MID-GESTATIONAL SERUM LEPTIN CONCENTRATION IN OBESE AND NON-OBESE GHANAIAN MOTHERS AND ITS RELATIONSHIP WITH GESTATIONAL OUTCOME.

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Abstract

Background: Leptin is produced abundantly in adipose tissue and in human placental trophoblast so serum leptin concentration in BMI matched pregnant women is higher than non-pregnant women. The aim of the study was to compare serum leptin concentration of obese and non-obese pregnant Ghanaian women and to match it with pregnancy outcome.

Method: This was a nested case control study, for which 80 antenatal women grouped into obese (cases) and non-obese (control) based on their booking body mass index (non-obese≤29.9kg/m^2<obese). The participants had their mid gestation (20-24 weeks) serum stored until delivery and serum leptin concentration of the first 20 cases and 20 controls who delivered at the study site were compared to examine if it had effect on gestational outcome. Correlation between leptin concentration, gestational age at delivery and birth weight were assessed using Spearman's correlation coefficient.

Results: The ages, median (range) 31(20-39) of cases and controls 32 (17-40) were not significantly different. There was no significant difference between the serum leptin concentration of cases 1.9 (0.5-50) ng/ml and controls 1.9 (1.5-50) ng/ml (P>0.05) and these had no correlation with maternal BMI or with baby's Apgar scores. Our study subsequently, found no correlation between maternal mid-gestational leptin concentration and gestational age at delivery, as well as with birth weight of neonates.

Conclusion: Mid-gestational leptin concentration did not correlate with BMI in pregnant Ghanaian women and our study failed to find correlation between mid-gestational leptin concentration and gestational age at delivery.

Key Words: *Body Mass Index, Leptin, Mid-gestation, Parturition*

Introduction

Leptin, the obese (*ob*) gene product, is a peptide hormone which is produced abundantly in adipose tissue and released into the blood circulation[1,2]. Leptin concentration is known to be higher in obese persons and well correlated with body fat mass and body mass index (BMI)[3,4]. Leptin plays extensive role in energy metabolism and works as a local mediator for glucose and lipid metabolism[5,6]. It suppresses appetite andincreases energy expenditure, thereby decreasing body weight[7].

Leptin is also produced in the placental trophoblast and secreted into maternal and fetal circulations, so serum leptin concentration in pregnant women are higher than non-pregnant women[8-10].

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It was previously reported that, increase in leptin concentration peaks in the second trimester, decreasing slightly in the third trimester but remaining higher above first trimester concentration[11]. Leptin concentration further increased during labour declines to pre-pregnancy levels in the postpartum period[12]. A positive umbilical venous-arterial difference of leptin concentration and its rapid decline after birth also suggests a possible contribution of placental leptin production on fetal metabolism during pregnancy and in labour[9]. The fetus depends on the mother for its metabolism, therefore defective maternal metabolism may adversely impact on fetal outcome, including birth weight. Obesity in pregnancy, a defect in maternal metabolism is found to be a risk factor for adverse fetal and maternal outcomes[13-15].

According to the Ghana Demographic Health Survey, 40% of women aged 15-49 years are overweight or obese indicating significant proportion of obesity in pregnancy in the country[16]. However, there is limited information regarding leptin, the obese (*ob*) gene product, and pregnancy outcomes in the Ghana. Leptin resistance is reported in pregnancies complicated by maternal obesity[17] and a recent study by Bawah *et al* in

Ghana, found a significantly higher first trimester leptin concentration among those who subsequently developed gestastional diabetes mellitus (GDM) as compared to those who did not (35.0434±8.700 21.9352±9.192 respectively[18].The aim of the study was to compare mid-gestatioan serum leptin concentration of obese and non-obese pregnant Ghanaian women and to correlate it with pregnancy outcomes such as gestational age at delivery, birth weight, Apgar scores at 1 and 5 minutes.

Methods

Study participants and design

This was a nested case control study involving mothers who booked for antenatal care at the Korle-Bu Teaching Hospital (KBTH) between 20-24 weeks of gestation from September15th to October 26th, 2015. All pregnant women with singleton gestation were eligible. We excluded those who had plans to deliver outside the study site. The sample size for the study was calculated based on previous studies indicating that the mean leptin levels in pregnant women is greater than 37ng/ml (37.5±5.8ng/ml) and that among non-pregnant women is less than 37ng/ml (20.3±4.5ng/ml)[12]. In non-pregnant women the leptin concentration is higher in obese as compared with non-obese women[19]. At the 95% confidence level and power of 90% based on EPI INFO version 4.3.1, a minimum sample size of 18 for each patient group (obese and non-obese pregnant women) was obtained. Accounting for losses to follow up and incomplete data, the sample size was increased by 10% giving 20 persons in each group. With information that about 40% of women aged 15-49 years are overweight or obese and another study reporting that 9%-37% of Ghanaian women of child bearing age are obese,[16,20] we recruited 80 pregnant women after written informed consent by a simple random sampling. The recruited participants were then grouped into obese (cases) and non-obese (controls) pregnant women, based on their booking body mass index (BMI) (Supplementary table 1) and followed up until delivery. Selection of final follow up (nested) participants were based on first 20 cases or controls who delivered at KBTH, until the desired sample size for each group was obtained: the first 20 obese (BMI>29.9kg/m^2) and 20 non-obese (BMI≤.29.9kg/m^2) parturients. The flow chart of patient's participation and follow up is shown in Figure 1. The research protocol was approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (Study protocol no: MS_ET/P3.1/2014-2015).

Maternal and infant demographics and anthropometry

All participants were interviewed with a structured questionnaire to obtain demographic information and anthropometry. The height of the mothers was measured using a stadiometer attached to a mechanical scale (SecaCE 0123) with maximum capacity of 200cm for height and 160kg for weight and graduation in 1mm and 0.5kg for height and weight respectively. In measuring the height, the women had no shoes on and any head gears were also taken off. In measuring the weight of the women, minimal clothing was ensured. These were used to determine the mother's BMI at the antenatal visit between 20 weeks and 24 weeks gestation.

The weight of the baby was determined after delivery using a mechanical scale (Kinlee with a maximum capacity of 20kg and a graduation of 0.1kg). Babies with birth weight<2.500kg (2500g) were classified as low birth weight and those with birth weight>4000g, obese. The gestational age was estimated using first trimester ultrasound scanned report.

Blood sampling and determination of leptin concentration

After the written informed consent, about 5mls of blood sample was drawn from the antecubital vein of each participant with sterile disposable needles and syringes. The blood sample was transferred immediately into a gel separator tube and gently inverted several times (5-10) for thorough mixing of blood with clot activator to separate the clot from the serum in the specimen bottle. Samples were then allowed to clot by leaving them standing for about 30 minutes, placed on ice and transported to the laboratory at the Department of Chemical Pathology of the School of Biomedical and Allied Health Sciences, University of Ghana where samples were centrifuged at 3,000 x g for 3 minutes at 4°C. The serum samples thus obtained were transferred into Eppendorf tubes and stored at -20°C until required for use.

ELISA analysis

Reagents

All reagents used for the study, were bought from MedPoint Medical Laboratory and Equipment Supplies, Kwabenya, Accra. Ghana.

Leptin assay was conducted at the Virology unit of Microbiology Department, School of Biomedical and Allied Health Sciences, also of the University of Ghana. Prior to the ELISA, all reagents were brought to room temperature (18-25°C). Standard samples of concentrations were prepared in duplicates according to protocol provided. The antibody-coated micro-plate module was affixed onto the frame. The blank wells, standard wells and test sample wells were set respectively. Prepared sample, standard and HRP-Conjugate reagent was added. 50µl of the standard prepared was pipetted into the pre-coated 96-wells plates in duplicates. 40µl of the sample diluents and 10µl of the sample was added. 50µl of horseradish peroxidase (HRP) were added into each well except the blank. The plates were sealed with adhesive cover and incubated for 60 minutes at 37°C. After incubation, the excess liquid was discarded and the wells washed five times with the washing buffer. 100µl of the anti-human antibody was then placed into each well. The micro-plate was then

incubated for 15 minutes at 37°C and 50µl of stop solution was added into each well. A colour change occurs and the absorbance is read at 450nm within 15 minutes. The Leptin concentrations were calculated using the standard curve. The detection limit of this assay is 1.0 ng/ml for serum leptin.

Data handling and analysis

Data were entered in excel spread sheet (Microsoft company, USA) and imported into SPSS version 22 (IBM SPSS, Chicago, Illinois, USA) for analysis. Descriptive analysis was performed and baseline characteristics of the participants were presented as median (range)for continuous variables.The normality assumptions of the continuous variables were assessed using Q-Q plots and confirmed with Shapiro-Wilk text. Generally, all the variables violated the normality assumptions and therefore nonparametric test (Mann-Whitney test) was used in the comparison of the variables between obese and non-obese mothers. Correlation between the circulating maternal serum leptin concentration and outcome variables was assessed using Spearman's correlation coefficient. Results of the analysis were considered statistically significant with a *P*-value of <0.05.

Results

The flow chart of study participants is shown in figure 1. The prevalence of obesity among the 80 baseline study participants was 41%. Table 1 outlines BMI characteristics at booking. Selection of cases to controls was 1:1 (obese (20) and non-obese (20).

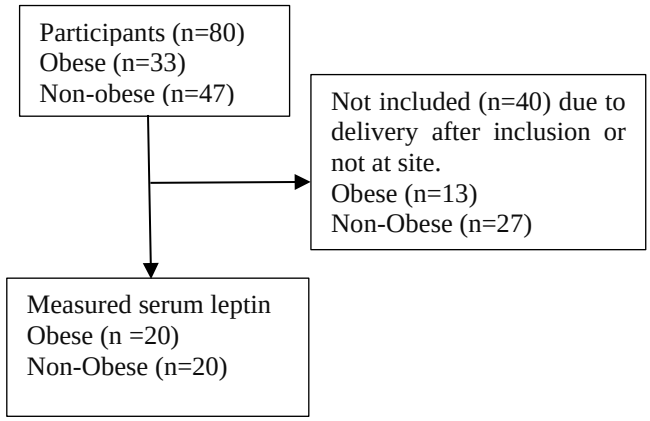

Fig 1. Flow chart of study participants

However, the obese mothers were of a significantly higher gravidity than the non-obese mothers 3(1-10) and (1-6) respectively,*P* < 0.05(Table 1). There was no significant difference in serum leptin concentration between obese and non-obesemothersobese 1.9 (0.5-50) ng/ml versus 1.9 (1.5-50) ng/ml respectively. Mid-pregnancy serumleptin concentration did not correlate with mid-pregnancy BMI and there was nosignificant inverse correlation between maternal leptin concentration and gestational age at delivery for both obese cases and non-obese controls(r_s=-0.07, n=20, *P*=0.75)(fig. 2A), r_s=-0.27, n=20, *P*=0.27)(fig. 2B).Birth weight also showed no significant inverse related with maternal leptin concentration both in cases and controls (r_s=-0.29, n=20, *P*=0.22)(fig. 3A). r_s=-0.02, n=20, *P*=0.49)(fig. 3B).

Table 1. Maternal and neonatal characteristics of non-obese and obese women

Category	**Non-obese (n=20) Median (Range)**	**Obese (n=20) Median (Range)**	***P*-value**
Age (yrs)	32 (17-40)	31 (20-39)	0.924
Gravidity	2 (1-6)	3 (1-10)	0.022
Parity	0 (0-2)	1 (0-3)	0.067
Weight (kg)	66 (53-79)	84.5 (76-137)	< 0.001
BMI (kg/m^2)	25.3 (19.4-29.3)	32.8 (30.5-53.5)	< 0.001
Leptin (ng/ml)	1.9 (1.5-50)	1.9 (0.5-50)	0.183
GAD (weeks)	38.5 (31-42)	37 (34-41)	0.0123
Birth Weight (g)	3.035 (1.100-3,755)	3.145 (2.075-4.090)	0.285
Apgar scores 1 min.	7 (2-8)	7 (5-9)	0.507
Apgar scores 5 mins.	8 (5-9)	9 (7-10)	0.159

Abbreviation: GAD; Gestational Age at delivery, n; number in a group, min.; minute.

Supplementary Table 1. Body mass index (BMI) characteristics of participants at booking

Variable	Non-Obese (n = 47) Median (range)	Obese (n = 37) Median (range)	P value
Gestational weeks	20 (20-24)	20 (20-24)	0.95
Weight	67(44-86)	91(68-137)	< 0.001
Height	1.61 (1.5-1.71)	1.6 (1.16-1.72)	0.43
BMI	25.28 (19.04-29.73)	33.26 (30.22-69.86)	< 0.001

Abbreviation: n; number in a group
N: number in group

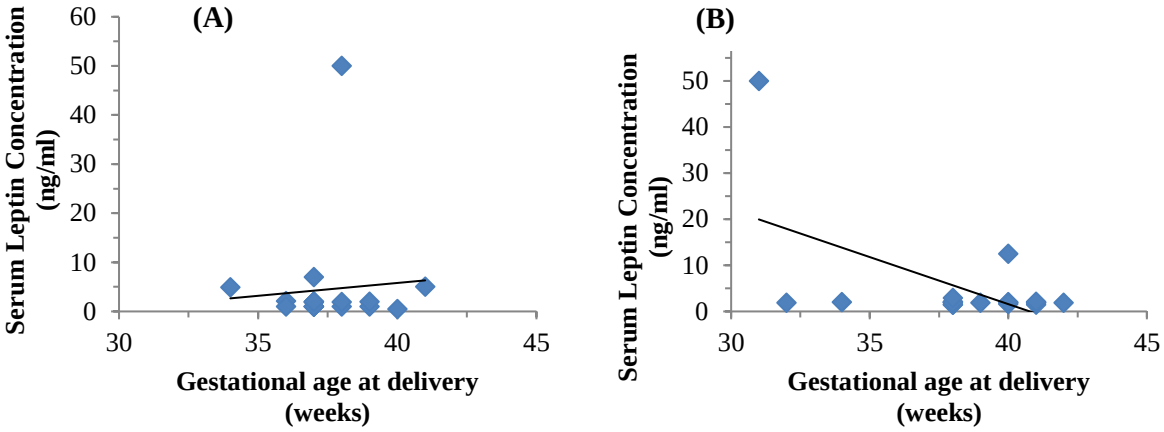

Fig 2. Correlation between maternal mid-gestational serum leptin concentration and gestational age at delivery. **(A)** Correlation in obese pregnancy. (**B**) Correlation in non- obese pregnancy. Correlation coefficient: r_s (95% confidence interval), is shown for twenty women in each group (n=20, *P*>0.05). Two women (1 non-obese, 1 obese) had Leptin concentration of 50ng/ml each.

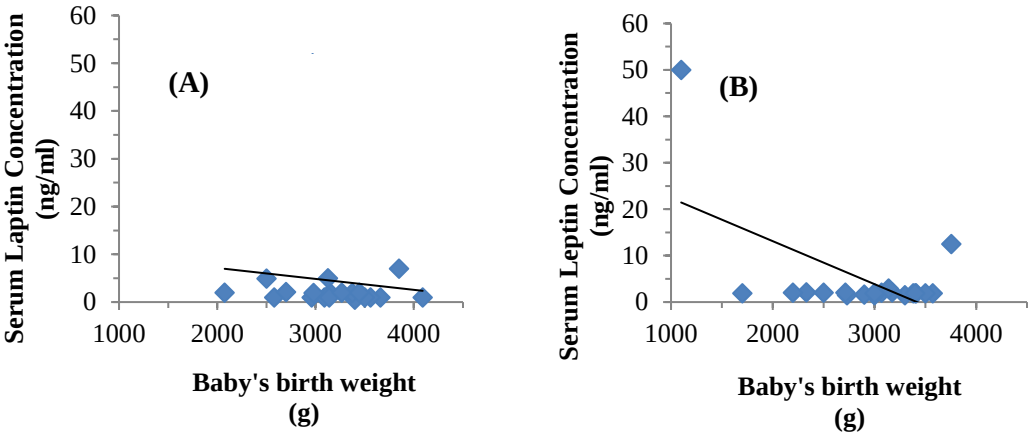

Fig. 3 Correlation between maternal mid-gestational serum leptin concentration and neonatal weight at delivery. **(A)** Correlation in obese pregnancy. (**B**) Correlation in non-obese pregnancy. Correlation coefficient: r_s(95% confidence interval), is shown for twenty women in each group (n=20, *P*>0.05). Two women (1 non-obese, 1 obese) had Leptin concentration of 50ng/ml each

Table 2. Maternal and neonatal characteristics of vaginal and cesarean deliveries

Category	VD (n=21) Median (Range)	CS (n=19) Median (Range)	P-value
Age (yrs)	30 (17-40)	34 (20-39)	0.132
Gravidity	2 (1-10)	3 (1-8)	0.1787
Parity	1 (0-2)	1 (0-3)	0.486
Weight (kg)	75 (56-137)	78 (53-100)	0.924
BMI (kg/m^2)	28.2 (19.4-53.5)	30.5 (21.6-40.0)	0.655
Leptin (ng/ml)	1.9(1.0-50)	1.9 (0.5-7)	0.134
GAD (weeks)	38 (31-41)	38 (34-42)	0.036
Birth Weight (g)	3,000 (1,100-3,755)	3,300 (2,075-4,090)	0.030
Apgar's score at 1 min	7 (5-8)	7 (2-9)	0.916
Apgar's score at 5 min	8.5 (7-9)	8 (5-10)	0.856

Abbreviation: VD; Vaginal Delivery, CD; Cesarean Delivery, GAD; Gestational age at delivery, n; number in a group.

The median birth weight for babies delivered by obese and non-obese mothers was also not significantly different (3.145g versus 3.035 respectively $P > 0.5$). Seven babies (17.1%) had low birth weight (LBW< 2500g), comprising 2 (9.5%) from obese; and 5 (25.0%) from non-obese mothers, but there was no significance difference in leptin concentration between women who gave birth to low and normal weight babies (Table1). Eleven (55%) obese mothers were delivered by cesarean section as compared with 8 (40%) non-obese mothers. Maternal age, BMI, gestational age at delivery, baby's weight and Apgar score were not significantly different between the vaginal and cesarean groups (Table 2). Leptin concentration was higher in the vaginal group 1.9 (0.5-50) ng/ml, n=21) as compared with the CS group 1.9 (0.5-7) ng/ml, n=19) but the difference did not reach statistical significance.

Discussion

The study provided the first leptin report on obesity in pregnancy in Ghanaian women at the Korle-Bu Teaching Hospital. The study observed high prevalence (41%) of obesity in pregnancy among the participants, confirming the previous observations of high prevalence of obesity in child bearing women in Ghana[16,20]. Obesity was significantly associated with high gravity in the present study as in another study reported in the African subregion[21].

We did not find any significant difference in the mid-pregnancy leptin concentration between obese and non-obese mothers. Several studies have attempted to examine association between maternal circulating leptin and BMI. One study reported significant association in early and late pregnancy[22], whereas others could not find significant association[23,24]. A recent study reported that at gestational week 29, the mean leptin concentrations are not significantly higher in women with obesity class II compared to women in obesity class I, in spite of the differences found in early gestation and in the postpartum[23]. This may reflect different physiological states or dysregulation of leptin during different gestational weeks and in pathological pregnacies[25-28].

More of the non-obese mothers delivered per vaginum (60%) than the obese mothers (45%), confirming previous studies that obese women have increased risk of complications during pregnancy and delivery[13,14,29-31].Obesity showed no significant association with neonatal outcome in our present study. In a similar study, Shroff et al studied mid-gestation maternal serum leptin levels which were significantly higher in the obese women compared to those with normal BMI but were markedly attenuated after adjustment for prepregnancy BMI[24]. In their study, mothers who delivered large-for-gestational age neonates had significantly higher levels of serum leptin and they concluded that mid-pregnancy leptin levels might correlate with fetal growth status. In our study, on the contrary, there was no significant difference between maternal serum levels and the birth weight.

A relatively higher leptin concentration was seen in the vaginal birth group but this did not reach statistical significance. However, this may have physiological significance and calls for further studies. Strengths of this study include the recruitment of a larger base eligible women and allowing natural self-selection in each study group through delivery at the study site to mitigate lost to follow up which is common at the study site. This is because many women comes for antenatal care at this tertiary referral center and go to deliver elsewhere. Possible limitations are the sample size of 40 which could contribute to lack of power to observe differences in outcomes; however, the sample size was relevant for meeting the objective of the study. It is plausible that, a longitudinal sampling of blood at several gestational ages would have given a broader spectrum in the changes of leptin concentration during the course of the pregnancy. Another limitation was the wide range of leptin values with extreme outliers with very low and very high values than reported values of leptin in pregnancy.[11,12,18,24] However, having extreme values equally distributed in both the cases and the controls, normalize the effect of bias that could have affected the difference seen in the patterns observed in this study. Also, non-parametric test was used to assess the statistical differences as the continuous variables were not normally distributed. Future research could take a longitudinal approach that could relate the dynamics of leptin changes with respect to weight gain and changes in the physiology of the pregnancy as it progresses. Nevertheless, the findings of this study has clinical and scientific significance in our sub-region, being the first leptin study comparing the obese and non-obese women in pregnancy in Ghana and suggesting a possible role of leptin in the metabolism of normal and obese human pregnancy at the Korle-Bu Teaching Hospital.

In conclusion,there was no significant difference between obese and non-obese pregnant women with respect to mid-gestationalserum leptin concentration. Our study subsequentlyfound no correlation between maternal mid-gestational leptin concentration and gestational age at delivery, as well as with birth weight of neonates, neither in the obese cases nor in the non-obese controls. This finding calls for more research of the role of leptin in human pregnancy outcome.

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