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Analysis of Hematological Indices and Serum Immunoglobulin Levels (IgG and IgM) in Preeclamptic and Normotensive Pregnancies

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ABSTRACT: Preeclampsia (PE) is a common complication during pregnancy, affecting 2–5% of pregnant women. It is believed to be associated with modulaton of immune response. This study investigated some hematological indices and serum levels of immunoglobulins- Ig G and IgM in pre-eclamptic and normotensive patients attending Ante-natal Clinic at the University of Benin Teaching Hospital Benin City, Nigeria. The study was carried out among 229 subjects consisting of 128 preeclamptic subjects and 101 healthy normotensive pregnant women. Blood samples were collected from pre-eclamptic and normotensive pregnant women. The hematological analysis was carried out using the blood hematology autoanalyzer while the samples for immunoglobin analysis for IgG and IgM was conducted using the ELISA method. The hematological parameters for all test subjects, revealed that mean counts of the neutrophil, lymphocyte and basophil as well as the packed cell volume (PCV) and platelets were significantly higher in pre-eclamptic than normotensive women. The immunoglobulin G (IgG) significantly decreased from 7.22 ± 0.10 ng/ml in the normotensive women to 5.48 ± 0.23 ng/ml in the pre-eclamptic women while immunoglobulin (IgM) weas observed to be higher in preeclamptic women than the normal pregnant women though not statistically significant ($p>0.05$).. In conclusion, the pre-eclamptic condition significantly affected some hematological indices and immunoglobulin G when compared to the normal preganant women in the study population and these could be used as important biomarkers in the diagnosis of the condition.

Keywords: Normotensive, Preeclampsia, Hematology, Immunoglobulins, Pregnancies

Introduction

In Nigeria, approximately 34% of pregnant women receive no antenatal care, putting them at higher risk of maternal mortality (Awoleke *et al.,* 2012). A recent, nationwide cross-sectional survey revealed that preeclampsia and eclampsia are the leading cause of maternal mortality in Nigeria and are responsible for 28.2% of maternal deaths; the other main contributors to maternal mortality are hemorrhage (24.4%) and pregnancy related infections/sepsis (14.2 %) (Abalos *et al.,* 2016). Preeclampsia is the most common pregnancy-specific complication that still ranks as one of obstetrics major problems. It is a placenta-dependent pregnancy disorder. Preeclampsia is associated with modulation of immune response and defective trophoblast invasion (Atiba *et al.,* 2014). Syndrome of preeclampsia is described as excessive maternal inflammatory responses, perhaps directed against foreign fetal antigens that induce a chain of events including surface trophoblast invasion, defective spiral artery remodeling, placental infarction and release of pro-inflammatory cytokines and placental fragments in the systemic circulation. Currently, preeclampsia is suggested to be caused by changes in immunity (Capolunghi *et al.,* 2013). Therefore, in addition to treating hypertension in preeclampsia, attempts of modifying immune responses may be a future treatment modality. There have been several observations on modulation of

immune responses in pregnancy, most of which are evidenced in the increase of lymphocytes and cytokines (Heyman and Shulman, 2016).
Hematological parameters are good indicators of the physiological status of human and animals (Khan and Zafar, 2005). According to Olafederan *et al*. (2010), examining blood for their constituents can provide for the diagnosis and prognosis of diseases.
Immunoglobulin M (IgM) is one of several isotypes of antibody that is produced by vertebrates. IgM is the largest antibody, and it is the first antibody to appear in the response to initial exposure to an antigen (Sörman *et al.,* 2014). In pregnant women and other mammals that have been studied, plasmablasts residing in the spleen are the main source for specific IgM production. Immunoglobulin G (Ig G) is a type of antibody (Ouchida, 2012). Representing approximately 75% of serum antibodies in humans, IgG is the most common type of antibody found in blood circulation, and second to be produced at the onset of an infection, IgG molecules are created and released by plasma B cells (de Haan *et al.,* 2019). Hence this study was aimed at assessing the hematological indices involved in cellular immunity and the levels of IgG and IgM in preeclamptic and normotensive pregnant women attending University of Benin Teaching Hospital Benin City, Nigeria.

Materials and methods

Study design and setting: Analytical cross-sectional design was utilized to conduct this study. The study area was the Obstetrics and Gynaecology (Ante-natal) Clinic, University of Benin Teaching Hospital (UBTH) Benin City.
Study population and ethical clearance: A total of 229 subjects comprising of 128 preeclamptic women and 101normal non-hypertensive, commonly referred to as normotensive, pregnant women (Control) attending Antenatal Clinic, University of Benin Teaching Hospital (UBTH), were recruited for this study.
A structured questionnaire was used to collect the maternal biodata, clinical characteristics and other relevant information.
Ethical approval was obtained from the University of Benin Teaching Hospital Ethics and Research Committee (Protocol No: ADM/E22/A/VOL.VII/14831138).
An oral informed consent was obtained from all the participants, after duly explaining the aim of the study. The subjects who met the inclusion criteria and consented to the study were recruited.
Inclusion criteria: Women with blood pressure ≤ 140/90 mmHg taken twice at least 4hrs apart, pregnant women of 20 weeks and above gestation and proteinuria of at least 1+..
Exclusion criteria: Non pregnant women, pregnant women with any immunological disease, diabetes mellitus, pre-existing renal disease, intrauterine fetal death, gestational diabetes, pregnant women on any type of antibiotic therapy.
Sample collection and storage: Blood samples (4.5 ml) were drawn from each participant with minimal stasis from the antecubital vein using a dry, sterile disposable syringe and needle. Whole blood was collected and used for the determination of the hematological indices using hematology Emma Full Blood Count Automation (Model PCE210N) while an aliquot of the blood was dispensed into sterile plain tubes. The specimens were labeled with the subject's identification number and were allowed to clot and centrifuged at 3000 rpm for 10min., the sera extracted was stored at -20 °C for analysis of IgG and IgM.
Immunoglobulin assay: The serum immunoglobulins were estimated by enzyme-linked immunosorbent assay (ELISA), as described by de Haan *et al.* (2019).
Data analysis: Analysis of variance in complete randomized design was done using the SPSS version 21 statistical software, All variables were expressed as means ± standard error of means. Differences between groups were tested with a nonparametric Kruskal–Wallis statistical test. For all statistics, the values ≤0.05 were considered significant.

Results

The clinical profile of all study subjects are presented in Table 1. It was observed that in this study that preeclampsia predominantly occurred in women of 31.11 ± 0.54 weeks gestational age. In terms of the blood pressure measurement, this study revealed that the systolic (111.19 ± 0.96 - 159.84 ± 1.91) mm/hg and diastolic blood pressure (71.98±0.76 - 102.36±1.01) mm/hg, were significantly higher in the preeclamptic women when compared with the control group (normotensive women). The pre-eclamptic women had high body mass indices (BMI) which ranged from 49.81- 55.17 kg/m.

Table 1: Clinical Profile of the preeclamptic and normotensive subjects attending UBTH, Benin City

Parameters	Normotensive women	Preeclamptic women	p-value
Gestational age (weeks)	29.18 ± 0.55	31.11 ± 0.54	0.014
Specific gravity (kg/m^3)	31.43 ± 17.38	1.02 ± 0.00	0.050
Systolic blood pressure (mm/hg)	111.19 ± 0.96	159.84 ± 1.91	<0.001
Diastolic blood pressure (mm/hg)	71.98 ± 0.76	102.36 ± 1.01	<0.001
Body mass index (kg/m)	49.81 ± 0.87	55.17 ± 0.90	<0.001

Values are mean ± statistical error mean

The hematological parameters for the study subjects, (preeclamptic and normotensive) are shown in Table 2. The results of the blood indices analyzed revealed that the mean counts of the neutrophil, lymphocyte and basophil as well as the packed cell volume (PCV) and platelets were significantly higher in preeclamptic than normotensive women ($p<0.05$), with the exception of white blood cell, monocyte and eosonophil counts..

Table 2: Hematological Parameters of Preeclamptic and Normotensive Women attending UBTH, Benin City

Blood cell counts	Normotensive women	Preeclamptic women	p- value
White Blood Cells (10^9/l)	7.13 ± 0.21	7.45 ± 0.20	0.282
Neutrophils (%)	0.67 ± 0.01	0.60 ± 0.01	<0.001
Lymphoctye (%)	0.24 ± 0.01	0.31 ± 0.01	<0.001
Monocyte (%)	0.05 ± 0.01	0.06 ± 0.00	0.095
Eosonophil (%)	0.03 ± 0.00	0.04 ± 0.00	0.539
Basophil (%)	0.01 ± 0.00	0.05 ± 0.00	0.001
Packed cell volume (%)	31.82 ± 0.32	34.19 ± 0.33	<0.001
Platelet (10^9/l)	231.11 ± 7.33	211.94 ± 5.35	0.032

Values are mean ± SEM.

The results of the serum immunoglobulin levels among the study population are presented in Table 3. It was observed that the mean immunoglobulin G significantly decreased from (7.22 ± 0.10 ng/ml) in the normotensive to (5.48 ± 0.23 ng/ml) in the preeclamptic women while IgM was observed to be slightly higher in the preeclamptiic women than the normal pregnant women though not statistically significant ($p>0.05$).

Table 3: Immunological Profile of the preeclamptic and normotensive subjects attending UBTH, Benin City

Serum Immunoglobulin	Normotensive women	Preeclamptic women	p-value
Immunoglobulin G (ng/ml)	7.22 ± 0.10	5.48 ± 0.23	<0.001
Immunoglobulin M (ng/ml)	11.46 ± 2.40	19.06 ± 4.90	0.200

Values are mean ± SEM.

Discussion

Preeclampsia increases the risk of undesirable outcomes for both the mother and the baby. If left untreated, it may result in seizures at which point it is known as eclampsia. Preeclampsia affects 2–8 % of pregnancies worldwide. Hypertensive disorders of pregnancy (which include preeclampsia) are one of the most common causes of death due to pregnancy. Generally it is known that preeclampsia is a multisystemic disorder of pregnancy, although to diagnose preeclampsia, hypertension and proteinuria are adequate, there is a great need for additional laboratory biomarkers, to predict, diagnose, or predict risk-stratification. Preeclampsia is a major contributor to maternal and fetal morbidity and mortality worldwide.

Preeclamptic women in this study population had a significantly elevated neutrophil counts. Neutrophils play key roles as effective or killer cells for many types of antigenic challenges. In this case, the maternal immune system may perceive the foetus as an invader or a foreign substance. It has been reported that neutrophil activation can result in vascular damage. Greer *et al*. (1991) reported that preeclampsia is usually associated with neutrophil activation, the mechanism underlying this activation remains unknown, but it has also been reported that concentrations of neutrophil elastase, a specific marker for neutrophil activation *in vivo*, are elevated in the peripheral circulation of women with preeclampsia.

The lymphocytes were also observed to be significantly higher in the preeclamptic women than the normal pregnant women. Lymphocytes are collectively T and B cells which function in presenting antigens to activate other cells of the immune system.

Packed cell volume (PCV), is the percentage of red bloo cells in the blood. It is involved in the transport of oxygen and absorbed nutrients in the body. Hence, decreased PCV in the preeclamptic women can result in poor transportation of oxygen in the system as well as anemia in the women. This finding was corroborated by Awodu *et al*., 2012, who also observed that PCV was also lower among preeclamptics than the controls. They explained that the decreased PCV may be due to the additional effect of hemoconcentration in preeclampsia. The hemoconcentration could be as a result of decreased osmotic pressure which can create a filtration imbalance, further displacing intravascular fluid. Also, endothelial damage can lead to pathologic capillary leakage of fluid further causing the hemoconcentration.

Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation will be prolonged resulting in excessive loss of blood in the case of an injury. Also, preeclampsia can result in thrombocytopaenia in pregnancy and it is as a result of uncontrolled intravascular platelets activation and fibrin deposition (Ganzevoort *et al*., 2014). Incidence of preeclampsia did not affect the concentrations of monocyte and white blood cells in this study. This is contrary to the findings by Bonet *et al*., 2020, who reported that the total WBC and monocyte count were elevated in pregnancy, and even elevated further during labour and the puerperium. This could also be as a result of poor immune response.

Our data revealed that immunoglobulins extracted from the serum of preeclamptic patients contain antibodies. These antibodies subside after the pregnancy is terminated. The mean value of IgG in preeclamptic women significantly decreased when compared to the normotensive pregnant women while for immunoglobulin M even though it was slightly higher in the preeclamptic women when compared to the normal pregnant women, it was observed not to be statistically significant (p value >0.05). Similar finding was obtained by Collins and Katherine (2013) but contrary to the findings of (Heyman and Shulman, 2016). Several health conditions including pregnancy and the presence of disease (s) affect the immune system of an individual. The decrease in the IgG level in the preeclamptic women may be attributed to mild suppression of the maternal immune system's response to allow for protection for the growing placenta and fetus. It could be suggested that for a successful outcome of pregnancy, the sensitivity of maternal immunity was reduced to tolerate the developing fetus which could be perceived as an allograft in this condition. However, the event of markedly increased or decreased immune responses in pregnancy may result in some forms of complexities. Reports have shown that not only is the immune function affected in disease conditions in non-pregnant states but much more severe during pregnancy, whichncan be attributed to hormonal and immune response changes, associated with pregnancy. However, the roles of immunoglobulins in preeclampsia remain unclear; in some cases, they develop protective mechanisms which, when overwhelmed or inadequate, allow preeclampsia to occur. In other cases, they can form part of the cascade of aggressions, leading to the abnormalities encountered in preeclampsia. This would also mean alterations in the immune-related complications in pregnancy macroglobulins, or due to depression in their synthesis (Abalos *et al.*, 2016). This study therefore revealed that human IgG significantly decreased as a result of the incidence of preeclampsia when compared to the normotensive pregnant women and IgM was not significantly influenced in the study population.

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References

Abalos E, Cuesta C, Grosso AL, Chou D, Say L: Global and regional estimates of preeclampsia and eclampsia. Eur J Obstet Gynecol Reprod Biol 170: 1–7. 2016.

Atiba AS, Abbiyesuku FM, Adekanle DA, Oparinde DP, Ajose OA, Niran-Atiba TA: Malondialdehyde and antioxidant enzymes in second and third trimesters of preeclamptic Nigerian women. Nigerian Postgrad Med J, 21(2): 150–154. 2014.

Awodu O A, Anyanwu BA, Ande BA, Famodu AA: Haemostatic Profile determinants of Rheology in Nigerian Preeclamptics. J Clin Med, 17(1):36-42. 2016.

Awoleke JO, Ajayi GO, Adegbola O: Prevalence of *Chlamydophila pneumoniae* antibodies in women with preeclampsia in Lagos, Nigeria. West Afr J Med, 31(4): 253– 258. 2012.

Bonet M, Brizuela V, Abalos E, Cuesta C, Baguiya A, Chamillard M: Frequency and management of maternal infection in health facilities in 52 countries (GLOSS): a 1-week inception cohort study. Lancet Glob Health, 8(5):661–671. 2020.

Capolunghi F, Rosado MM, Sinibaldi M, Aranburu A, Carsetti R: Why do we need IgM memory B cells. Immunol Lett, 152(2): 114–120. 2013.

Collins AM, Katherine, JL: A temporal model of human IgE and IgG antibody function. Front Immunol, 4: 235-246. 2013.

de Haan N, Falck D, Wuhrer M: Monitoring of immunoglobulin N- and O-glycosylation in health and disease. J Glycobiol, 30(4): 226–240. 2019.

Ganzevoort W, Rep A, Bonsel GJ, deVries JI, Wolf H: Plasma volume and blood pressure regulation in hypertensive pregnancy. J Hypertens, 22:1235-1242. 2014.

Greer IA, Haddad NG, Dawes J, Johnstone FD, Calder AA: Neutrophil activation in pregnancy-induced hypertension. Obstet Gynecol**,** 78:28–32. 1991.

Heyman B, Shulman MJ: Structure, function, and production of immunoglobulin M (IgM). J Immunol, 12(2): 1–14. 2016.

Khan TA, Zafar F: Hematological study in response to varying doses of estrogen in broiler chicken. Int J Poul Sci, 4(10): 748-751. 2005.

Müller R: High-resolution structures of the IgM Fc domainsreveal principles of its hexamer formation. Proc Natl Acad Sci USA, 110 (25): 10183–10188. 2013.

Olafedehan CO, Obun AM, Yusuf, MK, Adewumi OO, Olafedehan AO, Awofolaji AO, Adeniji AA: Effects of residual cyanide in processed cassava peel meals on hematological and biochemical indices of growing rabbits. Proc 35th Annual Conf Nig Soc Ani Prod, 212p. 2010.

Ouchida R: Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. Proc Natl Acad Sci USA, 109 (40): 2699–2706. 2012.

Raghupathy R: Th1-type immunity is incompatible with successful pregnancy. Immunol Today, 18 (10): 478-482. 2017.

Sayin M: Evaluation of natural coagulation inhibitor levels in various hypertensive states of pregnancy. Eur J Obstet Gynecol Reprod Biol, 123(2):183-187. 2015.

Vidarsson G, Dekkers G, Rispens T: IgG subclasses and allotypes: from structure to effector functions. Front Immunol, 5: 520-533. 2014.