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Gestational Diabetes and Adverse Pregnancy Outcomes: Role of HbA1c, Anemia and Other Risk Factors

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DECLARATION

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this thesis has not been submitted for higher degree to any other university or institution.

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DEDICATION

To the soul of my lovely mother, and to the supporting father.

To my husband and children….

To my family…

To my friends…

To my expert teachers.

To all the people who have supported and encouraged me.

Inas Adnan Hasan Saleh

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Gestational Diabetes and Adverse Pregnancy Outcomes: Role of HbA1c, Anemia and Other Risk Factors

Abstract

Background- Some women experience health problems during pregnancy such as gestational diabetes mellitus (GDM) and iron deficiency anemia. These complications can affect the health of the mother and fetus. Getting early detection can decrease the risk of adverse pregnancy outcomes.

It has been reported that on average, 2-6% of pregnant women develop temporary GDM. Glycated hemoglobin (HbA1c) which is used to diagnose diabetes mellitus is not used for diagnosing GDM and the gold standard for diagnosis is still the oral glucose tolerance test (OGTT).

Aims- To evaluate the role of HbA1c in screening and diagnosing GDM and its correlation with adverse pregnancy outcomes and assess its levels during and after pregnancy in the high-risk pregnant women to develop GDM. Among the risk factors, iron deficiency anemia and its correlation with adverse pregnancy outcomes will also be evaluated.

Settings and design- Prospective study in prenatal care units of the United Nations Relief and Works Agency for Palestine Refugees in the West Bank (UNRWA).

Methods- Data was collected by a structured questionnaire including age, gravidity, parity, BMI (Body Mass Index), family history for Diabetes Mellitus, and personal history of previous GDM. Blood pressure was taken on every visit. HbA1c, FPG (Fasting Plasma Glucose), and CBC (Complete Blood Cell count) were measured for all subjects, while 2 hour oral glucose tolerance test (OGTT) was done when required. GDM was diagnosed at pregnancy weeks 4-22 and 24-37 by the WHO (World Health Organization) criteria from 1999 as FPG $\geq$ 126 mg/dl or plasma glucose $\geq$ 140 mg/dl 2 hours after ingestion 75g glucose orally (OGTT), and the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (FPG $\geq$ 92 mg/dl or plasma glucose $\geq$ 153 mg/dl 2 hours after the glucose load). A total of 955 pregnant women participated in this study.

Statistical Analysis- Data was collected and analyzed using SPSS software version 23. Comparisons of the means, correlations and calculation of sensitivities and specificities for

diagnosing GDM by HbA1c and the prediction of adverse pregnancy outcomes were performed.

Results: Accumulated GDM percentage was 7.4% by WHO1999 criteria, and 45.8% by modified IADPSG criteria. The mean HbA1c1 value (HbA1c measured at first visit) in women with GDM1-WHO was significantly higher than women without GDM1-WHO (5.9 ± 0.6% compare to 5.4 ±0.4%, P = 0.000). In the same direction, a statistically significant difference was detected in the mean value of HbA1c1 between women who developed T2DM after delivery and those who didn't (5.9 ± 0.5% compare to 5.4 ± 0.4%, P = 0.000). A positive correlation was observed between a baby's birth weight and the baby's head circumference and HbA1c1 at first visit.

The optimal HbA1c1 threshold value at first visit for detecting GDM1-WHO was 5.75% with sensitivity 57.5% and specificity 85.1%. The HbA1c1 cutoff value ≥ 5.65% had a sensitivity of 31.1 % and specificity of 88.5% in detecting adverse pregnancy outcomes.

A significant relationship was observed between pre-abortion and GDM1-WHO (P = 0.001), 64.5% of participant who previously complained from abortion were at risk to develop GDM.

Four hundred and nine (43.6%) of the study population suffered from different adverse pregnancy outcomes, including abortion, perinatal death, having macrosomic babies, and cesarean delivery. The mean value of HbA1c1 (HbA1c measured at first visit) in women with adverse pregnancy outcomes was significantly higher than women without adverse effect (5.5 ± 0.44% compared to 5.3 ± 0.4%, respectively), as well as FPG1 (88.7 ± 10.8 compared to 86.1 ± 9.4) and OGTT1 (measured at first visit) (107 ± 27.5 compared to 101 ± 22.8), respectively.

A statistically significant difference was observed in the mean value of baby birth weight between GDM-IADPSG diagnosed and non-diagnosed at first and second visit, while baby birth weight was higher in GDM-WHO women diagnosed at the first and second visit but not statistically significant.

Serum ferritin concentration in GDM1-WHO women was (36.8 ± 24 µg/dL), in GDM1-IADPSG, it was (28 ± 21.6 µg /L) and in DM women diagnosed at first visit by IADPSG was (31.7 ± 24.3 µg /L) that were higher than those who didn't develop GDM1 or DM but not statistically significant.

The percentage of anemia in the study population at first visit was 13.8% according to WHO definition (hemoglobin < 11g/dL), 25.3% had IDA (Iron Deficiency Anemia)

according to WHO definition (serum ferritin < 12 µg /L), and the percentage of anemia at the second visit was 22.8% according to WHO definition (hemoglobin < 10.5g/dL at the second visit). 21.8% suffered from adverse outcomes related to anemia (baby birth weight < 2500g and preterm delivery < 37 gestational weeks) which were statistically significant with mean hemoglobin concentration at first visit (P = 0.033).

Conclusions- HbA1c level in early pregnancy is increased in GDM, however, it does not replace OGTT for diagnosing GDM. However, including HbA1c as part of the diagnostic criteria for diabetes during pregnancy in 2010 by IADPSG was important which is still not adopted in Palestine. HbA1c level was associated with various adverse pregnancy outcomes in high risk women. Anemia in early pregnancy was associated with preterm delivery and low birth weight baby. Serum ferritin level in early pregnancy was higher in GDM women in comparison to non-GDM women, but it was not statistically significant.

Keywords- HbA1c, GDM, OGTT, Ferritin, and adverse pregnancy outcomes.

Gestational Diabetes and Adverse Pregnancy Outcomes: Role of HbA1c, Anemia and Other Risk Factors

سكري الحمل ومضاعفات الحمل الضارة: دور السكر التراكمي وفقر الدم وعوامل الخطورة الأخرى

ملخص

مقدمة - تعاني بعض النساء من مشاكل صحية أثناء الحمل مثل داء سكري الحمل (GDM) وفقر الدم خاصة فقر الدم الذي يسببه نقص الحديد. يمكن أن تؤثر هذه المشاكل على صحة الأم والجنين على حد سواء. لكن الكشف المبكر عن هذه المشاكل يمكن ان يقلل من خطر حدوث مضاعفات غير مرغوبة بها للحامل وجنينها. هناك حوالي 2-6% من النساء الحوامل يصبن بمرض سكري الحمل. يستخدم فحص السكر التراكمي (HbA1c) لتشخيص مرض السكري بينما لا يستخدم لتشخيص سكري الحمل ولا يزال المعيار الذهبي للتشخيص هو اختبار (OGTT)

اهداف الدراسة - تقييم دور السكر التراكمي (HbA1c) في فحص وتشخيص سكري الحمل ومدى ارتباطه بمضاعفات الحمل الضارة للأم والطفل. وكذلك تقييم مستوى السكر التراكمي أثناء الحمل وبعده عند الحوامل اللواتي لديهن عوامل خطورة لمرض سكري الحمل. من بين عوامل الخطورة سيتم أيضًا تقييم فقر الدم الناجم عن نقص الحديد وارتباطه بمضاعفات الحمل الضارة.

الإعدادات والتصميم - دراسة استطلاعية في مراكز رعاية الامومة والطفولة التابعة لوكالة الأمم المتحدة لإغاثة وتشغيل اللاجئين الفلسطينيين في الضفة الغربية (الأونروا).

الطرق - تم جمع البيانات عن طريق استبيان يضم العمر وعدد الاحمال السابقة بشكل عام وعدد الاحمال الناجحة ومؤشر كتلة الجسم، والتاريخ العائلي والشخصي للإصابة بمرض السكري وسكري الحمل. تم أخذ ضغط الدم في كل زيارة وقياس تركيز السكر والسكر التراكمي في الدم وفحص تعداد الدم الكامل (CBC)، بالإضافة لعمل اختبار تحمل الجلوكوز (OGTT) عند الحاجة وذلك حسب المعايير التي تتبعها عيادات الاونروا. تم تشخيص سكر الحمل في أسابيع الحمل 22-4 و 37-24 وفقًا لمعايير منظمة الصحة العالمية لعام 1999 إذا كانت نسبة السكر ≤ 126 ملغم % أو ≤ 140 ملغم % بعد ساعتين من تناول 75 غم من الجلوكوز عن طريق الفم (OGTT) وكذلك تم تشخيص حسب الرابطة الدولية لمرضى السكري ودراسات الحمل (IADPSG) (تركيز السكر ≤ 92 ملغم % أو ≤ 153 ملغم % بعد ساعتين من تناول 75 غم من الجلوكوز عن طريق الفم OGTT). شارك ما مجموعه 955 إمرأة حامل في هذه الدراسة.

التحليل الإحصائي - تم جمع البيانات وتحليلها باستخدام برنامج التحليل الاحصائي SPSS إصدار 23. تم إجراء مقارنات بين الأوساط الحسابية وعمل الارتباطات وحساب sensitivities و specificities لتشخيص سكري الحمل بواسطة السكر التراكمي والتنبؤ بنتائج الحمل الضارة.

النتائج - بلغت نسبة الحوامل اللواتي ظهر عندهن سكر الحمل 7.4% وفقًا لمعايير منظمة الصحة العالمية لعام 1999، و 45.8% وفقًا لمعايير IADPSG المعدلة. كان المتوسط الحسابي للسكر التراكمي HbA1c1 لدى النساء المصابات بـسكري الحمل في الزيارة الأولى أعلى من النساء اللواتي لم يكن لديهن سكري الحمل (5.9 ± 0.6% مقارنة بـ 5.4 ±0.4% P = 0.000= 0.000). وكذلك تم الكشف عن فرق ذو دلالة إحصائية في متوسط قيمة السكر التراكمي بين النساء اللواتي اصبن بالسكري النوع ا لثاني T2DM بعد الولادة وأولئك اللواتي لم يصبن (5.9 ± 0.5% مقارنة بـ 5.4 ± 0.4% P = 0.000) . لوحظ وجود ارتباط إيجابي بين وزن الطفل المولود ومحيط رأسه مع نسبة السكر التراكمي في الزيارة الأولى.

ان القيمة المثلى للسكر التراكمي عند أول زيارة للكشف عن سكري الحمل كانت 5.75% مع sensitivity (57.5%) و specificity (85.1%) بينما كانت قيمة السكر التراكمي المثالية ≤ 5.65% لديها sensitivity 31.1% و specificity 88.5% في الكشف عن نتائج الحمل الضارة.

لوحظ وجود علاقة ذات دلالة إحصائية بين الإجهاضات السابقة وسكري الحمل (P = 0.001)، 64.5% من المشاركات اللواتي عانيين من اجهاضات سابقة كن من ضمن الفئات اللاتي اصبن بـسكري الحمل.

عانى أربعمائة وتسعة (43.6%) من مجتمع الدراسة من نتائج سلبية مختلفة للحمل، بما في ذلك الإجهاض، ووفيات المولود قبل الولادة، وأطفال وزنهم زائد عن الطبيعي، والولادة القيصرية. كان متوسط قيمة السكر التراكمي عند الزيارة الأولى لدى النساء ذوات نتائج الحمل الضارة أعلى من النساء اللاتي لم يعانين من نتائج الحمل الضارة (5.5% ±0.44 مقارنة بـ 5.3 ± 0.4% على التوالي) ، وكذلك نسبة السكر و OGTT في الزيارة الأولى كان متوسط القيم 88.7 ملغم % و 100.7 ملغم % على التوالي.

ولوحظ وجود فرق ذو دلالة إحصائية في متوسط قيم اوزان المواليد بين الحوامل المشخصات بسكري الحمل حسب IADPSG وغير المشخصات في الزيارة الأولى والثانية. بينما كانت اوزان المواليد أعلى عند الحوامل المشخصات بسكري الحمل حسب معايير WHO ولكنه ليس ذو دلالة إحصائية.

لوحظ أن تركيزً مخزون الحديد (ferritin) في الدم عند النساء اللواتي اصبن بسكري الحمل على الزيارة الاولى بحسب معايير منظمة الصحة العالمية لعام 1999 كان (36.8 ± 24 ميكروغرام / لتر) وبحسب معايير IADPSG كان (27.4 ± 22.6 ميكروغرام / لتر) و اللواتي شخصن بالسكري من النوع الثاني كان (31.7 ± 24.3 ميكروغرام / لتر) حيث كانت تلك القيم اعلى منها عند السيدات السليمات و لكن لم تكن ذات دلالة احصائية.

كانت نسبة فقر الدم في مجتمع الدراسة في الزيارة الأولى 13.8% وفقًا لتعريف منظمة الصحة العالمية (WHO) بان يكون تركيز خضاب الدم <11 غم، بينما بلغت نسبة فقر الدم الناتج عن نقص الحديد (IDA) ما نسبته 25.3% و ذلك حسب تعريف منظمة الصحة العالمية بان يكون مخزون الحديد (Ferritin) < 12 ميكروغرام / لتر ، و ترتفع النسبة المئوية للإصابة بفقر الدم في الزيارة الثانية لتصل 22.8% وفقًا لتعريف منظمة الصحة العالمية (WHO) بان يكون تركيز خضاب الدم < 10.5غم في الزيارة الثانية. عانت 21.8% من الحوامل المشاركات في الدراسة من نتائج سلبية تتعلق بفقر الدم مثل وزن الطفل المولود أقل من 2500 غرام او الولادة المبكرة (أقل من 37 أسبوعًا من عمر الحمل) والتي كانت ذات دلالة إحصائية في متوسط قيمة تركيز خضاب الدم في الزيارة الأولى (P = 0.033)

الاستنتاجات – كان هناك زيادة في مستوى السكر التراكمي في بداية الحمل عند النساء اللواتي تم تشخيصهن بسكر الحمل. ومع ذلك، فإن فحص OGTT لا يزال يستخدم لتشخيص سكر الحمل. وقد كان من المهم تضمين السكر التراكمي كجزء من المعايير التشخيصية لمرض سكري الحمل في عام 2010 من قبل IADPSG. ارتبط مستوى السكر التراكمي

بمختلف نتائج الحمل الضارة في النساء ذوات عوامل الخطورة للإصابة بسكري الحمل. ارتبط فقر الدم في بداية الحمل بالولادة المبكرة وانخفاض وزن المولود. كان مستوى مخزون الحديد في الدم (Ferritin) في بداية الحمل أعلى عند النساء اللواتي اصبن بسكري الحمل مقارنة مع النساء اللواتي لم يصبن بسكري الحمل لكنه لم يكن ذو دلالة إحصائية.

الكلمات الرئيسية- السكر التراكمي، سكر الحمل، اختبار تحمل الجلوكوز OGTT، مخزون الحديد (Ferritin)، نتائج الحمل الضارة.

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List of Abbreviation Used in This Thesis

2 hr. PP	Two Hour Post Prandial
2hr OGTT	2 Hour Oral Glucose Tolerance Test
ACOG	American Council of Obstetricians and Gynecologists
ADA	American Diabetes Association
AFR	Africa
BBW	Baby Birth weight
BHC	Baby Head Circumference
BMI	Body Mass Index
CBC	Complete Blood Cell Count
CDC	Centers for Disease Control and Prevention
CI	Confidence of Interval
CV	Coefficient of Variation
DBP1	Diastolic Blood Pressure at First visit
DBP2	Diastolic Blood Pressure at Second visit
DBP3	Diastolic Blood Pressure at Postnatal visit
DM	Diabetes Mellitus
EDTA	Ethylene Diamine Tetra Acetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nation
FAS	Folic Acid Supplementation
FH DM	Family History of Diabetes Mellitus
FPG	Fasting Plasma Glucose
FPG1	Fasting Plasma Glucose at First visit
FPG2	Fasting Plasma Glucose at Second visit
FPG3	Fasting Plasma Glucose at Postnatal visit
GAB	Gestational Age at Birth
GAD65	Glutamic Acid Decarboxylase
GDM	Gestational Diabetes Mellitus
GDM1-IADPSG	Gestational Diabetes Mellitus diagnosed by Modified IADPSG Criteria at First visit
GDM1-WHO	Gestational Diabetes Mellitus diagnosed by WHO 1999 Criteria at first visit
GDM2-IADPSG	Gestational Diabetes Mellitus diagnosed by Modified IADPSG Criteria at Second visit
GDM2-WHO	Gestational Diabetes Mellitus diagnosed by WHO 1999 Criteria at second visit
GDM-IADPSG	Gestational Diabetes Mellitus diagnosed by Modified IADPSG Criteria
GDM-WHO	Gestational Diabetes Mellitus diagnosed by WHO 1999 Criteria
HAPO	Hyperglycemia and Adverse Pregnancy Outcome
Hb	Hemoglobin
Hb1	Hemoglobin at First visit
Hb2	Hemoglobin at Second visit

Hb3	Hemoglobin at Postnatal visit
HbA1c	Glycosylated hemoglobin
Hct1	Hematocrit at First visit
Hct2	Hematocrit at Second visit
Hct3	Hematocrit at Postnatal visit
HPLC	High Performance Liquid Chromatography
HT	Hypertension
IADPSG	International Association of the Diabetes and Pregnancy Study Group
IDA	Iron Deficiency Anemia
IDF	International Diabetes Federation
IDF	International Diabetes Federation
LGA	Large for Gestational Age birth
MCV2	Mean Corpuscular Volume at Second visit
MCV3	Mean Corpuscular Volume at Postnatal visit
MENA	Middle East and North Africa
MOHPHC	Ministry of Health-Primary Health Care
N	Number
NAC	North America and the Caribbean
NDDG	National Diabetes Data Groups
NICE	National institution of Health and Care Excellence
NIH	National Institutes of Health
OGTT	Oral Glucose Tolerance Test
PCOS	Polycystic Ovary Syndrome
PDBB	Previous Delivery of a Big Baby
PDLB	Previous Delivery of a Low birth weight Baby
PH GDM	Personal History of Gestational Diabetes Mellitus
PPD	Previous Preterm Delivery
PROF	Preterm Premature Rupture of membranes
r	Pearson correlation
ROC	Receiver Operating Characteristics
SBP1	Systolic Blood Pressure at First visit
SBP2	Systolic Blood Pressure at Second visit
SBP3	Systolic Blood Pressure at Postnatal visit
SD	Standard Deviation
SEA	South East Asia
SGA	Small for Gestational Age birth
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
TD	Type of Delivery
UK	United Kingdom
UNRWA	United Nations Relief and Works Agency for Palestine Refugees
USA	United Stat of America
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Introduction

Diabetes in all its forms is a lifelong condition that imposes unacceptably high medical, social and economic costs in countries at all income levels. Recent estimates by the International Diabetes Federation (IDF) in 2017 showed that globally, 8.8% (424.9 million) of adults have diabetes with 80% of cases in low and middle-income countries [1]. The Middle East and North Africa (MENA) regions are disproportionately affected compared to other regions in the world (Figure 1). The prevalence of type 2 diabetes mellitus (DM2) in the MENA region, to which Palestine belongs, is the second highest in the world (10.8%) and this is expected to remain in the year 2045 (IDF 2017). As figure 1 shows, North America and the Caribbean (NAC) has the highest prevalence (11.0%) followed by MENA, then South East Asia (SEA) while Africa (AFR) has the lowest prevalence. The prevalence of DM2, however, is not only increasing among adults but also spreading into younger age groups and is being reported in adolescents and children as well.

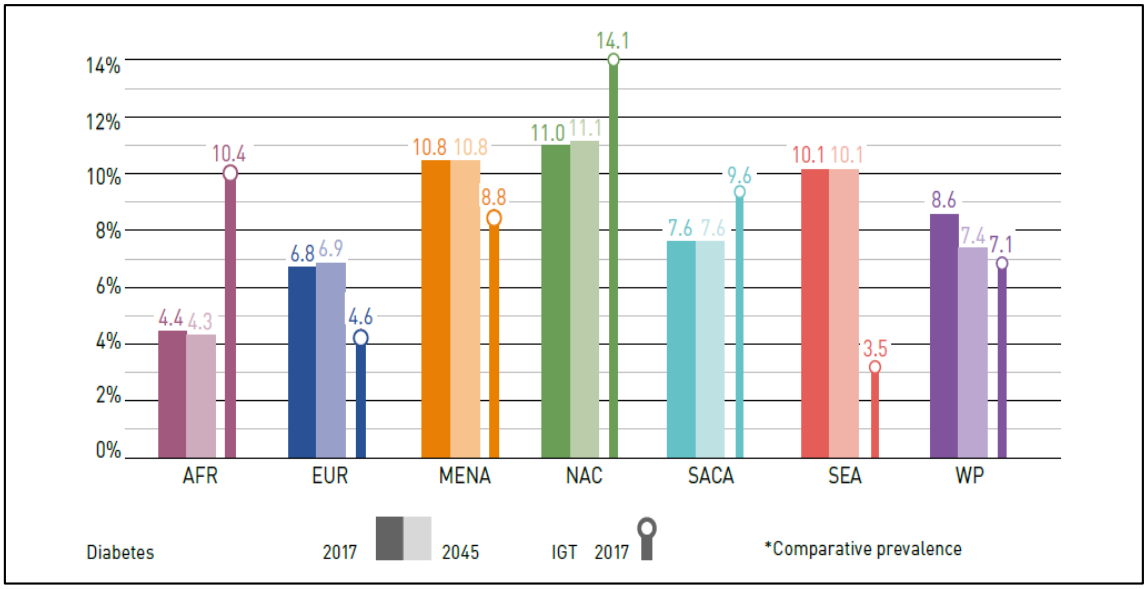

Figure 1: Prevalence* of diabetes and IGT (20-79) years by IDF Region, 2017 and 2045. AFR: Africa; EUR: Europe; MENA: Middle East and North Africa; NAC: North America and Caribbean; SACA: South and Central America; SEA: South East Asia; WP: Western Pacific **[1] .**

1.2 Definition of Diabetes Mellitus

a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The severity of symptoms is due to the type and duration of diabetes. Some diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease. Other patients with marked hyperglycemia especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia (excessive drinking as a result of thirst), polyphagia (excessive hunger or increased appetite), weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor (a state of impaired consciousness), coma and if not treated, death, due to ketoacidosis or rarely from nonketotic hyperosmolar syndrome (a serious condition caused by extremely high blood sugar levels) [2, 3].

1.3 Diagnostic Criteria for Diabetes Mellitus

Diabetes mellitus is diagnosed using either plasma glucose (FPG or OGTT) or HbA1c. Estimation of the cutoff values for glucose and HbA1c is based on the association of FPG or HbA1c with retinopathy. Fasting plasma glucose of ≥ 126 mg/dL (7.0 mmol/L), plasma glucose after 2-hr OGTT ≥ 200 mg/dL (11.1 mmol/L), HbA1c ≥ 6.5% (48 mmol/mol) or a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) along with symptoms of hyperglycemia is diagnostic of diabetes mellitus [3].

In addition to monitoring the treatment of diabetes, HbA1c has been recommended to diagnose diabetes by the International Expert Committee in 2009[4] and endorsed by ADA [5], the Endocrine Society, WHO [6] and many scientists and related organizations around the world. Jeon et al. form a retrospective study in 2011 concluded that HbA1c is an acceptable complementary diagnostic test for diabetes in Korean patients [7]. The advantages and disadvantages of the different tests used to diagnose diabetes have been reviewed by Sacks and his colleagues [8]. The advantages of using HbA1c over FPG to diagnose diabetes include greater convenience and pre-analytical stability, lower CV (Coefficient of Variation) (3.6%) compared to FPG (5.7%) and 2h OGTT (16.6%). In addition, HbA1c provides stronger correlation with micro vascular complications especially retinopathy, a marker for glycemic control and glycation of proteins which is the direct link between diagnosis of diabetes and its complications [9, 10]

1.4 Classification of Diabetes Mellitus

Although classification of diabetes is important and has implications for the treatment strategies, this is not an easy task and many patients do not easily fit into a single class especially younger adults [2, 11, 12] and 10% of those initially classified may require revision [13]. The classical classification of diabetes proposed by the American Diabetes Association (ADA) in 1997 as type 1, type 2, other types, and gestational diabetes mellitus (GDM) is still the most accepted classification and adopted by ADA [2, 14].

1.4.1 Type 1 Diabetes Mellitus (β-Cell destruction, usually leading to absolute insulin deficiency)

This type represents 5-10% of those with diabetes, and previously known as insulin-dependent diabetes or juvenile onset diabetes, is due to destruction of β -cells in the pancreas, due to of cellular mediated autoantibody, including islet cell autoantibodies, autoantibodies to GAD65 (glutamic acid decarboxylase), autoantibodies to insulin, or tyrosine phosphatases. One or more of these autoantibodies are present in 85-90% of type 1 diabetes. The remaining 10-15% patients show insulinopenia and are prone to ketoacidosis with no evidence of autoimmunity, most of them are of African or Asian ancestry [2].

1.4.2 Type 2 Diabetes Mellitus

This type ranges from predominantly insulin resistance with relative insulin deficiency to predominantly insulin secretory defect with insulin resistance [5]. More than 90-95% of diabetes patients belong to this type and most of them are adults. The number of youth (less than 20 years) with type 2 diabetes in the United States in the year 2009 was (0.46 in 1000) and accounted approximately for 20% of type 2 diabetes in this group [15]**.** The increased incidence of type 2 diabetes in youth is mainly due to the change in the lifestyle of the children in terms of a more sedentary life with less healthy food. Obesity is the major reason behind insulin resistance which is mainly responsible for type 2 diabetes [16, 17]. Insulin resistance in type 2 diabetes patients increases the demand for insulin in insulin-target tissues. In addition, the increased demand for insulin could not be met by the pancreatic β cells due to defects in the function of these cells [18]. Type 2 DM previously known as insulin independent diabetes mellitus, it differed from type 1 DM due to the absence of ketoacidosis in most type 2 diabetes patients and autoimmune destruction of β cells does not occur [2].

1.4.3 Other Types of Diabetes Mellitus

Diabetes has been found in patients with endocrine diseases that secrete excess hormones like growth hormone, glucocorticoids, glucagon and epinephrine and in patients with genetic syndromes such as Down syndrome, Klinefelter syndrome, Turner syndrome and Wolfram syndrome [2]. Disruption of β cell function or a reduction in their number due to genetic defect is known as monogenic diabetes [2, 3, 19] or damage of β cells due to pancreatic carcinoma, pancreatitis, infection, pancreatectomy, trauma and atrophy of the exocrine pancreas which leads to progressive loss of β cells [2, 3, 20], results in hypoinsulinemia and modest hyperglycemia to severe diabetes [2].

1.4.4 Gestational Diabetes Mellitus

Pregnant women have been evaluated for diabetes for more than 50 years: It is notwithstanding that 5 international workshops devoted to GDM have been held between 1979 and 2005, failed to reach on agreement concerning the optimal method to identify "any degree of glucose intolerance" [21], with no international consensus on how to screen for GDM [22]. It is still considered as one of the most serious health problems worldwide affecting 14% of all pregnant women according to IDF [23, 24]. Gestational diabetes mellitus (GDM) is a prevalent and potentially serious condition that may lead to adverse effects for both mothers and neonates (WHO 1999). The evidence suggests that early detection and treatment of this condition reduce the risks for mothers as well for their babies [25, 26]. Although the risks of complications caused by GDM are well established, there is considerable controversy regarding its diagnosis [27]. Some European countries recommend screening all pregnant women with OGTT, whereas others use selective screening based on risk factors or glucose challenge test [22].

1.4.4.1 Definition of GDM:

Gestational Diabetes Mellitus is a defect in glucose tolerance with onset or first recognition during pregnancy. This definition, proposed in 1998 during the 4th international conference on gestational diabetes [28], acknowledges the possibility that women may have previously undiagnosed diabetes mellitus, or may have developed diabetes concomitantly with pregnancy. Although the cause of GDM is not known, there are some theories as to why the condition occurs. The placenta supplies the growing fetus with nutrients and water and produces a variety of hormones to maintain pregnancy. Some of these hormones (estrogen, cortisol, and human placental lactogen) may have a blocking effect on insulin which usually begins about 20 to 24 weeks in pregnancy [29].

Hyperglycemia in pregnancy, whether in the form of type 2 diabetes diagnosed before or during pregnancy or in the form of gestational diabetes, has an increased risk of adverse maternal, fetal and neonatal outcome. Mothers with gestational diabetes and babies born to such mothers have an increased risk of developing diabetes later in life [30]. Additionally, there is evidence that women with GDM are less likely to breastfeed which indicates that breastfeeding improves the subsequent glucose tolerance of the mother and may reduce the risk of type 2 diabetes in children [31].

Hyperglycemia in pregnancy is responsible for fetal complications including macrosomia (large baby with birth weight $\geq$ 4.000 Kg, which leads to birth injuries [32]), shoulder dystocia (after the delivery of the head, the anterior shoulder of the infant cannot pass below), large for gestational age births (LGA, newborns with birth weight above the 90th percentile), small for gestational age (SGA, newborns with birth weight below the 10th percentile), stillbirth (children born dead after 20 week of gestation or at 140 days) and preterm birth (<37 weeks). Neonatal complications include neonatal hypoglycemia, neonatal mortality before the 28th day of life [21, 33, 34], neonatal hypocalcemia, neonatal hyperbilirubinemia and neonatal asphyxia [35]. In addition to, adverse pregnancy outcomes for the mothers include preeclampsia, increase risk for cesarean delivery due to large babies, hypertension during pregnancy, and significantly higher risk of subsequent type 2 diabetes [21, 33].

1.4.4.2 Epidemiology of GDM:

The prevalence of GDM generally ranges between 2 to 6%, with sometimes much higher values (10 to 22%) in specific populations with a general trend towards increasing prevalence depending on the population studied and the diagnostic tests employed [3]. For example, in India, the prevalence was 13.9% [36], while in the Middle East, it was 13.5% [37], while in Sardinia the prevalence reached 22.3% [38]. In Gaza Strip (Palestine) according to the annual report of the Ministry of Health-Primary Health Care (MOH-PHC 2006) the prevalence of GDM was 5.5%. (MOHPHC-Women Health, Health Status in Palestine 2005 October 2006). In 2018, 17.4% of registered pregnant women in MOH PHC clinics were referred to high risk pregnancy clinics for their medical condition including GDM, but there was no clarification about the prevalence of GDM alone. Even though GDM is a common disorder in pregnancy, it has been difficult to compare its frequency among various populations and estimate its global impact, due to the lack of uniform diagnostic criteria [39].

1.4.4.3 Risk Factors for GDM:

The risk factors of GDM used in selective screening guidelines vary between the different committees. The ADA guidelines would result in the highest rate of diagnosis and the lowest number of missed cases [40]. These risk factors include [3]:

- Overweight (BMI ≥ 25 kg/m^2).
- First-degree relative with type 2 diabetes.
- Member of a high-risk ethnic population (e.g., African American, Latino and American), and family origin with a prevalence of diabetes (e.g., South Asia, specifically India, Middle East specifically Saudi Arabia, United Arab Emirates, Iraq, Jordan, Syria, Oman, Qatar, Kuwait, Lebanon and Egypt).
- Previous macrosomic baby weighing ≥ 4.00 kg.
- Previously diagnosed with gestational diabetes.
- Maternal age ≥ 25 years old.
- Hypertension (≥ 140/90 mmHg or on therapy for hypertension).
- Polycystic ovary syndrome (PCOS).
- Multiple pregnancies.
- Exposures to toxic factors (tobacco, pesticide arsenic or treatment with 17 OH progesterone) [3].
- Socioeconomic factors
- Physical activity

A prospective study in the antenatal care clinic of Lampang Regional Hospital Arora et al [41], reported that 21.8% of GDM cases had no risk factors, while having one risk factor doubles the chance of having GDM.

1.4.4.4 Diagnostic Criteria for GDM:

Gestational diabetes has been diagnosed at 24-28 weeks of gestation in women not previously diagnosed with diabetes using two approaches. The first approach is based on the "one-step" International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus [42] recently adopted by WHO [43]. Gestational diabetes is diagnosed by this method by FPG ≥ 92 mg/dL (5.1 mmol/L), 1-hr plasma glucose after a 75 g glucose load ≥ 180 mg/dL (10.0 mmol/L) or 2-hr plasma glucose after a 75 g glucose load ≥ 153 mg/dL (8.5 mmol/L). This criterion is derived from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study [33], even though the HAPO study showed a

continuous relationship between hyperglycemia and adverse short-term pregnancy outcome with no threshold reported [44].

The second approach is used only in the United States and is based on the "two-step" NIH (National Institutes of Health) consensus [45]. In the first step 1-hr plasma glucose after a 50 g glucose load under non-fasting state ≥ 140 mg/dL (7.8 mmol/L) is followed by a second step under fasting conditions after a 100 g glucose load for those who screened abnormal in the first step. The diagnosis of gestational diabetes is made when at least two of the four plasma glucose levels are met. The four plasma glucose levels according to Carpenter/Coustan criteria are: FPG ≥ 95 mg/dL (5.3 mmol/L); 1-hr ≥ 180 mg/dL (10.0 mmol/L); 2-hr ≥ 155 mg/dL (8.6 mmol/L); and 3-hr ≥ 140 mg/dL (7.8 mmol/L) [2].

The IADPSG criteria in comparison with the Carpenter/Coustan criteria was associated with a 3.5-fold increase in GDM prevalence, significant improvement in pregnancy outcomes and was cost-effective [46]. In another retrospective cohort study of women diagnosed with gestational diabetes, Ethridge et al [47] have shown that newborns of women diagnosed of gestational diabetes by IADPSG approach have greater measures of fetal overgrowth compared with Carpenter-Coustan "two-step" approach neonates. A strategy using fasting plasma glucose as a screening test and to determine the need for OGTT is valid [48, 49]. According to Sacks et al [8], correlation of glucose concentrations and the risk of subsequent complications will eventually lead to universal guidelines.

The use of ADA/WHO cut off value of HbA1c ≥ 6.5% (48 mmol/mol) to diagnose gestational diabetes is not recommended by the "one step" IADPSC criteria or the "two-step" NIH criteria. Further investigation is required in light of recent reports on HbA1c in combination with OGTT and its usefulness to predict the adverse effects of gestational diabetes or obviate the use of OGTT in all women with gestational diabetes [34, 50].

1.4.4.5 Threshold Values for Diagnoses GDM:

The National Diabetes Data Groups (NDDG) in 1979 suggested measuring plasma blood glucose during a three hours oral glucose tolerance test (OGTT) with glucose levels of 105, 190, 165 and 145mg/dl (for fasting, one-hour, two-hour and three-hour post glucose load respectively) [51]. In 1982, Carpenter and Coustan proposed changing the values to 95, 180, 155 and 140mg/dl [52]. According to the NDDG and Carpenter and Coustan criteria, the diagnosis of GDM is established if two or more glucose values are higher than the defined cutoffs during a three-hour OGTT. In 1989, Sacks et al proposed the more inclusive criteria of 96, 172, 152 and 131 mg/dl, after calculating glucose concentrations in paired whole blood and plasma specimens of 995 consecutive pregnant women [53].

All the aforementioned diagnostic thresholds were based on data from women who were diagnosed with diabetes after gestation and not on any short-term adverse pregnancy outcomes. In 2010, the International Association of Diabetes and Pregnancy Groups (IADPSG) proposed a new set of criteria, based on the incidence of adverse perinatal outcomes, as assessed in the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study [33, 54]. According to these criteria, the diagnosis of GDM is made if at least one value of plasma glucose concentration is equal to or exceeds the thresholds of 92, 180 and 153 mg/dl (for fasting, one-hour and 2-hour post glucose load glucose values respectively), after performing a 75 g OGTT [42].

The American Diabetes Association (ADA) suggested that all pregnant women should be screened for GDM between the 24th and 28th weeks of gestation, unless they were of low risk status, those with no risk factors of GDM. For those women, there is no need to screen and they are less likely to benefit from any screening by the two-step approach for GDM [55]. Women are initially screened by measuring plasma glucose 1 hour after a 50 g glucose load; women with glucose concentration ≥140 mg/dl, undergo an 100 g OGTT on a separate day. In the one-step approach, the 100 g OGTT is performed directly without any initial screening. In both occasions, the diagnosis of GDM is established by the Carpenter and Coustan criteria.

The American Council of Obstetricians and Gynecologists (ACOG) also suggests screening of all women except for those of low risk status [56]. It supports the use of the 100 g OGTT and application of either NDDG or Carpenter and Coustan criteria. The World Health Organization (WHO) in 1999 recommends using the 75 g two-hour OGTT and the diagnostic thresholds of 126 mg/dl and 140 mg/dl for fasting and 2-hour glucose concentrations, respectively [57]. Finally, The IADPSG suggests screening all women at the first prenatal visit and a 75 g OGTT between the 24th and 28th week of gestation in those not already diagnosed with overt diabetes or GDM by early testing. One or more abnormal value (≥ 92, 180 or 153 mg/dl for fasting, 1-hour and 2-hour plasma glucose, respectively) after a 75 g OGTT is diagnostic of GDM [42]. The diagnostic threshold values of the various organizations are summarized in Table 1.

For the identification of overt diabetes during pregnancy and its distinction from GDM, the IADPSG recommends that fasting plasma glucose (FPG) or glycosylated hemoglobin (HbA1c) should be measured at the first prenatal visit on all or only high-risk women depending on the frequency of diabetes in the background population and on local circumstances [42]. Values equal to or above 126 mg/dl and 6.5% (for FPG and HbA1c,

respectively) establish the diagnosis of overt diabetes. Women with 92 ≤ FPG < 126 mg/dl are diagnosed with GDM, while those with FPG < 92 mg/dl should undergo a 75 g OGTT at 24 to 28 weeks of gestation, are shown in Table 2. Finally, the diagnosis of GDM by means of the 75 g OGTT is based on the aforementioned criteria (92, 180, and 153 mg/dl for fasting one-hour, and two-hour OGTT glucose concentrations, respectively) [42].

Table 1. GDM Diagnostic Threshold Values from Various Organization.

		Plasma Glucose Concentration Thresholds (mg/dl)			
Organization	**OGTT Glucose Load**	**FPG**	**1-hour**	**2-hour**	**3-hour**
ADA*	**100g**	**95**	**180**	**155**	**140**
ACOG*	**100g**	**105**	**190**	**165**	**145**
WHO-1999§	**75g**	**126**	**-**	**140**	**-**
IADPSG§	**75g**	**92**	**180**	**153**	**-**

*Diagnosis of GDM if two or more glucose values equal to or exceeding the threshold values
§Diagnosis of GDM if one or more glucose values equal to or exceeding the threshold values
GDM: Gestational Diabetes Mellitus, OGTT: Oral Glucose Tolerance Test, FPG: Fasting Plasma Glucose, ADA: American Diabetes Association, ACOG: American Council of Obstetricians and Gynecologists, WHO: World Health Organization, IADPSG: International Association of Diabetes and Pregnancy Study Groups.

Table 2. IADPSG Threshold Values in Diagnose Hyperglycemia in Pregnancy at First Antenatal Visit.

Measure of Glycemia	Threshold Values	Diagnoses
FPG	**< 92mg/dl**	**Do 75g OGTT at 24 Week Gestation**
FPG	**≥ 92mg/dl < 126mg/dl**	**GDM**
FPG	**≥ 126mg/dl**	**DM**
HbA1c	**≥ 6.5%**	**DM**

IADPSG: International Association of Diabetes and Pregnancy Groups, GDM: Gestational Diabetes Mellitus, OGTT: Oral Glucose Tolerance Test, FPG: Fasting Plasma Glucose, HbA1c: glycated Hemoglobin, DM**:** Diabetes Millets.

1.5 HbA1c

Hemoglobin A1c (HbA1c), also known as glycated hemoglobin, is a normal adult hemoglobin resulting from irreversible non-enzymatic glycation of the beta chain in hemoglobin A [58, 59]. HbA1c reflects the average plasma glucose level during the previous (8-12) weeks [60]. HbA1c values are directly proportional to the degree of glucose exposure over that period of time and further diabetic treatment can be adjusted depending

on the patients HbA1c data. HbA1c test can be performed at any time of the day, does not require fasting, and is not affected by acute events like stress or vigorous physical exercise. The pre-analytical stability and reproducibility is large and the coefficients of variation are lower than for FPG and oral glucose tolerance test [4, 61, 62].

1.5.1 Factors That Affect HbA1c Level

HbA1c values depend on genetic factors like hemoglobinopathies (HbS, HbC, HbD), age and ethnicity and non-glycemic factors that are associated with a decreased turnover of red blood cells, such as iron deficiency, renal failure, and vitamin B12 deficiency. These factors lead to higher HbA1c values while those associated with decreased life spans of red blood cells, like hemolytic anemia and chronic liver disease, lead to lower HbA1c values [61].

1.5.2 HbA1c in Pregnancy

Screening and diagnosis of GDM in pregnant women will aid in preventing the adverse pregnancy outcome by giving therapy at an early stage of pregnancy. Oral glucose tolerance test (OGTT) is the gold standard test for diagnosing GDM, but it is a cumbersome procedure for the patients and health care providers since it requires fasting state of the pregnant women, minimum two blood samples collection and at least two hours for samples to be collected. In 2011, the world health organization (WHO) and American diabetic association (ADA) have accepted HbA1c as a diagnostic tool for diagnosis diabetes mellitus with a threshold value $\geq 6.5\%$ [6, 63]. However, there is no recommendations available for the use of HbA1c as a diagnostic tool in GDM. Several studies were constructed to evaluate the role of HbA1c in screening and diagnosis of GDM and in prediction of adverse pregnancy outcomes, however, using HbA1c in screening for GDM is still debated.

The prevalence of diabetes in women of childbearing age has increased [64], and the poor pregnancy outcome associated with late diagnosis of preexisting diabetes also increased [62]. Due to this alarming observation the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) [42], ADA [2] and WHO [48] recommend early screening for diabetes in pregnancy at the first antenatal visit, however, the most appropriate test and threshold remain undefined. A recent study reported that GDM associated with fetal overgrowth starts early in pregnancy before the diagnosis of GDM, potentially demonstrating the need to identify pregnancies with glucose intolerance earlier in pregnancy [65]. HbA1c, a measure of glycated hemoglobin which serves as an indicator of blood glucose control in the prior 3-4 months, may save as good and convenient marker for earlier identification of women at risk for GDM.

In New Zealand, Ruth et al. reported that the optimal detection of HbA1c level in early pregnancy (before 20 weeks of gestational age) for GDM was ≥ 5.9% (41mmol/mol) [62]. Compatible results were seen by Balaji et al. where the mean HbA1c level of GDM women in first trimester was (5.96±0.63%) [66].

The first trimester is characterized by increased insulin sensitivity [67], pregnant women deplete their glycogen stores quickly due to the fetoplacental glucose demands, and switch from carbohydrate to fat metabolism within 12 hours, resulting in increased lipolysis and ketone production [68], led to decreased in fasting plasma glucose [69]. New erythrocytes formed will therefore be exposed to a lower time-averaged glucose concentration than those of non-pregnant women, and the degree of glycation might be less [70]. Erythrocyte lifespan is likely to be decreased in pregnancy [71], hence also reducing the HbA1c value. The second and third trimesters are characterized by insulin resistance due to an increase in placental growth hormone [72], reprogram maternal physiology to become insulin resistant to ensure an adequate supply of nutrients to the growing fetus [73].

1.5.2.1 HbA1c in First Trimester Pregnancy (< 24 Weeks Gestation)

In normal pregnancy, HbA1c shows biphasic changes with an initial gradual decline to a nadir level at 24 weeks due to decline in blood glucose levels, followed by a subsequent slow increment to peak near term. All these changes fell within the usual range of normal values for HbA1c [74, 75]. HbA1c measurement in early pregnancy (< 20 gestational weeks) helps in the diagnosis of gestational diabetes mellitus and predicts the adverse pregnancy outcome [62]**.** Since fetal malformation occurs before eight weeks of gestation (postmenstrual dates) when most pregnant women have not yet come to medical attention, diabetes women delivering infants with major congenital anomalies had high HbA1c values in early pregnancy [59]. It was associated with preeclampsia, shoulder dystocia, and perinatal death [62]**.** The rational of doing HbA1c in early pregnancy is that pregnant women with HbA1c ≥ 5.9% (41mmol/mol), could receive treatment with no need to wait another 2-3 months to do OGTT. In addition, more obese women may have non-diagnostic OGTT and give false-negative results. It may be that the nutrient load of their diet stresses the pancreas more than a 75 g glucose load [76].

Ruth and his colleagues offered HbA1c test in early pregnancy (≤ 140 days) to all pregnant women who visited Christchurch primary care setting in New Zealand between 2008 and 2010, and performed early pregnancy OGTT (≤ 20 weeks of gestation or ≤ 140 days) most likely to those who had HbA1c ≥ 5.9% (41mmol/mol), second OGTT after 24 weeks of gestation and postnatal OGTT only to women who had been referred to GDM management.

Three subgroups were classified depending on HbA1c values, [< 5.9% (41mmol/mol), 5.9-6.4%(41-46 mmol/mol) and ≥ 6.5% (≥ 48 mmol/mol)]. The pregnancy outcome data were analyzed to examine whether an HbA1c threshold of ≥ 5.9% (41 mmol/mol) was associated with increased adverse pregnancy outcome [62]. It was found that HbA1c value ≥ 5.9% in early pregnancy identified all women with diabetes, and between 5.9-6.4% was associated with an increased risk of adverse pregnancy outcomes, including major congenital anomaly, preeclampsia, shoulder dystocia, and prenatal death [62].

A study conducted by Balaji et al. in India, included 507 pregnant women who were screened for GDM by 75g-OGTT and HbA1c irrespective to their gestational age. The mean HbA1c level for women with normal OGTT was (5.36 ± 0.36%), and with GDM was (5.96±0.63%) at first trimesters, while the mean HbA1c level for women with normal OGTT was 5.3% and with GDM was 6% at different trimesters [66]. Granada et al. found that HbA1c in early pregnancy with cut point more than 5.9% may be predictive for postpartum DM [77].

1.5.2.2 HbA1c in Second Trimester Pregnancy at (24-28 Weeks Gestation)

Novel data was about clinically significant GDM in pregnant women with normal OGTT and HbA1c (> 40 mmol/mol, 5.8%) reported by Janet and her colleagues in New Zealand [78]. Those women were more obese and pacific than other women with GDM. Moreover, most women diagnosed with GDM by OGTT had an HbA1c ≤ 40 mmol/mol (5.8%), therefore, HbA1c measurement did not replace OGTT for diagnosing GDM. These authors found that pregnant women with HbA1c above reference range at delivery (≥ 39 mmol/mol, 5.7%) were more likely to have macrosomic infants than women with a lower HbA1c [78].

In India, Rajput and his colleagues found that HbA1c in combination with OGTT can obviate the need of OGTT in almost two thirds of women with GDM because the mean HbA1c value in women with GDM was significantly higher than women without GDM (5.73 ± 0.34% compared to 5.34 ± 0.35%) [79].

At the Holy family Hospital of Catholic University in Korea, 107 GDM women and their newborns were evaluated for gestational weeks and HbA1c level at time of diagnosis, and clinical outcomes of mothers and newborn babies by reviewing their medical records, Choi et al. recommended to screen for GDM no later than the 24th week of gestation, since the frequency of delivery of large for gestational age (LGA) infants was higher in mothers diagnosed with GDM after the 29th week of pregnancy or with HbA1c levels ≥ 7.0% [80].

1.5.2.3 HbA1c in Third Trimester Pregnancy

The third trimester HbA1c predicates the occurrence of macrosomia [81] and increased risk of preeclampsia [82]. Lowe et al. compared the association of maternal glucose and HbA1c measured at 24-32 weeks of gestation with adverse outcomes for pregnant women who underwent 75g-OGTT, and HbA1c between 24 - 32 weeks of gestation. A cord blood sample was collected at delivery to analyze for C-peptide and glucose which was used as an index for fetal β-cells function. Data about primary outcomes (birth weight, primary cesarean section, clinical neonatal hypoglycemia and C-peptide) and secondary outcomes which include preeclampsia, preterm delivery, sum of skinfolds and percent body fat were analyzed. Lowe. et al. found significant correlation of HbA1c with OGTT. There were also significant associations between higher levels of HbA1c and all primary and secondary outcomes. However, the adjustment for the composite with glucose measured HbA1c was not significantly associated with any of the neonatal anthropometric outcomes. Therefore, their finding suggested that the measurement of HbA1c was not a useful alternative to an OGTT in pregnant women [83]. One hundred forty-eight Caucasian women diagnosed with GDM between the 24th and the 28th week of gestation were screened for HbA1c at diagnosis and before the expected delivery date (approximately 36th gestational week). A significant correlation between HbA1c value at GDM diagnosis and individual BMI prior to conception was observed by Capula et al. who reported that HbA1c at diagnosis and before delivery was a good predictor of adverse pregnancy outcome [34].

Hashimoto et al. reported that not only the non-diabetic pregnant women, but also the diabetic ones show increase in HbA1c levels in late pregnancy due to iron deficiency [75].

1.5.2.4 HbA1c After Delivery

Ekelund and his colleague, found that 30% of 174 GDM women after five years, who had high fasting glucose and HbA1c levels during pregnancy (HbA1c≥ 5.7% and fasting blood glucose ≥ 5.2mmol/l) had developed diabetes [84].

ADA and WHO recommended diabetes screening with a 75 g oral glucose tolerance test (OGTT) at 6-12 weeks after delivery in women with GDM [85].There is no official recommendation for HbA1c use for diabetes screening in the postpartum period. HbA1c ≥ 48 mmol/mol (≥ 6.5%) at 6th week postpartum was recommended for screening of diabetes in GDM women by Mahesh and his colleges [86].

The UK National institution of Health and Care Excellence (NICE) and the American Diabetes Association (ADA) have proposed measuring HbA1c as an alternative, more user

friendly test, with HbA1c ≥ 5.7% indicating high risk of T2DM in women with recent GDM [87].

1.6 Anemia

According to the World Health Organization (WHO), anemia is the most common disease, affecting > 1.5 billion people worldwide and iron deficiency anemia (IDA) accounts for 50% anemia cases [88]. IDA is common during pregnancy and postpartum period, and can lead to serious maternal and fetal complications**.**

WHO expert committee defined nutritional anemia as a condition in which hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency [89]. Accordingly, the Joint FAO/WHO Expert Committee on Nutrition has called attention to the problem of anemia in pregnancy, which is considered to be a great public health concern.

1.6.1 Hematological Adaptation to Pregnancy

Normal pregnancy involves many changes in maternal physiology including alteration in hematologic parameters like expansion in maternal blood volume by an average of 40 to 50 % above non-pregnant level [90], and plasma volume, most predominantly in the second trimester as well as red blood cells mass, increases but to a lesser extent than plasma volume [91]. The increment of red cell mass is due to an increased need for oxygen transport during pregnancy. Serum erythropoietin levels increase about 50% throughout pregnancy from a mean of 22.8 mU/mL at booking to 43.7 mU/mL at 38 gestation weeks [92, 93]. These changes will lead to a decrease in hematocrit due to disproportional increase in the blood volume compared with the red cell mass [93]. During pregnancy, an increase in maternal iron is needed as a result of the demands for growing fetus, placenta and increased erythrocyte mass [94].

1.6.2 Diagnostic Criteria of Anemia in Pregnancy

In pregnant women, anemia is defined as hemoglobin concentration less than 11 g/dl in the first and third trimesters, and less than 10.5 g/dl in the second trimester [95]. The reason for these different values, during pregnancy, is that the plasma volume expansion of 40 to 50% exceeds the 20 to 25% increase in red cell mass leading to the physiological hemodilution [96]. Iron deficiency anemia (IDA) during pregnancy poses a number of maternal and fetal problems, including premature birth, intrauterine developmental retardation, placental problems, decrease in newborn iron storage, the risk of decrease in maternal blood reserves during birth, and the need for transfusion in cases of heavy blood

loss, cardiac stress, symptoms of anemia, prolonged hospital stay, decreased maternal breast milk production, and maternal depletion of iron stores during and after postpartum period [97, 98].

The most important factor in the diagnosis of iron deficiency anemia is laboratory testing. The classic laboratory findings of iron deficiency anemia include a decrease in hemoglobin (Hb) level, serum iron concentration, serum transferrin saturation, serum ferritin level, and an increase in total iron-binding capacity. In fact, it is adequate to study complete blood count (CBC) and serum ferritin for diagnosis. Serum ferritin concentration <30 µg /L together with Hb concentration <11 g/dl during the 1st trimester, <10.5 g/dl during the 2nd trimester, and <11 g/dl during the 3rd trimester are diagnostic for anemia during pregnancy [99]. Ferritin level of <12 µg /L is considered the gold standard for diagnosis of iron deficiency anemia in pregnancy [96]. Measurement of serum ferritin concentration is the most accurate test in patients without underlying inflammation, and serum ferritin level below the threshold value alone is adequate for diagnosis in the absence of other tests; however, physicians should be aware that serum ferritin is also an acute phase reactant and may be normal, even elevated, under inflammatory conditions despite the presence of anemia, and in such cases confirmation of the diagnosis may require additional tests [100, 101].

It is recommended to measure serum ferritin at least once early in pregnancy. It is not necessary to measure serum ferritin again later in pregnancy unless symptoms of anemia occur. On the other hand, Hb should be measured in each trimester because the probability of an increase in the need for iron and development of iron deficiency is always possible, even if the baseline value is normal. Moreover, Hb concentration during delivery is important because low maternal Hb can result in fetal problems, including mortality [102].

1.6.3 Anemia and Maternal Mortality and Morbidity

Increase risk of maternal mortality and morbidity is due to hemorrhage and late arrival at admission in sever anemic women, which is related to poor ability to withstand the adverse effects of excessive blood loss and increase risk of infections [103]. Theresa et al, reported that iron deficiency anemia in early pregnancy are associated with low birth weight (<2500g) and preterm delivery (<37 weeks gestation) [104].

1.6.4 Anemia and Fetus Complications

A prospective cohort study was conducted by Qiaoyi Zhang in China found that anemia in early pregnancy was associated with increased risk of stillbirth and preterm premature

rupture of membranes (PROF), but anemia in late pregnancy was associated with reduced risk of preterm birth and spontaneous preterm labor, potentially due to hemodilution. Theresa et al. reported that increased risk of preterm delivery (< 37 week gestation) was associated with maternal anemia or iron deficiency anemia in first trimester and high hemoglobin concentration (> 14.5 g/dl) irrespective to gestation age, thus give a U shaped distribution of hemoglobin while anemia in third trimester was no longer risk factor for preterm delivery [105]. High hemoglobin (> 13.0g/dl) and hematocrit (39%) concentration are associated with increased risk of fetal death, preterm delivery and low birth weight as reported by Garn et al [106].

1.6.5 Anemia and Infant Complications

Rusia et al found that the maternal hemoglobin concentration showed significant correlation with Apgar score [107], (Apgar score is the very first test given to newborns to evaluate their conditions, which include appearance or skin color, pulse or heart rate, Grimace response or reflexes and activity or muscle tone). Evidently, there is no correlation between hematocrit of infant and hematocrit of mother since the fetus requirement of iron are always met even in case of maternal iron deficiency [108].

1.6.6 Serum Ferritin Concentration in Early Pregnancy and Risk of Subsequent Development of GDM.

High serum ferritin levels have been demonstrated in many chronic disorders and vascular inflammation [109, 110], mildly elevated body iron stores have been associated with elevations in glucose hemostasis indexes [111]. A significant correlation between higher serum ferritin levels and insulin resistance syndrome has been shown [112], and greater levels of ferritin in women with impaired glucose tolerance test and GDM have been shown in epidemiologic studies [113, 114].

1.7 Problem Statement

- Utility of HbA1c in diagnosing GDM women, and its usefulness in providing a better prediction of adverse pregnancy outcomes.
- Anemia in early pregnancy, mainly iron deficiency anemia, is associated with adverse pregnancy outcomes.

1.8 Aims of the Study

The oral glucose tolerance test (OGTT) is a cumbersome test that is time consuming, labor intensive and often poorly tolerated by pregnant women. To date, glycated hemoglobin (HbA1c) is the most accepted measure of chronic glycemia outside of pregnancy. HbA1c is an uncomplicated test, less time consuming, does not require any specific patient preparation and is considered straightforward compared with OGTT. Therefore, we prospectively tested the utility of HbA1c when used as a screening tool in pregnancy for gestational diabetes mellitus (GDM) in high risk population, as well as determined the optimal HbA1c threshold for detecting diabetes in early pregnancy as defined by an early oral glucose tolerance test (OGTT) at < 24weeks gestation, and examined pregnancy outcomes related to this threshold.

The aims of this study are:-

1. To investigate the percentage of GDM and adverse pregnancy outcomes.
2. To investigate the role of HbA1c in diagnosing GDM and in prediction of the adverse pregnancy outcomes.
3. To investigate the ability of HbA1c to reduce a number of OGTT tests required.
4. To evaluate maternal anemia and its role in adverse pregnancy outcomes.

CHAPTER TWO: METHODS

2.1 GDM and HbA1c

To evaluate the significance and role of the HbA1c levels in diagnosing GDM. The variation of HbA1c will be examined across gestation and analyze differences between normal and GDM women diagnosed using OGTT.

2.2 HbA1c in pregnancy

The levels of HbA1c were examined for high risk pregnant women to develop GDM (according to UNRWA criteria which is listed in the appendix number 1) at their first antenatal visit (< 24 gestational weeks). The rationale of this approach is these women are unlikely to have normal glucose tolerance during pregnancy if they have high HbA1c value particularly as HbA1c decrease in early pregnancy in pregnant women who belong to high risk population. Adverse pregnancy outcome was followed by recording the screening tests at pregnant women during each visit including blood pressure, urine for albumin and sugar analysis and complete blood count (CBC) as well as performing FPG test. Pregnant women having FPG ≥ 126 mg/dl and HbA1c 6.5% (this value is the diagnostic criteria for DM) are diagnosed with DM according to IADPSG criteria at < 24 gestational weeks. For women with FPG ≥ 85 mg/dl, OGTT was performed earlier without need to wait for the second visit according to UNRWA protocol. At 24^{th}-37^{th} weeks gestation new sample was collected from the same candidates to repeat CBC, HbA1c and FPG, urine for albumin and sugar analysis. At 6^{th} weeks (42 days after delivery), additional blood collection from participant women was used to perform the same tests.

After delivery, neonatal information was collected particularly birth weight, gestational age of delivery, type of delivery, head circumference, gender and other related complications.

2.3 Anemia in the Study

Iron deficiency anemia in pregnant women was tested according to CDC definition (serum ferritin < 12μg/L) in their first trimester by CBC and serum ferritin. The association between adverse pregnancy outcomes like low birth weight (< 2500g), preterm delivery (< 37 weeks gestation) and iron deficiency anemia was evaluated.

2.4 Study Population

Nine hundred fifty five (955, 22% in Jenin,19.4% in Alamari, 2.7% in Hebron and 3.5% in Kalandia) pregnant women without previous DM (Diabetes Mellitus), PCOS (Poly Cystic Ovary Syndrome) or HT (Hypertension), who were referred to the prenatal care unit of the United Nations Relief and Works Agency (UNRWA) for Palestine Refugees in the West Bank, between October 2015 to May 2017 to do follow up due their first trimester, and have one or more risk factors were consecutively invited to participate in the study.

All women signed an informed consent form and answered a standardized questionnaire. Data from questionnaires and anthropometric measurement were collected by specially trained midwives according to a standard protocol at mean gestational week 10.6 ± 4.3 (SD) (first visit), mean gestational week 26.3 ± 2.2 (SD) (second visit), and 5.7 ± 1.7 (SD) week postpartum (postnatal visit). Participants with the following conditions, which are known to interfere with or lead to misinterpretation of HbA1c results were excluded from participation: chronic renal disease and/or presence of hemoglobinopathy. The enrollment of the study population is presented in Figure 2.

2.5 Ethical Considerations

The project was ethically approved by the institutional review board (IRB) committee of Al-Quds University (appendix 3). In addition, a permission to conduct the study was achieved by the UNRWA administration, by miss Fidaa Zedan (email: F.zedan2@UNRWA.org. Mobile number 0542168270). The staff of the UNRWA clinic were involved in collecting the data along with the researcher. Nine clinics were engaged in the study include (Jenin, Qalqilia, Asker, Balata, Tulkarem, Kalandia, Khamashta and Hebron). The project was fully explained to the patients by well-trained researcher and UNRWA clinic Nursing staff and then the participants were consented for voluntarily participating in the study. Each participant signed a consent form (appendix 2) that confirms her participation and assures the confidentiality of the obtained data that DNA and serum samples will not be used for other purposes. Moreover, every participant was assured the freedom to accept or refuse participation in the study without intimidations. In addition, patients' samples, the questionnaires and the databases were securely stored.

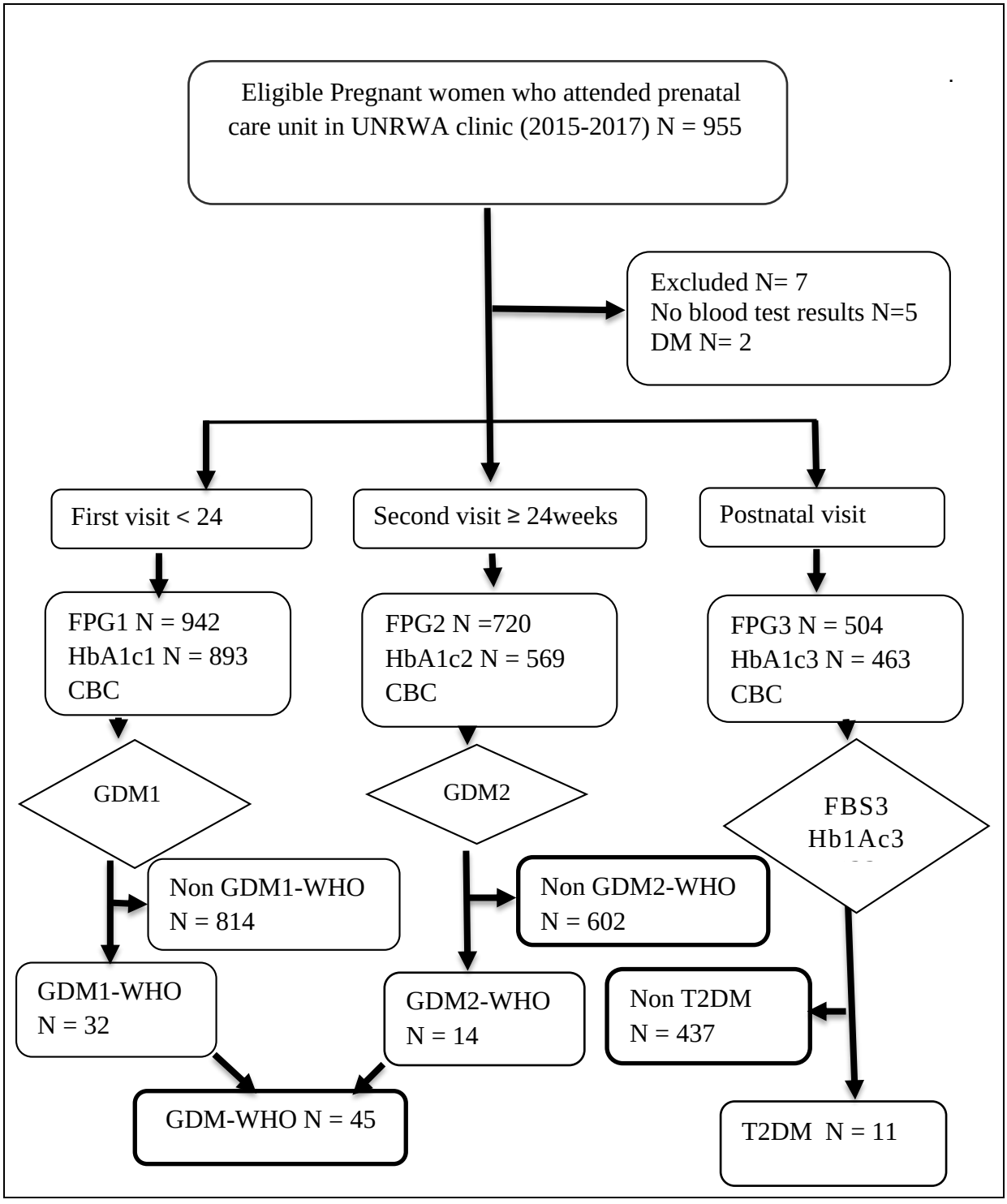

Figure 2. **Flow Diagram of The Study Participants**. The information in boxes show how many of the study participants that had completed data set for all potential predictors considered in the model. (DM: Diabetes Millets, GDM1: Gestational Diabetes Millets at first visit, GDM2: Gestational Diabetes Millets at second visit, FPG: Fasting Plasma Glucose, N: Number, CBC: Complete Blood Cell count, HbA1c: glycated hemoglobin, OGTT1: Oral Glucose Tolerance Test at first visit, OGTT2: Oral Glucose Tolerance Test at second visit, T2DM: Type 2 Diabetes Millets.

2.6 Diagnostic Criteria

Gestational diabetes mellitus (GDM) was diagnosed at 4-22 and 24-37 by the WHO criteria from 1999 using fasting plasma glucose (FPG) ≥ 126 mg/dl or plasma glucose ≥ 140 mg/dl 2 hour after ingestion 75g glucose orally (OGTT). After the study GDM was diagnosed according to the modified IADPSG criteria using fasting plasma glucose (FPG) ≥ 92 mg/dl or plasma glucose ≥ 153 mg/dl 2 hour after the glucose load [42, 115]. GDM diagnosed by the WHO criteria was named GDM-WHO and by modified IADPSG criteria was named GDM-IADPSG. WHO recommendation for anemia include hemoglobin < 12 g/dl for non-pregnant women and < 11 g/dl for pregnant women, and severe anemia in pregnancy as hemoglobin < 7 g/dl, and for very severe anemia hemoglobin < 4 g/dl [116], and serum ferritin concentrations < 12.0 μg /L [117].

2.7 Clinical Data

Maternal age at inclusion, gestational age, obstetric history including gravida and para were obtained from subjects' files. Parity was categorized as nulliparous or multiparous (≥ 1), referring to status before the current pregnancy. Family history of diabetes mellitus (DM) was reported and categorized as Yes or No if a woman's parents or siblings had diabetes, and personal history of previous GDM, baby birth weight and pre-term delivery were also reported. Maternal height was measured, and pregnancy weight was taken by electrical scale at registry, and body mass index BMI (kg/m^2) was calculated and categorized as underweight (BMI < 18.5 kg/m^2), normal weight (18.5 – 24.9 kg/m^2), overweight (25.0 – 29.9 kg/m^2), or obese (≥ 30.0 kg/m^2). Arterial blood pressure (systolic and diastolic mmHg) was measured with a validated electronic device at each visit, and a urine sample was taken each visit to detect protein and sugar levels. Data of adverse pregnancy outcomes were obtained from medical records. Gestational hypertension was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation in women with previously normal blood pressure and blood pressure levels that returned to normal postpartum. Preeclampsia was characterized by gestational hypertension and proteinuria (≥ 1+ on urine dipstick) with or without pathologic edema.

2.8 Laboratory Analysis

In early pregnancy, OGTT (< 24 weeks of gestation) was performed to women who had FPG ≥ 85mg/dl, same as second OGTT ≥ 24 weeks of gestation. Women who had 2hr OGTT ≥140 mg/dl were monitored by performing 2 hours postprandial at each visit. During postnatal visit, OGTT was only performed in a subset of women with previous GDM and returned for their follow-up visit (N = 2 of 45).

Following an overnight fast, blood samples were drawn to determine HbA1c levels, FPG and CBC at first and second trimesters and at sixth weeks after delivery, while serum ferritin was taken at registry. Oral glucose tolerance tested performed 75g anhydrous glucose, which was performed according WHO recommendation. Plasma glucose was determined by enzymatic method using plasma, glucose oxidase method (Chem Well, USA). Hemograms were performed by automated hematology analyzer (Sysmex, America). HbA1c levels were determined by ion-exchange high performance liquid chromatography (HPLC) on EDTA whole blood.

Serum ferritin was quantitatively measured by ELISA (enzyme-linked immunosorbent assay) using (Architect, USA).

2.9 Statistical Analysis

The study data were taken from the questionnaires then entered into a computer database by using SPSS software program, version 23 Windows (IBM, SPSS, Statistics). Continuous variables were descriptively expressed as the mean, minimum, maximum and standard deviation (SD). Proportions were determined for categorical variables using the Chi-squared test. To determine the association between HbA1c level and GDM, a receiver operating characteristic (ROC) curve was constructed. To investigate whether the HbA1c levels during pregnancy was significantly associated with adverse pregnancy outcomes, Pearson correlation was applied. Differences in mean HbA1c levels were determined between normal and adverse pregnancy outcomes using independent sample T test.

CHAPTER THREE: RESULTS

3.1 Baseline Characteristics of Participant

In this study, diagnosing of GDM was according to the criteria adopted by UNRWA in the West Bank (WHO 1999) unless otherwise indicated. Of the 955 pregnant women recruited, 7 were excluded (5 had no blood test results available and 2 were diagnosed as T2DM based on HbA1c value which was 8% and they started medication. Of the 948, who were included in the study, 12 of them gave birth to twins.

The baseline maternal characteristic of the study population is presented in Table 3. The mean maternal age of the study population was 28.5 (17- 45) years, 61.9% of participants were between (21- 30) years old. The mean pre-pregnancy BMI was 27.2 kg/m^2, and 24.6% were obese. Gravida (total number of confirmed pregnancies that a woman had, regardless of the outcome) ranged from 1 to 13, and nulliparous status (women who have never gave birth either by choice or for any other reason) was 17.9% (168/939). Family history of DM was 53% while the personal history of having previous GDM was 3.8%, and 11.1% previously gave birth to big babies.

3.2 Mean Values of the Laboratory Tests

Table 4 shows that among all the study subjects, 947 came before 24 weeks of gestation, 942 did FPG1 (mean FPG1 values were 87.3 mg/dL (58-136) ± 10.1 SD) and the mean FPG2 values at second visit were 83 mg/dL (60-125) ± 10.5 (SD). 893 did HbA1c1 (at first visit). Doing 2 hr postprandial (2 hr PP) as a monitoring test to those who were diagnosed with GDM-WHO, only 11 values are presented. The values of HbA1c1 before 24 weeks gestation ranged from 4.0% to 7.6% with mean value 5.4 ± 0.41% SD, while the HbA1c2 values (after 24 weeks gestation) ranged from 3.9% to 6.7% with mean value 5.3 ± 0.42% SD. Urine samples were taken each visit to detect protein and sugar levels by chemical stick test to exclude participants with hypertension.

Mean hemoglobin level before 24 weeks was within the normal range (12.1g/dl). Out of the 495 participants needed to do OGTT1 test depending on FPG1 values (≥ 85mg/dl) before 24 weeks gestation, only 401 complied. Only 2 of 45 GDM participants did OGTT test at postnatal visit and the results were < 140 mg/dl. Participants were assessed as to the presence or absence of GDM-WHO according to WHO1999 criteria.

Table 3. Baseline Characteristic of Participant.

Characteristic (N)		N	%
Age, years, (951)	≤ 20	50	5.3
	21-30	589	61.9
	31-40	297	31.2
	> 40	15	1.6
BMI, kg/m^2, (918)	Underweight (<18.5)	13	1.4
	Normal weight (18.5-24.9)	320	34.9
	Over weight (25-29.9)	359	39.1
	Obese ≥30	226	24.6
Gravida (940)	1	146	15.5
	2-5	607	64.6
	≥6	187	19.9
Para (939)	0	168	17.9
	≥1	771	82.1
Pre-abortion (939)	No	606	64.5
	Yes	333	35.5
FH DM (927)	No	436	47
	Yes	491	53
PH GDM (928)	No	893	96.2
	Yes	35	3.8
PDBB (928)	No	825	88.9
	Yes	103	11.1
PDLB (928)	No	848	91.4
	Yes	80	8.6
PPD (928)	No	882	95
	Yes	46	5

BMI: body mass index, FH DM: family history of diabetes millets, PH GDM: personal history of gestational diabetes mellitus, PDBB: previous delivery of a big baby, PDLB: previous delivery of a low weight baby, PPD: previous preterm delivery. Gravida: the total number of confirmed pregnancies that a woman had, regardless of the outcome. Para: the number of births that a woman had after 20 weeks gestation.

Table 4. Mean Values of Study Tests in Participating Subjects.

Test	Visit	N	Mean (CI)	SD
FPG1 mg/dl	FV	942	87.3 (58-136)	10.1
FPG2 mg/dl	SV	720	83 (60-125)	10.5
FPG3 mg/dl	PN	504	86 (57-137)	10.5
Hb1. g/dl.	FV	948	12.1 (8.2-14.9)	1.06
Hb2 g/dl.	SV	734	11.2 (7.5-14.5)	1.05
Hb3 g/dl.	PN	529	12.6 (8.3-136)	5.54
Hct1 %	FV	910	36. (23-46.3)	2.80
Hct2 % SV	SV	621	33.3 (24.7-44.1)	2.84
Hct3 % PN	PN	502	37.8 (27.1-47.1)	3.41
MCV1 fl	FV	911	82.9 (60.8-94.9)	5.94
MCV2 fl	SV	618	84.4 (60-97.3)	6.57
MCV3 fl	PN	503	83.4 (60.9-98)	6.22
HbA1c1%	FV	893	5.40 (4.0-7.6)	0.41
HbA1c2%	SV	569	5.3 (3.9-6.7)	0.42
HbA1c3%	PN	463	5.4 (4.0-7.0)	0.42
2hr.75g OGTT1	FV	401	103.9 (50-211)	25.2
2hr.75g OGTT2	SV	156	107.3 (56-181)	22.26
Ferritin µg /L	FV	447	27.5 (1-156)	22.31
SBP1	FV	909	111.1 (80-142)	10.48
DBP1	FV	909	67.6 (46-92)	8.01
SBP2	SV	702	111.1 (72-146)	10.39
DBP2	SV	703	66.9 (44-115)	8.33
SBP3	PN	536	113 (57-156)	11.36
DBP3	PN	535	70 (50-110)	8.67
2hr PPG	SV	11	100.2 (55-144)	27.70

FPG1: Fasting plasma glucose at first visit; FPG2: Fasting plasma glucose at second visit; FPG3: Fasting plasma glucose at postnatal visit; Hb1: Hemoglobin at first visit; Hb2: Hemoglobin at second visit; Hb3: Hemoglobin at postnatal visit; Hct1: Hematocrit at first visit; Hct2: Hematocrit at second visit; Hct3: Hematocrit at postnatal visit; MCV1: Main corpuscular volume at first visit; MCV2: Main corpuscular volume at second visit; MCV3: Main corpuscular volume at postnatal visit; 2hr. 75 g OGTT1: two hour glucose tolerance test at first visit; 2hr. 75 g OGTT2: two hour glucose tolerance test at second visit; HbA1c1: glycated Hemoglobin at first visit; HbA1c2: glycated Hemoglobin at second visit; HbA1c3: glycated Hemoglobin at postnatal visit; FV: first visit; SV: second visit; PN: Postnatal visit; SBP1: Systolic Blood Pressure at first visit; DBS1: Diastolic Blood Pressure at first visit; SBP2: Systolic Blood Pressure at second visit; DBP2: Diastolic Blood Pressure at second visit; SBP3: Systolic Blood Pressure at postnatal visit; DBP3: Diastolic Blood Pressure at postnatal visit. SD: Standard Deviation; and N; Number.

3.3 Percentage of GDM

Gestational Diabetes Mellitus (GDM1-WHO) occurred in 3.8% (32/846) of participants when diagnosed at first visit (before 24 weeks gestation) according to UNRWA protocol applied in their perinatal clinic, while it occurred in 2.3% (14/616) when diagnosed at 24 weeks gestation or more, so the percentage of GDM diagnosed by WHO1999 criteria was 7.4% (45/616) which represented the sum of cases at the two visits except one case which was diagnosed with GDM at first and at second visit.

The percentage of GDM1-IADPSG at first visit using modified IADPSG criteria was 32.1%, as well 1.2% diagnosed with DM and the percentage of GDM2-IADPSG at the second visit was 19.2% while the accumulative GDM-IADPSG percentage was 45.8% (358/781). Table 5 shows the comparison between WHO1999 criteria used in UNRWA clinics the and modified IADPSG criteria in diagnosing GDM at different times of visits during pregnancy.

Table 5. Percentage of GDM According to Different International Criteria.

MEASURE	**WHO 1999 & UNRWA at < 24 weeks gestation**	**WHO 1999 & UNRWA at ≥ 24 weeks gestation**	
FPG	≥ 126 mg/dl N = 3/942 (0.3%)	≥ 126 mg/dl N = 0	
	Or	Or	
2 hr. 75g OGTT	≥ 140 mg/dl N = 30	≥ 140 mg/dl N = 14	
GDM	32 (3.8%)	14 (2.3%)	
MEASURE	**IADPSG & WHO 2013 at < 24 weeks gestation.**	**IADPSG & WHO 2013 at < 24 weeks gestation.**	**IADPSG & WHO 2013 at ≥ 24 weeks gestation**
FPG	≥ 92 mg/dl < 126 mg/dl N= 306/942 (32.5%)	≥ 126 mg/dl N = 3/942 (0.3%)	≥ 92 mg/dl N = 136/720 (18.9%)
	&	Or	Or
HbA1c1	< 6.5% N = 882/893 (98.8%)	≥ 6.5% N = 11/893(1.2%)	
2 hr. 75g OGTT			≥ 153 mg/dl N = 6/156 (3.8%)
DM		1.2% (11/940)	
GDM	32.1% (302/940)		19.2% (138/720)

IADPSG: International Association of Diabetes and Pregnancy Groups; WHO: World Health Organization; GDM1: Gestational Diabetes Mellitus at < 24 weeks gestation; GDM2: Gestational Diabetes Mellitus at ≥ 24 weeks gestation; OGTT: Oral Glucose Tolerance Test; FPG: Fasting Plasma Glucose; HbA1c1: glycated Hemoglobin at first visit; DM**:** Diabetes Millets

3.4 BMI as a Risk Factor for GDM-WHO

The correlation between BMI as a risk factor for GDM diagnosed by GDM-WHO and glucose levels was analyzed. Mean BMI for 918 pregnant women was 27.2 ± 5.22 kg/m^2 (SD). The correlation between FPG1 and BMI revealed a significant but moderate positive correlation (r = 0.234. A significant positive correlation between BMI and glucose levels in the 2hr 75g OGTT1 test before 24 weeks gestation (r = 0.164) was observed which disappeared in 2hr 75g OGTT2 (P = 0.289). HbA1c1 and age were positively correlated with BMI.

3.5 GDM1-WHO Diagnosed at First Visit and the GDM Risk Factors

Thirty-two pregnant women (3.8%) tested positive for GDM1-WHO at < 24 weeks of pregnancy. Between 24 and 37 weeks of gestation, 14 (2.3%) women were diagnosed with GDM2-WHO.

Risk factors in participants including age, BMI, number of previous pregnancies, deliveries, abortions, family history of diabetes mellitus, GDM in previous pregnancy, previously giving birth a macrosomia, low birth weight babies and previous preterm delivery were assessed and presented in Table 6.

Chi Square analysis revealed significant correlation between age groups of pregnant women and the presence or absence of GDM1-WHO (GDM diagnosed by WHO criteria before 24 weeks gestation) revealed the presence of a significant relationship (P = 0.000). Pregnant women (31-40 years old) were more likely to develop GDM1-WHO than younger women (21-30 years old) (58.1% vs. 35.5% respectively). However, the number of women greater than 40 years (only 2) was small for statistical analysis. Also, there was a significant relationship between BMI groups and GDM1-WHO with (P = 0.020). Overweight and Obese women were more likely to develop GDM1-WHO than others. More than 83% of women with GDM1-WHO were overweight or obese. There was a significant relationship between gravida (the number of times a woman is or has been pregnant, regardless of the pregnancy outcome) and GDM1-WHO (P = 0.001). A significant relationship was also observed between pre-abortion and GDM1-WHO (P=0.001). Participants who previously experienced abortion were more like to have GDM1-WHO (64.5%). A significant relationship existed between family history of DM and GDM1-WHO (P=0.0357). Pregnant women whose parents or siblings had DM were more likely to develop GDM1-WHO than pregnant women whose family were DM free (71.0% vs. 29% respectively). Whereas, there were insignificant relationship between GDM1-WHO and preterm delivery, previously

gave birth to low weight or macrosomic babies, and number of pregnancies reaching viable gestational age (parity).

Table 6. Association Between GDM1-WHO (GDM Diagnosed at First Visit < 24 Weeks by WHO Criteria) and Risk Factors.

Risk factors (N)		GDM1-WHO < 24 weeks (31)		P Value
		Yes	No	
		N (%)	N (%)	
Age, years, (839)	≤ 20	0	46 (5.7%)	0.000
	21-30	11 (35.5%)	506 (62.6%)	
	31-40	18 (58.1%)	247 (30.6%)	
	> 40	2 (6.5%)	9 (1.1%)	
BMI, Kg/m^2 (818)	Underweight	1(3.3%)	10 (1.3%)	0.020
	Normal weight	4 (13.3%)	290 (36.8%)	
	Over weight	12 (40.4%)	300 (38.1%)	
	Obese	13 (43.3%)	188 (23.9 %)	
Gravida (833)	1	2 (6.5%)	130 (16.2%)	0.001
	2-5	15 (48.4%)	522 (65.1%)	
	≥ 6	14 (45.2%)	150 (18.7%)	
Para (832)	0	5 (16.1%)	147 (18.4%)	0.753
	≥ 1	26 (83.9%)	654 (81.4%)	
Pre-abortion (832)	Yes	20 (64.5%)	282 (35.2%)	0.001
	No	11 (35.5%)	519 (64.8%)	
FH DM (825)	Yes	22 (71%)	411 (51.8%)	0.036
	No	9 (29 %)	383 (48.2%)	
PH GDM (826)	Yes	6 (19.4%)	22 (2.8%)	0.000
	No	25 (80.6%)	773 (97.2%)	
PDBB (826)	Yes	5 (16.1%)	81 (10.2%)	0.288
	No	26 (83.9%)	714 (89.8%)	
PDLB (826)	Yes	3 (9.7%)	70 (8.8%)	0.867
	No	28 (90.3%)	725 (91.2%)	
PPD (826)	Yes	1 (3.2%)	41 (5.2%)	0.631
	No	30 (96.8%)	754 (94.8%)	

Underweight: BMI (<18.5); Normal weight: BMI (18.5-24.9); Over weight: BMI (25-29.9); Obese: BMI (≥ 30); DM: Diabetes Mellitus; FH DM: Family history of DM; PH GDM: Personal history of GDM; PDBB: Previous delivery of a big baby; PDLB: Previous delivery of a low weight baby; PPD: Previous preterm delivery; N: Number.

3.6 HbA1c1 at First Visit and the GDM Risk Factors

One way ANOVA for pregnant age, BMI, gravida and HbA1c1% (HbA1c measured before 24 weeks gestation) revealed the presences of statistically significant difference (P = 0.000) in the mean values of HbA1c between the groups as well as with pre-abortion (P = 0.000) and personal history of GDM when independent sample T test was used (P = 0.000). However, PDLB (Previous delivery of a low weight baby), and PPD (Previous preterm delivery) didn't show statistically significant difference between HbA1c1% mean values, as shown in Table 7.

Table 7. Association Between HbA1c1% at First Visit (< 24 Weeks Gestation) and GDM Risk Factors.

Risk Factors (N)		HbA1c1% at < 24 weeks gestation		P Value
		Mean	SD	
Age (886)	≤ 20	5.3	0.37	0.000
	21-30	5.4	0.39	
	31-40	5.5	0.43	
	> 40	5.7	0.37	
BMI Kg/ m^2 (854)	Underweight	5.3	0.28	0.000
	Normal weight	5.3	0.36	
	Overweight	5.4	0.39	
	Obese	5.5	0.46	
Gravida (875)	1	5.3	0.40	0.000
	2-5	5.4	0.40	
	≥6	5.5	0.44	
Para (874)	0	5.3	0.44	0.040
	≥1	5.4	0.41	
Pre-abortion (874)	Yes	5.48	0.44	0.000
	No	5.36	0.39	
FHDM (862)	Yes	5.44	0.42	0.001
	No	5.35	0.40	
PH GDM (863)	Yes	5.69	0.57	0.000
	No	5.39	0.40	
PDBB (863)	Yes	5.51	0.39	0.005
	No	5.38	0.42	
PDLB (863)	Yes	5.4	0.41	0.602
	No	5.4	0.42	
PPD (863)	Yes	5.28	0.38	0.068
	No	5.4	0.42	

Underweight: BMI (<18.5); Normal weight: BMI (18.5-24.9); Over weight: BMI (25-29.9); Obese: BMI (≥ 30); DM: Diabetes Mellitus; FH DM: Family history of DM; PH GDM: Personal history of GDM; PDBB: Previous delivery of a big baby ; PDLB: Previous delivery of a low weight baby; PPD: Previous preterm delivery, SD: Standard Deviation.

3.7 Levels of HbA1c Throughout Gestation

The changes in HbA1c values during pregnancy were obvious in figure 3 and table 8 which show that HbA1c tend to decrease in first and second trimester then increase in third trimester. The mean value of HbA1c at 4-13 weeks' gestation was 5.4% which was significantly higher than that at 14-22 weeks' gestation (5.3%), and at 24-29 weeks' gestation, (P = 0.002, and 0.000 respectively). A statistically significant higher mean values was observed between the mean value of HbA1c at 1-6 weeks' postnatal (5.4%) 14-22 weeks' gestation and 24-29 weeks' gestation, (P = 0.003 and 0.000 respectively). One way ANOVA for HbA1c mean values at different time visits were statistically significant (P = 0.000).

Table 8. HbA1c% Values Throughout Pregnancy and Postnatal.

Gestational Week	N	HbA1c%Mean (CI)	SD
4 -13 (weeks' gestation)	661	5.4 (4.0-7.6)	0.41
14 - 22 (weeks' gestation)	226	5.3 (4.0-6.5)	0.42
24 - 29 (weeks' gestation)	525	5.3 (3.9-6.6)	0.43
30 - 37 (weeks' gestation)	39	5.4 (4.7-6.7)	0.39
1 - 6 (weeks' postnatal)	403	5.4 (4.2-6.9)	0.41
7 - 13 (weeks' postnatal)	51	5.5 (4.9-7.0)	0.44

HbA1c: glycated Hemoglobin; SD: Standard Deviation; N: Number; CI: confidence intervals.

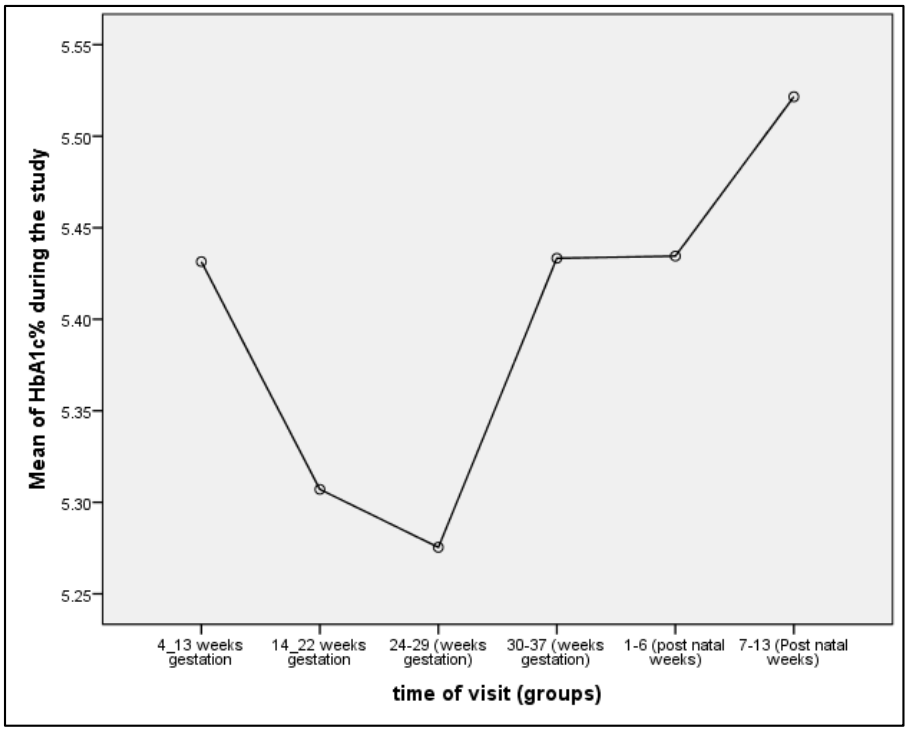

Figure 3. HbA1c % Values Throughout Pregnancy and Postnatal Visit.

A two-way analysis of variance tested the HbA1c values of pregnant women who had or didn't have GDM-WHO across different gestational age revealed that pregnant women who had GDM-WHO showed a significantly higher level of HbA1c value (P = 0.000) compared to those who did not (Table 9). Times of visits also showed significant differences in HbA1c values (P = 0.000) and the interaction between time of visit and GDM, was also significant (P = 0.006). Figure 4 Represents the HbA1c values throughout pregnancy in GDM-WHO and non GDM-WHO cases. The lowest HbA1c value was seen between 24-29 weeks (5.26% ± 0.42 SD) and the highest value was seen between 30-37 weeks (5.4% ± 0.34 SD) in non GDM cases.

Table 9. HbA1c % Throughout Pregnancy in GDM-WHO and non GDM-WHO Cases.

Time of visit	GDM-WHO	N	HbA1c	
			Mean	SD
4-13 (weeks' gestation)	No	629	5.41	0.38
	Yes	25	5.95	0.68
	Total	654	5.43	0.41
14-22 (weeks' gestation)	No	218	5.30	0.41
	Yes	5	5.94	0.30
	Total	223	5.31	0.42
24-29 (weeks' gestation)	No	501	5.26	0.42
	Yes	13	5.39	0.32
	Total	514	5.26	0.42
30-37 (weeks' gestation)	No	38	5.40	0.34

HbA1c: glycated Hemoglobin; GDM-WHO: Gestational Diabetes Mellitus diagnosed by WHO criteria, SD: Standard Deviation; N: Number.

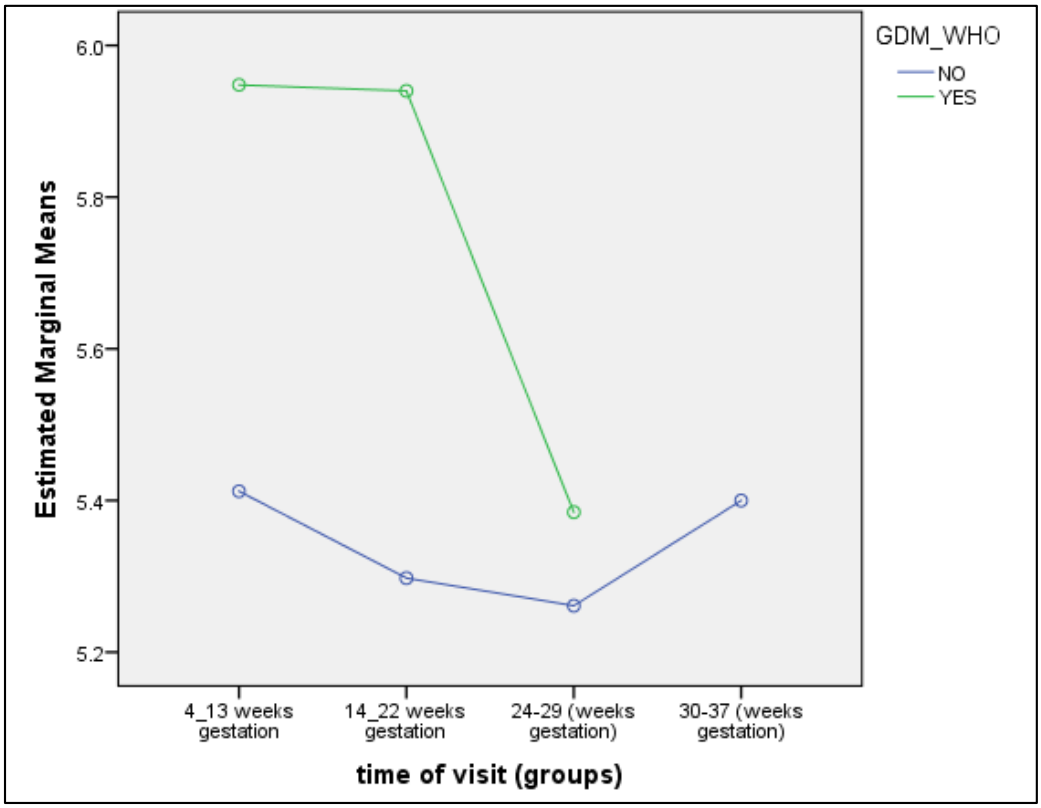

Figure 4. HbA1c % Values Throughout Pregnancy in GDM-WHO and non GDM-WHO. HbA1c: glycated Hemoglobin; GDM-WHO: Gestational Diabetes Mellitus diagnosed by WHO criteria.

Table 10 showed that there was a statistically significant difference in mean value of HbA1c1 at first visit between pregnant women who had GDM and those who didn't. The mean HbA1c1 value in women with GDM1-WHO was significantly higher than in women without GDM1-WHO (5.9 ± 0.6% compare to 5.4 ± 0.4%). A statistically significant difference was also observed in mean values of HbA1c1 between women who had GDM1-IADPSG and women who had DM in their first visit. In the same direction, a statistically significant difference in the mean value of HbA1c1 between women who developed T2DM after delivery and those who didn't (5.9 ± 0.5% compare to 5.4 ± 0.4%).

Table 10. Mean Value of HbA1c1% (at First Visit < 24 Weeks Gestation) and GDM

		N	HbA1c1% (at first visit)		P. Value
			Mean	SD	
GDM1-WHO	Yes	31	5.93	0.62	0.000
	No	764	5.38	0.38	
GDM2-WHO	Yes	14	5.52	0.37	0.202
	No	569	5.39	0.40	
GDM-WHO	Yes	44	5.81	0.59	0.000
	No	537	5.38	0.39	
GDM1-IADPGS	Yes	292	5.52	0.37	0.000
	No	581	5.32	0.37	
	DM	11	6.80	0.4	
GDM2-IADPGS	Yes	134	5.60	0.40	0.000
	No	550	5.37	0.39	
T2DM	Yes	11	5.87	0.50	0.000
	No	443	5.44	0.40	

GDM1-WHO: Gestational Diabetes Millets at first visit diagnosed by WHO; GDM2-WHO: Gestational Diabetes Millets at second visit diagnosed by WHO; GDM-WHO: Gestational Diabetes Millets diagnosed by WHO; GDM1-IADPSG: Gestational Diabetes Millets at first visit diagnosed by modified IADPSG; GDM2-IADPSG: Gestational Diabetes Millets at second visit diagnosed by IADPSG; T2DM: Type 2 Diabetes Millets; SD: Standard Deviation; DM: Diabetes Mellitus.

3.8 HbA1c as a Screening Test for GDM

Correlation analysis (Table 11) revealed a moderate positive correlation between HbA1c and FPG and between HbA1c and 2hr. 75g OGTT (r =0.341, and 0.303, respectively). Pregnant women who had higher FPG and higher 2hr. 75g OGTT values reported higher HbA1c value (at different time of first visit).

Table 11. Correlation Between HbA1c, FPG and 2hr OGTT Throughout Pregnancy.

HbA1c1 % N=893, Mean = 5.4, SD = 0.41					
Parameter	**N**	**Mean**	**SD**	**r**	**P value**
FPG1 mg/dl	942	87	10.1	0.341	0.000
FPG2 mg/dl	720	83	10.5	0.186	0.000
FPG3 mg/dl	504	86	10.6	0.132	0.003
2hr. 75g OGTT1	401	104	25.2	0.303	0.000
2hr.75g OGTT2	156	107	22.3	0.158	0.051
HbA1c2 % N=569, Mean = 5.3, SD = 0.42					
Parameter	**N**	**Mean**	**SD**	**r**	**P value**
FPG1 mg/dl	942	87	10.1	0.261	0.000
FPG2 mg/dl	720	83	10.5	0.236	0.000
FPG3 mg/dl	504	86	10.6	0.161	0.001
75g OGTT1-2hr	401	104	25.2	0.245	0.000
75g OGTT2-2hr	156	107	22.3	0.236	0.007
HbA1c3 % N=463, Mean = 5.4, SD = 0.42					
Parameter	**N**	**Mean**	**SD**	**r**	**P value**
FPG1 mg/dl	942	87	10.1	0.186	0.000
FPG2 mg/dl	720	83	10.5	0.200	0.000
FPG3 mg/dl	504	86	10.6	0.199	0.000
75g OGTT1-2hr	401	104	25.2	0.388	0.000
75g OGTT2-2hr	156	107	22.3	0.238	0.017

FPG1: Fasting Plasma Glucose at first visit; FPG2: Fasting Plasma Glucose at second visit; FPG3: Fasting Plasma Glucose at postnatal visit; HbA1c1: glycated hemoglobin at first visit; HbA1c2: glycated hemoglobin at second visit; HbA1c3: glycated hemoglobin at postnatal visit; OGTT1: Oral Glucose Tolerance Test at first visit; OGTT2: Oral Glucose Tolerance Test at second visit.

3.9 Optimal HbA1c Value to Detect GDM-WHO

Receiver operating characteristics (ROC) curve analysis for HbA1c is used to determine if HbA1c could be used to predict GDM-WHO when compared to the gold standard of using OGTT. Figure 5 was used to determine the sensitivity and specificity of HbA1c1 (at first visit < 24 weeks' gestation) in detecting GDM1-WHO. The area under ROC curve (AUC) of HbA1c1 to detect GDM1-WHO was 0.772 (95% CI 0.689-0.856). It was observed that an HbA1c1 cutoff value of ≥ 5.75% had a sensitivity of 57.5 % and a specificity of 85.1% in diagnosis GDM1-WHO.

In Figure 6 ROC curve analysis was also used to determine the sensitivity and specificity of FPG1 test (at first visit < 24 weeks gestation**)** in detecting GDM1-WHO. The area under ROC curve of FPG1 to detect GDM1-WHO was 0.88 (95% CI 0.836-0.923). The optimal cutoff value was ≥ 94.5 g/dl, with a sensitivity of 71.9% and a specificity 81.4%. Table 12 summarizes the AUC, sensitivities, specificities and cutoff values for HbA1c and FPG at different time of visit, ferritin, BMI, BBW, head circumference for detecting GDM1-WHO using OGTT1 (at first visit < 24 weeks of gestations), while Table 13 summarizing same variables but in detect GDM2-WHO (at second visit ≥ 24 weeks of gestation).

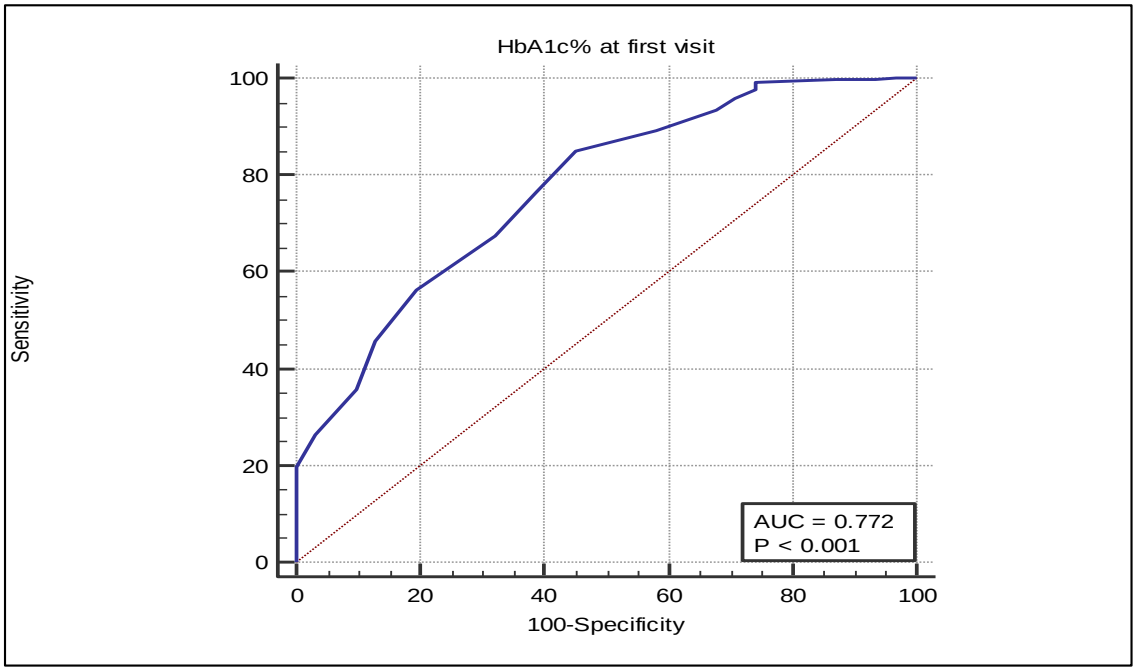

Figure 5. ROC curve Analysis of HbA1c1 % (at first visit) in detecting GDM1-WHO at < 24 weeks gestation. ROC: Receiver Operating Characteristics; HbA1c1: glycated hemoglobin at first visit; GDM1-WHO: Gestational Diabetes Millets diagnosed at first visit with WHO 1999 criteria.

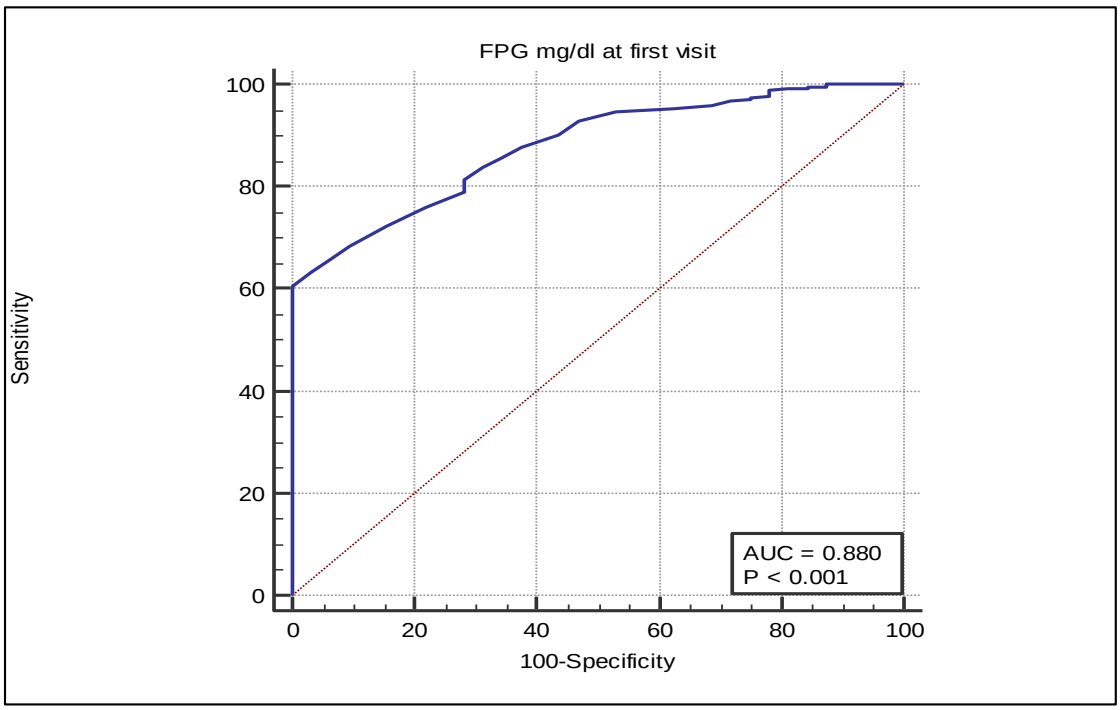

Figure 6. ROC Curve Analysis of FPG1 (at First Visit) in Detecting GDM1-WHO. ROC: Receiver Operating Characteristics; FPG1: Fasting Plasma Glucose at first visit; GDM1-WHO: Gestational Diabetes Millets diagnosed by WHO 1999 at first visit (< 24 weeks gestation).

Table 12. FPG, HbA1c, Ferritin, BBW, BHC and BMI Sensitivity, Specificity and Cutoff Values for GDM1-WHO at < 24 Weeks' Gestation.

GDM1- WHO					N =32		
scale variable	Cut-off	sensitivity	specificity	95% Confidence Interval		AUC	P. Value
				lower Bound	upper Bound		
FPG 1	94.5	71.90%	81.40%	0.836	0.923	0.880	0.000
FPG 2	88.5	62.50%	75.00%	0.634	0.891	0.762	0.000
FPG 3	94.5	33.30%	83.00%	0.429	0.682	0.555	0.360
HbA1c 1	5.75%	57.50%	85.10%	0.689	0.856	0.772	0.000
HbA1c 2	5.85%	34.80%	92.70%	0.634	0.847	0.741	0.000
HbA1c 3	5.85%	60.00%	86.40%	0.703	0.911	0.807	0.000
BBW g	3665	39.30%	81.30%	0.501	0.726	0.613	0.042
HC cm	35.35	30.80%	77.80%	0.461	0.671	0.566	0.252
BMI kg/m^2	30.7	43.30%	80.20%	0.563	0.764	0.664	0.002
Ferritin µg/L	36.43	57.10%	75.30%	0.456	0.831	0.643	0.193

GDM1-WHO :Gestational Diabetes Millets at first visit diagnosed by WHO 1999; FPG1: Fasting Plasma Glucose at first visit; FPG2: at second visit; FPG3: at postnatal visit; HbA1c1: glycated hemoglobin at first visit; HbA1c2: at second visit; HbA1c3: at postnatal visit; BBW: Baby Birth Weight; HC: Head Circumference; BMI: Body Mass Index; AUC: Area under the ROC curve

Table 13. FPG, HbA1c, Ferritin, BBW, BHC and BMI Sensitivity, Specificity and Cutoff Values for GDM2-WHO at ≥ 24 Weeks' Gestation.

GDM2- WHO					N =14		
scale variable	Cut-off	sensitivity	specificity	95% CI		AUC	P Value
				lower Bound	upper Bound		
FPG 1	94.5	50.00%	80.60%	0.521	0.815	0.668	0.031
FPG 2	91.5	78.60%	88.40%	0.878	0.942	0.910	0.000
FPG 3	91.5	61.50%	77.80%	0.49	0.82	0.655	0.058
HbA1c 1	5.65	42.90%	76.80%	0.466	0.759	0.613	0.075
HbA1c 2	5.55	38.50%	80.70%	0.474	0.779	0.627	0.119
$HbA1_C$ 3	5.65	50.00%	76.10%	0.483	0.836	0.659	0.085
BBW	4190	21.40%	97.50%	0.440	0.723	0.582	0.295
BHC	35.75	28.60%	77.60%	0.457	0.728	0.592	0.237
BMI	29.7	50.00%	76.70%	0.498	0.78	0.639	0.075
Ferritin µg/L	46.6	20.00%	84.20%	0.167	0.627	0.397	0.431

GDM2-WHO :Gestational Diabetes Millets at second visit diagnosed by WHO; FPG1: Fasting Plasma Glucose at first visit; FPG2: at second visit; FPG3: at post-natal visit; HbA1c1: glycated hemoglobin at first visit; HbA1c2: at second visit; HbA1c3: at postnatal visit; BBW: Baby Birth Weight; HC: Head Circumference; BMI: Body Mass Index; AUC: Area under the ROC curve; CI: confidence intervals.

3.10 Pregnancy Outcomes

The birth weight of 880 babies were obtained from their medical record files with mean value 3220g (760-5200), head circumference mean value was 34.4 cm (21-39), and the mean value for the gestational age was 38.8 weeks (24-42).While cesarean delivery represent 33.6% including (elective cesarean and cesarean section scheduled of a previous cesarean section, malpositioning or malpresentation of fetus) of all cases in the study population, 7.2% suffer from abortion during the study, 0.6% of the delivered babies had perinatal death, and 8.6% of them were considered to have macrosomia (baby birth weight ≥ 4000g), while 3.53% complain from different complications after delivery.

3.11 HbA1c and Pregnancy Outcomes

Table 14 shows a positive correlation between a baby's birth weight and baby's head circumference with HbA1c1 (at first visit) ($r = 0.149$, $p = 0.00$ and $r = 0.093$, $p = 0.008$, respectively).

A positive correlation was found between HbA1c2 (at second visit) with baby birth weight ($r = 0.159$, $p = 0.00$) while no correlation was found between HbA1c2 (at second visit) with a baby's head circumference.

Table 14. Correlations between HbA1c% and Baby Birth Weight & Baby Head Circumference.

Correlation Between	Pearson Correlation r	P value
HbA1c1& BBW	0.149	0.000
HbA1c2 & BBW	0.159	0.000
HbA1c3 & BBW	0.178	0.000
HbA1c1 & BHC	0.093	0.008
HbA1c2 & BHC	0.073	0.084
HbA1c3 & BHC	0.141	0.002

HbA1c1: Glycated hemoglobin at first visit; HbA1c2: Glycated hemoglobin at second visit; HbA1c3: Glycated hemoglobin at postnatal visit; BBW: Baby Birth Weight; BHC: Baby Head circumference.

Receiver operating characteristics (ROC) curve analysis for HbA1c is used to determine if HbA1c could be used to predict adverse pregnancy outcomes compared to OGTT and FPG. Figure 7 shows the sensitivity and specificity of HbA1c1 (at first visit < 24 weeks gestation) in detecting adverse pregnancy outcomes. The area under ROC curve of HbA1c1 to detect adverse pregnancy out comes was 0.589 (95% CI 0.551- 0.627). It was observed that an

HbA1c1 cutoff value was ≥ 5.65% had a sensitivity of 31.1 % and a specificity of 80.2% in detecting adverse pregnancy outcomes, while OGTT1 cutoff value was ≥ 119.5 mg/dl had a sensitivity of 28.4% and a specificity of 79.5%. This mean that HbA1c1 at first visit could predict of the adverse pregnancy outcomes better than OGTT1.

In Figure 8 ROC curve analysis was also used to determine the sensitivity and specificity of FPG1 (at first visit < 24 weeks' gestation) in detecting adverse pregnancy outcomes. The area under ROC curve of FPG was 0.565 (95% CI 0.836-0.923). The optimal cutoff value was ≥ 98.5 mg/dl, with a sensitivity of 16.9% and a specificity 90.7%. However, the area under ROC curve of 2hr OGTT was 0.558 (95% CI 0.501-0.615) and the 2hr OGTT cutoff value was 119.5 mg/dl with a sensitivity of 28.4% and a specificity of 79.5%. The AUC, sensitivities, specificities and cut off values for HbA1c, FPG and OGTT for detecting adverse pregnancy outcomes are shown in Table 15.

Table 15. FPG, 2hr. OGTT, and HbA1c Performance in Detecting Adverse Pregnancy Outcomes.

Adverse pregnancy outcomes				N = 409			
Test	**Cut-off**	**sensitivity**	**specificity**	**95% Confidence Interval**		**AUC**	**P Value**
				lower Bound	**upper Bound**		
FPG 1	98.5	16.90%	90.70%	0.527	0.602	0.565	0.001
FPG 2	104.5	4.60%	96.50%	0.508	0.595	0.551	0.021
FPG 3	93.5	25.20%	83.50%	0.506	0.607	0.556	0.031
2 hr. OGTT1	119.5	28.40%	79.50%	0.501	0.615	0.558	0.046
2 hr. OGTT2	118.5	33.30%	78.20%	0.445	0.63	0.538	0.424
HbA1c 1	5.65%	31.10%	80.20%	0.551	0.627	0.589	0.000
HbA1c 2	5.65%	22.90%	88.20%	0.529	0.626	0.578	0.002
HbA1c 3	5.85%	22.50%	88. 5%	0.541	0.646	0.594	0.001

FPG1: Fasting Plasma Glucose at first visit; FPG2: Fasting Plasma Glucose at second visit; FPG3: Fasting Plasma Glucose at postnatal visit; HbA1c1: glycated hemoglobin at first visit; HbA1c2: glycated hemoglobin at second visit; HbA1c3: glycated hemoglobin at postnatal visit; 2hr.OGTT1: 2 hour Oral Glucose Tolerance Test at first visit; 2hr.OGTT2: 2 hour Oral Glucose Tolerance Test at second visit; AUC: Area under the ROC curve; CI: confidence intervals.

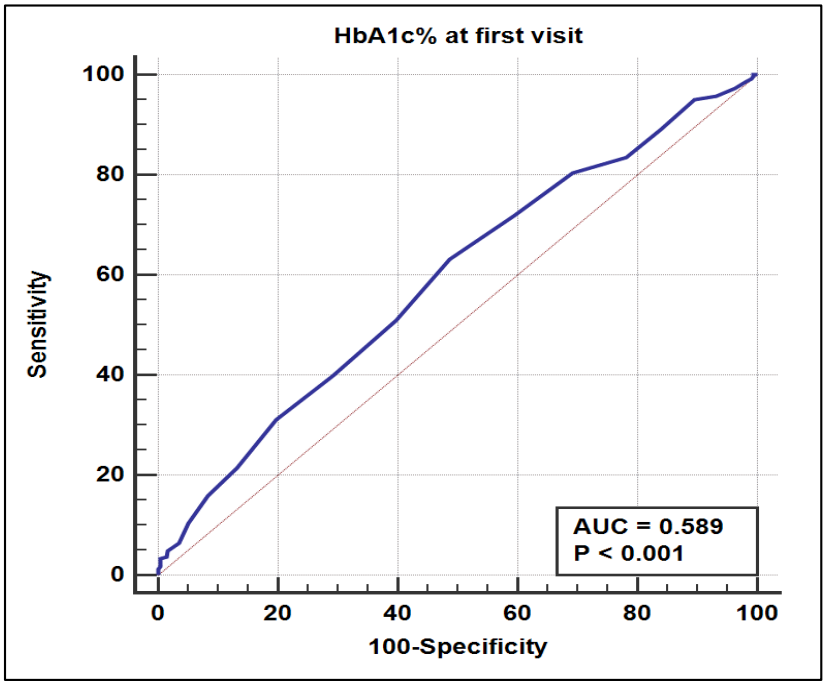

Figure 7. ROC Curve Analysis of HbA1c1 (at First Visit) in Prediction Adverse Pregnancy Outcomes. ROC: Receiver Operating Characteristics; HbA1c1: Glycated Hemoglobin at first visit (< 24 weeks' gestation).

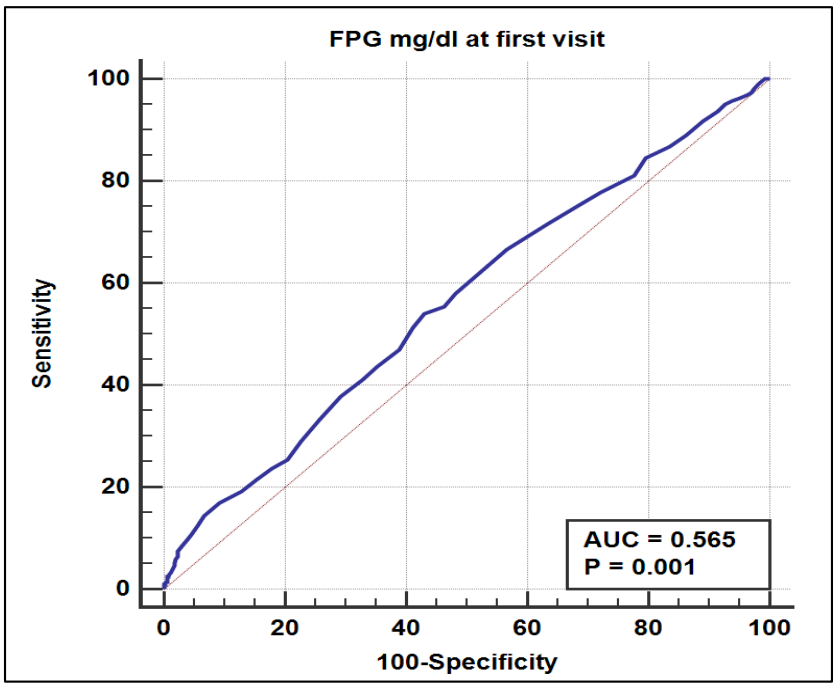

Figure 8. ROC Curve Analysis of FPG1 (at First Visit) in Prediction Adverse Pregnancy Outcomes. ROC: Receiver Operating Characteristics; FPG1: Fasting Plasma Glucose at first visit (< 24 weeks' gestation).

Table 16 showed that there was a statistically significant difference in mean values of HbA1c1 (at first visit) with baby birth weight (P = 0.001). The mean HbA1c1 value was 5.6%. in baby's with weight ≥ 4000g and 5.4% in baby's weight < 4000g. Also, a statistically significant difference was observed between HbA1c1 values in cesarean and normal deliveries at first visit (P = 0.001). However, there was no statistical difference in mean values of HbA1c1 levels (at first visit) between perinatal death and abortion during pregnancy.

Also, HbA1c2 (at second visit) was significantly different in mean value between baby's birth weight ≥ 4000g and <4000g (5.5% vs. 5.3%, p = 0.001, respectively. There was also statistically significant difference between HbA1c2 (at second visit) values in cesarean and normal delivery (5.34% vs. 5.26%, respectively). There was no significant difference in mean value of HbA1c2 (at second visit) between perinatal death and abortion during pregnancy.

Table16. HbA1c1% (at First Visit) and Adverse Pregnancy Outcomes.

Adverse pregnancy outcomes		N	HbA1c1% at first visit		P value
			Mean	SD	
Type of delivery	Cesarean	281	5.5	0.42	0.001
	Normal	546	5.4	0.41	
Perinatal death	Yes	5	5.4	0.24	0.95
	No	822	5.4	0.42	
Abortion during the study	Yes	56	5.5	0.51	0.057
	No	829	5.4	0.41	
BBW	≥ 4000 g	74	5.6	0.53	0.001
	< 4000 g	751	5.4	0.40	

HbA1c1: glycated Hemoglobin at first visit; N: Number; SD: Standard Deviation; BBW: Baby Birth Weight).

In the study subjects, 56.4% (528) of the participants had completed their pregnancy without any complication and 43.6% (409) suffered from cesarean delivery, abortion, perinatal death, and having a macrosomic baby or any other baby's abnormalities, but when the type of delivery was excluded from adverse pregnancy outcomes the percentage was 14.6%.

Table 17 depicted the overall levels of HbA1c, FPG and OGTT test in all adverse pregnancy outcomes. We found a statistically significant difference in mean value of HbA1c1 (at first visit) between women who had pregnancy without any complications and those who had adverse outcomes (P = 0.001). The mean values were 5.34% and 5.49% respectively. As well as at second visit (P = 0.001). Also, there was a statistically significant difference in mean values of FPG1 (FPG measured at first visit) between participants who had normal pregnancy and those who had not (P = 0.000) and the same as for FPG2 and FPG3 (FPG measured at second and postnatal visit). However, OGTT2 (OGTT measured at second visit) didn't show a statistically significant difference.

Table 17. HbA1c, FPG and OGTT Mean Values During Pregnancy and Pregnancy Outcomes.

Pregnancy Outcomes		N	Mean	SD	P value
HbA1c 1%	Normal	487	5.34	0.39	0.001
	Adverse outcomes	379	5.49	0.44	
HbA1c 2%	Normal	330	5.24	0.4	0.001
	Adverse outcomes	225	5.36	0.45	
HbA1c 3%	Normal	260	5.38	0.38	0.000
	Adverse outcomes	200	5.53	0.45	
FPG 1	Normal	517	86.1	9.41	0.000
	Adverse outcomes	402	88.7	10.82	
FPG 2	Normal	426	82.2	10.23	0.021
	Adverse outcomes	282	84.1	10.94	
FPG 3	Normal	279	85.1	10.03	0.022
	Adverse outcomes	222	87.3	11.07	
2hr. 75g OGTT1	Normal	210	101.1	22.81	0.022
	Adverse outcomes	183	107.0	27.5	
2hr. 75g OGTT2	Normal	87	106.5	22.39	0.440
	Adverse outcomes	66	109.3	22.17	

HbA1c1: Glycated hemoglobin at first visit; HbA1c2: Glycated hemoglobin at second visit; HbA1c3: Glycated hemoglobin at postnatal visit; FPG1: Fasting Plasma Glucose at first visit; FPG2: Fasting Plasma Glucose at second visit; FPG3 : Fasting Plasma Glucose at Postnatal visit; OGTT1: Glucose Tolerance Test at first visit; OGTT2: Glucose Tolerance Test at second visit; N: Number; SD: Standard Deviation.

3.12 GDM and Adverse Pregnancy Outcomes

Table 18 represents the mean values of baby birth weight (BBW) in GDM and non GDM women. A statistically significant difference in mean value of baby birth weight between GDM-IADPSG diagnosed and non-diagnosed at first and at second visit. The weight was higher in GDM-WHO women but not statistically significant. There was also no significant difference in head circumference and gestational age at birth between pregnant women who had GDM and those who had not.

Table 18. Mean Values of Baby Birth Weight in Gram in Different GDM Diagnostic Criteria.

BBW (g)		**N**	**Mean**	**SD**	**P. Value**
GDM1-WHO	Yes	28	3399	579	0.064
	No	763	3201	552	
GDM 2-WHO	Yes	14	3426	503	0.167
	No	599	3228	530	
GDM1-IADPSG	Yes	268	3331	517	0.000
	No	584	3163	567	
	DM	11	3473	626	
GDM2-IADPSG	Yes	137	3375	586	0.001
	No	579	3208	513	

GDM1-WHO: GDM diagnosed at first visit by WHO criteria; GDM2-WHO: GDM diagnosed at second visit by WHO criteria; GDM1-IADPSG: GDM diagnosed at first visit by modified IADPSG; GDM2-IADPSG: GDM diagnosed at second visit by modified IADPSG; BBW: Baby Birth Weight; SD: Standard Deviation; N: Number.

A significant relationship existed between adverse pregnancy outcomes and GDM1-WHO (P = 0.004). Pregnant women who suffered from different adverse pregnancy outcomes were more likely to have GDM1-WHO diagnosed before 24 weeks gestation (67.7%) as shown in Table 19, which presents also that 92.5% of non GDM1-WHO (diagnosed at first visit) their baby birth weight was < 4000g, and it is statistically significant (P = 0.007).

In our study population there was no significant relationship between type of delivery, abortion during the study and perinatal death in participants who had GDM1-WHO (at < 24 weeks gestation) and who hadn't.

0ut of 880 babies born, 8.4% were macrosomic, and 0.6% were perinatal death. While 33.6% of the participant had cesarean delivery and 7.2% suffered from abortion.

Table 19. Relation Between GDM1-WHO and Pregnancy Outcomes.

Adverse Pregnancy Outcomes		**GDM1-WHO**		**P. value**
		Yes N (%)	**NO N %**	
Pregnancy Outcomes	Normal	10 (32.3%)	463 (58.6%)	0.004
	Adverse effect	21 (67.7%)	327 (41.4%)	
TD	Normal	15 (53.6%)	510 (66.8%)	0.147
	Cesarean	13 (46.4%)	254 (33.7%)	
BBW	< 4000 g	22 (78.6%)	706 (92.5%)	0.007
	≥ 4000 g	6 (21.4%)	57 (7.5%)	
ADS	Yes	4 (12.9%)	45 (5.6%)	0.087
	No	27 (87.1%)	765 (95.4%)	
PD	Yes	0 (0.0%)	5 (0.7%)	0.668
	No	28 (100%)	759 (99.3%)	

GDM1-WHO: Gestational Diabetes Millets diagnosed at first visit by WHO 1999; TD: type of delivery; ADS: abortion during the study; PD: perinatal death; N: Number; BBW: Baby Birth Weight.

There was a strong evidence of a significant relationship between FPG1 ≥ 94.5 mg/dl and adverse pregnancy outcomes when we excluded cesarean delivery, 87.6% of pregnancies with FPG1 < 94.5 mg/dl had no adverse pregnancy outcomes, (p = 0.001). Likewise, 87.9% of pregnancies with HbA1c1 < 5.75% had no adverse pregnancy outcomes when cesarean delivery excluded too, while 55.8% of those whose HbA1c1 ≥ 5.75% had suffered from one or more adverse pregnancy outcomes including cesarean delivery which was statistically significant (P = 0.001), as shown in Table 20.

Table 20. Relationship Between GDM in Different criteria and Pregnancy Outcomes.

GDM according to different criteria	**GDM 1**			**Adverse Pregnancy outcomes 409 (43.6%)**			**Adverse Pregnancy outcomes (without cesarean) 138 (14.6%)**		
			N	**Yes – N (%)**	**No - N (%)**	**P value**	**Yes – N (%)**	**No - N (%)**	**P value**
WHO 1999	Yes – N (%)	32 (3.8%)	846	21 (67.7%)	10 (32.3%)	0.003	9 (29%)	22 (71%)	0.006
	No – N (%)	814 (96.2)		331 (41.5%)	466 (58.5%)		98 (12.2%)	708 (87.8%)	
IADPSG 2010	Yes – N (%)	302 (32.1%)	940	146 (49.3%)	150 (50.7%)	0.001	57 (19.3%)	238 (80.7%)	0.000
	No – N (%)	627 (66.7%)		245 (40.2%)	365 (59.8%)		72 (11.6%)	547 (88.4%)	
DM1	Yes- N (%)	11 (1.2%)		9 (81.8%)	2 (18.2%)		5 (45.5%)	6 (54.5%)	
HbA1c1 (≥5.75%)	Yes – N (%)	153 (17.1%)	893	82 (55.8%)	65 (44.2%)	0.001	39 (26.2%)	110 (73.8%)	0.000
	No – N (%)	740 (82.9%)		301 (41.5%)	425 (58.5%)		88 (12.1%	642 (87.9%)	
FPG1 (≥94.5 mg/dl)	Yes – N (%)	213 (22.6%)	942	102 (49%)	106 (51%)	0.081	46 (22.2%)	161(77.8 %)	0.001
	No – N (%)	729 (77.4%)		300 (42.2%)	411 (57.8%)		89 (12.4%)	631 (87.6%)	

GDM 1: Gestational Diabetes Millets at first visit; HbA1c: Glyated hemoglobin; FPG: Fasting Plasma Glucose; DM: Diabetes mellitus diagnosed at first visit; WHO: World Health Organization; IADPSG: IADPSG: International Association of Diabetes and Pregnancy Groups.

3.13 GDM and T2DM

Pregnant women who developed T2DM after delivery represent 2.4% (11/458) of the study population before 13 weeks postnatal visit by WHO criteria (FPG ≥ 126, or HbA1c ≥ 6.5%), 66.7% of T2DM were previously diagnosed with GDM-WHO, 6 (54.5%) of T2DM were previously diagnose with GDM-IADPSG, and 2 (18.2%) were diagnosed as having DM at first visit by IADPSG criteria. only 5.1% of the obese participants (6/117) had developed T2DM, 3.5% of participants whose FPG1 was between 85-125 mg/dl had developed T2DM while 50% of them with FPG1 greater than or equal 126 mg/dl had developed T2DM and 13.8% of GDM-WHO cases had developed T2DM. 28.6% of DM1 and 3.0% of GDM-IADPSG developed T2DM.

A significant relationship was seen between women who developed T2DM and gravida, women who had ≥ 6 previous pregnancies were at risk to develop T2DM. Also, older women (31-40) were more likely to develop T2DM.

There was a strong evidence of a relationship between GDM1-WHO diagnosed at first visit and T2DM (P = 0.000), but the correlation was weak (r = 0.164). While 3.3% (15/448) of the study population were at high risk of having or developing T2DM by using the combining FPG ≥ 100 mg/dl and HbA1c ≥ 5.7% [87] . 96.0% of women who didn't develop T2DM were GDM1-WHO free, and 81.8% (9/11) of T2DM had suffered from one of the adverse pregnancy outcomes as shown in Table 21.

Table 21. Association Between T2DM (Type 2 Diabetes Millets Diagnosed Before 13 Weeks at Postnatal Visit) and Risk Factors.

Risk factors (N)		T2DM at PN		P Value
		Yes	No	
		N (%)	N (%)	
Age, years, (458)	≤ 20	0	22 (4.9%)	0.02
	21-30	4 (36.4%)	276 (61.7%)	
	31-40	6 (54.5%)	145 (32.4%)	
	> 40	1 (9.1%)	4 (0.9%)	
BMI, Kg/m^2 (454)	Underweight	0	9 (2.o%)	0.112
	Normal weight	1 (9.1%)	157(35.4%)	
	Over weight	4 (36.4%)	166 (37.5%)	
	Obese	6 (54.5%)	111 (25.1 %)	
Gravida (458)	1	0	63 (14.1%)	0.018
	2-5	5 (45.5%)	292 (65.3%)	
	≥ 6	6 (54.5%)	92 (20.6%)	
Para (458)	0	0	74 (16.6%)	0.141
	≥ 1	11 (100%)	373 (83.4%%)	
Pre-abortion (458)	Yes	7 (63.6%)	165 (36.9%)	0.071
	No	4 (36.4%)	282 (63.1%)	
FH DM (456)	Yes	9 (81.8%)	247 (55.5%)	0.082
	No	2 (18.2%)	198 (44.5%)	
PH GDM (456)	Yes	1 (9.1%)	17 (3.8%)	0.375
	No	10 (90.9%)	428 (96.2%)	
GDM1-WHO (435)	Yes	3 (33.3%)	17 (4%)	0.000
	No	6 (66.7%)	409 (96.0%)	
GDM2-WHO (369)	Yes	1 (33.3%)	9 (2.5%)	0.001
	No	2 (66.7%)	357 (97.5%)	
GDM1-IADPSG (455)	Yes	5 (45.5%)	70 (8.8%)	0.000
	No	4 (36.4%)	277 (62.4%)	
	DM	2 (18.2%)	5 (1.1%)	
GDM2-IADPSG (438)	Yes	6 (60.0%)	92 (21.5%)	0.004
	No	4 (40.0%)	336 (78.5%)	
Adverse pregnancy outcomes (455)	Yes	9 (81.8%)	9 (81.8%)	0.009
	No	2(18.2%)	255 (57.4%)	

T2DM: type 2 diabetes millets; PN: postnatal visit; Underweight: BMI (<18.5); Normal weight: BMI (18.5-24.9); Over weight: BMI (25-29.9); Obese: BMI (≥ 30); DM: Diabetes Mellitus; FH DM: Family history of DM; PH GDM: Personal history of GDM; GDM1-WHO: GDM diagnosed at first visit by WHO criteria; GDM2-WHO: GDM diagnosed at second visit by WHO criteria; GDM1-IADPSG: GDM diagnosed at first visit by modified IADPSG; GDM2-IADPSG: GDM diagnosed at second visit by modified IADPSG; SD: Standard Deviation; N: Number.

3.14 HbA1c and T2DM

There was a statistically significant difference in mean value of HbA1c1 (at first visit) between pregnant women who developed T2DM and those who did not (P = 0.00). The mean value of HbA1c1was 5.87% in women who developed T2DM and 5.44% in women who did not develop T2DM. A significant difference was observed in mean HbA1c2 levels at second visit between pregnant women who developed T2DM and those who did not (P = 0.001). The mean value was 5.8% in women who developed T2DM and 5.31% in women who did not develop T2DM. On the other hand, the mean value of HbA1c3 at postnatal visit was significantly different between GDM and non GDM cases, HbA1c level was higher in GDM pregnant women than in non GDM pregnant women in our study population as shown in Table 22.

Table 22. The Main Values of HbA1c3 at Postnatal Visit in GDM Women Diagnosed by Different Criteria.

GDM1-WHO: GDM diagnosed at first visit by WHO criteria; GDM2-WHO: GDM diagnosed at second visit by WHO criteria; GDM1-IADPSG: GDM diagnosed at first visit by modified IADPSG; GDM2-IADPSG: GDM diagnosed at second visit by modified IADPSG; SD: Standard Deviation; N: Number.

HbA1c3% (at Postnatal Visit)					
GDM in Different Criteria		N	Mean	SD	P. Value
GDM1-WHO	Yes	20	5.95	0.49	0.000
	No	240	5.41	0.39	
GDM2_WHO	Yes	10	5.67	0.49	0.027
	No	364	5.40	0.38	
GDM1- IADPSG	Yes	169	5.47	0.42	0.000
	No	284	5.42	0.40	
	DM	7	6.1	0.61	
GDM2- IADPGS	Yes	98	5.54	0.44	0.002
	No	345	5.40	0.40	

3.15 Hematological Parameters Across Pregnancy

The mean value of Hemoglobin before 24 weeks gestation was 12.1±1.06 g/dl, which decreased to 11.2±1.06 g/dl after 24 weeks gestation, then return to almost the original value after delivery 12.3±1.32 g/dl. The mean value of serum ferritin was 27.5 ± 22.3 µg/L. Hemoglobin concentration at first visit, showed a positive correlation with serum ferritin (r = 0.165; P = 0.00) (Table 4).

3.16 Percentage of Anemia in the Study Populations and its Association with the Risk Factors

The percentage of anemia in the study population was 13.8% of the participants (131/948) according to WHO criteria with hemoglobin value < 11g/dl before 24 weeks gestation. The percentage reached 22.8% of the participants (167/734) by WHO criteria, hemoglobin < 10.5g/dl after 24 weeks gestation. Serum ferritin test was done to 447 participants at first visit, 25.3% have iron deficiency anemia (IDA) as defined by ferritin < 12 µg /L. Our data showed that 93.5% of the participants were committed to take folic acid at first visit, and 97.2% iron supplements at second visit, even though it didn't protect them from developing anemia.

Participants age, BMI, number of previous pregnancies, deliveries and abortions, previously giving birth to a low birth weight baby and previous preterm delivery, iron supplementation were assessed and presented in Table 23. There was a significant relationship between pregnant age and anemia diagnosed before 24 weeks gestation (P = 0.004). Pregnant participants whose age ranged between 21-30 years old represented 50% of the anemic population, while 1.1% of the non-anemic were > 40 years old. A significant relationship between the number of pregnancies (gravida) and anemia diagnosed before 24 weeks gestation (P = 0.020), with a 8.6% of anemia in primigravida (a woman who is pregnant for the first time). A significant relationship was observed between pre-abortion and anemia diagnosed before 24 weeks gestation (P = 0.020). Of the 147 anemic pregnant women, 44.9% previously suffered from abortion. There was no statistically significant relationship between BMI, previous preterm delivery and previously having a low birth weight baby with anemia.

Table 23. Association Between Participants Risk Factors and Anemia.

Risk factors (N)		Anemia According Hb. value		P value
		Anemic Hb < 11 g/dl	Non-anemic Hb ≥ 11 g/dl	
		N %	N%	
Age, years, (943)	≤20	8 (6.2%)	42 (5.2%)	0.004
	21-30	65 (50.0%)	520 (64.0%)	
	31-40	52 (40.0%)	242 (29.8%)	
	> 40	5 (3.8%)	9 (1.1%)	
BMI, kg/m^2, (911)	Underweight	5 (4.0%)	8 (1.0%)	0.07
	Normal weight	44 (35.2%)	274 (34.9%)	
	Overweight	45 (36.0%)	310 (39.4%)	
	Obese	31 (24.8%)	194 (24.7%)	
Gravida (932)	1	11 (8.6%)	134 (16.7%)	0.02
	2-5	83 (64.8%)	518 (64.4%)	
	≥6	34 (26.6%)	152 (18.9%)	
Para (931)	0	17 (13.4%)	150(18.7%)	0.15
	≥1	110 (86.6%)	654 (81.3%)	
Pro-abortion (931)	Yes	57 (44.9%)	275 (34.2%)	0.02
	No	70 (55.1%)	529 (65.8%)	
PDLB (920)	Yes	8 (6.3%)	71 (8.9%)	0.335
	No	118 (93.7%)	723 (91.1%)	
PPD (920)	Yes	3 (2.4%)	43 (5.4%)	0.147
	No	123 (97.6%)	751 (94.6%)	
FAS (920)	Yes	126 (98.4%)	734 (92.7%)	0.014
	No	2 (1.6%)	58 (7.3%)	

BMI: Body Mass Index; Underweight: BMI (<18.5); Normal weight: BMI (18.5-24.9); Over weight: BMI (25-29.9); Obese: BMI (≥ 30); PDLB: Previous delivery of a low weight baby; PPD: Previous preterm delivery; FAS: Folic acid supplementation at first visit; N: number.

3.17 Adverse Pregnancy Outcomes Related to Anemia

Table 24 represents pregnancy outcomes related to anemia. 8.6% of the delivered babies have birth weight ≤ 2500, whereas 17.6% were preterm delivery ≤ 37 weeks gestation. 1.4% of the participants had one of these different complications, such as macrocephaly, microcephaly, postpartum hemorrhage, abnormal baby for termination, deep venous thrombosis, fetal distress and early neonatal death, and 0.6% had perinatal death.

Table 24. Percentage of Adverse Pregnancy Outcomes Related to Anemia

Pregnancy Outcomes		**N (%)**
BBW	> 2500	783 (91.4%)
	≤ 2500	74 (8.6%)
GAB	>37 weeks	704 (82.4%)
	≤37 weeks	150 (17.6%)
Perinatal Death	No	853 (99.4%)
	Yes	5 (0.6%)
Other Complications	No	915 (98.6%)
	Yes	13 (1.4%)

BBW: Baby birth weight; GAB: Gestational age at birth; N: Number.

3.18 Relationship Between Anemia and Adverse Pregnancy Outcomes.

The relationship between anemia diagnosed at first and second visits and adverse pregnancy outcomes such as low birth weight baby and preterm delivery were statistically insignificant. A significant relationship was found between anemia diagnosed at first visit and perinatal death (P = 0.022) as shown in Tables 25. The same was found as the relationship between IDA and baby birth weight and preterm delivery were statistically not significant

Table 25. Relationship Between Baby Birth Weight (BBW), Gestational Age at Birth and Perinatal Death and Anemia Diagnosed at first and Second visit.

Adverse Pregnancy Outcomes		Anemia (Hb1)			Anemia (Hb 2)		
		< 11 g/dl	≥11 g/dl	P Value	< 10.5 g/dl	≥ 10.5 g/dl	P Value
		N (%)	N (%)		N (%)	N (%)	
BBW	>2500	106 (13.6%)	671 (86.4%)	0.376	151(23.1%)	502 (76.9%)	0.503
	≤ 2500	13 (17.8%)	60 (8.2%)		10 (18.5%)	44 (81.5%)	
GAB	≤ 37	27 (18%)	123 (82%)	0.119	32 (25.6%)	93 (74.4%)	0.412
	> 37	91 (13.1%)	606 (86.9%)		129 (22.2%)	452 (77.8%)	
PD	Yes	3 (60%)	2 (40%)	0.022	2 (40%)	3 (60%)	0.32
	No	116 (13.7%)	730 (86.3%)		159 (22.6%)	543 (77.4%)	

BBW: Baby birth weight; GAB: Gestational age at birth; N: Number. Hb: Hemoglobin measured at first visit; Hb2: Hemoglobin measured at second visit; PD: Perinatal Death.

Out of 865 pregnant women, 189 (21.8%) experienced adverse pregnancy outcomes which might be due the high percentage of cesarean delivery (33.6%). In general, there was insignificant relationship between pregnant women with IDA (by serum ferritin measured at first visit < 12 µg /L) and adverse pregnancy outcomes related to anemia (P = 0.271). The same was seen in anemia (Hb2 value at ≥ 24 weeks gestation) (P = 0.390). However, a statistically significant relationship was found between anemia diagnosed at first visit (hemoglobin values at < 24 weeks gestation) and pregnancy adverse outcomes (P = 0.042) as shown in Table 26.

Table 26. Relationship Between Anemia and IDA (Hemoglobin < 11g/dl and Ferritin < 12 µg /L) and Pregnancy Outcomes.

Anemic Parameters (N)		Pregnancy Outcomes			P value
		Adverse Outcomes	Normal	Total	
		N	N	N	
Ferritin µg /L (414)	< 12	27 (25.7%)	78 (74.3%)	105	0.271
	≥12	62 (20.1%)	247 (79.9%)	309	
Hb1 g/dl (858)	<11	35 (29.4%)	84 (70.6%)	119	0.042
	≥11	153 (20.7%)	586 (79.3%)	739	
Hb2 g/dl (713)	<10.5	40 (24.2%)	125 (75.8%)	165	0.390
	≥10.5	115 (21.0%)	433 (79.0%)	548	

Hb1: Hemoglobin measured at first visit; Hb2: Hemoglobin measured at second visit; N: Number.

3.19 Serum Ferritin in Early Pregnancy

The mean value of serum ferritin at first visit in pregnant women who developed GDM1-WHO was 36.8 µg /L, and in GDM1-IADPSG was (28 ± 21.66 µg /L). Those who were diagnosed with DM had ferritin levels of 31.7 µg /L, but wasn't statistically significant as presented in Table 27.

Receiver operating characteristics (ROC) curve analysis for serum ferritin was used to determine if serum ferritin could be used to predict high risk pregnant women to develop GDM1-WHO.The area under ROC curve of ferritin to detect GDM1-WHO was 0.643 (95% CI 0.456 - 0.831) but it was statistically insignificant (P = 0.193). The optimal cutoff value was ≥ 36.43 µg /L, with a sensitivity of 57.1% and a specificity 75.3%. The data were presented in Table 12 previously.

Table 27. Association Between Serum Ferritin in Early Pregnancy and GDM.

Ferritin µg /L		N	Mean	SD	P value
GDM1-WHO	Yes	7	36.8	24.04	0.275
	No	384	27.4	22.64	
GDM2_WHO	Yes	5	19.5	16.31	0.421
	No	291	27.9	23.33	
GDM1-IADPSG	Yes	108	28.0	21.66	0.875
	No	323	27.4	22.56	
	DM	6	31.7	24.32	
GDM2-IADPSG	Yes	5	19.5	16.31	0.275
	No	291	27.9	23.33	

GDM1-WHO: GDM diagnosed at first visit by WHO 1999 criteria; GDM2-WHO: GDM diagnosed at second visit by WHO 1999 criteria; GDM1-IADPSG: GDM diagnosed at first visit by modified IADPSG; GDM2-IADPSG: GDM diagnosed at second visit by modified IADPSG; SD: Standard Deviation; N: Number.

CHAPTER FOUR: DISCUSSION

The ongoing epidemic of obesity and diabetes has led to more type 2 diabetes in women of childbearing age, with an increase in the number of pregnant women with undiagnosed diabetes [64]. It is reasonable to test women with risk factors for GDM at their initial prenatal visit. According to ADA 2016, women with diabetes in their first trimester would be classified as having T2DM. GDM is diabetes diagnosed in the second or third trimester of pregnancy [118]. The percentage of overweight in our study population was 34.9%, and obesity was 24.6%. Accumulated GDM percentage was 7.4% by WHO 1999 criteria and 45.8% by modified IADPSG criteria.

There was a positive correlation between BMI and 2hr 75g OGTT1 measured at first visit, while no correlation was found between BMI and 2hr 75g OGTT2 measured at second visit.

GDM is considered as a risk factor to develop T2DM, Robinson et al. reported that all women with GDM must be screened for T2DM within 1-6 months after delivery [119]. In this study, pregnant women who developed type 2 diabetes mellitus (T2DM) before 13 weeks postnatal visit represented 2.4% (11/458), 33.3% of them were previously diagnosed with GDM1-WHO. These findings were similar to what was reported in a large systematic review involving 20 cohorts from different populations with different ethnic backgrounds [120]. Eades et al. in one region of Scotland found that 25% of women with GDM developed T2DM [121], while in this study 13.8% of women with GDM-WHO, and 3.0% with GDM-IADPSG developed T2DM. The proportion of T2DM in women with GDM2-IADPSG in this study was 60%, which is almost similar value to a study conducted in the Kingdom of Saudi Arabia by Mahzari et al. [122].

We found that the percentage of GDM diagnosed at first visit was higher than at the second visit in different diagnostic criteria, and some of those cases, GDM disappear at the second visit and new cases appear.

According to UNRWA protocols in Palestine followed in perinatal care unit is that any pregnant woman who has FPG $\leq$ 84 mg/dl, OGTT test is not required or obligatory. However, we found that 57.2% (246/430) at first visit and 57.1% (260/455) at second visit of them were overweight and obese, while 67.7% (245/362) of normal results of 2hr OGTT1 was also overweight or obese. This means that FPG in early pregnancy alone was not sufficient to exclude GDM.

In the present study, HbA1c levels in GDM women were higher than that in non GDM women. In this prospective study that included women without pre-existing medical conditions, we systematically examined HbA1c measured across pregnancy starting in the first trimester and its relation with GDM risk factors. Older women, multiparous, multigravida, obese one, those who had a family history of DM, and those who previously had GDM, were more likely to have high HbA1c1 value at the first visit, and it was statistically significant. We examined the distribution of HbA1c levels across pregnancy among women who did or didn't develop GDM. Women without GDM had lower first trimester HbA1c levels than women who developed GDM. HbA1c decreased slightly from the first to the second trimester and then tend to increase in the third trimester. This is intuitive and in line with the high erythrocyte turnover in pregnancy [123], and the decrease in insulin sensitivity with increase gestation [72]. Our findings were similar to other studies, such as Stefani et al, who reported longitudinal data across pregnancy of HbA1c value in each trimester [124].

In this study, the optimal cutoff value of the HbA1c level (with maximal sensitivity and specificity) to predict GDM was 5.75%. The area under the ROC curve of HbA1c level for the detection of GDM was 0.772. However, the sensitivity was 57.5% and the specificity was 85.1%. These findings confirmed the lack of adequate sensitivity and specificity as in other previous studies [125]. Osmundos and colleagues [126], reported that pregnant women with an HbA1c level of 5.7% - 6.4% was associated with low sensitivity (13%) and high specificity (94%) for the prediction of GDM [126]. In a study by Renz and colleagues, GDM was diagnosed by the World Health Organization (WHO) 1999 and the American Diabetes Association (ADA)/WHO 2013 criteria[127], They found that an HbA1c level of $\geq$ 5.8% exhibited 95% specificity but low sensitivity (26%). In Korean women, the HbA1c level at a cutoff point of 5.05% exhibited 91% sensitivity and 62% specificity [128]. In another study conducted by Soumya et al, the OGTT and HbA1c tests were performed in 500 pregnant women at 24-28 weeks of gestation, the cutoff point of the HbA1c level was 5.3%, which had a sensitivity of 95.6% and a low specificity (52%) [129].

Our findings demonstrated that HbA1c levels at different times of visits were associated with various adverse pregnancy outcomes in a continuous fashion. Outcomes like macrosomia and cesarean delivery were associated with HbA1c at first visit, while perinatal death and abortion during the study were not associated with HbA1c1. The results provided supporting evidence for recent reports that HbA1c level during pregnancy was associated

with adverse pregnancy outcomes [35, 62]. Thus, HbA1c level may be used as a prognostic biomarker for adverse pregnancy outcomes. The optimal cutoff value of HbA1c in adverse outcomes prediction with 0.589 AUC was 5.65%, while the sensitivity was low at 31.1% and specificity was moderately high at 80.2%.

In addition, compared with women with HbA1c ≥ 5.75% (the optimal cutoff point), the women with higher HbA1c1 level ≥ 5.75% had a higher risk of adverse pregnancy outcomes and this finding was similar to high risk Taiwanese women [130]. In the present study, it was observed that HbA1c levels in GDM pregnant women were significantly higher than that in normal pregnant women (5.93 ± 0.62% Vs. 5.38 ± 0.38%, p = 0.000), and this was similar to the findings of Balaji. et al. [66]. Pearson Correlation Coefficient showed that there was a positive correlation between FPG1 and HbA1c1 (0.341, p=0.000), this was consistent with the study by Ketema and Kibret [131].

In our study 66.7% of T2DM diagnosed before 13 weeks at postnatal visit were previously diagnosed with GDM-WHO. However the mean values of HbA1c at the first and second visit were significantly higher in those who developed T2DM (5.87% and 5.8%) compared to those who did not develop T2DM (5.44% and 5.31%, respectively), while pregnant women who had GDM had higher HbA1c level at postnatal visit than normal pregnant women, the mean value of HbA1c3 was 5.95% in GDM1-WHO, 5.67% in GDM2-WHO, 5.47% in GDM1-IADPSG and 5.54% in GDM2-IADPSG in agreement line with previous study [132]. As well, 81.8% (9/11) of T2DM in our study suffered from one of the adverse pregnancy outcomes. Postnatal HbA1c ≥ 5.7% is proposed as a cutoff value to identify women at risk of T2DM with previous GDM [133]. An HbA1c test is more feasible than OGTT as HbA1c now is the preferred diagnostic test for diabetes since adherence to a recommendation to use OGTTs postnatal is poor [134]. The concentration of HbA1c depends on glycemia and the life span of erythrocytes [135]. Lower levels of HbA1c in healthy pregnant women compared to non-pregnant women [136] could be explained by an increase in red blood cell turnover in pregnant women [135].

Increase risk of macrosomia in GDM is mainly due to the increased insulin resistance of the mother, higher amount of blood glucose passes through the placenta into the fetal circulation, which trigger fetal pancreas to make extra insulin that can cause the baby to grow too large, and also extra glucose in the fetus is stored as body fat causing macrosomia [137]. The percentage of macrosomia in our study was 8.4%, while the prevalence was 4.5% in Al Qassim Region of Suadi Arabia [138]. Higher prevalence was 20% in Nordic countries [139]. Macrosomia represent an obstetric challenge, and elective cesarean

delivery is the best option. 33.6% of the participant had cesarean delivery in this study, but this percentage not only due to macrosomia.

Gestational Diabetes Mellitus is associated with a variety of fetal effects including increase rate of spontaneous abortion, intrauterine fetal death, congenital anomalies, neurodevelopmental problems [140]. A significant relationship was found between pre-abortion and GDM cases and HbA1c in the present study. Greene et al. reported that the risk for spontaneous abortion was 12.45% with HbA1c lesser than or equal 9.3% [141].

Anemia is one of the most common hematological disorder encountered during pregnancy, the most frequent reason for anemia during pregnancy is iron deficiency. The Percentage of anemia in this study population was 13.8% of the participants diagnosed at first visit (<24 weeks gestation) according to WHO criteria with hemoglobin value < 11g/dl. This result was consistent with a study done in Nakhonsawan, Thailand 2010 [142], which reported 14.1%. While the percentage of anemia in this study was low value compared to the prevalence of anemia from Sudan, 2009 [143] and Uganda, 2013 [144] which reported 70% and 63.1% prevalence respectively. In 2018, records indicate that the percentage of anemia among registered pregnant women screened at the MOH PHC centers was 27.5%.

Anemia in pregnancy is related to different socio-demographic factors [145]. This study assessed socio-demographic variables associated with anemia. Group age of 21-30 years, multigravida and pre-abortion showed statistically significant association with anemia. This indicated a high percentage of anemia in women of reproductive age and a large number of children ever born. This finding was consistent with Bharati et al [146], and Suryanarayana, et al [147]. On the other hand Singh et al observed an insignificant association between anemia and gravida [148].

Anemia increased with the duration of pregnancy reaching 22.8% (at second visit) which is similar to Suryanarayana, et al [147]. Anemia seems to be higher among women with multiparity (86.6%) in the present study, but it was statistically insignificant. In contrast to a study conducted by Obse et al. in Ethiopia parity > 5 has a significant association with anemia [149].

A study in Southern Ethiopia demonstrated that undernourished pregnant women who had low BMI < 18.5 kg/m^2 were more likely to have anemia [150]. While in this study population the percentage of undernourished who were anemic (BMI < 18.5 kg/m^2) was 4.0%, which was statistically insignificant with anemia.

In the present study the percentage of iron deficiency anemia (IDA) was 25.3% (113/447) as defined by serum ferritin < 12 µg /L, which is the same the prevalence found in other

developing countries (25-35%) [151, 152], similar to the results from Southern Iran [153], where the prevalence of IDA was 28.5%. While the prevalence from industrial countries was (5-8%) [151].

A negative association between anemia and duration of gestation and low birth weight has been reported in the majority of studies according to a comparative study conducted by Allen et al [154], and also showed, similar to our findings, insignificant relationship between IDA and preterm delivery and low birth weight. Several potential biological mechanisms were identified through which anemia or iron deficiency could affect pregnancy outcome. Anemia (by causing hypoxia) and iron deficiency (by increasing serum norepinephrine concentrations) can induce maternal and fetal stress, which stimulates the synthesis of corticotropin-releasing hormone (CRH). Elevated CRH concentrations are a major risk factor for preterm labor, pregnancy-induced hypertension and eclampsia, and premature rupture of the membranes. CRH also increases fetal cortisol production, and cortisol may inhibit longitudinal growth of the fetus. An alternative mechanism could be that iron deficiency increases oxidative damage to erythrocytes and the fetoplacental unit. Iron deficiency may also increase the risk of maternal infections, which can stimulate the production of CRH and are a major risk factor for preterm delivery [154].

Results from some studies showed association between maternal IDA in early pregnancy and a great risk of preterm delivery [103, 155] in contrast to our study.

Maternal anemia is considered a risk factor for poor pregnancy outcomes, and it threatens the life of the fetus. In the present study about, about 21.8% of the study sample suffered from adverse pregnancy outcomes such as preterm delivery and low birth weight baby, 29.4% were anemic pregnant diagnosed at first visit which was statistically significant, similar to a study conducted by Nair et al. [156]. Even though 25.7% of them had IDA (Iron Deficiency Anemia) but the relationship wasn't significant.

Serum ferritin level was higher (30 µg /L) in GDM in comparison with the normal group, but it was not significant, this is in contrast to the finding of Soheilykhan et al. [157]. These authers reported that a high level of serum ferritin in early pregnancy was significantly associated with GDM. The limitation of this study was measuring ferritin once during pregnancy and did not assess the effect of the inflammatory marker of GDM.

Ferritin is a marker utilized to assess total body iron stores and it is also an acute phase reactant. The hormone hepcidin is the body's main regulator of systemic iron homeostasis,

and it is secreted in response to iron loading and inflammation[158]. Hepcidin has been associated with inflammatory states in humans [158]. The role of hepcidin in pregnancy is poorly understood, and only two studies have examined the association between GDM and hepcidin, both reporting increased hepcidin levels in GDM [159].

CHAPTER FIVE: CONCLUSION

HbA1c level in early pregnancy is increased in GDM. This may help in measuring glycemic control in women with GDM. It does not replace OGTT which is still considered the gold standard for diagnosing GDM. However, including HbA1c as part of the diagnostic criteria for diabetes during pregnancy in 2010 by IADPSG was important.

HbA1c level was associated with various adverse pregnancy outcomes in high risk women. However, it lacks adequate sensitivity and specificity to replace OGTT test to diagnose GDM. The current study was a prospective study. Additional randomized control design studies are required. However, OGTT was not a good predictor of adverse pregnancy outcomes and measuring HbA1c may help since HbA1c levels showed a significant relationship with adverse pregnancy outcomes but not all outcomes.

High percentage of women with elevated postnatal HbA1c, suggest that a substantial proportion of women had a high risk of future T2DM. More research is needed before general recommendation to replace the postnatal OGTT with the use of HbA1c $\geq$ 5.7% to identify women at high risk for T2DM.

The overall percentage of anemia in this study indicated that it is a moderate public health problem. Gravida status and para were important risk factors contributing to anemia in pregnant women, therefore increase awareness for family planning methods might have a contribution to reduce the risk of anemia.

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Appendix

1- ANTE-NATAL QUESTIONNAIRE

PERSONAL INFORMATION **Serial No._**

Contact number: ---------- NAME: ----------
-

1. Age :---------- years
2. Gravid (number of pregnancy: ----------
3. WEIGHT: _____ kg
4. HEIGHT: _____ cm

RISK ASSESSMENT

A. Factors related to past history
   5. Number of abortion
   6. Multiparity
      a. 1
      b. 2
      c. 3
      d. 4
      e. 5
   7. Family history of diabetes mellitus
      a. Yes
      b. No
   8. If yes,
      a. Father
      b. Mother
      c. Brother
      d. Sister
   9. Personal history of GDM
      a. Yes
      b. No
   10. Previous delivery of a big baby (> 4000gm).
      a. Yes
      b. No
   11. Previous delivery of a low birth weight baby (<2500gm).
      a. Yes
      b. No
   12. Previous preterm delivery
      a. Yes
      b. No

B. Factors related to present pregnancy

13. Gestational age (weeks). First visit ----------
14. Gestational age (weeks). Second visit ----------
15. Postnatal visit (weeks): ----------

16. Blood pressure: first visit: ----------
17. Blood pressure: second visit: ----------
18. Blood pressure: Postnatal visit: ----------

19. Protein in urine: first visit:
    a. Negative b. positive.
20. Protein in urine: second visit:
    a. Negative b. positive
21. Protein in urine: Postnatal visit:
    a. Negative b. positive
22. Sugar in urine first visit:
    a. Negative b. positive.
23. Sugar in urine second visit:
    a. Negative b. positive.
24. Sugar in urine Postnatal visit:
    a. Negative b. positive.

25. Fasting plasma glucose (mg/dL) – first visit ----------.
26. Fasting plasma glucose (mg/dL) – second visit ----------.
27. Fasting plasma glucose (mg/dL) – Postnatal visit ----------.

28. Hemoglobin (Hb) g/dl. First visit: ----------
29. Hemoglobin (Hb) g/dl. Second visit: ----------
30. Hemoglobin (Hb) g/dl. Pst partum: ----------

31. Hematocrit (Hct) % . First visit: ----------
32. Hematocrit (Hct) % . second visit: ----------
33. Hematocrit (Hct) % . postnatal visit: ----------
34. Mean corpuscular volume (MCV)fL – First visit: ----------
    ----
35. Mean corpuscular volume (MCV)fL – second visit: ----------
    -----
36. Mean corpuscular volume (MCV)fL – postnatal visit: ----------
    -
37. HbA1c % first visit : ----------
38. HbA1c % second visit : ----------
39. HbA1c % postnatal visit : ----------

40. Ferritin ng/mL: first visit : ----------
    -

41. 75g OGTT – 2 hr (mg/dL) first visit : ----------
    ----
42. 75g OGTT – 2 hr (mg/dL) second visit : ----------
    ----
43. 75g OGTT – 2 hr (mg/dL) postnatal visit : ----------
    -

44. Maternal weight (Kg): first visit: ----------
    ----
45. Maternal weight (Kg): second visit: ----------
    -----
46. Maternal weight (Kg): postnatal visit: ----------
    -

47. Iron supplementation: first visit:
    a. Yes
    b. No
48. Iron supplementation: second visit:

a. Yes
b. No

49. Iron supplementation: postnatal visit:
    a. Yes
    b. No

CHILD INFORMATION

Gender of the Child.	
Weight at Birth (gm).	
Head Circumference	
Gestational Age at Birth (Weeks).	
Type of Delivery (Vaginal /Cesarean).	
Perinatal Death	

2- Consent form

إقرار بالموافقة على عمل بحث
دور السكر التراكمي في تشخيص سكري الحمل والمشاكل الناتجة عنه

تهدف هذه الدراسة إلى قياس مستوى السكر التراكمي مقارنة مع مستوى السكر و OGTT في تشخيص سكري الحمل والمشاكل الناتجة للطفل والأم.

إن قياس سكر الدم بالطرق المختلفة يمكن أن يعطي تشخيصاً أفضل لسكري الحمل مقارنة مع الطرق المستخدمة حالياً (OGTT) ويمكن أن يساعد في التنبؤ في حصول المشاكل الناتجة عن الحمل للأم والطفل.

البحث لا يشكل أي خطورة على المريض وكل ما يتطلبه البحث هو استخدام جزء من عينة الدم التي سيتم سحبها للمريض لقياس نسبة السكر التراكمي بالإضافة إلى الفحوصات التي يتطلبها فحص الحامل وهذا يتطلب زيادة بسيطة في كمية الدم التي سيتم سحبها.

النتائج من هذه الفحوصات سوف يتم تزويد وكالة الغوث بها وهذه النتائج تعامل بخصوصية كاملة من قبل وكالة الغوث والجامعة ويتم المحافظة على سرية أسماء المرضى.

وعليه فإنني أوافق على قيام باحثين من جامعة القدس بالتعاون مع وكالة الغوث بعمل تحاليل طبية على جزء من عينة الدم التي سيتم سحبها ولا مانع لدي أن يتم عمل هذه الفحوصات في جامعة القدس بغرض الأبحاث.

_____	_____	_____
الاسم	التوقيع	التاريخ

3- Ethical approval

Al-Quds University
Jerusalem
Deanship of Scientific Research

بسم الله الرحمن الرحيم

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: December 10, 2016
Ref No: 5/REC/2016

Dear Dr. Akram Kharroubi,

Thank you for submitting your application for research ethics approval. After reviewing your application entitled **"Role of Hemoglobin A1c and other Risk Factors in Diagnosing and Predicting the Adverse Pregnancy Outcomes of Gestational Diabetes Mellitus"** the Research Ethics Committee (REC) confirms that it is in accordance with the research ethics guidelines at Al-Quds University.

Please inform us if there will be any changes in your research methodology, subjects, plan and we would appreciate receiving a copy of your final research report.

Thank you again and wish you productive research that serves the best interest of your subjects.

Dina M. Bitar PhD
Chairwoman of the Research Ethics Committee

cc. Prof. Imad Abu Kishek,- President of AQU
cc. Members of the committee
cc. file

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