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■ Original Research Article

Serum Pro-Inflammatory Cytokines; IL-6 and TNF-α Profiles and their Correlation with Severity of Pre-Eclampsia in Benin City, Nigeria

Osegi Nkencho[1], Ande Adedapo B.A[2], Onakewhor Joseph U.E[2], Idogun Sylvester E[2]

1Department of Obstetrics and Gynaecology, Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria.
2Department of Chemical Pathology, University of Benin Teaching Hospital, Benin. Edo State

ABSTRACT

Background: A generalised inflammatory response intricately involving placenta formation and immune maladaptation is considered the main pathology in pre-eclampsia. Pro-inflammatory cytokines: Interleukin-6 and Tumour necrosis factor-alpha, amongst several other factors have been implicated in the aetio-pathophysiology with their levels correlating to severity of pre-eclampsia. This study was undertaken to describe the pattern of expression of IL-6 and TNF-α, and their significance in determining severity of pre-eclampsia in Nigerian pregnant women. **Method:** This was a prospective case-control study among 96 pregnant women; 48 with pre-eclampsia and matched 48 without pre-eclampsia at the University of Benin Teaching Hospital, Benin City, Nigeria. Venous blood serum levels of IL-6 and TNF-α were determined using enzyme-linked immunosorbent assay procedures. Relevant socio-demographic, clinical variables, and serum levels of IL-6 and TNF-α levels were obtained and analysed using SPSS 17. The P value was set at <0.05. **Results:** Proteinuria was statistically different (p = < 0.001) between cases and controls. The mean serum concentrations of IL-6 and TNF-α for cases were 36.21 ± 60.18 pg/ml and 3.26 ± 2.14 pg/ml as against 8.24 ± 15.26 pg/ml and 3.45 ± 3.14 pg/ml for controls respectively. The P values were <0.001 and 0.167 respectively. The mean serum concentrations of IL-6 and TNF-α for mild pre-eclampsia were 13.89 ± 23.27 pg/ml and 3.29 ± 2.82 pg/ml as against 56.74 ± 75.36 pg/ml and 3.23 ± 1.31 pg/ml for severe pre-eclampsia respectively. The P-values were <0.001 and 0.160 respectively. The serum concentrations of IL-6 positively correlated with the severity of pre-eclampsia. **Conclusion:** Serum concentration of IL-6 depicts and positively correlates with severity of pre-eclampsia in our environment. TNF-α, though also associated with pre-eclampsia, its serum concentration is not positively correlated with severity of the disease from this study

Corresponaence:

Dr Osegi Nkencho
Phone:+2348033896601,
E-mail: oseginkencho@yahoo.com

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INTRODUCTION

Hypertensive disorders of pregnancy comprising pre-eclampsia, eclampsia, gestational hypertension, and chronic hypertension affect about 10% of all pregnant women around the world[1, 2]. They are important causes of severe acute morbidity, long-term disability and death among mothers and babies[1-3]. Nearly one tenth of all maternal deaths in Africa and Asia are associated with hypertensive disorders of pregnancy[3].

Pre-eclampsia, a pregnancy-specific disorder present in 2 - 8% of all pregnancies[2] stands out among the hypertensive disorders considering its impact on maternal and neonatal health. It is clinically characterized by hypertension with significant proteinuria (>300mg per-24-hour period) and exaggerated maternal systemic inflammatory response after 20 weeks of gestation. It contributes about 19% of the over 40,000 maternal deaths reported annually in Nigeria[4] and is documented to account for 12.4% maternal deaths in Benin city[5]. It is also a leading cause of preterm birth and intrauterine growth restriction, leading to increased perinatal morbidity and death[6].

Although the cause is uncertain, and it remains a disease of theories, it is clear that pre-eclampsia is triggered by factors released from the placenta, since it only occurs in pregnant women and placental tumours. Dysregulation of the maternal immune response against the foetus has been postulated as a possible causal factor[7] and an inflammatory response has been shown to occur in pre-eclampsia[8, 9]. A comprehensive model of pre-eclampsia[10] suggests that immune maladaptation at the implantation site contributes to impaired trophoblast invasion and subsequent incomplete transformation of the decidual spiral arteries and arterioles[11, 12]. The resultant reduced utero-placental blood flow produces placental ischaemia and hypoxia[13, 14]. The fore-mentioned is associated with maternal circulation containing reduced levels of angiogenic factors[15 - 17], elevated levels of anti-angiogenic factor[17 - 19], placental debris[20, 21], reactive oxygen species[22, 23] and pro-inflammatory cytokines[24-28]. Altered levels of these circulating factors promote endothelial cell activation and vascular damage leading to proteinuria and hypertension; the leading symptoms of the maternal syndrome of pre-elcampsia[10, 29].

Increased serum levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor - alpha (TNF-α), transforming growth factor beta 1 (TGF β1), and growth factors in pre-eclampsia have been postulated to be involved in the pathogenesis[30,31] and play an important role in the maternal vascular dysfunction observed in pre-eclampsia[32]. The increased levels of pro-inflammatory cytokines is thought to trigger the maternal endothelial cell dysfunction seen in pre-eclampsia[33]. It is postulated that women with pre-eclampsia show an exaggerated inflammatory response, characterized by aberrant cytokine production towards harmful Th1 type immunity[34 - 36]. The sources of the increased levels of pro-inflammatory cytokines in the circulation of pre-eclamptic women have not been fully determined.

Cytokines are proteins and peptides secreted by cells, which act as humoral regulators to modulate the intercellular actions of the cell[37]. Cytokines can be pro-inflammatory or anti-inflammatory. The pro-inflammatory cytokines identified include Interleukin-6 (IL-6) and Tumour necrosis factor-alpha (TNF-α), amongst several others.

IL-6, a glycosylated protein molecule produced mainly by the Th 2 lymphocytes but also by fibroblasts, endothelial cells, monocytes and macrophages is the major physiological mediator of the acute phase reaction[38]. Endothelial dysfunction and increased endothelial permeability, characteristic findings as pathophysiology of pre-eclampsia are thought to be due to IL-6 activity of increasing the permeability of endothelial cells[39] by changing the cell shape and rearrangement of intracellular action fibres[40]. IL-6 inhibits the cyclooxygenase enzyme[41] thus increase the thromboxane A_2 to prostacyclin ratio; an abnormality observed in pre-eclampsia. Oxygen free radicals are implicated in the pathogenesis of pre-eclampsia through endothelial damage, with reduction in nitric oxide synthesis and distortion of the balance of prostaglandin production induce synthesis of IL-6 by the endothelium[42]. IL-6 may be the most useful circulating marker of endothelial dysfunction and is observed in the arteries of women suffering from preeclampsia[2, 43].

TNF-α, a non-glycosylated protein is produced mainly by T-helper cells, monocytes, macrophages and neutrophils[44]. In healthy pregnant women, TNF-α is thought to modulate the

growth and invasion of trophoblasts in maternal spiral arteries[45]. It is thought to contribute to abnormal placental invasion[46] and endothelial cell damage[47]. TNF-α can stimulate IL-6 production[48] since IL-6 inhibits TNF-α release[49]. TNF-α secreted by placental residing macrophages has been suggested to collaborate with other molecules to limit extravillous trophoblast invasion of spiral arterial segments through apoptosis[50].

Studies on IL-6 levels in maternal serum generally show a significantly higher level in pre-eclamptic women[25, 26, 31, and 49]. Some studies have shown significant elevation of TNF-α in pre-eclamptic women [24, 31, 51], while others suggest non-significant difference with normotensive pregnant women[25, 26, and 52]. Elevated serum levels of IL-6 and TNF-α in patients with preeclampsia have been found to correlate with severity of the disesae[24, 54]. There may be ethnic/racial or regional differences in the levels of IL-6 and TNF-α during normal pregnancy and pregnancy complicated by pre-eclampsia[55].

Most studies implicating pro-inflammatory cytokines in the aetio-pathophysiology of pre-eclampsia were carried out among non-Africans and outside Africa. We decided to undertake this study considering the possibility of ethnic/racial or regional differences in levels of pro-inflammatory cytokines during normal and pre-eclamptic pregnancy[55]. Our study describes the pattern of expression of pro-inflammatory cytokines; IL-6 and TNF-α, and their significance in determining severity of disease in Nigerian pregnant women with pre-eclampsia in our environment. It is expected to establish a baseline for further research on this subject in our environment.

MATERIALS AND METHODS

This was a prospective case control study at the Department of Obstetrics and Gynaecology of the University of Benin Teaching Hospital, Benin City, Nigeria from October 2013 to March 2014. Approval for this study was obtained from the institution's Ethics and Research Committee.

The cases were consenting pregnant women in the third trimester (from 28 weeks' gestation) admitted for pre-eclampsia except those in the exclusion criteria. The controls were consenting normotensive and non-proteinuric women with uncomplicated pregnancy matched for gestational age, age, weight, and parity. The study population (cases and controls) were recruited consecutively until the desired sample size was obtained. The sample size of 48 subjects per group (96 total) was calculated based on case control study (for two sample t-test i.e., comparing 2 means) [56]. All patients were interviewed and relevant information and variables such as the biodata, social class, investigations and interventions prior to presentation was obtained. Social class was determined using the scoring system of Olusanya and colleagues[57].

The diagnostic criteria for pre-eclampsia was systolic blood pressure (SBP) of 140mmHg or higher or a diastolic blood pressure (DBP) of 90mmHg or higher on two occasions at least 6 hours apart occurring after 20 weeks of gestation in a pregnant woman with previously normal blood pressure and detectable urinary protein (≥1+ by dipstick). Severe preeclampsia was defined as SBP ≥ 160mmHg; DBP ≥ 110mmHg with a urine dipstick protein at least 3+. Other evidence of severe disease considered were elevated serum creatinine, eclampsia, pulmonary oedema, oliguria (urine less than 500ml/24hr), foetal growth restriction, oligohydramnios and symptoms suggesting significant end-organ involvement (headache, visual disturbance, or epigastric or right upper quadrant pain). Women who met the criteria of pre-eclampsia, but not severe pre-eclampsia were classified as mild.

Pregnant women with the following conditions were excluded from the study; multiple gestation, diabetes mellitus, renal disease, hypertension, infectious disease recognized in pregnancy, premature rupture of membrane, active labour, polyhydramnios, acute inflammatory diseases (tonsillitis, urinary tract infections chorioamnionitis), chronic inflammatory diseases (systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease), and any with fever defined as temperature > 37.2^0C.

Patients with preeclampsia and matched controls had 5ml of venous blood

collected into a sterile container. Blood samples from pre-eclamptic women were taken after diagnosis and admission to hospital, while that from matched controls were taken at the Antenatal Care Clinic. The blood sample was allowed to stand for 30 min to clot and then centrifuged at 2000xg for 10 min to clarify serum. Serum IL-6 and TNF-α concentrations were determined by enzyme linked immunosorbent assay (ELISA) procedures using kits by ABCAM, UK (manufacture date 23.01.2014, Lot no. GR154988-1, for IL-6; GR157122-1, for TNF- α).[58] The standard curves were drawn and used to find the appropriate concentrations of IL-6 and TNF-α from optical density. Quality Control (QC) was ensured by carrying precision studies to determine the co-efficient of variation (CV) which was 4 - 8% for IL-6 and 2-5% for TNF-α. QC materials provided in the kit as well as those available routinely in the laboratory were used and assayed along with the specimens. QC results were all within the acceptable range of ± 2SD.
Data was analysed using Statistical Package for the Social Sciences (SPSS) version 17. Continuous variables were presented as means with standard deviations and the differences were determined with the student's *t* test. P-value less than 0.05 was taken as significant.

RESULTS

Table 1 shows the socio-demographic and clinical characteristics of the study. A higher proportion of the study; cases (17, 35.4%) and controls (14, 29.2%) were in social class V. All the cases were proteinuric while none of the controls were. Twenty-one (43.8%) of the cases had ++, 13 (27.1%) had +, 12 (25.0%) had +++ while 2 (4.2%) had ++++ proteinuria.

Table 2: Means of socio-demographic and clinical variables

Variable	CASES (Mean ± SD)	CONTROLS (Mean ± SD)	t-test	P-value
Age (years)	30.67± 5.17	30.67±4.94	< 0.001	0.683
GA (weeks)	35.35± 3.40	35.35±3.37	< 0.001	0.799
Parity	1.04± 1.473	1.04±1.473	< 0.001	0.999
Weight (kg)	85.85±11.5	85.29±10.82	0.247	0.615
Social class	3.40 ± 1.46	3.21 ± 1.46	0.631	0.934
SBP (mmHg)	167.08± 24.66	105.62±10.09	15.979	< 0.001*
DBP (mmHg)	104.58± 14.87	65.21±6.84	16.668	< 0.001*
Proteinuria (+ to ++++)	2.06± .84	0.00± 0.00	17.102	< 0.001*

*Significant

Table 1: Socio-demographic and clinical variables

Variable	CLASSES	CASES [N (%)]	CONTROLS [N (%)]	t – test	P-value
Age (years)	21 – 25	8 (16.7)	7 (14.6)	<0.001	0.683
	26 – 30	15 (31.2)	18 (37.5)		
	31 – 35	14 (29.2)	14 (29.2)		
	36 – 42	11 (22.9)	9 (18.8)		
Parity	Nullipara	27 (56.2)	27 (56.2)	<0.001	0.999
	Primipara	7 (14.6)	7 (14.6)		
	Multipara	12 (25.0)	12 (25.0)		
	Grand multipara	2 (4.2)	2 (4.2)		
GA (weeks)	28 – 30	4 (8.3)	3 (6.2)	<0.001	0.799
	31 – 33	15 (31.2)	16 (33.3)		
	34 – 36	9 (18.8)	11 (22.9)		
	37 – 40	20 (41.7)	18 (37.5)		
Weight (kg)	69.0 – 78.9	13 (27.1)	14 (29.2)	0.247	0.615
	79.0 – 88.9	17 (35.4)	17 (35.4)		
	89.0 – 98.9	12 (25.0)	11 (22.9)		
	99.0 – 108.9	4 (8.3)	4 (8.3)		
	109.0 – 119	2 (4.2)	2 (4.2)		
Social class	Class I	6 (12.5)	7 (14.6)	0.631	0.934
	Class II	9 (18.8)	11 (22.9)		
	Class III	10 (20.8)	9 (18.8)		
	Class IV	6 (12.5)	7 (14.6)		
	Class V	17 (35.4)	14 (29.2)		
Proteinuria (Urinalysis)	NAD*	0 (0.0)	48 (100.0)	17.102	<0.001*
	+	13 (27.1)	0 (0.0)		
	++	21 (43.8)	0 (0.0)		
	+++	12 (25.0)	0 (0.0)		
	++++	2 (4.2)	0 (0.0)		
Severity of pre-eclampsia	Mild pre-eclampsia	23 (47.9)	0 (0.0)		
	Severe pre-eclampsia	25 (52.1)	0 (0.0)		
Total		48 (100.0)	48 (100.0)		

*NAD = No abnormality detected

The cases (n=48) were 23 with mild preeclampsia and 25 with severe preeclampsia. While age, gestational age, parity, weight and social class showed no statistically significant relationship between the cases and controls, proteinuria was statistically different (p = < 0.001).

The mean socio-demographic and clinical characteristics are shown in Table 2. The mean blood pressure (SBP; DBP) and proteinuria were different, with statistically significant relationship between cases and controls (p = < 0.001).

Table 3: Comparison of mean IL-6 and TNF-α serum concentrations in cases and controls

Variable	CASES (n = 48) (Mean ± SD) (Pg/ml)	CONTROLS (n = 48) (Mean ± SD) (Pg/ml)	t-test	P-value
IL-6	36.21 ± 60.18	8.24 ± 15.26	3.121	< 0.001*
TNF-α	3.26 ± 2.14	3.45 ± 3.14	0.333	0.167

*Significant

There was a statistically significant relationship between cases and controls in mean IL-6 serum concentration (p = < 0.001) which was not observed for TNF-α. (Table 3)

Table 4: Comparison of mean IL-6 and TNF-α serum concentrations with severity of pre-eclampsia

Variable	MILD PRE-ECLAMPSIA (n = 23) (Mean ± SD) (Pg/ml)	SEVERE PRE-ECLAMPSIA (n = 25) (Mean ± SD) (Pg/ml)	t-test	P-value
IL-6	13.89 ± 23.27	56.74 ± 75.36	2.613	< 0.001*
TNF-α	3.29 ± 2.82	3.23 ± 1.31	0.083	0.160

*Significant

There was a statistically significant relationship in the mean IL-6 serum concentrations with severe pre-eclampsia (p = <0.001) while there was none observed with TNF-α levels. From Tables 3 and 4, the mean serum concentrations (Pg/ml) of TNF-α for controls (3.45 ± 3.14), mild pre-eclampsia (3.29 ± 2.82) and severe pre-eclapmsia (3.23 ± 1.31) showed

a downward trend. The reverse was the case for IL-6; as the mean serum concentrations (Pg/ml) increased with increasing severity: 8.24 ± 15.26, 13.89 ± 23.27, and 56.74 ± 75.36 for controls, mild pre-eclampsia, and severe pre-eclampsia respectively with P-value <0.001.

DISCUSSION

The exact pathogenesis of pre-eclampsia, a disorder unique to pregnancy remains unknown. It has been suggested that pro-inflammatory cytokines such as interleukin – 6 (IL-6) and Tumour Necrosis Factor–alpha (TNF-α) have important roles in the pathogenesis of pre-eclampsia and may cause generalized endothelial dysfunction, a disorder widely seen in pre-eclampsia. There is paucity of publications, if any on pro-inflammatory cytokines profiles among pregnant women with pre-eclampsia in our environment.

Our study carried out at UBTH, Benin City, Nigeria showed a mean age for preeclampsia of 30.67 ± 5.17 years with most within 26 – 35 years. This reflects the peak reproductive age range and correlates with that found at cytokine mapping in sera of pre-eclamptic and normal pregnant women by Jonsson and colleagues[59]. The mean gestational age was 35.35 ± 3.40 weeks correlating the study by Xiao and colleagues[54] that had more patients with late onset pre-eclampsia. Majority of the cases in our study (56.2%) were nulliparous women, which was not surprising as most cases of pre-eclampsia occur in nulliparous women[5.] The social class for the cases and controls as determined by Olusanya and colleagues[57] scoring system were similar.

The mean level of IL-6 in the cases was elevated compared to the matched controls in our study. This was in consonance with studies done in Europe, some Asian countries and local studies[25, 26,51,54,60].

Our study showed that serum levels of IL-6 positively and consistently correlated with disease severity at the point of diagnosis as shown by other studies[28]. It however did not correlate with disease severity progression since a one-point blood sample was taken, which would have been taken care of, if a longitudinal study was done involving baseline and subsequent serial measurements of the serum cytokines (IL-6, TNF-ALPHA) levels at a later gestational age.

In effect, assays of IL-6 can be used both as a diagnostic indicator of pre-eclampsia and degree of severity at the point of diagnosis.

The high concentration of serum IL-6 seen in pre-eclamptic patients in our study population may be partly due to the significant role of inflammation and infection in the pathogenesis of pre-eclampsia in developing countries. The high incidence of chronic subclinical infections may contribute to the high incidence of pre-eclampsia. Since the study was carried out in women with established pre-eclampsia, it is not possible to determine whether the observed increased serum concentration of IL-6 is a cause or consequence of the disease.

The mean TNF-α serum concentration in the study population did not show statistically significant difference in the cases and controls. A similar finding has been documented in studies done in Iran and Turkey[52, 53]. The mean TNF-α serum concentrations however showed decreasing trend in controls, mild pre-eclampsia, and severe pre-eclampsia respectively though not statistically significant. This observation is not surprising since IL-6 inhibits TNF-α release[49]. The mean levels of TNF-α may be reducing with increasing severity of preeclampsia due to progressively elevated IL-6 levels.

The findings from this study suggest a significant role for IL-6, and the need for more studies to further elucidate the role of pro-inflammatory cytokines in the pathogenesis of pre-eclampsia in our environment. Studies on larger numbers of pregnant women from different populations of Nigeria and a longitudinal one would be necessary, more so to determine mean values for pro-inflammatory cytokines across the trimesters in normal pregnancy and pre-eclampsia.

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