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ASSESSMENT OF IMMUNOLOGICAL CORRELATES AMONG PRE-ECLAMPTIC WOMEN IN YENAGOA, SOUTH-SOUTH NIGERIA

Gloria T. Ogbede[1]*, Simeon G. George[2], Nelson E. Onitsha[3] and Ferdinand C. Ezeiruaku[4]

[1,2,3,4]Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

***Corresponding Author: Gloria T. Ogbede**
Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

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ABSTRACT

Background: The 5.69% prevalence of pre-eclampsia in Yenagoa, the South-South region of Nigeria is alarming and therefore calls for additional markers that will enhance the diagnosis of the disease. **Aim:** This study was aimed at assessing immunological correlates among pre-eclamptic women in Yenagoa, South-South Nigeria. **Method:** Ninety-one pregnant women with pre-eclampsia (gestation > 20 weeks, blood pressure ≥ 140/90 mmHg) and ninety-one normotensive pregnant women at gestation > 20 weeks were enrolled in the study. Venous blood was collected from each of the subjects, followed by assaying for Immunoglobulin- (Ig) G, IgM, Tumor Necrosis Factor – alpha (TNF-∝), Interleukin (IL)-6 and C-reactive protein (CRP) by ELISA (enzyme-linked immunosorbent assay). SPSS Windows Version 26 was used for all statistical analyses. Differences between groups was determined by the independent samples t-test. The association between demographic variables and cellular immune responses was determined using the Pearson correlation coefficient. Data was considered significant at $p < 0.05$. **Results:** Results showed that BMI, BP, educational level, family history of pre-eclampsia, and primipaternity were significantly associated with pre-eclampsia ($p < 0.05$). TNF-α and CRP levels were significantly elevated in pre-eclampsia cases ($p < .01$). Significant correlations were observed between TNF-α, SBP ($r = -0.231$, $p < 0.05$), DBP ($r = -0.214$, $p < 0.05$), BMI ($r = -0.277$, $p < 0.01$), IgM ($r = -0.683$, $p < 0.01$), and IL-6 ($r = -0.416$, $p < 0.01$). **Conclusion:** The correlation between pre-eclampsia demographic variables and each of TNF-α and CRP is a pointer to the predictive relevance of these markers in Yenagoa.

KEYWORDS: Pre-eclampsia, immunological parameters, systemic blood pressure, diastolic blood pressure.

INTRODUCTION

Pre-eclampsia is a pregnancy-related disorder that affects about 5-8% of all pregnant women worldwide.[1] It is characterized by high blood pressure, proteinuria (presence of protein in urine), and in severe cases, organ impairment.[2] The exact cause of pre-eclampsia is unknown, but it is believed to be associated with problems in the development of the placenta, which nourishes the fetus with nutrients and oxygen.[1] The condition usually occurs after 20 weeks of pregnancy and can lead to serious complications for both the mother and the baby if left untreated.

The prevalence of pre-eclampsia and eclampsia varies widely around the world, and understanding their epidemiology is important for improving maternal and fetal health outcomes. According to the World Health Organization (WHO), pre-eclampsia affects 2% to 8% of all pregnancies worldwide.[3] The incidence of pre-eclampsia is highest in developing countries, where it is a leading cause of maternal and fetal morbidity and mortality.[4] The incidence of pre-eclampsia in Africa varies significantly between countries and regions. A study conducted in Ethiopia reported a pre-eclampsia prevalence rate of 5.9%, while another study in Nigeria found a prevalence rate of 10.6%.[5,6] In Yenagoa, the prevalence of pre-eclampsia was determined as 5.69%.[7] In South Africa, the incidence of pre-eclampsia is estimated to be around 3.3%, with an eclampsia incidence rate of 0.07%.[8]; lower than that of Yenagoa.

The pathophysiology of pre-eclampsia is complex and not fully understood, but it is believed to involve a combination of maternal and fetal factors that lead to the dysfunction of the placenta.[9] One of the hallmarks of pre-eclampsia is the failure of the maternal spiral arteries to undergo normal remodeling, resulting in poor perfusion of the placenta and reduced oxygen and nutrient delivery to the fetus.[10] This placental ischemia is thought to trigger a series of events that ultimately lead to the development of pre-eclampsia. One of the first responses to placental ischemia is the release of anti-angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin (sENG), into the

maternal circulation.[11] These factors bind to and inhibit the action of vascular endothelial growth factor (VEGF) and other pro-angiogenic factors, leading to endothelial dysfunction, vasoconstriction, and hypertension.[12]

The release of anti-angiogenic factors also leads to the activation of the maternal inflammatory response, with the release of cytokines and chemokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1).[10] These inflammatory mediators further contribute to endothelial dysfunction, as well as the activation of the coagulation system, resulting in a pro-thrombotic state.[13] The combination of these factors leads to the characteristic symptoms of pre-eclampsia, including hypertension, proteinuria, oedema, and organ dysfunction.

Thus, understanding the role of immunological markers such as cytokines and chemokines in the onset of pre-eclampsia is expedient in managing patients with the disorder in our setting. This study was aimed at assessing immunological correlates among pre-eclamptic women in Yenagoa, South-South, Nigeria.

MATERIALS AND METHODS

Subjects, Design and Setting

The population of this study consisted of pre-eclamptic and healthy pregnant women attending antenatal clinic at the Federal Medical Centre, Yenagoa, Otuoke, and the Niger Delta University Teaching Hospital, Okolobiri, all in Bayelsa State. The sample size was 182 pregnant women, consisting of 91 women with pre-eclampsia (gestation > 20 weeks, blood pressure ≥ 140/90 mmHg) and 91 normotensive pregnant women at gestation > 20 weeks. All subjects were aged 18 years and above and were enrolled by cross-sectional study design.

Blood Sample Collection

A minimum of 4 mls of venous blood was collected from each of the subjects and dispensed into EDTA anticoagulated tubes.[14] Samples were then stored at 4 – 8 ^{o}C prior to analysis. Samples for whole blood analysis were made homogenous by inverting them severally before being used for testing. Serum samples were collected in serum separator tubes and allowed to clot undisturbed at room temperature for about 1 h. The supernatant serum was pipetted into another tube and centrifuged again at 1200 × g for 10 minutes to obtain pure serum. This was followed by storage at -20oC pending analysis.

Immunological Assay by the Enzyme-linked Immunosorbent Assay (ELISA) Method

Serum levels of immunoglobulins G and M.[15,16], Human Tumor Necrosis Factor – alpha (TNF-α)[17], Human Interleukin-6 (IL-6)[18], and C-reactive protein (CRP)[19] were determined by the Enzyme-Linked Immunosorbent Assay (ELISA) method, respectively.

Statistical Analysis

The statistical analyses were done using SPSS Windows version 26.[20] Data were grouped into pre-eclampsia and control subjects. The normality of the distributions was determined using the one sample Kolmogorov-Smirnov testing. Differences between the groups was determined by the independent samples t-test for normal data. The association between demographic variables and immune responses was determined using the Pearson correlation coefficient. Data was considered significant at $p < 0.05$.

RESULTS

A total of 182 subjects were enrolled in this study comprising a symmetrical sample size of 91 pre-eclampsia (PE) cases and 91 normal pregnancy women as control. All subjects were aged 20 to 44 years, with most of them falling between the 30 to 34 age group for both cases (55.30%) and control (44.70%). No significant association was observed between the age groups and pre-eclampsia ($p > 0.05, X^2 = 6.64$), and also between parity and pre-eclampsia ($p > 0.10, X^2 = 2.64$). Other details are captured in Table 1. Table 2 shows the effect of pre-eclampsia on immunological parameters of the study subjects. Comparison of immunological parameters between women diagnosed with pre-eclampsia and healthy control subjects showed significant differences for only tissue necrosis factor- alpha (TNF-α) ($t = 3.67$, $p < 0.01$) and C-reactive protein (CRP) ($t = 4.64$, $p < 0.01$). There were no significant differences observed for immunoglobin-M (IgM), interleukin-6, and immunoglobin -G ($p > 0.05$). Table 3 shows the correlation of blood pressure, body mass index (BMI), and cellular immune parameters among pre-eclampsia subjects. Significant correlations were observed between TNF-α and each of systemic blood pressure (SBP) ($p = 0.032$, $r = 0.225$), BMI ($p = 0.008$, $r = 0.277$), IgM ($p = 0.000$, $r = 0.683$), and IL6 ($p = 0.000$, $r = 0.416$). DBP correlated significantly with SBP ($p = 0.000$, $r = 0.539$) and BMI ($p = 0.007$, $r = 0.283$). IgM also showed significant correlation with BMI ($p = 0.005$, $r = 0.285$) and IL6 ($p = 0.001$, $r = 0.357$). IL6 also correlated significantly with IgG ($p = 0.000$, $r = 0.363$) and CRP ($p = 0.000$, $r = 0.361$). Other details are found in Table 3.

Table 1: Demographic Characteristics of Study Subjects.

Item	Characteristics	Frequency (%)		X^2	*P*	OR.
		Cases	Control			
Total number		91	91			
Age (yrs)						
	Frequency (%)					
	20 – 24	7 (7.70)	4 (4.40)			
	25 – 29	19 (20.90)	22 (24.20)			
	30 – 34	42 (46.20)	34 (37.40	6.64	0.16	nil
	35 – 39	15 (16.40)	27 (29.70)			
	40 – 44	8 (8.80)	4 (4.40)			
Parity	Frequency (%)					
	1-2	84 (92.30)	77 (84.60)	2.64	0.10	0.46
	≥ 3	7 (7.70)	14 (15.40)			
BMI (Kg/m^2)	Frequency (%)					
	18.5 – 24.9	5 (5.50)	20 (22.00)			
	25.0 – 29.9	24 (26.40)	38 (41.80)	21.01	0.00**	nil
	≥ 30	62 (68.10)	33 (36.30)			
BP (mmHg)	Mean ± SD			*T*	*p*	95%CI
	SBP	157.69 ± 16.55	105.27 ± 8.21	27.06	0.00**	48.59
	DBP	99.86 ± 8.43	66.04 ± 6.81	29.75	0.00**	31.57
Educational level	Frequency (%)			X^2	*p*	OR.
	Primary	4 (4.40)	0 (0.00)			
	Secondary	43 (47.30)	24 (26.40)	14.15	0.00**	nil
	Tertiary	44 (48.40)	67 (73.60)			

Key: ** = Significant at *p*< .01, * = Significant at *p*< .05 *T* = T-test Statistic, X^2 = Chi-square statistic, OR. = odds ratio.

Table 2: Effect of Pre-eclampsia on Immunological Parameters of Study Subjects.

Parameter	Mean ± SD		*t*	*p*-value
	Pre-eclampsia n = 91	Control n = 91		
IgM (µg/mL)	554.74 ± 125.51	568.51 ± 67.60	-0.92	0.36
TNF-α (ρg/ml)	1379.32 ± 405.57	1185.85 ± 297.29	3.67	0.00**
IL6 (ρg/ml)	6.64 ± 2.02	6.70 ± 0.94	-0.25	0.80
IgG (mg/mL)	137.37 ± 72.32	130.56 ± 16.72	0.88	0.38
CRP (mg/L)	6.19 ± 4.07	4.17 ± 0.85	4.64	0.00**

***Key:* SD = standard deviation, n = total number of subjects, *t* = T-test statistic, IgM = immunoglobulin-M, TNF-α = tissue necrosis factor – alpha, IL6 = interleukin-6, immunoglobulin-G, CRP = C-reactive protein, ** = Significant difference observed between Pre-eclampsia and control subjects, *p* < 0.01**

Table 3: Correlation of Blood Pressure and Body Mass Index (BMI), to selected immunological parameters among pre- Pre-eclampsia Subjects Attending Tertiary Hospitals in Bayelsa State.

		SBP	DBP	BMI	IgM	TNF-α	IL6	IgG	CRP
SBP	*r*	1							
	p								
	n	91							
DBP	*r*	0.539**	1						
	p	0.000							
	n	91	91						
BMI	*r*	0.085	0.283**	1					
	p	0.425	0.007						
	n	91	91	91					
IgM	*r*	0.200	0.130	0.285**	1				
	p	0.057	0.220	0.006					
	n	91	91	91	91				

TNF-α	*r*	0.225*	0.175	0.277**	0.683**	1			
	p	0.032	0.096	0.008	0.000				
	n	91	91	91	91	91			
IL6	*r*	0.081	-0.118	-0.106	0.357**	0.416**	1		
	p	0.446	0.264	0.318	0.001	0.000			
	n	91	91	91	91	91	91		
IgG	*r*	-0.035	0.035	0.051	0.064	0.195	0.363**	1	
	p	0.739	0.743	0.630	0.546	0.064	0.000		
	n	91	91	91	91	91	91	91	
CRP	*r*	0.021	-0.018	-0.024	-0.060	0.007	0.361**	0.158	1
	p	0.840	0.868	0.821	0.572	0.948	0.000	0.136	
	n	91	91	91	91	91	91	91	91

Key: *r* = correlation coefficient, *p* = error probability, *n* = total number, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, IgM = immunoglobulin-M, TNF-γ = tissue necrosis factor – gamma, IL6 = interleukin – 6, IgG = immunoglobulin-G, CRP = C-reactive protein, * = Significant correlation observed, $p < 0.05$, ** = Significant correlation observed, $p < 0.01$.

DISCUSSION

The 10.6% prevalence rate of pre-eclampsia in Nigeria[5,6] and 5.69 % prevalence rate in Yenagoa[7] calls for better diagnostic markers to flag off the disorder., and hence better management of these delicate group of patients, especially in South-South Nigeria with limited resources. Here, we assessed immunological correlates among pre-eclamptic women in Yenagoa, South-South Nigeria.

Although the enrolled subjects in this study were between the ages of 20 and 44 years, the predominant age group for both cases and control were between 30 – 34 years. Of the various demographic features of pre-eclampsia considered in this study, only body mass index (BMI), blood pressure (BP), educational level, family history of pre-eclampsia, and primipaternity showed significant association with pre-eclampsia ($p < 0.05$). 95.50% of the pre-eclampsia cases had BMI at booking ≥ 25 kg/m^2 ($X^2 = 21.10$, $p = 0.00$). A meta-analysis of observational studies by Thangaratinam *et al*.[21] found that obese women had a higher risk of developing pre-eclampsia than non-obese women. Moreover, the risk increased with increasing body mass index (BMI). Similarly, a systematic review and meta-analysis by Aune *et al*.[22] reported that overweight and obese women had a higher risk of pre-eclampsia than women with normal BMI. The association was stronger for severe pre-eclampsia than mild pre-eclampsia.

Although the underlying mechanism behind the association between pre-eclampsia and overweight is poorly understood, it seems that obesity is a protagonist variable for endothelial dysfunction and inflammation: both of which are characteristic features of pre-eclampsia. Obesity also correlates well with dyslipidemia and insulin resistance which can impede placental function, thereby increasing the risk of pre-eclampsia.[23, 24] Chronic hypertension is a known risk factor for pre-eclampsia, and several studies have investigated the relationship between pre-eclampsia and chronic hypertension. A systematic review and meta-analysis by Rolnik *et al*.[25] found that women with chronic hypertension had an increased risk of pre-eclampsia, with a pooled odds ratio of 5.15. Another study by Li *et al*.[26] reported that chronic hypertension was an independent risk factor for pre-eclampsia, with an odds ratio of 2.69.

The underlying mechanisms linking chronic hypertension to pre-eclampsia are not fully understood. However, it has been suggested that chronic hypertension may lead to impaired placental function and subsequent placental ischemia, which can trigger the development of pre-eclampsia.[27]

Pre-eclampsia, a pregnancy-related hypertensive disorder, has been shown to be associated with socio-economic disparities. A growing body of literature suggests that low socio-economic status (SES) is a significant risk factor for pre-eclampsia. Several studies have found that women of low SES have a higher risk of developing pre-eclampsia than women of higher SES. A population-based study in California by El-Sayed *et al*.[28] reported that women with Medicaid insurance, a proxy for low SES, had a higher risk of pre-eclampsia compared to those with private insurance. Similarly, a systematic review and meta-analysis by Aibar *et al*.[29] reported that women of low SES had a higher risk of pre-eclampsia than women of high SES.

In addition to income and insurance status, other indicators of SES, such as education and occupation, have also been shown to be associated with the risk of pre-eclampsia. A population-based study in Australia by Hsieh *et al*.[30] reported that women with lower educational attainment had a higher risk of pre-eclampsia than those with higher educational attainment. Similarly, a study by Ezebialu *et al*.[31] found that women in manual labour occupations had a higher risk of pre-eclampsia than those in non-manual labour occupations.

The mechanisms underlying the association between pre-eclampsia and SES are not fully understood. However, several factors have been proposed, including limited access to prenatal care, poor nutrition, and chronic stress.

A study by Anderson *et al*.[32] found that women of low SES were less likely to receive timely prenatal care, which may contribute to the development of pre-eclampsia.

Furthermore, TNF-α levels in this study, were significantly increased in pre-eclampsia cases, when compared with normotensive control subjects ($t = 3.67$, $p < 0.01$, Table 2). This result agrees with that of Iavazzo *et al*.[33] who showed significant increases in TNF-α levels in pre-eclampsia at antepartum and postpartum in comparison with normotensive control subjects. Multiple lines of evidence have suggested that ischemic placenta contributes to endothelial cell activation or dysfunction of maternal circulation by promoting the synthesis of cytokines like TNF-α.[34] It has been demonstrated in a previous study that a double-fold increase in plasma concentration of TNF-α caused a significant increase in arterial pressure; placental, aorta, and kidney pre-proendothelin-1 expression; and renal vascular resistance in pregnant rats.[35] Also, in support to the results of the study by Iavazzo *et al*.[33], there was no significant difference in the concentration of the immunological marker, IL-6 between pre-eclampsia cases and controls ($t = -0.25$, $p > 0.05$). This was not the case with the C-reactive Protein (CRP).

In this study, CRP levels were significantly increased in the pre-eclampsia women when compared with the control subjects ($t = 4.64$, $p < 0.01$). Several studies have investigated the relationship that exists between CRP and pre-eclampsia. A meta-analysis by Zhang *et al*.[36] examined the association between CRP and pre-eclampsia in various global populations. The meta-analysis included 28 studies with a total of 2,630 cases of pre-eclampsia and 2,842 controls. The researchers found that serum CRP levels were significantly elevated in women with pre-eclampsia than in the control subjects. The meta-analysis also found that the association between CRP and pre-eclampsia showed stronger effect in early-onset pre-eclampsia (before 34 weeks of gestation) than in late-onset pre-eclampsia (after 34 weeks of gestation). The meta-analysis concluded that elevated CRP levels could be a useful biomarker for predicting the development of pre-eclampsia in various populations worldwide.

A study by Gallo *et al*.[37] investigated the association between CRP and pre-eclampsia among Italian women. The study enrolled 190 women with pre-eclampsia and 190 normotensive pregnant women. The researchers found that serum CRP levels were significantly higher in women with pre-eclampsia than in the control group. The study also found that serum CRP levels were significantly higher in women with early-onset pre-eclampsia than in those with late-onset pre-eclampsia. The study concluded that CRP could be a useful biomarker for predicting the development of pre-eclampsia in Italian women.

On the contrary, a study by Mert *et al*.[38] found no significant difference in CRP levels between women with pre-eclampsia and normotensive pregnant women. The study involved 60 women with pre-eclampsia and 60 normotensive pregnant women. However, the study had a small sample size, which might have limited its statistical power. The CRP is a significant marker of inflammation, which is suggestive of alterations in the immune system that occur in pre-eclampsia.[39]

Moreover, in this study, significant correlations were observed between tumor necrosis factor-alpha (TNF-α) and each of systemic blood pressure (SBP) ($r = -0.231$, $p < 0.05$), diastolic blood pressure (DBP) ($r = -0.214$, $p < 0.05$), BMI ($r = -0.277$, $p < 0.01$), immunoglobulin-M (IgM) ($r = -0.683$, $p < 0.01$), and interleukin-6 (IL-6) ($r = -0.416$, $p < 0.01$) among the pre-eclampsia subjects Table 3). The importance of the cytokines TNF-α and IL-6 in blood pressure homeostasis among pre-eclampsia subjects have been highlighted in other study.[40] In their study, they also found that significantly elevated levels of TNF-α were associated with increased risk of pre-eclampsia; and a significantly negative correlation between TNF-α and IL-6, in-line with this study.

CONCLUSION

The findings from our study show that tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) levels were elevated in pre-eclampsia cases. Interleukin-6 (IL-6) levels, however, showed no significant difference between pre-eclampsia cases and controls. The correlation between pre-eclampsia demographic variables and each of TNF-α and CRP is a pointer to the predictive relevance of these markers. We, therefore, recommended a larger cohort study that would establish a predictive function for severity of pre-eclampsia in our study.

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