RESEARCH ARTICLE

MATERNAL ADIPOSITY AND SERUM VISFATIN LEVELS DURING FIRST TRIMESTER AMONG PREGNANT WOMEN WITH PREECLAMPSIA.

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Abstract

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Background: Visfatin is produced in the adipose tissue and has glucose lowering effects; however during pregnancy the placenta produces more visfatin which has been reported to increase in insulin resistant states such as type 2 diabetes mellitus and obesity and thus vistafin levels has invariably been linked to preeclampsia. Several studies have however provided conflicting results about the role of visfatin in preeclampsia.

The aim of this study was to determine whether serum visfatin concentration and body fat are significantly altered during the first trimester in pregnancies and whether such changes can leadto the development of PE.

Methods: This work involved participants who were purposively selected for a longitudinal study,consisting of 314 pregnant womenwith pregnancy complications attending antenatal visit between 11 and 13 weeks of gestation at the Volta Regional Hospital, Ho, Ghana.

Maternal serum visfatin levels and body fat percent were measured between 11 and 13 weeks of gestation. First trimester Body mass index (BMI) and lipid profile of all participants were also determined.

Results:- First trimester serum visfatin level (ng mL^{-1}), BMI (Kg/M^2) and body fat were significantly higher in those who developed PE than those who did not, while the TG (mmol L^{-1}), TCHOL (mmol L^{-1}), LDL (mmol L^{-1}), HDL (mmol L^{-1}) and VLDL (mmol L^{-1}) did not show any significant difference between the two groups.

Conclusion:- Visfatin levels increased significantly during the first trimester of pregnancy in women with PE and in women with higher fat percentage than those with normotensive pregnancies and women with less fat percentage. Hypervisfatinaemiais possibly an earlier event in the pathogenesis and potentially precedes preeclampsia.

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Visfatinis expressed abundantly in adipose tissue of both humans and mice and plasma levels increases during the development of obesity (Miehle *et al.*, 2012). Although abundantly expressed in the adipose tissue, visfatin is not tissue specific and can be produced in placenta, foetal membranes (Kendal-Wright *et al.*, 2008) and myometrium(Fasshauer *et al.*, 2002). It is also expressed in bone marrow, liver, muscle, heart lung, and the kidney (Samal *et al.*, 1994).Conflicting results have been published on visfatin levels during pregnancy complicated by preeclampsia (PE). Some researchers reported increased visfatin levels in PE (Fasshauer *et al.*, 2008) while other investigators reported decreased levels (Hu *et al.*, 2008)or values similar to that in normal pregnancy (Mazaki-Tovi *et al.*, 2010).

There is emerging evidence that the first trimester assessment is likely to be the basis for a new approach to antenatal care, whereby data from the maternal characteristicswill be combined with biochemical analysis to evaluate the patient-specific risk for various pregnancy complications, including foetal aneuploidies and other abnormalities; miscarriage, stillbirth, preeclampsia, gestational hypertension, preterm delivery, and birth of small- or large-for-gestational-age neonates(Nicolaides, 2011).

Several risk factors have been suggested as contributing to the development of PE. These include: nulliparity, family and previous history of preeclampsia, diabetes mellitus, higher BMI and excessive body fat, multiple pregnancies, maternal age (less than 20 and greater than 35 years), renal disease, hydatidiform mole, hydrops foetalis, oocyte donation or donor insemination, chronic hypertension and chronic autoimmunedisease (Dekker and Sibai, 2001; Mostello *et al.*, 2008). Twin pregnancy, foetal congenital abnormality(Barton and Sibai, 2008) and high altitude has also been shown to increase the incidence of pre-eclampsia.

The main aim of this study was to determine whether first trimester visfatin concentration, lipids and body fat percentage are altered in pregnancies that subsequently lead to PE.

Materials and methods:-
This work involved participants who were purposively selected for a longitudinal study,consisting of 314 pregnant women,with pregnancy complications attending antenatal visit between 11 and 13 weeks of gestation at the Volta Regional Hospital, Ho, Ghana.

Written informed consent was obtained from each participant and ethical clearance was obtained from joint Committee on Human Research Publication and Ethics of the School of Medical Science, Kwame Nkrumah University of Science and Technology and the KomfoAnokye Teaching Hospital, Kumasi (CHRPE). Questionnaires were administered and information on maternal characteristics and medical history of participants obtained.

Anthropometric parameter measurements:-
Blood pressure was measured using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been made to rest for at least 5 min in accordance with the guide lines of the American Heart Association (Kirkendall *et al.*, 1967). Triplicate measurements were taken with at least 5 min rest interval between measurements and the mean Blood Pressure was recorded to the nearest 2.0 mm Hg.

Height of subjects without shoes were measured using a wall-mounted ruler to the nearest 0.5cm with study participants standing upright and heels put together and the head in the horizontal plane.Weight of subjectswearing light clothing was measured in kilograms with a bioimpedance analyzer (BIA)(BSD01, Pure Pleasure, Cape Town South Africa. www.purepleasure.co.za).

The BIA was used to obtain BMI, body fat percentage, percentage of muscle mass, water, bone, and calories according to the manufacturer's instruction.

Biochemical analysis:-
Fasting blood samples were taken and sera and plasma separated and stored in several aliquots at -21^0C. Visfatin levels were measured in 26 cases that subsequently developed PE and the 286 unaffected participants.

Maternal serum visfatin concentration was measured quantitatively by Sandwich-ELISA technique using VF (Human VF) kit (ElabscienceBiotechnology co ltd WuHan P.R.C). The lowest limit of detection of the assay was 0.094ng/mL. The intra assay and inter assay CV ranged from 2.5% to 9.5% and from 4.7% to 8.3%, respectively.

Lipid profile was done using the Vitros dry chemistry analyzer (Ortho-Clinical Diagnostics, Johnson & Johnson, 50-100 Holmers Farm Way, High Wycombe, Buckinghamshire, HP124DP, United Kingdom). None of the samples in this study were previously thawed and refrozen.

Diagnosis of PE:-
Blood pressure measurement was repeated and urine protein determined after 20 weeks of gestation. Diagnosis of PE was based on systolic blood pressure of 140mmHg or more, diastolic blood pressure of 90mmHg or more (or both) and proteinuria of 2+ or 3+ on semiquantitative examination.

Statistical analysis:-
Data was first entered unto a Microsoft Office Excel 2007 and GraphPadPrism 3.02 program was used to analyze the data. The values were expressed as mean plus/minus standard deviations (mean ± SD). Student *t*-test was used for comparison of means of variables between case and control subjects. The level of statistical significance was set at $p<0.05$ for all tests and at 95% confidence interval (CI).

Results:-

A total of 314 subjects were recruited for this study. The mean age of participants was 29.01 ± 5.90 years with minimum and maximum age of 15.00 and 46.00 years respectively. The mean BMI of the respondents was $25.70\pm4.50Kg/M^2$ while the mean body fat percent was $30.5 \pm 7.5\%$. The mean percentage of body water was $48.70\pm 7.20\%$ and the mean muscle mass percent was $30.3\pm3.8\%$ while the amount of available calories was 1337.10 ± 215.30. The mean gestational age at the start of the study was 11.70 ± 0.80 weeks. The mean first trimester systolic and diastolic blood pressure were111.6 ± 8.4, 69.90 ± 8.90 mmHg respectively (Table 1).The mean triglycerides, total cholesterol, HDL, LDL and VLDL were 1.60 ± 0.80, 5.8 ± 0.90, 1.50 ± 0.90, 3.90 ± 1.80 and 0.70 ± 0.40 mmol/l respectively (Table 2). When the baseline demographics, lipid and visfatin characteristics were stratified by preeclampsia (Tables 3 and 4), the mean age of those with PE was significantly higher than those without PE $(34.50\pm5.20$vrs 28.60 ± 5.70; $p<0.001)$, BMI was also significantly higher in those who developed PE than those who did not$(32.3\pm2.7$ vrs25.1 ± 4.1; $p <0.0001)$. There was no significant difference in the bone density of the PE group and the controls $(p=0.167)$. The mean first trimester SBPandDPBdid not also show significant difference between the PE group and those without PE. There were however significant differences in the body fat% $(p<0.0001)$, body water % $(p<0.0001)$, muscle mass%, $(p<0.0001)$ and body calories $(p=0.003)$ when the PE group was compared to those without PE.

The lipid profile parameters did not show any significant difference between the PE group and those without PE.However, Visfatin levels, was significantly higher $(8.6 \pm 4.2$ vrs 4.4 ± 2.7 ngmL$^{-1})$, $p<0.0001)$ in the PE group compared to those without PE.

Table 1:-Antepartum characteristics, of the study respondents at first trimester of pregnancy.

Variables	Mean ± SD	Min	Max
Age (years)	29.1 ± 5.9	15	46
Mass (squared)	2.5 ± 0.2	2.2	2.9
Weight (kg)	64.8 ± 11.3	43.9	94.0
Height (m)	1.6 ± 0.05	1.5	1.7
BMI (kg m^{-2})	25.7 ± 4.5	18.9	38.4
Bone Density	2.1 ± 0.3	1.5	3.3
Body Fat (%)	30.5 ± 7.5	18.9	49.2
Body water (%)	48.7 ± 7.2	33.0	60.0
Muscle Mass (%)	30.3 ± 3.8	21.0	36.2
Calories	1337.1 ± 215.3	1036.0	3081.0
Gestational age (weeks)	11.7 ± 0.8	11.0	13.0
SBP (mmHg) (First Trimester)	111.6 ± 8.4	90.0	130.0
DBP (mmHg)aq(First Trimester)	69.9 ± 8.9	50.0	90.0

Data are presented with descriptive statistics. Min-Minimum; Max-Maximum; BMI-body mass index; DBP-diastolic blood pressure; SBP-systolic blood pressure.

Table 2:-Antepartum lipid and adipokine status of the study respondents at first trimester of pregnancy.

Variables	Mean ± SD	Min	Max
Lipids			
TG (mmol L^{-1})	1.6 ± 0.8	0.50	3.70
TC (mmol L^{-1})	5.8 ± 1.8	2.40	9.70
HDL (mmol L^{-1})	1.5 ± 0.9	0.04	3.42
LDL (mmol L^{-1})	3.9 ± 1.8	0.16	8.2
VLDL (mmol L^{-1})	0.7 ± 0.4	0.19	1.71
Adipokine			
Visfatin (ng mL^{-1})	4.7 ± 3.0	0.33	19.48

Data are presented with descriptive statistics. Min-Minimum; Max-Maximum; TG-triglyceride; TC-total cholesterol; HDL-high density lipoprotein; LDL-low density lipoprotein; VLDL-very low density lipoprotein

Table 3:-Comparison of baseline demographics of the study respondents stratified by pre-eclampsia.

Variables	With PE (N=26)	Without PE (N=286)	p-value
Age (years)	34.5 ± 5.2	28.6 ± 5.7	< 0.0001
Mass (squared)	2.49 ± 0.2	2.5 ± 0.2	0.182
Weight (kg)	80.1 ± 4.7	63.4 ± 10.7	< 0.0001
Height (m)	1.57 ± 0.05	1.59 ± 0.05	0.179
BMI	32.3 ± 2.7	25.1 ± 4.1	<0.0001
Bone Density	2.2 ± 0.1	2.1 ± 0.3	0.167
Body Fat (%)	41.6 ± 2.7	29.6 ± 7.0	<0.0001
Body water (%)	38.2 ± 2.9	49.6 ± 6.7	<0.0001
Muscle Mass (%)	24.8 ± 1.3	30.9 ± 3.5	<0.0001
Calories	1439 ± 50.5	1322 ± 197.0	0.003
Gestational age (weeks)	11.7 ± 0.8	11.7 ± 0.8	0.642
SBP (mmHg)(First Trimester)	113.1 ± 7.9	111.5 ± 8.4	0.363
DBP(mmHg) (First Trimester)	68.9 ± 7.1	70.1 ± 9.0	0.491

Data are presented as means±SD and proportions. PE-Preeclampsia; BMI-body mass index; DBP-diastolic blood pressure; SBP-systolic blood pressure; CS-Caesarean operation.

Table 4:-Comparison of baseline biochemical characteristics, of the study respondents stratified by PE.

Variables	With PE (N=26)	Without PE (N=286)	p-value
Lipids			
TG (mmol L^{-1})	1.58 ± 0.8	1.61 ± 0.8	0.860
TC (mmol L^{-1})	6.0 ± 1.7	5.8 ± 1.8	0.562
HDL (mmol L^{-1})	1.37 ± 1.0	1.47 ± 0.9	0.588
LDL (mmol L^{-1})	4.2 ± 1.7	3.9 ± 1.8	0.487
VLDL (mmol L^{-1})	0.71 ± 0.3	0.74 ± 0.4	0.654
Adipokine			
Visfatin (ng mL^{-1})	7.3 ± 3.6	4.5 ± 2.8	<0.0001

Data are presented as means±SD and proportions. PE-Preeclampsia TG-triglyceride; TC-total cholesterol; HDL-high density lipoprotein; LDL-low density lipoprotein; VLDL-very low density lipoprotein
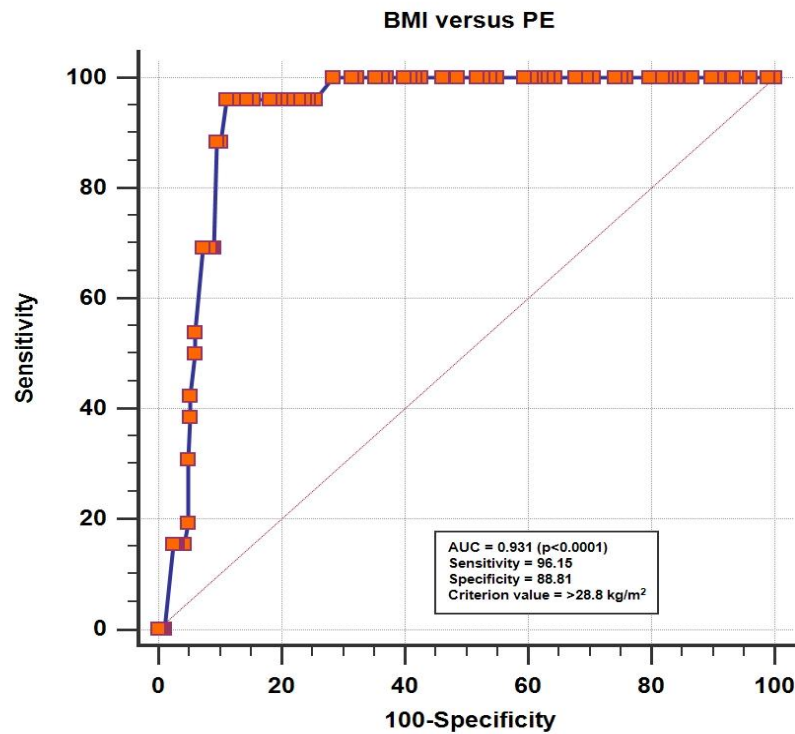
Fig 1:-AUC of BMI versus PE.

Analysis of ROC curve of BMI and PE showed that BMI of $28.8Kg/M^2$and above has a sensitivity of about 96 and specificity of 88.8% in the prediction of PE (p<0.0001).

Discussions:-

Our study shows that visfatin is increased significantly during PE. The increase in visfatin during PE is evident from the first trimester indicating the possible role of visfatin in the pathogenesis of PE. Visfatin is expressed abundantly in adipose tissue as well as in the placenta and foetal membrane(Kendal-Wright *et al.*, 2008). Median concentrations of visfatin during the second and third trimester of normal pregnancy have been reported to be higher than in the first trimester(Mastorakos *et al.*, 2007) further supporting the fact that this protein is produced by the placenta and the foetal membrane. Thus, it is possible that this normal production of visfatin is regulated in such a way as to support the growing foetus, however in PE this supportive role ofVF is disrupted leading to PE. Results of this study is similar to a report by(Fasshauer *et al.*, 2007) which indicated that higher visfatin levels are found in the PE compared to normal pregnancy. Other reports indicated no significant difference between normal and pregnancies complicated bypreeclampsia(Mazaki-Tovi *et al.*, 2010)while some reported lower levels (Hu *et al.*, 2008). The differences in visfatin levels during pregnancy as reported by different researchers could be due to differences in sampling methods, ethnic or geographical differences.Our study suggests a rise in visfatin concentration before the onset of preeclampsia. The potential of visfatin as marker of preeclampsia will require further evaluation using larger sample size. Such studies could even provide useful information on prediction of this condition in order to help initiate interventionprogrammes to mitigate the effect of PE on the maternal and foetal morbidity and mortality. This study did not show any significant difference in lipid profile parameters between women who subsequently developed PE compared to their peers who did not. The finding in this study is contrary to a report by researchers in Brazil who reported significant difference in TG rich proteins (VLDL 1) and small dense lipoprotein(LDLIII) in women with PE compared to normal pregnant women(Lima *et al.*, 2011). The difference could be due to differences in sample size and period of gestation during which sampling was done. Whereas their sample size was 8 each for controls and samples, our study involved 26 cases with PE and 286 without PE. Additionally our samples were taken before the onset of PE. Furthermore our research did not measure those specific TG-rich proteins and that could also be the reason for the differences in our findings and what they reported. Other studies have also reported lipid abnormalities in preeclamptic women compared to their normotensive counterparts(Sattar *et al.*, 1997; Belo *et al.*, 2002). Our study shows general dyslipidaemia in both pregnant women with and without PE though no

significant differences exist between them. The fact that first trimester lipids did not show any significant difference between those who subsequently developed PE and those who did not, suggests that the atherogenic lipid profile generally seen in pregnant women may be insufficient in predicting the likelihood of developing PE.

Our study showed that obese women are likely to develop PE during the course of theirpregnancy and corroborates an earlier study which indicated that the risk of developing PE increases about two-to-three folds in obese women (Bodnar *et al.*, 2005) and also similar to a study which associated maternal morbid obesity to a number of pregnancy complications including PE(Cedergren, 2004). Analysis of the AUC, point to the fact that higher BMI($\geq$28.8Kg/m^2) has high accuracy of determining pregnancies that are likely to develop PE. This suggests that excessive fat accumulation in the body contributes substantially to the pathogenesis of preeclampsia.

Conclusions:-
Visfatin iselevatedin obese pregnant women and also during first trimester of pregnancies that subsequently develop preeclampsia and could potentially provide a predictive role in the pathogenesis of preeclamptic conditions. The dyslipidaemia observed in normal pregnancies is not significantly different from that seen in PE.Body Mass Index and percentage of body fat are better indicators of pregnancies that are likely to develop PE than lipids. Hypervisfatinaemia precedes preeclampsia.

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