

Original Research Article

Influence of Hormones in Pregnant Women with Hypertensive Disorders from Ekiti and Oyo States, Nigeria

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Abstract

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This study was aimed at investigating the influence of hormones in pregnant Nigerian women with hypertensive disorders. The study was a prospective cohort study. The participants were pregnant women attending the clinics for antenatal care in four different tertiary health facilities in Nigeria. A total of 521 participants were enrolled in the study out of which 34 developed different types of hypertensive disorder of pregnancy (HDP). After an overnight fast, about 12 millilitres of venous blood sample was collected aseptically from the antecubital vein of each participant without HDP at baseline, second trimester, third trimester and those with HDP at point of development of hypertension respectively. Placental growth factor (PLGF), leptin and insulin were assayed using Enzyme-Linked Immunosorbent Assay (ELISA). The mean values for insulin was increasing from first to second and third trimester while that of PLGF and leptin decreased from first to second trimester and peak in the third trimester. The findings of this study showed that placental growth factor, a vascular endothelial hormone promoting angiogenesis is an early biomarker and predictor of hypertensive disorders in pregnancy. To the best of our knowledge, this hormone has not been estimated in Nigerian pregnant women. Early screening of leptin and insulin may be useful in early prediction of hypertensive disorders in pregnancy in Nigeria pregnant women who may develop hypertensive disorders. Defective placental implantation which lead to endothelial dysfunction and reduced perfusion revealed PLGF as a biomarker and predictor of HDP in the second trimester of pregnancy. Elevated serum leptin and insulin which led to increase in placental-associated factors resulting in maternal disease appear to be strong biomarkers and predictors of HDP in the first and second trimesters of pregnancy.

Keywords: Hypertensive disorders in pregnancy, Insulin, Leptin, Placental growth factor

INTRODUCTION

Hypertensive Disorders of Pregnancy (HDP) is generally regarded as a multisystem disorder specific to pregnant women. They are characterized by widespread endothelial damage which originates from the uteroplacental

circulation but ultimately involves a variety of other organs such as the kidney, liver and brain (Airaodion et al., 2019). The onset may predate a pregnancy or develop during the antenatal, intrapartum or postpartum

course (Shaba and Siziya, 2015).

The pathogenesis of HDP is not completely clear. It is a multifactorial disease and its central pathogenesis seems to involve the systemic activation and injury of maternal endothelial cells, which manifest as raised blood pressure, proteinuria, systemic inflammatory response and accumulation of anti-angiogenic factors, which seem to cause the disease by depriving the glomerular endothelial cells of essential growth factors (Sachan et al., 2013). Features reported are uteroplacental hypoperfusion and foetal ischaemia caused by inadequate vascularization of placenta essential for foetal-maternal circulation. There is also inadequate embryonal trophoblastic cell invasion of uterine wall and spiral arteries secondary to failure of cytotrophoblast epithelial-to-endothelial transformation and subsequent lack of adhesion molecules, integrins, and cadherins (Krane and Hamrahian, 2007).

Normal pregnancy is a carbohydrate-intolerant state characterised by a progressive increase by the dose response of insulin to glucose, suggesting that women who are pregnant become insulin resistant with the duration of gestation. Pre-existing hyperinsulinaemia and/or hyperglycaemia has been documented in early or mid-pregnancy, before the development of preeclampsia, gestational hypertension or both women with pregnancy induced hypertension during the third trimester of pregnancy displayed marked hyperinsulinism in response to an oral glucose tolerance test (OGTT) compared with normotensive controls (Kayemba-kay's et al., 2013).

Studies have identified leptin, an adipocyte-derived hormone that promotes satiety and suppresses appetite, as a marker of increased risk for cardiovascular disease. Levels of biologically active leptin are increased significantly in preeclampsia mothers (Garovic and Hayman, 2007). Elevated leptin levels are associated with insulin resistance, even independent of the recognized association with body mass index. Higher leptin levels independent of obesity have been described in pregnant women with preeclampsia in case-control studies (Solomon and Seely, 2001). Anim-Nyame (et al., 2000), in his longitudinal study showed that plasma leptin level in early gestational age precedes the significant risk of preeclampsia with rise in maternal serum leptin concentration and can be used as a marker of preeclampsia. Increased leptin levels may in part reflect maternal adiposity and have also been hypothesized to reflect placental insufficiency. Leptin might also contribute to endothelial dysfunction by increasing free fatty acid oxidation (Yamagishi et al., 2001).

Placental growth factor (PLGF) is a member of the vascular endothelial growth factor family. It is produced mainly by the placenta and has potent proangiogenic effects. In normal uncomplicated pregnancy, PLGF levels rise until approximately pregnancy week 32 and then fall until delivery. In pregnancies complicated by preeclampsia before the 37th week with or without intra-

uterine growth restriction, PLGF levels are significantly lower (Molvarec et al., 2013). Maternal serum levels of PLGF at 11–13 weeks' gestation are decreased in pregnancies with foetal aneuploidy and those with impaired placentation resulting in preeclampsia and delivery of small-for-gestational-age neonates. Serum levels of PLGF are also reduced in the second and third trimesters of pregnancies that develop preeclampsia or deliver small for gestational age neonates (Tsiakkas et al., 2015). Molvarec et al. (2013) in their study found that free PLGF measured before 35 weeks of pregnancy may predict preterm delivery in all forms of HDP. Benton et al. (2016) reported that placental biomarkers such as placental growth factor present in the maternal circulation, may provide an additional clinical tool for identifying placental foetal growth restriction antenatally. Similarly, they reported that low circulating levels of PLGF may characterize pregnancies complicated by foetal growth restriction associated with significant placental pathology but larger studies are required to elucidate its clinical utility. Shen et al. (2006) in their findings suggested that the abnormal expression of PLGF in placentas is related to the pathogenesis of HDP.

Leptin is a hormone that plays an important role in several physiological processes, including the regulation of endocrine function, immune function, inflammation, reproduction, and angiogenesis (Miehle et al., 2012). The main source of leptin is adipose tissue, but during pregnancy, leptin is also produced by the placenta. In normal pregnancy, placental leptin expression is increased compared with non-pregnant women and suggested to support implantation, human chorionic gonadotrophin production, placental growth, amino acid uptake, and mitogenesis (Hauguel-de Mouzon et al., 2006). Thus, a dysregulation in leptin levels may indicate or lead to maternal disease (Handler et al., 2005).

In addition, there is evidence that leptin may play a direct role in preeclampsia pathogenesis. Increased leptin leads to hypertension in mouse models and has been shown to increase blood pressure through sympathetic activation and nitric oxide synthesis (Ibrahim et al., 2013). Furthermore, leptin may have proinflammatory properties and inflammation is associated with preeclampsia. Although, epidemiological studies have shown significant associations between leptin and preeclampsia, some studies have found no association after adjustment for maternal characteristics, including body mass index (Dalamaga et al., 2011). Indeed serum leptin is increased with obesity and increasing body mass index has been shown to be linked with preeclampsia. However, it is possible that leptin partly mediates this association. This may occur as a result of an increase in placental leptin resistance and a dysregulation of leptin function, which is observed in obese women (Hauguel-de Mouzon et al., 2006; Iftikhar et al., 2010). The aim of this present study is to investigate the influence of hormones in

hypertensive disorders in pregnant women from Ekiti and Oyo states, Nigeria.

MATERIALS AND METHODS

Study Design

The study was a prospective cohort study. The participants were pregnant women attending the clinics for antenatal care in four different tertiary health facilities in Nigeria, namely: Ekiti State University Teaching Hospital, Ado-Ekiti, Federal Medical Centre, Ido-Ekiti, University College Hospital, Ibadan and Adeoyo Maternity Hospital, Ibadan. The hospitals are the major referral centres and therefore attract people from different part of the area. Participants were recruited from June 2011 to October 2012 and involved women at first visit (booking day) without hypertension in their first or second trimester of pregnancy and were followed up to delivery.

Inclusion Criteria

Inclusion criteria include women first seen at first or second trimester (< 20 weeks at booking) with systolic blood pressure below 140 mm/Hg and diastolic blood pressure below 90 mm/Hg and participants that gave consent.

Exclusion criteria

Exclusion criteria include pregnant women first seen at ≥ 20 weeks of pregnancy, women who are already hypertensive at entry into the study or had proteinuria by the dipstick measurement greater than 300 mg/L (1+).

Ethical Consideration

The ethical approval for the study was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Committee Ibadan, Oyo State, Nigeria. (UI/UCH EC Registration Number: NHREC/05/01/2008a; UI/UCH Ethics Committee assigned number: UI/EC/10/0195). A written informed consent was obtained from each participant before recruitment into the study.

Study population

A total of 521 participants were enrolled in the study out of which 34 developed different types of HDP. The remaining 487 which were referred to as censored (those who did not develop HDP till the end of the study period, those whose outcome of pregnancy were not known till

the end of the study period, those who were lost for follow up and those who dropped out from the study for reasons unrelated to the study), 50 were lost for follow-up whose outcomes of pregnancy were not known. The remaining 437 were normotensive till the end of the study period. The various trimester of follow-up for both hypertensive and normotensive women are shown in table 1.

Socio-demographic characteristics of the study population- age, place of residence, marital status, educational background, occupation, ethnic group, diet history and social history, family history, past medical history/medication and gynaecological/obstetrical history were obtained from each participant through a semi pretest questionnaire.

Sample Collection

After an overnight fast (10-12 hrs), about 10 millilitres of venous blood sample was collected aseptically from the antecubital vein of each participant without HDP at baseline, second trimester third trimester and those with HDP at point of development of hypertension respectively. Prior to their scheduled second and third trimester visits, reminder telephone calls were made to each participant. Samples were dispensed into fluoride oxalate bottles, EDTA-containing sample bottles, citrate-containing sample bottles and plain sample bottles to obtain plasma and serum after centrifugation at 4000 rpm for 5 minutes.

Determination of Hormones

Placental growth factor (PLGF), leptin and insulin were assayed using Enzyme-Linked Immunosorbent Assay (ELISA) according to the methods described by Imagawa et al. (1998) and Flier et al. (1979).

Statistical Analysis

Statistical Package of Social Sciences (SPSS) software version 22.0 (SPP, Inc, Richmond, CA) was employed for analysis of data from study population. Paired student's t-test was used to test the significance of difference between mean values. Analysis of variance (ANOVA) was used to test the significance of variations among group means. Post-Hoc was used for comparison of multiple variable. The relationship between all the variables was assessed by Pearson correlation coefficient. Chi square analysis was used for comparison of means for qualitative (non- quantitative) variables. Survival analysis (time to event analysis) was employed using Cox proportional hazard regression model analysis as the technique to measure the survival and hazard function. A two sided probability value $p < 0.05$ was considered statistically significant. Values are reported as

Table 1. Summary of Participant's Recruitment

Event	Normotensive n=487	Hypertensive n=34	Total n=521
Yes	0	34 (100.0%)	34
No (Normotensive)	437 (89.7%)	0	437
Lost for follow-up	50 (10.3%)	0	50
Trimester			
1, 2 & 3	69 (14.2%)	9 (26.5%)	78
2 & 3	64 (13.1%)	8 (23.5%)	72
1 & 2	26 (5.3%)	3 (8.8%)	29
1 & 3	69 (14.2%)	4 (11.8%)	73
1	158 (32.4%)	0	158
2	101 (20.7%)	10 (29.4%)	111

Values are in number of participants with percentage in parenthesis, % = percent, n= number of participants, HDP = hypertensive disorders, 1= first trimester, 2= second trimester, 3= third trimester

mean \pm standard deviation or standard error of mean as appropriate.

RESULTS

Table 2 shows the hormonal concentration of hypertensive women in the three trimesters. Significant differences were observed in all the parameters when compared using ANOVA. None of the parameters was statistically significant when comparing first and second trimesters. The mean values for insulin was increasing from first to second and third trimester while that of PLGF and leptin decreased from first to second trimester and peak in the third trimester.

Table 3 shows the hormonal concentration of normotensive women in the three trimesters. Significant differences were observed in all the parameters using ANOVA. Comparing first and second, second and third with first and third trimesters, none of the hormones was observed to be significant except insulin when first and second trimesters were compared. The mean values for PLGF and insulin were increasing from first to second and declined at third trimester while that of leptin was decreasing.

Tables 4 – 6 show the hormonal concentration in hypertensive and normotensive women in the first, second and third trimester. Significant differences were observed in leptin and insulin when hypertensive and normotensive were compared in the first trimester. The mean values of leptin and insulin were significantly higher in the normotensive than hypertensive in the first trimester. In the second trimester, only insulin was observed to be significantly different when hypertensive and normotensive participants were compared. The mean value of insulin was lower in hypertensive than normotensive in the second trimester ($p=0.032$).

Table 7 shows adjusted cox regression of the hormonal concentration in women with HDP during first trimester. After controlling or adjusting for all the

parameters, significant differences were observed in insulin and leptin ($p= 0.010$ and 0.014). Development of HDP was reduced by 15.1% [$100\% - (100\% \times 0.849)$] and 7.5% [$100\% - (100\% \times 0.925)$] for each additional unit in insulin and leptin respectively (HR =0.849 and 0.925). Negative B coefficient means that higher values of insulin and leptin in HDP women will be associated with lower development of HDP and therefore longer survival (B coefficient = -0.164 and -0.078).

Table 8 shows adjusted cox regression of the hormonal concentration in women with HDP during second trimester. Development of HDP was reduced by 28.0% [$100\% - (100\% \times 0.720)$] and 38.3% [$100\% - (100\% \times 0.617)$] and 22.8% [$100\% - (100\% \times 0.772)$] for each additional unit in insulin, leptin and PLGF respectively. (HR =0.720, 0.617 and 0.772). The negative B coefficient means that lower values of insulin, leptin and PLGF in HDP women will be associated with higher development of HDP in the second trimester of pregnancy (B coefficient = -0.329, -0.483 and -0.259).

Tables 9 and 10 shows adjusted cox regression of the hormonal concentration in women with HDP during second and third trimesters respectively. No significant difference was observed in all the parameters.

In table 11, adjusted cox regression of the hormonal concentration in the women with HDP during third trimester is shown. After controlling or adjusting for all the parameters, No significant difference was observed in all the parameters.

Table 12 shows un-adjusted cox regression of the hormonal concentration in women with HDP. After individual analysis on all the parameters at first, second and third trimesters of pregnancy, leptin at 1st trimester, and insulin at 1st and 2nd trimesters were observed to be statistically significant. Development of HDP was reduced by 4.9% [$100\% - (100\% \times 0.951)$], 12.3% [$100\% - (100\% \times 0.877)$] and 3.7% [$100\% - (100\% \times 0.963)$] for each additional unit in leptin at 1st trimester, insulin at 1st and 2nd trimester respectively (HR =0.951, 0.877 and 0.963). The negative B coefficient means that higher values of

Table 2. Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy

Index	1 st trimester n=10	2 nd trimester n=10	3 rd trimester n=10	P1	P2	P3	P4
PIGF (pg/mL)	11.2±2.9	7.5±2.9	10.1±6.1	0.005*	0.288	0.734	0.853
LEP (ng/mL)	9.8±3.5	9.0±1.8	16.1±3.8	0.001*	0.778	0.075	0.219
INS (µi.u/mL)	2.6±1.0	7.0±1.7	8.1±2.3	0.001*	0.066	0.618	0.066

Values are reported as means ± standard error of mean, P1 =values obtained from ANOVA, P2=values compared between 1st and 2nd trimester, P3=values compared between 2nd and 3rd trimester, P4=values compared between 1st and 3rd trimester, PLGF= Placental growth factor, LEP = Leptin, INS = Insulin, n=number of participants, * = significant at p < 0.05 (2-tailed), p = significant level.

Table 3. Hormonal Concentrations in Normotensive Pregnant Women

Index	N	1 st trimester	2 nd trimester	3 rd trimester	P1	P2	P3	P4
PIGF (pg/mL)	59	14.3±5.5	19.4±3.2	18.2±4.1	0.000*	0.439	0.777	0.564
LEP (ng/mL)	57	15.8±1.8	17.2±2.0	18.3±2.4	0.000*	0.556	0.683	0.329
INS (µi.u/mL)	56	9.8±1.7	18.0±2.5	12.5±2.3	0.000*	0.010*	0.073	0.375

Values are reported as means ± standard error of mean, P1 =values obtained from ANOVA, P2=values compared between 1st and 2nd trimester, P3=values compared between 2nd and 3rd trimester, P4=values compared between 1st and 3rd trimester, PLGF= Placental growth factor, LEP = Leptin, INS = Insulin, n=number of participants, * = significant at p < 0.05 (2-tailed), p = significant level.

Table 4. Hormonal Concentrations in Hypertensive and Normotensive Women during First Trimester

Index	N	Non- hypertensive	N	Hypertensive	t-test	p-value
PIGF (pg/mL)	76	18.3 ± 5.1	21	14.7 ± 6.0	0.352	0.726
LEP (ng/mL)	75	14.1 ± 1.5	21	6.9 ± 1.9	2.402	0.018*
INS (µi.u/mL)	73	10.5 ± 1.5	21	1.5 ± 0.5	3.197	0.002*

Values are reported as means ± standard error of mean, PLGF= Placental growth factor, LEP = Leptin, INS = Insulin, n=number of participants, * = significant at p < 0.05 (2-tailed), p = significant level.

Table 5. Hormonal Concentrations in Hypertensive and Normotensive Women during Second Trimester

Index	N	Non- hypertensive	N	Hypertensive	t-test	p-value
PIGF (pg/mL)	77	19.6 ± 2.6	20	14.4 ± 5.2	0.897	0.372
LEP (ng/mL)	74	16.9 ± 1.8	19	10.1 ± 2.8	1.783	0.078
INS (µi.u/mL)	74	17.5 ± 2.1	19	7.9 ± 3.2	2.182	0.032*

Values are reported as means ± standard error of mean, PLGF= Placental growth factor, LEP = Leptin, INS = Insulin, n=number of participants, * = significant at p < 0.05 (2-tailed), p = significant level.

Table 6. Hormonal Concentrations in Hypertensive and Normotensive Women during Third Trimester

Index	N	Non- hypertensive	N	Hypertensive	t-test	p-value
PIGF (pg/mL)	73	20.9 ± 3.8	15	9.5 ± 4.3	1.320	0.190
LEP (ng/mL)	74	18.7 ± 2.2	16	10.5 ± 3.0	1.690	0.095
INS (µi.u/mL)	74	12.6 ± 1.9	16	6.2 ± 1.6	1.513	0.134

Values are reported as means ± standard error of mean, PLGF= Placental growth factor, LEP = Leptin, INS = Insulin, n=number of participants, * = significant at p < 0.05 (2-tailed), p = significant level.

Table 7. Adjusted Cox Regression of Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy during 1st Trimester

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
Insulin (μ i.u/ml)	-0.164	0.849	0.749	0.962	0.010*
Leptin (ng/ml)	-0.078	0.925	0.869	0.984	0.014*
PLGF (pg/ml)	-0.006	0.994	0.977	1.011	0.468

*= significant at $p < 0.05$, p= significant level, PLGF = Placental growth factor,

Table 8. Adjusted Cox Regression of Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy during 2nd Trimester

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
Insulin (μ i.u/ml)	-0.329	0.720	0.571	0.907	0.005*
Leptin (ng/ml)	-0.483	0.617	0.428	0.889	0.009*
PLGF (pg/ml)	-0.259	0.772	0.637	0.935	0.008*

*= significant at $p < 0.05$, p= significant level, PLGF = Placental growth factor,

Table 9. Adjusted Cox Regression of Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy during 2nd Trimester

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
Insulin (μ i.u/ml)	-0.057	0.945	0.865	1.031	0.204
Leptin (ng/ml)	-0.071	0.931	0.795	1.091	0.380
PLGF (pg/ml)	-0.019	0.982	0.913	1.056	0.618

*= significant at $p < 0.05$, p= significant level, BMI = Body mass index, PLGF = Placental growth factor, AST = Aspartate transferase, ALT = Alanine transferase, HDL = High density lipoprotein, LDL = Low density lipoprotein

Table 10. Adjusted Cox Regression of Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy during 2nd Trimester

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
Insulin (μ i.u/ml)	-0.014	0.986	0.935	1.040	0.607
Leptin (ng/ml)	-0.084	0.919	0.834	1.013	0.091
PLGF (pg/ml)	-0.015	0.986	0.935	1.038	0.585

*= significant at $p < 0.05$, p= significant level, PLGF = Placental growth factor,

Table 11. Adjusted Cox Regression of Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy during 3rd Trimester

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
Insulin (μ i.u/ml)	0.017	1.017	0.886	1.168	0.810
Leptin (ng/ml)	-0.109	0.897	0.773	1.040	0.148
PLGF (pg/ml)	-0.048	0.953	0.847	1.073	0.426

*= significant at $p < 0.05$, p= significant level, PLGF = Placental growth factor

Table 12. Adjusted Cox Regression of Hormonal Concentrations in Women with Hypertensive Disorders in Pregnancy during 3rd Trimester

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
Insulin (μ i.u/ml)	-0.018	0.983	0.904	1.068	0.680
Leptin (ng/ml)	-0.004	0.996	0.927	1.070	0.909
PLGF (pg/ml)	-0.039	0.962	0.899	1.029	0.260

*= significant at $p < 0.05$, p= significant level, PLGF = Placental growth factor,

Table 13. Un-adjusted Cox Regression of Hormonal Concentrations in Women with HDP

Index	B coefficient	Hazard ratio	Confidence interval:		p-value
			Lower	Upper	
PLGF (pg/ml) 1	-0.002	0.998	0.986	1.010	0.700
PLGF (pg/ml) 2	-0.011	0.989	0.966	1.012	0.343
Leptin (ng/ml) 1	-0.051	0.951	0.905	0.999	0.046*
Leptin (ng/ml) 2	-0.038	0.963	0.926	1.002	0.060
Insulin (μ i.u/ml) 1	-0.132	0.877	0.786	0.978	0.018*
Insulin (μ i.u/ml) 2	-0.038	0.963	0.930	0.997	0.033*

*= significant at $p < 0.05$, p= significant level, 1= first trimester, 2= second trimester, 3= third trimester, PLGF = Placental growth factor

leptin at 1st trimester, insulin at 1st and 2nd trimester will be associated with lower risk of development of HDP and therefore longer survival (B coefficient = -0.051, -0.132 and -0.038).

DISCUSSION

Placental growth factor (PLGF) is a member of the vascular endothelial growth factor and is implicated in angiogenesis and trophoblastic invasion of the maternal spiral arteries (Tsiakkas et al., 2015)]. In the pathogenesis of HDP, inadequate trophoblast invasion leads to defects in spiral arteries remodelling resulting in imperfect oxygen placental perfusion (Duhig et al., 2014; Wantania et al., 2015). An imbalance of angiogenic and growth factors at the maternal-foetal interface and a consecutive imbalance of these factors in maternal blood might lead to the clinical symptoms of hypertension and proteinuria (Schmidt et al., 2009; Lai et al., 2014). An imbalance between the factors promoting angiogenesis such as vascular endothelial growth factor or placental growth factor (PLGF) and the factor antagonizing angiogenesis such as soluble fms-like tyrosine kinase 1 (sFLT1) plays a fundamental role in the pathogenesis of preeclampsia (Schmidt et al., 2009; Lai et al., 2014).

Several studies of pregnancies affected by HDP have indicated that PLGF levels are decreased in the first and second trimesters as well as at the stage of clinical onset of the disease (Tsiakkas et al., 2015; Lai et al., 2014; Khosravi et al., 2014; Cowans et al., 2010). In this study, the level of PLGF reduced at second and third trimester of pregnancy in HDP women while significant increase

was observed at second trimester, which declined at third trimester of pregnancy in normotensive women. This finding agrees with previous studies that PLGF concentrations in normotensive pregnancies show a steady increase starting at first trimester of pregnancy with a peak at second trimester and a consistent decline thereafter while in HDP pregnancies PLGF serum levels are significantly lower from first to third trimester (Duhig et al., 2014; Wantania et al., 2015; Schmidt et al., 2009; Lai et al., 2014; Khosravi et al., 2014).

In this study, PLGF became a biomarker, protective factor and predictor of HDP in the second trimester of pregnancy. After adjusting for anthropometric indices in the second trimester, it was observed that the development of HDP is reduced by 22.8% (Hazard ratio = 0.772; C.I.= 0.64-0.94) for each additional unit of PLGF. The lower value of PLGF observed is associated with significant increase in the development of HDP. The significantly reduced value of PLGF observed is associated with higher rate of HDP development in the second trimester of pregnancy (B coefficient = -0.259, $p = 0.008$). This observation agrees with previous studies that reduced serum level of PLGF were observed in women with hypertension during pregnancy (Duhig et al., 2014; Wantania et al., 2015; Schmidt et al., 2009; Lai et al., 2014).

Leptin was initially discovered as a regulator of food intake and energy expenditure (Airaodion et al., 2019), but is now characterized as a pleiotropic molecule involved in a wide range of physiological and pathological functions (Rytlewski et al., 2012). It is a product of the Ob gene and its production takes place mostly in adipose tissue, and then is secreted into the circulation. Leptin is

also synthesized by the placenta during pregnancy – its levels increase proportionally during the pregnancy and decrease postpartum (Rytlewski et al., 2012). Studies have shown that hypertensive subjects frequently have higher leptin levels than normotensive subjects and a positive relationship between serum leptin level and blood pressure has been reported (Ibrahim et al., 2013; Rytlewski et al., 2012; Taylor et al., 2015). The findings in this present study agree with these previous studies that leptin level is elevated in hypertensive pregnant women. Serum leptin value was significantly increased at second and third trimester of pregnancy among the women who developed HDP while a decrease was observed at second and third trimesters among normotensive women (Tables 2 and 3).

Elevated placental leptin expression in HDP is consistent with the accepted model that placental dysfunction leads to an increase in placental-associated factors released into the maternal circulation, resulting in maternal systemic disease (Taylor et al., 2015). One hypothesis is that leptin is increased as a result of placental stress to increase nutrient delivery to the foetus (Taylor et al., 2015). Alternatively, excessive maternal inflammation coupled with other placental factors may mediate excessive leptin expression in preeclamptic women (Redman and Sargent, 2009; Jones et al., 2006). Although, epidemiological studies have shown significant associations between leptin and HDP, some studies have found no association after adjustment for maternal characteristics, including body mass index (Dalamaga et al., 2011; Taylor et al., 2015).

In this present study, after adjustment for anthropometric indices including body mass index and other biochemical parameters, development of HDP significantly reduced by 15.4% for each additional unit of leptin in the first trimester of pregnancy ($p=0.001$, HR = 0.846; C.I.= 0.77-0.94) and 38.3% in the second trimester ($p= 0.009$, HR = 0.617; C.I.= 0.43-0.89). The high value of leptin in HDP women is associated with lower development of HDP (B coefficient = -0.167 and -0.483). After adjustment for all the biochemical parameters without the anthropometric indices, development of HDP is significantly reduced by 7.5% for each additional unit of leptin in the first trimester of pregnancy ($p = 0.014$, HR = 0.925; C.I.= 0.87-0.98). The higher value of leptin in HDP women is associated with lower development of HDP (B coefficient = -0.078). On the other hand, in this study, development of HDP is significantly reduced by 4.9% for each additional unit of leptin in the first trimester of pregnancy ($p <0.05$, HR = 0.951; C.I.= 0.91-0.99) for unadjusted leptin. The higher value of leptin in HDP women is associated with lower development of HDP (B coefficient = -0.051). Hence, leptin became a positive (protective) factor, biomarker and predictor of HDP according to the findings from this study.

Leptin is suggested to play a role in angiogenesis, immunomodulation, and fatty acid metabolism in early

placentation (Miehle et al., 2012; Taylor et al., 2015). Reduced placental perfusion is hypothesized to increase placental expression of leptin, which may increase nutrient delivery to the foetus (Taylor et al., 2015). Studies show that leptin released from the placental can stimulate system A amino acid transport, possibly influencing foetal growth (System A is a highly regulated sodium-dependent transport system capable of transporting small, non-branched amino acids such as alanine, glutamine, glycine, and serine). It is regulated by many effectors, including insulin, glucagon, cortisol, pH and oxygen levels (Jones et al., 2006). Thus, leptin may be a coping mechanism for reduced placental perfusion and a marker of placental insufficiency. Alternatively, an increase in maternal leptin expression may be a result of other stimuli (Taylor et al., 2015; vonVersen-Hoyneck et al., 2009).

Insulin is an anabolic hormone that plays an important role in the regulation of glucose, lipid homeostasis and energy storage through its metabolic effects on classic insulin-responsive tissues (Schulman and Zhou, 2009). Specifically, insulin promotes the storage of glucose as glycogen in liver and skeletal muscles, and facilitates deposition of fatty acids in the form of triglycerides in adipose tissue (Soeters et al., 2012). During insulin resistance, insulin-mediated anabolic metabolic effects are inhibited in the classic insulin-responsive tissues. In physiological condition insulin stimulates endothelial nitric oxide production to exert a vasorelaxation and anti-inflammatory effect (Zhou et al., 2014). Whereas, in the state of insulin resistance, the insulin-stimulated nitric oxide pathway is selectively impaired and the compensatory hyperinsulinemia may activate Nitrogen-Activated Protein Kinase (MAPK) pathway, resulting in enhancement of vasoconstriction, pro-inflammation, increased sodium and water retention and the elevation of blood pressure (Zhou et al., 2014). The increased blood pressure by insulin resistance may contribute to increased blood perfusion to the foetus during pregnancy (Zhou et al., 2014; Zhou et al., 2010).

During normal pregnancy, there is an increase in the number of pancreatic beta cells resulting in increased insulin secretion and an initial increase in insulin sensitivity followed by progressive insulin resistance (Gongora and Wenger, 2015). In this study, the value of insulin significantly increased from first to second and third trimesters among the hypertensive women when compared to the normotensive women ($p<0.05$). The value of insulin peaks in the second trimester and declined in the third trimester among the normotensive women in this study. These findings are consistent with the previous studies that hypertensive disorder during pregnancy is characterized with increase in insulin (Solomon and Seely, 2001; Magon et al., 2011).

In this present study, insulin was observed to be a positive factor, biomarker and predictor of hypertensive disorders of pregnancy in the first and second trimesters

of pregnancy. After adjustment for anthropometric indices including body mass index, percentage body mass and other biochemical parameters. The development of HDP is significantly reduced by 21.4% and 28.0% for each additional unit of insulin in the first and second trimesters of pregnancy ($p=0.003$ and 0.005 , HR = 0.786 and 0.720). The lower value of insulin in HDP women is associated with higher risk of development of HDP (B coefficient = -0.241 and -0.329). Also in this study, after adjustment for all the biochemical parameters without the anthropometric indices, development of HDP is significantly reduced by 15.1% for each additional unit of insulin in the first trimester of pregnancy ($p = 0.010$, HR = 0.849, B coefficient = -0.067).

However in this study, for unadjusted insulin lower value in HDP women is associated with higher risk of development of HDP ($p = 0.018$ and 0.033 , HR = 0.877 and 0.963, B coefficient = -0.132 and -0.038). Hence, insulin became a biomarker and predictor of HDP in the first and second trimesters according to the findings of this study. Our findings agree with previous studies that reported insulin levels as significant predictors of hypertensive disorders in pregnancy (Solomon and Seely, 2001; Sunitha et al., 2012; Nagrato et al., 2009).

CONCLUSION

The findings of this study showed that placental growth factor, a vascular endothelial hormone promoting angiogenesis is an early biomarker and predictor of hypertensive disorders in pregnancy. To the best of our knowledge, this hormone has not been estimated in Nigerian pregnant women. Early screening of leptin and insulin may be useful in early prediction of hypertensive disorders during pregnancy in Nigerian women who may develop hypertensive disorders. Defective placental implantation which lead to endothelial dysfunction and reduced perfusion revealed PLGF as a biomarker and predictor of HDP in the second trimester of pregnancy. Elevated serum leptin and insulin which led to increase in placental-associated factors resulting in maternal disease appear to be strong biomarkers and predictors of HDP in the first and second trimesters of pregnancy.

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