FIRST TRIMESTER GLYCATED HAEMOGLOBIN AND PLACENTAL PEPTIDES AS MARKERS OF GESTATIONAL DIABETES

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By

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Introduction

DECLARATION

I, PETER GMAGNA**,** hereby declare that this thesis is my own work towards the award of a Master of philosophy degree and that, it does not contain any material previously submitted by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in text.

…… Date: ……

PETER GMAGNA

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SUPERVISORS DECLARATION

I hereby declare that this thesis was supervised and scrutinized by me in accordance with the regulations laid down by the Kwame Nkrumah University of Science and Technology.

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PROFESSOR FRANCIS A. YEBOAH

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DEDICATION

I wish to first of all dedicate this work to the Almighty God for guiding and protecting me throughout this period. I also dedicate it to my father Mr Peter Gmagna Senior, My lovely wife Mrs Patience Gmagna and all my children Begrini, Hannah, Mary and Diana Gmagna. Finally to my aunty Mansu Laatib who provided me the love, care and support during my stay on campus.

ABSTRACT

Background: In most pregnant women there is a counter effect of increased insulin secretion to normalize glucose levels in their bodies. However, when the capacity of insulin secretion is not large enough to overcome the effect of insulin resistance, glucose intolerance develops resulting in gestational diabetes mellitus (GDM).

Aim: The main objective of this study is to assess the role of first trimester glycated hemoglobin and placental peptides as potential predictors of gestational diabetes.

Research design and methods: After 12 to 16 hours fasting, venous blood samples were taken from 200 pregnant women (150non-diabetic and 50 type 2 diabetics) into labeled yellow top gel-separator vacutainer tubes and centrifuged to separate serum from blood cells. All serum samples were analyzed for lipid profile, insulin and β-HCG, Progesterone, estradiol and human placenta lactogen, whilst glucose levels were measured immediately with glucometer. The A_1 fast fraction – cation exchange method was used to estimate the level of glycated hemoglobin. OGTT was performed for the non-diabetic pregnant women at the 24^{th}-28^{th} week of gestation for the diagnosis of Gestational Diabetes mellitus (GDM).

Results: Blood glucose, Glycated hemoglobin (HbA1c), and BMI were significantly ($P< 0.05$) increased in the pregnant women with diabetes as compared to the non-diabetics. Insulin, β-HCG and HPL were higher in the diabetics than the non-diabetics, however, the difference in insulin and HPL levels between control and diabetics (newly diagnosed GDM) was statistically significant (P<0.05). A prevalence of 8% GDM was observed in the non-diabetic pregnant women with 58.3% of them aged between 30-39years.Among study subjects with GDM, 25% (3) had family history of diabetes. The risk of developing GDM is high with ageing (OR= 1.02, P=0.757) and overweight (OR=1.76, P=0.370). The area under the ROC curve for FBG-1 (first trimester test) was 0.49 suggesting that fasting plasma glucose is a poor test for predicting GDM in the first trimester. However, AUC for

FBG-2 was 0.99 (24wks-28 wks) showing that FBG-2 is a very good test and insulin, ßHCG, P4, E2 and HPL averagely good for diagnosing GDM.

Conclusions: The overall prevalence of GDM in this study was 8.0 %. Fasting plasma glucose may be useful as a screening test for GDM on account of its high Specificity; however, an additional test may be necessary to decrease the false negative test results. HbA1c, insulin, β-HCG, P4, E2 and HPL are fair tests to predict GDM.

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ABBREVIATIONS

- A.D.A – American Diabetic Association
- ADIPS _ Australian Diabetes in Pregnancy Society
- AGE _ Advanced Glycation End products
- AUC – Area Under The Curve
- BMI – Body Mass Index
- C I – Confidence Interval
- CDA – Canadian Diabetic Association
- CGR _ C-peptide-to-glucose Ratio
- CHRPE _ Committee on Human Research, Publications and Ethics
- CVD _ Cardiovascular Diseases
- DBP – Diastolic Blood Pressure
- EDTA – Ethylen Diamine Tetra Acetic Acid-
- ESRD – End Stage Renal Failure
- FBG – Fasting Blood Glucose
- FBG 1 – Fasting Blood Sugar in the First Trimester
- FBG 2 – Fasting Blood Sugar between 24-28th week of Gestation
- GDM _ Gestational diabetes mellitus
- HAPO _ Hyperglycemia and Pregnancy Outcome
- HbA1c _ Glycosylated hemoglobin
- hCS _ human Chorionic Somatomamotrophin
- HDL – High Density Lipoprotein
- HGH – Human Growth Hormone
- HIV/AIDS _ Human Immuno deficiency Virus
- hPL _ Human Placenta Lactogen
- I R – Insulin Resistance
- I.D.F. – International Diabetic Federation
- IADPSG _ International Association of the Diabetes and Pregnancy Study Groups
- IDDM _ Insulin-dependent diabetes mellitus
- IGF _ Insulin-like Growth factor
- KATH – Komfo Anokye Teaching Hospital

- K.N.U.S.T _ Kwame Nkrumah University of Science & Technology
- LADA _ latent autoimmune diabetes in adults
- LDL – Low Density Lipoprotein

- LGA – Large for Gestational Age
- mmHg – Millimeters Mercury
- NICE, – National Institute for Health and Clinical Excellence
- OGTT – Oral Glucose Tolerance Test
- PAP-A – Pregnancy Associated Plasma Protein
- PGDM – pre-gestational diabetes mellitus
- R O C – Receivers Operation Curve
- RCT – Randomized Control Trial
- RDS – Respiratory Distress Syndrome
- S D – Standard Deviation
- SBP – Systolic Blood Pressure
- SMHS – School of Medical and Health Science
- SMS – School of Medical Sciences
- SPSS – Statistical Package for Social Science
- T2DM – Type 2 Diabets
- TC – Total Cholesterol
- TG – Triglyceride
- TNF – Tumor Necrosis Factor
- VDL C – Very Low Density Lipoprotein
- W.H.O. – World Health Organization
- β-HCG – βeta Human Chorionic Gonadotropin

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Pregnancy is a physiological condition that coexist with insulin resistance (Dahlgren, 2006) as a result of decrease in accepted levels of insulin concentration in the body(Baban *et al.*, 2010). Most pregnant women exhibit a counter effect of increased insulin secretion to offset high glucose levels in fetal development. However, impaired insulin secretion or its low level during gestation could lead to high glucose levels in the body and subsequently the development of gestational diabetes mellitus (Shalayel *et al.*, 2011). Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of varying levels of severity usually during the second or third trimester of pregnancy (Shalayel *et al.*, 2007; Kaaja and Ronnemaa, 2008; Shalayel *et al.*, 2010). Diagnosis of an individual with previous glucose intolerance condition in pregnancy is usually confirmed when glucose tolerance test is normal at postpartum (Metzger *et al.*, 2007). GDM may present with longterm implications, such as subsequent development of type 2 diabetes and the risk of obesity or glucose intolerance in the mother and offspring respectively (Barbour *et al.*, 2007).

In Sub-Saharan Africa, as in the developed world, there is an increasing prevalence of diabetes and cardiovascular diseases as well as other non-communicable diseases. With this increasing awareness of the disease, studies conducted on the issue are limited, especially in Ghana. Prevalence of GDM ranges from 4-10% of all pregnancies globally. In Sub-Saharan Africa prevalence has been reported from 0% among pregnant women in Tanzania to 9% in Ethiopia. Incidence however differs due to variations in nutritional lifestyle and variances in genetic patterns between populations (Metzger *et al.*, 2007).

Unlike type 1 diabetes, GDM comes with high levels of insulin secretion in pregnant women. In pregnancy, the partial inhibitory effect of peptides secreted by the placenta, such as oestrogen, cortisol and human placental lactogen on insulin, could lead to GDM(Page *et al.*, 2002).The placental peptides inhibitory role on insulin is a physiological adaptation to provide adequate nutritional requirements including glucose to the growing fetus in pregnancy (Kautzky-Willer *et al.*, 2001). This adaptation or role of placental peptides may lead to the development of gestational diabetes. There is evidence pointing to a justification of this relationship (Kautzky-Willer *et al.*, 2001). It has been shown that, women with gestational diabetes, leptin levels are increased during and after pregnancy, likewise babies born to these mothers (Kautzky-Willer *et al.*, 2001).

Many of the tests used currently for gestational diabetes provide only an estimation of risk and therefore it is very important to develop new prenatal screening tests that are more reliable and specific. Efforts for first trimester detection of gestational diabetes is emerging to relief individuals from the psychological anxiety and pathological trauma faced by prospective parents which will require the use of biomarkers may for early detection and diagnosis. Although peptides are very promising candidates, there remains much to be learnt (Page *et al.*, 2002). Placental peptides increase in levels at various stages of pregnancy and play a role in augmenting the onset of gestational diabetes. Maternal factors such as age, parity and previous birth weight among others also have an effect on the development of gestational diabetes.

Therefore, 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 here in Ghana, it would in turn allow medical practitioners to better manage patients and prevent many pregnancies associated complications. The current study aims at assessing the role of

placental peptides and maternal factors as potential predictors of gestational diabetes among pregnant women in the Tema metropolis of Ghana.

1.2 PROBLEM STATEMENT

Between 2-5% of pregnancies are complicated by diabetes, of which 90% are classified as gestational diabetes mellitus (Ben-Haroush *et al.*, 2004).

. These complications are associated with adverse maternal and infantile outcomes (Ben-Haroush *et al.*, 2004).Ability to properly manage and improve outcome is largely dependent on the identification of potential markers that may prove more reliable and specific in their diagnostic value and may be useful for identifying patients at risk.A case for early first trimester diagnosis is emerging to help the situation and peptide markers may be able to fill this niche.

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1.3 JUSTIFICATION

Sub-Saharan Africa, like the rest of the world, is experiencing an increasing prevalence of diabetes alongside other non-communicable diseases. With this increasing awareness of the disease, studies conducted on the issue are limited, especially in Ghana. From 19992011 two studies were identified, one in Ethiopia (Seyoum *et al.*, 1999) and one in South Africa (Mamabolo *et al.*, 2007). Three other relatively older studies have also been identified and prevalence ranges have been reported in a range from 0% among pregnant women in Tanzania to 9% in Ethiopia. There is therefore an indication that further studies are warranted in the subject area in Africa, to assess prevalence and risk factors which will enable a better understanding and help in its prevention and management.

Gestational Diabetes Mellitus is characterized by glucose intolerance during pregnancy(Reece, 2010), of which the onset or discovery of glucose intolerance during pregnancy is usually in the second or third trimester (Shalayel *et al.*, 2007). The timing at

which this is usually detected carries long-term implications on management as well as for the subsequent development of type 2 diabetes in the mother and increased risk of obesity and glucose intolerance in the offspring. Placental peptides with good diagnostic value could be useful for identifying patients at risk. Placental peptides may have a role to play in the augmentation of gestational diabetes and this can be harnessed as predictive markers for the development of gestational diabetes. A case for early first trimester diagnosis is emerging to help reduce the psychological anxiety and pathological trauma faced by prospective mothers. Peptide markers could be explored to fill this niche (Page *et al.*, 2002). Also, there is a gap in the fight to early detection of gestational diabetes in Ghana. The most widely used approach is diagnosis and management, a non-preventive approach. Moreover, incidence of gestational diabetes is influenced by nutritional habits and differences in genetic patterns between populations. Furthermore, 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 here in Ghana, it would in turn help medical practitioners to better manage patients and prevent the many associated complications in pregnancy

1.4 MAIN OBJECTIVE

The main objective of this study is to assess the role of first trimester glycated hemoglobin, placental peptides, and maternal factors as potential predictors of gestational diabetes mellitus (GDM).

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1.5 SPECIFIC OBJECTIVES

- To determine the levels of glycated haemoglobin and placental peptides in pregnant Ghanaian women.
- To determine the role of placental peptides in the augmentation of gestational diabetes.

- To determine maternal factors that may be associated with the onset of gestational diabetes.
- To determine the prevalence of dyslipidaemia among pregnant women.
- To determine the relationship between placental peptides and serum lipids in the augmentation of gestational diabetes.

1.6 NULL HYPOTHESIS:

Glycated hemoglobin and placental peptides are not markers of gestational diabetes.

1.7 ALTERNATIVE HYPOTHESIS:

Glycated hemoglobin and placental peptides are markers of gestational diabetes.

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETES

Diabetes is the condition in which the body is unable to properly process food for use as energy. In humans, carbohydrates and proteins consumed are converted into glucose for our bodies to use as fuel for energy in the body cells (W.H.O, 2006). Insulin, the most pivotal in glucose and amino acids metabolism; is synthesized by the pancreas, an organ that is positioned near the stomach. In diabetes the body is either unable to produce enough insulin or is not responsive to the hormone in the blood circulation, leading to hyperglycemia. Diabetes can lead to deleterious health complications including coronary disease, blindness, kidney failure, and lower-extremity amputations(Nayak and Roberts, 2006).

The ADA(American Diabetes Association, 2005)grouped diabetes into four clinical classes*:*

- Type 1 diabetes (β-cell destruction hence, no moreinsulin production leading to its deficiency)
- Type 2 diabetes (progressive insulin secretory problems due to insulin resistance)
- Other specific types of diabetes due to other causes, e.g., genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced (such as in the treatment of HIV/AIDS or after organ transplantation)"
- "Gestational diabetes mellitus (GDM) (diabetes detected during pregnancy that is not clearly overt diabetes)(Kim *et al.*, 2002).

According to the Center for Disease Control and Prevention, blacks are 1.7 times as likely to develop diabetes as whites, with a prevalence of 2.3 million people above the age of 20(CDC, 2011). Diabetes condition is characterized by one or more of these conditions; frequent urination, excessive thirst, unexplained weight loss, extreme hunger, abrupt vision deviations, itchy or emotionlessness in hands or feet, feeling very tired most of the time, very dry skin, wounds that are slow to heal and more infections than normal(Moore, 2004; American Diabetes Association, 2005; Mbanya *et al.*, 2010).

2.2 GLOBAL STATE OF DIABETES

The International Diabetes Federation estimated that approximately 194 million people around the world had diabetes in 2003. This figure is expected to surge to 333 million by 2025, which means that 6.3% of the world's population will be living with diabetes, debilitating the overall work force. Diabetes as a whole, is gradually reaching epidemic proportions globally. (Nayak and Roberts, 2006).

The upsurge in the prevalence of type 2 diabetes is due to increase in life expectancy, obesity and sedentary lifestyles. Of particular concern is the dramatic rise of type 2 diabetes in children born of diabetic's mother in the adolescent's life(Moore, 2004).

India and China are classical examples of countries with upsurge in diabetes prevalence's. The largest diabetic population in the world is in India with an estimated number of 35 million people, which forms 8% of the total population. Whereas, in China 2.7% of the adult population is affected with type 2 diabetes, this figure is expected to go over 50 million within the next 25 years(A.D.A, 2014).

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2.3 DIABETES IN AFRICA

About 7 million people are living with diabetes in Africa. However, this is expected to increase to two times (15 million) by 2025) (Sierra, 2009). Nigeria has recorded highest number of diabetes cases in Africa (Approximately 1,218,000 people affected) (Danaei *et al.*, 2011). This could be attributed to the fact that Nigeria has the highest number of people populating the African continent, coupled with impaired glucose tolerance, estimated as 3.85 million. It has been estimated that undiagnosed diabetes in the early state accounts for 60% of those with the disease in Cameroon, 70% in Ghana and over 80% in Tanzania. In Ghana, the prevalence is estimated at 3.35% of the adult population (Amoah *et al.*, 2002; Ruhembe *et al.*, 2014).

A comparative study of urban/rural ratio in Tanzania indicated 5:1 ratio and a similar study in Cameroon indicated that people living with diabetes were in a ratio of 2:1(Levitt *et al.*, 2000; Wild *et al.*, 2004).However, the ratios clearly point at imbalance diabetes prevalence's between urban and the rural setting, possibly owing to the variations in levels of exposer to the risk factors of diabetes, obesity and sedentary lifestyles.

2.4 COMPLICATIONS OF DIABETES

Inadequate insulin production and action result in sugar accumulation in the blood leading to chronic hyperglycemia. This can result in both short and long term complications and may lead to various degrees of complications, which, if not prevented but left untreated, can be fatal.

Some of the short-term effects of diabetes include:

- **Recurrent infections**: When blood glucose level is elevated, defenses against infection do not function properly.
- **Weight loss**: the body starts to burn protein and fat instead of glucose. The most common long-term complications associated with diabetes are:
- **Cardiovascular disease** - affects the heart and blood vessels resulting in the malfunctioning of the heart. This may cause fatal complications such as heart attack and stroke (a common cause of disability and death in people with diabetes). Diabetes patients are two to four times more likely to develop cardiovascular disease than people without diabetes(Bloomgarden, 2003).
- **Ketoacidosis**: This is a metabolic disorder as a result of high levels of blood glucose and ketones. When insulin levels fall far low for an extensive period of time, the body begins to break down its fat stored at adipose tissues as an alternative source of energy. This causes the body to release ketones into the blood. Ketoacidosis can make people feel disorganized, become sick, extremely desiring for water, tired or short of breath. It may also result in coma leading to death.
- **Diabetic neuropathy** (nerve disease) - damage to the nerve fibers primarily affecting the legs and feet. Ulceration of the feet are common indicators of diabetic neuropathy. Infections in these wounds may ultimately result in amputation of the foot and lower leg. Research has shown that, 70% of all lower limb which have been amputated were related to diabetes(Sinnreich *et al.*, 2005).
- **Diabetic nephropathy** (kidney disease) - may result in total kidney failure and in the need for dialysis or kidney replacement. Diabetes is the major cause of kidney failure in

the developed world and accounts for approximately 35 to 40 % of new cases of End Stage Renal Disease (ESRD) each year(Mbanya and Sobngwi, 2003).

- **Diabetic retinopathy** (eye disease) - damage to the retina of the eye which can lead to vision loss. The incidence of blindness is 25 times higher in people with diabetes than in the general population(Mbanya and Sobngwi, 2003).

2.5 TYPES OF DIABETES

2.5.1 TYPE 1 DIABETES

This type of diabetes was previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. It may account for 5 to 10 % of all diagnosed cases of diabetes(CDC, 2011). Type 1 diabetes mostly occurs when the immune system mistakenly attacks and kills the beta cells of the pancreas resulting in no or very little insulin production. As a result, glucose builds up in the blood instead of being used as energy. Type 1 diabetes generally develops in childhood or adolescence (hence the term juvenileonset diabetes), but can develop in adulthood(Levitt, 2008).Risk factors for Type 1 diabetes are less defined as compared to Type 2 diabetes, but autoimmune, genetic, and environmental factors are involved in the development of this type of diabetes(Goldstein *et al.*, 2004; Cooke, 2008).

Type 1 diabetes is always treated with insulin. Meal planning also helps with keeping blood glucose at the right levels. Another form of diabetes referred to as latent autoimmune diabetes in adults (LADA), this diabetes describe a small number of people with apparent type 2 diabetes who appear to have immune-mediated loss of pancreatic beta cells. LADA is also considered as Type 1 diabetes(Mamabolo *et al.*, 2007; Levitt, 2008).

2.5.2 TYPE 2 DIABETES MELLITUS

This form of diabetes is considered as a metabolic disorder that is characterized by hyperglycaemia resulting from insulin resistance and relative insulin deficiency (Kumar *et al.*, 2005). Insulin resistance is the inability of cells to respond adequately to normal levels of insulin. It occurs primarily within tissues that require insulin to utilize glucose, including the liver and fat tissues. Insufficient production of the insulin is mostly as a result of beta-cells failure of the pancreas, characterising the pathophysiology of Type 2 diabetes (T2DM). The key mechanisms of T2DM, are beta-cell failure, insulin resistance in the liver and insulin resistance in the muscle (DeFronzo, 2009).(Pettitt *et al.*, 1988;

Catalano *et al.*, 2003). Studies in women from India revealed that, 45% of the children of women with Type 2 diabetes had developed diabetes compared with 1.4% of the

offspring of non-diabetic women (Pettitt *et al.*, 1988).

Diabetes in pregnancy may contribute to the upsurge of T2DM (Silverman *et al.*, 1998; Dabelea *et al.*, 2000). It is hypothesized that an exposure to a diabetic environment *in utero* enhances adolescent and adulthood obesity Populations with very high rates of Type 2 diabetes are known to have the highest prevalence of diabetes in women of childbearing age, and many women with gestational diabetes may have had pre-existing, undiagnosed Type 2 diabetes (Feig and Palda, 2002).

2.5.3 GESTATIONAL DIABETES MELLITUS (GDM)

This is a temporary condition that occurs during pregnancy and is defined as carbohydrate intolerance that begins or is first recognized during pregnancy (W.H.O., 1999). Although it is a well-known complication of pregnancy, its prevalence may vary greatly due to difference in screening programs and diagnostic criteria. This difference in screening programs has made comparison of prevalence between populations difficult (Ben_Haroush *et al.*, 2004). However, It is a complication in about 5% pregnancies and poses as a long

term risk to T2DM to both the mother and fetus (Silverman *et al.*, 1998; Ben_Haroush *et al.*, 2004; Ferrara *et al.*, 2004).

The majority of diabetes cases seen in pregnancy are gestational diabetes mellitus (GDM). In Nigeria 61% of all cases of diabetes reported in pregnancy are GDM (Ozumba *et al.*, 2004).This findings were reported by Ben_Haroush *et al* in 2004; though in the absence of other predisposing risk factors, the prevalence of GDM is low (Ben_Haroush *et al.*, 2004). Moreover, ethnicity has been identified as an independent risk of GDM. However, in a particular population or ethnic group, the prevalence of GDM is directly proportional to the prevalence of T2DM (Ben_Haroush *et al.*, 2004).

Women with GDM experience twice the number of urinary tract infections than women who do not have GDM. This increased infection incidence is thought to be as a result of increased amount of glucose in the urine beyond the normal glycosuria that is present in pregnancy (Asare-Anane *et al.*, 2013). In support of the above findings, Gilmartin *et al*., reported that there is an increased risk of pyelonephritis and asymptomatic bacteriuria (Gilmartin *et al.*, 2008).

In a normal pregnancy, sensitivity to insulin reduces with advancing gestation (Barbour *et al.*, 2007). This is a physiological programming that shift metabolic fuel to the fetus for development(King, 2006). In this physiological state, insulin sensitivity in pregnant non-diabetics is reportedly reduced (Buchanan and Xiang, 2005).

*2.6*GLUCOSE METABOLISM IN NORMAL PREGNANCY

Glucose metabolism is altered during pregnancy. Although there is increased secretion of insulin in the pregnancy state, postprandial glucose concentrations are still higher than in non-pregnant individuals (Metzger *et al.*, 2007). This high insulin levels is partially contributed by the suppressed insulin sensitivity in the skeletal muscle and liver tissue,

exacerbating the hyperglycemic condition by basal hepatic glucose production (Lain and Catalano, 2007).

In the pregnancy state, as part of a physiological adjustment, the increase levels of insulin production is partly due to estrogen and progesterone hormone counter secretion to inhibit insulin action and augment enough and available glucose for the growing fetus. The effects of this hormones are implicated in either the enlargement of the islets of Langerhans cells or hyperplasia of the pancreatic b-cells (Ramiya *et al.*, 2000). Other reasons for changes in glucose metabolism may include a dilution effect, increased glucose utilization from the placenta to the fetus and inadequate production of glucose during pregnancy (Catalano *et al.*, 1992; Lain and Catalano, 2007).

Insulin resistance in pregnant women is also known to be contributed by maternal adiposity and insulin-desensitizing effects of the placental hormones; progesterone and placental growth hormone (PGH) (McIntyre *et al.*, 2000). This resistance is compensated by increased in insulin production during normal pregnancy (Lain and Catalano, 2007).This physiological condition also results in the reduction in insulin sensitivity as pregnancy progresses, such that, by the third trimester it is about 33-78% as compared to that of non-pregnant women (Catalano *et al.*, 1993; Lain and Catalano, 2007).

2.7 GLUCOSE METABOLISM AND PATHOGENESIS OF GDM

Glucose metabolism in pregnancy is very crucial for the wellbeing of the prospective mother and the fetus development throughout the gestation period. However, in pregnancies complicated by GDM, there is higher than normal fasting glucose levels as compared to normal pregnancies at similar basal hepatic glucose production(Marshall, 2005). Study has indicated that in late pregnancy, insulin resistance is increased by about 40% in patients with severe GDM as compared to normal pregnancies (Lain and Catalano, 2007). GDM mostly occurs when the pancreatic ß-cells fails to produce enough insulin to

offset the persisting insulin resistance in the pathological state of this condition in pregnant women (Buchanan and Xiang, 2005). Obesity and chronic insulin resistance are the most common factors that contributes and exacerbates ß-cell dysfunction during pregnancy (Buchanan *et al.*, 2007). Genes could also be implicated and contributed to the insulin secretion deficiency and predisposes pregnant women to the development of GDM as in the case of type 2 diabetes (Mao *et al.*, 2012).

2.8 THE HUMAN PLACENTA IN GDM

The placenta is a foeto-organ complex that fulfills pleiotropic roles during the growth of the foetus. The placenta is in contact with the maternal and foetal surfaces with adequate blood circulation in normal pregnancy. The actively growing surface that is involved in early phase of implantation, syncytiotrophoblast, is exposed to the maternal circulation whiles the endothelium is in contact with the foetal blood (Kaufmann *et al.*, 2004). Because of this unique position, the placenta is exposed to the regulatory influence of hormones, cytokines, growth factors, and substrates present in both circulations and, hence, may be affected by changes in any of these. The placenta can also produce molecules that could affect both the mother and fetus independently. All known cytokines could be expressed by the human placenta, such as tumor necrosis factor (TNF), resistin, and leptin, which are also produced by the adipose cells (Osmond and Barker, 2000; Desoye and Hauguel-de Mouzon, 2007). Some of these adipokines play different roles in the regulation of insulin and has therefore lead to the suggestion that there is a possible interaction between the placenta and adipose tissue in pregnancy-induced insulin resistance. The interplay between the two systems becomes more evident in gestational diabetes mellitus (GDM) (Hahn *et al.*, 2001).

The placenta undergoes a variety of structural and functional changes in pregnancy with diabetes. The nature and extent of these changes depend on a range of variables, such as, the quality of glycemic control achieved during the critical periods in placental

development, the modality of treatment, and the time period of a complete sift from excellent metabolic control of a non-diabetic environment (Saldeen *et al.*, 2002). The development of the placenta is characterized by three distinct phases; at the beginning of gestation, a series of critical proliferation and differentiation processes predominantly of the trophoblast eventually result in the formation of villous and extra villous structures. The extra villous structures anchor the placenta in the uterus and modify the uterine spiral arteries into low resistance vessels. The newly formed villi differentiate sequentially to maturation. Gestation is associated with placental mass expansion mostly at the end. Moreover, in the first half of gestation, trophoblast is the key tissue that undergoes the most successive modifications, whereas the major angiogenesis activities and vascularization occur in the second half of gestation, i.e., the endothelium is the site of the more prominent processes, although there is overlap. This developmental phase is also accompanied by most of the vascular remodeling and stabilization of the vascular bed (Jirkovska *et al.*, 2002).

Just as in the case of pre-gestational diabetic pregnancies, diabetic insults at the beginning of gestation may have long-term effects on the placenta normal functioning for normal foetal development. These responses (adaptive in nature) of the placenta may help limit the growth of the fetus within a normal range, however if the duration or extent of the diabetic insult, including maternal hyperglycemia, hyperinsulinaemia or dyslipidemia, exceeds the placental capacity to mount adequate responses, then excessive foetal growth may ensue(Doshani and Konje, 2009). Diabetic insult at later stages in gestation, such as may occur in gestational diabetes, will foremost lead to short-term changes in a variety of molecules for key functions including gene expression. The diabetic environment can be regarded as a network of substances (hormones, nutrients, cytokines) with altered concentrations. The current view in most research areas today is that the abnormal maternal metabolic environment may generate stimuli within the adipose tissue and the placental

cells resulting in the increased production of inflammatory cytokines whose expression is minimal under normal pregnancy. One of the leading hypotheses is that changes in circulating TNF, adiponectin, leptin, and resistin link inflammation to metabolic changes by enhancing insulin resistance in the mother. Likewise, the fetal environment is also changed in diabetes, and elevated levels of insulin, leptin, and other cytokines have been well documented (Desoye and Hauguel-de Mouzon, 2007).

2.9 ROLE OF HORMONES IN THE ONSET OF GDM

Hormonal imbalance, involving estrogen, progesterone, cortisol and especially high levels of human placenta lactogen in pregnancy and its inhibitory role on insulin as a normal physiological adaptation for glucose mobilization for foetal development may contribute to the insulin resistance state in normal pregnancy. However, this hormonal action may consequently lead to the development of GDM in favor of the growing foetus. Moreover, the resistance of cells to insulin in pregnancy is shown to be characterized by a postreceptor defect which results in the decreased ability of the insulin to bring about mobilization of SLC2A4 (GLUT4) from the interior of the cell to the cell surface (Catalano, 2010). This receptor defect is severally been proposed to be as a result of increase in the plasma levels of one or more of pregnancy associated hormones, such as, estrogen, progesterone, cortisol, and placental lactogen (Kühl, 1991; Kirwan *et al.*, 2002). These are produced mainly by the feto-placental unit. High level of insulin resistance inpregnant women has been indicated to be associated with GDM as compared to normal insulin function in non-diabetic pregnant women. This insulin resistance is seen to persist even in the post-partum period (Buchanan and Xiang, 2005).

Normally, the β-cellis shown to increase insulin secretion to compensate for the insulin resistance in pregnancy (Catalano *et al.*, 1993). A defect in pancreatic β-cell function has been proposed as one of the possible etiology for GDM. This defect results in an inability

of the β-cell to increase insulin secretion to meet the increased demands of insulin posed by pregnancy. It has been shown that elevated maternal insulin secretion in early pregnancy in women without diabetes leads to gestational weight gain and weight retention post-partum (Scholl *et al.*, 2002; Buchanan and Xiang, 2005).

2.10 HUMAN PLACENTA LACTOGEN (HPL) AND GDM

The skeletal muscle is the final disposal of most of the body glucose as well as the adipose tissue for lipid storage purposes. In later half of pregnancy, the skeletal muscle and adipose tissue becomes severely resistant to insulin. In a normal pregnancy there is a 50% decrease in insulin-mediated glucose distribution into the skeletal muscles and destination of its need in humans (Abraham, 2009; McDonald *et al.*, 2009). Kühl also posited that insulin secretion to maintain euglycemia in pregnant mother increases by 200–250% (Kühl, 1991). The phenomenon described above, may contribute significantly to GDM. Studies conducted point to the fact that placental-derived hormones could be a major determinant in reprogramming maternal physiology to arrive at insulin-resistant condition (Barbour *et al.*, 2007). Kirwan and coworkers stated that with the exception of tumor necrosis factor (TNF)-α, alteration in placental hormones in pregnant mothers do not have positive correlation with changes in maternal insulin resistance(Kirwan *et al.*, 2002).

Human placenta lactogen is a single polypeptide chain bonded by disulphide bonds. It has 96% similarities to human growth hormone (HGH), but functionally only 3% of HGH activity. Glass and Kase, opined that the half-life of HPL is short and therefore, suggested that it should be used as an index of placenta problems (Glass and Kase, 1984). HPL, which is the product of the HPL-A and HPL-B genes, is secreted into both the maternal and foetal circulations after the sixth week of pregnancy (Handwerger and Freemark, 2000). The level of HPL in the maternal circulation is correlated with foetal and placental weight, plateauing in the last 4 weeks of pregnancy. Therefore, measurement of HPL levels

is used as a screening test for foetal distress and neonatal asphyxia (Letchworth and Chard, 1972; Redman, 1991).

During pregnancy the maternal level of HPL can be altered by changing the circulating level of glucose. HPL is elevated with hypoglycemia and depressed with hyperglycemia (Kühl, 1991; Barbour *et al.*, 2007). The metabolic role of HPL to mobilize glucose, lipids and free fatty acids cannot be underestimated. It has been shown that in the fed state, there is abundant glucose available, leading to increased insulin level, lipogenesis, and glucose utilization. This is associated with decreased gluconeogenesis, and a decrease in the circulating free fatty acid levels, as the free to deposit storage packets of triglycerides fatty acids are utilized in the process of lipogenesis (Kim and Felig, 1971; Glass and Kase, 1984).

Human placental lactogen (HPL) increases up to 30-fold throughout pregnancy and induces insulin release from the pancreas in pregnancy(Brelje *et al.*, 1993). A study which was not related to pregnancy, showed that HPL can cause peripheral insulin resistance although the results have been consistent (Ryan, 1988).

In the second half of pregnancy, HPL level rises approximately 10 folds. HPL stimulates lipolysis leading to an increase in circulating free fatty acids in order to provide a different fuel for the mother so that glucose and amino acids can be conserved for the fetus. The increase in free fatty acid levels, in turn directly interferes with insulin-directed entry of glucose into cells. Therefore, HPL is considered as a potent antagonist to insulin action during pregnancy (Glass and Kase, 1984; Mills *et al.*, 1985). Furthermore, HPL and placental growth hormone act in concert in the mother to stimulate insulin-like growth factor (IGF) production and modulate intermediary metabolism, resulting in an increase in the availability of glucose and amino acids to the fetus (Handwerger and Freemark, 2000).

2.11 PLACENTA ß–HCG AND GDM

Gestational diabetes mellitus (GDM) has multiple predisposing factors, which are all significant for equal attention in order to salvage GDM condition in pregnant mothers. These adverse effects may lead pregnancy into complications and sometime the loss of the will be mothers life in the process. It is therefore, important to investigate the placenta hormonal markers in pregnant women who are potentially prone to GDM. One of such placenta markers, β-HCG, was initially reported to have no difference in levels between free β-HCG in PGDM women and non-diabetic controls (Spencer *et al.*, 2005). However, several but current studies have shown that β-HCG levels decreased in women with pregestational diabetes (De Groot *et al.*, 2012; Savvidou *et al.*, 2012)

Pellitero *et al*., and Madsen *et al*., reported that β-HCG concentrations among other markers such as pregnancy associated plasma protein-(APAP-A) have been shown to be inversely related to glycosylated hemoglobin (HbA1c) in non-pregnant diabetic women. This could explain the low levels of PAPP-A and β-HCG levels in diabetic pregnancies in their studies(Pellitero *et al.*, 2007) and (Madsen *et al.*, 2012). Several reports on first trimester HCG concentrations have shown either normal or reduced levels in diabetic women (Spencer *et al.*, 2005; Madsen *et al.*, 2012) in those studies, the findings indicated that the levels of maternal serum PAPP-A and total beta HCG which were used in first trimester aneuploidy screening, reportedly decreased in women with pre-gestational diabetes mellitus (PGDM) receiving insulin. Furthermore, the false positive rates of first trimester screening were higher in pregnancies with PGDM compared to non-diabetic women when these differences are not taken into consideration.

Several studies reported low levels of total β-HCG in women with PGDM. These reports showed a significant reduction by 18%(Spencer *et al.*, 2005; Madsen *et al.*, 2012; Gurram *et al.*, 2014). These results contrast with a current study, which reported that there was no difference in free β-HCG in women PGDM(Gurram *et al.*, 2014). This report stated

categorically that only one study reported a reduction in free β-HCG and therefore, pregestational diabetes has effect on total free β-HCG. Lambert-Messerlian *et al.*, also postulated that total free β-HCG is not changed in women with PGDM(Lambert-Messerlian *et al.*, 2009), suggesting the need for further clarification of this controversy. It has been well acclaimed that HbA1c is considered to be a good reflection of glucose control in diabetic patients; however, hormonal markers could be of use for predicting the onset of PGDM.

2.12 PREVALENCE OF GDM

GDM prevalence is approximated to be around 10% globally, however it could vary from 1 to 14% depending on factors such as, differences in test method used, criteria, ethnicity of the population and environmental factors (American Diabetes Association, 2007). Studies have shown that there is a higher incidence of GDM in African and Asian women than in Caucasian women (Chawla *et al.*, 2006). Apart from ethnicity, the risk of GDM rises with a family history of type 2 diabetes or GDM, increased maternal age, parity, previous GDM or macrosomic child, polycystic ovary syndrome (PCOS) and especially obesity with increased insulin resistance (Torloni *et al.*, 2009; Reece, 2010).

Analysis of over 600 000 women showed that the risk of GDM was two times higher in overweight women (BMI 25-29 kg/m2), 3 times higher in obese (BMI >30 kg/m2) and 6 times higher in severely obese women (BMI >35 kg/m2) as compared to women with normal pre-pregnancy BMI(Torloni *et al.*, 2009). Moreover, women with previous GDM have a risk of 30-84% of recurrent GDM (Kim *et al.*, 2007).

2.13 SCREENING AND DIAGNOSIS OF GDM

There are many varieties of screening and diagnostic strategies globally.

GDM is usually diagnosed by oral glucose tolerance test (OGTT).

This test procedure can be performed by a one-step approach or two step approaches. OGTT is usually performed at 24-28 gestational weeks, as study have shown that insulin sensitivity decreases as pregnancy advances (Lain and Catalano, 2007).

Table 2.1: Cut-off values recommended internationally for diagnosing GDM using OGTT

Recommendation	Screening	Diagnostic OGTT and Cut-off values				
	Universal 50g glucose screen, cutoff value (mmol/l)	Glucose load (g)	Fasting (mmol/l)	1-hour (mmol/l)	2-hour (mmol/l)	3-hour (mmol/l)
ADA:one- or two- step (ADA 2013)	≥7.2 or 7.8	100	≥5.1	≥10.0	≥8.5	≥7.8
CDA:two-step (CDA 2008)	≥7.8	75	≥5.3	≥10.6	≥8.9	
WHO:one-step (Alberti and Zimmet 1998)		75	≥5.8	None	≥7.8	
ADIPS: one-step (Nankervis *et al*. 2013)		75	≥5.1	≥10.0	≥8.5	
NICE:one- or two- step (NICE 2008)	≥7.8	75	≥7.0		≥7.8	
Finland:one-step (Gestational diabetes: Current Care Summary, 2013)		75	≥5.3	≥10.0	≥8.6	
IADPSG:onestep (Metzger *et al*. 2010)		75	≥5.1	≥10.0	≥8.5	

American Diabetes Association (ADA), Canadian Diabetes Association (CDA), World Health Organization (WHO),International Association of Diabetes and Pregnancy Study Group (IADPSG), National institude for clinical excellence (NICE) England and Wales. Modified from Melzter et al, 2010

Screening for GDM can be universal or selective for women with a higher risk (Evensen, 2012). Universal screening is routinely performed widely because it is easier and a common approach for treating diagnosed GDM, as it is widely agreed to be beneficial

(Hillier *et al.*, 2008; Horvath *et al.*, 2010). Furthermore , the recommended approach is that pregnant women at high risk of pre-existing diabetes are screened for GDM early in pregnancy (Evensen, 2012).

In the one step diagnostic procedure, either all pregnant women, or women with risk factors for GDM (ethnicity, maternal age, obesity, parity, family history of type 2 diabetes, PCOS) undergo the OGTT (Torloni *et al.*, 2009; Reece, 2010). However, it is also recommended that the diagnoses of all pregnant women without any diabetes screening test should be performed by the OGTT diagnostic method in order not to have pregnant women with GDM go undetected. The OGTT cut off values (Table 2.1) rely on the results of the Hyperglycemia and Pregnancy Outcome study (HAPO)(Ratner *et al.*, 2008). A diagnosis of GDM is conclusive, if one or more values are out of range. When this procedure is used with these cut off-values, the incidence of GDM is approximately 18% (Coustan *et al.*, 2010).

2.14 GLYCATED HAEMOGLOBIN AND IT'S ROLE IN DIAGNOSIS OF GDM

Hyperglycemia has been confirmed by epidemiology as the most important factor in the onset and progress of diabetes complications in all types of diabetes. A study has indicated the link between hyperglycemia and long term complications of diabetes, using nonenzymatic glycation processes (Lyons and Jenkins, 1997). Non-enzymatic glycation is a process by which glucose is chemically bound to amino acids groups or amino acids of proteins without the involvement of enzymes. This occurs through a series of chemical reactions, as described by Maillard in 1912. These reactions are complex and multi layered. The final phase of the reactions consists of a complex polymerization, which results in the formation of heterogeneous structures called advanced glycation endproducts (AGEs) (Vlassara *et al.*, 1994; Singh *et al.*, 2001).

Until recently, high glucose (sugars precursors) concentrations were believed to be the primary mechanisms involved in the Maillard reactions; study has shown that, other intermediary metabolites, such as α-Oxo aldehydes, are also involved in non-enzymatic glycation reactions.

An important product of non-enzymatic reaction is glycated hemoglobin (GHb), which is also referred to as glycosylated hemoglobin, glycohemoglobin, HbA_{1C}, HbA1, or A1C.

GHb is a term used to describe a series of stable minor hemoglobin components formed slowly and non-enzymatically from hemoglobin and glucose. The rate of formation of GHb is directly proportional to the ambient glucose concentration(Ahmad, 2005). In the study of diabetes, the importance of glycosylation processes are manifested in two essential issues:

1. Effect of protein glycation on changes in their structure and function and
2. Use of glycated protein levels as a parameter of integrated glycaemia (Bucala and Cerami, 1992; Brownlee, 2000).

Since erythrocytes are freely permeable to glucose, the level of GHb in a blood sample provides a glycemic history of the previous 120 days, which is the average erythrocyte lifespan. GHb accurately reflects the previous 2−3 months of glycemic control. However, a recent study has indicated that plasma glucose levels contributes considerably about 50% to the level of HbA_{1C} in 3 – 4 weeks earlier (recent glycation) than a 3 – 4 months (passed glycation) with about 10% plasma glucose levels contribution to the HbA1c(Rohlfing *et al.*, 2002). Therefore, the measure of glycated protein has added a new dimension to the assessment of glycaemia. Blood and urine glucose and urine ketone tests cannot provide the patient and health care team with an objective measure of glycaemia over an extended period of time. However, with a single measurement, glycated proteins can quantify average glycaemia over weeks and months, thereby complementing day-to-day testing of

blood and urine glucose and urine ketones(Singer *et al.*, 1989). It also provides an additional advantage because GHb values are free of day-to-day glucose fluctuations and are unaffected by exercise or recent food ingestion.

Although glycosylated hemoglobin is not usually used clinically to diagnose GDM but it could be useful in diagnosing pre-existing diabetes in early pregnancy (Sacks *et al.*, 2011). Fructosamines are glycosylated proteins in the serum and they reflect the glycemic balance during the previous 2-3 weeks i.e. a shorter period than HbA1c (Li and Yang, 2006). Li and Yang have studied the value of measuring Fructosamines during pregnancy in patients with abnormal glucose tolerance. They have observed that the mean level of Fructosamines decreases with gestational age, and that the level of Fructosamines was similar in GDM and non-GDM subjects in their 16-20 gestational weeks. This suggests that not all glycosylated hemoglobin are useful in the diagnosis of GDM.

2.15 MATERNAL RISKS OF GDM

2.15.1 SHORT-TERM RISKS

There are several risks associated with GDM in pregnancy. Study has shown that there is about 2-3 times increased risk for induced hypertension and pre-eclampsia (Schmidt *et al.*, 2001) and a 2 folds risk for caesarean deliveries in expected mothers with GDM (McDonald *et al.*, 2009) as compared to non-GDM patients.

2.15.2 LONG-TERM RISKS

GDM is observed to be a major contributor to the high prevalence rate of type 2 diabetes today (Kim *et al.*, 2002; Malcolm, 2012), this may be partly due to high population growth rates in the world. Furthermore, study has also shown that about 7-fold risk of type 2 diabetes was common with GDM patients as compared with pregnant women without GDM (Bellamy *et al.*, 2009). The relationship between duration of GDM and the development type 2 diabetes indicated a 9 fold risk of type 2 diabetes in pregnant women

with the reoccurrences GDM for over 9 years as compared to pregnant women without GDM for the period(Feig and Palda, 2002). A cohort study among Danish, has shown a 41% prevalence's of type 2diabetes in pregnant women with GDM within a period of 10 years after being diagnosed with GDM (Lauenborg *et al.*, 2009). In industrialized world such as the USA, it has been estimated that about 30% of pregnancy with GDM will have diabetes or impaired glucose metabolism postpartum (England *et al.*, 2009). Several studies have revealed the association of metabolic syndrome and GDM. It was reported that pregnant women with GDM have about 3-fold higher risk developing metabolic syndrome within 10-11 years after delivery as compared to subjects without previous GDM diagnosis (Verma *et al.*, 2002; Lauenborg *et al.*, 2009). The prevalence increases over 4-fold if the GDM mother is obese (Lauenborg *et al.*, 2009). In another study the relative risk of the metabolic syndrome was 2.4 in GDM patients independently of obesity (Gunderson *et al.*, 2009).

A history of GDM raises the risk of cardiovascular diseases (CVD) but the major underlying risk factor is diabetes, which emerges after delivery (Shah *et al.*, 2008). The risk of CVD among pregnant women with GDM over 11 years, increased to about 13% after being diagnosed of GDM when adjusted for diabetes. Several studies have also reported that the risk of CVD among12 years diagnosed GDM was about 1.7-fold as compared to patients without a history of GDM (Shah *et al.*, 2008; Retnakaran and Shah, 2009).

2.16 FOETAL AND NEONATAL RISKS OF GDM

There are several risks for the fetus and the new born of GDM patients. These risks can be classified as either short or long term risks.

2.16.1 SHORT-TERM RISKS

A recent study reported the associated short term risks of gestational diabetes with the fetal or neonate out-comes during parturition as: fetal macrosomia, shoulder dystocia, birth injuries (brachial plexus palsy and bone fractures), hypoglycemia, respiratory distress syndrome (RDS) and hyperbilirubinemia (Reece, 2010) (Table 2.2)

Table 2. 2: Foetal and neonatal short-term risks.

Outcome	Incidence	Study
Macrosomia* or LGA**	14-40 % 4-5 x more common in insulin treated GDM patients than in diet treated patients (Suhonen *et al.*, 2008)	(Jensen *et al.*, 2000; 2003; Ehrenberg *et al.*, 2004; Surkan *et al.*, 2004; Langer *et al.*, 2005; Metzger *et al.*, 2008)
Shoulder dystocia	2-11%	(Esakoff *et al.*, 2009)
Brachial plexus palsy	2.4-2.7 %	(Suhonen *et al.*, 2008)
Hypoglycemia	3-24 % 3 x increased in insulin treated GDM patients and 10 x increased in GDM patients without treatment compared with non-GDM patients (Langer *et al.*, 2005)	(Jensen *et al.*, 2000; Metzger *et al.*, 2008; Esakoff *et al.*, 2009)
RDS	1.5-4 %	(Esakoff *et al.*, 2009)
Hyperbilirubinemia	2-13%	(Metzger *et al.*, 2008; Esakoff *et al.*, 2009)

*birth weight ≥ 4000g or ≥4500g

** birth weight > 90th percentile or >2SD

Poor Neonatal and Foetal outcomes in GDM are mostly due to the hyperglycemic state of the mother. Glucose usually passes through the placenta to the foetus inducing excessive foetal insulin production leading to macrosomia. This revelation was first indicated by Jorgen Pedersen in the 1920s when he formulated his hyperglycemia-hyperinsulinemia hypothesis (the Pedersen hypothesis), which is used to explain foetal macrosomia as a condition associated with hyperglycemia(Catalano and Hauguel-De Mouzon, 2011). Insulin, an anabolic hormone, acts as a growth factor for the foetus due to its anabolic effect in the body(Ouzounian *et al.*, 2011). This metabolic effect is as a result of the

excessive intrauterine nutrient environment (available glucose) coupled with the maternalfoetal hyperinsulinemic status, predisposing the growing foetus to macrosomia. Macrosomia is usually seen as a disproportion in growth between the head and the body of the foetus (Nold and Georgieff, 2004).

Macrosomia is also reported in overweight and obese non-diabetic pregnant women to be around 16-28% (Owens *et al.*, 2010). Several reports implicated maternal overweight and obesity or excessive weight gain during pregnancy as independent risk factors for fetal macrosomia (Ehrenberg *et al.*, 2004; Langer *et al.*, 2005; Cheng and Caughey, 2008).

2.16.2 LONG-TERM RISKS

Although there are several controversies surrounding the proposed association between GDM of the mother and disturbances in glucose metabolism and obesity of the young child (Chu *et al.*, 2007; Catalano *et al.*, 2009; Pirkola *et al.*, 2010), Pirkola *et al*., poised that GDM is not an independent risk factor for childhood obesity but rather pre-pregnancy BMI (Pirkola *et al.*, 2010). However, on the contrary, several works reported the incidences of the metabolic syndrome among children born by GDM mothers to have increased to about 3.5-4 fold as compared to children of non-GDM mothers (Boney *et al.*, 2005; Clausen *et al.*, 2009; Vääräsmäki *et al.*, 2009).

Boney *et al.* also held the view as was earlier proposed by Pirkola *et al*. They reported that gestational diabetes is not an independent risk factor for the metabolic syndrome in childhood but in association with fetal macrosomia. This constitutes a significant risk for the newborn of the metabolic syndrome in childhood (Boney *et al.*, 2005). They had the view that metabolic syndrome could be associated with heredity, environment or both. Epigenetic mechanisms could explain whether hyperglycemic prenatal environment have some effect on metabolic dysregulation in children born to GDM patients (FernandezMorera *et al.*, 2010; Buchanan *et al.*, 2012).

2.17 TREATMENT

The primary goals of the management of GDM are to prevent macrosomia and to detect and prevent pregnancy complications (Evensen, 2012). The effects of the treatment of GDM have shown enormous improvement. This was reported in two large randomized control trial (RCTs)(Crowther *et al.*, 2005; Landon *et al.*, 2009) and one systematic review and a meta-analysis of RCTs comparing usual care with specific treatment of GDM patients (Horvath *et al.*, 2010). A study conducted in Australia involving 1000 GDM patients comprising an intervention group and a control group reported that the intervention group had a lower rate of serious perinatal complications but had higher rates of admission to neonatal nursery and labor induction than the control group(Landon *et al.*, 2009).

Treatment of mild GDM reduces the risk of maternal weight gain and also the incidence of blood pressure problems during pregnancy (Landon *et al.*, 2009). Several researchers have established a connection between the several complications of GDM and high fasting glucose values (Naylor *et al.*, 1996; Suhonen *et al.*, 2008).

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CHAPTER THREE

METHODOLOGY

3.1 STUDY DESIGN

A case-control study carried out on 200 pregnant women among which: 150 were non diabetic (subjects) and 50 pre diabetic pregnant women (controls) were recruited for the study. This study was conducted at the Tema General Hospital and Provita Specialist Hospital from March 2014 to March 2015.

3.2 SAMPLE SIZE JUSTIFICATION

The following formula was used:

$$N = \frac{t^2 x\ P\ (1 - P)}{m^2}$$

Where;

N: sample size, **t**: confidence interval of 95% (standard value of 1.96), **P**: prevalence rate (9.0%), **m**: margin of error (standard value of 0.05). Hence **N=** 126(minimum**).** A sample size of 200 was thus chosen for this study.

3.3 STUDY SITE

The study was carried out at the antenatal clinic of the Tema General Hospital and Provita Specialist Hospital in the Greater Accra Region of Ghana. The Tema General Hospital is a Metropolitan Hospital and a major referral center located in the Tema Metropolis. It

offers both general and specialist care services in all the major clinical disciplines including Internal Medicine, General Surgery, Pediatrics, Obstetrics and Gynecology, Out-patient attendance is high and thus provides adequate numbers required for the study. Provita Specialist Hospital is an invitro fertilization Hospital (I. V. F) also in Tema community 6.

3.4 ETHICAL CONSIDERATIONS AND CONSENT PROCESS

- Ethical clearance was obtained from the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences (SMS), Kwame Nkrumah University of Science & Technology (KNUST).
- Informed Consent of subjects: Clients who reported at the antenatal clinic during the study period were asked to give written informed consent after detailed explanation of the study procedures have been given, before recruitment into the study. Guardians of participants below 18 years of age gave consent on their behalf.

3.5 ELIGIBILITY CRITERIA

Participants must be pregnant and attending antenatal clinic of the Tema General Hospital and Provita Specialist Hospital. Participants must be in good overall health without glucose intolerance or gestational diabetes, and others with pre-gestational diabetes. Non pregnant women, persons with recent or chronic conditions that could affect concentrations of the markers such as lipids, cortisol and others, persons taking cholesterol-lowering medication were all excluded from the study. In addition, pregnant women with uremia, iron deficiency anemia, haemoglobinopathies were also excluded from the study.

3.6 SAMPLE COLLECTION AND ANALYSIS

Primary data was collected with reference to W.H.O STEPS approach for noncommunicable diseases risk factor assessment with particular emphasis on steps 1, 2 and

3.

STEP 1 was used to capture information relating to age, sex occupation tobacco use, alcohol consumption, as well as history of raised blood pressure and diabetes and many others with the use of questionnaires.

STEP 2 was also used to capture information on weight, height, blood pressure level (systolic blood pressure (SBP) and diastolic blood pressure (DBP) and BMI (Body Mass Index). All these were accomplished by the use digital weighing scale, standiometers and standard digital sphygmomanometer with standard cuff.

STEP 3 involved taking 6mls blood by the standard vacutainer technique into yellow top gel tubes during the first trimester and again between the 24th and 28th week of gestation after an over night fast for 12-14hrs. The standard protocol of fasting, timing and appropriate positioning of the pregnant women was duly followed before blood samples were drawn. These samples were allowed to freely clot, span 3000 rpm for 15 minutes and then sera separated into appropriate tubes and frozen at -20ºC until analysis were done. Tests conducted included serum insulin, glycosylated hemoglobin, progesterone, estradiol, human placental lactogen and beta HCG.

To determine if gestational diabetes was present in pregnant women, a standard OGTT was Performed between the 24th and 28th week of gestation after overnight fasting (8-14 hours) by giving 100 g anhydrous glucose in 250-300ml water. Gestational age was also determined on the basis of the woman's last normal menstrual period if it coincided within 1 week of the date determined by ultrasound done between 16 and 20 weeks of gestation, otherwise the ultrasound estimates was used.

The homeostasis model assessment index-insulin resistance (HOMA-IR), which is based on fasting insulin and glucose measured in a single blood sample, were used frequently to calculate insulin resistance(Matthews *et al.*, 1985).The HOMA-IR yields an equation where insulin resistance = [fasting insulin (μIU/ml) x fasting glucose (mmol/L)]/ 22.5.

Abnormal HOMA-IR was defined as that above the upper limit of ≥25μIU/ml.

3.7 QUESTIONNAIRE ADMINISTRATION

A guided questionnaire was administered to all pregnant women who consented to enroll onto the study for information on demography, habits and clinical history. Samples of the questionnaire were pretested before the commencement of the study. Strict confidentiality was ensured throughout the administration of questionnaire.

3.8 BIOCHEMICAL MEASUREMENTS

The frozen serum samples were removed from the freezer and allowed to thaw at room temperature before being analyzed for triglyceride, total cholesterol, HDL-cholesterol, using Envoy® 500 reagents (Vital Diagnostics, USA) according to the manufacture's specification on BT 5000® Random Access Chemistry Analyzer (Biotecnica, Italy).

3.9 PRINCIPLES OF BIOCHEMICAL TESTS;

3.9.1 GLUCOSE TEST

There is enzymatic oxidation of glucose by the enzyme glucose oxidase (GOD) to gluconic acid and hydrogen peroxide (H_2O_2). Another enzyme peroxidase (POD) then aids in the reaction involving H_2O_2, phenol and 4-aminophenazone (AP) producing a complex dye with maximum absorbance at 505 nm according to the following scheme:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4AP + phenol \xrightarrow{POD} 4H_2O + 4 - aminophenazone$$

3.9.2 TRIGLYCERIDE TEST

Triglyceride is enzymatically hydrolyzed to glycerol and this is in turn converted to glycerol phosphate in the presence of adenosine triphosphate (ATP) and glycerol kinase

(GK), an enzyme. The resulting glycerol phosphate is then oxidized in the presence of glycerol phosphate oxidase (GPO) to produce hydrogen peroxide (H_2O_2). Finally, the H_2O_2reacts with p-chlorophenol and 4-aminoantipyrine (4-AAP) in the reagent to produce a red dye which absorbs at 510 nm. Potassium Ferro cyanide in the reagent reduces interference by bilirubin.

$$Triglyceride + 3H_2O \xrightarrow{lipase} Glycerol + Fatty\ acids$$

$$Glycerol + ATP \xrightarrow{glycerol\ kinase} Glycerol - 3 - phosphate + ADP$$

$$Glycerol - 3 - phosphate + O_2 \xrightarrow{GPO} \text{Dihydroxyacetone} + H_2O_2 + \text{Glycerol}$$

$$2H_2O_2 + \text{p} - \text{Chlorophenol} + 4 - \text{AA} \xrightarrow{PO} 4H_2\text{4O} + 4 - \text{p} - \text{benzoqunoneimine}$$

3.9.3 TOTAL CHOLESTEROL TEST

The enzymatic oxidation of cholesterol by cholesterol esterase (CHOD,EC 1.1.3.6) coupled with hydrolysis of its esters with a fungal lipase hydrogen peroxide released produces the oxidative coupling of phenol with 4-aminophenazone(4AP) by means of a reaction catalyzed by peroxidase(POD,EC 1.11.1.7),yields quinoneimine which is read at 500-505nm.

$$Cholesterol\ ester + H_2O \xrightarrow{cholesterol\ esterase} Cholesterol + Fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{cholesteroloxidase} Cholest - 4 - en - 3 - one + H_2O_2$$

$$H_2O_2 + 4 - aminophenazone + Phenol \xrightarrow{PO} 4H_2O + 4 - (\text{p} - \text{benzoquinoneimine})$$

3.10 PRINCIPLE OF GLYCATED HAEMOGLOBIN (HBA1C)

This uses Cat ion Exchange Resin method for the selective estimation of glycated hemoglobin (HBA1C) in human whole blood.

A hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weak binding cat ion –exchange resin. During this time, HBA binds to the resin. The non

glycated hemoglobin binds to the resin leaving the GHb free in the supernatant containing the glycated hemoglobin. After the mixing period, a filter is used to separate the supernatant containing the glycated hemoglobin from the resin. The Glycated hemoglobin is determined by measuring the absorbance at 415nm (405-420) of GHb fraction and the total Hb fraction using a semi auto spectrophotometer. The ratio of the two absorbance gives the percentage of glycated hemoglobin.

CALCULATIONS:

$$GHb\% = \frac{\text{Absorbance of glycated hemoglobin (GHb)} \times 5.2 \text{ (assay factor)}}{\text{Absorbance of Total hemoglobin (THb)}}$$

3.11 THE PRINCIPLE OF PLASMA INSULIN TEST (CLIA METHODS)

The Insulin CLIA, by MAGLUMI is a sandwich immune luminometric assay. It uses an anti-insulin monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, calibrator or control with ABEI label, FITC label and Nano magnetic micro beads coated with sheep anti-FITC are mixed thoroughly and incubated at 37 degrees forming a sandwich, after sediment in a magnetic field, decant the supernatant, and then cycle washing it for one time. Subsequently, the starter 1 and 2 reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of insulin present in samples. This is measured spectrophotometrically at 450 nm. The intensity of the color generated is directly proportional to the amount of insulin in the sample.

3.12 PRINCIPLES OF PROGESTERONE (P4) TEST

This uses the principle of competitive chemiluminescence immunoassay.

By this technique, ABEI is used to label an anti-PRG monoclonal antibody, also FITC to label a purified PRG antigen and again an anti-FITC antibody to coat magnetic microbeads.

The samples (or calibrator/control if applicable), ABEI label, FITC label and magnetic microbeads are mixed thoroughly together and incubated at 37°C, forming antigen-antibody complexes: after precipitation in a magnetic field, the supernatant is decanted and then a cycle wash performed. Subsequently, the starter 1 and 2 is added to initiate a chemiluminescence reaction.

The light signal is measured by a photomultiplier within 3 seconds as Relative light unit (RFU) which is directly proportional to the concentration of PRG present in samples.

3.13 PRINCIPLE OF ESTRADIOL (E2) ESTIMATION (MAGLUMI 600 ANALYZER)

This also uses the principle of competitive chemiluminescence immunoassay.

It uses ABEI to label an anti-E2 monoclonal antibody, and also a purified E2 antigen to coat magnetic microbeads. The sample (or calibrator/control, if applicable), ABEI label and magnetic microbeads are mixed thoroughly and incubated at 37°C foaming antigenantibody complexes. After precipitation in a magnetic field, the supernatant is decanted And then a wash cycle is performed. Subsequently, the starter 1 and 2 is added which ultimatedly initiates a chemiluminiscence reaction. The light signal produced is measured.

By a photomultiplier within 5 seconds as RLU which is proportional to the concentration of E2 present in the sample.

3.14 BETA CHORIONIC GONADOTROPHIN (Bhcg) (BY MAGLUMI 600 ANALYZER)

SANDWICH CHEMILUMINISCENCE IMMUNOASSAY

An ABEI is used to label anti-HCG monoclonal antibody, and also another anti-HCG monoclonal antibody to coat microbeads. The sample (or calibrator/control, if applicable), and microbeads are mixed thoroughly and incubated at 37c, and the a cycle wash is performed. Then ABEI label is added, mixed well and incubated again at 37c to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then another wash cycle performed. Subsequently, the starter 1 and 2 are added to initiate a chemiluminiscence reaction and the light signal generated is measured by photomultiplier within 3 seconds as relative light unit (RLU) which is proportional to the concentration of HCG present in the sample.

3.15 PRINCIPLE OF HUMAN PLACENTA LACTOGEN (HPL)

SANDWICH ELISA TECHNIQUE

The microelisa strip plate are precoated with antibodies specific to HPL. The samples or standards are added to the microelisa strip and this combines with the specific antibody. Then a horseradish peroxidase (HRP) conjugated antibody specific for HPL is added to each microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain HPL and HRP conjugated HPL antibody will appear blue in colour and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. The OD values is proportional to the concentration of HPL A standard curve is plotted and the concentration is calculated by comparing the OD of the samples to the standard curve.

3.16 STATISTICAL ANALYSIS

Results were expressed as mean ± S.D. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc.) and Graph Pad prism 5 for Windows. Normal distribution and

homogeneity of the variances were tested using Kolmogorov-Smirnov and Levène 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.

CHAPTER FOUR

RESULTS

4.1 GENERAL CHARACTERISTICS OF THE PARTICIPANTS

A total of 200 women who were pregnant and attending antenatal clinic at the TGH were recruited for the study. These included 150 non-diabetic and 50 diabetic pregnant women. Table 4.1 presents the baseline characteristics of both groups comparing their demographics. The mean ages of the non-diabetic and diabetic pregnant women were 29.08 ±5.26 and 27.78 ± 4.65 respectively. 13.3% and 34% of the non-diabetic and diabetic pregnant women respectively, had significant family history of diabetes, whilst family history of obesity was low in both groups (Table 4.1). Developing pregnancy complications previously was significant (P= 0.001) between both groups with 6.7% and 22% pre-mature births occurring in the non-diabetic and diabetic pregnant women respectively.

Table 4. 1: Baseline characteristics of non-diabetic pregnant women (Cases) and diabetic pregnant women (control)

Variable	Cases (n = 150)	Controls (n = 50)	P-value
Age (Mean ± SD)	29.08 ± 5.26	27.78 ± 4.65	0.136
Age group n (%)			0.494
<20	5 (3.3)	2 (4.0)	
20-29	74 (49.3)	30 (60.0)	
30-39	69 (46.0)	18 (36.0)	
40-49	2 (1.3)	0 (0.0)	
Marital status n (%)			**0.040**
Single	27 (18.0)	3 (6.0)	
Married	123 (82.0)	47 (94.0)	
Occupation n (%)			0.496
None	32 (21.3)	7 (14.0)	
Informal	99 (66.0)	37 (74.0)	
Formal	19 (12.7)	6 (12.0)	
Educational status n (%)			0.215
None	12 (8.0)	0 (0.0)	
Basic	81 (54.0)	30 (60.0)	
Secondary	40 (26.7)	13 (26.0)	
Tertiary	17 (11.3)	7 (14.0)	
Family History of other conditions			**<0.0001**
None	107 (71.3)	18 (36.0)	
Diabetes	20 (13.3)	17 (34.0)	
Obesity	1 (0.7)	2 (4.0)	
Hypertension	5 (3.3)	6 (12.0)	
Thyroid	17 (11.3)	7 (14.0)	
History of Full term delivery			0.141
Yes	104 (69.3)	29 (58.0)	
No	46 (30.7)	21 (42.0)	
History of Abortions			0.115
Yes	24 (16.0)	13 (26.0)	

No	126 (84.0)	37 (74.0)	
History of Miscarriages			0.749
Yes	10 (6.7)	4 (8.0)	
No	140 (93.3)	46 (92.0)	
Pregnancy Complications			**0.001**
None	137 (91.3)	34 (68.0)	
Pre-eclampsia	1 (0.7)	3 (6.0)	
Pre-mature birth	10 (6.7)	11 (22.0)	
Still birth	2 (1.3)	2 (4.0)	

Comparison between means was done using un-paired t-test. p < 0 .05 was considered statistically significant, n (%); number and percentages.

4.2 CLINICAL CHARACTERISTICS OF NON-DIABETIC AND DIABETIC PREGNANT WOMEN

The mean values for blood glucose, Glycated haemoglobin, and BMI were significantly (*P< 0.05)* increased in the pregnant women with diabetes as compared to the non-diabetics (Table 4.2). Overweight and obesity was observed as 32.0% and 2.0% respectively, in the non-diabetic pregnant women and also 36.0% and 8.0% respectively, in the diabetic pregnant women. The mean serum TC and TG were not significantly (*P*> 0.05) increased in the diabetic pregnant women than the non-diabetics (Table 4.2). Insulin, insulin resistance, β-HCG and HPL were higher in the diabetics than the non-diabetics, however, the mean value for insulin resistance (<0.0001), insulin levels (P= 0.003) and HPL (<0.0001)in the diabetics (controls) increased with significant difference. P4 and E2 levels were significantly (<0.0001) increased in the cases than the controls (Table 2)

Table4. 2: Biochemical characteristics of non-diabetic pregnant women (Cases) and diabetic pregnant women (control).

Variable	Cases	Controls	P-value

	(n = 150)	(n = 50)	
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**
P4	60.50 ± 21.91	44.86 ± 14.82	**<0.0001**
E2	4249.94 ± 1592.88	2470.52 ± 1297.95	**<0.0001**
HPL	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

4.3 PREVALENCE OF GDM IN NON-DIABETIC PREGNANT WOMEN

Table 4.3 presents the prevalence rate of 8.0% GDM in the non-diabetic pregnant women group. However, the lower extreme age groups of the entire non-diabetic pregnant women indicated that there was no GDM in the age group <20yrs, whilst about 8.3% of GDM among the upper extreme age group of the non-diabetic pregnant women (ages between

40-49yr) was recorded. Pregnant women in their thirties showed a remarkable increase in GDM prevalence rate of about 58.3% among the age group (30-39yr) than those in the age group 20-29yr (33.3%).

Table4. 3: Prevalence of GDM in non-diabetic pregnant women

Status	N	(%)
GDM	12	8
No GDM	138	92

GDM=Gestational Diabetes Mellitus, the percentage is out of n= 150

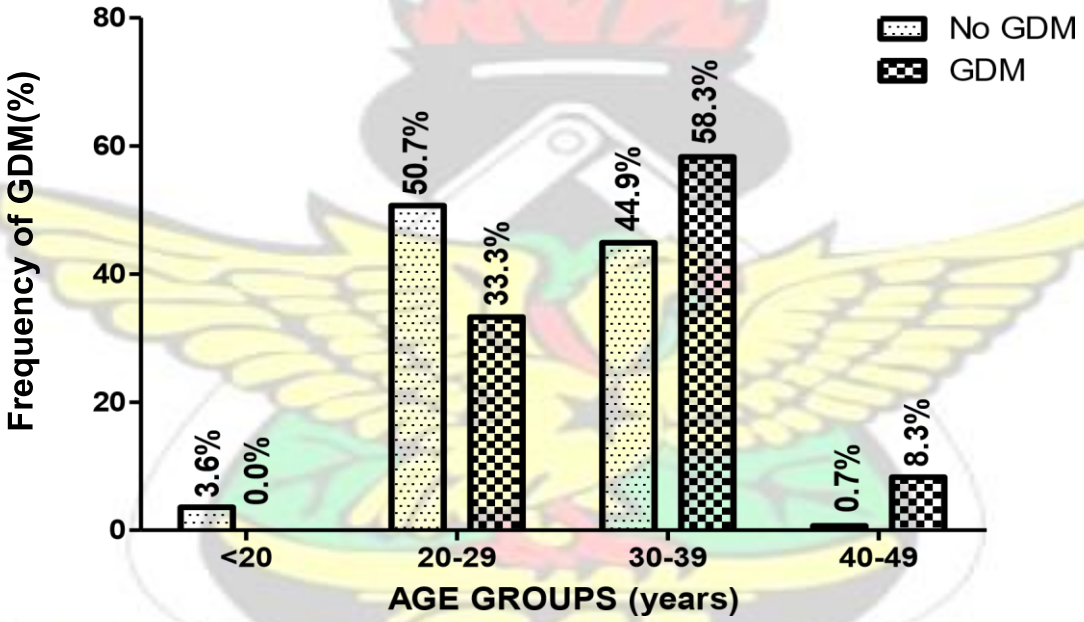


Figure 4. 1: Age prevalence of GDM in non-diabetic pregnant women.

4.4 HISTORICAL CHARACTERISTICS AMONG PREGNANT WOMEN WITH GDM

The pregnant women with gestational diabetes mellitus did not have history of GDM (0.0%). Among the patients with GDM, 25% (3) had family history of diabetes and 8.3% (1) had family history of thyroid disease whereas these were indicated as 12.3% and 11.6%

in those with no GDM (Table 4.4). The history of abortions and miscarriages were observed as 25% and 8.3% respectively among pregnant women without GDM, however, these was also recorded as 15.2% and 6.5% in pregnant women with no GDM accordingly. All the pregnant women who developed GDM (100%) were not aware of diabetes (Table 4.4). A history of pre mature births was indicated as 8.3% in GDM compare to 6.5% in participants without GDM.

Table4. 4: Historical characteristics among pregnant women who developed GDM and those without GDM

Variable	GDM (n = 12)	No GDM (n = 138)	P-value
History of GDM			0.767
Yes	0 (0.0)	1 (0.7)	
No	12 (100)	137 (99.3)	
Family History of other conditions			0.736
None	8 (66.7)	99 (71.7)	
Diabetes	3 (25.0)	17 (12.3)	
Obesity	0 (0.0)	1 (0.7)	
Hypertension	0 (0.0)	5 (3.6)	
Thyroid disease	1 (8.3)	16 (11.6)	
History of Full term delivery			0.389
Yes	7 (58.3)	97 (70.3)	
No	5 (41.7)	41 (29.7)	
History of Abortions			0.375
Yes	3 (25.0)	21 (15.2)	
No	9 (75.0)	117 (84.8)	
History of Miscarriages			0.809
Yes	1 (8.3)	9 (6.5)	
No	11 (91.7)	129 (93.5)	

History of Pregnancy Complications			0.957
None	11 (91.7)	126 (91.3)	
Pre-eclampsia	0 (0.0)	1 (0.7)	
Pre-mature birth	1 (8.3)	9 (6.5)	
Still birth	0 (0.0)	2 (1.4)	
Awareness of diabetes			0.762
Yes	0 (0.0)	4 (2.9)	
No	12 (100)	134 (91.1)	

4.5 CLINICAL CHARACTERISTICS OF PREGNANT WOMEN WITH GDM (CASES) AND THOSE WITH DIABETES (CONTROLS)

Blood glucose in the pregnant women with diabetes (control group) was significantly ($p=0.014$) increased than the pregnant women who developed GDM (cases group). Difference in Glycated hemoglobin were no significant ($P< 0.05$) with Poor glycemic control observed in 16.7% of those who developed GDM and in 6.0 % of those with diabetes (control group). BMI comparison showed significant differences ($p= 0.009$), with overweight in 41.7% of those who developed GDM. However, obesity was observed in 8.0% of the pregnant women with diabetes (control group). Non-significant increased levels ofTG, β-HCG and decrease levels of TC, IR, and Insulin was observed in the pregnant women who developed GDM (cases group) compared to the pregnant women with diabetes (control group). However, significant (<0.0001) increased levels ofP4, E2 and decreased levels of HPL was also observed in the pregnant women who developed GDM (cases group) compared to the pregnant women with diabetes (control group). (Table 4.5).

Table 4. 5: Clinical characteristics of pregnant women who developed GDM (cases) and those with Diabetes (controls).

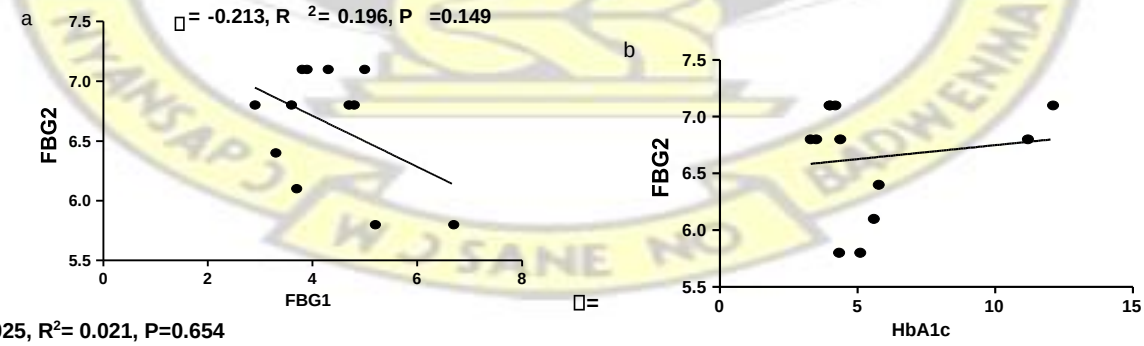
Variable	GDM (n = 12)	Controls (n = 50)	P-value
FBG (mmol/l)	4.33 ± 1.03	5.86 ± 2.03	**0.014**
HB (mg/dl)	9.22 ± 2.83	10.32 ± 1.72	0.086
HBA1c (%)	5.62 ± 2.92	5.56 ± 1.11	0.900
Glycemic control			0.223
≤ 7%	10 (83.3)	47 (94.0)	
> 7%	2 (16.7)	3 (6.0)	
Blood Pressure (mmHg)			
SBP	115.00 ± 4.99	115.34 ± 6.59	0.868
DBP	82.42 ± 7.45	82.88 ± 7.78	0.853
BMI n (Kg/m^2)	21.80 ± 2.85	24.75 ± 3.51	**0.009**
BMI n (%)			0.153
Underweight	1 (8.3)	0 (0.0)	
Normal	6 (50.0)	28 (56.0)	
Overweight	5 (41.7)	18 (36.0)	
Obese	0 (0.0)	4 (8.0)	
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
P4	72.76 ± 11.54	44.86 ± 14.82	**<0.0001**
E2	4502.01 ± 1338.39	2470.52 ± 1297.95	**<0.0001**
HPL	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; hemoglobin, HBA1c; glycated hemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, TC; total cholesterol, TG; triglyceride, β-HCG; betahuman chorionic gonadotropin, IR: insulin resistant.

4.6 LINEAR REGRESSION ANALYSIS OF PREDICTORS OF GDM

Linear regression graphs of FBG-2 with FBG1, HbA1c, Insulin, BMI, TC, TG, among the subjects who developed GDM showed that for a mmol/L decrease in FBG-1 (β= -0.213, r^2 = 0.196, P = 0.149), a significant decrease in Insulin (β= -0.035, r^2 = 0.612, P = 0.002), there was a corresponding mean mmol/L increase in blood glucose estimated at the 24-28th week of gestation (FBG-2) (Figure 4.2). However, for a percentage increase in HbA1c (β=0.025, r^2 = 0.021, P = 0.654), there was a corresponding increase in FBG-2. In addition, BMI and P4 showed a negative relationship with FGB-2 while TC, TG, E2 and HPL were positively correlated.



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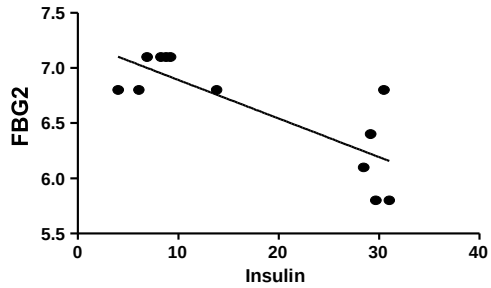

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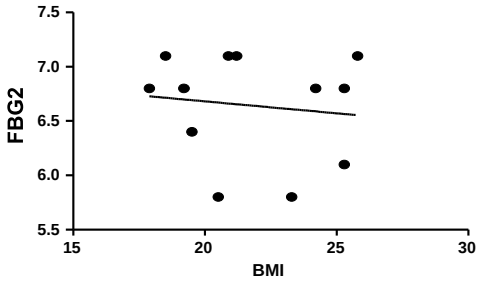
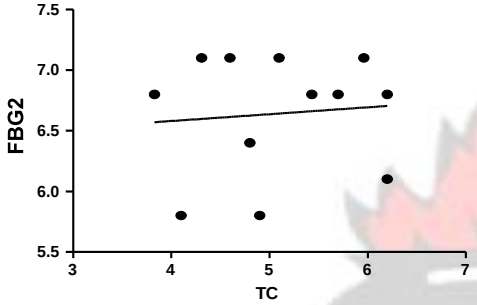
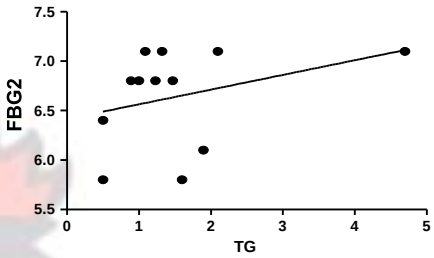


□= 0.057, R^2= 0.009, P=0.775

e

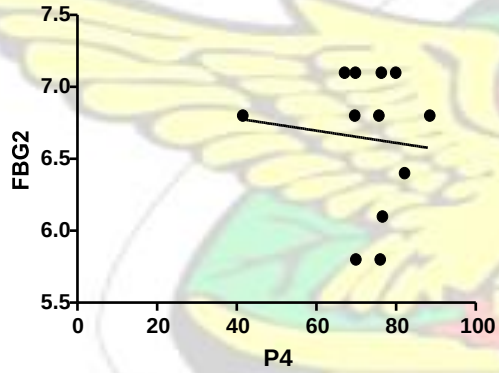


□= 0.149, R^2= 0.111, P=0.289 f



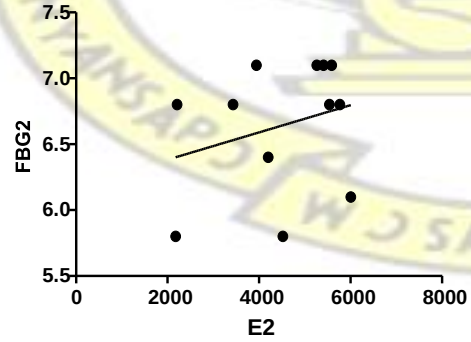
□= -235.5, R^2= 0.010, P=0.760

g



□= 9666, R^2= 0.078, P=0.380

h



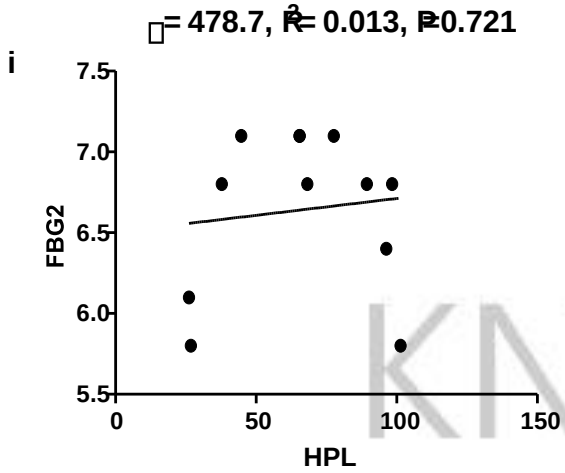


Figure 4.2: Linear Regression analysis between FBG2 and FBG1, HbA1c, Insulin, BMI, TC, TG, P4, E2 and HPL among pregnant women who developed GDM.

4.7 CLINICAL CHARACTERISTICS OF PREGNANT WOMEN WHO DEVELOPED GDM

Generally, Table 4.7 shows that study participants had good glycemic control scores as 83.3% for GDM as well as 97.8% for the non GDM group, contrary, participants without GDM were obese and recorded high body mass index than those with GDM. The group with the pathological condition (GDM) showed increase in mean values for TC, TG,IR, insulin and ß-HCG as compared to those who did not develop the condition, though there were no significant p-value was recorded (Table 4.7). There was an increase in the mean values of blood glucose, Glycated hemoglobinand P4 levels with significant difference (P< 0.05) among subjects that developed GDM as compared with those that did not. However, a similar increased was realized in subjects that developed GDM when hemoglobin levels were considered than those who did not.

Table 4. 6: Comparison of numerical variable in those who developed GDM and those who did not

Variable	GDM (n = 12)	No GDM (n = 138)	P-value

FBG (mmol/l)	4.33 ± 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/m^2)	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 (µIU/mL)	17.17 ± 11.38	14.30 ± 7.37	0.221
IR (µIU/mL)	3.50 ± 2.75	2.68 ± 1.64	0.120
P4	72.76 ± 11.54	59.43 ± 22.30	**0.043**
E2	4502.01 ± 1338.39	4228.02 ± 1615.44	0.569
HPL	66.45 ± 27.43	76.50 ± 23.02	0.155

p < 0 .05 was considered statistically significant, FBG; fasting blood glucose, HB; hemoglobin, HBA1c; glycated hemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, TC; total cholesterol, TG; triglyceride, β-HCG; beta-human chorionic gonadotropic, P4; Progesterone, E2; Estradiol, HPL; Human Placenta Lactogen

4.8 LOGISTIC REGRESSION FOR PREDICTORS OF GESTATIONAL DIABETES MELLITUS

The risk of developing GDM is higher with overweight (OR=1.76, P=0.370). Family history of diabetes also increases the odds of developing GDM (OR=2.18, P=0.282) (Table 4.8). Pregnant women with history of abortion, miscarriage and pre-mature birth as complications have higher risk of developing GDM.

Table 4. 7: 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	-	-

4.9 RELATIONSHIP BETWEEN GLYCATED HEMOGLOBIN, PLACENTA PEPTIDES AND DYSLIPIDEMIA

Body mass index was indicated to have significant and positive correlation with Glycated hemoglobin (.702*) but an inverse and strong correlation with diastolic blood pressure (.821*) among the subjects who later developed gestational diabetes among the non GDM group. Glycated hemoglobin, the glycemic control index marker was inversely associated with diastolic blood pressure (-.637*) as shown in (Table4.9). High blood sugar levels

(FBS) also has an inverse and strong significant correlation (-.801**) with insulin levels among this group (GDM).Among the control group (diabetics at the lower left corner of Table4.9) depict a significant but negative correlation of ß-HCG with glycated hemoglobin (-.303*) and fasting blood sugar levels (-.469**).Insulin resistant among the diabetes control was positively associated with the high insulin and sugar levels than the newly diagnosed (12) GDM of the 150 subjects (cases).

Table 4. 8: Relationship between glycated hemoglobin, Placenta peptides and dyslipidemia among pregnant women with GDM (upper portion) and those with Diabetes (lower portion).

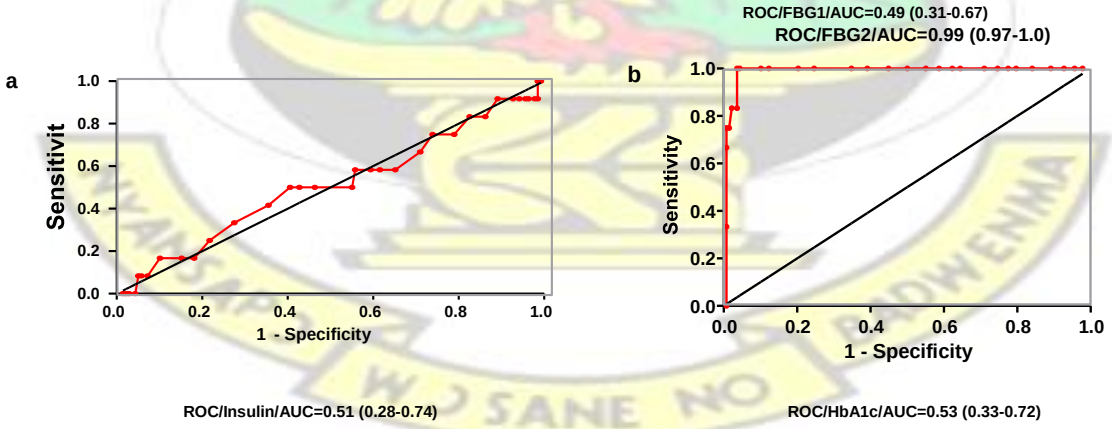
Variables		HbA1c	FBG	β-HCG	Insulin	IR	BMI	P4	E2	HPL	TC	TG
HbA1c	r	1	0.25	.a	-0.096	-0.087	.702*	0.073	0.352	-0.167	-0.104	0.555
	P-value		0.434	.	0.766	0.788	0.011	0.821	0.261	0.604	0.748	0.061
FBG	r	.510**	1	.a	0.427	.714**	0.163	-0.409	-0.13	-0.518	-0.369	0.244
	P-value	0		.	0.166	0.009	0.613	0.187	0.686	0.084	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	-0.448	-0.314	-0.144	-0.238
	P-value	0.388	0.921	0.622		0	0.763	0.356	0.144	0.32	0.655	0.457
IR	r	0.193	.545**	-0.172	.803**	1	-0.084	-0.357	-0.455	-0.403	-0.223	-0.159
	P-value	0.178	0	0.231	0		0.796	0.254	0.138	0.194	0.486	0.623
BMI	r	-0.008	-0.105	-0.237	-0.17	-0.176	1	0.142	0.576	-0.161	0.024	0.438
	P-value	0.958	0.47	0.097	0.239	0.221		0.659	0.05	0.618	0.942	0.154
P4	r	-0.144	-.298*	0.162	0.237	0.02	-0.092	1	0.235	0.524	0.003	-0.212
	P-value	0.318	0.035	0.261	0.098	0.891	0.525		0.461	0.08	0.994	0.509
E2	r	0.142	0.066	0.133	-0.206	-0.155	0.155	0.014	1	-0.282	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	-0.219	1	-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	0.138	0.126	1	0.413
	P-value	0.211	0.407	0.318	0.372	0.318	0.232	0.851	0.341	0.384		0.182
TG	r	0.186	0.011	-0.027	-0.011	-0.004	0.052	-0.203	.314*	0.041	0.187	1
	P-value	0.196	0.938	0.852	0.941	0.98	0.717	0.158	0.026	0.777	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), [a]not be computed because at least one of the variables is constant. FBG; fasting blood glucose, HB; hemoglobin, HBA1c; glycated hemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, TC; total cholesterol, TG; triglyceride, βHCG; beta-human chorionic gonadotropin; P4; Progesterone, E2; Estradiol, HPL; Human Placenta Lactogen

4.10 USE OF FBG, HBA1C, INSULIN, ß-HCG, P4, E2 AND HPL AS PREDICTORS OF GDM

Figure 4.3 shows the receiver operator curve for fasting blood glucose, glycated hemoglobin, β-HCG and insulin levels. The area under the ROC for FBG-1 (at first trimester) was 0.49 suggesting that fasting plasma glucose level in the first trimester was a poor diagnostic test for gestational diabetes mellitus. However, AUC for FBG-2 (between 24wks-28 wks.) was 0.99 showing that FBG-2 is a very good diagnostic test for diagnosing GDM between 24wks-28 wks. Whereas, Glycated hemoglobin, Insulin, β-HCG and E2 have indicated averagely as diagnostic test markers for GDM as compared with FBS-2, HPL and P4 as markers used for GDM in this study.



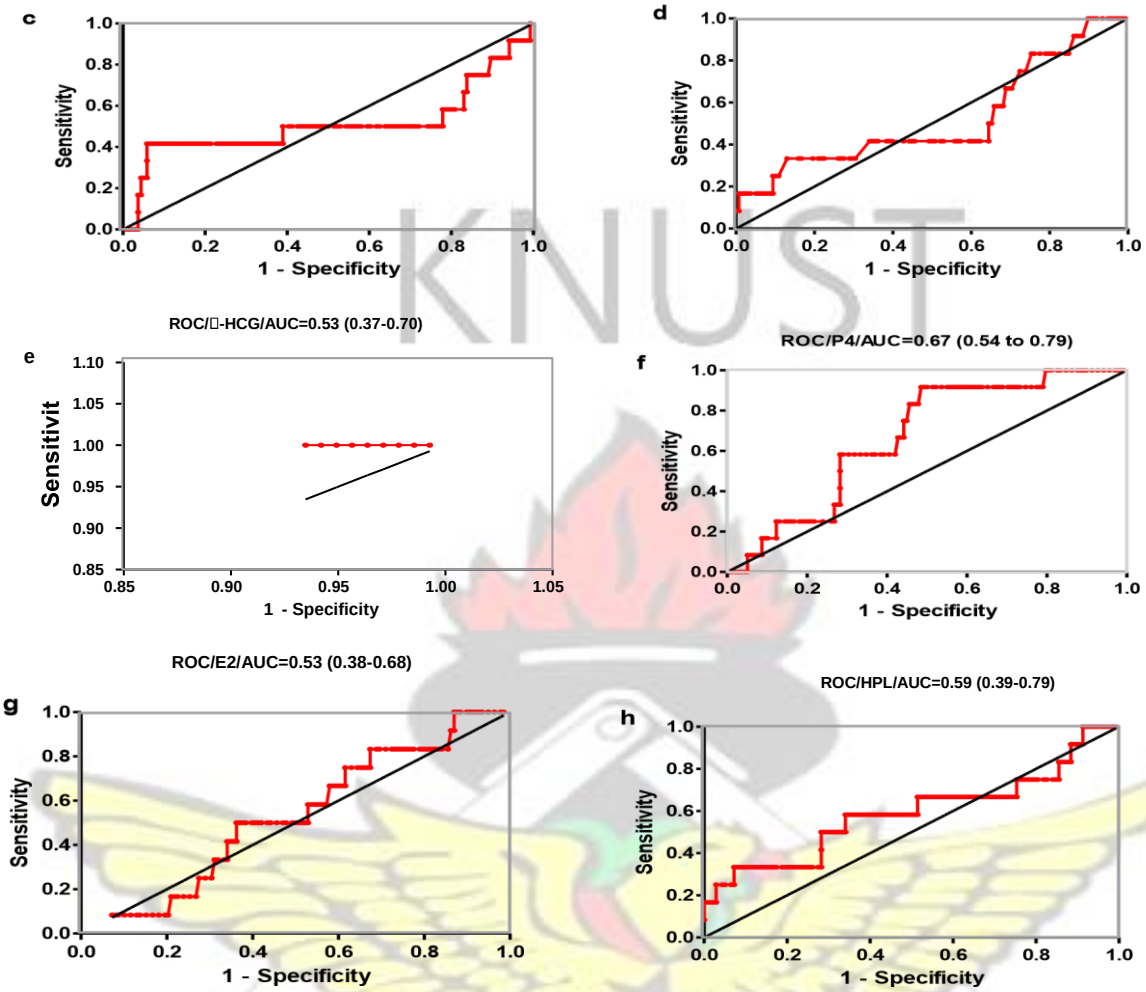


Figure 4.3: Receiver Operator Curve and Area under the curve (AUC) for FBG-1, FBG-2, HbA1c, Insulin, β-HCG, P4, E2 and HPL.

4.11 ACCURACY OF FIRST TRIMESTER FBG-1, FBG-2, HBA1C, INSULIN, BHCG, P4, E2 AND HPL AS MARKERS OF GESTATIONAL DIABETES

Table 4.8 shows the Accuracy of first trimester FBG-1, FBG-2, HbA1c, Insulin, β-HCG, P4, E2 and HPL. The diagnostic value of 3.75 mmol/L forFBG-1 had a sensitivity of 33.3% and Specificity of 72.0% as marker for GDMand 5.6 mmol/L diagnostic value with significant 100% sensitivity and 95.7% Specificity for 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 sensitivity of 63.0% and 71.7% at 5256 and 65.61 respectively.

Table 4.8: 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
B-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)	0.052
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

CHAPTER FIVE

DISCUSSION

5.1 GENERAL CHARACTERISTICS OF THE PREGNANT WOMEN

The rising trend of diabetes in pregnancy poses a considerable maternal and perinatal morbidity and mortality (Clausen *et al.*, 2008). This study was aimed at determining the prevalence of GDM among pregnant women attending antenatal clinic and to assess the role of placental peptides and maternal factors as potential predictors of gestational diabetes. Epidemiological studies comparing diabetic and non- diabetic mothers have clearly demonstrated adverse outcomes in diabetic mothers(Alur *et al.*, 2002; Genuth *et al.*, 2003). Furthermore, perinatal mortality and neonatal mortality rates are markedly higher among diabetic, compared to non-diabetic, pregnancies(Östlund *et al.*, 2003).There were no significant difference in mean ages between groups in this study (non-diabetic and diabetic pregnant women).

Outcomes of pregnancy in diabetic women have improved dramatically over the years with temporal trends showing a decline in rates of spontaneous abortions in diabetic mothers (Greene, 1998). However, diabetic mothers still carry a higher risk for fetal morbidity and mortality. It was observed in the present study that 6%, 22% and 4.0% of the diabetic pregnant women had history of pre-eclampsia, pre-mature birth and still birth as pregnancy complications respectively (Table 4.1). A recent prospective study has shown that in spite of planned pregnancies with good glycemic control, diabetic mothers still had higher rates of maternal and perinatal complications (Evers *et al.*, 2004).

Lipid profile changes in normal pregnancy are characterized by marked elevations of total plasma cholesterol and triglyceride levels as a result of increased liver synthesis of triglycerides (TG) and Very Low Density Lipoprotein cholesterol (VLDL-C) in response to elevated estrogen levels (Beigel *et al.*, 1998). Reduction in Lipoprotein lipase (LPL)

activity due to the down regulation of LPL gene expression by estrogen during pregnancy decreases the clearance of VLDL-C (Asare-Anane *et al.*, 2013). Furthermore, insulin inhibits lipoprotein lipase in adipose cells preventing lipolysis of TG (Denison *et al.*, 2010) hence the increase in TG and probably in TC levels as observed in the diabetic women in this present study. These factors may partly account for the increase in BMI accounting for 8.0% obesity observed among the diabetic women (Table 4.2).

This study showed significantly ($P<0.001$) increased glycated hemoglobin levels in the diabetics compared with non-diabetic pregnant women, which are also supported by Jeffcoate (Jeffcoate, 2004). HbA1c is formed by a non-enzymatic irreversible process with combination of aldehyde group of glucose and the amino terminal valine of β chain of hemoglobin. As plasma glucose is consistently elevated, there is increase in non-enzymatic glycation of hemoglobin(Chandalia and Krishnaswamy, 2002). Therefore, the increased in HbA1c among gestational diabetes mellitus subjects in our study participant is expected (Table 4.5).

Early pregnancy is characterized by normal glucose tolerance or slightly improved and peripheral (muscle) sensitivity to insulin and hepatic basal glucose production (Catalano *et al.*, 1992; Catalano *et al.*, 1993). These could be caused by the increased maternal estrogen and progesterone in early pregnancy which increases and promote pancreatic ß-cell hyperplasia, causing an increased insulin release (Carr and Gabbe, 1998; Rieck and Kaestner, 2010). This explains the rapid increased in insulin level in our study, in response to insulin resistance. This report therefore accounts for the comparable and significant difference in blood glucose and insulin with increased levels in the diabetic pregnant women mainly attributable to insulin resistance as observed in diabetics in this study. Beta HCG was also slightly increased in the diabetics than the non-diabetics. Increases in peripheral glucose utilization and glycogen storage with a concomitant reduction in hepatic

glucose production result in lower maternal fasting glucose levels (Dinsmoor and Forrest, 2002).

5.2 GESTATIONAL DIABETES MELLITUS AND ITS ASSOCIATED CHARACTERISTICS AMONG PREGNANT WOMEN

5.2.1 PREVALENCE OF GDM

Gestational diabetes affects between 7%-18% of pregnancies with rates varying by age, ethnicity, family history of diabetes, and obesity among other risk factors (CDC, 2011). The prevalence of gestational diabetes mellitus in this study using the WHO diagnostic criteria was 8.0% (Table 4.3). This is higher than the 1.7% that was obtained in a study in Enugu, south eastern Nigeria but lower than 13.9% that was obtained in Ibadan, south west Nigeria (Nwaoguikpe and Uwakwe, 2008; Hall *et al.*, 2011). However, the prevalence of GDM in this study is within the range quoted for Sub-Saharan Africa (Ugege *et al.*, 2015). Prevalence rates in different regions vary on account of the type of criteria used for diagnosis (Harris, 1995). Higher relative increases in GDM in younger women as observed in about 58.3% of the pregnant women aged 30-39 years, suggesting that the prevalence of risk factors for GDM may have increased in younger women than in older women as reported in a previous study (Ferrara, 2007). Generally, 50% of women with GDM are expected to develop type 2 diabetes within 5 years of the index pregnancy(Kim *et al.*, 2002). Recent clinical trials have shown that health behaviors such as diet and physical activity prevent or delay the onset of diabetes (Tuomilehto *et al.*, 2001; Knowler *et al.*, 2002). Such behavioral interventions have been shown to be cost-effective at a higher level than a pharmacological intervention (Zhang *et al.*, 2004). Therefore, clinicians will increasingly have to promote plasma glucose testing and encourage the practice of improved health behaviors at postpartum visits of women who had GDM to prevent development of diabetes and recurrent GDM.

5.2.2 HISTORICAL CHARACTERISTICS ASSOCIATED WITH GDM

Several investigations have widely reported the association of family history of DM with the development of gestational diabetes mellitus. The prevalence of family history of DM in first-degree relative was found to be 36.2%, 86%, 11%, and16.6% (Clausen *et al.*, 2008; Wahi *et al.*, 2011). According to this study the prevalence was 25.0% (Table 4.4). All these studies express that the role of family history of diabetes in first-degree relatives was highly significant and this could be because of some genetic factors transmitting from generation-to- generation among the families(Amraei and Azemati, 2007). Histories of full term delivery, abortions and miscarriages were also observed in this study as 58.3%, 25.0% and 8.3% respectively among the women who developed GDM (Table 4.4), which was supported by Xiong *et al.,* 2001. In their study, it was indicated that mothers with GDM were at increased risk of presenting with pre-eclampsia, premature rupture of membranes, and preterm delivery(Xiong *et al.*, 2001). Infants born to mothers with GDM were at higher risk of being macrosomic or large-for-gestational-age(Saydah *et al.*, 2005).

5.2.3 OBESITY AMONG WOMEN WHO DEVELOPED GDM

Obesity in pregnancy is a great health issue and all effort geared toward its reduction is a laudable idea. There exists significant relationship between overweight/obesity and GDM as established by previous studies (Abrams and Parker, 1987; Wahi *et al.*, 2011). Several studies reveals that a significant proportion of subjects with GDM were overweight and obese(Abrams and Parker, 1987; McMahon *et al.*, 1998). In a report by Amraei and Azemati, 31.06% of pregnant women with GDM were overweight and 27.2% were obese (Amraei and Azemati, 2007), however, in this present study, 41.7% of women who developed GDM were overweight (Table 4.6). In pregnancy, weight gain might be a better useful predictor of GDM. In another reported work, GDM plays a crucial role in the

increasing prevalence of diabetes and obesity(Ferrara, 2007). Infants of women with GDM or diabetes are at increased risk of developing obesity, impaired glucose tolerance, and diabetes as children or young adults (Pettitt *et al.*, 1988; Silverman *et al.*, 1998), and the increased risk may be independent of genetic factors(Dabelea *et al.*, 2000). Maternal obesity has been shown to correlate with adverse pregnancy outcomes, such as preeclampsia, gestational diabetes, fetal macrosomia and caesarean deliveries (Cnattingius *et al.*, 1998; Sebire *et al.*, 2002).

5.2.4 DYSLIPIDEMIA AMONG WOMEN WHO DEVELOPED GDM

In this study, we reported increased mean values of total cholesterol and triglycerides in subjects who developed GDM as compared to the pregnant women with no GDM (Table 4.6). These findings are in agreement with a recent report (Asare-Anane *et al.*, 2013). During pregnancy, there is increased synthesis of triglyceride from the liver in response to elevated estrogen levels resulting in hyperlipidemia. There is also an elevation in plasma concentrations of placental hormones like progesterone and human chorionic somatomamotrophin as well as cortisol (Asare-Anane *et al.*, 2013). These changes are designed to provide energy and nutrition for the developing fetus by providing more glucose for the fetus, resulting in deregulation of glucose metabolism (McMahon *et al.*, 1998).

5.3 VARIATIONS IN INSULIN, ß-HCG, P4, E2 AND HPL

Mothers with gestational diabetes mellitus (GDM), as is defined as carbohydrate intolerance first diagnosed in pregnancy, experience an additional decrease in whole-body insulin sensitivity by late pregnancy (Lain and Catalano, 2007). This may however account for the increase in the levels of insulin and insulin resistance in the women who developed GDM than those without GDM in this study, indicating that most women who develop

GDM have chronic insulin resistance, as reported in a related study (Homko *et al.*, 2001). Decreased hepatic insulin sensitivity is more pronounced in women with GDM compared with weight-matched controls (Xiang *et al.*, 1999). This phenomenon in gestational diabetes mellitus women account for the increase in hepatic glucose production and hyperglycemia. Insulin secretion increases, but less so than the increase seen among normoglycemic pregnant women, because of inadequate β-cell function in mothers with GDM (Buchanan and Xiang, 2005). Additionally, defects in insulin signaling on a background of chronic insulin resistance are thought to lead to the development of GDM, however, the underlying mechanisms (particularly the genetics) of maternal GDM are yet to be fully elucidated(Buchanan and Xiang, 2005; Lain and Catalano, 2007). As pregnancy progresses and the placenta grow larger, hormone production also increases and so does the level of insulin resistance. This process usually starts between 20 and 24 weeks of pregnancy. At birth, when the placenta is delivered, the hormone production stops and so does the condition, strongly suggesting that these hormones cause GDM (Ryan and Enns, 1988; Buchanan and Xiang, 2005)

Reports on first trimester β-HCG concentrations have shown either normal or reduced levels in diabetic women and in those that subsequently develop gestational diabetes(Spencer *et al.*, 2005; Prats *et al.*, 2012). In this study, we hold contrary view because β-HCG levels were increased in the diabetic pregnant women and in those that subsequently developed gestational diabetes. Prats *et al*. has further solidified our stand by stating categorically that only one study reported a reduction in free β-HCG and therefore, pre-gestational diabetes has effect on total free β-HCG(Prats *et al.*, 2012). As pregnancy progresses, increased levels of human chorionic somatomamotrophin (hCS), cortisol, prolactin, progesterone, and estrogen lead to insulin resistance in peripheral tissues (Dinsmoor and Forrest, 2002).

During pregnancy the maternal level of HPL can be altered by changing the circulating level of glucose. HPL is elevated with hypoglycemia and depressed with hyperglycemia (Kühl, 1991; Barbour *et al.*, 2007). This is consistent with the observation from this study in that HPL is lower in the non-diabetic pregnant women and increased in the diabetic pregnant women (Table 4.2). The metabolic role of HPL to mobilize glucose, lipids and free fatty acids cannot be underestimated. It has been shown that in the fed state, there is abundant glucose available, leading to increased insulin level, lipogenesis, and glucose utilization.

5.4 RISK FACTORS ASSOCIATED WITH GDM

Risk factors for GDM are family history of DM, advanced maternal age, obesity, pregnancy weight gain and previous history of poor obstetric outcome. Generally, the results of this study agree with some of the available literature on the association between these risk factors and GDM.

In this study, GDM was associated with family history of diabetes mellitus more especially with Odds of 2.18 (Table 4.7). This study was in line with earlier studies that indicated that GDM is common in women with family history of diabetes mellitus (Abrams and Parker, 1987; Flack *et al.*, 2010). These results suggest that the development of GDM during pregnancy could be linked to genetic predisposition to diabetes mellitus especially type 2 DM (Buchanan *et al.*, 2007).An increase in GDM prevalence also has implications for the upsurge of type 2 diabetes in women who have had GDM previously, because about 50% of women with a history of GDM develop diabetes within 5–10 years after delivery(Metzger *et al.*, 2008). After postpartum, this period offers an opportunity to both screened women at an early stage for preexisting diabetes to be counseled on type 2 diabetes prevention(Metzger *et al.*, 2008).

The odds (OR=1.76) of developing GDM was higher for pregnant women who were overweight in the present study (Table 4.7). Consistent with this finding is the report by Chu *et al.*, indicating that the risk of developing GDM is about 2 and 4 times higher among overweight and obese women respectively, as compare with normal-weight pregnant women(Chu *et al.*, 2007). The public health implications are significant because the potential adverse consequences associated with obesity and GDM, including higher risk of adverse infant outcomes, higher risk of diabetes for the mother later in life, and a higher risk of diabetes and overweight for the offspring(Casey *et al.*, 1997; Xiong *et al.*, 2001).

5.5 FBG, HbA1c, INSULIN, ß-HCG, P4, E2 AND HPL AS PREDICTORS OF GDM

In the study by(Sharma *et al.*, 2011), the Area under the curve (AUC), (95% CI) for fasting plasma glucose using the WHO criteria was 0.69 (0.67-0.71). The AUC (95% CI) for fasting plasma glucose in another study by (Ugege *et al.*, 2015)was 0.72 (0.42-1.00) suggesting that FPG is a fair test for the diagnosis of Gestational diabetes mellitus. This is comparable to the results of this study where AUC (95% CI) for fasting plasma glucose in the 24^{th}-28^{th} week was 0.99 indicating a strong diagnostic value for FBG (Figure 4.3b). However, the AUC (95% CI) for fasting plasma glucose measured in the first trimester was 0.49, suggesting poor diagnostic value of sugar measurement in the first trimester.

In addition, HbA1c, insulin, β-HCG, P4, E2 and HPL could be of use but have fair diagnostic tests values for GDM, since the AUC (95% CI) were >5.0 (Figure 4.3). However, there are not much supporting evidence to these findings, hence the need for a closer consideration of these markers for diagnosis of GDM. Although glycosylated hemoglobin is not usually used clinically to diagnose GDM, it is however useful in diagnosing pre-existing diabetes in early pregnancy(Sacks *et al.*, 2011).Li and Yang have also indicated in their study the value of measuring Fructosamines during pregnancy in patients with abnormal glucose tolerance(Li and Yang, 2006). They found that the mean

level of Fructosamines decreases with gestational age, and that the level of Fructosamines is similar in GDM and non-GDM patients in 16-20gestational weeks. Therefore, not all glycosylated hemoglobin are useful in the diagnosis of GDM(Li and Yang, 2006).

CHAPTER SIX

6.1 CONCLUSION

The overall prevalence of GDM in this study was 8.0 % and a prevalence of 58.3 % occurring between the ages of 30-39 years. The placenta peptides estimated in this study were increased in the gestational diabetes mellitus women and also in the non-diabetics who subsequently developed GDM.

Results of blood sugar, HbA1c, TC and TG were also increased in the gestational diabetes mellitus.

It was found out that factors such as increased age of pregnant women, overweight and family history of diabetes mellitus predispose non-diabetic pregnant women to GDM. Fasting plasma glucose may be useful as a screening test for GDM in the 24-28 gestation weeks on account of its high Specificity; however, an additional test may be necessary to decrease the false negative test results. HbA1c, insulin and β-HCG are fair tests to predict GDM.

6.2 RECOMMENDATION

1. Further research could be carried out to measure even more placental peptides longitudinally in each trimester during pregnancy.
2. Also a follow up study could be made on the GDM cases to monitor them for every complication and also the mode of delivery.
3. The sample size be increased to identify more GDM cases
4. The GDM mothers and babies could be monitored for about 12 months after birth

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