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RESEARCH ARTICLE

SERUM HIGH SENSITIVITY C-REACTIVE PROTEIN, SOLUBLE FMS-LIKE TYROSINE KINASE-1 AND URIC ACID IN PREGNANCY INDUCED HYPERTENSION IN A TERTIARY HEALTH CENTRE

[1,*]Agbara, J.O., [2]Gbadegesin, A.A., [3]Oshodi, Y.A. and [4]Sobande, A.A.

[1]Lecturer, Department of Obstetrics and Gynaecology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
[2]Senior Lecturer, Obstetrics and Gynaecology, Department of Obstetrics and Gynaecology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
[3]Senior Lecturer, Obstetrics and Gynaecology, Department of Obstetrics and Gynaecology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeri
[4] Professor, Obstetrics and Gynaecology, Department of Obstetrics and Gynaecology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria

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ABSTRACT

Background: Hypertensive disorders are common complications in pregnancy and may be associated with adverse fetal and maternal outcomes. Current research interests focus on the identification of biomarkers with the potential to offer major advances in the diagnosis and management of preeclampsia

Objective: To compare maternal serum high sensitivity C-reactive protein (Hs-CRP), soluble fms-like tyrosine kinase-1 (sFlt-1) and uric acid in pregnancy induced hypertensives and normotensive pregnant controls.

Method: Prospective case-control study was conducted in Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital, Lagos, Nigeria. One hundred and fifty two subjects with hypertensive disorders of pregnancy and 152normotensive control had serum levels of Hs-CRP and sFlt-1were and Uric acid assayed. Data obtained were analyzed using SPSS version 19and p-value of <0.05 was considered significant at confidence interval of 95%.

Results: The median serum level of Hs-CRP in preeclampsia was 0.090mg/ml (IQR 0.068, 0.10), gestational hypertension 0.056mg/ml (IQR 0.044, 0.073) and the normotensive 0.040mg/ml (IQR 0.021, 0.06) showed statistically significant difference (P<0.001). The median serum level of sFlt-1in preeclampsia was 4875pg/L (IQR 3266.5, 5703.5), gestational hypertension 1749pg/L (IQR 1639, 1825) and the control 1400.75pg/L (IQR 1480, 1541.75 and also showed statistically significant difference (P<0.001). Uric acid was elevated in all the women with hypertension in pregnancy compared with the controls.

Conclusion: Both Hs-CRP and sFlt-1 were elevated in black women with pregnancy induced hypertension.

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INTRODUCTION

Hypertensive disorders constitute a major cause of maternal, fetal and neonatal morbidity and mortality and affect 4-10% of all pregnancies (Rocella, 2000 and Roberts, 2005). Research interest is focused on the identification of biomarkers with the potential to offer major advances in the early diagnosis and management of pregnancy specific conditions like preeclampsia.

Corresponding author: **Agbara, J.O.,**
Lecturer. Department of Obstetrics and Gynaecology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
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Current hypotheses focus on the maladaptation of the maternal immune responses and defective trophoblastic invasion (Salto, 2007). Evidence from clinical and biochemical findings suggest that disturbance of normal endothelial cell function and inflammation may be central to its pathogenesis. Endothelial dysfunction is accompanied by elevated level of inflammatory markers. Some of these markers have been found to be elevated in the serum of pre-eclamptic than normotensive pregnant women (Sharma, 2007 and Redman, 1998). The biomarkers of interest in this study include high sensitivity C - reactive protein and soluble fms-like tyrosine kinase-1. C-reactive protein (CRP) is a component of the innate immune system.

It is primarily produced by the liver as an acute phase reactive protein in response to severe tissue damage or injury, microbial infections, systemic autoimmune disease and malignant tumors (Mehandale, 2008). Elevated serum CRP provides a sensitive biomarker of chronic systemic inflammation, an independent predictor of future cardiovascular events (Ridker, 2007). Studies have documented elevated levels (of CRP) in preeclampsia, increasing with severity of disease which may precede clinical symptoms and signs of the disorder (Tavana, 2011 and Ghazavi, 2003). Recent studies hypothesize that altered expression of placental angiogenic-antiangiogenic factors play a role in the widespread endothelial dysfunction and clinical manifestation of pre-eclampsia (Noori, 2010 and Maynard, 2008). The soluble form of vascular endothelial growth factor (VEGF) receptor-1 also known as fms-like tyrosine kinase-1 (sFlt-1) is an antagonist of non-soluble VEGF and placental growth factor (PIGF). sFlt has been observed to be elevated in pregnancies complicated by preeclampsia with concomitant reduction in circulating levels of VEGF and PIGF (Maynard, 2010).

Maynard and colleagues (Maynard, 2008), postulated that this increase may disturb the quiescent endothelial permeability of normal vascular tissue maintained by VEGF, and to a lesser degree P1GF, under the increased inflammatory stress of pregnancy. The altered levels of these factors in pre-eclampsia was also documented by other authors (Woolcock, 2008). The alteration in the level of these factors in preeclampsia is said to occur several weeks before the onset of clinical disease (Levine, 2006 and Levine, 2004). This imbalance has been suggested to promote proteinuria, glomerular endotheliosis and hypertension which are hallmarks of preeclampsia (Lu, 2007). The association of Hs-CRP and sFlt-1 with disorders of pregnancy like preeclampsia has been largely documented in Caucasians and none in West Africa. Hypertensive disorders of pregnancy is one of the 5 major causes of maternal mortality in Nigeria including haemorrhage and infection. The absence of studies on these biomarkers in pregnancy induced hypertension in Nigeria thus inform the basis for the study.

MATERIALS AND METHODS

This prospective case-control study was conducted from September, 2014 to August, 2015 in the Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital, Lagos, Nigeria. Ethical clearance was obtained from the Health Research and Ethics Committee of the hospital. The sample size was calculated using formula $N= (r+1/r)\ \sigma^2(Z_\beta +Z_{\alpha/2})^2/(\mu_1-\mu_0)$ (Azmi, 2013). Using the study conducted by von Versen-Hoeynnk and co-workers (Von Versen-Hoeynk, 2009), and the average mean uric acid in normal uncomplicated pregnancy was 5.0, the average mean of uric acid complicated pregnancy (gestational hypertension and preeclampsia) was 6.35. The number calculated was approximately 38. In view of the small sample size, this number was doubled to give 76 gestational hypertension and 76 preeclampsia and 152 controls so as to increase the power of the study and take care of possible attrition. The study populations included consenting pregnant women newly diagnosed with pregnancy induced hypertension at gestational ages 20weeks and normotensive pregnant women who presented at the emergency room and antenatal clinics on same day within the study period. Gestational age was determined using the last menstrual period (LMP) and /or an early ultrasound scan. Women with diabetes, any infectious disease, and premature rupture of membranes, preterm labour, multiple pregnancies/ gestations, renal disease, chronic hypertension, or any other medical condition were excluded. On enrolment into the study, a structured proforma was used to obtain information on socio-demographic data, weight, height, obstetric history, medical and family history and examination findings at diagnosis. Ten millilitres (10mls) of venous blood was collected from the antecubital vein into plain bottles at diagnosis and left for 2 hours to clot and retract. Thereafter centrifugation was done at 1500rpm for 5 minutes. Serum samples were extracted and stored at -20^{o}c. Protein estimation in the urine was also done using urine strips. Gestational hypertension was diagnosed when blood pressure was equal to or greater than 140/90mmHg, at least on two occasions more than 6 hours apart and no proteinuria. Preeclampsia was diagnosed when blood pressure equal to or greater than 140/90 mmHg, at least on two occasions more than 6 hours apart and proteinuria with positive dipstick test 1+ or higher than 300 mg/ 24hr were observed after the 20th week of pregnancy. Severe preeclampsia was defined by blood pressure of ≥160/110mmHg with ≥ 1+ proteinuria or other evidence of severe disease such as HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) or symptoms of significant end organ involvement (like cerebral or visual involvement, epigastric or right upper quadrant pain). Women who met the criteria for pre-eclampsia but not for severe pre-eclampsia were categorized as having mild pre-eclampsia.

The Hs-CRP AccuDiag^{TM} kit (Cortez Diagnostics Inc. California, USA) was used to assay for hs-CRP. Calibration curve was auto- plotted by the auto-analyzer and concentrations of Hs-CRP in sample and control sera were determined at 450 wavelength. The assay sensitivity (detection level) is 0.01mg/ml. Soluble Flt-1 was assayed using the Biosensis ELISA kit (*biosensis*® Thebarton, South Australia 2015). The concentration of the antigen-antibody complex formed was read at 450nm with a microplate reader. Assay sensitivity was <30pg/mL. Uric acid was assayed using the COBAS Uric Acid version 2 (COBAS® Diagnostics, Mannheim, Germany 2014) assay. The lowest detection limit in serum was 0.20mg/dl. Data entry was done with Statistical Package for Social Sciences (SPSS) version 19. Means, standard deviation, median, interquartile range and standard deviation of numeric variables were determined. The distribution of the outcome variables was determined. Mann-Whitney-U and Kruskal Wallis tests were used to compare numeric variables. Chi square was used to compare categorical variables. A p-value of <5% was considered significant at a confidence interval of 95%.

RESULTS

A total of three hundred and four (304) pregnant women were recruited into this study comprising of 76 women with pre-eclampsia, 76 with gestational hypertension and 152 normotensive pregnant controls. The socio-demographic data of these subjects is shown in Table I. There were statistically significant differences between the two groups with regards to booking status and family history of hypertension with $p<0.05$. The test of normality of the biomarkers studied showed that the distribution was not normal so that serum levels were reported as median values with inter-quartile ranges (IQR). The median serum levels of the serum marker, sFlt-1, Hs-CRP and Uric acid were significantly higher in the subjects with pregnancy complicated with hypertension (cases) than those who were normotensive (control) with p-value <0.05. This finding is shown in Table II.

Table I. Socio-Demographic characteristics of respondents studied

Variables	Study n = 152 (%)	Control n = 152(%)	Test	p
Age group (years)				
<25	8 (5.3)	15 (9.9)	2.878#	0.411
25 – 29	57 (37.5)	47 (30.9)		
30 – 34	54 (35.5)	70 (46.0)		
≥ 35	33 (21.7)	20 (13.2)		
Booking status				
Booked	111 (73.0)	144 (94.7)	18.427#	<0.001
Unbooked	41 (27.0)	8 (5.3)		
Parity				
Nulliparous	88 (57.9)	98 (64.5)	0.087#	0.768
Multiparous	64 (42.1)	54 (35.5)		
Proteinuria				
Absent	62 (40.8)	152 (100.0)	61.60#	<0.001
Present	90 (59.2)	0 (0.0)		
History of PIH				
None	137 (90.1)	148 (97.4)	2.766#	0.096
Yes	15 (9.9)	4 (2.6)		
Family history of PIH				
None	142 (93.4)	152 (100.0)	4.368#	0.036
Yes	10 (6.6)	0 (0.0)		
Trimester at sampling				
Second	7 (4.6)	20 (13.2)	4.464#	0.043
Third	145 (95.4)	132 (86.8)		
Mean BMI	29.8±6.8	28.2±5.8	1.638##	0.103

NB # = chi Square
= student t test

Table II. Median level of serum Hs-CRP (mg/ml), sFlt – 1(pg/ml) and Uric acid (µmol/L) in cases and controls

Variable	Cases Median (IQR) n = 152	Control Median (IQR) n = 152	U	p
Hs-CRP	0.070 IQR (0.049, 0.093)	0.048 IQR (0.021, 0.069)	2626.5	<0.001
Uric acid	284.90 IQR (223.6, 351.3)	231.90 IQR (196.28, 257.05)	436.4	<0.001
sFlt – 1	1925.05 IQR (1734.0, 4904.5)	1400.75 IQR (1466.0, 1541.75)	2342.0	<0.001

NB: U=Mann-Whitney U test

Table III: Median level of serum hs-CRP (mg/ml), sFlt – 1 (pg/ml) and uric acid (µmol/L) in women with preeclampsia, gestational hypertension and control

Variable	Pre-Eclampsia Median (IQR) n = 76	Gestational hypertension Median (IQR) n = 76	Control Median (IQR) n=152	H	p
Hs-CRP	0.090 IQR (0.068, 0.100)	0.056 IQR (0.044, 0.073)	0.048 IQR (0.021, 0.069)	57.69	<0.001
Uric acid	336.0 IQR (278.25, 419.40)	242.15IQR (207.50, 292.5)	231.90 IQR (196.28, 257.05)	66.420	<0.001
sFlt -1	4875.0 IQR (3266.5,5703.5)	1749.0 IQR (1639.0,1825.0)	1400.75 IQR (1466., 1541.75)	153.768	<0.001

NB: H = Kruskal Wallis test
IQR = Interquartile range

Table IV. Median level of serum hs-CRP, sFlt – 1 and uric acid in women with mild and severe preeclampsia

Variable	Severe pre-eclampsia Median (IQR) n = 63	Mild pre-eclampsia Median (IQR) n = 13	U	P
Hs-CRP	0.091 IQR (0.070, 0.102)	0.079 IQR (0.051, 0.087)	188.5	0.004
Uric acid	343.75 IQR (287.05, 461.48)	305.90 IQR (268.35, 379.50)	304.5	0.218
sFlt – 1	4979.5 IQR (3484.25, 5779.0)	2997.0 IQR (1629.5, 3520.5)	96.0	<0.001

NB: U = Mann Whitney U test

Table III showed the median serum levels and inter-quartile range of sFlt-1, Hs-CRP and Uric acid in preeclampsia, gestational hypertension and the normotensive controls. These differences was statistically significant, at p-value <0.001. Compared with mild preeclampsia, patients with severe preeclampsia, had a statistically significant higher median serum sFlt-1, p<0.05 but not Hs-CRP or Uric acid levels as p>0.05 (Table IV). The median serum level and inter-quartile range of Uric acid was significantly higher in women with severe preeclampsia than those with, mild preeclampsia, gestational hypertension and the controls as shown in Table 4 (p value < 0.001).

DISCUSSION

The determination of maternal serum level high sensitivity C-reactive protein (Hs-CRP), soluble fms-like tyrosine kinase-1 (sFlt-1) receptor and uric acid in gestational hypertension and preeclampsia was the focus of this study. It was observed that when compared with normotensive pregnant women (control), there was increased level of Hs-CRP in all the groups studied (reference- normal <3mg/L; Low risk <1mg/L, Intermediate risk 1.0-3.0mg/L, High risk >3mg/L).This was consistent with the fact that normal pregnancy is characterised by a mild systemic inflammatory response and increases as pregnancy progresses. However, there was a significantly higher level of Hs-CRP in preeclampsia. The finding of a higher level in preeclampsia is consistent with the finding documented by Vijayalakshmi and colleagues (Vijayalakshmi, 2015). Serum sFlt-1 has been documented to increase in pregnancy complicated with preeclampsia. Maynard and colleagues (Maynard, 2008 and Maynard, 2003), showed that placenta derived sFlt-1 and placental growth factor (PIGF) is up regulated in preeclampsia leading to increased levels in circulation. Elevated levels of sFlt-1in preeclampsia was demonstrated in preeclampsia in this study compared with the control. A serum level of 4875pg/mL and 1400.75pg/mL in severe preeclampsia and control respectively documented in this study was comparable with 4382pg/mL and 1643pg/mL respectively reported by Levine and co-workers (Levine, 2004).

Similar findings was also documented by other researchers (Carmen, 2016 and De Vivo, 2008). The serum level of sFlt-1 was however, observed to be comparable between women with gestational hypertension and those who were normotensive. This finding was different from that documented by Suseela et.al (Suseela, 2010) and Leanas-Miranda et.al (Leanas-Miranda, 2017), who documented elevated levels in severe gestational hypertension. The difference in finding may be because the gestational hypertension was not subdivided to mild or severe in this study.

This study demonstrated an increased level of uric acid in both gestational hypertension and preeclampsia compared with the control. The degree of elevation was most marked in those with severe preeclampsia. Current focus is on elevated uric acid as pathogenic for preeclampsia with a hypothesis that elevations in circulating uric acid in preeclamptic women contribute to the pathogenesis of the disorder, in part, through attenuation of normal trophoblast invasion and spiral artery vascular remodelling. The serum levels of all the biomarkers studied was observed to be significantly elevated in all the women who developed maternal complications. Uric acid and sFlt-1 were elevated in pregnancies complicated with adverse maternal and perinatal outcomes in this study.

Conclusion

Our study showed a higher levels of s-Flt-1, Hs CRP and Uric acid in hypertensive pregnancies than the normotensive controls. The degree of elevation of sFlt-1 varied with disease severity being most marked with severe preeclampsia. A larger, more robust, multicentre trial will be of value to validate our results.

Limitation of Study

The size of the population studied was small and may limit generalization of findings from this study.

Conflict of Interest statement: The authors report no conflict of interest.

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