ASSESSMENT OF THE UTILITY OF HbA1C COMPARED TO ORAL GLUCOSE TOLERANCE TEST IN DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS ATKENYATTA NATIONAL HOSPITAL

PRINCIPAL INVESTIGATOR:

MRS. DEBORAH MAIKO MOGI

BACHELOR OF SCIENCE IN MEDICAL LABORATORY SCIENCES

DEPARTMENT OF HUMAN PATHOLOGY

SCHOOL OFMEDICINE, COLLEGE OF HEALTH SCIENCES

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STUDENT DECLARATION

I, Mrs. Deborah Maiko Mogi declare that this research is my original work under the guidance of my supervisors and has not been submitted to the University of Nairobi or any institution for an award.

Signature: _____ Date: 24/11/2021

Mrs.Deborah M. Mogi,Bsc. Medical Laboratory Sciences (Mount Kenya University),

Master of Science, Clinical Chemistry

Department of human Pathology, School of Medicine,

College of Health Sciences, University of Nairobi,

P.O.Box 19676 -00202,

Nairobi, Kenya.

O723833714

Deborah.mogi@uobi.ac.ke

SUPERVISORS' DECLARATION

This dissertation has been submitted with our approval as university supervisors

Prof. Christine Sekadde Kigondu,

Professor, Thematic Unit of Clinical Chemistry

Department of Human Pathology, College of Health Sciences

University of Nairobi,

Signature…… Date…24/11/2021…

Dr. George Wandolo Odero

Lecturer, Department of Human Pathology

University of Nairobi,

Signature…… Date…24/11/2021…

DEDICATION

This research work is dedicated to my parents, the late Bishop Angaya Wemali and Mrs. Beatrice Angaya, who taught me to live by Faith in God and sacrificed for my education. To my dear husband Andrea M. Mogi, my children, Amos, Caleb and Naomi for giving me humble time during my studies and supporting in many different ways.

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LIST OF ABBREVIATIONS

ACOG- American College of Obstetrics and Gynecology

ADA-American Diabetes Association

ANC-Ant- Natal Clinic

DCCT-Diabetes Control and Complication trial

DGGT- Decreased Gestational Glucose Tolerance

DIPSI-Diabetes In Pregnancy Study India

DM- Diabetes Mellitus

GDM-Gestational Diabetes Mellitus

HAPO- Hyperglycemia and Adverse Pregnancy Outcome

Hb- Hemoglobin

HbAlc-Glycated hemoglobin AI Content

IADPSPG- International Association of Diabetes in Pregnancy Study Group

KNH Kenyatta National Hospital

KNH/UON/ERC-Kenyatta National Hospital/ University Of Nairobi/ Ethical Research Committee

MBG- Mean Blood Glucose

MPG-Mean Plasma Glucose

NGSP-National Glycohemoglobin Standardization Program

OGTT- Oral Glucose Tolerance Test

RDS- Respiratory Distress syndrome

ROC- Receiver Operating Curve

SENS-Sensitivity

SPEC- Specificity

SPSS- Statistical Package for Social Sciences

WHO- World Health Organization

PPV- Positive Predictive Value

NPV-Negative Predictive Value

AUC- Area Under the Curve

TPR-True Positive Rate

FPR-False Positive Rate

TNR- True Negative Rate

FNR- False Negative Rate

LR- Likelyhood Ratio

HbA1c- Glycated Haemoglobin

ABSTRACT

Background information: GDM stands for gestational diabetes mellitus, which is diabetes diagnosed during the second and third trimesters of pregnancy and is not obviously type 1 or type 2 diabetes prior to pregnancy. During pregnancy and after birth, it has negative consequences for both the mother and the fetus. The Oral Glucose Tolerance Test is the gold standard test for diagnosing GDM but is time consuming and expensive. It involves fasting, ingestion of a high concentrated glucose solution and multiple invasive sample collection. Glycosylated hemoglobin (HbA1c) test is a single, simple, random, non- fasting, noninvasive, time saving and economical test that can be performed instead of OGTT, however the cut off value for pregnant mothers has not been established.

Objectives: To determine the utility of glycosylated hemoglobin compared to oral glucose tolerance tests in diagnosis of GDM (OGTT, IADPSG).

Materials and Methods: Descriptive cross-sectional study was employed among pregnant women (139) at 24-28 weeks gestational period, receiving ante-natal care services at Kenyatta National Hospital. Oral Glucose Tolerance Test (IADPSG) and HbA1c tests (Clover A1c^{TM} analyzer system) were performed. The Receiver Operating Characteristic (ROC) curve was prepared. The area under the curve (AUC) was calculated. The performance of HbA1c for detection of GDM were evaluated against the gold standard (OGTT).

Results: Between March and July 2020, 139 participants were enrolled. Gestational age was 24 – 28 weeks. The prevalence of GDM was as follows:- 72(51.8%) using OGTT (IADPSG criteria), 9 (6.47%) using HbA1c cut off of $\geq$ 6.5%, 113(81.29%) using HbA1c cut off of $\geq$4.95% and 101 (77.8%) using HbA1c cut off of 5.1%.The HbA1c cut off of $\geq$6.5% had low sensitivity of 11.2% while the cut-off of $\geq$4.95% had a high sensitivity of 88.9%. Both had low PPV (88.9%, 56.6%) and NPV (51.5%, 69.2%) respectively. ROC generated an AUC of 0.598 and cutoff of $\geq$4.95%. There was a significant difference between the two variables with p= 0.003 which is less than 0.05.There was an agreement between results generated by IADPSG and WHO 2013, OGTT criteria.

Conclusion: It was found out that HbA1c cannot be used as a diagnostic tool for GDM because the cut off value of $\geq$ 6.5% had low sensitivity while the cut off value of $\geq$4.95% generated by the ROC is too low and is within the reference range for pregnant women.

CHAPTER 1

1.1 INTRODUCTION

Diabetes that is diagnosed in the second and third trimesters of pregnancy and is not definitely type 1 or type 2 diabetes prior to pregnancy is characterized as gestational diabetes mellitus(1)(2).It is one of the Glucose metabolism disorders classified by American Diabetes Association(2) as Diabetes Mellitus type I, type II, Gestational Diabetes Mellitus and diabetes due to other causes (2). Diagnosis of GDM is important because it can avert or reduce incidences of complications associated with it. Macrosomia, neonatal hyperglycemia, hyperbilirubinemia, shoulder dystocia (trauma), respiratory distress syndrome, obesity, diabetes, and mortality are among the baby's complications due to GDM (4). The complications in the mother include Cesarean section delivery, ill health in pregnancy and birth, miscarriage, hydramniosis, hypertensive disorders, development of permanent type II diabetes and death (4). Perinatal mortality increases in untreated GDM (5). GDM is the cause of 90% of cases of DM in pregnancy (1). Fetal and neonatal morbidity caused by GDM are preventable if diagnosed early and prompt, effective treatment therapies are used.GDM can be managed with diet and exercise. To attain normal blood glucose levels, insulin and other hypoglycemic medications are utilized. (1) The gold standard test for diagnosing GDM is standard WHO OGTT. (6)There are various OGTT criteria worldwide (3). OGTT is restricted to high risk individuals because of its disadvantages of being cumbersome due to fasting overnight, time consuming, heavy labor, poor reproducibility and costly. Pregnant women do not tolerate it and it also involves multiple pricking since sampling is done at different stages. It involves ingestion of glucose which causes nausea and vomiting. (7) Glycosylated hemoglobin is used as a diagnostic tool for Type I and II DM (3). It has the advantage of having less biological variations, high reproducibility, no need of fasting, non-invasive, uncomplicated, better sample stability and less time consuming. Because of its limited sensitivity and specificity, it has not been approved as a GDM diagnostic tool. (7). If used for diagnosis, a number of patients will be left out in diagnosis of GDM. This has led to recommendation by a number of investigators for further investigations which may lead to optimization of the test prior to its application (8).

HbA1c level of $\geq$ 6.5% which is the recommended cut off value for diabetes Type I and type II is based on data from non- pregnant women. The cut off value in pregnancy is lower

because HbA1c levels fall in the first trimester (9). A study by Hughes found a cut off value of 5.9% as best in detecting gestational diabetes Mellitus with a sensitivity of 100% and specificity of 98.4% for early GDM. It ad PPV of 52.9% and a NPV of 92.8% (5). It was pointed out that a HbA1c result of >6.5% would rule out around half of all pregnant women with GDM. This study was done on low-risk Caucasian population; therefore more studies need to be done to find out how the HbA1c threshold performs in other populations. Since Indian population was diverse and variable, using it to judge international values may not be conclusive, and further comparative studies should be done using different diagnostic criteria (9). (3) According to a study looking for the optimal HbA1c cutoff value to use as a screening tool in Iranian women with gestational diabetes mellitus, pregnant women with HbA1c of more > 5.05 % should take the OGTT but suggested that more studies be done to find out the diagnostic and screening value of HbA1c. It was also recommended that there is need of developing more effective and simpler strategies of universal screening of GDM by which performing OGTT can be avoided(2). Another study showed that if the cut off mark is lowered to 5.3%, In around half of the women now advised for diagnosis by oral glucose tolerance test, HbA1C can be utilized in screening to avoid OGTT, meaning that a HbA1c cutoff of > 6.5 percent or more would miss nearly half of the women with gestational diabetes. (9). Correlation between HbA1c and OGTT (IADPSG, OGTT criteria), provided the lowest HbA1c value that gave positive results with OGTT.HbA1C cut off point with best sensitivity and specificity for pregnant women in KNH was established leading to utilization of HbA1c test as a diagnostic tool.

CHAPTER 2

2.1 LITERATURE REVIEW.

2.2 Physiology of normal pregnancy.

Pregnancy is a physiological state of insulin resistance that causes beta-cell stress. Insulin resistance emerges in the 2nd trimester and continues throughout to 3rd trimester, leading to hyperglycemia in the mothers,(11). Normally, insulin resistance causes increase of insulin secretion and euglycemia. Demands of the developing fetus and increase in transplacental nutrition transfer leads to lower levels of glucose in pregnant mothers compared to healthy non-pregnant women.(11)

2.3 Gestational Diabetes MellitusGDM leads to adverse maternal, perinatal outcome and future diabetes (1). It also affects their children and therefore there are two generations at risk. (1) GDM is linked to several negative maternal outcomes, including high blood pressure, metabolic abnormalities preeclampsia, urinary tract infection, hydramniosis, increased instances of operation and future diabetes mellitus. Fetal and neonates outcomes include macrosomia, congenital anomalies, Respiratory distress syndrome (RDS) and obesity and death in childhood and adolescent age (12). Screening, diagnosing earlier and promptly treating hyperglycemia in pregnancy can prevent the complications.GDM has a lot of controversy in its screening, diagnosis and cost- effectiveness (12).

2.3.1 Pathogenesis of gestational diabetes mellitus. GDM is a hyperglycemic metabolic condition that is discovered for the first-time during pregnancy. It is generally regarded as pre-type II diabetes (11). Many mothers with GDM have metabolic syndrome and are obese. Increased waist body mass index, hypertension, abnormally elevated cholesterol, polycystic ovary syndrome, advanced age, history of diabetes in the family and ethnic status are risk factors. (11) Nutritional stress caused by maternal under-nutrition and over-nutrition or persistent maternal hyperglycemia change offspring metabolism.GDM comes in when there is impaired insulin secretion in the pancreas leading to inability to control the glucose levels, leading to hyperglycemia (11). Human placental lactogen (HPL) produced in the placenta causes insulin resistance in pregnancy by rendering the body less sensitive to insulin.

2.3.2 Epidemiology and Prevalence of GDM Prevalence of GDM worldwide as per WHO 2014 (13) was 8.5% and 7.1% in African region, 422 and 25 million respectively. Global prevalence by ADA (14) was 9.2%. (4) In a study by Djelmis et al (15), Prevalence estimate according to two diagnostic criteria, IADPSG and NICE criteria at Croatia between 2012-2014 was, IADPSG criteria: 17.8% and NICE criteria: 23.1%. In Morocco as per Macaulay et al (16) was 7.7% while in Nigeria by Anzaku(17) was 8.3%.In Kenya, prevalence of gestational diabetes was shown to be 2.9% in a research done in Moi Teaching and referral hospital (IADPSG criteria) (18). Prevalence of GDM in Kenya as per Nyakundi et al(19) in Kenyatta National Hospital was 8.9% by WHO 1999 criteria (20) and 23% (40)

2.4 Diagnosis of GDM (Different types of OGTT procedures and the controversies).

2.4.1 World Health Organization procedure for GDM (ADA, 2014).

This procedure is performed by loading the patient with 75g glucose in 250ml of water after fasting overnight for 8-12 hours. Glucose concentration is determined, at fasting, one hour and after 2 hrs. In the WHO 2013 diagnostic criteria, if one or more of the following anomalies are found: fasting glucose >5.1 Mmol/L, 1 hour plasma glucose > 10.0 Mmol/L, 2-hour glucose > 8.5 Mmol/L, GDM is diagnosed. (21).

2.4.2. International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria.

There is overnight fast, glucose ingestion of 75 g in water and GDM labeled positive if any one of the following cut-off point is encountered. Fasting, or 1-hour,or 2-hour blood glucose concentrations are≥ 5.1mmol/l,≥10 Mmol/l),≥ 8.5 Mmol/l),respectively.(20) One disadvantage of IADPSG criteria is that it is based on one value of glucose above the cut off and also uses a low fasting blood glucose cut off . This leads to increased number of false positive GDM cases compared to Carpenter and Coustan method which requires two or more glucose values above the cut off for diagnosis. (3)

2.4.3 O'Sullivan test

(23) It is one step non-fasting challenge test performed by loading the subject with (50g glucose in 250ml) and blood glucose levels determined after one hour. A concentration of above 7.2Mmol/L is considered GDM positive. Normal women with adequate and brisk insulin response maintain euglycaemic state even after ingestion of glucose while those with

GDM will develop hyperglycemia with glucose challenge because of impairment in secretion of insulin or because of presence of Human placental lactogen (HPL).(5),(24)

2.4.4 The American College of Obstetricians and Gynecologist (ACOG, 2014)

It is a non-fasting, two-step method that begins with a 50-gram glucose load and ends with a blood glucose concentration of >7.8mmol/l, which indicates GDM leading to Step 2, a 3-hour, 100-gram load OGTT for confirmation of the above diagnosis. Fasting, Serum glucose levels of (5.3 - 5.8 mmol/l), (10.0 - 10.6 mmol/I), (8.6 - 9.2 mmol/I), and (7.8 - 8.0 Mmol/l) were measured after one hour, two hours, and three hours, respectively. It has the disadvantages of taking too long, requiring two separate trips, and necessitating numerous readings. (25).

2.4.5 DIPSI (Diabetes in Pregnancy Study Group India).

It is a one step screening procedure, non-fasting, acceptable and simple (2).It is performed by loading with 75g glucose in 250ml of water and blood glucose concentration determined after 2 hrs(2) glucose concentration ≥ 7.8Mmol/l is taken as feasible GDM and a value of ≥ 11.0 Mmol/L as DM and 6.7- 7.7Mmol/L as decreased gestational glucose tolerance (DGGT) (3) (24)

Table 1. Different criteria for diagnosis of GDM with their respective glucose cut offs.

Guidelines	Fast. PG (mmol/l)	Glucose Challenge	1hr (mmol/l)	2-hr PG (mmol/l)	3-hr PG (mmol/l)
WHO, 1999[1]	≥7.0	75g	7.8	N/A	N/A
ACOG [2] (Bener et al., 2011)	≥5.3	100g	≥ 10.0	≥ 8.6	≥ 7.8
Canadian Diabetes Association[3] (Bener et al., 2011)	≥ (5.3)	75 g	≥(10.6)	≥(8.9)	N/A
IADPSG[4] (Benhalima et al., 2012)	≥5.1	75g	≥10.0	≥ 8.5	N/A
DIPSI[5]Benhalima et al., 2012)	N/A	75g	N/A	≥7.8	N/A
	5.1 -6.9	75g	≥10.0	8.5 -11.0	N/A
O' Sullivan[7] (Molina et al., 2018)	_	50g	≥7.2	_	_

Table 1 shows different criteria for diagnosis of GDM with their respective glucose cut offs.

1. 1 value sufficient for diagnosis,
2. 2 or more values
3. 2 or more values,
4. 1value used for diagnosis
5. 1 value
6. 1 or more values
7. 1 value is sufficient for diagnosis

All these criteria and guidelines prove that a universal cost-effective screening and diagnostic method is needed, to provide correct diagnosis and prompt treatment (12). This may help to prevent negative maternal perinatal outcomes and the eventual development of diabetes in both the mother and the baby. A simple one-step noninvasive test can be used to accomplish this.

2.5 CORRELATION BETWEEN HbA1C AND OGTT GDM is a serious condition that posses risk to expectant women and new born. The diagnostic test is oral glucose tolerance test. (3)(22) In these studies, HbA1C and OGTT findings were compared to see if the HbA1C test might be used as a diagnostic tool comparing with the gold standard method, OGTT (22)In a study of four hundred and eighty pregnant women it was found(12) out that, the percentage of those with GDM by using OGTT criteria was (11.9%). A sensitivity of 61 % and specificity of 68 % with a negative predictive value of 93 % was found when using a HbA1c cut-off value of 5.1 %, compared to a sensitivity of 27 % and specificity of 95 % with an NPV of 91 % when using a HbA1c cut-off value of 5.4 %, with the conclusion that pregnant women with HbA1c of > 5.4 % should undertake an oral glucose tolerance test. (12)and this significantly reduced the testing burden on both mothers, staff and resources**.** It was recommended that more investigations be performed before integrating and optimizing the HbA1c is a test done without fasting, single, not invasive screening test for GDM (6). (26)Another study showed that the upper limit of HbA1c in early pregnancy is significantly below levels found in non- pregnant women (5.7%) and in late pregnancy (5.6%). (6)In a study of 246 non-diabetic pregnant women with normal Hb levels, discovered HbA1c reference intervals specific to trimesters: The first, and third trimester ranges were 4.8–5.5%, 4.4–5.4% and 4.4–5.4% respectively. It was concluded that HbA1c can be useful in diagnosing and confirming GDM. (11). (9) Hughes et al studied more than 16,100 pregnant women, the mean HbA1c was 5.3%. Thirty-three women (0.2%) had HbA1clevels of $\geq$6.5%.From 2001 to 2012, various research utilizing various diagnostic criteria for GDM and different HbA1c cut-off values advocated HbA1c testing with conventional tests concurrently(11). Researchers from New Zealand, New York found out HbA1c level $\geq$5.9 percent as optimal value for detection of GDM. (3).Women with HbA1c $\geq$5.9% had more than a twice increased risk adverse outcome, above three-times increased risk of fetus r neonate death; and above 15-times increased risk of giving birth before term,

compared with women who had HbA1c below 5.9%. It was recommended that other researchers are needed to confirm this results in different ethnic groups (9). In a study of Nordic Caucasian women, Odsaeter et al. (2016) found that using HbA1c for GDM screening reduces the frequency of OGTT tests (6). The study aimed to find out the utility of HbA1c for diagnosing GDM in India, Rajput concluded that pregnant mothers with an HbA1c concentration of 5.45% - 5.95%, undergo oral glucose tolerance test to ascertain the positivity of the test results. 85.7 percent of the cases were detected and only 2.8 percent was false positive. This obviated an OGTT in 61.8% (two thirds) of those with GDM (6). Renz et al, in their study made a conclusion that a threshold of 5.8 percent can help to avoid the unpleasant and labor demanding oral glucose tolerance test in one-third of cases (27).
It is increasingly needful to find out a universally simpler, acceptable, and accessible test. (9) HbA1c is the accepted measure of glycemic status in women without pregnancy. The mean plasma glucose over a period of 120 days is correlated with glycosylation. This is a single test that does not involve fasting. It provides a reflection of blood glucose concentration within a period of one hundred and twenty days. (28) It has less pre-analytical variations (6). When compared to fasting blood glucose and two-hour postprandial glucose, HbA1c exhibits less intra-individual variance. Fasting, diurnal variation, meals, acute stress, or variety of common medication agencies which affect metabolism of glucose, do not affect HbA1c (29).

2.5.1 Present and future utility of HbA1c as a diagnostic tool for GDM

Rajput et al (6) concluded HbA1c cannot be used to diagnose GDM instead of an oral glucose tolerance test at the moment, but they can be combined to spare a large number of pregnant women from OGTT and its disadvantages(30). Various studies from different regions suggest that population-based values for HbA1c should be established instead of using a single value from a single population for ruling out GDM.(6)

Table 2. Studies on with utility of glycated hemoglobin in diagnosing GDM

Authors	Cut off value	Sensitivity	Specificity	PPV	NPV
Rajput et al.,2012, ADA	a) 5.95% b) 5.45%	28.6% 85.7%	97.2% 61.1%	- -	- -
Soumya et al.,2015, WHO	a) 5.3% b) 5.7% c) 6.15	95.6% 73.7% 46.7%	51.6% 75% 95%	16% 21.5% 47.6%	99% 92.2% 94.6%
Ryu et al., 2015, ADA	a) 5.25% b) 5.35% c) 5.55%	95.6% 87.2% 50.5%	51.6% 70.9%% 90.2%	43.6% 58.3% 70.5%	93.6% 92.2% 79.6%
Hughes et al., 2014	5.9%	100%	98.4%	-	-
Kwon et al., 2015	a) 5.05% b) 5.25% c) 5.55%	91.3% 73.6% 78.6%	62% 77.2% 72.5%	- - -	- - -
Khalafallah et al.,2015 & 2016	a) 5.1% b) 5.4%	61% 27%	68% 95%	- -	- -

Table 2 shows a number of studies on utility of glycated hemoglobin in diagnosing GDM. The lowest cut off value has been 5.05%

STUDY JUSTIFICATION:

GDM can result in major difficulties for the mother, fetus, and newborns, as well as long-term issues such as type 2 diabetes and mortality. The gold standard test for diagnosing GDM is the OGTT, yet there are several diverse techniques that lead to disagreements. OGTT is time consuming and costly and involves ingestion of glucose, fasting and invasive sample collection which is cumbersome for both mother and health workers. Because of these reasons, the test has been left for high risk mothers only, leading to delays in early diagnosis, treatment and appropriate follow up of mothers with GDM. Therefore cheap, non invasive, time saving diagnostic tools that do not interfere with patients' routine are needed. The existence of a single, non-fasting, cost effective test that could accurately identify GDM will be helpful in providing timely onset of appropriate management. HbA1C presents as such a test. Together with IADPSG OGTT can be used as exploratory tests for GDM. This may reveal HbA1c sensitivity, specificity and cut off value that may allow its use for diagnosis. Utilization of HbA1c may avoid the need for performing an OGTT.

HbA1c test is a one step glucose value test. It has least disturbance to patients' routine activities. Adverse maternal and perinatal outcomes, as well as future diabetes in both mother and child, can be avoided if GDM is successfully tested, diagnosed. This study will provide information which will improve our understanding of GDM and apply early diagnosis, prompt follow-up and cheap methods of management of GDM, thus reducing economic burden on the individuals and the nation. It will encourage universal screening for GDM as opposed to high risk screening which is practiced in this set up.

This will enhance the achievement of the beyond zero campaign from maternal death spearheaded by the first lady of Kenya, Her Excellency Mrs. Margaret Kenyatta.

RESEARCH QUESTION:

1. What is the utility of HbA1c compared to oral glucose tolerance test in diagnosis of gestational diabetes mellitus?

2.8 BROAD OBJECTIVE:

To determine the utility of HbA1C compared to OGTT in diagnosis of GDM at KNH.

2.8.1 Specific objectives:

1. To determine OGTT results (IADPSG procedure) in all the participants.
2. To determine HbA1c levels in all the participants.
3. To correlate the HbA1C results with OGTT results in the participants.
4. To determine the HbA1c cut off value for diagnosis of GDM.
5. To determine sensitivity and specificity of HbA1C for diagnosis of GDM.
6. To compare IADPSG criteria results with WHO 2013criteria results.

CHAPTER 3

3.1 STUDY DESIGN, METHODS AND MATERIALS.

3.2 STUDY DESIGN:

Descriptive-cross-sectional study was employed.

3.3 STUDY SITE

The research was conducted at Kenyatta National Hospital which is located approximately 3.7 kilometers from Nairobi Central Business District. It is the largest teaching and referral hospital in Kenya and attends to 70,000 inpatients and about 550,000 outpatients annually with a bed capacity of 2000 beds. It serves patients from all over the country and parts of East and Central Africa. It is a training and research centre for different cadres of healthcare professionals including pharmacists, medical doctors, dentists, medical laboratory technologists, nurses and many others. It has different specialized clinics including the antenatal clinic, No.18 which serves 80 - 100 mothers per day from Monday to Thursday in the morning hours. The clinic is run by highly skilled physicians and other medical professionals ie nurses, Medical doctors, Clinical officers and laboratory technologists. It offers preventive, curative, and diagnostic health services. Being the largest referral hospital and serving patients from across the country makes it a suitable study site because the results can be generalized to all pregnant mothers. Pregnant mothers are scheduled to have four visits to the clinic before delivery.

3.4 STUDY POPULATION

Pregnant mothers over the age of 18 who sought antenatal treatment at Kenyatta National Hospital between 24 and 28 weeks of pregnancy were included in the study. In this study all participants underwent OGTT and HbA1c testing. One hundred and thirty nine participants were involved. They were aged between 20 and 49years. One hundred and twenty seven

were married. Eighteen had attained primary education, forty one had secondary education and eighty had tertiary education. Occupationally, three were students, forty two were unemployed, fifty nine were self- employed and thirty five were employed.

3.5 INCLUSION CRITERIA

1. Pregnant mothers attending KNH antenatal clinic at 24 – 28 weeks gestational period.
2. Pregnant mothers who gave written informed consent.
3. Pregnant mothers with normal Hemoglobin levels (10 - 14 g/dl (found in the file)

3.5.1 EXCLUSION CRITERIA

4. Pregnant mothers with18 years and above.

1. Known diabetic pregnant women were excluded
2. Pregnant mothers with Hemoglobin levels below 10g/dl and above 14g/dl.

3.6 SAMPLE SIZE DETERMINATION

Fischer's formula was used to calculate sample size(31) with a GDM prevalence of 8.9% (19)

$$N = \frac{Z^2 P (1-P)}{e^2}$$

n = Desired sample size

$Z^2 = (1.96)^2$ for 95% confidence interval (Area under normal curve)

P = prevalence of GDM of 8.9% (19)

e^2 = Maximum tolerance error for the prevalence estimate (0.05)

$$N = \frac{1.96 \times 1.96 \times 0.089 \times 0.911}{0.05 \times 0.05} = \frac{3.8416 \times 0.089 \times 0.911}{0.0025} = \frac{0.311473086}{0.0025} = 124.5892346$$

Sample size: 125 participants plus ten percent. One hundred and thirty nine pregnant mothers participated.

3.7 SAMPLING METHOD

Recruitment was done using consecutive sampling of every eligible pregnant woman. The

files for those who went through the procedure were identified using a sticker to avoid contamination by reparticipation. Sampling continued until desired sample size was achieved. Those who refused to sign informed consent were excluded.

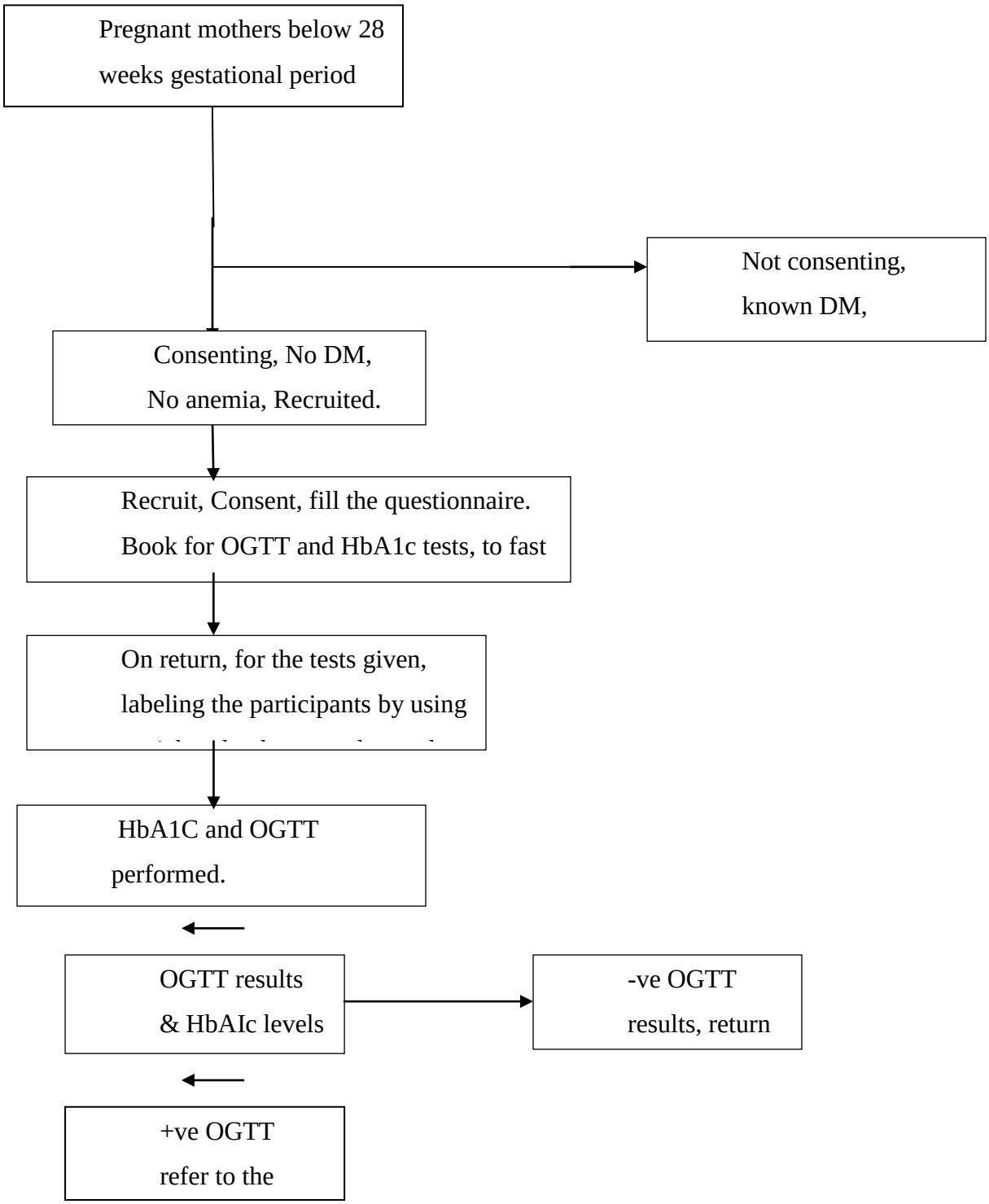
3.8 CASE DEFINITION

Gestational Diabetes Mellitus was defined as per standard (IADPSG) OGTT if fasting,1 hour and 2 hour glucose levels were $\geq$5.1,$\geq$10.0 and $\geq$8.5Mmol/L, respectively.

3.9 RECRUITMENT OF STUDY PARTICIPANTS AND CONSENTING PROCEDURE

The Principal investigator (PI), talked to all the mothers attending the clinic on the particular day about the study in general. A research assistant 1, (A KRCHN nurse who works in the ANC clinic was chosen, talked to and trained by the PI), chose the files for mothers who were at 24 - 28 weeks gestational period and non diabetic. The nurse took their clinic attendance cards and presented them to the PI, who explained all about the study and screened them using a pro forma (Appendix 1) to select those suitable for participating. They were booked for the test on their convenient day on which they came after fasting overnight. The PI consented them, signed the consent form,(Appendix 4) and assisted them to fill out the questionnaire (Appendix 2) for demographic characteristics. The principal investigator performed the tests (OGTT and HbA1c) at Ante- natal clinic clinic No.18). Results were recorded and interpreted. A copy of the results was given to the participant to present to the doctor who used it and filed. Those who were positive for OGTT were immediately presented to the nutritionist on duty by the PI. The nutritionist started the interventions immediately. Those who were negative were given a copy of the results for filing.

Figure 1: WORK FLOW DIAGRAM FOR RECRUITMENT OF STUDY PARTICIPANTS



3.10. DATA COLLECTION

3.10.1 Clinical methods

A trained research assistant (Nurse) and the principal investigator (PI) employed a screening form to collect medical information and determine study eligibility, as well as a study questionnaire to collect demographic information from the recruited individuals.

3.10.2 Laboratory methods

3.10.3 Sample collection and Test procedure for OGTT.

The procedure was explained to the participants in the laboratory by the PI.

(The glucose was weighed and kept in small bags ready for use).The PI confirmed that the participant had fasted. Fasting blood glucose level was obtained by sterilizing the finger using methylated spirit, pricking firmly using a lancet to obtain a free flow of blood. The first drop of blood was wiped out, the subsequent drop was touched by the glucose strip inserted into the glucometer sampling area and the glucose concentration determined. Those participants with fasting blood glucose below 7.0Mmol/l proceeded on. The participant ingested a glucose solution (75g in 250 ml of water) within five minutes. The participant reported immediately they finished drinking and timing begun and recorded on the report form. The participant was informed of the exact time to come back for another glucose testing. Blood glucose concentrations were determined after 1 hour and after 2 hours using a glucose meter.

3.10.4 Quality assurance for OGTT

Pre analytical

The equipment (Glucometer) was brought to the right temperature before use. A firm prick was performed to obtain enough and free flow of blood to avoid errors due to insufficiency of blood.

Analytical

The manufacturer's guidelines for glucose analysis were followed..The glucose meter was calibrated by the manufacturer. Failed internal adjustment would have lead to an error message appearing on screen. The results of the participants were frequently compared with the results from the laboratory in antenatal clinic

Post analytical

Transcriptional errors were ruled out by counterchecking the results from the glucometer memory by the principal investigator and documenting the results in a note book and on the laboratory report form.

3.10.5 Sample collection and handling for HbA1c test

No special diet and no fasting was required. Five microlitres of whole blood or (one large drop) was obtained from the prick mentioned above and used for HbA1c determination and results were seen on screen after five minutes using a reagent that had been stored at room temperature. This was performed at the antenatal clinic. HbA1c tests were performed by the laboratory technologist (PI) using Clover AIc Self analyzer.

3.10.6 Principle of the test

The CLOVER A1cTM self system is a completely automated boronate affinity test that uses a fluorescent immune chromatography analysis method to determine HbA1c.. Test results were displayed as %A1C (A1C/total Hb x 100).

3.10.7 Quality assurance procedures for HbA1c

Pre-analytical:

Measures were taken to ensure that adequate samples are collected. All tests were carried out by a trained laboratory technologist immediately.

1. The professional Procedure Guide was read carefully to ensure proper test performance.
2. Reagent and Monitor were brought to the room temperature for 1 hour.
3. If the foil pouch was damaged reagent was not used.

Analytical: The manufacturers' guidelines (SOPs) for HbAlc analysis were followed. We checked that the analyzer's optical and operating systems were in good working order. Every morning, the daily checks were carried out. The Clover AIc Analyzer had been calibrated. During the first power-up and each assay, the equipment adjusts itself automatically. An error appears on LCD if Analyzer does not make appropriate internal adjustment. The Analyzer usesan NGSP certified method and standardized to DCCT assay.

Post-Analytical:

For recording and statistical analysis, all results were entered into a pre-designed data

sheet.The used cartridges and cotton wool soiled with blood were disposed off according to the standard laboratory bio-safety guidelines. Transcriptional errors were avoided by counterchecking results from the Clover AIc Self analyzer to ensure correctness of the records, by the principal technologist and recorded in a book.

3.11 ETHICAL CONSIDERATIONS The Kenyatta National Hospital/University of Nairobi Ethics and Research Committee granted permission to perform this study. (KNH/UON ERC) and from KNH research unit. Permission was sought for from the department of Obstetrics and Gynaecology. Following permission, all respondents were told of the study's goal and protocol before it began. All responders were given the assurance that their responses would be kept private. The individuals' complete names were not included in the questionnaires since they were coded. The people that were recruited gave their consent. They were told that they would not be victimized or face any consequences if they did not participate or withdraw from the study.

3.12 DATA MANAGEMENT AND ANALYTICAL ANALYSIS

The statistical analysis was carried out in accordance with the study's objectives. Data was collected, tabulated, validated, cleaned, placed into a data entry sheet, and statistically evaluated using suitable tests. Data entered onto the computer was password locked for privacy reasons. The prevalence of GDM was calculated. The association between HbA1c and OGTT findings was explored using Spearman's correlation. The prevalence of GDM, as determined by a positive standard IADPSG test, was assessed and reported as a percentage with a 95% confidence level.HbA1c results were determined in all the participants. The HbA1c results were correlated with OGTT results using the two by two tables. The software Statistical package for social sciences, version 20.0, was used to conduct statistical analyses. At various cutoff settings, the performance characteristics of HbA1c were calculated..We calculated sensitivity, specificity, predictive values, false positives, and false negatives. The prevalence of GDM was computed at various HbA1c cutoff values, and the results for IADPSG criteria were compared to the results for WHO, 2013.The significance level for all statistical tests was set at 5%. Tables, graphs, and pie charts were used to summarize the data. The optimal HbA1c cutoff values were determined using receiver operating characteristic curves.

CHAPTER 4

RESULTS

The primary objective of this study was to determine the utility of HbA1c compared to OGTT in diagnosis of GDM in pregnant mothers in Kenyatta National Hospital (KNH). The study period was from March 2020 to July 2020. In this study, 345 pregnant women were recruited and given appointments .One hundred and thirty nine participants were booked and came back for the test while 206 never came back for the test. One hundred of the participants had different experiences like vomiting, nausea, dryness of the mouth (Thirst), very hungry, sleepy, sweating, general tiredness, discomfort and struggled to finish drinking the highly concentrated glucose solution.

4.1 Socio-demographic characteristics of the 139 study participants

Table 3 shows the demographic characteristics of the study participants as follows: One hundred and thirty nine pregnant mothers at the Kenyatta National Hospital qualified for the analysis. The ranged of age was 20 to 44 years with a mean age of 31.62yrs. Fifty four participants (38.8%) of the cohort ranged between 20 to 29 years, 54% (n=75) between 30 and 39, and 7.2% (n=10) between 40 to 49 years. The study showed that 91.4% (n=127) of the participants were married while 8.6% (n=12) were single. While evaluating the education level of the cohort, it was established that 57.6% (n=80) were educated beyond secondary education, 29.5% (n=41) up to secondary level while the remaining 12.9% (n=18) were up to primary school. On occupation, majority of the respondents were self-employed; covering a 42.4% (n=59) followed by Unemployed 30.2% (n=42), then employed 25.2% (n=35), and lastly students covering 2.2% (n=3).

Table 3. Demographic characteristic and obstetric history of 139 study participants.

Variables	n= 139	%
A. Obstetric history& gestational Period (Weeks) (Mean)		
≤ 26	58	41.7
>26	81	58.3
Social democraphic characteristics(Mean)		
Age (Years)		
20-29 (24.5)	54	38.8
30-39 (34.5)	75	54.0
40-49(44.5)	10	7.2
Marital status and occupation		
Married	127	91.4
Single	12	8.6
Education		
Tertiary	80	57.6
Secondary	41	29.5
Primary	18	12.9
Employed	35	25.2
Self-employed	59	42.4
Unemployed	42	30.2
Student	3	2.2

Table 3 shows demographic characteristic of 139 study participants.

The study population was, pregnant mothers, above18 years who sought antenatal care at Kenyatta National Hospital at 24 to 28 weeks gestational period. In this study all participants underwent OGTT and HbA1c testing. One hundred and thirty nine (139) participants were involved. They were aged between 20 and 49 years. Those who were married were 127(91.4%) while 12(8.6%) were single. For the educational status, 18(12.9%) had attained primary education, 41(29.5%) had secondary education and 80(57.6%) had tertiary education. Occupationally,3(2.2%) were students, forty two were unemployed, 59(42.4%) were self- employed and 35(25.2%) were employed.

Table 4: Results for OGTT test on pregnant mothers at KNH to determine GDM (n=139)

Glucose level	Negative	Positive
Fasting glucose (≥5.1 mmo1/L)	84 (60.4%)	55 (39.6%)
1 hour (≥10.0 mmo1/L)	111 (79.9%)	28 (20.1%)
2 hours (≥8.5 mmo1/L)	106 (76.3%)	33 (23.7%)
At least one OGTT glucose level(Fasting, 1 hour or 2 hours)	67 (48.2%)	72 (51.8)

Table 4 shows OGTT test results for pregnant mothers at KNH, determine GDM (n=139)

4.2 OGTT test on pregnant mothers at KNH using fasting, one hr and two hr blood glucose test to determine the GDM. From table 4, it is clear that 39.6% of the cohort tested positive on fasting glucose level, 20.1% on one hr and 23.7% on two hr time. Based on one or two or three of the three blood glucose levels (fasting, 1hour and 2 hour) 51.8% (n=72) had their values equal or above the cutoff of 5.1mmol/l at fasting, 10mmol/l at one hour or 8.5mmol/l at two hours. Prevalence of GDM was found out to be 51.8%.

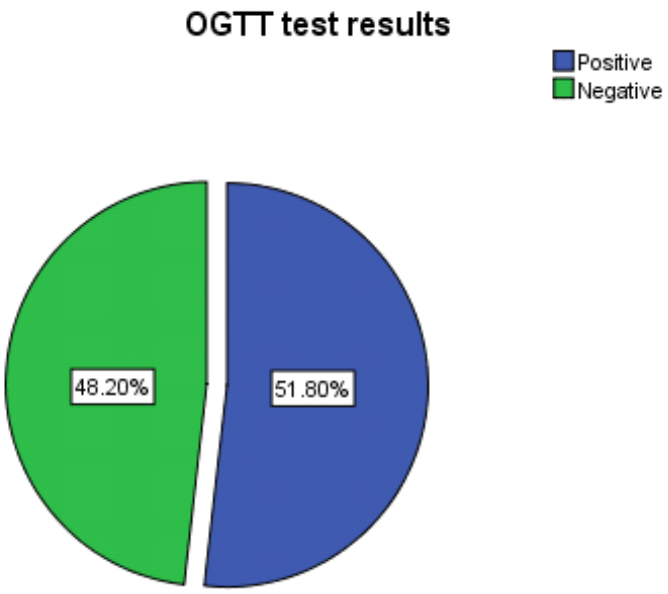


Figure 2. Shows results of GDM in 139 participants in KNH using OGTT (IADPSG)

4.3 Prevalence of GDM in 139 participants in KNH using OGTT (IADPSG)

Figure 2 shows that 51.8% (*n*=72) were positive and 48.2% (*n=67*) were negative respectively. This was calculated by counting all participants that had at least one, two or three of their results at fasting, 1hour or 2hour being above their respective cut off values

4.4 Results for GDM in 139 participants in KNH using HbA1c cut-off of $\geq$ 6.5%

Examination of (GDM) using the cut of $\geq$6.5%, the study established that 93.53% (*n*=130) had HbA1c within the reference range and 6.47% (*n*=9) had diabetes as shown on figure 2. Of 6.47% positive with GDM, 22.2% aged 20-29 years while the remaining 77.78% aged (30-39) years. Of 93.53% negative tests with GDM, 40% aged(20-29) years, 52.3% aged (30-39) years and 7.7% aged (40-49) years.

Figure 3. Results for GDM in 139 participants in KNH using HbA1c cut off of>6.5%

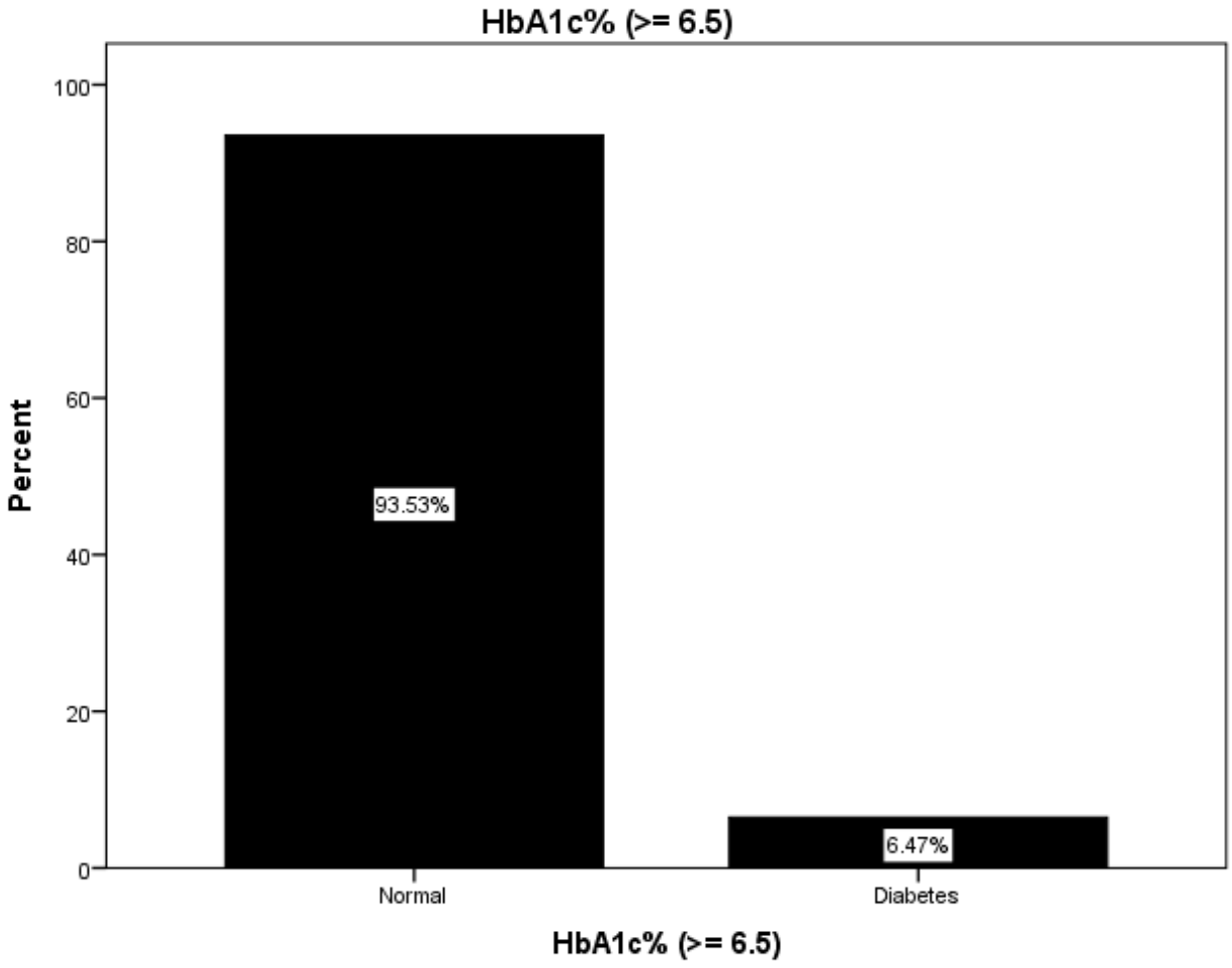


Figure 3 shows Results for GDM in 139 participants in KNH using HbA1c cut off of≥6.5%

Table 5: The relationship between HbA1c% (≥6.5) and OGTT prevalence

HbA1C % (≥6.5) and OGTT results Cross-tabulation				
		OGTT results		Total
		Positive	Negative	
HBA1C % (≥6.5)	Positive	8	1	9
	Negative	63	67	130
Total		71	68	139

Table 5 shows the relationship between HbA1c% (≥6.5) and OGTT prevalence

4.5 The relationship between HbA1c% (>6.5) and OGTT prevalence

Table 4 shows that out of 139 samples, 8 (5.8%) were both positive in OGTT and HbA1c, 63 (45.3%) were negative on HbA1c and positive on OGTT, 67 (48.2%) were negative on both tests while 1 (0.7%) of the tests was positive on HbA1c and negative on OGTT. Upon testing whether there was a relationship between the HbA1c and OGTT results, it was established that there was a significant difference between the two variables with p= 0.003 which is less than 0.05. On the other hand, Spearman's correlation inferred that there was a weak positive correlation between HbA1c and OGTT which was statistically significant with r_s=0.254and p = 0.003.

4.6 HbA1c test performance in GDM diagnosis at cut off value of ≥6.5%

These are calculated from table 4

Sensitivity = 11.2%

Specificity = 98.5%

True positive rate (TPR) = (8/ (8+63)) x 10 = 8/71 x100 =11.2%

True negative rate (TNR) = (67/ (67+1)) x100 = 67/68 x 100 =98.5%

False negative rate (FNR) = (63/ (63+8)) x 100 = 63/71 x 100 = 88.7%

False positive rate (FPR) = (1/ (1+67)) x 100 = 1/68 x 100 = 1.5%

Positive predictive value (PPV) = (8/ (8+1)) x 100 = 8/9 x 100 =88.9%

Negative predictive value (NPV) = (67/ (67+63)) x 100 = 67/130 x 100 = 51.5%

Youden Index = sensitivity + specificity – 1 = 0.112+ 0.985 – 1 = 1.097-1 = 0.097

Receiver Operating Characteristic (ROC) curve

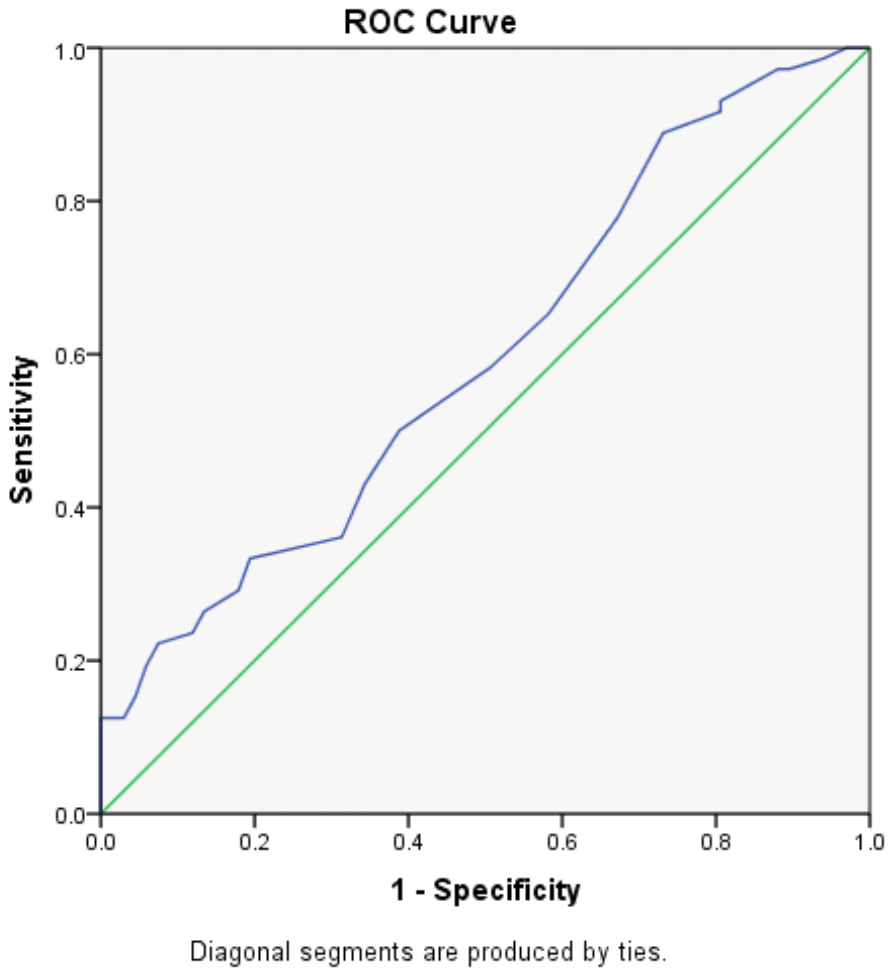


Figure 4: (ROC) curve using ≥ 6.5% cut off value

The ROC curve generated provided an area under the curve (AUC) of 0.598. The best cut off value of HbA1c as generated by the curve was 4.95% with a high sensitivity of 88.91% and low specificity of 26.9% (TNR). (1-specificity is 73.1 %).

Table 6 shows Area Under the Curve using ≥ 6.5% cut off value

Test Result Variable(s): HBA1C %				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.598	.048	.047	.504	.691

Table 6 shows Area Under the Curve using ≥ 6.5% cut off value

4.7 Area under the Curve (AUC) using ≥ 4.95%cut off value

The adjusted ROC test results revealed that there was no statistical difference between the two tests with AUC adjusted: 0.421, 95% CI (0.326-0.517), $p = 0.109$. Using HbA1c test cut off value of ≥ 4.95, the true positive value is 64 (46.04%) and true negative is 18 (12.95%).

Figure 5. Prevalence of GDM using HbA1c (> 4 .95%)

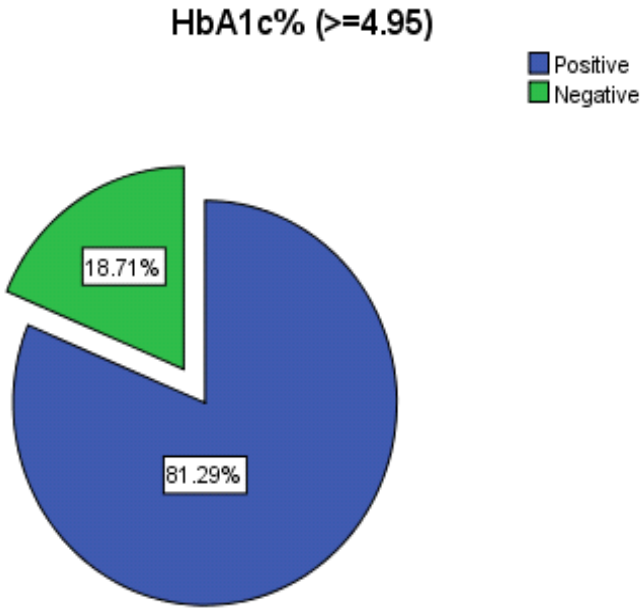


Figure 5: HbA1c% (using ≥ 4.95) test results

4.7.1. HbA1c% (> 4.95) test results

Figure 5 shows that 81.29% (*n*=113) were GDM positive while 18.71% (*n*=26) were negative.

Table 7 Relationship between HbA1c (>4.95%) and OGTT results Cross –tabulation (IADPSG)

Count

		OGTT results		Total
		Positive	Negative	
HbA1c% (≥4.95)	Positive	64	49	113
	Negative	8	18	26
Total		72	67	139

Explanation of table 7

Use of the 4.95% cut off value labeled forty nine participants as positive while they did not have the disease. (False Positive).Sixty four out of seventy two were true positive, only eight were labeled negative when they are supposed to be positive (False Negative).Eighteen were true negative.

4.7.2 HbA1c performance characteristics at cut off value of > 4.95%

Sensitivity = 88.9%

Specificity = 1-0.731 = 26.9%

TPR = 88.9%

TNR = 26.9%

FNR = 11.1%

FPR = 73.1%

PPV = 56.6%

NPV = 69.2%

		OGTT results		Total
		Positive	Negative	
HbA1c (>=5.1%)	Positive	56	45	101
	Negative	16	22	38
Total		72	67	139

Explanation:

The results for GDM using an HbA1c cut off value of ≥5.1% revealed fifty six (TP),sixteen (FN), Forty five (FP) and twenty two (TN). In comparison to the best cut off value in this study, the TP has reduced while the TN has increased and FP rate has decreased.

4.7.3 Performance characteristics of HbA1c using cut off value of ≥5.1%

Sensitivity = 77.8%

Specificity = 32.8%

TPR =77.8%

TNR=32.8%

FNR =22.5%

FPR = 67.2%

PPV =55.4%

NPV = 57.9%

.

Table 9 Comparison of sensitivity and specificity of HbA1c across different cut offs

Cut off value	Sensitivity	Specificity	PPV	NPV
≥ 6.5%	11.2%	98.5%	88.9%	51.5%
≥4.95%	88.9%	26.8%	56.6%	69.2%
≥5.1%	77.8%	32.8%	55.4%	57.9%

Table 9 shows HbA1c sens. and spec. of HbA1c across different cut off values. As the HbA1c cut off value increases, the sensitivity decreases and specificity increases

4.8 Comparison of sens. and spec. of HbA1c at different cut off values

Reduction of the cut off value from ≥ 6.5% to 4.95% increased the sensitivity by eight fold from11.2% to 88.9% while reducing specificity from 98.5% to 26.8% which is four fold too. Similar results were found out in a study by a study by Rajput et al (2012).

Table 10 Comparison of prevalence between OGTT and HbA1c at different cut off values in this study (IADPSG criteria)

Test	Positive/ Prevalence No. (%)	Negative No. (%)	Total No. (%)
IADPSG (OGTT)	72 (51.8)	67 (48.2)	139 (100)
WHO (OGTT)	64 (46.0 %)	67(48.2)	131 (100)
HbA1c (≥ 6.5%)	9 (6.47)	130 (93.53)	139 (100)
HbA1c (> 4.95%)	113 (81.5)	26 (18.7)	139 (100)
HbA1c (5.1%)	101 (77.8)	38 (32.8)	139 (100)

4.9 Comparison of prevalence between OGTT and HbA1c at different cut off values.

OGTT produced a prevalence of 51.8 %. HbA1c (≥ 6.5%) produced a lower prevalence of 6.47%.

HbA1c (≥ 4.95%) produced a high prevalence of 81.3%. HbA1c (≥ 5.1% had a high prevalence of 77.8%. In general, a good new test should agree with the gold standard in order for it to be accepted for use in place of the gold standard. With a cut off of 6.5%, only 9 (8 TPR and 1 FPR) were detected as GDM positive. 64 participants (88.9%) would have been left out. This is true with findings of Dr. Hughes who pointed out that a cut off of> 6.5% will leave more than 50% of the participants suffering from GDM undetected. Hughes et al (2016)(9)

4.10 Interpretation of results using WHO 2013 criteria

In WHO 2013 criteria, GDM is diagnosed if one or more of the following criteria are met:
WHO 2013

Fasting glucose level is 5.1 – 6.9 mmol/l

1-hour glucose level is $\geq$ 10 mmol/l following a 75g oral glucose load.

2-hour glucose level is 8.5 -11.0 mmol/l following a 75g oral glucose load.

Diabetes mellitus in pregnancy is diagnosed if one or more of the following criteria are met:

WHO 2013

Fasting glucose level $\geq$ 7.0 mmol/L

2- Hour glucose level $\geq$ 11.1 mmol/L following a 75 g oral glucose load.

Random blood glucose $\geq$ 11.1 mmol/L in the presence of diabetes symptoms.

Table 11 OGTT test on pregnant mothers at KNH using(determine the GDM) (WHO 2013 criteria).

(n=139)

Glucose level	Negative	Positive	Diabetes mellitus
Fasting glucose (>5.1 - <6.9mmo1/L)	84 (60.4%)	52 (37.4%)	3 (2.2%)
1 hour (>10.0 mmo1/L)	111 (79.9%)	28 (20.1%)	
2 hours (≥8.5 - ≤11 mmo1/L)	106 (76.3%)	27(19.4%)	6 (4.3%)
At least one OGTT glucose level(Fasting, 1 hour or 2 hours)	67 (48.2%)	64 (46.0%)	8 (5.8%)

4.11. Performing OGTT test on pregnant mothers at KNH using to determine the GDM

From table 13, it is clear that 37.4% of the cohort tested positive on fasting glucose level, 20.1% on 1 hour and 19.4% on 2 hour time. Based on one or two or three of the three blood glucose levels (fasting, 1hour and 2 hour) 46.0% (*n*=64) had their values equal or between the cutoff of 5.1-6.9mmol/l at fasting, equal or above the cutoff of 10mmol/l at one hour or, equal or between the cutoff of 8.5 - 11mmol/l at two hours. Prevalence of GDM was found out to be 46.0%.

Figure 6 Prevalence of GDM in 139 participants in KNH using WHO 2013criteria

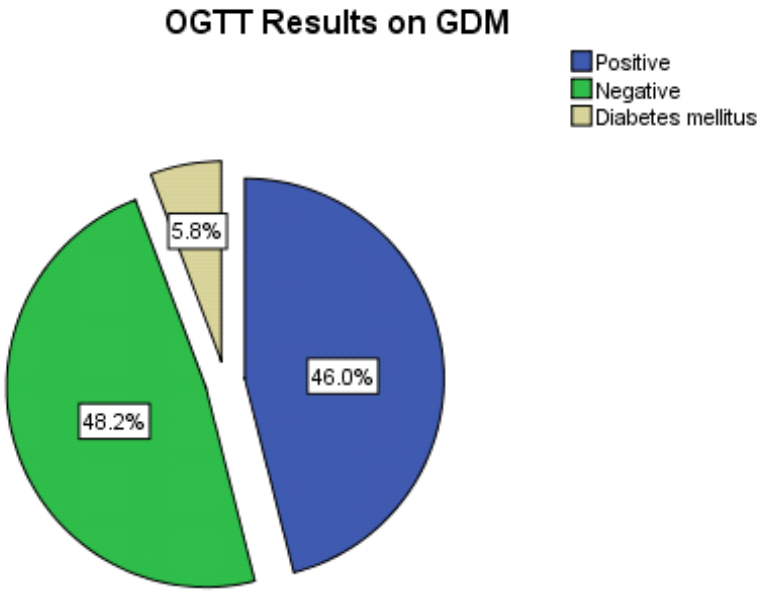


Figure 6 shows prevalence of GDM in 139 participants in KNH using WHO 2013criteria

4.12 Prevalence of GDM in 139 participants in KNH (WHO 2013 criteria)

Figure 6 shows that 48.2% (*n*=67) were GDM negative, 46.0% (*n*=64) were GDM positive while 5.8% (*n*=8) had diabetes mellitus.

Table 12 The relationship between HbA1c% (≥6.5) and OGTT (WHO 2013 criteria) prevalence

HbA1c (≥6.5%) * OGTT Results on GDM Cross-tabulation

		OGTT Results on GDM			Total
		Positive	Negative	Diabetes mellitus	
HbA1c (>=6.5%)	Positive	5	1	3	9
	Negative	58	67	5	130
Total		63	68	8	139

Table 12 shows the relationship between HbA1c% (≥6.5) and OGTT (WHO 2013 criteria)

4.13. The relationship between HbA1c% (≥6.5) and OGTT prevalence

The study established that out of 139 participants, 5 (3.6%) were positive in both OGTT and HbA1c tests, 67 (48.2%) were negative on both test, 58 (41.7%) were negative on HbA1c and positive on OGTT, while 1 (0.7%) of the tests was positive on HbA1c and negative on OGTT.

Upon testing whether there was a relationship between the HbA1c and OGTT results, it was established that there was a statistically significant difference between the two variables with p= 0.010 which is less than 0.05. On the other hand, Spearman's correlation inferred that there was a weak positive correlation between HbA1c and OGTT which was statistically significant with r_s=0.224 and p = 0.010.

Table 13 HbA1c test performance in GDM diagnosis based on cut off value of >6.5% (WHO, 2013 criteria)

HbA1c% (≥6.5%) *OGTT Results on GDM Cross-tabulation				
		OGTT Results on GDM		Total
		Positive	Negative	
HbA1c (>=6.5%)	Positive	5	1	6
	Negative	58	67	125
Total		63	68	131

Table 13 shows HbA1c test performance in GDM diagnosis based on cut off value of ≥6.5%.

From the table 14 above, five participants tested positive for both OGTT and HbA1c(TP),58 tested negative for HbA1c and positive for OGTT (FN),67 tested negative for both HbA1c and OGTT (TN) and 1 tested positive for HbA1c and negative for OGTT(FP). The number of true negative and false positives were the same as the results in IADPSG. The true positive and false negative is the same except that the values for DM have been subtracted.

4.14. Performance characteristics of HbA1c at ≥ 6.5% cut off value using WHO criteria

Sensitivity = 7.9%

Specificity = 98.5%

True positive rate (TPR) = (5/ (5+58)) x 100 = 5/63 x 100 =7.9%

True negative rate (TNR) = (67/ (67+1)) x 100 = 67/68 x 100 =98.5%

False negative rate (FNR) = (58/ (58+5)) x 100 = 58/63 x 100 =92.1%

False positive rate (FPR) = (1/ (1+67)) x 100 = 1/68 x 100 = 1.5%

Positive predictive value (PPV) = (5/ (5+1)) x 100= 5/6 x 100=83.3%

Negative predictive value (NPV) = (67/ (67+58)) x100 = 67/125 x 100 = 53.6%

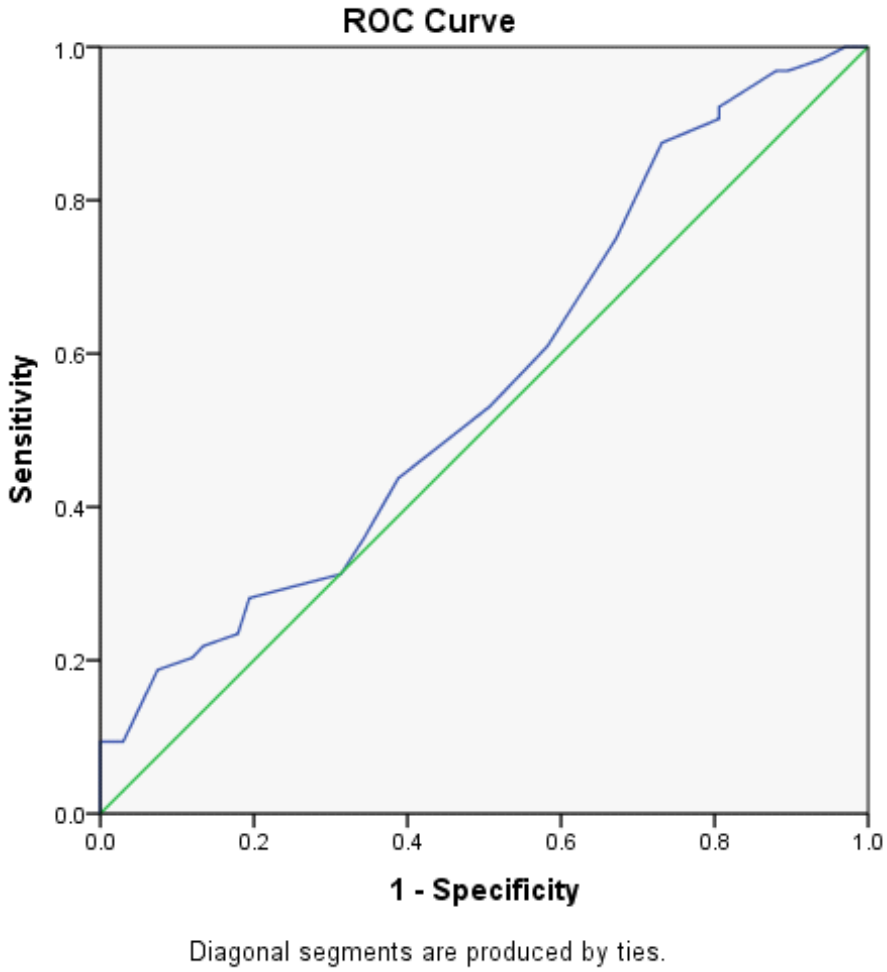


Figure 7 Receiver Operating Characteristic curve (WHO 2013 criteria)

The relationship between HbA1c (≥6.5) and OGTT (WHO criteria)

Table 14 Area Under the Curve

Test Result Variable(s): HBA1C %				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.563	.050	.215	.464	.661

4.15 Receiver Operating Characteristic (ROC) curve at a cut off value of ≥6.5% using WHO criteria

The study evaluated the relationship represented by the ROC curve. The area under the curve (AUC) was 0.563 (56.3%), 95% confidence interval: 0.464 – 0.661, *p*= 0.215. The ROC curve and the corresponding AUCimplied that there was agreement between the two tests in diagnosis of GDM. In reference to table 15, the cut-off point of HbA1c value of ≥ 4.95% present the best equilibrium sensitivity of 87.5% and 1-specificity of 73.1%(TNR=26.9%).

Using IADPSG criteria, the AUC was 0.598, TP was 64(46.04%) and TN was 18(12.95%).

Using WHO criteria, the AUC was 0.563, TP was 5(7.9%) and TN was 67 (98.5%). The OGTT criteria produced the best cut off value of 4.95% which was the same as the one produced by IADPSG criteria.

Figure 8 Prevalence of GDM using HbA1c (≥ 4 .95%) WHO criteria

Figure 8 shows prevalence of GDM using HbA1c (≥ 4 .95%) WHO, which is 81.3 %.

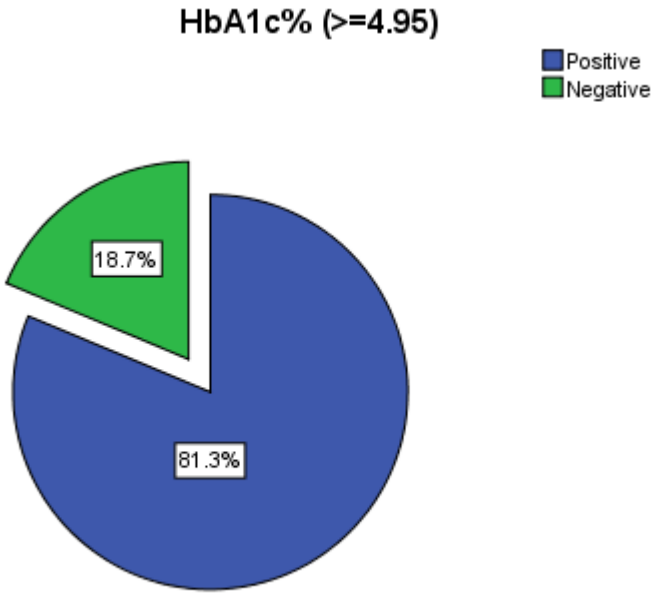


Figure 8 shows prevalence of GDM using HbA1c (≥ 4 .95%) WHO, which is 81.3 %.

Table 15 HbA1c% (>=4.95) * OGTT (WHO 2013) Results Cross-tabulation

Count

		OGTT Results on GDM		Total
		Positive	Negative	
HbA1c% (>=4.95)	Positive	56	49	105
	Negative	8	18	26
Total		64	67	131

Table 15 shows HbA1c% (>=4.95) * OGTT (WHO 2013) Results (Perfomance characteristics of HbA1c at $\geq$ 4.95%. Fifty six participants tested positive for both HbA1c and OGTT, (TP) 8 participants tested negative with HbA1c but positive for OGTT (FN), 18 participants tested negative for both HbA1c and OGTT (TN) and 49 participants tested positive for HbA1c and negative for OGTT). In this case, the number of false positive, false negative and true negative are the same as the results for IADPSG.

4.16 HbA1c test performance characteristics in GDM diagnosis at cut off value of $\geq$ 4.95%

Sensitivity = 87.5

Specificity =1-0.731 = 26.9%

TPR = 87.5%

TNR = 26.9%

FNR = 12.5%

FPR = 73.1%

PPV = 53.3%

NPV = 69.2%

It was noted that the sensitivity for IADPSG and WHO were 88.91% and 87.5% respectively and Specificity for both criteria were 26.9%

DISCUSSION

The purpose of this study was to evaluate the value of HbA1c as a screening tool in comparison to the 75g OGTT by determining the performance characteristics of HbA1c as a screening tool (IADPSG). The goal was to find the best HbA1c cut-off value for diagnosing gestational diabetes mellitus in comparison to the current cut-off of > 6.5 percent and to evaluate the prevalence of GDM in this setting. OGTT (IADPSG) was used as the gold standard to detect GDM in the participants using cut off values of $\geq$5.1, $\geq$10, and 8.5mmol/l at fasting, 1hr and 2hrs respectively.

1. Oral Glucose tolerance test (IADPSG) result in 139participants.

Out of the 139 participants, 72 (51.8%) were positive and 67 (48.2%) were negative, using the OGTT (IADPSG) . This produced a prevalence of 51.8% for GDM in this set up. This is higher than the prevalence of 23% in a study done in KNH using 75g OGTT (32). Another study in KNH showed a prevalence of 8.9% (20).It was also higher than the prevalence of 17.8% (IADPSG) and 23.1% (NICE) reported in a study among women in Croatia.(15) .The higher prevalence may be due to the use of one level of glucose reading in interpretation as compared to two or three. When IADPSG was utilized, Widness found a drop in HbA1c levels from the first half of pregnancy to the third trimester, as well as a 2-3fold rise in the prevalence of GDM (18). Another study found a 3.5-fold rise in GDM prevalence when IADPSG criteria were used. (33). The high prevalence may also be attributed to the Stress caused by the COVID 19 pandemic because stress leads to increased glycemic status (34). The bias may be due to the fact that during this period, only patients with complications were allowed into the clinic. Our higher prevalence may be due to lower diagnostic threshold of

the IADPSG criteria and the low cut off values. Prevalence of GDM in Kenya (Moi referral hospital) by Pastakia et al (2017) was 2.9% (18). At KNH, it was 8.9%, (19) and 23%, (40).

2. HbA1c results in the participants at various cut off points

Using HbA1c cut off point of ≥ 6.5%, 9 (6.47%) were positive while 130 (93.53%) were negative for GDM. This study showed that the prevalence of GDM using glycated hemoglobin cut off value of ≥ 6.5% was 6.47%. This prevalence is slightly lower than 8.9% (20) and 23% reported at KNH (32) (40) and 17.8 reported at Croatia. Using HbA1c cut off of≥ 4.95%, 113 (81.3%) were positive for GDM and 26(18.7%) were negative for GDM. This prevalence was higher than the prevalence for the Gold standard. It is far much higher than the highest prevalence that has been reported in Pakistan, 44% (39).

2. Correlation between OGTT and HbA1c

The results for HbA1c at a cut off value of ≥ 6.5% were correlated with the results for OGTT (IADPSG). It was found out that 8(5.8%) were true positive, 63(45.3%) were false negative, 67(48.2%) were true negative and 1(0.7%) were false positive. This meant that 63 participants were labeled as negative yet they had the disease. The use of ≥ 6.5% HbA1c cut off value in diagnosis of GDM would leave out many undetected. The same was reported by Hughes et al (9).The results for HbA1c at a cut off value of ≥ 4.95% were correlated with the ones for OGTT (IADPSG). It was found out that 64 (88.9%) were true positive, 8(11.1%) were false negative, 18(26.9%) were true negative and 49(73.1%) were false positive. This meant that 49 participants were labeled as positive yet they did not have the disease. The use of HbA1c cut of value of ≥ 4.95% in diagnosis of GDM would lead to over diagnosis and unnecessary interventions (9). Lene et al (2004) (41) found out the reference range of HbA1c in women who are not pregnant was 4.7 - 6.3%, and 4.3 – 5.7% in early gestational period and 4.4 – 5.6% in late gestational period (37). This means that the 4.95% cut off value generated in this study is within the reference range for pregnant women. The use of this HbA1c cut off value as a diagnostic tool may lead to over diagnosis (3) and false alarm in both the treating physician and patients(6).

4. Determination of the best cut off value for diagnosis of GDM

According to the ROC curve, the best cut off value for diagnosing GDM was ≥ 4.95%.This produced a high GDM prevalence of 113 participants (81.3%) which is higher than the 44% realized in Pakistan, (39).The prevalence produced by this cut off value is higher than the prevalence produced by the Gold Standard ,(51.8%). Both IADPSG and WHO generated the similar cut off value of ≥ 4.95%.(33)

5. Sens. and spec. of HbA1c for diagnosis of GDM.

A low sensitivity of 11.2 % and a high specificity of 98.5 % were achieved using a cut off value of > 6.5 percent. A cut off of≥ 6.5% had a low sensitivity which rules it out. The similar findings were realized in a study by Hughes (9).

As per curve, the best HbA1c cutoff value was ≥ 4.95% with a high sens. of 88.91% and low spec. of 26.9% (TNR). (1-specificity is 73.1 %). These results do not agree with the results for the gold standard. This cut off value was lower than the one reported in South Korea which gave a cut off value of 5.05% with sens. of 91.3% and spec. of 62% .(35). In a study done in Iran a best cut off value of 5.1% with sens. of 61% and spec. of 68% was realized (8). This study's cut-off value was lower than that reported by Khalafallah et al. (2016) (8) who had a cut off of 5.4% with a low sens. of 27% and high spec. of 95%.(8) This is different from one done on Iranian women and reported a cut off of 5.35% with a high sens. and high spec. of 87.2% and 70.9% respectively (26). The cut off value in this study is comparable to one that gave a cut off value of 5.0% with a sens. of 89.7% and spec. of 32.6% (36). A cut off value of 5.1% had a sens. of 61% and spec. of 68% (8).In this study using two HbA1c cut off values, the 4.95% cut off had a high sens. of 88.91%, low, spec. of 26.9%, and prevalence of 81.29 while a cut off of ≥6.5 produced a low sens. of 11.2%, high spec. of 98.5% and prevalence of 6.47%.A decrease of HbA1c cut off value from 6.5% to 4.95% increased sens.

eight times and decreased spec. four times.A HbA1c cutoff value of > 5.1 percent resulted in a low sensitivity of 77.8% and a low specificity of 32.8 % . This indicates a lower sens. and a lower spec. The same cut off value produced sens. of 61% and a spec. of 68% (8). The Area under the curve (AUC) of the ROC curve created using a cutoff value of > 6.5 percent was 0.598, which is unacceptable. The AUC generated by cut of value f ≥ 4.95% was The Area

under the curve (AUC) of the ROC curve created using a cutoff value of > 6.5 percent was 0.598, which is unacceptable. The AUC generated by cut of value f ≥ 4.95% was 0.563 which is also unacceptable. In general, an Area under the Curve of 0.7-0.8 is regarded adequate, 0.8–0.9 is great, and more than 0.9 is exceptional.

6. Comparison of OGTT, (IADPSG) criteria results with WHO 2013 criteria results.

Prevalence of GDM using IADPSG was 51.8% and using OGTT (WHO, 2013) criteria was 46% which is comparable to the prevalence reported in Pakistan, 44% (39).

HbA1c cut off of≥ 6.5% gave a true positive of 8 participants and WHO 2013 criteria gave the number with Diabetes Mellitus as 8 participants. This indicates that HbA1c cut off value of ≥ 6.5% detects only those who have DM but not those who have GDM.(9)

Prevalence of GDM by different tests were as follows: OGTT (IADPSG) was 51.8%,

HbA1c cut off value of ≥6.5%, had prevalence of 6.47%, cut off value of 5.1% had 77.8 % and ≥4.95% had a prevalence of 81.5% and WHO 2013 gave a prevalence of 7.9%.

Using HbA1c cut off of≥4.95%, true positive value was 64(46.04%) and true negative was 18 (12.95%). A cut off value of 4.95% gave a PPV of 56.6% and NPV of 69.2%. This suggests that 56.6 % of the subjects were appropriately diagnosed with GDM, while 69.2% were correctly identified as not having GDM. In 43.3 %, there would be an over-diagnosis. The cutoff value of ≥ 4.95% PPV of 56.6 % and NPV of 69.2% in this study are low .This is comparable to Ryu et al who had PPV of 43.6% and 58.3% in different studies. (26).A good test should have higher values tending towards 100%.

Using WHO 2013 criteria, the prevalence was 46.0% which is comparable with the prevalence 51.8% in IADPSG and the best cut off value generated by the ROC curve was ≥ 4.95% which is the same as the one for IADPSG in this study. The Area Under the curve was 0.563 using WHO and 0.598 in IADPSG at a cut off value of ≥ 6.5%. At a cut off of≥4.95% the sens. was 87.5% (WHO) and 88.9% (IADPSG) and a spec. of 26.9% in both IADPSG and WHO. At a cut off of≥ 6.5% (IADPSG), the low sens.11.2% and high spec. of 98.5% and WHO had a low sens. of 7.9% and high spec. of 98.5%.In this study, the IADPSG results and WHO results are comparable and in some cases, they are similar.(33)

CONCLUSION

1. The OGTT (IADPSG) results produced a high prevalence. The prevalence produced in this study is very high which may be due to the COVID 19 pandemic and stress that it produced The bias may be due to the fact that during this period, only patients with complications were allowed into the clinic. The high prevalence may be attributed to use of one reading in interpretation and the low cut off value at fasting. A larger percentage of the positive results was in the fasting for both IADPSG (40%) and WHO 2013 (37.4%) which may imply that the participants may not have fasted as required, leading to the high prevalence. Prevalence of GDM by IADPSG criteria was 51.8% while it was 46.0% by WHO 2013 criteria. Only 8 participants were diagnosed with DM.

2. The GDM prevalence using HbA1c cut off value of $\geq$ 6.5% was low and was the same as the prevalence for DM in WHO criteria. This may imply that this cut off only detects DM and not GDM.

The prevalence for GDM using HbA1c cut off of $\geq$ 4.95% (generated best cut off value) was far much higher than the prevalence for Gold standard, therefore cannot be adopted as the cut of value for pregnant women.

3. Correlation between HbA1c results and OGTT results at cut off value of $\geq$ 6.5% produced a low sens. and high spec. while the HbA1c cut off value of $\geq$ 4.95% produced a high sens. and low spec.. Both are ruled out due to under-detection and over-detection of GDM respectively. This study rules them out due to their low PPV and NPV.

4. In this study, HbA1c cannot be used in diagnosis of GDM because the best cut off value has not been established. The cut off in this study does not agree with the Gold Standard results.

5. The comparison between the results for IADPSG criteria and WHO 2013criteria were comparable at both HbA1c cut off values of$\geq$ 6.5% and $\geq$ 4.95%, the specificities were similar and sensitivities had slight differences. This indicates that IADPSG criteria is an acceptable criteria for detection of GDM because the results from the two criteria agree.

RECOMMENDATIONS

Further studies should be done to find out the best cut off value for HbA1c for screening and diagnosing GDM using a larger sample size, from different facilities, different communities and at an appropriate time when there is no pandemic.

Testing for GDM should be performed using IADPSG or WHO, 2013 criteria because there's an agreement in both results.

Further studies are recommended to find out if the high prevalence of 51.8% (IADPSG) and 46% (WHO 2013) represent an increased GDM cases in the community.

There's need of establishing local reference range for HbA1c in pregnant women.

All pregnant women should undergo OGTTduring second trimester to detect GDM and prevent its complications both in mother and child during and after pregnancy.

LIMITATIONS

The invasive, multiple sample collection caused discomfort for participants.

The cost of performing these tests was high because of expensive reagents and equipment.

The study was done during the Covid-19 and this might have led to raised glycemic status because most of the patients who came to the clinic then were those with complications, the rest had been told to stay at home.

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APPENDICES

APPENDIX 1: SCREENING PROFORMA

Characteristic	Response (Tick)	
	YES	NO
Age (Above 18)	☐	☐
Gestational period (24 to 28 weeks)	☐	☐
Not Diabetic	☐	☐
Consenting	☐	☐
Hb 10 to 14(g/dl)	☐	☐
Not overt DM	☐	☐
Agrees to take glucose	☐	☐

FOR OFFICIAL USE

If all yes, recruitment will take place.

		Recruited	
		YES	NO
Study number	☐	☐	☐
Results for OGTT	POSITIVE ☐	NEGATIVE	☐
HbA1c results	☐		

APPEDIX 2: QUESTIONNAIRE

Study number `

A. Social demographic characteristics

Age (yrs)

Date of birth

Marital Status: Single Married Divorced

Occupation

Self employed Employed

Unemployed ing/ Student

Level of Education

None Primary school Secondary School College

B. Pregnancy history

Gestational period (weeks)

APPENDIX 3: LABORATORY REPORT FORM

STUDY NUMBER: ……

TEST	RESULTS	REFERENCE	OGTT +VE	
			YES	NO
Fasting blood sugar After glucose load 1 hr blood glucose 2hr blood glucose	Mmol/L Mmol/L	≤5.1Mmol/L ≤10Mmol/L ≤8.5Mmol/L		
HbA1C LEVEL	%	-	-	

Interpretation as per standard who OGTT

One or more value above the following cut off values

Fasting < 5.1, one hour < 10.0 and two hour< 8.5 Mmol/L. …… NO GDM

Fasting ≥ 5.1, one hour ≥ 10.0 and two hour ≥ 8.5 Mmol/L. …… GDM

APPENDIX 4: PATIENT INFORMATION AND CONSENT FORM

This informed consent form has two parts:

1. Information sheet (to share information about the study)
2. Certificate of consent

PART 1: INFORMATION SHEET

My name is MRS. DEBORAH M. MOGI. I am a postgraduate student in the Department of Human Pathology at the University of Nairobi. I am conducting this study to assess the usefulness of HbA1c and OGTT as diagnostic tests for GDM. You are invited to participate in this study. In case you do not understand any words used in this information sheet and have any questions please ask me to stop and explain. I shall be assisted by the nurse to explain the study and consent form.

Purpose of the Study:

The purpose of the study is to check your glucose level. Glucose (sugar) is attached to red blood cells over a period of time. The higher your blood sugar the larger the amount of glucose is attached. This is measured, as a percentage, in the laboratory using a test called glycated hemoglobin (HbAlc). The test is used to estimate your blood sugar level over the past 3 months. It can be used to diagnose and monitor Gestational diabetes.

Participant Selection:

Adult women in the health ANC clinic in Kenyatta National Hospital, in their 24 - 28 weeks of gestational period. Total number will be 139 participants.

Voluntary Participation:

Your participation in this research is entirely voluntary and it is your choice whether to participate or not. Whether you choose to participate or not, all the medical care you receive will not change.

Specimen collection:

A drop of blood from a finger will be collected. You will feel a little pain (like a prick) which will be over in about one minute. Sterile lancets will be used which will be disposed of after the test. The blood will then be used to measure the amount of sugar using a glucose meter and HbA1c using Clover AIcself equipment.

Side Effects: The study involves ingestion of glucose which may cause nausea. There are no further side effects in this study.

Risks: There will be minimal risks expected with this study**.** There may be little discomfort because of multiple pricking since sampling is done at different stages. It also involves ingestion of glucose which may cause nausea and vomiting to some individuals. We do not anticipate infection because sterile equipments will be used.

Benefits: We will be able to diagnose whether or not you have gestational diabetes and refer you to the proper physicians for better care. Your participation will also help in the overall care of patients with diabetes by improving on testing procedures for GDM.

Reimbursements: You will not be given any money or gifts to take part in this research. However for those who opt to come back for the test on another day apart from the appointment (clinic) day will be reimbursed bus fare at a rate of ksh. 200/=.

Confidentiality: All participants will be identified using a number (names will not be used). All information shared by you during this study will be viewed by the researchers only.

Sharing the results: The results obtained during this study will be forwarded to your doctor. The results will be published so that other interested people may learn from it. However your identity will never be revealed.

Request to participate in the study: You have been explained to about the study and if you are willing to join the study, I kindly request you to fill the consent certificate provided.

Right to refuse: Should you decline to participate in this study, this will not affect your treatment in any way. You will still have all the benefits that you would have otherwise.

Who to contact: If you have any questions regarding this study at any time you may contact the principal investigator: MRS. MOGI DEBORAH (0723833714) or any of my supervisors below: **Supervisors:**

1.Prof. C. S. Kigondu.P O. BOX 19676-00202, Nairobi. Telephone, 0727862993.

2. Dr. Wandolo George. P O. BOX 19676-00202, Nairobi. Telephone, 0721563947.

You can also contact the Ethics and Research Committee at Kenyatta National Hospital **(KNH/UON-ERC):** PO. BOX 20723-00202, Nairobi. Telephone +254 020 726300-9 (Ext. 44102)

PART II: CERTIFICATE OF CONSENT

I have read the forgoing information, or it has been read to me. I have the opportunity to raise any questions about my participation in the study, and the questions I asked have been answered to my satisfaction. My rights have been explained to me and I consent voluntarily to participate in this study.

Print Initials of Participant: …….

Signature of Participant: …….Date: ……

CERTIFICATE OF CONSENT FOR ILLITERATE PARTICIPANTS: A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumbprint as well. I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of Witness: …….

Signature of Witness: ……..Date: ……

Thumb print of Participant: ……

FOMU YA IDHINI KICHWA CHA UTAFITI:

KIPIMO CHA DAMU KUCHUNGUZA UGONJW A WA SUKARI KWA WAMAMA WAJAA WAZITO

Jina langu ni **Bi. Deborah M. Mogi,** mwanafunzi wa chuo kikuu cha Nairobi idara ya Pathologia ya wanadamu. Ningependa kufanya utafiti ambao nitawaelezea. Tafadhali soma ujumbe ufuatao kwa makini. Ujumbe huu utaelezwa kwa njiaya Kiingereza na Kiswahili. Una uhuru wakuchagua lugha ambayo utaelewa vyema.

Maelezokwaufupi: Kipimo cha damu kuchunguza ugonjwa wa sukari kwa wamama wajaa wazito katika hospitali ya Kenyatta. Tutachukua damu yako kutoka kwa mkono (kidole) na kupima kiwango cha sukari (ugonjwa wa sukari kwa mtambo unaoitwa ‘glucose meter and HbA1c using Clover AIcself’.

Faida na tatizo za utafiti Huu: Damu yako itapimwa kuchunguza ugonjwa wa sukari. Utanufaika kwa kupata matokeo ya damu kuhusu ugonjwa wa sukari. Lsipokuwa uchungu

kidogo wa kudungwa, hakuna madhara ama tatizo lingine kubwa.

Taratibu wa kushiriki: Watakaoshiriki katika uchunguzi huu itakuwa kwa njia ya hiari bila kushurutishwa. Ukiamua kutoshiriki, hautapoteza kwa njia yoyote bali utaendelea kuhudumiwa unavyostahili. Majibu ya uchunguzi huu utapewa daktari wako wakati wa kufuata kliniki yako ya kawaida.

Idhiniyamshiriki: Watakaoshiriki katika utafiti huu itakuwa kwa hiari bila kusurutishwa. Una uhuru wakutoshiriki au kutojibu swali lolote kwenye dodoso au kukatiza kipindi cha maswali iwapo hautaridhika na Jambo lolote. Pia waweza kutamatisha ushirika wako kwenye utafiti huu bila kupoteza haki yako ya kutibiwa katika hospitali hii.

Anwani: Mchunguzi **Bi. Deborah.M.Mogi**, Chuo Kikuu Cha Nairobi SLP 19676-00202 Nairobi Nambari ya simu 0723833714. Pia unaweza kutafuta wasimamizi wafuatao:

Wasimamizi

1. Prof. C. S. KigonduS.L.P. 19676-00202, Nairobi, Nambari ya simu, 0727862993.

2.**Dr. Wandolo George.** S.L.P. 19676-00202, Nairobi, Nambari ya simu, 0721563947

IdhiniyaMshiriki

Kama utashiriki tafadhali tia sahihi yako kwenye pengo lililoachwa hapa chini

Mimi……nimesoma na nimeelewa nia ya uchunguzi huu, utaratibu utakaotumika kuchukua kipimo, faida na madhara yanayohusika na uchunguzi huu. Nimekubali kushiriki kwa hiari bila kushurutishwa.

SahihiyaMshiriki …….Tarehe ……

Sahihiya Shahidi ……..Tarehe ……

Statement of the researcher/person taking consent:

I have accurately read the information sheet to the potential participant, and to the best of my ability made sure that the participant understands what the research is all about.

I confirm that the participant was given an opportunity to ask questions about the study and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent and the consent has been given freely and voluntarily.

A copy of this document has been provided to the participant.

Print Name of Researcher/person taking the consent ……

Signature of Researcher/person taking the consent ……..…….. Date …….

Mpelelezi/ Mchunguzi

Mimi nimemsomea mshiriki na kumwelezea juu ya uchunguzi huu na kwakuelewa kwangu nimehakikisha ya kwamba ameelewa yote juu ya uchunguzi huu.

Nathibitisha ya kwamba mshiriki alipewa nafasi ya kuuliza maswali juu ya uchunguzi na akajibiwa kikamilifu.

Nathibitisha ya kwamba mshiriki hakushurutishwa kutia sahihi bali amekubali kwa hiari yake na kwa kujitolea.

Nakala kwake mshiriki.

Jina la mpelelezi/Mchunguzi ……..

Sahihi ya mpelelezi/Mchunguzi …… Tarehe ……

APPENDIX 5: PRINCIPLES OF THE PROCEDURES

The *CLOVER A1c™Self* system is a fully automated boronate affinity assay for the determination of the percentage of Hemoglobin A1c (HbA1c %) in whole blood. The Test Cartridge is composed of a cartridge and a reagent pack containing the reagents necessary for the determination of hemoglobin A1c, with a sample collecting area for blood sample collection.

The reagent pack is pre-filled with reaction solution and washing solution. The reaction solution contains agents that lyse erythrocytes and bind hemoglobin specifically, as well as a boronate resin that binds cis-diols of glycated hemoglobin. The blood sample (4uL) is collected at the sample collecting area of the reagent pack, then the reagent pack is inserted into the cartridge, where the blood is instantly lysed releasing the hemoglobin and the boronate resin binding the glycated hemoglobin.

The assembled cartridge is inserted into the *CLOVER A1c™Self* Analyzer and rotated so that the blood sample mixture is placed at the measurement zone of the cartridge, where the amount of total hemoglobin in the blood sample is photo metrically measured by the diffused

reflectance of the optical sensor composed with LED (Light Emitting Diode) and PD (Photo Diode).Then, the assembled cartridge is rotated so that the washing solution washes out non-glycated hemoglobin from the blood sample, thus the amount of glycated hemoglobin can be photo metrically measured.

The ratio of glycated hemoglobin with respect to total hemoglobin in the blood sample is calculated as follows:

HbAlc%= Ax [HbA1c/Total Hemoglobin x100] +B

Where 'HbA1c' and 'Total Hemoglobin' are the signal obtained from the CLOVER A1c™ Self system, 'A' and 'B' are the slope and intercept factor to correlate the value for DCCT calibration.

Expected Values:

The American Diabetes Association's most recent Clinical Practice Recommendation for diabetes specifies a treatment goal of less than 7.0%, and suggests additional action when the HbA1c level is above 8.0%.

HbA1c%	Mean Plasma Glucose mg/dL	Mean Plasma Glucose mmol/L
6	135	7.5
7	170	9.5
8	205	11.5
9	240	13.5
10	275	15.5
11	310	17.5
12	345	19.5

Limitation of Procedure:

The *CLOVER A1c™Self* assay gives accurate and precise results over a range of total hemoglobin of 7 to 20 g/dL. Most patients will have hemoglobin concentrations within those values. However, patients with severe anemias may have hemoglobin concentrations lower than 7 g/dL, and patients with polycythemia may have hemoglobin concentrations above 20 g/dL. Patients known to have these conditions should be assayed by a test employing a different assay principle if their hemoglobin concentrations are outside of the acceptable range.

CLOVER A1c™Self-Test Cartridge:

When the Test Cartridges are produced, each production lot undergoes a thorough analysis and characterization before they are released. Test method of the *CLOVER A1c™ Self* system is National Glyco-hemoglobin Standardization Program (NGSP) certified, thus the values of calibration parameters determined to provide an optimal reagent performance, are based on DCCT reference method. The values for the calibration parameters are on the Test Cartridge label provided with each lot of Test Cartridge. As soon as the Test Cartridge is inserted into the analyzer, the system automatically recognizes the code printed on the cartridge. This accesses the appropriate calibration parameter values (calibration curve) for the particular lot number of reagent packs in use.

IFCC Standardization

Values have been assigned using material supplied by the IFCC network. The correlation of the HhAlc results from NGSP network and IFCC network have been evaluated and a master equation has been developed. IFCC results are consistently 1.5-2.0% lower than NGSP results. Users may convert their NGSP values to IFCC values using the following equation:

IFCC value = [1.093 x NGSP value] -2.350

Recently, all IFCC Network mark their IFCC HbAlc result in mmol/mol as from the 1st of October 2008. The equation is as follows in case that HbAlc results are marked in %.

IFCC-HbA1c (mmol/mol) = [DCCT-HbAlc (%)-2.15] x 10.929 Introduction to the Your Blood Glucose Monitoring System

GOLD - ACCU BLOOD GLUCOSE MONITORING SYSTEM

The Gold-Accu Blood Glucose Monitoring System is designed for the quantitative measurement of glucose in fresh capillary whole blood samples taken from the finger tip or venous whole blood samples or arterial whole blood samples.

The Blood Glucose Monitoring System is for use outside the body only (in vitro diagnostic use) for self-testing and professional use as an aid in the management of diabetes.

The Gold-Accu Blood Glucose Monitoring System is intended for in vitro diagnostic use and should not be used for the diagnosis of or screening of diabetes.

The Gold-Accu Blood Glucose Monitoring System includes:

Gold-Accu blood glucose meter, Gold-Accu blood glucose test strip, blood glucose control Solution.

The Gold-Accu meter display blood glucose results in mmol/L or mg/dL.
Each meter only displays one unit of measurement. The unit is preset and you can't change the setting. Please confirm the unit before testing.

Test Principle
A glucose test is based on the measurement of electrical current caused by the reaction of glucose with the FAD glucose dehydrogenase on the electrode of the strip. The blood or control solution sample is drawn into the tip of the test strip through capillary action.

Glucose in the sample reacts with the FAD glucose dehydrogenase and generates electrons, which produce an electrical current. The Gold-Accu blood glucose meter measures the electrical current and calculates the glucose result. The glucose result is displayed by the meter as mg/dL or mmol/L.

APPENDIX 8: APPROVAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/37

30th January 2020

KENYATTA NATIONAL HOSPITAL APPROVED 30 JAN 2020 KNH/UoN-ERC P. O. BOX 20723 - 00202 NAIROBI

Deborah Maiko Mogi
Reg.No.H58/7959/2017
Dept. of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Deborah

RESEARCH PROPOSAL: UTILITY OF HbA1C AND ORAL GLUCOSE TOLERANCE TEST IN DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS AT KENYATTA NATIONAL HOSPITAL (P746/08/2019)

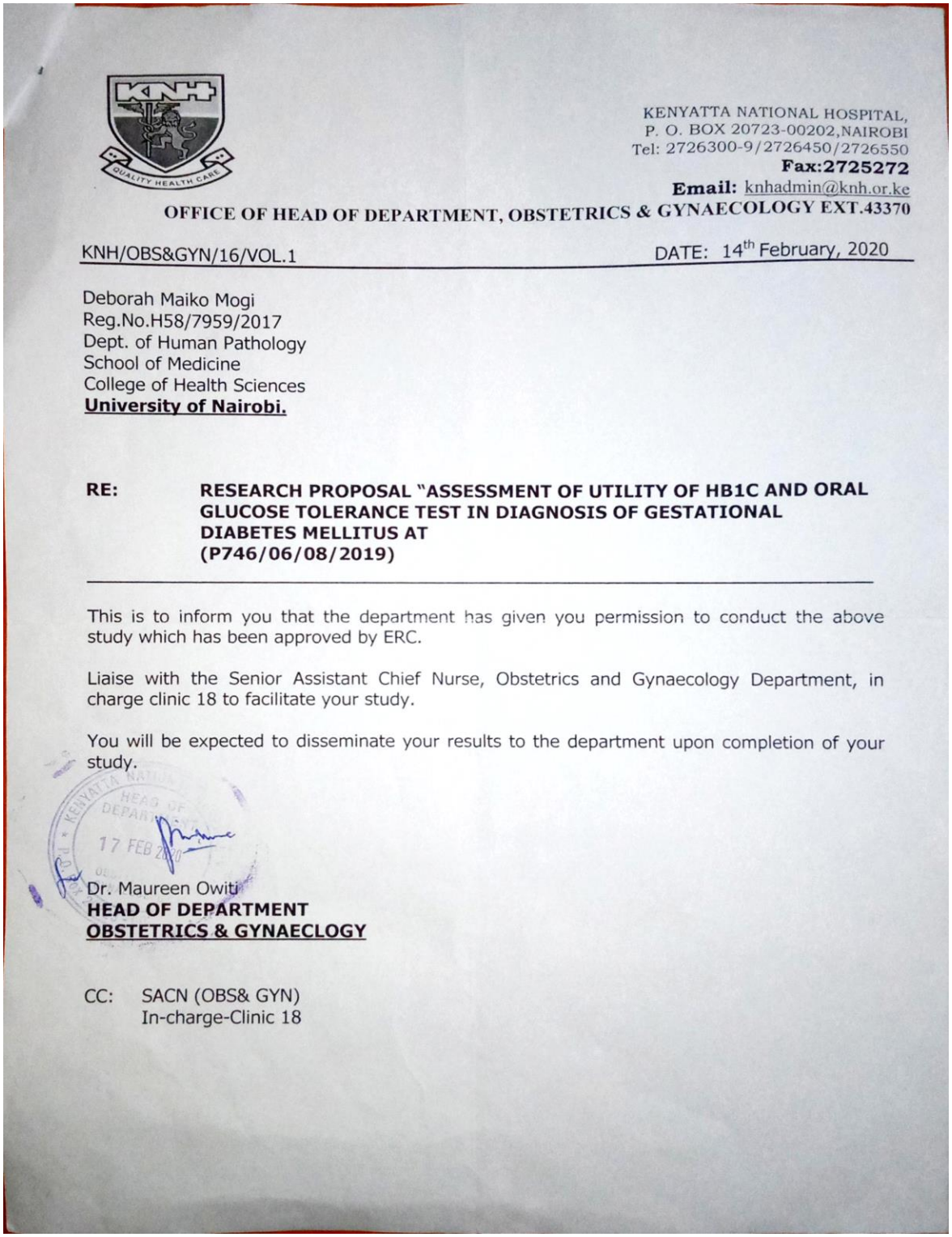
This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 30th January 2020 – 29th January 2021.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

KNH-UON-ERC APPROVAL LETTER



KENYATTA NATIONAL HOSPITAL,
P. O. BOX 20723-00202,NAIROBI
Tel: 2726300-9/2726450/2726550
Fax:2725272
Email: knhadmin@knh.or.ke

OFFICE OF HEAD OF DEPARTMENT, OBSTETRICS & GYNAECOLOGY EXT.43370

KNH/OBS&GYN/16/VOL.1 DATE: 14th February, 2020

Deborah Maiko Mogi
Reg.No.H58/7959/2017
Dept. of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi.

RE: RESEARCH PROPOSAL "ASSESSMENT OF UTILITY OF HB1C AND ORAL GLUCOSE TOLERANCE TEST IN DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS AT (P746/06/08/2019)

This is to inform you that the department has given you permission to conduct the above study which has been approved by ERC.

Liaise with the Senior Assistant Chief Nurse, Obstetrics and Gynaecology Department, in charge clinic 18 to facilitate your study.

You will be expected to disseminate your results to the department upon completion of your study.

17 FEB 2020

Dr. Maureen Owiti
HEAD OF DEPARTMENT
OBSTETRICS & GYNAECLOGY

CC: SACN (OBS& GYN)
In-charge-Clinic 18

APPROVAL LETTER FROM DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY