Original Research Article

SERUM VISFATIN IN PREECLAMPSIA AND NORMAL PREGNANCY IN LAGOS, SOUTH-WESTERN NIGERIA.

ABSTRACT

Background: Adipocytokines have been recently implicated in the pathogenesis of preeclampsia. Visfatin is one of such adipokines .

Objective: To determine the association between serum visfatin levels and preeclampsia

Methods: A prospective, case-control study was carried out in 160 pregnant women 80 pre-eclamptics and 80 normotensive controls, matched for age and parity during the third trimester. Maternal serum visfatin levels were determined in both groups using a visfatin (Human) enzyme- linked immunosorbent assay. Serum Visfatin levels were compared between the groups and correlated to the blood pressure, proteinuria and fetal birth weight and Apgar scores in both groups.

Results: The mean serum visfatin level was significantly higher 10.3±6.9ng/ml in preeclampsia than (7.4±4.4ng/ml) in the control group *(p=0.001*). The mean serum visfatin level was higher in severe pre-eclamptics (10.8±8.9ng/ml) compared to 9.6±5.8ng/ml in mild preeclamptics and this was statistically significant (*p=0.021)*. Visfatin levels showed a negative and non-significant correlation with both systolic (r= -0.011 and p=0.924), diastolic blood pressure(r= -0.012, p=0.913) and body mass index (r= -0.142, p=0.209) in both study and control groups. Mean fetal birth weight was significantly lower in the preeclampsia (2.8±0.25kg) compared to the control group (3.2±0.31kg) P=0.000. The mean fetal weight was lower in severe preeclampsia (2.7±0.25kg compared to 2.9±0.39 in mild preeclamsia. There was no significant correlation between the visfatin levels and Apgar score at 5minutes and birth weights in both groups (*P*=>0.05).

CONCLUSION: This study showed a significant increase in the level of visfatin in preeclampsia compared to their normo-tensive controls. However, this increased level was not consistent with the severity of the disease.

Keywords: pre-eclampsia, visfatin, normotensive, fetal outcome

INTRODUCTION

Preeclampsia is a human pregnancy-specific hypertensive multisystem disorder, characterized by hypertension and proteinuria in the second half of pregnancy. It has been reported to complicate about 4-7% of all pregnancies globally.[1] It affects 5.8% of all primigravidae and 0.4% of second pregnancies[2]. It is responsible for about 50-75 thousand maternal deaths annually in Africa[3]. Preeclampsia-related fatalities occur three times more often in black women than in white women[4]. These racial difference in morbidity and mortality from preeclampsia may probably be as a result of disparities in health status, as well as access to, and quality of prenatal care[4]. In Nigeria, the reported incidence varies between 4 and 17%[3,5,6**].** Regional variations in the incidence exist, with higher values being reported in the Northern compared to the Southern part of Nigeria[7-10]. Seasonal variations have also been reported in the incidence, with higher values during the rainy season in most parts of Nigeria[10].

Numerous factors have been proposed as being implicated in the pathogenesis of preeclampsia including genetic, immunologic, endothelial, behavioural and environmental factors[11]. Pathologic variations in preeclampsia in nulliparous women may be different from those in women with preexisting cardiovascular disease, twin gestation, diabetes mellitus, chronic hypertension, or various thrombophilias[12]. In addition, the pathophysiology of early-onset preeclampsia may be different from that of preeclampsia developing at term, during labour, or in the postpartum period[13]. Unfortunately the only

solution to abort the disease progression has remained the timely delivery of the fetus and placenta[8,12].

Preeclampsia has recently been shown to share many pathophysiologic features of the metabolic syndrome [14] including insulin resistance, low grade systemic inflammation and atherosclerosis [15]. Endothelial dysfunction is one of the early stages of atherosclerosis, and insulin resistance and low grade systemic inflammation might contribute to the pathogenesis of endothelial dysfunction. The causes of endothelial dysfunction which plays an important role in the progression and aetiopathogenesis of preeclampsia involve dysregulation of multiple complex pathways. There are various studies about the regulation of substances secreted by adipose tissue (adipokines) such as tumour necrosis factor (TNF-α), leptin, adiponectin, resistin and interleukin (IL-6) in normal and complicated pregnancies. Some of these studies suggest that adipocytokines play an important role in the pathogenesis of preeclampsia in low grade systemic inflammation, atherosclerosis and insulin resistance [16].

Adipokines may directly or indirectly influence the function of endothelial cells thus plays a role in the pathogenesis of preeclampsia [7]. One of such adipokines is visfatin - a 52 kilodalton protein expressed in fatty tissues, liver, muscle, macrophages, placenta and fetal membranes [17,18]. Visfatin is also expressed in amniotic epithelium, cytotrophoblast and deciduas, and is over expressed when fetal membranes are exposed to mechanical stress[19] and /or pro-inflammatory stimuli. Some recent studies have demonstrated that visfatin levels are elevated in pregnant women with intrauterine growth restriction (IUGR)[20] and pre-eclampsia [21-24].

This present study was carried out in Nigerian (black African) pregnant women in whom preeclampsia is relatively common and where there is paucity of publications concerning

this biochemical marker locally. It seeks to determine the association between serum visfatin levels and preeclampsia.

METHODOLOGY

The study was a prospective case-control study involving pregnant women with preeclampsia and healthy normotensive controls conducted between January and June 2016 at the obstetric unit of Lagos State University Teaching Hospital, Ikeja, Lagos. Our institutional ethics and research committee approved the protocol for the study. Sample size was determined using the formula for single proportion with prevalence and precision rate of 5% each which gave 72.93, addition of 7 to account for attrition gives total sample size of 80 adopted for each group. All consecutive pregnant women who were diagnosed with preeclampsia (study) using the International Society For The Study Of Hypertension (ISSHP) criteria together with normotensive (controls) who presented at the antenatal and emergency unit of the hospital and fulfilled the inclusion criteria were recruited after due counseling, and informed consent was taken to participate in this study.

The study subjects were further sub-divided into mild and severe preeclampsia subgroups. Preeclampsia was diagnosed in the presence of hypertension (blood pressure of 140/90 mmHg or higher, on at least two occasions, at least 6 h apart, after 20th week of gestation) and proteinuria (300mg in a 24-h urine collection or ≥1+ by dipstick or more).

Severe preeclampsia was diagnosed if one or more of the following were present: blood pressure of 160/110mmHg or higher, excretion of 5g or more of protein in 24-h urine sample or a urine dipstick showing ≥ 2+ in a random urine sample, oliguria of less than 500ml in 24-h, pulmonary oedema or cyanosis, visual or cerebral disturbance, pain in the epigastric area or right upper quadrant, impaired liver function, thrombocytopenia and HELLP syndrome. Subjects were recruited at the point of diagnosis of preeclampsia before the commencement of anti-hypertensive medication.

The control group consisted of pregnant women who were normotensive, matched at the time of recruitment for gestational and maternal age. Exclusion criteria were gestational diabetes, infectious diseases, premature rupture of membrane, any other medical disease and being in the labour. The body mass index was calculated as weight in kilograms divided by height in meters squared. The height was measured using a secanthropometer and weight by omiron$^{R)}$ weighing scale. The blood pressure was measured according to standardized approach.

About 3ml of maternal venous blood samples was collected from the antecubital fossa of the right arm of each participant before administration of any medication or intervention for those with preeclampsia. The blood sample was processed to obtain the supernatant serum which was kept frozen at -70^{o}C until batch analysis was done. Determination of visfatin levels were performed by enzyme-linked immunosorbent assay (ELISA) using Visfatin C-Terminal (Human) EIA (Cat.No:EK-003-80)(Range:0.1-1000ng/ml). ELISA kits were obtained from Phoenix Pharmaceuticals Inc. (Belmont, CA, USA). The assay was conducted according to the ELISA kit manufacturer's instruction. Minimum detectable concentration of 2.62ng/ml; intra-assay co-efficient of variation of <10% and inter-assay co-efficient of variation of <15% were used.

The data were analysed statistically using SPSS software version 18. Descriptive data was summarized as mean, standard deviation, median and percentages as appropriate.

Comparison between continuous variables was evaluated using student's t-test for continuous variables or the chi-square test for categorical variables. Correlation between variables was evaluated using Pearson's correlation co-efficient. P-value of < 0.05 was considered significance at 95% confidence level.

RESULTS

Socio-demographic characteristics of the participants were shown in Table I which revealed no significant difference between the preeclampsia and the normotensive control. Most of them were Yorubas (55% versus 52.5% in cases and controls respectively). Also about 75% and 59% of the respondents in the control and study group respectively had tertiary education. While all the subjects in the control group were booked, only 70% of the study group was booked.

Table II depicted the clinical characteristics of the respondents. There was no statistical difference between the mean age of subjects in the study (29.6±3.8) and control (29.6±4.2) P= 0.938. The mean gestational age at sampling between the study and the control group were (36.5±2.9) and (37.3±1.6) respectively, P=0.360. The systolic, diastolic blood pressures and body mass index were significantly higher in women with preeclampsia as expected compared to control group (P=0.000, P=0.001) respectively. Serum visfatin was significantly higher in the preeclampsia women 10.3 ±6.9 than the control group (7.4±4.4ng/ml), P=0.001.

Table III showed the comparison of mean visfatin levels among the groups. Maternal serum levels of visfatin were 7.41±4.4ng/ml in the normotensive, 9.6±5.8 in mild

preeclampsia and 10.8±8.9ng/ml in severe preeclampsia respectively indicating statistically significant difference(P=0.002). Furthermore, serum visfatin level was significantly higher in severe preeclampsia (10.8±8.9ng/ml) than mild preeclampsia (9.6±5.8) P=0.021.

TableIV showed the correlation of visfatin with the clinical features of preeclampsia and normotensive pregnant subjects, there was a weak correlation but insignificant difference between visfatin levels and age, blood pressure body mass index, proteinuria and gestational age at sampling in both the study and control groups (P=>0.05).

There was no significant correlation between visfatin levels and Apgar score at 5 minutes in both study and control group (p = 0.677). Eight of the women in the study group did not deliver in our facility leading to attrition and difference in number analysed. Four of the women had intrauterine fetal deaths which were due to abruptio placentae in two, and severe IUGR in the remaining two. Eleven of the babies delivered by women in preeclampsia group were admitted into the special care baby unit compared to six babies delivered in the normotensive group. Table V

The mean birth weight of babies delivered by subjects in the normotensive group was significantly higher than those in the preeclampsia group. P = 0.000 (Table VI). There was significant difference in the mean birth weight of babies born to normotensive subjects and subjects with mild and severe preeclampsia. P = 0.000 (Table 7). There was no significant difference between the mean birth weight of babies delivered by subjects with mild(2.9kg) and severe preeclampsia(2.7kg) P = 0.292 while the mean Apgar score at 5minutes between the preeclampsia and normotensive control group was statistically significant (7.82±0.86 vs 8.04±0.45) P=0.019, (Table 8).

TABLE 1: SOCIO DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

Variable	Preeclampsia n = 80 (%)	Control n = 80 (%)	p value
Age group (yrs)			
Less than 20	0 (0.0)	3 (3.8)	0.938
20 – 24	9 (11.3)	5 (6.3)	
25 – 29	32 (40.0)	24 (30.0)	
30 – 34	29 (36.3)	41 (51.3)	
35 – 39	9 (11.3)	7 (8.8)	
≥ 40	1 (1.3)	0 (0.0)	
OCCUPATION			
Low Income	21 (26.3)	20 (25.0)	0.350
Middle Income	55 (68.7)	51 (63.7)	
High Income	4 (5.0)	9 (11.3)	
TRIBE			
Igbo	22 (27.5)	30 (37.5)	0.2128
Yoruba	44 (55.0)	42(52.5)	
Hausa	3 (3.8)	0 (0.0)	
Others	10 (12.5)	8 (10.0)	
EDUCATIONAL LEVEL			
None	1 (1.3)	0 (0.0)	0.1140
Primary	1 (1.3)	0 (0.0)	
Secondary	31 (38.8)	20 (25.0)	
Tertiary	47 (58.8)	60 (75.0)	
RELIGION			
Christianity	67 (83.8)	72 (90.0)	
Islam	13 (16.2)	8 (10.0)	
BOOKING STATUS			
Unbooked	24 (30.0)	0 (0.0)	0.2418
Booked	56 (70.0)	80 (100.0)	
PARITY			
Nullipara	47 (58.8)	39(48.8)	0.3942
Primipara	11 (13.8)	16 (20.0)	
Multipara	22 (27.4)	25 (31.2)	

TABLE II: M±S.D OF CLINICAL CHARACTERISTICS OF THE RESPONDENTS

Variables	Preeclampsia n = 80	Control n=80	p value
Age (years)	29.6 ±3.8	29.6±4.2	0.938
Gestational age (weeks)	36.5 ±2.9	37.3 ±1.6	0.360
Systolic blood pressure (mm/Hg)	169.8±24.5	111.8 ±9.4	0.000
Diastolic blood pressure (mm/Hg)	107.5 ±14.6	69.2 ±7.9.6	0.000
Body mass index (kg/m^2)	32.1±5.1	29.5 ±4.1	0.001
Visfatin (ng /ml)	10.3 ±6.9	7.4 ±4.4	0.001

*M±SD = Mean±Standard deviation

TABLE III: COMPARISON OF MEAN VISFATIN LEVELS IN NORMOTENSIVE, MILD PREECLAMPTIC AND SEVERE PREECLAMPTIC SUBJECTS

Categories (ng/ml)	M±SD	Range	Median
Normotensive (n=80)	7.41±4.4	0.21-35.0	6.35
Mild preeclampsia (n=19)	9.6±5.8	4.8 – 42	8.2
Severe preeclampsia (n=61)	10.8±8.9	0.6 – 50.0	8.0

f statistic = 6.166 (anova) P = 0.002

TABLE IV: CORRELATION OF VISFATIN WITH THE CLINICAL FEATURES OF PREECLAMPTIC AND NORMOTENSIVE PREGNANT SUBJECTS

VARIABLES	PREECLAMPSIA		NORMOTENSIVE	
	VISFATIN		VISFATIN	
	r	p	r	p
Age	0.128	0.259	-0.041	0.721
Systolic blood pressure	- 0.011	0.924	0.069	0.546
Diastolic blood pressure	- 0.012	0.913	0.069	0.703
Body mass index	- 0.142	0.209	0.171	0.128
Proteinuria	0.028	0.809		
Gestational age	0.139	0.219	- 0.054	0.633

r = Pearson's correlation

TABLE 5: CORRELATION BETWEEN VISFATIN, BIRTH WEIGHT AND APGAR SCORE IN BOTH GROUPS

VARIABLE	PREECLAMPSIA			NORMOTENSIVE		
	n	r	p	n	r	p
Birth weight	72	-0.010	0.935	80	-0.097	0.413
Apgar score at 5min	68	0.116	0.335	70	-0.050	0.677

TABLE 6: FETAL OUTCOME IN PREECLAMPSIA AND NORMOTENSIVE

Variables		**Preeclampsia n= 72 M±SD**	**Control n =80 M±SD**	**p value**
BIRTH WEIGHT (KG)	72	2.80±0.25	3.2±0.031	0.000[X]
APGAR SCORE AT 5 MIN	68	8.04±0.45	7.82±0.866	0.019[X]
ADMISSION TO SCBU[&]	11	(16.17%)	6(8.57%)	0.209*

[&] SCBU - special care baby unit

[X] = student t text * = chi square

TABLE 7: ASSOCIATION BETWEEN THE MEAN BIRTH WEIGHT IN MILD AND SEVERE PREECLAMPSIA

GROUP	N	MEAN	T	DF	P
MILD	11	2.9±0.39	1.063	70	0.292
SEVERE	61	2.7±0.25			

TABLE 8: M±SD APGAR SCORE IN PREECLAMPSIA AND NORMOTENSIVE

VARIABLE	PREECLAMPSIA (73)	NORMOTENSIVE (71)	T	DF	P
APGAR SCORE	8.04±0.45	7.82±0.86	2.376	142	0.019

DISCUSSION

This study did not show any significant difference with respect to the socio-demographic characteristics of the patients with preeclampsia and control. Maternal serum visfatin assay was significantly increased in women with preeclampsia in the third trimester 10.3±6.9ng/ml compared to normotensive (7.4±4.4ng/ml). This finding corroborates that of Fasshauer et al[21] with values of 7.66±0.28ng//ml, 2.66±0.41ng/ml and 2.81±0.37ng/ml for severe pre-eclampsia, mild pre-eclampsia and normotensive control respectively. Adali et al [22] in their study also reported values of 21.58±0.46ng/ml, 19.17±0.44ng/ml and 14.91±0.43ng/ml for severe preeclampsia, mild pre-eclampsia and normotensive control respectively. Other workers have also reported similar findings to this study [23,25]. However, the serum visfatin in the mild preeclampsia sub-group 9.6±5.8ng/ml was lower than that of severe preeclampsia but higher than normotensive control (7.4±4.4ng/ml). This was similar to findings of other workers [21,22]. While Wensheng in China[26] reported decreased plasma levels of visfatin in preeclampsia compared to the controls, Makazi-Tovi et al[27] showed no significant increase in patients in preeclampsia compared with normal pregnancies.

A mechanism by which visfatin might be associated to preeclampsia is that of endothelial dysfunction. A study by Adali et al[22] in Turkey showed that serum visfatin and leptin increased markedly with severity of the disease in preeclamptics when compared with normotensive controls of similar gestational ages. They also observed that maternal visfatin concentrations in preeclamptic patients with abnormal umbilical and uterine artery Doppler velocimetry were significantly higher than those in preeclamptics with normal Doppler

velocimetry. Pathological utero-placental perfusion predicts a high risk for fetal growth restriction originating from deteriorating placental function.

Though the mean birth weight in the preeclampsia group was significantly lower than of normotensive control, there was a negative but insignificant correlation between the visfatin levels and birth weight in both groups. Serum visfatin level was also insignificantly correlated with the 5minutes apgar score in both the cases and controls. While the mean birth weight in both groups were within normal limit, Fasshauer et al[21] found that visfatin levels were significantly elevated in women with fetal growth retardation compared to those with normal birth weight neonates. This probably showed that the pathology determining the fetal outcome may be from the underlying effect of preeclampsia on the fetus rather than a direct effect of visfatin. The proinflammatory property of visfatin can result in abnormal utero-placental perfusion which may affect the fetal weight if the condition is prolonged [21].

Though majority of subjects in the study were recruited as late-onset preeclampsia, early-onset preeclampsia occurs at the gestational age of less than 34 weeks and are usually accompanied by severe complications for both the mother and fetus due to placental factors. Late-onset preeclampsia occurs at the gestational age of 34 weeks onward and the disorder is accompanied by mild complications derived from maternal abnormalities [28, 29].

The study was limited by its cross-sectional institutional based case-control design and restricted the inferences that could be derived from the results with regards to the role of visfatin as a marker of severity in preeclampsia. However, this study demonstrated higher visfatin levels in preeclampsia compared to normal pregnancy but failed to demonstrate a significant correlation of serum visfatin with severity of the disease. There was no significant correlation between the serum visfatin level and birth weight and apgar score at 5minutes in

both study and control groups. Longitudinal study among normal pregnant subjects whose serum visfatin levels will be monitored from second half of pregnancy before preeclampsia develops may indicate or refute the usefulness of visfatin as a marker in predicting development of preeclampsia and possibly its severity.

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