufficient trophoblastic invasion, oxidative stress, hypoxia, and the production

of chemicals that promote endothelial dysfunction, inflammation, and other possible responses are thought to occur. The clinical symptoms of vasoconstriction, end-organ ischemia, and general endothelial dysfunction of PE (4).

One of the most notable differences is that EO-PE can be complicated by intrauterine growth restriction (IUGR), but LO-PE does not. EO-PE placentas exhibit apparent pathology, but LO-PE placentas are often benign on regular clinicopathological testing. Uteroplacental malperfusion lesions are part of the pathogenesis of EO-PE. During early pregnancy (8–18 weeks, insufficient placentation), the spiral arteries between the uterus and the placenta get too small and tight to support the high-volume, low-pressure flow that the third-trimester placenta needs. As a result, oxidative stress and even infarction harm placental tissue. The spiral arteries can be clogged by acute atherosclerosis or an atherosclerosis-like lesion that produces arterial thrombosis and infarctions.

PE has been linked to chronic or latent infections such as urinary tract infection (UTI), periodontal disease, cytomegalovirus (CMV), Epstein:–Barr: virus, herpes simplex: virus type 2, adeno:-associated virus 2, and *Chlamydia: pneumonia* (*C. pneumonia*). These infections: are thought: to induce PE: by activating the innate immune: system and enhancing: the inflammatory: environment of pregnancy. No one knows how this happens, what effect it has on the development of trophoblasts, or how these things contribute to the development of PE. Many of the traditional risk factors for PE, such as young age at conception, nulliparity, a new sexual partner, and a sexual partner: who has previously fathered a PE-affected: pregnancy, can also be interpreted as risk factors for acquiring infections, particularly: those spread through close contact (5, 6).

A significant component in the start of atherogenesis appears to be chronic infection (7). CMV and *C. pneumoniae* have been identified within atheromatous plaques (7, 8). In epidemiological studies, pneumonia infection has been linked to atherosclerotic cardiovascular disease (8). Two proposed paths amplify the systemic inflammatory response or the direct local effects of infectious agents on endothelium (on vascular smooth muscle cells and/or macrophages within the atherosclerotic lesion). These data support the theory that chronic infection is associated with later atherosclerosis in PE, especially considering the increased sensitivity to chronic infection during pregnancy due to reduced cell-mediated immunity.

C. pneumoniae belongs to the group of gram-negative bacteria that are obligate intracellular energy parasites that initially colonize the respiratory tract epithelial cells. Monocytes disperse them throughout the body, which can be found in atherosclerotic artery plaques, synovia, nerve tissue, and even trophoblasts (9). Droplet infection is how the disease spreads from person to person. *C. pneumoniae* infection of the placenta is not uncommon, and it has been linked to an increased risk of obstetric problems such as PE due to placental malfunction. As a result, we wanted to see if C. pneumonia infection of extravillous trophoblast cells reduces cell viability and/or inhibits cell invasion and (ii) if *C. pneumoniae* infection of the placenta is linked to PE. Anti-*C. pneumonia* immunoglobulin G (IgG, not IgA or IgM) seroprevalence has recently been reported to be higher in PE than in normal pregnancy (10). Only C. pneumonia showed these changes, neither C. trachomatis nor *C. psittici*. The absence of detectable antibodies several weeks after the onset of infection does not rule out a diagnosis of acute C. pneumonia since the IgM antibody response in primary infections can take up to 6 weeks and the IgG antibody response up to 8 weeks (11).

1.1. Aim of the Study

This study aimed to determine the role of chronic *Chlamydia pneumoniae* in the pathogenesis and onset of preeclampsia.

2. Materials and Methods

One-year case-control research was undertaken at Al-Yarmouk Teaching Hospital in Baghdad's Department of Obstetrics and Gynecology (October 1, 2018, to

September 30, 2019). The study included 140 pregnant women split into two groups: 70 women who met the clinical criteria for PE but were admitted to the obstetric ward for evaluation, and 60 women who did not meet the clinical criteria for PE but were admitted to the obstetric ward for evaluation. They were further classified into the following categories:

•Early-onset PE (Group I): included 35 singleton pregnant women who presented with clinical onset of PE<34 weeks of gestation, with BP>140/90, proteinuria +1.

•Late-onset PE (group II): included 35 singleton pregnant women who presented with clinical onset of PE≥34 weeks of gestation, with BP>140/90, proteinuria +1. Control group (Group III): included seventy normotensive pregnant women who were admitted with signs and symptoms of labor.

Inclusion criteria: 1. Singleton viable pregnancy 2. Gestational age ≥28 weeks; it was confirmed by the last menstrual period and early ultrasound.

Multiple pregnancies are an exclusionary factor. 2. Women with a medical condition such as diabetes, hypertension, or metabolic, nutritional, or hemorrhagic problems. 3. abnormal Placentation. 4. Substance abuse (alcohol, cigarettes, illegal drugs, etc.) and aspirin usage history. 5. Renal illness that has been present for some time.

The following conditions were imposed on the participants: 1. They were informed about the study's purpose and were only invited to join if they agreed. All pregnant women in the study gave their verbal consent. 2. Data on the patient's age, gestational age, parity, and previous medical, surgical, and obstetric history were collected. 3. All patients underwent general and obstetric examinations. 4. All participants' blood pressures were taken. 5. All subjects were subjected to routine and particular investigations, depending on their condition. A. Abdominal ultrasound, complete blood count, coagulation profile, liver function test, renal function test Patients with equal or more than +1 albumin in urine dipstick were included in the study. B. Midstream urine dipstick examination for albumin in urine and patients with equal or more than +1 albumin in urine dipstick were included in the study. In the labor room and intensive care unit, the patients were inpatients.

Venipuncture was used to obtain 5 mL of blood from each patient. The material was centrifuged for 10 minutes at 2000RPM; then, the plasma was collected and refrigerated at - 20°C until the IgG levels were determined. *Chlamydia pneumoniae* Detection of IgG levels

•Dilution of the Sample 2 mL frozen blood sample that has been thawed at 4°C and is ready for testing. All samples were diluted 1+100 with IgG Sample Diluent before being assayed. To make a 1+100 dilution, pour 10l and 1ml IgG Sample Diluent into tubes and carefully mix with a Vortex.

Procedure for performing an assay the temperature in the incubator is set at 37°C + 1°C. Control samples of 1.100l were distributed and diluted into their respective wells. Leave well A1 blank for the substrate. 2. The foil provided in the kit covers the wells. 3. Incubated at 371°C for 1 hour and 5 minutes. 4. After incubation, remove the foil, aspirate the contents, and wash each well three times with 300l of Washing Solution, each wash cycle taking >5sec. Except for the blank well (e.g., A1), 5.100l of C. Pneumonia IgG conjugate was distributed in all wells. 6. Incubated at room temperature for 30 minutes. Any blue color that develops throughout the incubation process fades to yellow.

•Measurement using the substrate blank in well A, zero the ELISA Microwell Plate Reader, subtract, measure the absorbance of all wells at 450 nm, and enter the absorbance values for each control and patient sample in the distribution and identification plan.

•Results Interpretation If the absorbance value is more than 10% greater than the cut-off, the sample is declared POSITIVE. Absorbance values of 10% above or below the cut-off should not be deemed unambiguously positive or negative. If the absorbance

value is less than 10% below the cut-off, the sample is declared NEGATIVE. If the IU/ml result for C. Pneumonia is less than 0.41, it is declared negative.

2.1. Statistical Analysis

A serial identification number was assigned to each patient. Statistical Package for Social Sciences (SPSS) version 20 was used to analyze the data. The frequency and percentages are used to present the categorical data. The connection between the categorical data was tested using Pearson's chi-square and Exact 1 tests. - The Shapiro-Walk test was performed to determine the distribution type (either normally or not normally). Where appropriate, the continuous variables were displayed as mean, standard deviations, median, and inter-quartile ranges. Analysis of variances (ANOVA) and Kruskal-Wallis non-parametric tests were utilized to compare continuous variables, depending on the distribution. -LSD and Dunnett T3 posthoc tests were employed to compare mean differences across research groups to determine the true significance based on data homogeneity. - The non-parametric Mann-Whitney U test was used as a post-hoc test for non-parametric variables. This investigation used a *P*-value of less than 0.05 to determine significance.

3. Results

Table 1 displays the demographic characteristics of the study groups included in the research; the results indicated that Group I patients were generally younger than the other two groups, with an average age of 26.9 years, compared to 28.9 years for group II and 29.5 years for Group III. However, these differences did not reach statistical significance. Regarding the gestational age at the time of delivery, the average for group I was the shortest at 33.3 weeks, followed by 37.5 weeks and 37.4 weeks for groups II and III, respectively; these differences were found to be statistically significant ($P<0.001$). Hypertension was present in 18/30 (60%) of Group I, 14/30 (46.7%) of group II, and 35/60 (58.3%) of Group III, with no significant association between BP and BMI. The parity of the study groups also showed no significant association with the condition of the patients. The mean arterial pressure of group I and group II were 124 mmHg, while it was only 84 mmHg for Group III, with a highly significant increase in the patients compared to group 3 ($P<0.001$).

Comparing the median titer of *C. pneumonia* IgG tests showed that group I had the highest median level, at 0.3 U/ml, compared to 0.09U/ml for Group II and 0.19U/ml for Group III. These differences were statistically significant ($P=0.001$), as shown in table 2.

Group II showed a significantly lower median level of *C. pneumonia* IgG test ($P<0.001$) as compared to Group I as well as Group III ($P=0.016$). In contrast, no significant difference was shown between Group I and group III, as shown in table 3.

Table 1. Maternal demographic characteristics included in the study

Variables	Group I (n=35)	Group II (n=35)	Group III (n=70)	*P-value*
Maternal age (years) Mean ± SD	26.9±8	28.9±5.3	29.5±5.5	0.128 [a]
Gestational age at delivery (weeks) Mean ± SD	33.3±2.5	37.5±0.6	37.4±3.0	<0.001*[a]
Body mass index >25 kg/m2 No. (%)	18 (60)	14 (46.7)	35 (58.3)	0.5
Parity				
Nulliparous	15 (50)	13 (43.3)	24 (40)	0.15 *P*
Para (1 - 4)	13 (43.3)	15 (50)	36 (60)	
Para ≥5	2 (6.7)	2 (6.7)	0 (0)	
Mean arterial pressure (mmHg) Mean ± SD	124±6.6	124±5.9	86.3±6.8	<0.001*[a]
Positive proteinuria No. (%)	30 (100)	30 (100)	6 (10)	<0.001**P*

SD= standard deviation, [a] ANOVA test, P Pearson's chi-square test, * Significant at 0.05 level

Table 2. Comparison among the study groups regarding *Pneumonia*IgG levels

Parameters	Group I (n=35) Median (IQR)	Group II (n=35) Median (IQR)	Group III (n=70) Median (IQR)	*P*-value
Pneumonia IgG level (U/mL)	0.3 (0.15-2.9)	0.09 (0.05-0.14)	0.19 (0.06-3.1)	0.001*

IQR= inter-quartile range, Kruskal-Wallis test, * Significant at 0.05 level

Table 3. Post-hoc comparison between the study groups regarding *C.Pneumonia*IgG levels

Study groups	*C. pneumoniae* IgG level (U/mL) Median (IQR)	*P*-value
Group I (n=35) Group II (n=35)	0.3 (0.15-2.9) 0.09 (0.05-0.14)	<0.001*
Group I (n=35) Group III (n= 70)	0.3 (0.15-2.9) 0.19 (0.06-3.1)	0.111
Group II (n=35) Group III (n=70)	0.09 (0.05-0.14) 0.19 (0.06-3.1)	0.016*

IQR= inter-quartile range, Mann-Whitney U test, * Significant at 0.05 level.

4. Discussion

After 20 weeks of pregnancy, preeclampsia (PE) is a pregnancy disease characterized by high blood pressure and a substantial amount of protein in the urine (3). PE increases the chances of both the mother and the baby having a poor outcome (12).

The distinction between early and late-onset PE is a more recent idea. It is commonly understood that these two entities have distinct etiologies and should be treated as distinct diseases (13, 14). Early-onset PE (before 34 weeks) is frequently linked to uterine artery Doppler abnormalities, IUGR, and poor maternal and newborn outcomes (15). On the other hand, late-onset PE is typically associated with a normal or minor increase in resistance index, a low likelihood of fetal involvement, and better perinatal outcomes (after 34 weeks) (15, 16). Jim and Karumanchi (17) recently established that early-onset PE and IUGR are placenta-mediated diseases that share fundamental commonalities (17, 18).

Many investigators have found that reactivation of chronic or latent infection is linked to PE, and C. pneumonia may play a role in increasing endothelial dysfunction. The current study found that women with early-onset PE had the highest median levels of C. pneumonia IgG compared to late-onset PE and normotensive non-complicated term pregnant women. These differences were significant (*P*=0.001). These findings were consistent with several previous studies, including a case-control study conducted by von Dadelszen, Magee (19) in 2003 to evaluate the levels of *C. pneumoniae* in early-onset PE. Early-onset PE, late-onset PE, normotensive IUGR (birth weight less than the third centile), and matched normal pregnancy were all included in the study. In addition, Mokhtari, Yaghmaei (20) conducted a cross-sectional study on 176 women with normal pregnancy outcomes, patients with late-onset PE, and patients with early-onset PE from 2004-2005. The ELISA method was used to check for anti-*C. pneumonia* IgG and its titer in the patients and controls. There was a significant difference in IgG titers between the three groups (*P* 0.0001), early-onset and late-onset PE (*P* 0.0001), and early-onset PE and the control group (*P* 0.0001), but no differences between late-onset PE and the control group (*P*=0.98), according to this study. The findings of this investigation suggested that C. pneumonia infection could be linked to the beginning of early-onset PE (20).

Two hundred seventy women were involved in the study, supported by one of the most extensive investigations by Zbinden, van den Brandt (21) to elucidate the relationship between infection and immunity in pregnancy and PE (early-onset PE, late-onset PE, and healthy pregnancy controls). The researchers discovered a link between anti-*C. pneumoniae* IgG seroprevalence and concentration, as well as the PE maternal syndrome. According to the findings, *C. pneumoniae* infection could be a risk factor in the etiology of PE. It is likely that the apparent higher link between anti-*C. pneumonia* IgG in the mid-trimester and early-onset PE is due to a closer temporal association between blood collection and clinical disease onset (21). The study found no significant association between body mass index, maternal age, and parity of the study groups' conditions (19-21).

According to this study, early-onset PE had a higher *C. pneumonia* IgG titer than late-onset PE and healthy non-complicated term pregnancy. This significant increase was directly associated with the start of PE, indicating a pathophysiological relationship between the reactivation of chronic or latent infection and PE.

Authors' Contribution

Study concept and design: H. A. M.
Acquisition of data: H. A. M.
Analysis and interpretation of data: H. A. M.
Drafting of the manuscript: H. A. M.
Critical revision of the manuscript for important intellectual content: H. A. M.
Statistical analysis: H. A. M.
Administrative, technical, and material support: H. A. M.

Ethics

The human study was approved by the Al Iraqi University, College of Medicine, Baghdad, Iraq Review Board and written informed consent obtained from each participant.

Conflict of Interest

The authors declare that they have no conflict of interest.

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