

Pregnancy Specific Beta-1 Glycoprotein, Pro- and Anti-inflammatory Cytokines in Eclampsia in Kaduna State, Nigeria

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Abstract: Eclampsia (EC), a human pregnancy-specific-syndrome is the life-threatening occurrence of convulsion (s) in association with signs of preeclampsia (hypertension and proteinuria). Eclampsia has remained a significant public health threat, contributing to maternal and perinatal morbidity and mortality in Nigeria. However, the pathogenic mechanism of the disease is not fully understood. Disturbance of the cytokine equilibrium has been accused for many pathological disorders including EC. Thus the aim of this study was to analyze the maternal cytokines and pregnancy specific beta-1 glycoprotein in EC and compared results with those of normal healthy pregnant controls. Enzyme linked immunosorbent assay (ELISA) was used to measure levels of pro-inflammatory cytokines (Tumor necrosis [TNF]- α , and interleukin [IL] -2), anti-inflammatory cytokines [IL-4 and IL-10] and pregnancy specific beta-1 glycoprotein (PSG-1) in the peripheral blood of patients with EC (n=38), normal healthy pregnant women [PC] (n=25) and compared with healthy non pregnant women controls [NPC] (n=25). Women with malaria and human immunodeficiency virus infections were excluded from the study. The overall results (Mean \pm SD) were: TNF- α (2.34 \pm 0.13 pg/ml) in EC was significantly higher than the mean values (2.25 \pm 0.07pg/ml and 2.24 \pm 0.10 pg/ml) in PC and NPC respectively. Furthermore, EC had higher TNF- α mean value compared with NPC (P<0.05). There was no statistical difference in mean IL-2 value between EC (1.69 \pm 0.17 Pg/ml), PC (1.71 \pm 0.09Pg/ml) and NPC (1.72 \pm 0.13Pg/ml) (P>0.05). The mean value of IL-10 was noted to be lower in EC (1.28 \pm 0.54Pg/ml) compared with: PC (1.58 \pm 0.61 Pg/ml) and NPC (2.06 \pm 0.08Pg/ml). No significant difference in IL-4 mean value exist between EC (2.45 \pm 0.10 Pg/ml) and NPC (2.45 Pg/ml) (P>0.05) but significant difference exist between EC and NPC (2.40 \pm 0.0 6Pg/ml) (P<0.05). The serum PSG-1 levels in EC (2.5 \pm 0.11 Pg/ml) and PC (2.5 \pm 0.03 Pg/ml) were similar and significantly higher than in NPC (0.06 \pm 0.020 Pg/ml) P<0.05. While a pro-inflammatory cytokine environment was demonstrated in EC, and decreased anti-inflammatory reactivity, EC was not associated with low levels of PSG-1. Further research is advocated to discover how anti-inflammatory cytokines could be exploited as a therapeutic agent for women at high risk of EC.

Keywords: Eclampsia, Interleukin, Pregnancy Specific Beta-1 Glycoprotein, Tumor Necrosis Factor- α , Pro-inflammatory Cytokines, Anti-inflammatory Cytokines

1. Introduction

In the recent decades, reports show that Eclampsia (EC) has assumed a public-health-threat dimension, both in the developing and developed countries [1]. It is the most common medical complication of pregnancy accounting to about 2-8% of all pregnancies [2]. Unfortunately, there has been little progress in preventing EC compared to progresses made in eliminating other major obstetric complications [3]. The incidence of EC continues to increase worldwide and especially, in Nigeria due to inadequate and poor utilization of maternal health care facilities. Globally, EC accounts for 50,000 maternal deaths in the world annually [4]. In Nigeria the impact of the disease is alarming. Thirty seven thousand (37,000) women in Nigeria die annually due to EC and eclampsia-related complications. In northern Nigeria, up to 40% of maternal death is due to EC [5].

Eclampsia, a human pregnancy-specific-syndrome is the life-threatening occurrence of convulsion (s) in association with signs of preeclampsia (hypertension and proteinuria) after 20 weeks gestation, during labor or 7days after delivery [4].

Although intensive research efforts had been made in the past, the etiology of EC is still not fully understood. The only definitive cure for EC is the delivery of the placenta which has been identified as the major culprit. Many hypotheses have been proposed to explain the mechanism of the disease and disturbance of the cytokine equilibrium has been accused for many pathological disorders including EC. The current theory is that women who developed EC or its precursor preeclampsia (PE), have abnormal immunological response to the fetoplacental unit, and that hypertension and proteinuria represent clinical signs of a mild form of fetal rejection, while severe forms of PE/EC represent spontaneous abortion and fetal demise [6].

The maternal immune system plays a critical role in the establishment of a healthy pregnancy. Normal successful pregnancy is associated with T-helper (T_h) 2 phenomenon due to shift in cytokine pattern from T_h 1 (Interferon- γ , TNF- α , TNF- β , interleukin (IL)-2) to Th2 (IL-4, IL-5, IL-10, and IL-13) [7]. This shift is thought to contribute to maternal tolerance to the fetus by suppressing the anti-fetal cell-mediated immune response. Various cells and molecules of the immune system are key players in the development and function of the fetoplacental unit. Although the specific mechanisms to utilize to achieve successful pregnancy are not well understood, pregnancy specific beta-1 glycoprotein contributes immensely towards Th2 cytokine production. A fine balance between pro-inflammatory and anti-inflammatory influence is required for good pregnancy outcome. It is believed therefore that Th 1/T_h 2 balance defines the welfare of the organism [8]. The T_h 1 and T_h 2 are the major subsets of CD4 T⁺ helper cells with different cytokine production profile and therefore very critical in maternal immune response to foreign antigen. T helper-1 cells secrete pro-inflammatory cytokines which activate macrophages and cell-mediated reactions relevant to

cytotoxic reactions and delayed-type hypersensitivity [6]. On the other hand T_h 2 secretes anti-inflammatory cytokines that strongly induce humoral immunity. Anti-inflammatory cytokines block or suppress the intensity of the pro-inflammatory cascade. For example IL-4, IL-10, IL-13 and TGF- β suppress the production of IL-1, TNF- α , IL-8 and vascular adhesion molecules. It is therefore thought that the balance between the pro-inflammatory and inflammatory cytokines determines the outcome of a disease. Furthermore, pregnancy specific beta-1glycoprotein (PSG-1), a major secretion of the placenta detectable in the maternal serum as early as three days post fertilization, coinciding with the attachment of the blastocyst to the uterine wall, is thought to play a crucial role in supporting gestation and fetus protection against maternal immune system [9]. Sack *et al.* [10] proposed that pregnancy-specific factors induce the suppression of a specific arm of the maternal response and assumes the role in maternal immunological adaptation that spares the fetus from being damage by the cytotoxic effects of the immune system. Report shows that PSG-1 could modulate the immune response by inducing the secretion of anti-inflammatory cytokines such as IL-10, IL-6 and TGF- β by human and murine cells [11]. Experimental studies reported abnormal levels of PSG-1 in pregnancy and their importance in the maintenance of successful pregnancies [12].

What are the alterations or changes that may occur in pro-inflammatory cytokines, anti-inflammatory cytokines and PSG-1 levels in human pregnancies complicated by EC? This question therefore, forms the basis of this study. The specific objective of this study was to measure and analyze cytokines (TNF- α , IL -2, IL-10 and IL-4) and PSG-1 in the peripheral blood of eclamptic women and to compare the data obtained with values in normal pregnant women and normal healthy non-pregnant controls with the hope of understanding the importance of these immune components in the pathogenesis of EC for possible therapeutic interventions.

2. Materials and Methods

This was a comparative cross sectional study, conducted in Gynecology and Obstetrics Departments of Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria, Hajiya Gambo Sawaba General Hospital (HGSGH), Zaria, Barau Dikko Teaching Hospital (BDTH) Kaduna, Yusufu Dantsoho Memorial Hospital (YDMH) and General Hospital (GH) Kafanchan. Patients and controls were enrolled as they present.

2.1. Ethical Approval

Ethical clearances were obtained from the Scientific and Health Research Ethics Committee of the Ahmadu Bello University teaching hospital Shika– Zaria and the Kaduna State Ministry of Health and human services (KSMOH& HS) before commencing the study. Patients retained the right to deny consent for or opt out of the study at any stage. Patient confidentiality was maintained throughout the study.

2.2. Clinical Evaluation and Selection of Patients

All the participants were briefed about the nature of the study and an informed consent was taken from all the recruits and blood pressure measured using a simple mercury sphygmomanometer on right hand arm in a supine position after 10 min. rest by the collaborating clinician at the antenatal clinic (ANC)s of the Obstetrics and Gynaecology Department of the respective hospitals. To perform dipstick urine analysis, combi-2 Medi-test strips were used. Clients who fulfilled the entry criteria were enrolled for the study. Client's personal data such as age and parity, etc were sourced from each subject in addition to the data resulting from the clinical and laboratory examination and were entered into the study profoma.

2.2.1. Inclusion Criteria

For the women with EC : third trimester women with identifying features of high blood pressure ($\geq 140/90$), proteinuria (2+ dip stick testing of random urine) and tonic-clonic convulsion, who were previously normotensive and nonproteinuric after 20 weeks of gestation were included in the study [13].

For the controls: third trimester healthy pregnant women (normotensive and nonproteinuric) age and parity matched with EC above and non pregnant healthy (normotensive and nonproteinuric) age matched with EC and PC [14].

2.2.2. Exclusion Criteria

Participants that refused consent or opt out, tested seropositive for human immunodeficiency virus (HIV), blood smear positive for malaria test, or any known clinical disorder.

2.3. Specimen Collection

A total of 5mls of blood were drawn from each research participants into pre-belled plain tubes after confirmation of diagnosis and before the administration of any drugs. From the plain tubes serum samples were extracted and stored in pre-labeled serum vials containing drops of trasylol (aprotonin)-Sigma USA and stored at -20°C to inhibit degradation of the cytokine before immunoassay.

2.4. Thick Blood Film for Detection of Malaria Parasite

Malaria plasmodium parasites were detected by standard hematological procedure outlined by Dacie and Lewis [17] as obtained in the respective hospital Standard Operating Procedures (SOPs).

2.5. Laboratory-Based HIV Screening Test

Nigerian established National algorithm for rapid double HIV screening was used to rule out HIV infections in participants in this study. Commercially acquired test kits: DetermineTM HIV1/2 (Trinity Biotech, Japan) and Uni-Gold Recomigen HIV1/2 (Trinity Biotech, Ireland) parallel tests were used according to manufacturer's instructions as obtained in the respective hospital's Standard Operating

Procedures (SOPs).

2.6. Cytokine Assay

Cytokines (TNF- α , IL-2, IL-4, IL-10) and PSG-1 were assayed on batched serum samples stored at -20°C in trasylol (SIGMA, USA) by ELISA kits following the outlined protocol by Pathare *et al.* [15]. Frozen (-20°C) serum samples were thawed once and brought to room temperature at the time of assay. Serum samples were dispensed along side with dilutions of standards (recombinant TNF- α , IL-2, IL-4, IL-10 or PSG-1) into wells of the micro titre ELISA plates pre-coated with monoclonal antibodies against the human cytokines (IL-2, IL-4, IL-10, TNF- α or PSG-1) to be assayed and incubated at room temperature for 2 hours. The plates were washed four times with buffer (phosphate buffer saline-0.05% and tween 20). Conjugates (polyclonal antibody against IL-2, IL-4, IL-10, and TNF- α or PSG-1 to horseradish peroxidase-HRP) were added and incubated for 2 hours at room temperature. Unbound enzymes were washed out while the bound enzymes were then detected by incubation in the dark with substrate solutions (stabilized hydrogen peroxides and tetramethylbenzidine-TMB). The plates were scanned using a microplate reader (Bio-Rad, USA) set at 450 nm with wave length correction set at 570nm. A standard curve was then generated from the known standards. Concentrations of IL-2, IL-4, IL-10, and TNF- α or PSG-1 in the specimen was determined by comparing sample optical density with the values on the standard curve.

2.7. Statistical Analysis

The data obtained was entered into computer to generate a database for subsequent analysis. Computation was made using the SPSS version 20.0 (Chicago, USA) and Graph Pad Prism 6.0. Results were expressed as mean \pm standard deviation. Kruskal Wallis test was used to evaluate the difference between non parametric data (IL-2, IL-4, IL-10, TNF- α , and PSG-1). Comparisons were made between EC, PC and NPC. Test was carried out at 0.05 level of significant and $p < 0.05$ was considered significant.

3. Results

3.1. Demographic and Clinical Features of Eclamptic Women and Controls

The mean age and standard deviation of the groups were similar: EC (25.0 \pm 5.9 years), PC (24.9 \pm 5.7years) and NPC (25.1 \pm 5.9 years) Table 1.

The mean gestational age and parity were also similar: EC (37.2 \pm 2.2 weeks and 1.4 \pm 2.4) and PC; (37.1 \pm 2.0weeks and 1.5 \pm 2.5) respectively Table 1

The mean BMI and standard deviation recorded was: EC (26.4 \pm 4.3 Kg/m²), PC (25.8 \pm 3.7 Kg/m²) and NPC (25.2 \pm 4.3 Kg/m²). There was no statistical difference between EC, PC and NPC ($P > 0.5$) (Table 1).

In table 1, the mean values of blood pressures (systolic; diastolic) and standard deviation were noted to be higher in EC (171.6±25.2mmHg; 110.0±10.7mmHg) compared with PC, (111.6±7.1mmHg; 79.5±11.3 mmHg) and NPC, (109.6±6.6 mmHg; 84.2±6.4 mmHg). There was significant differences between EC, PC and NPC ($p < 0.05$).

Most of the eclamptic women (55.3%) were not booked in the facility at the time of study. While (100%) of the pregnant women control had been booked who served as controls. Table 1.

Majority of the unbooked patients were said to have registered at private Clinics, Nursing homes or Primary Health Care Centers (PHC), but there were no records to authenticate their claims. ABUTH Zaria had the highest number of unbooked cases being a major referral centre (tertiary institution) serving a larger community (Table 1).

In table 1, urinary proteins (albumin), $\geq 2+$ were recorded in all eclamptic women and non in PC and NPC and tonic-clonic convulsions that occurred antepartum and intrapartum; 16 (42.1%) and 22 (57.9%) in the eclamptic women, respectively.

Other clinical features of EC recorded were pitting edema, blurred vision, and severe frontal headache.

Table 1. The Demographic and Clinical Characteristics of Women included in the Eclampsia Study (Mean and \pm SD).

Characteristics	EC (n = 38)	PC (n = 38)	NPC (n =38)
Age (years)	25.0±5.9	24.9±5.7	25.1±5.9
Gestational age (wks)	37.2±2.2	37.1±2.0	-
Parity	1.4±2.4	1.5 ± 2.5	-
BMI (Kg/m ²)	26.4±4.3	25.8±3.7	25.2±4.3
Systolic BP (mmHg)	171.6±25.2*	111.6±7.1	109.6±6.6
Diastolic BP (mmHg)	110.0±10.7*	79.5±11.3	84.2±6.4
Proteinuria	$\geq 2+$	Not detected	Not detected
Antenatal booking	55.3%	100.0%	NA
Antepartum convulsion	16 (42.1%)	NA	NA
Intrapartum convulsion	22 (57.9%)	NA	NA

EC= Eclampsia, PC=Healthy Pregnant Control, NPC=Healthy Non Pregnant Control, BMI=Body Mass Index, BP= Blood pressure, NA= not applicable
*Blood pressure in eclampsia is significantly different from both controls at $p < 0.05$ using ANOVA.

3.2. Mode and Outcome of Delivery of the Eclamptic Women in the Study

Thirty three (33) 86.84% Pregnant women with EC delivered their babies by spontaneous vaginal deliveries (SVD), while 4 (10.5%) delivered by caesarean section (CS). One (1) 2.6% eclamptic patient was discharged against medical advice therefore the mode of delivery for the patient was not known (Table 2).

Twenty nine (76.3%) eclamptic women delivered live babies while 3 (7.9%) and 5 (13.2%) had fresh still births and macerated still births respectively. One (2.6%) eclamptic patient was discharge against medical advice so the outcome of delivery for the patient was not known. There was no maternal death recorded, Table 2.

Table 2. Mode and Outcome of Delivery of the Women with EC.

Mode of delivery	Number	Percentage (%)
SVD	33	86.9
CS	4	10.5
Not known	1	2.6
Total	38	100
Outcome of delivery	Number	Percentage (%)
Live birth	29	76.3
Fresh still birth	3	7.9
Macerated still birth	5	13.2
Not known	1	2.6
Total	38	100.00

SVD=Spontaneous Vaginal Delivery, CS=Caesarean Section.

3.3. Pregnancy Specific Beta-1 Glycoprotein Levels in Women Included in the Eclampsia Study

In table 3, the mean serum (log value) of PSG-1 EC was 2.53±0.11 pg/ml, while for the PC and NPC; it was 2.56±0.03 pg/ml and 10.62±0.20 pg/ml respectively.

While there was no significant difference between EC and PC ($P > 0.05$), women with EC had significantly higher mean serum values compared to NPC $p < 0.05$.

Table 3. Pregnancy Specific beta-1 glycoprotein levels in EC Study (Mean and SD).

Parameter	EC (n =38)	PC (n = 25)	NPC (n = 25)	P-value
Log PSG-1 (pg/mL)	2.53±0.11	2.56±0.03	0.62±0.20	
Kruskal-Wallis	56.46	57.82	13.00	$< 0.001^*$

PSG-I= Pregnancy Specific beta-1 glycoprotein, EC=Eclampsia, PC= Healthy Pregnant Control,
NPC= Healthy Non-Pregnant Control.

*PSG-1 levels in eclampsia and PC are significantly different from NPC at $p < 0.05$ using Kruskal-Wallis.

3.4. Pro-inflammatory and Anti-inflammatory Cytokines in the Study Participants (Kruskal-Wallis Test)

In the table 4, the mean serum levels of TNF- α (2.34 ±0.13 pg/ml) in EC was significantly higher than those in PC and NPC (2.25±0.07pg/ml and 2.24±0.10 pg/ml) respectively. Furthermore, EC had higher TNF- α mean value compared with NPC $P < 0.05$ (Kruskal-Wallis test).

The mean serum levels (log mean \pm SD) of IL-2 in women with EC and PC were 1.69±0.17 pg/ml and 1.71±0.09 pg/ml respectively, while that of NPC was 1.72±0.13 pg/ml. There was no significant difference between EC, PC and NPC at $p > 0.05$ (Kruskal-Wallis test).

The mean serum value of IL-4 and standard deviation in EC, PC and NPC were: 2.45±0.10 pg/ml, 2.40±0.06 pg/ml and 2.45±0.06pg/m respectively. There was statistical significant difference between EC and PC compared with NPC at $P < 0.05$ (Kruskal-Wallis test).

The mean serum (log value) levels of IL-10 in EC (1.28±0.54 pg/ml) was significantly lower between PC (1.58±0.61 pg/ml) and NPC (2.06±0.08 pg/ml) at $P < 0.05$ (Kruskal-Wallis test). There was further significant decrease in mean level of IL-10 value in EC compared with NPC ($P < 0.05$).

Table 4. Pro-inflammatory and Anti-inflammatory Cytokines levels in EC Study.

Women's Characteristics	EC (n = 38) mean ±SD	PC (n = 25) (mean ±SD)	NPC (n = 25) (mean ±SD)	P-Value
LogTNF-α (pg/mL)	2.34±0.13#	2.25±0.07	2.24±0.10	
Kruskal-Wallis	56.71	34.42	36.02	< 0.001**
Log IL-2 (pg/mL)	1.69±0.17	1.71±0.09	1.72±0.13	
Kruskal-Wallis	41.66	46.80	46.52	0.645
Log IL-4 (pg/mL)	2.45±0.10	2.40±0.06	2.45±0.06	
Kruskal-Wallis	47.49	33.56	50.90	0.035*
Log IL-10 (pg/mL)	1.28±0.54#	1.58±0.61	2.06±0.08	
Kruskal-Wallis	29.21	42.00	70.24	< 0.001**

EC=Eclampsia, PC= normal healthy pregnant control, NPC=Healthy non-pregnant control

*Significantly different in IL-4 exists between EC, PC and NPC at $p < 0.05$ using Kruskal-Wallis

**Significantly different in TNF-α exists between EC, PC and NPC at $p < 0.01$ using Kruskal-Wallis

4. Discussion

The levels of cytokine and PSG-1 in the blood of a pregnant woman may define the health or pathological state of the woman, hence may have a prognostic character for therapeutic intervention.

The study revealed significant increased in pro-inflammatory cytokine (TNF-α) level in EC, while the level of IL-2 component of the pro-inflammatory cytokine showed no significant difference in EC compare to the pregnant and non-pregnant controls. A significant decrease in anti-inflammatory cytokine (IL-10) level in EC was also documented compared to normal healthy pregnant and non-pregnant controls, while IL-4 component of the anti-inflammatory cytokine did not increase in EC compared to healthy pregnant control.

This result agrees with the findings of Musa *et al.* [16] and Anim-Nyame *et al* [17] who reported higher levels of TNF-α and decreased levels of IL-10 in the sera of women with EC compared with pregnant and non pregnant controls. These researchers, however, did not assay for IL-2 and IL-4 in their studies.

Redman *et al.* [18] observed that production of pro-inflammatory cytokines occurs in pregnancy but under strict regulatory control. Pro- and anti-inflammatory cytokines and counter regulate each other whereby pro-inflammatory cytokines immuno-suppresses anti-inflammatory cytokine and vice-versa to achieve a maternal physiological cytokine level compatible with the requirement of the fetus for a healthy growth

Normal healthy successful pregnancy is associated with highly controlled immune responses, the fetus being a semi-allograft. Implantation, placental and fetal development required some form of immune responses; inflammatory and/or anti-inflammatory response depending on the immunological phase of the pregnancy. For example the implantation of the blastocyst require a strongly inflammatory response to ensure the adequate remodeling of the uterine epithelium and removal of cellular debris

following the implantation of the blastocyst, while an anti inflammatory state is necessary for fetal growth and development.

In pregnancy, the primary source of cytokines is the activated leukocytes. Cytokines may be produced by many other cell types as well. The exact mechanisms controlling the activation of T cells and the release of cytokines in pregnancy are not known but the subsets of CD4+ T cell activated by a particular major histocompatibility complex (MHC) viral, bacterial, fungal or fetal-antigen fragment complex will determine the type and amount of cytokine produced. Malaria, HIV and other infections complicating pregnancies are prevalent in this environment and induce the production of excess TNF-α, the non inclusion women with EC in this study. The equilibrated balance of TNF-α versus and IL-10 determine the pregnancy outcome. A shift towards Th 2-type immune response away from Th 1-type (cytotoxic) response detrimental to the baby with its products like IL-2, IL-12, Interferon-γ and TNF-α occurs in normal healthy pregnancy. Redman *et al.* [18] proposed that PE/EC arises from an exaggeration of vascular inflammatory response due to maternal inflammatory responses in the course of pregnancy.

Tumor necrosis factor is a cytokine involve in system inflammation and is a member of cytokines that induces T cells apoptosis and stimulate acute phase reaction during immune response to foreign agents. Excessive production of TNF-α may serve as immunoreactive agent responsible for fetal death recorded in women with EC in this study. In normal healthy pregnancy, TNF-α is low in the first trimester and subsequently increases with advancing gestation in a finely control manner [19]. Dysregulation of TNF-α production may be responsible for EC in women in this study. Since this study is carried out among women with established EC at third trimester, it is difficult to determine whether excessive production of proinflammatory cytokines is a cause or the consequences of EC

The source of high concentration of circulating levels of inflammatory cytokines, including TNF-α, in women with EC has not yet been identified. It is likely however, that the placenta and invasive trophoblast are involved in its production. Another likely source of TNF-α are the activated monocytes/macrophages since they are the major producers of cytokines and can be good candidates for excessive TNF-α synthesis in EC. Activated neutrophils are another source of TNF-α in normal and pathological pregnancy. Also deciduas macrophages and neutrophils can be the source of TNF-α, these cells being activated and in higher number in PE [20]. Banda *et al.*, [21] in their study, recorded significant increases in neutrophils among the eclamptic women. Although not analyzed in this study, increase in neutrophils might have contributed to elevated levels of TNF-α among the eclamptic women in this study

In this study participants with HIV, malaria and any known clinical disorders were excluded, hence eclampsia may be solely responsible for the activation of immune cells leading to exaggerated production of TNF-α in pregnant woman with EC.

Tumor necrosis factor- α directly controls endothelial dysfunction, this cytokines are thought to be involved in the pathogenesis of PE/EC [22].

Again, the immunomodulator, PSG-1 levels in EC and NPC were similar and significantly lower than the mean serum values in non-pregnant controls.

This result also agrees with findings of Onyemelukwe *et al.*, [23] who studied 71 normal healthy pregnant women in northern Nigeria. They documented a low rise in PSG-1 up to 24 weeks, then a steep rise up to 36 weeks followed by a gradual fall near term. These researchers in Nigeria did not however have the privilege of measuring PSG-1 levels in pregnancy complicated by EC. This study however, did not support the findings of Silver *et al.* [24] who reported low PSG levels in PE.

In this study, the slight decreased mean PSG-1 value in the eclamptic women compared to normal healthy pregnant women group might in part be associated with the pathogenesis of EC. Lower levels of PSG-1 have been associated with certain human, pathological conditions such as autoimmune disease, spontaneous abortion and EC [25]. In contrast, Arnold *et al.* [26] found no effects of PSG-1 on T cell proliferation using purified recombinant PSG-1a or PSG II molecules.

Pregnancy Specific beta-1 glycoprotein is an excellent immunomodulator that provides the needed environment for a favorable pregnancy outcome by redirecting immune cells to secrete anti-inflammatory cytokines that favors fetal healthy growth and development.

5. Conclusion

While a pro-inflammatory cytokine environment was demonstrated in EC in this study, as illustrated by increased (TNF- α /Th1) and decreased anti-inflammatory (IL-10/Th2) reactivity and may explain the fresh and macerated still births recorded in this study, EC was not as associated with low levels of PSG-1. There is need for further longitudinal studies on transforming growth factor (TGF)- β , Interferon (IFN)- γ and other cytokines involved in the Th1/Th2 model for better understanding of the pathophysiology of EC for possible therapeutic interventions.

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