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Full Length Research Article

SERUM LEVELS OF TUMOR NECROSIS FACTOR ALPHA IN THIRD TRIMESTER ECLAMPTIC WOMEN ATTENDEE OF SELECTED HOSPITALS IN KADUNA STATE, NIGERIA

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ABSTRACT
Eclampsia, a life-threatening occurrence of convulsion(s) in association with hypertension and significant proteinuria in pregnancy has remained an important public health problem, contributing to significant maternal and perinatal morbidity and mortality in Nigeria. The aim of this was to determine serum levels of tumor necrosis factor alpha in eclampsia, and to compare with those of healthy pregnant and non-pregnant controls. Enzyme-linked immunoassay was used to measure the levels of tumor necrosis factor alpha in the sera of eclamptic women (n=38), normal healthy pregnant women (n=25) and healthy non pregnant controls (n=25). Data was analyzed using SPSS ver. 21.0 (Chiga, USA) and p< 0.05 is considered to be significant. The overall result of the level of tumor necrosis factor alpha (232.10±135.87 pg/ml) in eclamptic women was significantly higher than the mean values (180.58±29.18 pg/ ml and 178.38±36.12 pg/ml) in pregnant and non-pregnant controls respectively. Furthermore, eclampsia had higher level of tumor necrosis factor alpha mean value compared with non-pregnant controls (P<0.05). Elevated tumor necrosis factor alpha in the maternal circulation might play a central role in the excessive systemic inflammatory response, as well as the generalized endothelial dysfunction characteristics of the maternal syndrome of eclampsia. Excessive production of tumor necrosis factor alpha may serve as immunoreactive agent responsible for clinical symptoms and fetal death recorded in women with eclampsia in this study. A further longitudinal study involving a larger population is strongly advocated to captures dangerous alterations in the levels of tumor necrosis factor alpha as they manifest in the course of pregnancy.

Keywords: Eclampsia, Enzymes-linked immunosorbent assay, nonpregnant control, pregnant controls, Tumor necrosis factor alpha.

INTRODUCTION

Eclampsia (EC), a fatal human pregnancy-specific disease that affects the mother and the fetus, has remains a major cause of perinatal morbidity and mortality (Ghulmiyyah and Sibai, 2012; Dimitriadis *et al.,* 2023). Globally, EC is the most common medical complication of pregnancy accounting to about 2% -8% of pregnancies (Ghulmiyya and Sibai 2012; Dimitriadis *et al.,* 2023). The impact of EC is felt more in the developing countries including Nigeria) than the developed countries of the world. Studies revealed that approximately 600, 000 women died annually of pregnancy disorder, of this, 50,000 is due to EC related diseases globally (WHO, 2013) while in Nigeria, 37,000 die yearly (Olaoye *et al.,*2019).

Eclampsia is the occurrence of convulsion(s) and sign of pre-eclampsia (hypertension and significant proteinuria) in pregnancy in the absence of epilepsy or other convulsive disorders in pregnancy (Poom *et al.,* 2019). Eclampsia and pre-eclampsia (PE) are not different pregnancy disorders but the manifestation of the clinical features of the same syndrome (Fondjo *et al.,* 2019). Report show that EC is associated with immunopathology and also that susceptibility varies from one woman to another, indicating genetic and immune factor (Lokki *et al*., 2018; Steinthorsdottri *et al.,* 2020; Ismail *et al.,* 2023).

Despite tremendous research progress made in the field of obstetrics and gynecology, the exact mechanism of EC is still not fully elucidated. Several theories have been advanced to explain the mechanism of the diseases (Githiram and Moodley, 2016; Chang *et al*., 2023). These include generalized endothelial dysfunction, inadequate trophoblast invasion at the feto-placental junction and inappropriate maternal inflammatory responses among others (Boeldt and Bird, 2018; Echeverria *et al*., 2020; Opichka *et al.,* 2021). Disturbance of the cytokine equilibrium with increased serum level of tumor necrosis factor alpha, has been accused for many pathological disorders including eclampsia. The current theory is that women who developed EC or its precursor (PE) have abnormal immunological response to the feto-placental 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 (Spradley *et al*., 2015; Andronikidi *et al.,* 2024; Kornacki *et al.,* 2024).

The maternal immune system plays a vital role in the establishment of a healthy pregnancy. Normal successful gestation is associated with T-helper (Th) 2 phenomenon due to shift in cytokines pattern from T h1. This shift is thought to contribute to maternal tolerance to the fetus by suppressing anti-fetal cell mediated immune response. A fine balance between Th1(IL-2, TNF-α etc.) and Th2(IL-4, IL-5, IL-10 etc.) cytokines is required for good pregnancy outcome. It is believed therefore that Th1/Th2 balance defines the welfare of an organism (Abu-Raya *et al.,* 2020; Wang *et al.,* 2020; Graham *et al.,* 2021).

Determination of the maternal serum level TNF-α will be useful in the prediction of EC and the understanding of the pathogenesis of EC. Some anti-TNF-α drugs are already in use and have potential benefits in the prevention of eclampsia and eclampsia-related diseases.

What are the changes that may occur in the serum levels of TNF-α in eclamptic pregnancy? This question therefore, form the basis of this study. The specific objective of this study was to measure and analyze TNF-α 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 this immune factor in the pathogenesis of EC for possible therapeutic interventions.

MATERIALS AND METHODS

Study Area and study population

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

For the women with EC: third trimester women with identifying features of high blood pressure (≥140/90), proteinuria (2+ dip stick testing of random urine) and tonic-clinic convulsion, who were previously normotensive and nonproteinuric after 20 weeks of gestation (Ramsey *et al.,* 2003). 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. Participants that refused consent or opt out, tested sero-positive for human immunodeficiency virus (HIV), blood smear positive for malaria test, or any known clinical disorder were excluded from the study.

Clinical Evaluation and selection participants

All the participants were briefed about the nature of the study and written informed consent was taken from all the recruits. Blood pressure was 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. Participant's personal data such as age and parity, etc. were sourced from each participant in addition to the data resulting from the clinical and laboratory examination and entered into the study.

Blood Sample Collection

A total of 5mls of blood were drawn from each research participants, after confirmation of diagnosis and before the administration of any drugs into plain tubes. Sera were extracted and stored in pre-labelled serum vials containing drops of trasylol (aprotonin)-Sigma USA and stored at −20˚C to inhibit degradation of cytokines (TNF-α)

Laboratory-Based HIV Screening Test

Nigerian established National algorithm for rapid serial HIV screening was used to rule out HIV infections in participants in this study. Commercially acquired immunochromatic test kits: Determine™ HIV1/2 Trinity Biotect, Japan Stat-Pak and Uni-Gold Recomigen HIV1/2 Trinity Biotech, Ireland serial tests were used according to manufacturer's instructions as obtained in the hospital's Standard Operating Procedure (SOP). Serum samples reactive for HIV by these tests were excluded from this study (FMOH 2008).

Malaria Screening

Malaria plasmodium parasites were detected by standard haematological procedure outlined in the standard text book of Dacie and Lewi (1999) as obtained in the respective Hospitals Standard Operating Procedure (SOP). Blood samples of participants that were blood smear positive for malaria plasmodium organisms were excluded from the study.

Tumor necrosis alpha Assay

Serum TNF-α was assayed on batched serum samples by quantikine ELISA kits following the outlined protocol by Pathare *et al*. (2004). Frozen (−20˚C) serum samples were thawed once and brought to room temperature at the time of assay. Serum samples (EC; n = 38, PC; n = 25, NPC; n = 25) were dispensed alongside with dilutions of standards (recombinant TNF-α) into wells of the micro titre

ELISA plates pre-coated with monoclonal antibodies against the human TNF-α 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 TNF-α 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 TNF-α in the specimen were determined by comparing sample optical density with the values on the standard curve.

Statistical Analysis

Data obtained was entered into computer to generate a data base for subsequent analysis. Computation was made using SPSS ver. 21.0 (Chigo USA). Results were expressed as mean ± standard deviation as tables. Pair wise comparison using t-test was made. Comparison was made between EC, PC and NPC. Test was carried out at 0.05 level of significant and $p< 0.05$ is considered to be significant.

RESULTS

The demographic and clinical Characteristics of Women with EC, PC and NPC

From Table 1, the mean age and standard deviation of the groups were similar: EC (25.03±5.91 years), PC (25.28±5.33 years) and

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NPC (25.44±5.43 years). The mean gestational age of pregnancies of patients and controls were also similar: EC (37.21±2.18 weeks) and PC; (37.48±1.71) respectively. Similarly, the mean BMI and standard deviation recorded was: EC (26.38±4.33 Kg/m$^{2)}$, PC (25.68±3.25 Kg/m$^{2)}$ and NPC (25.62±4.99 Kg/m$^{2)}$. There was no statistical difference between EC, PC and NPC (P > 0.5). However, the mean values of blood pressures (systolic; diastolic) and standard deviation were noted to be higher in EC (171.58±25.20 mmHg; 110.01±0.65 mmHg) compared with PC, (111.56±8.01 mmHg;78.60±12.71 mmHg) and NPC, (110.60±6.93 mmHg; 84.12±5.75 mmHg). There were significant differences between EC, PC and NPC ($p < 0.05$). Urinary proteins (albumin), ≥2+ (100.0%) were recorded in all eclamptic women and non in PC and NPC. 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. Similarly, tonic-clonic convulsions that occurred antepartum (22(57.9%) and intrapatum (16(42.1%) were recorded in the eclamptic women and non in the pregnant and non-pregnant controls.

Table1: The Demographic and Clinical Characteristics of the EC, PC and NPC (Mean ± SD)

Characteristics	EC (n=38)	PC (n=25)	NPC (n=25)
Age (yrs.)	25.03±5.91	25.28±5.33	25.44±5.43
Gestational age (wks.)	37.21±2.18	37.48±1.71	
BMI(Kg/m2)	26.38±4.33	25.68±3.25	25.62±4.99
Systolic BP(mmHg)	171.58±25.20*	111.56±8.01	110.60±6.93
Diastolic BP(mmHg)	110.01±0.65*	78.60±12.71	84.12±5.75
Urinary protein (albuminuria)	≥2+ (100.0%)	Not detected	Not detected
Antenatal booking	21(55.3%)	100.0%	
Antepartum convulsion	22(57.9%)		
Intrapartum convulsion	16(42.1%)		

EC= Eclampsia, PC=Pregnant Control, NPC=Non-Pregnant Control, BMI=Body Mass Index, BP= Blood Pressure *eclampsia is significantly different from both controls at $p < 0.05$ using ANOVA

Twenty-nine; 29 (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. Outcome of Delivery of the Women with EC

Mode 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

Serum TNF-α Levels in EC, PC and NPC

Results showed a significantly high mean serum level of TNF-α in EC (232.10±135.87 Pg/mL) compared to both controls [PC; 180.58±29.18 Pg/mL and NPC; 178.38±36.12Pg/mL] (P<05). Table 2

There was further statistically significant increase of the TNF-α levels in EC (232.10±13587) compared to the PC (180.58±29.18). Table 3

Table 3: Tumor Necrosis Factor-α Level in EC , PC NPC

Group	TNF-α Pg./mL) (Mean ± SD)	95% C. I.	F-test	p-value
EC(N=38)	232.10±135.87a	202.56-284.84	3.462	0.036
PC(N=25)	180.58±29.18b	169.46-192.76		
NPC(N=25)	178.38±36.12b	163.38-191.95		

Mean with different superscripts in the same column are significantly different at p<0.05, EC=Eclampsia, PC=Pregnant Control NPC=Non Pregnant Control.

Pair wise comparison using t-test, EC vs PC = 0.047, EC vs NPC = 0.049, PC vs NPC = 0.813

DISCUSSION

We report significant increase in TNF-α levels in EC. This agrees with finding of Ahmed *et al.* (2019) in Egypt who reported higher levels of TNF-α in EC compared with NPC. Redman *et al.* (1999) observed that production of proinflammatory cytokines (TNF-α) occurs in pregnancy but under strict regulatory control. Tumor necrosis factor alpha have been shown to cause microvascular protein leakage and hypertension which are the major determinants of PE/EC (Belo *et al.*, 2003). TNF-α derived from the placenta, trophoblast and immune cell induces functional alteration in endothelial cells (Pober and Cotran 1990) and is a major contributor in the pathogenesis of PE/EC (Muzammil *et al.,* 2005) Elevated TNF-α in the maternal circulation might play a central role in the excessive systemic inflammatory response, as well as the generalized endothelial dysfunction characteristics of the maternal syndrome of EC.

A further longitudinal study involving a larger populations is strongly advocated to captures debilitating alterations in the levels of TNF-α as they manifest in the course of pregnancy The study revealed significant increase in pro-inflammatory cytokine (TNF-α) level in EC, This result agrees with the findings of Musa *et al.* (2012) and Anim-Nyame *et al.* (2003) who reported higher levels of TNF- and decreased levels ofIL-10in 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*. (1999) 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 verse-visa 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 requires 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-ϒ and TNF-αoccurs in normal healthy pregnancy. Redman *et al*. (1999) 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 (Redmam 1999). 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, deciduous macrophages and neutrophils can be the source of TNF-α, these cells being activated and in higher number in PE (Banda *et al*., 2019).

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.
Anti-TNF-α drugs have been developed and used in varieties of diseases such as rheumatoid arthritis. This has not been tested for eclampsia because pregnant and breastfeeding women are exempted from clinical trials.

Conclusion

A pro-inflammatory cytokines environment was demonstrated in this study by the elevated levels of TNF-α in the eclamptic women and may be responsible for clinical symptoms and fetal death recorded in women with EC. A further longitudinal study involving a larger population is strongly advocated to captures dangerous alterations in the levels of tumor necrosis factor alpha as they manifest in the course of pregnancy.

REFERENCES

Abu-Raya, B., Michalski, C., Sadarangani, M. and Lavoie, P.M. (2020). Maternal immunological adaptation during normal pregnancy. *Front. Immunol.*, 11:575197.

Ahmed, M.A., Alqosaibi, A.I., Mohamed, M.A. and Soliman, M.G. (2019). Evaluation of some cytokines and gene expressions in preeclampsia. *Pak. J Biol. Sci.*, 22:148-153.

Andronikidi, P.E., Orovou, E., Mavrigiannaki, E., Athanasiadou, V., Tzitiridou-Chatzopoulou, M., Iatrakis, G. and Grapsa, E. (2024). Placental and Renal Pathways Underlying Pre-Eclampsia. *Int. J. Mol. Sci.,* 25(5): 27-41.

Anim-Nyame, N., Gamble, J., Sooranna, S.R., Johnson, M.R. and Steer, P.J. (2003). Microvascular permeability is related to circulating levels of tumour necrosis factor-α in preeclampsia. *Cardiovasc Res.*, 58:162-169.

Banda, J.M. Onyemelukwe, G.C. Musa, B.O.P., Shittu, S,O., Babadoko, A,A. Bakari, A.G., Mammam, A.I., Sarkin-Pawa, A. and Junaid, S.A. (2019). T-lymphocyte subpopulations in normal pregnancies and those complicated by eclampsia in Kaduna State, Nigeria. *Open J Immunol*., 6(3): 93-100. http://www.scrip.org/journal/ojihttp://dx.doi.org/10.4236/orji.2016.63010. 6:93-100.

Belo, L., Santos-Silva, A., Caslake, M., Cooney, J., Pereira, L., Quintanilha, A. and Rebelo, I. (2003). Neutrophil activation and C-reactive protein concentration in preeclampsia. *Hypertens Pregnancy*., 22:129–141.

Boeldt, D.S. and Bird, I.M. (2018). Vascular adaptation in pregnancy and endothelial dysfunction inn preeclampsia. *J. Endocrinol.*, 232(1): 27 – 44.

Bowen, J.M., Chamley, L., Mitchell, M.D. and Keelan, J.A. (2002). Cytokines of the placenta and extra-placental membranes: biosynthesis, secretion and roles in establishment of pregnancy in women. *Placenta*, 23:239–256.

Chang, K., Seow, K. and Chen, K. (2023). Preeclampsia: Recent advances in predicting, preventing, and managing the maternal and fetal life-threatening condition. *Int. J. Env. Res. Pub. Health,* 20: 2994 – 3021.

Dacie, J.V. and Lewis, S.M. (1991). *Practical Haematology*. 7th Edition, Churchill Livingstone, Edinburgh, 54-79.

Dimitriadis, E., Rolnik, D.L., Zhou, W., Estrada-Gutierrez, G., Koga, K., Francisco, R.P.V., Whitehead, C., Hyett, J., Costa, F.S., Nicolaides, K. and Menkhorst, E. (2023). Pre-eclampsia. *Nature Rev. Dis. Prim.,* 9(8): 1-22.

Echeverria, C., Eltit, F., Santibanez, J.F., Gatica, S., Cabello-Verrugio, C. and Simon, F. (2020). Endothelial dysfunction in pregnancy metabolic disorders. *Mol. Basis Dis.,* 1866(2): 165414 – 165426.

Federal Ministry of Health (2008). Sero-Prevalence Sentinel Survey among Pregnant Women Attending Antenatal Clinics in Nigeria. A Technical Report, Federal Ministry of Health, National AIDS Control Programmes, 1-85.

Fondjo, L.A., Boamah, V.E., Fierti, A., Gyesi, D. Owiredu, E. (2019). Knowledge of preeclampsia and its associated factors among pregnant women: a possible link to reduce related adverse ourcome. *BMC Preg. Childbirth*, 19: 456 – 462.

Gathiram, P. and Moodley, J. (2016). Pre-eclampsia: Its pathogenesis and pathophysiology. *Cardiovasc. J. Afr.,* 27(2): 71 – 78.

Graham, J.J., Longhi, M.S. and Heneghan, M.A. (2021). T helper cell immunity in pregnancy and influence on autoimmune disease progression. *J. Autoimmun.*, 121: 102651.

Han, X., Ghaemi, M.S., Ando, K., Peterson, L.S., Ganio, E.A., Tsai, A.S., Gaudilliere, D.K., Stelzer, I.A., Einhaus, J., Bertrand, B., Stanley, N., Culos, A., Tanada, A., Hedou, J., Tsai, E.S., Fallahzadeh, R., Wong, R.J., Judy, A.E., Winn, V.D., Druzin, M.L., Blumenfeld, Y.J., Hlatky, M.A., Quaintance, C.C., Gibbs, R.S., Carvalho, B., Shaw, G.M., Stevenson, D.K., Angst, M.S., Aghaeepour, N. and Gaudilliere, B. (2019). Differential dynamics of the maternal immune system in healthy pregnancy and pre-eclampsia. *Front Immunol.*, 10:1305.

Ismail, H., Khaliq, O. and Ngene, N.C. (2023). The role of genetics in maternal susceptibility to preeclampsia in women of African ancestry. *J. Repr. Immunol.*, 160: 104139.

Kornacki, J., Olejniczak, O., Sibiak, R., Gutaj, P. and Wender-Ożegowska, E. (2024). Pathophysiology of Pre-Eclampsia - Two Theories of the Development of the Disease. *Int. J. Mol. Sci.,* 25(1): 307-324.

Lokki, A.I., Heikkineh-Eloranta, J.K. and Laivuori, H. (2018). The immunogenetic conundrum of preeclampsia. *Front. Immunol.,* 9: 2630 – 2637.

Musa, B., Onyemelukwe, G., Olatunji, O., Odogwu, K., Hambolu, J. and Kene, T. (2012). Serum cytokine levels and T lymphocytes subsets in pregnant women with eclampsia. *Open J. Immunol.*, 2: 116-124.

Muzammil, S., Singhal, U., Gulati, R. and Bano, I. (2005). Serum Tumor Necrosis Factor–α in Preeclampsia. *Indian J Physiol Pharmacol*., 49 (2): 236–240.

Opichka, M., Rappelt, M.W., Gutterman, D.D., Grobe, J.L. and McIntosh, J. (2021). Vascular dysfunction in preeclampsia. *Cells*, 10(11): 3055 – 3080.

Pathare, A., Al Kindi, S., Alnaqdy, A., Daar, S., Knox-Macauly, H. and Dennison, D. (2004). Cytokine profile of sickle cell disease in Oman. *Am. J. Hematol.*, 77: 323-328.

Pober, J.S. and Cotran, R.S. (1990). Cytokines and endothelial cell biology. *Physiol Rev*., 70: 427–451.

Poon, L.C., Shennan, A., Hyett, J.A., Kapur, A., Hadar, E., Divakar, H., McAuliffe, F., Costa, F.S., von Dadelszen, P., McIntyre, H.D., Kihara, A.B., Di Renzo, G.C., Romero, R., D'Alton, M., Berghella, V., Nicolaides, K.H. and Hod, M. (2019). The International Federation of gynecology and obstetrics (FIGO) initiative on preeclampsia: A pragmatic guide for first trimester screening and prevention. *Int. J. Gynaecol. Obstet.*, 145 (1):1-33.

Ramsey, J.E., Jamieson, N., Greer, J.A. and Sasar, N. (2003). Paradoxical elevation of adeponectin concentration in women preeclampsia. *Hypertension*, 42: 891-894.

Redman, C.W., Sack, G.P. and Sargent, I.L. (1999). Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am. J. Obstet. Gynecol.*, 180:499-506.

Redman, C.W.G., Sacks, D.P., Sargent, I.L. (1999). Preeclampsia: an excessive maternal response to pregnancy. *Am. J. Obstet. Gynecol*., 180:499–506.

Roberts, J.M., Taylor, R.N., Musci, T.J., Rodgers, G.M., Hubel, C.A. and McLaughlin, M.K. (1999). Preeclampsia: An endothelial cell disorder. *Am. J. Obstet. Gynecol*., 54:133-142.

Sarkin-Pawa, Z., Abdul, M.A., Bolanle, Musa, O.P. and Banda, J.M., (2021). Serum Levels of tumor necrosis factor alpha and Interleukin 10, and their clinical correlates in women with preeclampsia/ eclampsia in Ahmadu Bello University Teaching Hospital. *Arch Int Surg*., 10(1): 11-16.

Spradley, F.T., Palei, A.C. and Granger, J.P. (2015). Immune mechanisms linking obesity and preeclampsia. *Biomolecules*, 5(4): 3142 – 3176.

Steinthorsdottri, V., McGinnis, R., Williams, N.O., Stefansdottir, L., Thorleifsson, G., Shooter, S., Fadista, J., Sigurdsson, J.K., Auro, K.M., Berezina, G., Borges, M., Bumpstead, S., Bumpstead, S., Bybjerg-Grauholm, J. and Colgiu, I. (2020). Genetic predisposition to hypertension is associated with preeclampsia in European and Central Asian women. *Nature Communications*, 11:5976.

Wang, W., Sung, N., Gilman-Sachs, A. and Kwak-Kim, J. (2020). T helper (Th) cell profiles in pregnancy and recurrent pregnancy losses: Th1/Th2/Th9/Th17/Th22/Tfh cells. *Front. Immunol.*, 11: 2025.