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ORIGINAL ARTICLE

Prevalence of *Chlamydophila pneumoniae* Antibodies in Women with Pre-Eclampsia in Lagos, Nigeria

Prevalence des Anticorps Anti Chlamidiophila Pneumoniae Chez des Femmes en Pre Eclampsie a Lagos, Nigeria

J. O. Awoleke*, G. O. Ajayi‡, O. Adegbola†

ABSTRACT

BACKGROUND: **The precise aetiology of pre-eclampsia has remained unknown. It is still a major contributor to maternal and perinatal morbidity and mortality.**

OBJECTIVE: **To determine the relationship between pre-eclampsia and immunoglobulin G (IgG) antibodies to *Chlamydophila pneumoniae*.**

METHODS: **One hundred and eleven parturients in a tertiary hospital in Lagos, Nigeria comprising 49 women with pre-eclampsia and 62 women without pre-eclampsia were studied.Peripheral blood was obtained for *Chlamydophila pneumoniae* antibodies which were measured using a solid-phase enzyme-linked immunosorbent assay and maternal diastolic blood pressure, perinatal morbidity and mortality were also assessed.**

RESULTS: **The cases (N = 49) and controls (N = 62) were evenly matched with respect to age and parity. The women with pre-eclampsia delivered at significantly lower gestational ages than those with normotensive gestations.**

66.7% of all the subjects were seropositive for *Chlamydophila pneumoniae* antibodies.

38 out of the 49 cases (77.6%) were positive for the *Chlamydophila pneumoniae* antibodies compared with 36 out of the 62 controls (58.1%) [p<0.05].Higher antibody titres were found in parous women with a previous history of pre-eclampsia compared with those without a previous history (p = 0.0308).However, there was no significant association between antibody titres and pregnancy outcome (p > 0.05).

CONCLUSION: **Results from this study suggest a link between *Chlamydophila pneumoniae* IgG antibodies and pre-eclampsia. Further prospective studies with larger sizes are needed to verify this association and identify therapeutic options that will effectively prevent the onset or progression of pre-eclampsia. WAJM 2012; 31(4): 253–258.**

Keywords: **Pre-eclampsia, *Chlamydophila pneumoniae*, Seroprevalence, IgG antibodies.**

RÉSUMÉ

CONTEXTE: L'étiologie précise de la pré eclampsie demeure inconnue. Elle est encore un contributeur majeur de la morbidité et de la mortalité périnatales.

OBJECTIF: Déterminer la relation entre la pré ecclampsie et les anticorps anti Chlamophila Pneumoniae de type Immunoglobuline G (IgG).

MÉTHODES: Nous avons étudié cent onze parturientes d'un hôpital tertiaire de Lagos, Nigeria incluant 49 femmes avec pré ecclampsie et 62 femmes sans pré eclampsie. Le dosage du taux d'anticorps anti Chlamophila Pneumoniae a été réalisé sur du sang périphérique par la méthode d'essai immuno absorbant à enzyme lié en phase solide. De plus la pression artérielle diastolique, la morbidité et la mortalité péri natales ont été évaluées.

RÉSULTATS: Les cas (N = 49) et les témoins (N = 62) ont été croisés en tenant compte de l'âge et de la parité. Les femmes en pré eclampsie avaient accouché à des âges gestationnels significativement plus bas que les femmes gravides normotendues. Une séropositivité anti Chlamophila Pneumoniae a été notée chez 66.7% de toutes les femmes. Tente huit des 49 cas (77.6%) étaient séropositives à Chlamophila pneumoniae comparés à 36 des 62 témoins (58.1%) [p<0.05]. Des titres d'anticorps plus élevés ont été retrouvés chez les parturientes ayant un antcédent de pré eclampsie comparées aux femmes sans antécédents de pré eclampsie (p = 0.0308). Toutefois, il n'y avait pas d'association entre les taux d'anticorps et les résultats de la grossesse (p > 0.05).

CONCLUSION: Les résultats de cette étude suggèrent un lien entre les anticops IgG anti Chlamophila Pneumoniae et la pré ecclampsie. Des études prospectives à plus grande taille d'échantillon sont nécessaires pour vérifier cette association et identifier les options thérapeutiques qui vont efficacement prévenir l'installation ou la progression de la pré ecclampsie. **WAJM 2012; 31 (4): 253–258.**

Mots clés: Pré-eclampsie, Chlamydophila pneumoniae, Séroprévalence, anticorps IgG.

Department of *Obstetrics and Gynaecology, University Teaching Hospital, P.M.B. 5355, Ado-Ekiti, Ekiti State, Nigeria. ‡Prenatal Diagnosis & Therapy Centre, Department of Obstetrics & Gynaecology, College of Medicine, University of Lagos,†Obstetrics and Gynaecology, College of Medicine, University of Lagos/Lagos University Teaching Hospital, P.M.B. 12003, Idi-Araba, Surulere, Lagos, Nigeria.

Correspondence: **Dr. med. G. O. Ajayi,** Department of Obstetrics & Gynaecology, College of Medicine, University of Lagos, P. M. B. 12003, Idi-Araba, Surulere, Lagos, Nigeria. E-mail: bisijacob@yahoo.co.uk; goiajayi@yahoo.com

Abbreviations: IgG, Immunoglobulin G antibodies; **HRP,** Horse-Radish Peroxidase; **LUTH,** Lagos University Teaching Hospital; **PE,** Pre-eclampsia; Uarb, Arbitrary Units.

INTRODUCTION

Pre-eclampsia (PE) is a multisystem disorder that is unique to human pregnancy and remains a major cause of maternal and fetal morbidity and death.[1] It is defined as systolic blood pressure of ≥140mmHg and or diastolic blood pressure of ≥90mmHg on two occasions ≥6hours apart after 20 weeks of gestation, with proteinuria of ≥2+ (dipstick method) or 0.3gm/24hours. It is severe when the systolic blood pressure is ≥160mmHg or diastolic blood pressure is ≥110mmHg on two occasions ≥6hours apart, and a proteinuria ≥5gm/24hours or ≥3 +by dipstick testing on at least two separate occasions.[2]

Pre-eclampsia complicates about 2-8% of all pregnancies.[3] The incidence of PE is 23.6 cases per 100 deliveries (approximately 5% of all pregnancies) in the United States (US).[4] Studies done from Nigeria[5] and Kenya[6] revealed a prevalence of 5.6% and 5.4% respectively. It is one of the most common and dangerous complications of pregnancy.[3, 7]

As the third leading cause of pregnancy-related deaths (after haemorrhage and embolism), PE accounts for an estimated 790 maternal deaths per 100,000 live births in the US.[4] It also affects the fetus adversely resulting in fetal growth restriction, preterm delivery, birth asphyxia, fetal demise[5,8] and increased need for instrumental vaginal delivery.[9, 10]

Although the mechanisms and primary precipitant for this disorder are unclear, the consequences include widespread endothelial dysfunction, maternal vasoconstriction, hypercoagulability, platelet dysfunction and placental hypoxia.[11, 12]

Various theories have been postulated to explain the entire process. However, because of the findings of inflammatory cytokines, similarities between the acute atherosis of PE and atherosclerosis, and the association between chronic infection and atherogenesis, a potential infectious trigger has been suggested for the occurrence of PE.[13]

Following publication of studies establishing the role of *Chlamydophila pneumoniae*, an obligate intracellular bacterial pathogen in many chronic inflammatory conditions and the finding of the microbe within plaques in the walls of coronary arteries[14–16] (thereby linking infection with the pathogen with atherosclerosis and heart problems), recent studies have also found a higher prevalence of antibodies to *Chlamydophila pneumoniae* in women with history of PE.[17–20]

However, the evidence for the causal role of *Chlamydophila pneumoniae* infection in PE is currently far from overwhelming. In addition, there has been no documented Nigerian study addressing this issue. This study was therefore designed to investigate whether serological markers of *Chlamydophila pneumoniae* infection are associated with PE in a tertiary healthcare centre in Lagos, Nigeria.

SUBJECTS, MATERIALS AND METHODS

Design

This was a cross-sectional, single-blind case-control study.

Setting

The study was conducted at the Labour Ward of the Lagos University Teaching Hospital (LUTH), which is one of the three main referral hospitals in metropolitan Lagos, Nigeria. The Lagos University Teaching Hospital (LUTH) is the teaching hospital of the College of Medicine of the University of Lagos and was founded in 1962. It is situated in the heart of the Lagos mainland, which has a population of over 1,157,320 inhabitants. It acts mainly as a referral hospital for other hospitals in the state. The LUTH Department of Obstetrics and Gynaecology has a total of 134 beds – 74 for Obstetrics in 3 wards and 60 for Gynaecology in 2 wards. The labour ward has 14 private suites with easy communication to the central nurses' centre within the complex. The neonatal unit is within the labour ward complex. It is for the neonates delivered in LUTH labour ward and it has 58 neonatal cots including incubators. Also within the complex are two standard operating theatres and four doctors' rooms shared by resident obstetricians, anaesthetists and neonatologists.

The parturients seen were the booked antenatal patients of LUTH and unbooked patients referred from various maternity homes, private and other government hospitals. About 120 deliveries were conducted monthly.

Study Population

All pregnant women admitted into the labour ward for delivery were eligible for the study if they satisfied the following inclusion criteria:

1. Not been diagnosed hypertensive outside of pregnancy
2. No documented hypertension prior to the 20th week of index gestation.
3. The index pregnancy was not a multi-fetal gestation and the fetus was not known to be congenitally anomalous.
4. Have given informed verbal consent.

A parturient was excluded from the study if (i) she was a known hypertensive or had documented hypertension before the 20th week of the index gestation, (ii) the index pregnancy was multifoetal or the foetus was congenitally abnormal, or (iii) she with-held her consent to participate.

Data Collection

Selection of Cases

The cases were consecutive parturients admitted into the labour ward who had hypertension (defined as systolic blood pressure of ≥140mmHg and or diastolic blood pressure of ≥90mmHg on two occasions ≥6 hours apart after 20weeks of gestation), significant proteinuria (≥2 + by dipstick testing on a mid-stream or catheter-obtained urine specimen) and also fulfilled the inclusion criteria above.

Selection of Controls

For every case, a control was recruited from the same population using the following inclusion criteria:

i. No documented hypertension in previous or index pregnancy
iii. Had no significant proteinuria during the antenatal period or from urine testing during admission into the labour ward.
iv. Delivered on the same day as the case or not more than 24 hours later (if there were not enough parturients).

Study Procedure

The purpose of the study,which was done between 1st November, 2006 and 31st March, 2007, was explained to the patients and informed verbal consent was obtained. Using a proforma, the socio-demographic data, parity, last menstrual period, calculated estimated gestational age at delivery, past obstetric and medical histories were recorded. Also, a detailed review of the available antenatal records was done, and later the birth weight and perinatal outcome were documented.

3–4 millilitres of a peripheral venous blood sample was obtained from the participants and transported to the laboratory in special vacutainers (BD Vacutainer Systems, Plymouth) after labeling them with a serial number. The identity of and the distinction between the cases and controls was not revealed to the laboratory scientists until all the samples were processed.

Laboratory Methods

Blood samples of cases and control subjects were centrifuged at 2000g for 20 minutes at room temperature and the sera stored and kept frozen in 2-ml aliquots at –20^{0}C until they were analysed. Icteric or haemolysed samples were not used as they can give false results in the assay. Titres of immunoglobulin G (IgG) antibody to *Chlamydophila pneumoniae* were then analyzed with an enzyme-linked immunosorbent assay kit (DIA.PRO, Milano-Italy) according to the manufacturer's instructions. The kit included a microplate already precoated with an immunodominant species-specific polypeptide derived from the *Chlamydophila pneumoniae* major outer-membrane antigen. After diluting the samples 1:101 (10µl sample + 1000µl sample diluent), the microplate was treated with the diluted samples and incubated for 1 hour at 37^{0}C. Any anti-*Chlamydophila pneumoniae* IgG present was captured by the solid phase.

After washing out all the other components of the samples, 100µl of the conjugate (containing specific anti-IgG antibody labeled with horseradish peroxidase (HRP) enzyme) was added to the sample wells of the microplate, which was incubated again for 60 minutes at 37^{0}C. This was to identify the bound anti-*Chlamydophila pneumoniae* IgG. After washing again, 100µl of a Chromogen/ Substrate mixture was added to all the wells and the microplate incubated again at room temperature for 20 minutes. The HRP enzymes acted on the mixture to generate an optical signal that was proportional to the amount of anti-*Chlamydophila pneumoniae* IgG antibodies present in the sample. The enzymatic reaction was stopped by the addition of 100µ1of an acid stop solution.Test results were obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microplate reader at 450nm. The concentration of IgG in each sample was obtained by means of a standard curve calibrated in arbitrary units per millilitre (Uarb/ml) as no international standard is available. The value of 5 Uarb/ml was used to discriminate the IgG-negative from the positive population.The discriminatory value of 5 Uarb/ml was chosen in line with the instructions in the kit's manual.

Statistical Analyses

The data obtained was analysed using the Epi Info version 3.4.1 statistical software package. Analyses included chi-square and Fisher exact test where appropriate. Mann-Whitney U test was used to identify differences in the mean values between two groups. Odds Ratio was calculated for comparison of cases and controls. A p value of <0.05 was considered significant.

Ethics

The study was carried out after obtaining approval from the Ethics Committee of the Lagos University Teaching Hospital. Informed consent was also obtained from the patients prior to sample collection. Laboratory tests were carried out at no cost to the patients.

RESULTS

The data obtained from a total of 111 women (49 cases and 62 controls) were analyzed. The 49 women with pregnancies complicated by pre-eclampsia were not significantly different from the 62 women with unaffected pregnancies with respect to age [mean age (years) of cases: 30.48 ± 5.36 versus controls: 30.75 ± 5.10; p = 0.9853] and parity [mean parity of cases: 1.55 ± 1.58 versus controls: 1.11 ± 1.10; p = 0.3333] (Table 1). Not unexpectedly, a significantly higher proportion of women with pre-eclampsia delivered preterm

Table 1: Demographic and Obstetric Characteristics of the Cases and Controls

	Cases (n =49)	Controls (n = 62)
Age (Years)		
<20	3	0
20 – 24	2	8
25 – 29	14	21
30 – 34	20	17
35 – 39	8	13
≥40	2	3
	χ^2 = 8.22; df = 5; p = 0.1443 (NS)	
Parity		
0	17	21
1	11	23
2	7	10
3	6	6
≥4	8	2
	χ^2 = 7.36; df =4; p =0.1179 (NS)	
Gestational Age (Weeks)		
28 – 33	13	4
34 – 36	13	2
≥37	23	56
	χ^2 = 25.44; df = 2; p = 0.000003 (S)	

***S**=Significant; **NS** –Not Significant; χ^2 = Chi square*

Table 2: Relationship between Antibody Titres and Past/Present Incidence of Pre-Eclampsia

IgG Antibody Titre	Cases	Controls	Total
Negative	11	26	37
Positive (≥5 Uarb/ml)	38	36	74
Total	**49**	**62**	**111**
OR = 0.40 (95% C.I. 0.16 – 0.99); χ^2 = 4.68, p = 0.0306 (S)			
History of Pre-eclampsia			
IgG Antibody Titre	**Yes**	**No**	**Total**
Negative	3	24	27
Positive (≥5 Uarb/ml)	13	33	46
Total	**16**	**57**	**73**
OR = 0.32 (95% C.I. 0.06 – 1.39); χ^2 = 2.92, p = 0.0873 (NS)			
Antibody Concentration (Uarb/ml)			
<30	11	52	63
30 – 60	4	5	9
>60	1	0	1
Total	**16**	**57**	**73**
χ^2 = 6.96, p = 0.0308 (S)			

OR=Odds Ratio; C.I. = Confidence Interval; Uarb/ml = Arbitrary Units per millilitre.

Table 3: Antibody Levels Versus Pregnancy Outcome

Antibody Titre	Perinatal Condition		Total
	Still Birth	Live Birth	
Negative	1	36	37
Positive	7	67	74
Total	**8**	**103**	**111**
OR = 0.27 (95% C.I. 0.01 – 2.31); χ^2 = 1.68, p = 0.1849 (NS)			
Antibody Concentration (Uarb/ml)			
	<30	**30 – 60**	**>60**	**Total**
Apgar Score at 5 minutes				
Good	88	11	1	100
Fair	2	1	0	3
Poor	8	0	0	8
Total	**98**	**12**	**1**	**111**
χ^2 = 2.6699, p = 0.6145 (NS)				
Diastolic Blood Pressure (mmHg)				
90 – 109	21	6	2	29
≥110	28	4	1	33
Total	**49**	**10**	**3**	**62**
χ^2 = 1.48, p = 0.4768 (NS)				
Birth Weight (grammes)				
<2500	9	2	0	11
≥2500	89	10	1	100
Total	**98**	**12**	**1**	**111**

Fisher Exact Test p = 0.0650 (NS)

compared with those with normotensive pregnancies [mean gestational age (weeks) for cases: 35.95 ± 3.28 versus controls: 38.48 ± 2.29; p < 0.0001].

Of the 111 women whose data were analysed, 74 (66.7%) were found to be seropositive for *Chlamydophila pneumoniae* infection.

Using a discriminatory value of 5 arbitrary units/millilitre (Uarb/ml) to determine seropositivity (as highlighted in the Methodology), a higher proportion of women with pre-eclampsia (38 of 49 (77.6%)) were found to be positive for *Chlamydophila pneumoniae* antibodies compared with the controls (36 of 62 (58.1%)). And this observation reached significant levels (p = 0.0306). (Table 2). Table 2 also shows that the proportion of women with a history of pre-eclampsia having positive antibody titres was higher than those without a previous episode of pre-eclampsia although this was not statistically significant (p = 0.0873). However when the concentration of the antibodies were considered, women with a previous history were found to have significantly higher levels of immunoglobulin G (IgG) antibodies than women with a previous obstetric history not complicated by pre-eclampsia (p = 0.0308).

No significant association was found between increasing antibody titres and adverse perinatal outcome or worsening maternal diastolic blood pressure (p > 0.05) (Table 3) as far as this study was concerned.

DISCUSSION

Pregnant women with pre-eclampsia were found to be more commonly seropositive for IgG antibodies to *Chlamydophila pneumoniae* than normotensive pregnant women (p < 0.05) (Table 1). This is in agreement with findings from other studies[18–21] and suggests a possible link between *Chlamydophila pneumoniae* infection and pre-eclampsia. Although the possibility of cross-reactivity from the antibodies to other chlamydial species is an issue for consideration, the use of the solid phase enzymes immunoassay with species-specificity in this study makes it unlikely that cross-reactivity to other pathogens might explain the relationship

found between pre-eclampsia and *Chlamydophila pneumoniae*.[19]

A possible explanation could be proffered. In pregnancies uncomplicated by pre-eclampsia, the maternal spiral arteries undergo extensive remodeling.[22] This remodeling is deficient in pre-eclamptic women with resultant reduction in placental perfusion. Also, women who develop pre-eclampsia may have an inflammatory response more intense than healthy pregnant women[23] and this may be caused by a concurrent or preceding inflammatory stimulus such as infection. One of the pathognomonic findings in the placenta from a pre-eclampsia-complicated pregnancy is acute atherosis, with a pathogenesis similar to that of atherosclerosis especially regarding inflammation and endothelial cell damage.[18,21] Since *Chlamydophila pneumoniae* is thought to contribute to athero-sclerosis by bringing monocytes to the areas of arterial damage to form foam cells leading to plaque and thrombosis,[24] it may also be an initiator of the inflammatory process in pre-eclampsia. On the other hand, it may be an agent that has affinity for atherogenetic lesions.[21, 25]

Further, but limited, evidence of the link between *Chlamydophila pneumoniae* infection and pre-eclampsia was from studies suggesting that antibiotic use in pregnancy may reduce the risk of pre-eclampsia. [26-27]

The mismatch between the gestational ages of the cases and controls (Table 1) could be as a result of the fact that pre-eclampsia could lead to complications such as fetal growth restriction, intrauterine fetal demise, difficulties with controlling the blood pressure, abruptio placentae, eclampsia[5,8] necessitating delivery before term to save mother, baby or both.

There was a significant association between *Chlamydophila pneumoniae* infection and having a previous history of pre-eclampsia in this study (Table 2). This is similar to the findings in the study by Goulis *et al.*[19] However, because many women with elevated antibody titres had no history of pre-eclampsia, it could be that the association between *Chlamydophila pneumoniae* infection and pre-eclampsia is a relationship of concern. This association is not necessarily straight-forward or simple and may not follow a cause-and-effect pattern.[19, 21]

The seroprevalence of IgG antibodies to *Chlamydophila pneumoniae* from this study was 66.7%. The study done by von Dadelszen *et al*[18] also found a 53% prevalence of Chlamydophila pneumoniae IgG seropositivity for normal pregnancy and 67% for early-onset pre-eclampsia. Generally, the observation is that the prevalence of serum antibodies starts to rise steadily from school age, and it is estimated that between 50–70% of adults are seropositive.[28] This rising trend could be because following the primary infection, cycles of re-infection are common.[29, 30]

The lack of association between antibody concentration and pregnancy outcome could be because pre-eclampsia is a disease with multifactorial aetiology and *Chlamydophila pneumoniae* infection may be one of the factors modulating pregnancy outcome in these patients.

Limitations

There were limitations to this study. One is that it may not have sufficient power to reflect significant association or differences within the study population and the other is its cross-sectional design. The latter could make it possible to include women with undiagnosed essential hypertension or renal disease while the former may make generalisation unrealistic. Also for unbooked patients, the history of pre-eclampsia in previous deliveries was not rigorously ascertained making that analysis susceptible to bias.

The fact that urinary tract infection (UTI) was not excluded in the proteinuric parturients may be one of the limitations of the study. However, the definition of pre-eclampsia that was employed in the study is valid and does not require that UTI be excluded before it can be applied. Also, since there are other less common causes of proteinuria in pregnancy, excluding UTI may imply that those other causes should be excluded too.
However, the use of a proven method for serological evaluation of antibodies to *Chlamydophila pneumoniae* added quality to the study.

CONCLUSION AND RECOMMENDATION

This study has shown that seroprevalence of IgG antibodies to *Chlamydophila pneumoniae* was more common among women with pre-eclampsia than among women with normotensive pregnancies.

Also higher titres of antibodies to *Chlamydophila pneumoniae* were found in women with a previous history of pre-eclampsia. These preliminary data suggest a possible link between infection with Chlamydophila pneumoniae and pre-eclampsia in our environment.

It is recommended that before antibiotics are added to the present prophylactic measures against pre-eclampsia, additional studies with larger sizes are needed. If this association is confirmed, then careful prospective studies with selected therapeutic interventions would be needed to see if they would effectively prevent the onset or progression of pre-eclampsia.

Also those women with positive IgG antibody titres should have their IgM (immunoglobulin M), IgA (immunoglobulin A) antibodies and Chlamydophila pneumoniae DNA assay done too to further verify this observed link and ascertain its reproducibility.

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