

RESEARCH ARTICLE

Placental peptides metabolism and maternal factors as predictors of risk of gestational diabetes in pregnant women. A case-control study

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Abstract

Background

Gestational diabetes is a risk factor for perinatal complications; include shoulder dystocia, birth injuries such as bone fractures and nerve palsies. It is associated with later development of type 2 diabetes, the risk of macrosomia and other long-term health effects of infants born to diabetic mothers. The study assesses placental peptides and maternal factors as potential predictors of gestational diabetes among pregnant women.

Material and methods

A total of 200 pregnant women were recruited for the study, 150 pregnant women without pre gestational diabetes including 50 women with low risk factors of diabetes as controls and 50 other pregnant women with pregestational diabetes as control. Fasting blood glucose and the lipid profile were determined by enzymatic methods using Envoy[®] 500 reagents (Vital Diagnostics, USA). Glycated haemoglobin was assessed using the Cation Exchange resin method. Leptin and the Human Placenta Lactogen were assayed using the Sandwich-ELISA technique. Beta chorionic gonadotrophin, insulin, progesterone and estradiol were determined using chemiluminescence immunoassay technique on MAGLUMI 600 analyzer. Anthropometry, including BMI and blood pressure were also measured.

Results

Fasting plasma glucose (FBG), insulin, insulin resistance, glycated haemoglobin and Human Placenta Lactogen(HPL)were significantly ($p < 0.0001$) increased in the pregestational diabetic women whereas progesterone and estradiol were significantly decreased. In the second trimester however, there was no significant difference ($p > 0.05$) in estradiol, insulin, insulin resistance and HPL between the pregnant women who developed gestational diabetes and those who did not. Leptin, progesterone and FBG were significantly increased

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in those who developed GDM. The risk of developing gestational diabetes increased with overweight (OR = 1.76, P = 0.370) and family history of diabetes (OR = 2.18, P = 0.282).

Conclusion

Leptin, progesterone, estradiol estimated in this study were increased in the gestational diabetes mellitus women and fairly predicted gestational diabetes in the non-diabetics pregnant women. Obesity, aging and family history of diabetes were strongly predictive of gestational diabetes.

Introduction

In a normal pregnancy, sensitivity to insulin reduces with increasing gestation age [1]. It is a physiological programming designed to shift metabolic fuel to the fetus for development [2]. In this state, insulin sensitivity in pregnant non-diabetics is reportedly reduced [3]. The decrease in insulin sensitivity with progression in pregnancy is due partly to the inhibitory effect of placenta peptides (C-reactive protein, leptin and human placental lactogen etc) on insulin secretion [4,5]. The effects of these placenta peptides and hormones cause the enlargement of the islets of Langerhans cells and or the hyperplasia of the pancreatic β -cells [6] to increase the secretion of more insulin, resulting in compensated hyperinsulinaemia [7]. Obesity and chronic insulin resistance during pregnancy are most common factors that exacerbate β -cell dysfunction [8]. Gestational diabetes mellitus (GDM) occurs when the pancreatic β -cells are unable to produce sufficient insulin to offset the persisting insulin resistance in pregnant women [3].

Similar frequencies of HLA-DR2, DR3 and DR4 antigens in GDM as in healthy pregnant women have been reported and this has been found to be associated with low prevalence of autoimmune destruction of β -cells and therefore rules out the possibility that GDM is a disease of autoimmune origin [9].

Gestational diabetes mellitus is reported in 2 to 9 percent of all pregnancies, [10] it is a risk factor for adverse perinatal complications that may later result in the development of type 2 diabetes. [11]. The long-term health effects on infants born to mothers with gestational diabetes include impaired glucose tolerance, [12] obesity [13] and impaired intellectual ability [14].

Pregnant women with high risk factors for GDM or showing clinical signs of diabetes are usually subjected to an oral glucose tolerance test (OGTT) at first trimester and then again between 24 and 28 weeks if the initial tests were negative. At weeks 24–28 of gestation, it is recommended that all women undergo an OGTT. GDM is defined as a fasting plasma glucose level ≥ 5.5 mmol/l and/or a 2-h plasma glucose level ≥ 8.5 mmol/l after a fasting 75-g OGTT [15]. Treatment of gestational diabetes reduces perinatal morbidity and may also improve the woman's health-related quality of life [16].

Screening for GDM is limited, the standard screening procedure for diabetes and pre-diabetes, such as fasting blood glucose and glycated haemoglobin, are not recommended for screening for GDM. At first trimester FBG alone does not distinguish between GDM, impaired glucose tolerance or preexisting diabetes, also HbA1c predicts glucose metabolic state over a period of 3 months and therefore does not distinguish between preexisting diabetes and GDM. However, the recommended method is an oral glucose tolerance test (OGTT) [17] and this is expensive, invasive, and requires hospital visit and multiple blood draws.

The development of abnormal glucose metabolism after gestational diabetes can be predicted readily by means of available clinical and anthropometric measurements.[18, 19] Similarly, if there were a proper assessment of the various risks as well as markers that may predict the onset of gestational diabetes among pregnant women, it would in turn allow practitioners to better manage patients and prevent many pregnancy associated complications. The current study therefore was aimed at assessing the roles of placental peptides and maternal factors as potential predictors of gestational diabetes among pregnant women in the Tema Metropolis of Ghana.

Methods

A cross-sectional case-control study was carried out at the Tema General Hospital, Tema Polyclinic and Provita Specialist Hospital all in Ghana, from March 2014 to March 2015. A total of 200 pregnant women aged between 17–45 years and mean age of 28.43 ± 4.95 years were recruited for the study, comprising of 150 pregnant women without pregestational diabetes (subjects) including 50 women in the group with low risk factors of diabetes as controls. Fifty (50) other pregnant women with pregestational diabetes were recruited as controls.

Study participant selection

To determine gestational diabetes in pregnant women, a standard OGTT was performed between the 24th and 28th week of gestation after an overnight fast (8–12 hours), by administering 75 g anhydrous glucose in 300 ml water. Women were advised to follow a normal diet 48 hours before the oral glucose-tolerance test (OGTT). However, pregnant women belonging to high risk diabetes population were screened during the first trimester in order to detect a previously undiagnosed diabetes mellitus. Gestational age was also determined on the basis of the woman's last normal menstrual period and if it coincided within 1 week of the date determined by ultrasound done between 16th and 20th weeks of gestation, otherwise the ultrasound estimates was used. A pretested guided questionnaire was administered to collect demographic data of family history of diabetes, parity, miscarriages, and complications of pregnancy. Anthropometric parameters including BMI and blood pressures were measured. Pregnant women who reported at the antenatal clinic were included; unless they opted out of the study, non pregnant women seeking fertility advice were excluded from the study.

Ethical clearance approval

Ethical clearance was obtained from the Committee on Human Research and Publications Ethics (CHRPE)(REF) of the School of Medical Sciences (SMS), Kwame Nkrumah University of Science & Technology (KNUST).

Informed consent of subjects

Clients who reported at the antenatal clinic and agreed to participate in the study gave written informed consent after detailed explanation of the study procedures had been given to them before the recruitment into the study. Guardians of participants below 18 years of age gave consent on their behalf.

Sample collection

An over-night (8–12hr) fasted sample of 6ml venous blood sample was taken from subjects during the first trimester and between the 24th and 28th week of gestation. The sample was dispensed into gel separator tubes (yellow top), EDTA (mauve top) and Fluoride tubes (for

glucose) for analysis. Blood samples were allowed to clot. After clotting, samples were centrifuged at 3000 g for 4 min and the sera separated into appropriate tubes and frozen at -20°C until analysis. Metabolites analyzed for included total cholesterol, triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), fasting blood glucose serum insulin, glycosylated haemoglobin, leptin, estradiol, progesterone, Human placenta lactogen and beta HCG. The homeostasis model assessment index-insulin resistance (HOMA-IR), based on fasting insulin and glucose measured in a single blood sample, was used to calculate insulin resistance [20].

Biochemical analysis

The frozen serum samples were thawed to room temperature before being analyzed.

Lipid profile

Triglycerides, total cholesterol and HDL-cholesterol were assayed using enzymatic methods with Envoy[®] 500 reagents (Vital Diagnostics, USA) according to the manufacturer's specification on BT 5000[®] Random Access Chemistry Analyzer (Biotecnica, Italy).

Glycated haemoglobin

The Cation Exchange Resin method for the selective estimation of glycated haemoglobin (HbA1c) method in human whole blood was used, on Microtech 3000 semi auto analyzer.

Leptin

Maternal serum leptin concentration was measured quantitatively by Sandwich-ELISA technique using Leptin (Human LEP) kit (Elabscience Biotechnology 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.

Human placenta lactogen (HPL)

Maternal serum Human placenta lactogen concentration was measured quantitatively by Sandwich-ELISA technique on Human Reader HS, a semi auto analyzer.

Progesterone (P4) and estradiol (E2)

Progesterone and Estradiol, were determined using chemiluminescence immunoassay technique on MAGLUMI 600 analyzer. The method uses ABEI label anti- E2/anti-prog antibody and a purified E2/ Prog antigen to coat magnetic microbeads. The ABEI labeled microbeads were incubated at 37°C forming antigen antibody complex which was precipitated in a magnetic field and the supernatant discarded. The addition of starter 1 and 2 initiates chemiluminescence reaction, and the light signal produced measured.

Beta chorionic gonadotrophin (β -HCG) and Insulin assay

Beta chorionic gonadotrophin and insulin were also determined using MAGLUMI 600 analyzer. The sandwich chemiluminescence immunoassay technique was used, adhering to the manufacturers' protocol.

Statistical analysis

Results were expressed as mean ± S.D and statistical analysis performed using SPSS version 20.0 (SPSS Inc.) and GraphPad prism 5 for Windows. Normal distribution and homogeneity of the variances were tested using Kolmogorov-Smirnov and Levene tests, respectively. Student t-test was used to compare the significance of the difference in the mean values of any two groups and chi square analysis was used to compare frequency between the two groups. Linear regression analysis was used to study the correlation between the parameters. Correlations between parameters were analyzed using the Pearson R test for variables with normal distribution and ROC to determine the sensitivity and specificity of FBG, HbA1c, Insulin and β-HCG as markers of GDM. P<0.05 was considered statistically significant.

Results

Table 1 show 50 women with pre-gestational diabetes (controls) and 150 pregnant women without diabetes (cases) during first trimester, 50 of which have very low risk of developing diabetes. Fasting plasma glucose, insulin, insulin resistance, glycated haemoglobin, and Human Placenta Lactogen were significantly increased (p<0.0001) in the pregestational

Table 1. Biochemical characteristics of non-diabetic pregnant women (Cases) and pregnant women with regestational diabetes (control) in the first trimester.

Variable	Cases (n = 150)	Controls (n = 50)	P-value
Age (Mean± SD)	29.08±5.26	27.78±4.67	0.136
FBG (mmol/l)	4.28 ± 0.91	5.86 ± 2.03	<0.001
HB (mg/dl)	10.28 ± 1.83	10.32 ± 1.72	0.886
HbA1c (%)	4.70 ± 1.53	5.56 ± 1.12	<0.0001
Blood Pressure (mmHg)			
SBP	114.91 ± 6.07	115.34 ± 6.59	0.669
DBP	82.66 ± 7.82	82.88 ± 7.78	0.861
BMI n (Kg/m ²)	23.29 ± 3.40	24.75 ± 3.51	0.010
BMI n (%)			0.161
Underweight	2 (1.3)	0 (0.0)	
Normal	97 (64.7)	28 (56.0)	
Overweight	48 (32.0)	18 (36.0)	
Obese	3 (2.0)	4 (8.0)	
TC (mmol/L)	4.97 ± 1.36	5.25 ± 1.02	0.194
TG (mmol/L)	1.32 ± 0.79	1.45 ± 0.89	0.354
β-HCG	4880.07 ± 554.13	4923.04 ± 390.67	0.612
Insulin (μIU/mL)	14.43 ± 7.76	18.51 ± 9.30	0.003
IR (μIU/mL)	2.75 ± 1.76	4.81 ± 2.90	<0.0001
Leptin (ng mL ⁻¹)	24.12 ± 9.6	22.89 ± 9.4	0.232
P4 (ng/ml)	60.50 ± 21.91	44.86 ± 14.82	<0.0001
E2 (pmol/L)	4249.94 ± 1592.88	2470.52 ± 1297.95	<0.0001
HPL (μg/ ml)	75.70 ± 23.46	105.11 ± 21.35	<0.0001

p < 0.05 was considered statistically significant

FBG;fasting blood glucose, HB; haemoglobin, HbA1c; glycated haemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, TC; total cholesterol, TG; triglyceride, β-HCG; beta-human chorionic gonadotropin, IR; insulin resistant, P4; Progesterone, E2; Estradiol, HPL; Human Placenta Lactogen

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diabetic controls, whereas, progesterone and estradiol were significantly increased in the non-diabetic pregnant women. BMI was also significant ($p < 0.05$) increased in pregnant women with pregestational diabetes (Table 1).

After screening for gestational diabetes among the pregnant women without diabetes ($n = 150$), between 24–28 week gestation, 12 of them developed GDM giving a prevalence of 8% GDM. Fasting plasma glucose, glycated haemoglobin, leptin, and progesterone were significantly ($p < 0.0001, p = 0.029, p < 0.0001, p = 0.043$ respectively) increased in the pregnant women who developed GDM. Estradiol and HPL were non significantly changed in the GDM women, whilst plasma haemoglobin was higher in the women without GDM (Table 2).

When the pregnant women were stratified according to age, pregnant women less than 20yrs of age did not present with GDM, whilst, 33.3% of them in the age range of 20–29yrs had GDM. The highest prevalence of 58.3% GDM occurred at 30–39yrs, whilst only 8.3% GDM was found in the 40–49yrs range (Fig 1)

Pregnant women who developed GDM had significantly ($p < 0.01$) raised fasting blood glucose, ($p < 0.014$), BMI ($P = 0.022$), leptin, progesterone, estradiol ($p < 0.0001$) than the pregnant women with low risk of diabetes (Table 3) but significantly low ($p < 0.0001$) human placenta lactogen levels.

Table 2. Comparison of biochemical variables in case subjects who developed GDM and those without GDM in the second trimester.

Variable	GDM (n = 12)	No GDM (n = 138)	P-value
FBG (mmol/l)	6.33 ± 1.03	4.27 ± 0.90	<0.0001
HB (mg/dl)	9.23 ± 2.83	10.37 ± 1.70	0.035
HbA1c (%)	5.62 ± 2.92	4.62 ± 1.33	0.029
Glycemic control			0.007
≤ 7% (Good)	10 (83.3)	135 (97.8)	
> 7% (Poor)	2 (16.7)	3 (2.2)	
Blood Pressure (mmHg)			
SBP	115.00 ± 4.99	114.90 ± 6.17	0.955
DBP	82.42 ± 7.45	82.68 ± 7.88	0.913
BMI n (Kg/m²)	21.8 ± 2.85	23.41 ± 3.42	0.116
BMI n (%)			0.118
Underweight	1 (8.3)	1 (0.7)	
Normal	6 (50.0)	91 (65.9)	
Overweight	5 (41.7)	43 (31.2)	
Obese	0 (0.0)	3 (2.2)	
TC (mmol/L)	5.09 ± 0.81	4.96 ± 1.40	0.751
TG (mmol/L)	1.53 ± 0.11	1.31 ± 0.77	0.363
β-HCG	5000.0 ± 0.00	4869.65 ± 576.70	0.436
Insulin (0μIU/mL)	17.17 ± 11.38	14.30 ± 7.37	0.221
IR (μIU/mL)	3.50 ± 2.75	2.68 ± 1.64	0.120
leptin (ng mL⁻¹)	36.5 ± 9.2	19.8 ± 9.0	<0.0001
P4 (ng/ml)	72.76 ± 11.54	59.43 ± 22.30	0.043
E2 (pmol/L)	4502.01 ± 1338.39	4228.02 ± 1615.44	0.569
HPL (μg/ ml)	66.45 ± 27.43	76.50 ± 23.02	0.155

$p < 0.05$ was considered statistically significant

FBG; fasting blood glucose, HB; haemoglobin, HbA1c; glycated haemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, TC; total cholesterol, TG; triglyceride, β-HCG; beta-human chorionic gonadotropin, P4; Progesterone, E2; Estradiol, HPL; Human Placenta Lactogen

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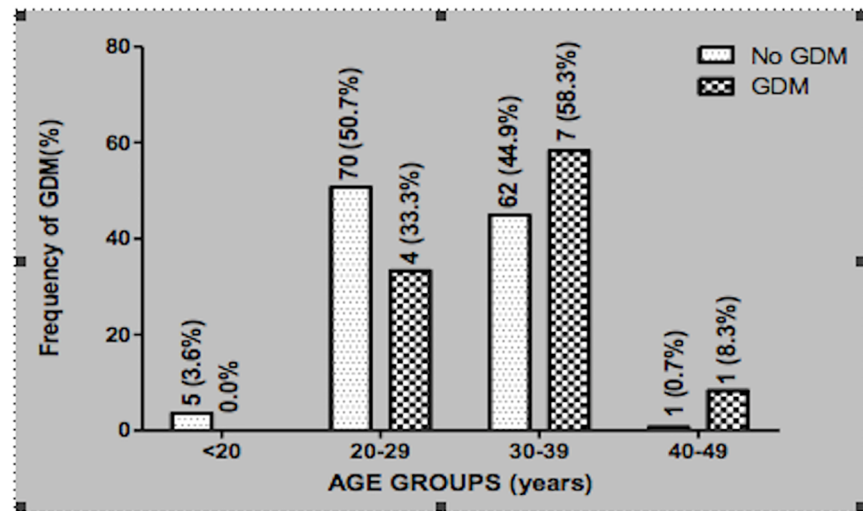


Fig 1. Prevalence of GDM in the various age groups of the pregnant women.

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Table 3. Biochemical comparison between pregnant women who developed GDM (cases) and pregnant women with low risk of diabetes (controls).

Variable	GDM (n = 12)	Controls (n = 50)	P-value
FBG (mmol/l)	5.86 ± 2.03	4.33 ± 1.03	0.014
HB (mg/dl)	9.22 ± 2.83	10.32 ± 1.72	0.086
HbA1c (%)	5.62 ± 2.92	5.56 ± 1.11	0.900
Glycemic control			0.223
≤ 7%	10 (83.3)	47 (94.0)	
> 7%	2 (16.7)	3 (6.0)	
Blood Pressure (mmHg)			
SBP	115.00 ± 4.99	115.34 ± 6.59	0.868
DBP	82.42 ± 7.45	82.88 ± 7.78	0.853
BMI n (Kg/m ²)	21.80 ± 2.85	20.12 ± 2.40	0.022
BMI n (%)			0.153
Underweight	1 (8.3)	5 (10.0)	
Normal	4 (33.3)	30 (60.0)	
Overweight	5 (41.7)	15 (30.0)	
Obese	2 (16.6)	0 (0.0)	
TC (mmol/L)	5.09 ± 0.812	5.25 ± 1.23	0.630
TG (mmol/L)	1.53 ± 1.11	1.45 ± 0.89	0.799
β-HCG	5000.00 ± 0.00	4923 ± 390.67	0.500
Insulin (μIU/mL)	17.17 ± 11.38	18.51 ± 9.30	0.668
IR (μIU/mL)	3.50 ± 2.75	4.81 ± 2.90	0.163
leptin (ng mL ⁻¹)	37.5 ± 7.2	18.8 ± 6.0	<0.0001
P4 (ng/ml)	72.76 ± 11.54	44.86 ± 14.82	<0.0001
E2 (pmol/L)	4502.01 ± 1338.39	2470.52 ± 1297.95	<0.0001
HPL (μg/ml)	66.45 ± 27.43	105.11 ± 21.35	<0.0001

Comparison between means was done using un-paired t-test. p < 0.05 was considered statistically significant

FBG; fasting blood glucose, HB; haemoglobin, HbA1c; glycated haemoglobin SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, TC; total cholesterol, TG; triglyceride, β-IR; insulin resistant. β-HCG; beta-human chorionic gonadotropin, P4; Progesterone, E2; Estradiol, HPL; Human Placenta Lactogen

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Table 4. Accuracy of first trimester glycated haemoglobin and placental peptides as markers of gestational diabetes.

Threshold values	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	P-value
GDM				
FBG 1 (mmol/L) 3.75	33.3% (9.93–65.1)	72.0% (64.2–79.7)	0.49 (0.31–0.67)	0.928
FBG 2 (mmol/L) 5.6	100% (73.5–100)	95.7% (90.8–98.4)	0.99 (0.97–1.00)	<0.0001
HbA1c (%) 5.6	33.3% (9.93–65.1)	87.0% (80.2–92.1)	0.53 (0.33–0.72)	0.8004
Insulin (µIU/mL) 28.5	41.7% (15.2–72.3)	94.1% (88.7–97.4)	0.51 (0.28–70)	0.9244
β-HCG 4947	100% (73.5–100)	7.65 (3.0–12.0)	0.53 (0.37–0.70)	0.7084
P4 74.95	58.3% (27.7–84.8)	71.0% (62.7–78.4)	0.67 (0.54–0.79)	0.050
E2 5256	50.0% (21.1–78.9)	63.0% (54.4–71.1)	0.53 (0.38–0.68)	0.737
HPL 65.61	50% (21.1–78.9)	71.7 (63.5–79.1)	0.59 (0.39–0.79)	0.305

FBG;fasting blood glucose, **HB**; haemoglobin, **HbA1c**; glycated haemoglobin, **SBP**; systolic blood pressure, **DBP**; diastolic blood pressure, **BMI**; body mass index, **TC**; total cholesterol, **TG**; triglyceride, **β-HCG**; beta-human chorionic gonadotropin, **IR**;insulin resistant, **P4**; Progesterone, **E2**; Estradiol, **HPL**; Human Placenta Lactogen

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Table 4 shows the Accuracy of diagnosis of first trimester FBG-1, FBG-2, HbA1c, Insulin, β-HCG, P4, E2 and HPL.

The diagnostic value of 3.75 mmol/L for first trimester fasting blood glucose (FBG-1) had a sensitivity of 33.3% and Specificity of 72.0% as marker for GDM and 5.6 mmol/L diagnostic value with significant ($p < 0.0001$) 100% sensitivity and 95.7% specificity for second trimester fasting blood glucose (FBG-2). First trimester HbA1c with sensitivity of 33.3% and specificity of 87.0% had a threshold value of 5.6%. β-HCG showed 100% sensitivity and specificity of 7.65% at 4947. E2 and HPL showed similar sensitivity of 50.0% but difference in specificity of 63.0% and 71.7% at 5256 and 65.61 respectively.

The risk of developing GDM increased with age (OR = 1.02, P = 0.757). Overweight (OR = 1.76, P = 0.370) and family history of diabetes (OR = 2.18, P = 0.282) (Table 5). Pregnant women in the first trimester, with a history of abortion, miscarriage and pre-mature birth as complications had higher risk of developing GDM. Also the risk of developing GDM increased with increasing plasma progesterone.

Body mass index was significantly and positive correlated with Glycated haemoglobin (.702*). Insulin resistance was positively associated with insulin concentration (.922**) and FBG (.714**) in the women with GDM. Glycated haemoglobin was positively associated with fasting blood glucose (-.510**) but negatively associated with β-HCG (-.303*) (Table 6) and fasting blood glucose levels negatively associated with β-HCG (-.469**) and progesterone (-.298*)

Discussion

Pregnancy has been identified as a diabetogenic condition characterized by the development of insulin resistance and hyperinsulinemia [7]. It has been proposed that pregnancy-induced insulin resistance reveals the onset of β-cell defects associated with insulin resistance and hyperglycemia, hyperlipidaemia [21], which underlie GDM. GDM is diabetes restricted to pregnant women in whom the development of glucose intolerance was first diagnosed during pregnancy, distinguishing it from preexisting glucose intolerance and diabetes induced by pregnancy [21, 22]. This study was aimed at assessing placental peptides and maternal factors as potential predictors of the development of gestational diabetes among pregnant women.

The pre-gestational diabetic pregnant women had significantly higher plasma glucose, insulin and insulin resistance than the non diabetic pregnant women (Table 1). The plasma glucose

Table 5. Logistic regression for predictors of gestational diabetes mellitus.

Predictors	OR (95% CI)	P-value
Age (years)	1.02 (0.91–1.13)	0.757
SBP (mmHg)	1.00 (0.91–1.10)	0.963
DBP (mmHg)	0.99 (0.92–1.07)	0.897
BMI n (%)		
Underweight	15.17 (0.84–27.35)	0.065
Normal*	Reference	
Overweight	1.76 (0.51–6.10)	0.370
Obese		
Family History of other conditions		
None*	Reference	
Diabetes	2.18 (0.53–9.06)	0.282
Obesity	-	-
Hypertension	-	-
Thyroid disease	0.77 (0.09–6.01)	0.814
History of Full term delivery		
Yes	0.59 (0.18–1.97)	0.393
No*	Reference	
History of Abortions		
Yes	1.86 (0.45–7.43)	0.382
No*	Reference	
History of Miscarriages		
Yes	1.30 (0.15–11.25)	0.810
No*	Reference	
History of Pregnancy Complications		
None	Reference	
Pre-eclampsia	-	-
Pre-mature birth	1.27 (0.15–10.99)	0.826
Still birth	-	-
β -HCG	1.12 (0.00–4.85)	0.994
Insulin (μ U/mL)	1.04 (0.97–1.12)	0.256
P4 (ng/ml)	1.04 (1.00–1.09)	0.050
E2 (pmol/L)	1.00 (1.00–1.00)	0.707
HPL (μg/ ml)	0.98 (0.95–1.01)	0.105

SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, β -HCG; beta-human chorionic gonadotropin P4; Progesterone, E2; Estradiol, HPL; Human Placenta Lactogen

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and insulin resistance in the non diabetic pregnant women were significantly low probably due to the early stage of pregnancy. However, it has been shown that insulin resistance is associated with normal pregnancy and may not develop into GDM [23]. The mechanisms leading to insulin resistance in normal pregnancy are explained by several theories including; the estrogen and progesterone hormone counter effect on insulin action by their inhibitory effect on the skeletal muscle β -subunit of the insulin receptor and insulin receptor substrate-1 [23] and thereby making available enough glucose for the growing foetus. [24]. These changes in insulin signaling reduces insulin-mediated glucose uptake in the muscle, the major tissues for whole-body glucose disposal [25]. However, insulin resistance is terminated soon after delivery and normal glucose metabolism returns within 1 year postpartum [26].

Table 6. Relationship between glycated haemoglobin, placenta peptides and dyslipidemia among pregnant women with GDM (upper portion) and those without diabetes (lower portion).

Variables		HbA1c	FBG	β-HCG	Insulin	IR	BMI	P4	HPL	E2	TC	TG
HbA1c	r	1	0.25	.a	-0.096	-0.087	.702*	0.073	-0.167	0.352	-0.104	0.555
	P-value		0.434	.	-0.766	0.788	0.011	0.821	0.604	0.261	0.748	0.061
FBG	r	.510**	1	.a	0.427	.714**	0.163	-0.409	-0.518	-0.13	-0.369	0.244
	P-value	0		.	0.166	0.009	0.613	0.187	0.084	0.686	0.237	0.445
β-HCG	r	-.303*	-.469**	.a	.a	.a	.a	.a	.a	.a	.a	.a
	P-value	0.033	0.001	
Insulin	r	-0.125	-0.014	0.071	1	.922**	-0.097	-0.293	-0.314	-0.448	-0.144	-0.238
	P-value	0.388	0.921	0.622		0	0.763	0.356	0.32	0.144	0.655	0.457
IR	r	0.193	.545**	-0.172	.803**	1	-0.084	-0.357	-0.403	-0.455	-0.223	-0.159
	P-value	0.178	0	0.231	0		0.796	0.254	0.194	0.138	0.486	0.623
BMI	r	-0.008	-0.105	-0.237	-0.17	-0.176	1	0.142	-0.161	0.576	0.024	0.438
	P-value	0.958	0.47	0.097	0.239	0.221		0.659	0.618	0.05	0.942	0.154
P4	r	-0.144	-.298*	0.162	0.237	0.02	-0.092	1	0.524	0.235	0.003	-0.212
	P-value	0.318	0.035	0.261	0.098	0.891	0.525		0.08	0.461	0.994	0.509
E2	r	0.142	0.066	0.133	-0.206	-0.155	0.155	0.014	-0.282	1	0.076	0.372
	P-value	0.325	0.651	0.357	0.152	0.284	0.282	0.924	0.374		0.814	0.234
HPL	r	0.114	-0.063	0.077	-0.114	-0.117	0.058	0.149	1	-0.219	-0.247	-0.551
	P-value	0.431	0.666	0.593	0.431	0.42	0.69	0.3		0.126	0.439	0.064
TC	r	0.18	0.12	-0.144	0.129	0.144	0.172	0.027	0.126	0.138	1	0.413
	P-value	0.211	0.407	0.318	0.372	0.318	0.232	0.851	0.384	0.341		0.182
TG	r	0.186	0.011	-0.027	-0.011	-0.004	0.052	-0.203	0.041	.314*	0.187	1
	P-value	0.196	0.938	0.852	0.941	0.98	0.717	0.158	0.777	0.026	0.193	

R = Correlation coefficient

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

^anot be computed because at least one of the variables is constant.

FBG;fasting blood glucose, **HB**; haemoglobin, **HbA1c**; glycated haemoglobin, **SBP**; systolic blood pressure, **DBP**; diastolic blood pressure, **BMI**; body mass index, **TC**; total cholesterol, **TG**; triglyceride, **β-HCG**;beta-human chorionic gonadotropin; **P4**; Progesterone, **E2**; Estradiol, **HPL**; Human Placenta Lactogen

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This phenomenon of insulin resistance in normal pregnancy was observed in this study. There was no significant difference, in plasma insulin, insulin resistance, β-HCG (P = 0.436) and the lipid profile between pregnant women diagnosed with GDM and pregnant women with no diabetes at the early weeks of gestation (Table 2). This seems to suggest that, these peptides and the lipids may not play significant roles in the aetiology of GDM.

However, as the gestation progresses the effect of placenta peptides; Progesterone, estradiol, leptin and glycated haemoglobin on insulin predisposes the women to glucose intolerance and eventually to overt GDM [27, 28, 29]. Progesterone, and estradiol were significantly (p<0.0001) increased in the cases compared to the controls (Table 1). Progesterone, estradiol and leptin were also significantly higher in women with GDM than women with low risk of developing GDM (Table 3). This phenomenon may account for the role of these placenta peptides in the aetiology of GDM. Women with GDM have a combination of acquired and chronic insulin resistance and are more insulin resistant compared to the normal women during late pregnancy. The phosphorylation of insulin receptor tyrosine is implicated in the transmission of insulin signal to enable glucose uptake³⁰. In women with GDM, there is a significant decrease

in insulin receptor tyrosine phosphorylation in the skeletal muscle, and a decrease in insulin-stimulated glucose uptake [30]. Therefore, pregnancy can unmask slight defects that induce GDM. It has been proposed that these peptides interfere with insulin phosphorylation during pregnancy and impair glucose uptake [29].

The mean plasma leptin in the non-diabetic pregnant women was nonsignificantly raised (Table 1), probably due to the smaller number of the women (12/150) who developed GDM had high leptin levels. This assertion is verified in Table 3, where leptin levels in GDM women is significantly higher than in women with low risk of GDM. Leptin concentration in amniotic fluid at 15–17 weeks of gestation is higher in women who subsequently developed GDM than in women with normal glucose tolerance during pregnancy [31]. Leptin enhances peripheral insulin sensitivity and improves pancreatic β -cell function [32]. These normal functions of leptin are disrupted in leptin resistance state of pregnancy inducing insulin resistance and impaired glucose tolerance in pregnancy [33,34]. The placenta produces leptin during pregnancy resulting in further hyperleptinaemia. This is exacerbated by hyperleptinaemia due to fat accumulation, and subsequent insulin resistance, giving rise to GDM in women who are unable to compensate for the physiological dysregulation.

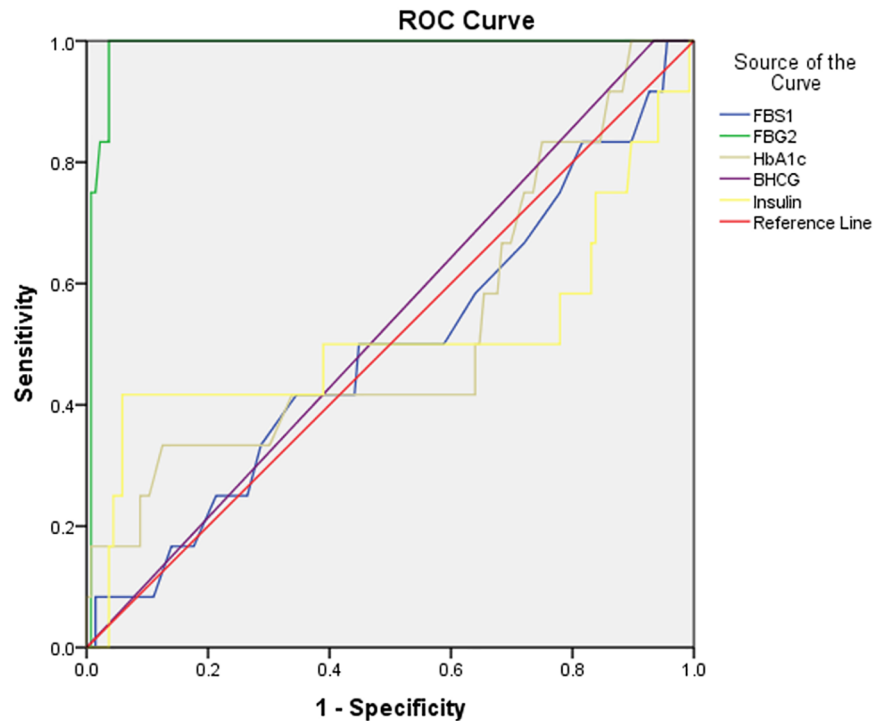
Anthropometric changes have been implicated in the aetiology of GDM. BMI was significantly ($p = 0.022$) higher in GDM women compared to pregnant women with low risk of GDM (Table 3). The risk of developing gestational diabetes increased with overweight (OR = 1.76, $P = 0.370$) (Table 5). Similar findings reported by Chu *et al.*, shows that the risk of developing GDM is about 2 and 4 times higher among overweight and obese women respectively, as compared to normal-weight pregnant women [35].

Aging is a major confounding factor associated with the development of GDM. Even though the odd ratio of developing GDM due to ageing was low (OR = 1.02, $P = 0.757$) (Table 5), women within the age range of 30–39 had the highest probability of developing GDM (58%), whilst those at 20 yrs and below had the lowest probability of 0.0%. (Fig 1). As women advance in age, there is a tendency to increased BMI, adiposity, multiparity and high blood pressure, and these are all confounding factors of GDM [36].

Apart from age, and BMI, other confounding factors that predisposes pregnant women to the development of GDM, include, family history of diabetes, ethnicity, adiposity and parity [37]. In a North American population, higher rates of GDM were reported among blacks, Hispanics, and Asians compared to non-Hispanic whites [38, 39]. A family history of diabetes increased the odds of developing GDM (OR = 2.18, $P = 0.282$). Pregnant women with a history of abortion, miscarriage and pre-mature birth as complications had higher risk of developing GDM (Table 5). The risk of developing GDM increased with increasing plasma insulin (OR 1.04 $P = 0.256$) and progesterone (OR 1.04 $P = 0.05$). Also the placental peptides were significantly higher in women who developed GDM than those who had low risk for the development of GDM (Table 3). Progesterone also had a strong sensitivity and specificity in diagnosing GDM, whilst β -HCG, estradiol and HPL were poor predictors of GDM.

Glycated haemoglobin, fasting blood glucose and fasting plasma insulin, are the key markers of diabetes. None of the placenta peptides and hormones had a significant specificity and selectivity as a marker of GDM in the first trimester. However, plasma glucose of diagnostic value of 5.6 mmol/l had significant specificity and selectivity as marker of GDM in the second trimester (Table 4).

Body mass index was significantly and positively correlated with glycated haemoglobin (.702*). Fasting blood glucose had an inverse and strong significant correlation (-.801**) with insulin levels among GDMs (Table 6). Though, HbA1c, insulin and β -HCG are useful metabolites for diagnoses and monitoring of diabetes mellitus, yet had fair diagnostic test values for GDM, since the AUC (95% CI) were >0.05 . Glycosylated haemoglobin is not usually used



Diagonal segments are produced by ties.

Fig 2. The receiver operator curve for fasting blood glucose, glycated haemoglobin, β -HCG and insulin levels. Receiver operator curve and area under the curve (AUC) 95% CI. The area under the ROC for FBG-1 (at first trimester) was 0.49. However, AUC for FBG-2 was F 0.99 (0.97–1.00), for week 24–28. HbA1c = 0.53 (0.33–0.72), Insulin = 0.51 (0.28), β -HCG = 0.53 (0.37–0.70).

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clinically to diagnose GDM, it is however useful in diagnosing pre-existing diabetes in early pregnancy [40].

The area under the ROC for FBG-1 (at first trimester) was 0.49 suggesting that fasting plasma glucose level in the first trimester is a poor diagnostic test for gestational diabetes mellitus. However, AUC for FBG-2 was 0.99 implying that FBG-2 is a good diagnostic test for diagnosing GDM between 24wks-28 wks, whereas, glycated haemoglobin/insulin and β -HCG were poor diagnostic test markers for GDM as compared to FBS-2 (Fig 2).

Conclusion

The crude prevalence of GDM in the metropolis was 8.0%. The plasma level of the placenta peptides, Leptin, Progesterone and Estradiol estimated were increased in the women who developed gestational diabetes mellitus. These peptides were fair in predicting GDM. However, progesterone level in the first trimester was outstanding in predicting the onset of GDM. Underweight, overweight, age and history of diabetes were strong predictors of GDM. HbA1c, insulin and β -HCG are fair tests for predicting GDM.

Limitations

The effect of some confounding factors, ethnicity, adiposity on gestational diabetes were not independently determined.

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