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Investigating Genetic Predisposition to Gestational Diabetes Mellitus Among Black Women Residing eThekwini, KwaZulu-Natal, South Africa

BY

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Investigating Genetic Predisposition to Gestational Diabetes Mellitus Among Black Women Residing eThekwini District, KwaZulu-Natal, South Africa.

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PREFACE

The Study and experimental lab work described in this thesis were conducted at Genetics Laboratory by Miss Angeline Nozipho Moloi at the School of Laboratory Medicine and Medical Sciences (Physiology), College of Health Sciences and Genetics Research Laboratory, School of Molecular Genetics, Microbiology and Engineering, College of Life Science at the University of KwaZulu-Natal, Durban, South Africa, from June 2018 to September 2022, under the supervision of Dr. K.R.Xulu, Dr. M. Ghai and Dr. H.P. Mbongwa.

This work has not been submitted to other institutions of higher learning for examination purposes towards any qualification; besides the acknowledgments of references used in the text, this work represents the original work.

Signature……  …….Date…….18/09/2022……

Angeline N. Moloi (Student)

PLAGIARISM DECLARATION

I, **Miss Angeline Nozipho Moloi**, declare that:

1. The study reported in this thesis is my own original work.
2. This thesis has not been submitted for examination at any other tertiary institution.
3. This dissertation does not contain data, pictures, graphs, or additional information unless expressly acknowledged as being sourced from other persons.
4. This does not contain other person`s writing unless expressly acknowledged as being sourced from other researchers. Where other written sources have been quoted, then.
    a) Their words have been re-written, but the general information attributed to them has been referenced,
    b) Where their exact words have been used, and their writing has been placed inside quotation marks and referenced.
5. Where I have used material for which publications followed, I have indicated my role in the work.
6. This dissertation is primarily a collection of material prepared by me.
7. Unless expressly acknowledged, this thesis does not contain any text, graphics or tables copied and pasted from the internet.

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Dr. Hlengiwe P. Mbongwa (Co-Supervisor)

DEDICATION

I dedicate this dissertation to my Heavenly Father, GOD the Creator (LORD GOD), my source of strength.

To my loving daughter Dineo Hope Moloi for her support and understanding during hard times.

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To my Heavenly Father, God the Creator, who arrange this skillful team to journey with me. I thank you.

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Prathna – Postgraduate Officer in the Department of Health Science, Physiology (Medical School)

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My friend, Tutu

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ABBREVIATIONS

ADA	American Diabetes Association
ADHD	Attention Deficit Hypertensive Disorder
AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Care
ARV	Antiretroviral
ART	Antiretroviral Therapy
BREC	Biomedical Research Ethical Committee
CAF	Central Analytical Facility
CARMMA	Campaign for Accelerated Reduction of Maternal Mortality in Africa
CDC	Centres for Disease Control and Prevention
CHCs	Community Health Centres
DIP	Diabetes in Pregnancy
DM	Diabetes mellitus
DOH	Department of Health
DNA	Deoxyribonucleic acid
FFAs	Free Fatty Acids
FHD	Family History of Diabetes
GDM	Gestational diabetes mellitus
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human immunodeficiency virus
HPCSA	Health Professions Council of South Africa
IDF	International Diabetes Federation
IGFBP- 1	Insulin -like growth factor -binding protein 1
IGF2BP2	Insulin -like growth factor 2 mRNA binding protein 2
KEVIII	King Edward VIII Tertiary Hospital
MDGs	Millennium Development Goals
MMR	Maternal mortality rate
MTNR1B	Melatonin receptor 1B
NCDs	Non-communicable diseases
NDFS	National Diabetes Fact Sheet
NHRD	National Health Research Department

NRF	National Research Foundation
OGTT	Oral Glucose Tolerance Test
PI	Protease Inhibitors
PMTCT	Prevention of mother-to-child -transmission
PCR	Polymerase chain reaction
PPARα	Peroxisome Proliferator -Activated Receptor -Alpha
UKZN	University of KwaZulu-Natal
UNAIDS	United Nations Programme on HIV/AIDS
US	United States
RFLP	Restriction Fragment Length Polymorphism
SA	South Africa
SAT	Subcutaneous Adipose Tissue
SNP	Single nucleotide polymorphism
SCN	Suprachiasmatic nucleus hypothalamus
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 Diabetes Mellitus
TCF7L2	Transcription factor 7 -like-2
VAT	Visceral Adipose Tissue
WHO	World Health Organization

STUDY OUTLINE

This master`s dissertation is presented in manuscript format, with five chapters which is formatted as follows; Chapter 1: introduction, Chapter 2: literature review, Chapter 3: manuscript 1, Chapter 4: manuscript 2, Chapter 5: synthesis and conclusions, followed by appendices. The abstract stated the background, method, main findings and conclusion of the current study. In chapter one the researcher highlighted the main aim, problem statement of the study and the overview of the methodology. The research objectives were presented with the research questions which cross-examine the proposed objectives. Chapter two has a full detailed literature review regarding this study. The search was positioned around genetics, obesity, and GDM stemming from local and international literature. The reviewed literature assisted the researcher in demonstrating the gap analysis on which the study was based. Chapter three identified GDM prevalence, and associated risk factors among pregnant women -analysed, discussed, and conclude the results of objective one. Chapter four investigated genetic profiles and associated them with SNPs genotypes *MTNR$_1$B* (rs1387153), *TCF$_7$L$_2$* (rs12255372), *PPARα* (rs4253778), in pregnant and non- pregnant women who had been previously diagnosed with GDM in their previous pregnancies. The SNP genotype and BMI in women with and without GDM was also investigated. In this chapter, we compared genotyped and allele frequencies for statistical significance between pregnant and control groups. Chapter 3 (manuscript 1) and Chapter 4 (manuscript 2) will be written in a submission format according to the authors guidelines for the journal. Discussion and conclusion of results were performed based on the research objectives. Chapter five discussed the link between chapter three and chapter four and the overall research findings. Again, included challenges, and limitations of the study, conclusion, and recommendations for future studies.

ABSTRACT

Background

Gestational Diabetes Mellitus (GDM) is regarded as a "silent killer" determined by an abnormal glucose tolerance firstly recognised at any time during pregnancy and disappear after delivery. Carrying a large baby (>4000g), being obese (BMI: .>40kg), HIV positive and multiple pregnancy may increase the risk of postpartum haemorrhage (Hadley *et al.*, 2021). Postpartum haemorrhage is a leading cause of maternal death , affecting 75% of maternal death worldwide (Maternal Mortality, Who Fact sheet, February 2023). Women previously diagnosed with GDM are at higher risk of subsequent type 2 diabetes mellitus (T2DM), and development of GDM in the early gestational week of pregnancy. Babies born from GDM mothers may develop T2DM, and GDM (Farahvar *et al.*, 2019) and become obese or overweight in their young and adolescence life (Egeland & Meltzer, 2010; Lowe *at al.*, 2019; Martinez-Cruz *et al.*, 2021). Previous studies have shown that genetic polymorphisms, obesity /overweight, and environmental risk factors may predispose women to GDM. However, the data in KwaZulu-Natal is limited. Furthermore, screening of GDM in women previously diagnosed with GDM has become compulsory every third year to help those mothers who may have pre-existing DM during pregnancy. Therefore, in this study, we selected black African women previously diagnosed with GDM and aimed to determine the prevalence rate and associated risk factors of GDM in the eThekwini district. Again, we investigated the association between SNP genotypes (*$MTNR_1B$* rs1387153, *PPARα* rs4253778, and *TCF_7L_2* rs12255372) and the development of GDM and obesity.

Methods

Firstly, primary data- the self-data report (a well-structured questionnaire) was performed to determine the GDM prevalence amongst black SA women living eThekwini district. Pregnant and non-pregnant women were randomly recruited from three local health district facilities: KwaMashu CHC, KwaDabeka CHC, and KEVIII Tertiary hospital in KwaZulu-Natal. This study used 87 black South African women with GDM history which included experimental group (twenty-five women with GDM) and control group (sixty-two women without GDM); aged 15-45 years of age, residing in eThekwini district and, attending clinics from the first to the third trimester of pregnancy. The GDM confirmation was performed by the relevant antenatal care clinic on women with GDM, using a standard procedure of 2hr- 75g OGTT as per the Guideline

for maternity Care in SA, (2016:98). Blood samples between 2-4ml were collected from each participant into vacutainer EDTA tube (BD Diagnostic, SA) for molecular analysis. The blood samples were collected for DNA extraction to perform the genetic polymorphisms' investigation and GDM and quantitative metabolic traits in pregnant and non-pregnant women within eThekwini district and its impacts on maternal health. The secondary data was obtained from the healthcare registry system for the pregnant women in the antenatal care clinic. The aim was to measure the initial maternal data of antenatal visits and compare those data with the existing data during the research collection. Secondly, the data was analysed using R. Statistical Computing Software of the R. core Team, 2020, version 3.6.3. Women with a previous diagnosis of GDM were regarded as current GDM and analysed as dependent variables and risk factors as independent predictive variables. Thirdly, BMI was measured as kg/m^2, and the following genetic variants: *MTNR$_1$B* (rs1387153), *PPARα* (rs4253778), and *TCF$_7$L$_2$* (rs12255372) were genotyped for each participant using the PCR-RFLP technique. Sanger Sequencing confirmed results at Central Analytical Facilities (CAF), Stellenbosch University, SA. All results of p-value <0.05 were considered statistically significant.

Results

Approximately 25 women reported GDM, and sixty two had no GDM. GDM prevalence rate is estimated at 28.7 %. GDM was significantly associated with older age above 36 years ($p<0.05$), family history of diabetes mellitus ($p<0.05$), women with 1 or 2 children ($p<0.01$), pre-existing diabetes mellitus ($p<0.01$). BMI (≥ 25 kg/m^2) odds ratio: 6.9; 95%CI; 1.35-5.48; p=0.03, ARV treatment (OR: 3.3 95%CI: 1.10-11.310; p=0.010), and pre-existing DM (OR: 0.23; 95CI: 0.07-0.71; p=0.014) remained risk factors for GDM. All pregnant women with and without GDM had a homozygous G-allele of *TCF$_7$L$_2$* rs12255372. Genetic polymorphism C-allele of *MTNR$_1$B* (rs1387153) and *PPARα* (rs4253778) were not associated with the risk of GDM and obesity ($p>0.05$). After the combination of three SNPs profiles (rs1387153, rs12255372, 4253778), genotype CC (rs4253778), CC (rs1387153), and GG (rs12255372) were significantly higher in the pregnant women without gestational diabetes mellitus and obese participants ($p<0.05$).

Conclusion

The GDM prevalence rate was 28.7%, and associated risk factors were as follows: age, parity, pre-existing DM, and family history of diabetes mellitus. ARV treatment, pre-existing DM, and overweight were independent risk factors of GDM. In this study G homozygous of *TCF$_7$L$_2$*

rs12255372 was a genetic marker in the population of black SA women in eThekwini, KwaZulu-Natal. Women with CC and GG genotype are at high risk of developing GDM and obesity. This study shows that SNP genotypes CC *MTNR$_1$B* rs1387153, *PPARα* rs4253778 CC genotype, and GG genotype of *TCF$_7$L$_2$* rs12255372 are susceptible to women developing obesity.

Key words: Gestational diabetes mellitus, genetic polymorphism, obesity.

CHAPTER 1: INTRODUCTION

1.1 Introduction

A relationship between a mother and her baby is the most intimate of human relationships. The foetus shares every breath that the mother breathes and every meal that the mother eats. A foetus acquires nutrients and disposes of its wastes through mother`s blood. Despite - this, in most human pregnancies and animal pregnancies, there is a delicate relationship between foetal demands and the maternal supply of nutrients (Bowman *et al.,* 2021). The fragile relationship is due to the maternal obesity, environmental factors including lifestyle diet, genetic differences that coexist between the mother and the foetus. In other words, the mother and her foetus do not share identical genetic profiles. In some cases, this maternal-foetal relationship can lead to complications in pregnancy due to abnormalities, which can occur at the uteroplacental interface, at the level of foetal-maternal signalling, and in the placental vascular system (Espinoza, 2016; Muglia *et al.,* 2022).

Some of the outcomes or consequences associated with abnormal maternal-foetal relationships include complications in pregnancy such as gestational diabetes mellitus (GDM), preeclampsia, gestational hypertension, pre-term labour, caesarean -section (c-section) and in extreme cases foetal and maternal death. Their offspring are at higher risk of developing both short and long - term complications, such as neonatal cardiac dysfunction, macrosomia, foetal growth restriction (FGR), obesity, impaired glucose tolerance (IGT), and diabetes in their early and late adulthood, hypoglycaemia which leads to mental and personality disorders, and a recurrence of seizures (Kamana *et al.,* 2015; Farahvar *et al.,* 2019; Muglia *et al.,* 2022). Therefore, achieving a successful pregnancy is contingent on a balanced relationship between foetal-maternal demand for nutrients as a measured maternal investment to safeguard her reproductive future (Haig, 1993; Hanson *et al.,* 2015; Bowman *et al.,* 2021). However, inadequate, harmful, or poor maternal - foetal interaction can lead to a conflict of interest between a mother and the foetus, when eventually results in complications in pregnancy (Pijnenborg *et al*., 2008; Muglia *et al.,* 2022).

The process of "foetal-maternal conflict" -, which is also known as maternal -foetal relationship is therefore, a conceptual framework in which foetal growth and development occur to the detriment of the maternal well-being (Fowden *et al*., 2011; Pijnenborg *et al.,* 2008; Constancia *et al.,* 2002; Haig, 1993; Muglia *et al.,* 2022). Foetal -maternal conflict is a complex mechanism

through relationships, that could give rise to complications in pregnancy such as GDM, preeclampsia, preterm labour, FGR, macrosomia, c-section, neonatal and maternal death.
Genome -wide association study (GWAS) and meta-analysis of candidate genes, the link between GDM and type2 diabetes mellitus (T2DM) was established based on the following genes: Glucokinase (*GCK*), Transcription factor 7-like 2 (*TCF_7L_2*), Potassium Channel Inwardly Rectifying Subfamily J Member 11 (*$KCNJ_{11}$*), Cyclin -dependent Kinase -5 Regulatory Channel Subunit-Associated Protein -1-like 1 (*$CDKAL_1$*), potassium voltage -gated channel Subfamily Q member 1(*$KCNQ_1$*), Insulin Receptor Substrate 1 (*$IRIS_1$)*, Melatonin receptor 1B *($MTNR_1B$)*, and insulin -Like Growth Factor 2m RNA Binding Protein 2 (*IGF_2BP_2*) (Lowe *et al*., 2016). This suggested that both GDM and T2DM share a common genetic structure to an extent. Furthermore, HK domain -dominating protein-1(*$HKDC_1$*) and Beta-Site App -Cleaving Enzyme 2 (*$BACE_2$*) were associated with maternal metabolic traits (Lowe *et al.*, 2016).

Genome -wide association studies and prospective case-control studies demonstrating the significant association between genetic polymorphism and GDM are higher to Caucasian and Asian (Popejoy *et al.* 2016; Cho *et al*., 2009; Lauenborg *et al*., 2009; Wang *et al*., 2011; Huopio *et al.*, 2013; Popova *et al.*, 2021), but less in the black African women, where the prevalence of DM, GDM and obesity is high (Macaulay *et al.*,2014; International Diabetes Federation, 2021; World Health Organization, 2014). Therefore, this study aimed to investigate the genetic variants of *MTNR1B* (rs1387153), *PPARα* (rs4253778), and *TCF_7L_2* (rs12255372) in the genetic background of pregnant and non-pregnant women in the black SA population.

This study wants to check if the selected genetic variants that were previously shown to be associated with GDM and obesity could be found in black South African women, who may be susceptible to developing GDM. We hypothesised that knowing the targeted genetic variants also called single nucleotide polymorphisms (SNPs) genetic profile in black SA women can influence the maternal risk of developing GDM, maternal obesity, maternal hypertension, preeclampsia, and possibly other pregnancy-related complications. Therefore, due to maternal health and mortality; and its relationship to biological and social determinants, this study attempted to find a beneficial solution towards improving maternal and child health and ultimately decreasing maternal mortality within the eThekwini Health District, KwaZulu-Natal, South Africa.

1.2 Research Problem

Gestational diabetes mellitus is a severe pregnancy complication that may lead to maternal and neonatal mortality during pregnancy (Wahabi *et al*., 2017; Lagese *et al*., 2016; Tavera *et al.*, 2022). Maternal mortality refers to the death of a woman while pregnant or within 42 days of childbirth, or termination of pregnancy, irrespective of the duration and site of the pregnancy from any cause related to or aggravated by the pregnancy. The maternal mortality ratio (MMR) measures the number of maternal deaths from pregnancy-related causes per 100 000 live births during pregnancy (WHO, 2019). Many countries have improved MMR from 2015 to 2017 globally (UNICEF, World Bank Group). In the US (19 to 17.4), Burma (18 to 250), Italy (4 to 2), Spain (5 to 4), South Korea (11 to 9.9), South Africa (138 to 119), Tanzania (398 to 578). Figure 1-1. showed that Burma, South Africa, and Tanzania are still experiencing high maternal death rate.

According to Centres for Diseases Control and Prevention (2019), and the National Institute for Health and Care Excellence (NICE) guideline pathway (2021), black African women (41/ 100 000) are 3 times more likely to die due to pregnancy-related complications than white women (13/ 100 000). Approximately 75% of all maternal deaths are due to difficulties during labour, severe bleeding (mostly bleeding after childbirth), and pre-eclampsia (high blood pressure) (Maternal Mortality, WHO Fact Sheet, February 2023; Lagese *et al.,* 2016). These difficulties do not exclude young girls (15- 19 years) who are dying globally due to pregnancy and childbirth complications (Maternal Mortality, WHO Fact Sheet, Jan 2020). However, severe haemorrhage has been observed as a leading cause of maternal death in the black African population (Kyi *et al*., 1988; Khan *et al*., 2006; Legesse *et al*., 2017; Manyeh *et al*., 2018; Lancaster *et al.,* 2020; Sabato *et al.,* 2020). Researchers suggested that most women who developed GDM had underlying conditions such as pre-gestational diabetes, pre-pregnancy obesity, and overweight/obesity (Jaffe *et al.,* 2020; Lautredou *et al.,* 2022), and this is higher among black African women than white women (Centres for Diseases Control and Prevention, 2018; Njete *et al.,* 2018; Tiwari *et al.,* 2022). Furthermore, the risk for early onset of delivery is higher among pregnant women with DM and GDM, when compared to women without DM and GDM (Tavera *et al.,* 2022). Therefore, this study aims at identifying the prevalence rate of GDM and associated risk factors in black African women.

While current literature suggests that there has been a decline in maternal mortality in South Africa, it remains unclear whether pre-existing conditions (biological and social) during pregnancy are a crucial factor in determining maternal mortality in the country. More specifically, according to WHO, 830 women die every day worldwide because of complications during pregnancy and childbirth (WHO, 2017; Maternal Mortality, WHO Fact sheet, February 2023). South African Department of Health (DoH) estimated 72% of these maternal deaths in Africa (DoH, 2018). An effort known as the Campaign for Accelerated Reduction of Maternal Mortality in Africa (CARMMA) was implemented in May 2012 at Osindisweni Hospital in South Africa to reduce the maternal death rate by 75% in 2015. Regardless of the measures that have been put into place, maternal mortality remains a public health concern within the South African context.

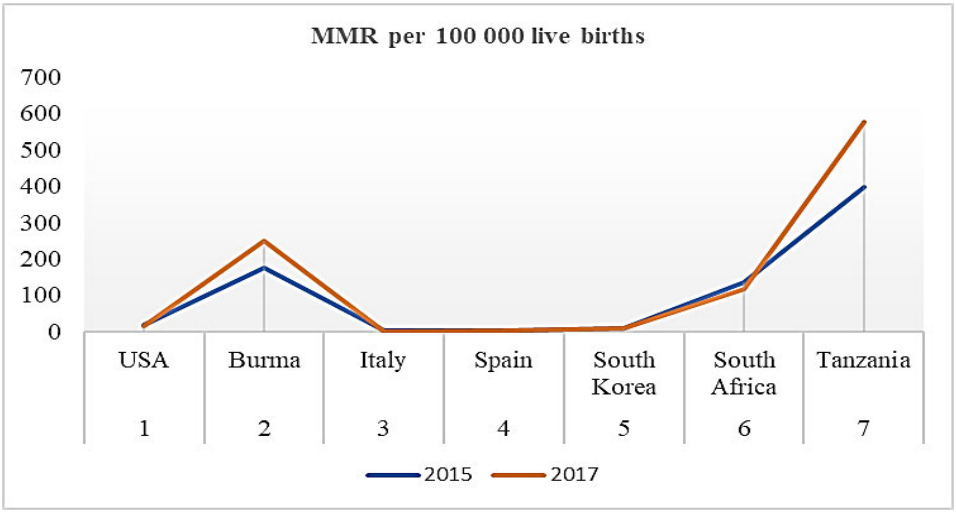

Figure 1-1: Illustrating increase prevalence of maternal mortality in African women.
*MMR-maternal mortality ratio. Source: WHO, UNICEF, UNFPA, World Bank Group, the United Nations populations Division (September 2019).

1.3 Aims

The aim of this study was to examine the association between the following genetic variants, *MTNR$_1$B* (rs1387153), *TCF$_7$L$_2$* (rs12255372), *PPARα* (rs4253778) and their predisposition to develop GDM and obesity in the eThekwini District, KwaZulu-Natal, South Africa.

1.4 Objectives

The research objectives of this study were:

- To determine a pilot of the prevalence of gestational diabetes mellitus in women with a previous history of GDM and identify associated risk factors among pregnant women attending antenatal clinic in eThekwini district, KwaZulu-Natal, South Africa.
- To investigate the relationship between SNP genotypes of *$MTNR_1B$ (rs1387153), TCF_7L_2 (rs12255372), PPARα* (rs4253778), and risks of GDM, and obesity in the study population.

1.5 Research Questions

When addressing this research problem statement, the following research questions were relevant for investigation:

- What is the prevalence of gestational diabetes mellitus, and the associated risk factors among women attending antenatal care (ANC) clinic in eThekwini district, KwaZulu-Natal?
- What is the associations of SNP genotype *$MTNR_1B$ (rs1387153), TCF_7L_2 (rs12255372), PPARα (rs4253778)* and GDM and obesity in the study population?

1.6 Overview of the Methodology

This research was a prospective case-control study. The study was conducted between the end of January 2020 -till the middle of March 2020. The study was conducted in three (3) sub-district healthcare facilities, namely: King Edward VIII Tertiary Hospital Antenatal Care (ANC), KwaDabeka Community Health Centre (CHC), and KwaMashu CHC in Durban, South Africa. One hundred and three participants were randomly recruited (seventy–three pregnant, and thirty non-pregnant women) at their respective sites. However, sixty-two women had non-GDM, and twenty-five had GDM.

The main reason the researcher chose this municipal area was because of the high African population (74%), dominated by females (50.4%), and 61% were below 35 years old (eThekwini Health District Plan, 2018/19 -2020/21). Participants were included if the following criteria were met: Signed informed consent form, black South African female aged between 15 – 45 years old, on ARV treatment, suffering from metabolic syndrome, pregnant, and visited ANC clinic for the first time in their gestational age from first to the third trimester of pregnancy at CHCs approved by the districts. Patients were excluded from the study if non-black SA females were not attending CHC approved by the districts and were aged below 15 years old and above 45 years old. The

biomedical research ethics committee approved the study -(BREC: BE 378/19), the Department of Health (DoH), National Health Research Department (NHRD: KZ_201909_040).

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CHAPTER 2: LITERATURE REVIEW

2.0 Literature Review Summary

Gestational diabetes mellitus (GDM) is significantly affecting pregnant women; it is more likely to become highly prevalent since obesity rates are rising globally. GDM development and complications can be accurately predicted in early pregnancy with the help of effective interventions employed. Clinical risk factors for GDM have already been established; however, they are not enough to accurately predict GDM risk. Thus, this chapter presented different biomarkers for GDM that further contributed to understanding of its pathogenesis. Again, this chapter discussed the link between genetic variants and GDM. Overall, this study has selected three genes, namely: the *MTNR$_1$B* gene, *TCF$_7$L$_2$* gene, and *PPARα* gene, for investigating, and predisposition to developing GDM and obesity.

2.1 Introduction

This chapter covers the background and pathophysiology of Gestational diabetes mellitus (GDM), risk factors, and genetics. The literature will help us to find the gaps we aim to close. The focus on this review will be more on gestational diabetes mellitus, but firstly, the pregnancy needs to be clarified.

The period of 40 weeks from conception to birth when a woman carries one or more foetus developing in the uterus is called the pregnant state. The non-pregnant state is a woman without any developing foetus in the womb. Pregnancy is divided into three trimesters namely: The first trimester starts from conception 1 to 12 weeks (embryo and placenta formation). During this stage it is common that pregnant women lose their babies (miscarriages) (Pontius & Vieth, 2019) because of delicate organs being formed during this stage (Thomas, 2020). The second trimester starts from 13 to 28 weeks (Foetus begins to move), and the third trimester begins at 29 to 40 weeks (Thomas, 2020).

A foetus is considered a full term when it reached 37 to 42 weeks (Thomas, 2020). Due to complications in pregnancy, some pregnant mothers develop GDM, hypertensive disorders including high blood pressure and preeclampsia, ended up delivering their babies before 39 weeks (early term), and/or before 37 weeks (preterm labour) depending on the severity of the diseases.

Attending Antenatal Care (ANC) may help pregnant women to prevent severe complications in pregnancy that may lead to macrosomia (≥4.5kg), preterm infants, intrauterine foetal growth and even death (Lagase *et al*., 2016; Tavera *et al.*, 2022). Women who attend full ANC schedules are less likely to develop severe complications than those who did not (Khambule, 2016; Bountogo *et al.*, 2021). Therefore, pregnant women are recommended by WHO to attend ANC visits to prevent severe complications in pregnancy such as diabetes mellitus.
The development of severe complications in pregnancy diabetes mellitus and the maternal-foetal conflict between the mother and the foetus became the pathophysiological centre of this study.

2.1.1 Diabetes Mellitus

Diabetes mellitus (DM) is described as a "metabolic disorder" characterised by chronic hyperglycaemia with the disturbance of carbohydrate, lipids, and protein metabolism resulting from a defect in insulin secretion, insulin action, or both (American Diabetes Association, 2020; World Health Organization, 2013; WHO Fact sheet April 2023). Globally, diabetes Mellitus is a leading cause of premature death in people below 60 years of age (International Diabetes Federation (IDF), diabetes atlas, 2021-10th edition). Furthermore, it has claimed 416 thousand people`s lives in the African region, and more than 50% of people living with DM remained undiagnosed (IDF, diabetes atlas, 2021 -10th edition; Grundlingh *et al.*, 2022)

In South Africa, diabetes mellitus has shown an increase from 1.9m -4.2m when compared to Nigeria (3.1m -3.6m); United Republic of Tanzania (472.900 -2.9m); Ethopia (1.4m – 1.9m) and Democratic Congo (730.700 -1.9m) (IDF diabetes atlas , 2021-10th edition) . Diabetes mellitus is affecting more females than males (WHO, 2016; Grundlingh *et al.*, 2022). In KwaZulu-Natal, it has affected more than 50 % of people, and most of the population is black (71%) compared to other populations, such as Indians (16.3%), and coloureds (2.2%) (eThekwini Health District, 2018/19).

Diabetes Mellitus is categorised into several types such as type 1 diabetes mellitus (T1DM) – insulin dependent type due to insulin deficiency- affects children, teenagers, and young adults. Type2 diabetes mellitus (T2DM) – non-insulin dependent, as a result of insulin insufficient supply- known to affects adults but know is more common among children, adolescents, teenagers and young adults due to the increased rate of overweight and obesity in the United States , and South Africa (Nadeau *et al*., 2016; Negash *et al*., 2017, Otitoola *et al*., 2021) . Gestational

diabetes mellitus (GDM) is a type of DM that is "only" triggered by complications in pregnancy and become T2DM 5 years later after delivery (Kim *et al.,* 2002; Farahvar *et al.,* 2019). Our review will focus more on the pathophysiology of GDM.

2.1.2 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a consequence of maternal -foetal abnormal relation during pregnancy. This relationship is based on the maternal obesity, environmental factors and genetic exchanges between a mother and a foetus at the placenta. Here, gestational diabetes mellitus, high blood pressure, iron -deficiency anaemia, maternal obesity, severe nausea, and vomiting are common complications that are associated with pregnancy (Chortatos *et al.,* 2015; American Diabetes Association, 2020; Fejzo *et al.,* 2019). Women with previous diagnosis of T1DM, T2DM and GDM in their previous pregnancies are more at risk of developing GDM in their first trimester of pregnancy. Approximately 16.7% (21.1 million) women of live births globally are affected by hyperglycaemia during pregnancy, of which 80.3% were GDM, 10.6% pre-existing diabetes mellitus, and 9.1% diabetes (type1 and type2) first detected in pregnancy (IDF diabetes atlas, 2021-10th edition). Although GDM disappears within 6 weeks of delivery, about 50% of women that are diagnosed with this metabolic condition are projected to develop type 2 diabetes mellitus (T2DM) at a later stage of their lives – between 10 to 50 years after delivery (Lee *et al.,* 2007; NDFS, 2011; Li *et al.,* 2020). Infants born from mothers with GDM are at high risk of developing T2DM or impaired glucose tolerance (IGT) (Kampman *et al.,* 2015; Farahvar *et al.,* 2019). Uncontrolled GDM may lead to hypertension and preeclampsia, which can lead to more severe complications, including miscarriages in the first trimester, and foetal macrosomia which can cause severe complications. These complications may lead the mother to undergo caesarean section (c-section), induction of labour, stillbirth, or death of a mother. Foetal severe complications may include neonatal -cardiac dysfunction, hypoglycaemia, a neurological disorder (mental retardation), recurrence seizure activities, developmental delays, personality disorder, obesity, impaired glucose tolerance (IGT), ADHD and T2DM in early or late adulthood (NDFS, 2011; Kamana *et al.,* 2015; Farahvar *et al*., 2019; Muglia *et al.,* 2022).

2.1.3 Screening and Diagnosis of GDM

The World Health Organization (WHO) defined GDM as a carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy (WHO, 1999). According to WHO, (2013) gestational diabetes mellitus is regarded as any degree of

glucose intolerance detected for the first time, either early or later during pregnancy. This includes undiagnosed women with T2DM in an early gestational week (1st trimester), and actual or true GDM which develops later in pregnancy. The revision of screening and diagnostic criteria was done to improve the severe complications in pregnancy including macrosomia, hypertension, and preeclampsia (WHO, 2013). Table 2-1 illustrated the difference between the two screening methods as revised by WHO and other health experts.

Gestational diabetes mellitus is diagnosed when one or two blood glucose values are high (WHO, 2013). There is still a debate among health professionals regarding the screening and diagnostic criteria (Metzger *et al*., 2010; Luewan., 2018; Chi *et al*., 2018; Cheung *et al.,* 2019; Vince *et al.*, 2020). The adoption of 2013 WHO criteria without the 1-hour glycaemic value, decrease the rate of GDM. However, lowering of fasting glucose threshold identified women who may benefit from treatment. Moreover, the raising of 2-hour threshold may fail to identify women at high risk of adverse pregnancy outcome and future metabolic diseases (Chi *et al.,* 2018; Cheung *et al.,* 2019). The use of one value for the diagnostic criteria misses 15-20% of women with GDM (Metzger *et al*., 2010; Sacks *et al.,* 2012; Coustan *et al.,* 2021; Vince *et al.*,2020), suggesting that the use of a one -step approach may not be recommended in a busy antenatal setting (Luewan *et al.,* 2018).

The gold standard test for diagnosing GDM is the use of a 75g, 2-hour, Oral Glucose Tolerance Test (OGTT) for all pregnant women visiting ANC for the first time (American Association of Diabetes (ADA), 2018; ADA, 2020). Furthermore, for women who had no pre-existing diabetes with no GDM testing, a repeat must be conducted between 24 -28 weeks gestation, and finally checked between 6 – 12 weeks after delivery. However, glucose screening is prioritised in pregnant women at high risk for GDM, such as those who had a history of GDM, a new-born larger than 4.5 kg, stillborn infant, miscarriages, abortion, or a baby with birth defects and family member (mother/father) with T2DM. Women with a history of GDM should be screened every 3 years, and if pre-existing DM (T1DM or T2DM) is detected, treatment as part of the intervention is considered to prevent further complications in pregnancy (ADA, 2018; ADA, 2020). Therefore, follow -up studies are needed to screen women previously diagnosed with GDM.

Table 2-1: The screening and diagnostic criteria for GDM globally

Diagnostic criteria	Screening Method	Expected Results
World Health Organization - (1999)	1-step, 75 g 2 hours, OGTT	
		Impaired glucose tolerance
	Fasting	<7.0 mmol/L
	2 hours	≥7.8 mmol/ L
		Diabetes
	Fasting	≥7.0 mmol/L
	2 hours	≥11.1 mmol/L
World Health Organization	1 -step, 75g, 2hours, OGTT	
(2013) – Revised version	Fasting	≥ 5.1 mmol/L
	1 hour	≥10.0 mmol/L
	2 hours	≥8.6 mmol/L

***Please note:** Venous blood for plasma glucose is drawn after 1 hour and 2 hours of ingestion of 75 g oral powder glucose.

2.1.4 The prevalence of GDM globally

The prevalence of GDM is increasing globally (Figure:2-1), with an increase in the prevalence of obesity (National Centre for Health Statistics, 2018; Centres for Disease Control and Prevention (CDC), 2018).

In 2019, International Diabetes Federation (IDF) reported a GDM prevalence of 15.8%, with GDM prevalence ranging from 7.5% to 27% globally. According to the (2021) IDF estimates, GDM affects 16.7% of hyperglycaemia in pregnancy-representing 21.1 million live births annually, worldwide. These GDM prevalence increase was previously projected for the year 2030 and 2045 in the IDF (2019) Atlas 9th edition. The latest International Diabetes Federation report (IDF, 2021 Atlas , 10th edition) has shown that the prevalence of GDM is increased from 8.6% to 28.0% worldwide. Moreover, the prevalence /affected live births is still higher to women in SEA (25.9%/6.8m); WP (14.0%/3.9m), and Africa (13.0%/4.1m). However, the majority (87.5%) of cases of hyperglycaemia in pregnancy is noted in low and middle -income countries where access to antenatal care is limited.

The variations in the GDM prevalence could be environmental factors such as patten of lifestyle, diet, ethnicity, overweight/obesity and genetic polymorphisms (Nguyen *et al.,* 2018; Adam and

Rheeder, 2017; Harper *et al.*, 2016; Lauring *et al.*, 2018; Corrado and Pintaudi, 2018; Mizger *et al.*, 2021; Wan *et al.*, 2019; Wang *et al.*, 2021; Wang *et al.*, 2022). The variation of GDM was observed in the African continent such as Sub-Saharan Africa, and South Africa (Muche and Gete, 2019; Dias *et al.*, 2019; Mamabolo *et al.*, 2007; Adam and Rheeder, 2017; Macaulay *et al.*, 2018).

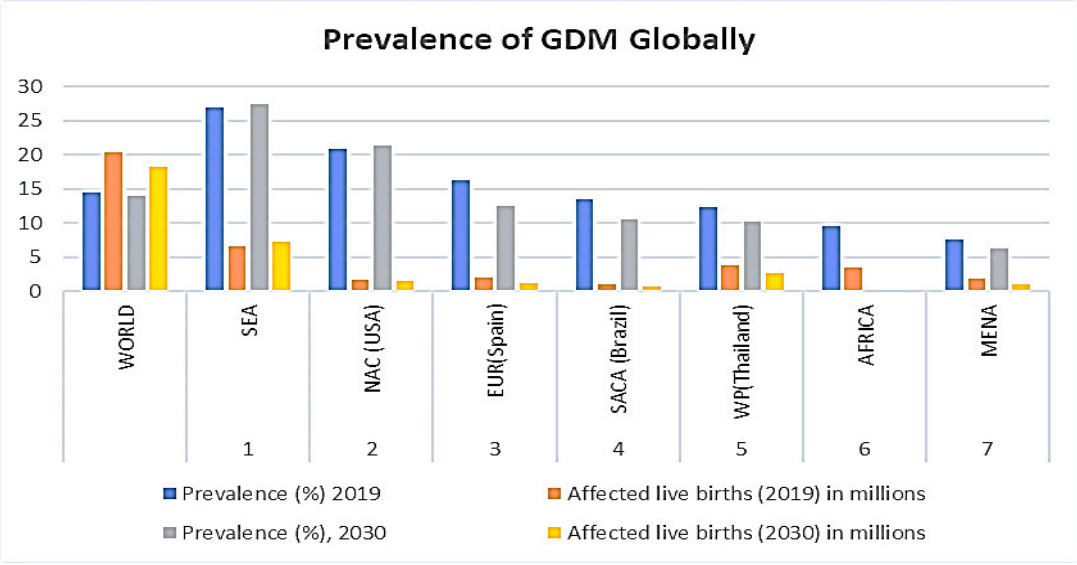

Figure 2-1: Estimation of GDM in women aged between 20-49 years worldwide (IDF, diabetes Atlas, 2019 9th edition)

*SEA-Southeast Asia, NAC -North America and the Caribbean, EUR – Europe, SACA – South and Central America, WP-Western Pacific, AFR -Africa, MENA – Middle East, and North Africa.

2.2 The Pathophysiology of Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus is defined by the relationship between maternal obesity, genetic makeup, and environmental risk factors (Rodrigo & Glastras, 2018; Shaat and Groop, 2007). Rodrigo and Glastras (2018) found that clinical risk factors of GDM comprise obesity/overweight, family history of diabetes, ethnicity, and age; however, these established clinical risk factors lack specificity for GDM development. Thus, using biomarkers to predict models of GDM can enhance the capacity to identify women predisposed to GDM before its development.

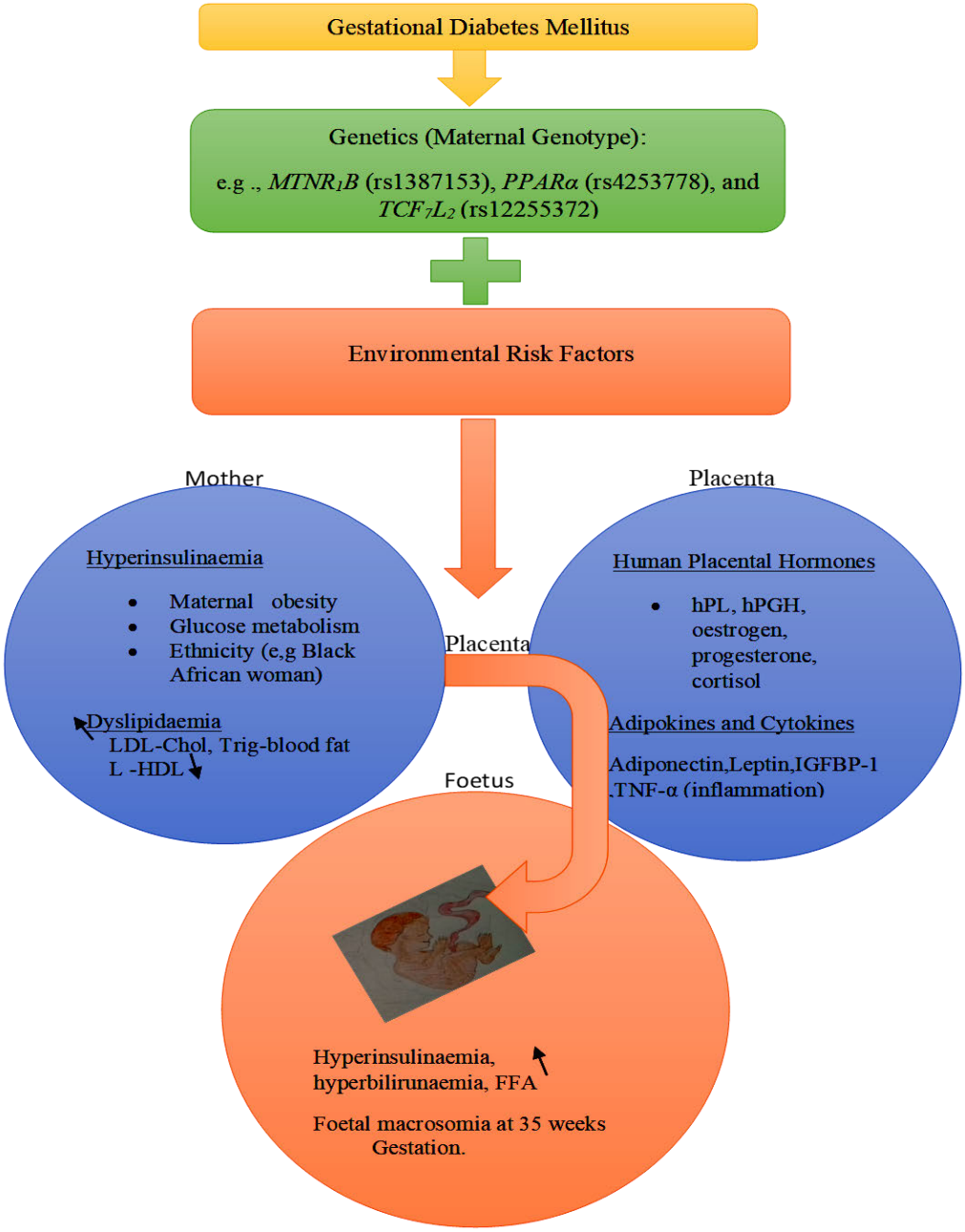

Figure 2-2: An illustration showing a pathophysiologic mechanism of gestational diabetes mellitus. *Reproduced (Rodrigo & Glastras, 2018)

2.2.1 Glucose Metabolism and GDM

Developing GDM during pregnancy has been associated with insulin resistance (IR) and hyperglycaemia (Alfadhli, 2015; Ara *et al.*, 2022). High maternal blood glucose level increases the risk of having foetal macrosomia (Sesmilo *et al.*, 2020).During pregnancy, the maternal metabolic adaptation for pregnant mothers is to maintain blood glucose level of 5.1 mmol/l before a meal, 1-hour after a meal: ≥10.0 mmol/l and 2-hour after a meal: 8.5 mmol/l (WHO, 2013). This metabolic adaptation is important to meet the energy demand of the foetus and to prepare a mother for delivery and lactation (Zeng *et al.*, 2017). The pregnant mothers who fail to meet the insulin demand develop GDM (Napso *et al.*, 2018), but the exact cause of GDM development and the conflict between the mother and the foetus remain unclear.

During pregnancy, the placenta plays an important role in transporting maternal nutrients and glucose to the developing foetus. However, the excess amount of glucose transferred through the placenta may lead to foetal overgrowth. "Maternal -foetal conflict" begin when the glucose metabolism of pregnant women changes to meet the nutritional needs of both the mother and the foetus (Lain and Catalano, 2007). This mechanism makes the fasting glucose concentrations of women with normal glucose tolerance decreases as gestation progresses, which then results in insulin resistance to some extent in all pregnant women, and by the late pregnancy (Jovanovic and Pettitt, 2001). Furthermore, the insulin level of pregnant women decreases to one- third as compared to non-pregnant women (Lain and Catalano, 2007).Therefore, the increase in insulin resistance in pregnant women facilitates a continuous glucose transfer through the placenta to the foetus (Robitaille and Grant, 2008).

2.2.2 Human Placental Hormones and GDM

Normal pregnancy is considered a state of insulin resistance due to "Diabetogenic hormones" (human placental hormones). In the early pregnancy, the placenta releases human placental hormones, to take over normal regulatory pathways. The increased levels of progesterone, prolactin, cortisol, and placental lactogen contribute to the development of insulin resistance in pregnant rate, whether they have the same effect in human pregnancy remains unclear-but diabetogenic hormones released by the placenta block mother`s insulin to increase the insulin resistance (Ryan *et al*., 2011).

Human Chorionic gonadotrophin (hCG) released earlier secretes the corpus luteum to produce progesterone hormone ensuring the alignment of the uterus during pregnancy (Wadhwani *et al*., 2017). Human Placental Lactogen (hPL) hormone increases blood glucose levels so the foetus can get the required amount of nutrients from maternal extra blood glucose. Human placental lactogen and prolactin play a role in the elevation of blood glucose by upregulating islets of Langerhans during pregnancy (Parsons *et al.,* 1992; Sorenson *et al*., 1997). The hPL is 10 times higher in the second trimester (Kamana *et al*., 2015). In the maternal fasting state, hPL hormone secretes lipolysis increasing free fatty acids to supply the mother and foetus with amino acids (Kampmann *et al.,* 2019). The placental lactogens are associated with GDM and foetal macrosomia (Sibiak *et al.,* 2020)

In normal pregnant state, approximately at 20 weeks gestational age, Growth hormone (GH)-pituitary which is found in the non-pregnant state is replaced by human placental growth hormone (hPGH) during pregnancy (Kampmann *et al.,* 2019). As the pregnancy progresses, the level of placental hormones also increases and contributes to the decreasing insulin sensitivity in adipocytes and skeletal muscles by interfering with insulin receptor signalling. The impairment of insulin action increases in the skeletal muscle than in adipose tissues, indicating the development of GDM during the second and third trimesters. Again, level of insulin resistance rises especially during the third trimester of pregnancy (Barbour *et al.,* 2007; McIntyre *et al.,* 2009). Excessive mother`s blood glucose in the circulation crosses the placenta to cause foetal overgrowth. Interestingly, placental hormones dropped with GDM after delivery. High progesterone level, prolactin level in the early pregnancy were identified as risk factors for GDM (Park *et al.,* 2013; Li *et al.,* 2020).

2.2.4 Adipokines, Cytokines, pro-inflammatory, growth factors and GDM

Glucose is an important source of nutrition in the red blood cells and the brain in the central nervous system of the mother and foetus (Hatfield & Lynyak, 2011; Reece *et al*, 2011). Most of the mother`s blood supply (nutrients, vitamins, glucose, and oxygen) is transported through the placenta to the foetal circulation (Michelsen *et al.,* 2019). Overnutrition or undernutrition may affect the developing foetus. Maternal obesogenic nutrition induces insulin resistance and increased foetal blood glucose levels (Ford *et al*., 2009). These events need to be clarified. The human placental hormones, adipokines, cytokines, growth factors, inflammations and lipid are all secreted by the placenta during pregnancy. They are found abundantly and increasing in the

maternal circulation from first to the third trimester and decreases after delivery (Hardie *et al.*, 1997; Stock and Bremme, 1998; Hills *et al.,* 1996; Wang *et al.,* 1995). Still, contribute to the development of GDM that lead to LGA (Mazaki *et al.,* 2005; Kirwan *et al.,* 2002; Ategbo *et al.,* 2006). However, the metabolic adaptation of obese pregnant women is the same as in normal pregnant state. Here, the increased glucose utilization by the placenta, skeletal muscle and adipose tissue become less sensitive to insulin during pregnancy (Kampmann *et al.,* 2019).

2.2.5 Adipokines

Adiponectin (AdipoQ) is an adipocytokine polypeptide that regulates glucose levels and fatty acids oxidation/breakdown (Catalano *et al.,* 2006; Huang *et al.,* 2019). Low plasma adiponectin concentration correlates strongly with insulin resistance in obesity, T2DM, and GDM during pregnancy (Catalano *et al.,* 2006; Retnakaran *et al.,* 2004; Worda *et al.,* 2004). Lower adiponectin concentration and GDM development was observed in obese pregnant women (Nien *et al.,* 2007), non-Caucasian women (Thyfault *et al*., 2005), pregnant women with pre-pregnancy obesity in their early pregnancy (lliodromiti *et al.,* 2016), and in normal pregnant women in their early gestational week (11 -13 weeks) (Weerakiet *et al* ., 2006).

Leptin is a protein hormone related to the bulk of fat stores. It acts as a glucose sensor in the third trimester. Women with a history of GDM continue to experience a high level of leptin, insulin resistance, and glucose tolerance (Saucedo *et al*., 2011), but this remains unknown. The leptin levels reduce glucose metabolism in early pregnancy indicating the involvement of adipose tissue for later impairments in metabolic flexibility in women at GDM risk (Bozkurt *et al.,* 2018). Leptin and association with GDM are seen in early pregnant women (Qui *et al*., 2005), and in different ethnic/race groups (Yang *et al.,* 2016; Soheilykhah *et al.,* 2011;). Leptin and GDM risk were noted in women with lipid profile, increased BMI, previous miscarriages, stillbirth, and history of C-sections in the African population (Bawah *et al.,* 2019). Therefore, leptin can be used in identifying women at risk for GDM in early to late gestational weeks of pregnancy.

2.2.6 Growth Factors

Insulin-like growth factor-binding protein 1 (IGFBP-1) is a peptide hormone expressed in the decidual endometrium during pregnancy. Insulin-like growth factor -binding protein 1 binds with Insulin-like growth factor 1 (IGF-1), leaving a small fraction of IGF-1 circulating in the maternal plasma. The increase and decrease maternal serum levels of IGF-1 and IGFBP-1 were observed

in severe complication in pregnancy include GDM, obesity, FGR, premature labour, and preeclampsia (Hills *et al.,* 1996; Wang *et al.,* 1993; Ramirez *et al*., 2014; Qiu *et al*., 2005).

2.2.7 Cytokines

Tumour necrosis factors (TNFs) are kinds of proteins that are expressed primarily by immune cells to regulate immune response, inflammation, proliferation, differentiation, apoptosis, and embryogenesis (Elsevier *et al.,* 2016). Tumour necrosis factors are implicated in different diseases such as T1DM by killing beta cells in the pancreas (Thomas *et al*., 2014; Luo *et al.,* 2020). Tumour necrosis factors play a pivotal role in the reallocation of energy reserves, by promoting the breakdown of muscle and fat. Tumour necrosis factors are produced by adipocytes that induce insulin resistance associated with T2DM by decreasing insulin signalling and overriding glucose transport. Cytokines (IL-6, TNF-α) were associated with macrosomia and the risk of GDM in women aged 19 to 42 years (Ategbo *et al.,* 2006).

Tumour necrosis factor-α (TNF -α) is a kind of protein that interferes with insulin receptor signalling and β-cell function having a greater influence on hyperglycaemia. Tumour necrosis factor -α impairment in insulin action associates with TNF-levels, when measured along with hCG, oestradiol, hPL, prolactin, and progesterone. Tumour necrosis factor -α remains the only significant predictor of the change in insulin sensitivity in late pregnancy. Though the placenta can produce TNF-α, over 90% of the circulating TNF-α is of maternal origin. The rise in cytokines is associated with the enlargement of the maternal fat mass (Kirwan *et al.,* 2002). Higher levels of TNF-α are seen in pregnant women developing GDM and macrosomic babies (Zhang *et al.,* 2017; Mohammed and Aliyu, 2018).

Interleukin – 6 (IL-6) is a pro-inflammatory multifunctional cytokine that is significantly higher in women with GDM, compared with healthy pregnant women, independent of adiposity (Amirian *et al.,* 2020). Interleukin -6 is secreted by T cells, and macrophages to stimulate immune response during inflammation and infection (Talaat *et al.,* 2016). Furthermore, they contribute to the chronic inflammatory response in adipose tissue, and the development of pregnancy-induced insulin resistance (Zhang *et al.,* 2017). Interleukin -6 is an independent risk factor in the pregnant women development GDM when assessed in the first trimester (Siddiqui *et al.,* 2019). Serum levels of IL-6 and placental biomarkers are significantly different between healthy group and pregnant women developing GDM in Mongolian population (Zhang *et al.* 2017).

2.2.8 Dyslipidaemia, and Gestational Diabetes Mellitus

Dyslipidaemia is defined as a high level of lipid density lipoprotein (LDL-bad cholesterol) and triglycerides (blood fat), low -high -density lipoprotein (L-HDL), which cause cardiovascular disease (CVD). Cardiovascular disease is one of the severe cases of pregnancy complications that lead to maternal and child mortality worldwide. In the United States, has affected 15.5% of women of which the majority 14% are middle -aged black African women. Dyslipidaemia (high cholesterol) is common among teenagers and young adults, globally. Untreated dyslipidaemia can lead to coronary artery diseases (CAD) which can lead to atherosclerosis, heart attack, and stoke. The mortality rate for CAD, hypertension, and stroke is higher in black women than in women white women (William *et al*., 2009), but black women are unaware of the mortalities from GDM to CVD (Lister *et al.*, 2019).

Lipid metabolism predisposes women to develop GDM during pregnancy. Maternal free fatty acids (FFAs) and triglycerides (TG) levels are independent risk factors for GDM and foetus Larger than Gestational Age (LGA), and newborn birth weight ≥4.5 kg (Schaefer-Graf *et al.*, 2008). Lipid profiles are different between GDM and non-GDM women suggested that women with GDM increase triglycerides and reduce HDL compared with non-GDM (Layton *et al*., 2019). Lipid abnormalities are risk factors for GDM from early to late trimester. Still, increased TG, lower HDL-C, and an increased TG: HDL-Cholesterol ratio were reported in women with GDM, previous history of GDM with macrosomia, and obesity (Jin *et al*., 2016., Wang *et al*., 2018; O'Malley *et al.*, 2020; Rao and Ping, 2021; Hu *et al.*, 2022; Rahnemaei *et al*., 2022). Therefore, lipid abnormalities can be used as a predictor for early and later development of GDM and foetal macrosomia during pregnancy.

2.3 Risk Factors for Gestational Diabetes Mellitus

Gestational diabetes mellitus risk factors are listed according to the guideline selected. The American Diabetes Association, ADIPS, NICE, and SA guidelines (SEMDSA and The National Guideline for Maternity Care in South Africa). South African guidelines include BMI ≥35 kg/m^2, Age ≥40 years old, previous history of GDM, family history (1st degree relative with DM), previous history of unexplained intrauterine foetal death, macrosomia baby of ≥ 4.5 kg.

Women at high risk of developing GDM involve Age ≥25 years, being overweight/ obese (BMI ≥25kg/m^2; ≥30kg/m^2), ethnicity/Race including women from African American, American Indian, American Asian, Hispanic, or Latino, and Pacific Islander, having a first-degree family history of diabetes mellitus, history of GDM from previous pregnancies, history of a newborn with macrosomia (birth weight ≥4.5kg), stillbirth, abortion, miscarriages, parity and gravidity, c-section, premature labour, poor diet and exercise, CVD (hypertension and preeclampsia) and genetic polymorphism (Zhang *et al*., 2021; Filardi *et al*., 2018; Shaat *et al.,* 2007).

2.3.1 Age

Maternal age is a type of risk factor that shows an increase as you get older (Ewenighi *et al.*, 2013). Again, as you get older complications in pregnancy become more severe (National Centre for Health Statistics, 2016).The adolescents, younger women, age ≥ 25 years, ≥35 years and middle-aged women are having a higher risk of developing GDM (ADA, 2018; IDF, 2019; NICE guideline). Maternal age ≥ 25 , and ≥35 years old are independent risk for GDM in United States, China, South Africa, Thailand, Cameroon, and Nigeria (Makgoba *et al*; 2012; Njete *et al.,* 2018; Macaulay *et al.,* 2018; Yong *et al*., 2020; Zhang *et el*., 2011; Zhang *et al.,* 2021; Boriboonhirunsarn *et al.,* 2021).

2.3.2 Body Mass Index (BMI) / Obesity

Obesity is defined as an extra fat sitting in the stomach and birth weight from previous complications in pregnancy, resulting in macrosomia (Lim *et al.,* 2007). During pregnancy, fat distribution shifts from the lower abdomen to the upper abdomen. These effects increased the accumulation of central, visceral adipose tissue and abdominal fat throughout pregnancy and in healthy people (Crowther, *et al.,* 2012).

Fat distribution differs according to ethnicity, sex, and cut-offs in measuring techniques (Crowther *et al.,* 2012; Murphy *et al.,* 2014). Table 2-2 is demonstrating the measuring technique of overweight and obesity worldwide.

In African women fat distribution is shown in lower levels with higher subcutaneous fat mass compared to women in Europe (Crowther *et al*., 2010). People from lower socioeconomic status (SES) are more obese than individuals from higher SES, and quality of food is the main issue (Volaco *et al*., 2018). Environmental factors such as, unhealthy diet, physical inactivity, ethnicity, increased insulin resistance (IR). Moreover, IR contribute to the development of obesity associated with T2DM in pregnant and non-pregnant women (Liu *et al.*, 2008; Volaco *et al.*, 2018;

do Nascimento *et al.,* 2019). In early gestational week of pregnancy, physical inactivity is associated with high risk of GDM among low -in-come pregnant women (do Nascimento *et al*., 2019).

Table 2-2: BMI classifications according to World Health Organisation (WHO).

Category	kg/m^2
Underweight	< 18.5
Normal weight	≥ 18.5 – 24.9
Overweight	≥ 25 – 29.9
Obese	≥ 30

*BMI-Body Mass Index.

Obesity is a predominant risk factor for the development of GDM. Obesity and overweight are the most common findings in women of childbearing age. Overweight and obese people are 10 times more compared to GDM women (Yogev and Catalano, 2009). Young adults exposed to maternal GDM in utero had a higher Body Mass Index (BMI) average (p=0.01) and a high BMI growth trajectory (p=0.008) compared with unexposed adolescents (Crume *et al*., 2011; Miao *et al*., 2017; Yeshaw *et al*., 2020; Feng *et al*., 2019). Maternal obesity was observed in pregnant women developing GDM with macrosomic infant associated with shoulder dystocia and severe bleeding, birth defects (Thepampan *et al.,* 2021; Nguyen *et al.,* 2015), and maternal death (Black *et al.,* 2013; Santangeli *et al.,* 2015; Lim and Mahmood, 2015).

Although these factors significantly contribute to the GDM epidemic outcomes, intrauterine exposures impact the GDM epidemic (Veeraswamy *et al.,* 2012). Therefore, this calls for coordinated and collaborative efforts that provide better perinatal care and postpartum diabetes prevention strategies to escape the vicious cycle.

Genetics and environmental factors are playing an important role in the development of obesity and GDM. People in the same environment can eat the same food and get affected differently. This effect includes obese and non-obese individuals, suggesting that genetics are having a critical role in the development of obesity and GDM.

There are two types of obesity, monogenetic and multifactorial. Monogenic obesity focuses on a specific genetic polymorphism inherited from the family member. However, multifactorial obesity is influenced by several genetic variants, consumption of food carrying high calories, and physical inactivity (Volaco *et al.,* 2018). Adipocyte- C1q – and Collagen domain-containing (*ADIPOQ*) gene, encoding the adipocyte complement -related protein (ACRP) with 30kDa. This gene regulates glucose and lipid metabolism, low adiponectin levels lead to GDM (Takeshi & Aboualizadeh, 2015). Genetic variation of *ADIPOQ* (rs266729) associates with increased fasting blood glucose concentration (Dias *et al*., 2021). However, genetic variants (rs266729 and rs17300539) have not been associated with GDM risk among black South African women (Dias *et al*., 2021).

The increased body fat is associated with *SEC16B* (rs543874) polymorphism and obesity (Lu *et al*., 2016). Genetic variant *SEC16B* rs543874 is a genetic marker of obesity risk in the black South African population (Sahibdeen *et al.,* 2018).
Melanocortin 4 receptor (*MC4R*) stimulates the appetite when bound by the alpha-melanocyte-stimulating hormone. Polymorphisms rs99399609 on the *FTO* gene and rs12970134, rs 17782313 *MC4R* were significantly associated with obesity in children and adolescents (Resende *et al*., 2021; Almeida *et al.,* 2018).

Leptin (*LEP*) is produced by fat cells, and when it is bound by leptin receptor (*LEPR*) it inhibits appetite. Polymorphism rs1137101 on gene *LEPR* had no significant effect on children`s obesity (Almeida *et al.,* 2018). Leptin plays a vital role in maternal glucose metabolism. Leptin is a marker for insulin resistance and obesity. Leptin is associated with a risk of GDM among Iranian women (Soheilykhah *et al*., 2011), linked with insulin resistance in Australian women (Kautzky – Willer *et al*., 2001).

Protein Convertase Subtilisin / Kexin type1 (*PCSK1*) regulates insulin biosynthesis (Hayes *et al*., 2013). Genetic variants rs13179048, rs17085593, and rs6235 of the *PCSKI* gene were associated with fasting plasma glucose levels and GDM in European, Thai, Afro-Caribbean, and Hispanic pregnant women (Hayes *et al.,* 2013). Botha, (2020) found no association between the *PCSKI (*rs17085593) variant and the risk of GDM in black South African women.

2.3.3 Diet

Diet is one of the primary environmental risk factors that control adult-onset disease outcomes throughout the human lifespan. Likewise, the impact of nutritional exposure during pregnancy, and early postnatal periods, can extremely result in short -and long- term phenotypic changes in the generations. For example, maternal intrauterine stimulant affects the foetuses. The response of future predictive factors can lead to permanent transformation in the foetus`s genome and may improve the adaptation to the postnatal environment (Indrio *et al.,* 2017; Lee *et al.,* 2015; Parlee & MacDonald, 2014; Li *et al*., 2014).

In addition, maternal diet controls the foetal life; thus, a complication that results in a loss of appetite can lead to foetal growth restriction due to the decrease of nutrient flow to the baby. This complication increases the possibility of preterm labour and a low -birth -weight baby (Burton and Jauniaux, 2018). The difficulties during pregnancies are commonly associated with the maternal-foetal relationship which is an abnormal foetal-maternal relation (Espinoza, 2016; Muglia *et al.,* 2022). Therefore, taking prenatal multivitamin (folic acid, Iron, vitamin A&D, Omega 3, Amino acids, and calcium) as advised by the clinic professionals during ANC visits may reduce the risk of foetal defects such as low birth weight, neural tube, and help the mother in reducing the risk of severe haemorrhage during and after birth (Hanson *et al*., 2015 ; Thomas, 2020).

2.3.4 Ethnicity

People of certain ethnic /racial are more likely to develop T2DM and or GDM. In the United State, Black, African, Hispanic, American Indian, Native American, and Asian women have a higher risk of developing GDM. Type 2 diabetes mellitus affects 13.2% of African Americans, 12.8% of Hispanics, 9.0% of Asians, and 7.6% of Non-Hispanic Whites (CDC, 2018). Non-Hispanic black women with a history of GDM are more at risk of developing GDM compared with non-Hispanic white women (CDC, 2019). Gestational diabetes mellitus was noted in Asian ethnicity (Filardi *et al*., 2018).

In addition, genetic polymorphism can predispose women to GDM. The increased risk of GDM varies by racial/ethnic group. Transcription factor -7-like 2 genes (*TCF_7L_2*), located on chromosome 10q25.3, is expressed in human pancreatic β-cells. The T minor allele frequency of genetic variant rs7903146 of *TCF_7L_2* gene is predominantly investigated in Caucasian and Asian

in many countries but less in African pregnant women, where GDM prevalence is high. The T allele rs7903146 was associated with GDM in Scandinavian Caucasian, Greek Caucasian women (Shaat *et al.,* 2007; Pappa *et al*., 2011), but not for Southern Polish Caucasian women (Michalak *et al.,* 2016). Again, the relationship between T allele rs7903146 of *TCF7L2* gene and *T2DM* was observed in Algerian general people, and SA Zulu people (Ouhaibi *et al*., 2014; Pirie *et al.,* 2010), but not in Cameroon population (Nguimmo *et al*., 2017). Therefore, genetic polymorphism *TCF7L2* rs7903146 (T allele) can be used to predict women developing GDM.

2.3.5 Family history of Diabetes Mellitus (T2DM)

A family history of diabetes mellitus (FHD) is defined as a first-degree relative (parent and /or siblings) with diabetes mellitus (Annis *et al.,* 2005; Monod *et al.,* 2023). Pregnant mothers who had a family history of diabetes mellitus contribute to the degree of insulin resistance. The glycaemic level during pregnancy is possible because of the compensatory hyperinsulinaemia. Same as in normal pregnancy, insulin resistance increases, and insulin sensitivity decreases in the early pregnancy. The difference here, is that pregnant women with pre-existing DM already has insulin resistance. Here, the insulin demand is doubled compared to women without pre-existing DM. Pregnant women who cannot meet the demands of insulin secretion to overcome insulin resistance develops GDM. However, GDM mothers who inherited T2DM from one of the parents are at higher risk of offspring susceptibility to develop T2DM in their adulthood (Armod *et al.,* 2017). Family history of diabetes mellitus is associated with T2DM and GDM (Hariri *et al.,* 2006; Jang *et al.,* 2011; Monteiro *et al.,* 2016; Di Cianni *et al.,* 2003; Kuti *et al.,* 2011; Erem *et al.,* 2015; Monod *et al.,* 2023).

Furthermore, the family history of type 2 diabetes mellitus is known to share genetics and environmental factors. Type 2 diabetes mellitus and gestational diabetes mellitus shared similar genetic profiles (Kwak *et al*., 2012). Since they share the same background, their risk factors are also expected to be the same. Type 2 diabetes mellitus is genetically found in families that are affected by it. A strong genetic element of predisposition was demonstrated by high incidence in certain ethnic/racial groups, and first-degree family relatives among people with T2DM. The pathophysiology of diabetes mellitus, pathways that are involved in the development of the disease, and the search for the genetic variants susceptible to the development of the disease have been investigated. The association between genetic variants of *MTNR1B* rs 1447352, rs1387153, and GDM was demonstrated in women with a family history of diabetes (first-degree relative)

(Shen *et al.*, 2020). Therefore, family history of diabetes mellitus can be used as screening tool to identify those at risk develop GDM and T2DM.

2.3.6 Pre-existing Diabetes Mellitus

Pre-existing diabetes mellitus is an impaired glucose tolerance (IGT) which is considered an undiagnosed DM either T1DM or T2DM or GDM (ADA, 2018; IDF, 2019, IDF, 2021). Here, blood glucose level is higher than normal but not high enough to detect DM (WHO, 1999, WHO, 2013). Type1 diabetes mellitus people are insulin-dependent because of insulin deficiency from β-cell destruction caused by autoimmune. The disease is more common to children. Therefore, they require more insulin to modulate their glucose levels the prevention of disease progression (IDF, 2012).

Individuals with type 2 diabetes mellitus are non-insulin dependent. The disease develops due to progressive loss of pancreatic β-cell insulin secretion in the background of insulin resistance (Prasad, 2014).

Approximately, 9.1% of diabetes mellitus during pregnancy is detected as T1DM or T2DM globally (IDF, diabetes Atlas , 2021 10^{th} edition). The risk of adverse pregnancy outcome is higher in women with T1DM than general population (Colstrup *et al.,* 2013). People with IGT are at higher risk of developing T2DM and GDM (IDF, 2021).Women undiagnosed with T2DM develop GDM in their early gestational age, and their babies are prone to develop obesity, T2DM or IGT in their childhood (Kuti *et al*., 2011; Aberg *et al.,* 2001).

Pre-existing diabetes mellitus is a contributing factor to pregnant women developing GDM and pregnancy outcome (Xiang *et al.*, 2018), but the way these diseases develop in the early gestational age is not understood. Placenta, human placental hormones, glucose metabolism and BMI in the first trimester, play a critical role in the level increased of insulin resistance, and maternal hyperglycaemia. Again, the amino acids from maternal plasma may have a greater influence on foetal macrosomia, Central nervous system (CNS) birth defects in pregnant women with T1DM and GDM (Anderson *et al.,* 2005; Gallo *et al.,* 2017; Sesmilo *et al.,* 2020). Pre-existing diabetes mellitus was observed in pregnant women with GDM (Lee *et al*., 2020; Njete *et al.*; 2018).

2.3.7 Gravidity and Parity

Gravidity is described as the number of times that a woman has been pregnant, whereas parity is defined as the number of times that a woman has given birth to a foetus with a gestational age of 24 weeks or more regardless of whether the child was stillborn or born alive. For example, gravida 1, para 1(G1P1) means a woman with one pregnancy and full-term delivery. The evidence showed that women born in 1982 have had slightly fewer children (average of 1.02) by their 30th birthday than women born in 1967 who had an average of 1.16 children by the same age.

Multiparous (more than one child) women are at higher risk of subsequent GDM than primiparous (one child) or nulliparous (never been pregnant) women (Luo *et al.*, 2020; Zhang *et al*., 2020). Parity was observed in non-Hispanic black women compared with non-Hispanic white women (Schwartz *et al*., 2015). Therefore, parity is considered a risk factor for GDM in black women.

2.3.8 Macrosomia and other pregnancy outcome

The most severe complication in pregnancy-related to perinatal and mortality is macrosomia. Macrosomia is an independent risk factor for Large gestational age (LGA) neonates ≥4.5kg corresponds to birth weight ≥90th (figure 2-3) for the corresponding gestational age in pregnant women with GDM. Foetal Macrosomia is associated with shoulder dystocia related to labour complications following heavy bleeding (Powe *et al.,* 2016; Thepampan *et al.,* 2021). The prevalence ranges from 2.3% to 22.1 %. Tanzania (2.3%), SA-KwaZulu-Natal (3.4%), Korea (4.3%), Nigeria (4.7%), Italy (6.0%), China (22.1%) Ghana (6.5%), (Said and Manji, 2016; Naicker, 2014; Bordin *et al.*, 2020; Ogunfowora *et al.*, 2019; Bedu -Addo *et al*., 2020; Harvey *et al.,* 2021). However, the mechanism by which the foetus increased in size in the uterus requires an explanation.

The major cause of foetal macrosomia is an increased level of insulin resistance due to maternal hyperglycaemia, dyslipidaemia, obesity, and placental human placental hormonal changes in early and late gestational. Foetal maternal complication begins when excess maternal blood glucose in the mother`s circulation passes through the placenta, adding to the foetal circulation, thereby stimulating the foetus to secrete insulin which leads to hyperinsulinaemia. Extra foetal glucose is then stored as body fat causing the foetus to grow faster and larger (>4kg) than the gestational age with shoulder dystocia in the uterus, which causes more difficulties during labour.

The larger the amount of foetal waste (polyhydramnios fluid) in the womb, the larger the foetus for that gestational age (Kampmann *et al.,* 2019; Kamana *et al.,* 2015).

Furthermore, major complications occur during a vaginal delivery when foetus shoulders are stuck in the birth canal, leading to prolonged labour, excessive bleeding, uterus rupture, and birth trauma including vaginal injuries (Jenner *et al.,* 2018). Foetal complications can lead to brachial plexus injury (nerve connected to the spine of the shoulder and arms), fractures in the upper arm bones and the collarbone (Jenner *et al*., 2018). Although prevalence rate is 2.3% of macrosomia in Tanzania, but postpartum haemorrhage, and shoulder dystocia were reported among cases of maternal death (Said and Manji, 2016).

Uncontrolled macrosomic infants can lead to hypoglycaemia ,which may cause long-term complications, including brain damage, mental retardation, recurring seizures, personality disorder, childhood obesity, metabolic syndrome, and T2DM. Gestational diabetes mellitus, pre-existing DM , undiagnosed diabetes mellitus, obesity before and during pregnancy, multiparity, multigravida, preeclampsia, and history of macrosomic babies may increase the risk of foetal macrosomia (Ovesen *et al.,* 2011; Mohammadbeigi *et al*., 2013; Demirel *et al.,* 2020). Large gestational age (LGA) foetus or foetal macrosomia in the early, and late gestational age is an independent risk factor of GDM and adverse outcome (Sesmilo *et al*., 2020; Jin *et al.,* 2016). Foetal macrosomia was noted in Nigerian women with GDM and pre-existing GDM (Anzaku and Musa, 2013; John *et al.,* 2019), women with previous history of foetal macrosomia (Fuka *et al*., 2020; Mohammadbeigi *et al*., 2013), Cameroonian women with a history of macrosomia and unexplained stillbirth (Egbe *et al.* 2018), in pregnant women presented the history of birth defects, and stillbirth in Turkey (Damirel *et al*., 2020). Therefore, history of macrosomia (≥4.5kg) can be used in predicting women developing GDM in different countries.

Figure 2-3: Showing newborn delivered with weight >4kg. Copied [Medscape: Obstetrics& Gynaecology (Patel, 2020)].

2.3.9 Gestational hypertension

Hypertension (HTN) during pregnancy or gestational hypertension (GH) is defined as the condition noticed for the first time after 20 weeks of pregnancy and disappears after delivery even though the patient might be at higher risk of developing the HTN in the future (Vest and Cho, 2014). The diagnosis during pregnancy is confirmed by two readings six hours apart (systolic ≥140 or ≥90 mmHg (Veerbeek *et al.,* 2015). Gestational hypertension can be a pre-existing and or pregnant induced (preeclampsia) (Vest and Cho, 2014). Gestational hypertension affects 6 to 7% of pregnant women worldwide. Uncontrolled gestational hypertension reduces blood flow in the placenta leading to severe complications, such as IUGR, even to death of both the mother and unborn baby (Solomon and Greene, 2015). Caesarean section – or premature labour can be used as an option to save the life of a mother and the foetus.

Women with pre-existing gestational diabetes mellitus are at higher risk of developing hypertension compared with those without pre-existing GDM. Still, gestational hypertension or subsequent T2DM is an independent risk factor for GDM (Tobias *et al.,* 2011). Gestational hypertension was associated with GDM among Egyptian women in EL-Minya (El Sagheer and Handi, 2018), and Indian women (Mohan and Chandrakumar, 2016). Therefore, gestational hypertension is regarded as a marker for GDM risk.

Table 2-3: Diagnostic criteria for the Blood Pressure. (www.health.harvard.edu)

Blood Pressure Stages

Blood Pressure Category	Systolic mm Hg (upper #)		Diastolic mm Hg (lower #)
Normal	less than 120	and	less than 80
Elevated	120-129	and	less than 80
High Blood Pressure (Hypertension) Stage 1	130-139	or	80-89
High Blood Pressure (Hypertension) Stage 2	140 or higher	or	90 or higher
Hypertensive Crisis (Seek Emergency Care)	higher than 180	and/or	higher than 120

Source: American Heart Association

2.3.10 Pre-eclampsia

Pre-eclampsia (PE) is a major cause of maternal and perinatal mortality worldwide Approximately 2-8% of pregnant women are affected by preeclampsia. Seventy-six thousand women die yearly because of pre-eclampsia; furthermore, 500 000 children die yearly due to pre-eclampsia (Bennan, 2021). The cause of the disease is not known. The presence of protein in the urine and the development of hypertension after 20 weeks of gestational age confirms pre-eclampsia (Turpin *et al.*, 2015). The early development of symptoms such as swelling of the feet, face, ankles, hands, and severe headaches indicates the severity of the disease. This condition (PE) affects the development of the foetus by reducing the oxygen and nutrients supply through the placenta to the unborn baby.

Pre-eclampsia occurs during pregnancy when the placenta is detached prematurely from the wall of the foetus due to hypertension, and back to normal after delivery (Anthony *et al.*, 2016). Most cases are presented in the late preterm (≥37 weeks) period rather than early, below 37 weeks. Pre-eclampsia is associated with maternal obesity, and death (Hansson *et al.*, 2015). People die because of heavy bleeding from placental previa (Solheim *et al.*, 2011). Incident rates for PE are estimated at 15% for pre-term labour, and 25% IUGR. Risk factors include higher BMI, history of PE, multiple pregnancies, maternal age <20 or >35 years old, and GDM. Mothers with GDM have a significant increased risk of developing PE (John *et al.*, 2019). Pre-eclampsia was linked with GDM in Indian women living in the rural Telangana (Reddy *et al.*, 2017). Therefore, it can be used as a predictor for women developing GDM.

2.3.11 ARV Treatment /HAART and GDM

Human immune virus or acquired immune deficiency syndromes (HIV/AIDS) is a pandemic disease that leads to death worldwide (The Lancet, 2016). Antiretroviral Therapy (ART) was initiated to prolong the life of people infected with the diseases (Wang *and* Yang, 2016). Infected individual need to take ARV treatment as instructed by the doctor, but the knowledge behind the treatment is not well understood.

Highly Active Anti-Retroviral Therapy (HAART) was implemented to suppress the viral load, improve CD4 counts, and reduce the mortality rate in HIV-infected people, including pregnant and non-pregnant women. Although recent data indicated that there is an improvement in SA pregnant women on ART treatment (WHO HIV statistics, 2021), studies have shown that certain drugs for HIV infection may predispose women to T2DM (Timmerman *et al.*, 2005), and GDM in HIV pregnant women (Gonzalez-Tome *et al*., 2008).

The development of gestational diabetes mellitus occurs because of HAART, has been reported to induces insulin resistance by causing dysregulation of human placental hormones such as oestrogen, hPL, and hPGH in pregnant women with HIV infection (Jao *et al.*, 2013). The highly active antiretroviral therapy, which is used to prevent mother-to-child transmission (PMTCT) has been demonstrated to alter maternal haemostatic profile. Protease Inhibitor -PI (Indinavir and ritonavir), a class of therapy, is known to increase hepatic glucose. In contrast, Nucleoside Reverse Transcriptase Inhibitors (Stavudine) increase insulin resistance through GLUT-4 transporter reducing insulin secretion through β-cell function. The increased amount of visceral fat with subcutaneous fat creates increased levels of inflammatory cytokines. This effect increases insulin resistance, leading to impaired glucose tolerance and diabetes mellitus (Timmerman *et al.*, 2005). Protease Inhibitor (ARV treatment) is associated with GDM in pregnant women infected with HIV (Biadgo *et al*., 2019; Marti *et al*., 2007; Gonzalez-Tome *et al.*, 2008). Therefore, ARV treatment (PI) is a marker for women with GDM.

2.3.12 Genetics of Maternal – Foetal Interaction

Genetic mutations, maternal obesity and environmental risk factors can predispose women to develop GDM. Gestational diabetes mellitus develops because of pancreatic β-cells losing their ability to compensate for increased insulin resistance during pregnancy (Kwak *et al.*, 2012).

Pregnancy serves as a state in some women who are already genetically predisposed to develop diabetes mellitus (Reece *et al.,* 2009). Genetic variants in the *TCF_7L_2* gene , *$KCNJ_{11}$* gene, *GCK* gene , *HNF1α* Gene, *CDK5-CDKAL1* gene , *$MTNR_1B$* gene were found to be associated with an increased risk of GDM among pregnant women (Shaat & Groop, 2007; Shaat *et al.,* 2007; Kwak *et al.,* 2012). Furthermore, the findings from the *$CDKAL_1$* and *$MTNR_1B$* gene among Korean women further provided evidence that GDM and T2DM shared a similar genetic profile, glucose metabolism, insulin resistance , insulin secretion, and β-cell function (Kwak *et al*., 2012; Kim *et al*., 2011). Here, *$CDKAL_1$* signals molecules on β-cells responsible for β-cell survival Daval *et al.,* 2011), while MTNR*$_1$B* gene is expressed on β-cells to regulates insulin secretion (Lyssenko *et al.,* 2009).

Therefore, considering the pathogenesis role of inadequate insulin secretion in GDM, *$CDKAL_1$* and *$MTNR_1B$* genetic variants serves as potential markers for predicting GDM development.

Since then, more studies were conducted (Table 2-4). Further confirmation was that a single nucleotide polymorphism (SNP) that predispose an individual to GDM can also predispose a patient to T2DM and obesity. Table 2-5 is showing that a risk allele of a certain disease and association with a specific genetic polymorphism of a particular gene may vary in different ethnic / race groups in many countries. The current review will focus on three selected genes and their polymorphisms namely: *$MTNR_1B$* (rs1387153), *PPARα* (rs 4253778), and *TCF_7L_2* (rs12255372).

Genetic predisposition and GDM

The pathophysiology of the most studied maternal genes is presented in **Table 2-4**. Review results for the selected genetic variants are shown in **Table 2-5** for the genetic predispositions.

Table 2-4: The pathophysiology of common maternal genes involved in β-cell function, insulin secretion and GDM globally.

	Gene	Full Name	Common SNPs	Location	Pathophysiology
1.	*CDKAL1*	*Cyclin-dependent Kinase-5 Regulatory Subunit - Associated protein 1- like -1*	rs7756992 rs10440833 rs7754840	6q22.3	Signals molecules on β-cell responsible for β-cell survival (Daval *et al.,* 2012)
2.	*FTO*	Fat Mass and Obesity Associated gene	rs9939609	16q12.2	Forms adiposity that leads to pre-pregnancy obesity and GDM (Khella *et al*., 2017; Beysel *et al.,* 2019)
3.	*IgF2BP2*	Insulin-Like Growth Factor 2mRNA	rs4402960 rs1470579	3q27.2	Play a role in the insulin function (Cho *et al*., 2009; Wang *et al*., 2011)
3.	*KCNJ11*	Potassium Channel Inwardly Rectifying Subfamily J Member 11	rs5219	11p15.1	Plays a crucial role in the insulin secretion by reducing ATP sensitivity of the K-ATP channel subunit (Kir6.2), leading to the reduction of insulin secretion (Lasram *et al*., 2014; Rastegari *et al*., 2015; Shaat *et al.,* 2005).
4.	*MTNR1B*	Melatonin receptor 1B	rs1387153 rs10830963 rs7936247 rs2166706 rs4753426 rs1447352	11q14.3	Encodes melatonin receptor MT2, a G -protein coupled receptor-expressed in the pancreatic β-cell to regulate insulin secretion and glucose metabolism (Lyssenko *et al.,* 2009; Jia *et al*., 2020; Hayes *et al*., 2013)
5.	*PPAR (A)* *PPAR (G)*	Peroxisome Proliferator Activated Receptor (alpha & gamma)	rs1801282 rs13559 rs1800206 rs4253778	3p25.2	Plays a critical role in adipocyte differentiation by causing insulin resistance, obesity, and T2DM (Shaat *et al*., 2007; Engwa *et al*., 2018; Vergotine *et al*., 2014) Expressed in the skeletal muscles, liver, heart, pancreatic

					β-cells to regulate fatty acids oxidations. Also, have an impact on exercises capacity through modulation of cardiac and hepatic lipid metabolism (Muoio *et al*., 2002).
6.	*SLC30AB*	Soluble Carrier Family 30 Member 8	rs13266634 rs3802177	8q24.11	Selectively expressed in the pancreatic βeta cells to regulate insulin secretion in human pancreas (Chimieti *et al.,* 2005)
7.	*TCF7L2*	Transcription factor 7- like - 2	rs7903146 rs12255372 rs7091695	10q25.3	Modulates pancreatic islet β-cell function by affecting the Wingless/Integrated (WnT) signalling pathway Resulting to insulin secretion during pregnancy (Shaat & Groop, 2007; Shaat *et al.,* 2007).

Table 2-5: Summary of genetic variants (rs1387153, rs12255372, rs4253778) previously studied for GDM predisposition in the world population.

Gene	SNPs	Country/ Ethnic group	Race	Case/ Control	MAF	T2DM	GDM	Obesity	Author
MTNR1B	rs1387153	S. Arabian	A	200/200	T		Yes	No	Alharbi *et al, 2019*
		Russian	C	278/179	T		Yes		Popova *et al*., 2017
		S. Chinese	A	753/676	T		Yes		Jia *et al.*, 2020
		USA/Danish	C	2636/6036	T		Yes		Ding *et al*., 2018
		Finnish	C	530/407	T		Yes		Huopio *et al*., 2013
		Greek	C	77/98	T		No		Vlassi *et al.*, 2012
		Korean	A	928/990	T		Yes		Kim *et al.*, 2011
		Russian	C	688/454	T		Yes		Popova et al., 2021
PPARα	rs4253778	Chinese	A	26/24	C		Yes		Iyidir *et al*., 2015
		Chinese	A	30/30	C		Yes		Kalabay *et al.*, 2002
		Chinese	A	74/74	-		No		Kralisch *et al*., 2017
		Chinese	A	135/135	C		Yes		Jin *et al.*, 2020
TCF7L2	rs12255372	Mexico (USA)	C	94/98	T		Yes	Yes	Watanabe *et al.*, 2007
		Mexico	C	92/108	T		Yes	Pre	Reyes-Lopez *et al.*, 2014
		Egypt	A	114/114	T		Yes		Shalabi *et al.*, 2021
		SEA	C	125/125	T		Yes	Yes	Klein *et al.*, 2017
		Euro-Brazilian	C	200/200	T		No		de Melo *et al*., 2015
		Korean	A	869/632	T		No		Cho *et al*., 2009
		Swedish	C	826/1185	T		Yes		Papadopoulos *et al*., 2011
		Greek. /Czech	C	261/376	T		Yes		Vcelek *et al.*, 2012
		Cameroon (p)	A_1	60/60	T	Yes			Nanfa *et al.*, 2015
		Cameroonian	A_1	35/30	T			No	Ngwa *et al.*, 2015
		Egyptian	A_1	180/210	T	Yes			El-Lebedy *et al.*, 2016
		Egyptian (p)	A_1	60/60	T	No			Mandour *et al.*, 2018
		USA-AA	A	545/391	T	No			Dabelea *et al.*, 2011

*A: Asian, A_1: African C: Caucasian, U: Unknown, MAF: Minor allele frequency, P: Pilot

2.4 Reference

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CHAPTER 3: IDENTIFICATION OF PREVALENCE AND RISK FACTORS ASSOCIATED WITH GESTATIONAL DIABETES MELLITUS IN ETHEKWINI DISTRICT KWAZULU-NATAL

Manuscript 1

This manuscript will be submitted to the Journal. The manuscript will be written in a submission format in line with the authors guidelines for the journal. The manuscript consists of a brief introduction of gestational diabetes mellitus, material, and method section followed by the results and conclusion sections. This paper focuses on the prevalence and GDM risk factors that are associated with pregnant women living eThekwini district in KwaZulu-Natal.

Abstract

Introduction: Gestational diabetes mellitus (GDM) is a non-communicable disease (NCD) that is affecting pregnant women and their babies during pregnancy. This study aimed to identify the prevalence and risk factors associated with GDM among black women in eThekwini District, South Africa.

Method: A prospective case-control study was carried out in the SA black population, eThekwini between January and March 2020. Eighty-seven (87) women in their previous pregnancy history were used from three local health district facilities. Sixty-two women presented non-GDM (control group), and twenty-five women had GDM (experimental group). All women with and without GDM history completed their questionnaires. The data was analysed using R. Statistical Computing Software of the R Core Team, 2020, version 3.6.3. Women with GDM and non – GDM (control group) were analysed as dependent variables and risk factors as independent predictive variables.

Results: The prevalence of GDM was 28.7% (25/87). Women with age above 36 years old showed significantly higher incidence of GDM ($p<0.05$). Women with GDM had been pregnant more often and given birth 1 or 2 children previously as compared to the non-GDM controls ($p<0.01$). Additionally, family history of diabetes mellitus was observed in women with GDM ($P<0.05$). Furthermore, a statistically significant association between GDM and ARV treatment ($p=0.563$), hypertension ($p=0.891$), and BMI ($P=0. 284$) was not observed. After adjusting for

confounders, women on ARV treatment were associated with a 3.3- folds increased odds of GDM (OR: 3.3; 95%CI: 1.10 -11.310; p=0.004). Participants with pre-existing diabetes mellitus showed a 69% reduced risk of developing GDM (OR: 0.23; 95%CI: 0.07- 0.71; p=0.014); furthermore, overweight was associated with 6.9 folds increased odds of GDM (OR: 6.9; 95%CI: 1.35-5.48; p=0.033) than obese women (p=0.546). Age >20 years, family history of diabetes mellitus, parity, hypertension, and previous history of GDM were dependent risk factors (p>0.05). The odds ratio (OR) below one was calculated as (1- 0.31=0.69) and expressed as a percentage.

Conclusion: The prevalence of GDM was high (28.7%) in black SA women living in KwaZulu-Natal, eThekwini District. Age, family history of diabetes mellitus and parity were associated with increased cases of GDM. ARV treatment, BMI ≥25 kg/m^2 (overweight), and pre-existing diabetes mellitus remained predictors of GDM in this study. Screening before pregnancy may minimize complications in pregnancy and ultimately reduce the maternal death rate in the country and globally.

Keywords: Gestational diabetes mellitus, risk factors, Africa.

3.1 Introduction

Gestational diabetes mellitus (GDM) is defined as hyperglycaemia first detected at any time during pregnancy (WHO, 2013). Women with a history of GDM are subsequent to develop T2DM 10 years later after delivery (Wang and Yang, 2016; Li *et al.,* 2020; Diaz-Santana *et al.,* 2022), and their children are more likely to develop T2DM and become overweight or obese later in life (Lowe *et al.,* 2019; Martinez -Cruz *et al.,* 2021). Hyperglycaemia affects approximately 16.7% of live births globally, of which 80.3% cases are GDM (IDF diabetes Atlas, 2021 10th edition). Developed countries have shown an increase of 15 to 20% (Koyanagi *et al.,* 2013). In Africa, the prevalence of 7.5%, 9.1 %, and 14.9% , respectively, was reported in Nigeria, South Africa, and Algeria.

Gestational diabetes mellitus usually disappears after delivery and possible, reappear in women having a history including , GDM, diabetes mellitus (type1 or type2), gestational hypertension, pre-eclampsia, delivered macrosomic baby (≥4.5kg), C- section, premature birth, neonate birth defects, miscarriages, stillbirth, and unexplained neonatal death, and in ≥2 children (Tobias *et al*., 2011; El Sagheer and Hamdi, 2018; Reddy *et al.,* 2017; Lee *et al.,* 2018; Zhang *et al*., 2021). Other risk factors such as, maternal age ≥25 years, ethnicity, overweight or obese (BMI ≥25 or ≥30 kg/m^2), ARV treatment, family history of DM (FHD), genetic polymorphism may predispose women to develop GDM (Biardgo *et al.,* 2019; Shaat *et al.,* 2007). Therefore, uncontrolled GDM may lead to maternal death globally (Dudley *et al.,* 2007; Lagese *et al*., 2016; Leggesse *et al*., 2017; Tavera *et al.,* 2022)

Pregnancy is associated with insulin resistance (IR) and hypergycaemia (Alfadhli, 2015; Ara *et al.,* 2022). Maternal obesity, envronmental factors, and genetics may predispose women to develop GDM in normal pregnant women. The placenta plays a crucial role in transporting maternal nutriemts , and glucose to the developing foetus. Overnution may contribute to the foetal overgrowth during pregnancy. Therefore, a successful pregnancy is determine by maternal wellbeing (Fowden *et al.,* 2011; Pijnenborg *et al.,* 2008).

The Glucose metabolism (glycemic level), dislipidaemia, “Diabetogenic” hormones (HCG, hPL, hPGH, Prolactin, progesterone, cortisol), adipokines (leptin, adiponectin, IGFBP-1), and cytokines (TNF-α), growth factors contribute to the level increased of insulin resistance, decreased insulin sensitivity and the development of GDM during the first, second and third trimester in pregnancy (Barbour *et al.,* 2007; McIntre *et al*., 2009). However, the abnormal conflict between the mother and the unborn child need a clarity.

The abnormal "maternal -foetal conflict" begins when the glucose metabolism of pregnant women changes to meet the nutritional needs of both the mother and the foetus (Lain and Catalano, 2007). This mechanism makes the fasting glucose concentrations of women with normal glucose tolerance to decrease as gestation progresses, which then results in insulin resistance to some extent in all pregnant women (Jovanovic and Pettitt, 2001) and by the late pregnancy, "the insulin level of pregnant women decreases to one-third as compared to non-pregnant women" (Monteiro *et al*., 2015:54). Therefore, the increased in insulin resistance in pregnant women facilitates a continuous glucose transfer to the foetus (Robitaille and Grant , 2008).

In South Africa, GDM prevalence is rising in parallel with obesity and overweight. In 2007, Mamabolo *et al*. found 8.8%, Adam and Rheeder *et al*. (2017) showed 25.8%, Macaulay *et al.* (2018*)* had 9.1%, and currently, IDF report – 2021 showed 11%. The results showed inconsistency, possibly due to the screening and diagnostic criteria used when diagnosing GDM.

According to the guideline for Maternal Care in South Africa, (2016: 98), "All pregnant women should go through screening for GDM at their respective site's clinics or community health centres (CHCs) during pregnancy". This includes testing for DM /GDM in all undiagnosed pregnant women attending a clinic for the first time, using a standard method at 6-12 weeks after delivery (ADA, 2018; ADA, 2020). In addition, women with GDM history should be screened every three years, and if pre-existing DM is detected, treatment should be considered as an intervention to prevent severe complications (ADA, 2018; ADA, 2020). This current study aims to identify the prevalence of GDM and associated risk factors among black SA pregnant women with a history of GDM. This has been considered a start in women who already had GDM from previous pregnancies without confirmation of OGTT standard procedure in eThekwini District, KwaZulu-Natal.

3.2 Material and Methods

3.2.1 Study Participants

This prospective case-control study was analysed on pregnant women who received antenatal care at a prenatal clinic in KwaDabeka, KwaMashu CHCs, and King Edward VIII Hospital (KEH) for high-risk pregnancies and non-pregnant women attending family planning clinic in KwaDabeka, and KwaMashu CHCs, Durban, South Africa. The inclusion criteria included: All women who

signed a written informed consent form -pregnant and non-pregnant women with a history of GDM. Pregnant women first to the third trimesters of pregnancy at the CHCs approved by the districts. Pregnant and non-pregnant black South African women between 15 and 45 years old included women with metabolic syndromes, HIV and AIDS. Pregnant women were visiting the clinic for the first time in their gestational age. Exclusion criteria were as follows: – Women were excluded from the study if they were below 15 and above 45 years old, non-black South African women, not residing in the area approved by the districts. Pregnant women who were not visiting for the first time in the respective area were excluded.

3.3 Ethical Approval

The District Head Officer granted permission to conduct the study at the CHCs for Durban, SA, the NHRD: ethics clearance certificate: KZ_201909_040. The permission at King Edward VIII hospital ANC was obtained from the Head of the Department of Obstetrics and Gynaecology and the Hospital Medical Officer. Full ethical approval was finally granted by the University of KZN Biomedical Research Ethical Committee before the resume of the study. Ethical clearance certificate: BE 378/19. All three ethical approval letters are attached to this thesis Appendix. A researcher explained the procedure to the participants in the language (IsiZulu), and all women willing to participate signed the written informed consent. The investigation started on the 30th of January 2020 and ended on the 13th of March 2020.

3.4 Clinical Data Collection

3.4.1 Questionnaire

The researcher (MSc Med and registered HPCSA member) collected questionnaire data. The data was collected during the participant's enrolment appointments, gestational age (pregnant women), and family planning (non-pregnant women) in their respective study sites. All procedures and processes involved were explained orally in the language of their choice (isiZulu). Participants who volunteered were asked to sign the written informed consent.

3.4.2 Socio-demographic Data

A well-trained antenatal clinic sister collected the data in each antenatal visit, data included: blood pressure(bp) (mmHg), Systolic(upper) / diastolic(lower), bp ranges were Normal= <120/<80; Elevated = 120-129 / <80; Hypertension (stage 1) = 130-139 / 80-89; Hypertension (stage 2) =

140+ / 90+; Crisis 180+ / 120+.The same antenatal clinic sister collected weight (kg), height (cm), and calculated BMI. As per the WHO, BMI ranges and classifications are as follows: normal weight - ≥18.5 – 24.9kg/m^2, Underweight < 18.5 kg/m^2, Overweight ≥ 25.0 – 29.9 kg/m^2, Obese ≥30 kg/m^2. Information was collected from antenatal cards of pregnant women, including Age, marital status, parity, gravidity, gestational age at the first visit, previous pregnancy history, weight (kg), height (cm), BMI, blood pressure (mmHg), ARV treatment, family history on diabetes and previous history on complications in pregnancy. Non-pregnant women who were attending family planning clinic were asked the same questions as above which applied to them except the duration of pregnancy.

3.5 Statistical Analysis

Data analysis was performed with the help of a statistician in the Department of Biostatistics at UKZN. All analyses were performed using R statistical computing software of the R Core Team, 2020, version 3.6.3. The socio-demographic of the study population was evaluated, and categorical variables were presented as numbers (n) and percentages (%), continuous variables as means (± standard deviations), and comparison between two groups (pregnant women and healthy control). The Chi-square and Fisher's tests were applied to analyse the associations between two categorical variables (women with or without GDM history). Median differences in at least three groups were analysed using Kruskal-Wallis. A multivariable logistic regression analysis was performed. This test was done to calculate odds ratios (OR) and 95% confidence intervals (95%CI) to check whether there were differences between the demographic data, whether the risk factors age, parity, gravidity, ARV treatment, previously diagnosis with DM, family history of DM was associated with GDM. The backward step logistic regression modelling procedure was used to select the final report for related risk factors. The final model report added all variables of interest (age, gravidity, ARV treatment, previously diagnosed with DM, BMI, family history of DM). GDM history was considered as a dependent variable, and associated risk factors were regarded as independent GDM. All reported p-values deemed statistically significant at $p < 0.05$. $p>0.05$ considered no effect on the study.

3.6 Results

3.6.1 Participant's Characteristics

A total of n=103 women were randomly recruited in the study. One was excluded from the study after she volunteered as a negative control, discovering positive for pregnancy testing. She never

submitted the information back. The final sample size remained at n=102 participants. Table 3-1(a-e) presents socio-demographic characteristics as frequencies and percentages.

Of the total of n=102 women whose information was eventually recorded, the majority, 71.6% (n=73) of them, were pregnant. About 37.3% (n=38) were from KEVIII Tertiary Hospital situated in the eThekwini Central Heath district, while 30.4% (n=31) and 32.4% (n=33) participants were from KwaMashu CHC in the North and KwaDabeka CHC in the West region of eThekwini Health District, respectively. Since KEVIII Tertiary Hospital is a referral for high risks individuals, therefore, our study showed that most pregnant women were already identified as high risk for various complications in pregnancy. All the participants were from KwaZulu-Natal province. The mean age of study participants was 27.7 ± years. Most, 23.5% (n=24) respondents were between age 31 – 35 years. Majority, 83.3% (n=86) respondents were single. Approximately 53.9% (n=55) had one or two children. Interestingly, 29.4% (n=30) participants started their antenatal clinic between 13-28 weeks gestation, while 27.5% (n=26) participants started between 29 -40 weeks gestation. The mean (SD) BMI was 29.0 ±7.31 kg/m^2; median 28.3kg/m ^{2}; range 17.9 -51.3 kg/m^2. Most, 37.3% (n=38) respondents were obese and 27.5% (n=28) overweight. Majority, 77.5% (n=79) had a normal blood pressure, while 18.6% (n=19) participants showed an elevated. Participant on ARV treatment lesser 46.1% (n=47) than the participants not on ARV treatment 53.9% (n=55). This has shown a decrease in women who are infected with HIV within the region. Unemployed respondents were higher 79.4% (n=81) than employed respondents 20.6% (n=21). Fewer than 21.6% (n=22) participants showed a history of miscarriages. Interestingly, the majority, 72.5% (n=74) participants, had no previous history of miscarriages. All women who responded to the questions are shown in below (Table 3-1a).

Table 3-1a Socio-demographics for the study participants (n=102)

Variables	FREQUENCIES	PERCENTAGES
	n=102	100 %
	n or Median (IQR)	%
Pregnant Status		
No	29	28.4%
Yes	73	71.6%
Clinic Name		
KwaMashu CHC	31	30.4%

KwaDabeka CHC	33	32.4%
King Edward VIII	38	37.3%
Sub-District		
eThekwini Central	38	37.3%
North	31	30.4%
West	33	32.4%
Clinic Location		
Urban	38	37.3%
Township	64	62.7%
Age (years)		
15-20	20	19.6%
21-25	22	21.6%
26-30	20	19.6%
31-35	24	23.5%
36+	16	15.7%
Marital Status		
Single	86	83.3%
Married	6	5.9%
Living together	10	9.8%
Parity		
0	35	34.3%
1-2,	55	53.9%
3+	12	11.8%
Gravidity		
0	7	6.9%
1-2,	55	53.9%
3+	40	39.2%
Gestational Age		
Not pregnant	29	28.4%
1-12 weeks	17	16.7%
13-28 weeks	30	29.4%
29-40 weeks	26	25.5%
BMI Group (kg/m^2)		
Normal	30	29.4%
Underweight	2	2.0%

Overweight	28	27.5%
Obese	38	37.3%
Missing	4	3.9%
BMI (kg/m^2)		
Mean & plus mean SD (CV%)	29.0±7.31 (25.2)	
Median (Q1-Q3)	28.3 (23.1-33.2)	
Min -Max	17.9 – 51.8	
Missing	4	3.9%
Blood Pressure		
Normal: SB<120 &DB<80	79	77.5%
Elevated :120-129 & DB<80	19	18.6%
Stage II: SB>140 & DB >90	3	2.9%
Missing	1	1.0%
ARV treatment		
Yes	47	46.1%
No	55	53.9%
Employment status		
Yes	21	20.6%
No	81	79.4%
Pregnancy history		
Miscarriage	22	21.6%
Stillbirth/birth defect	2	2.0%
Neonatal death	4	3.9%
None of the above	74	72.5%

Sample size (n), BMI -Body Mass Index, CHC- Community Health Centre. ARV- Antiviral Therapy.

3.6.2 Knowledge About Diabetes Mellitus

The proportion of diabetes mellitus was higher at 53.9% (n=55) among participants previously undiagnosed than participants previously diagnosed at 44.1% (n=45). The majority, 55.9% (n=57) participants were never tested or diagnosed with diabetes mellitus, while 37.3% (n=38) participants who were tested, had normal blood sugar levels. In a total of n=102 women (pregnant and non-pregnant), 24.5% (n=25) had GDM history, while 60.8% (n=62) had no-GDM history. Women currently pregnant were n=73. Out of 24.5% (n=25) GDM women, 21.9% (n=16) were pregnant, and 32.1% (n=9) non-pregnant. Our study demonstrated that the majority, 7.8% (n=8) of women were diagnosed later between 29 -40 weeks gestation (3rd trimester) for GDM, while

5.9% (n=6) women had a GDM diagnosis between 13-28 weeks gestation (2ⁿ trimester), and the least, 1.0% (n=1) were diagnosed between 1-12 weeks gestation (1st trimester). Participants with a family history of DM demonstrated a lesser proportion 41.2% (n=42) than those with no family history of DM 52.9% (n=54). However, 26.5% (n=27) of participants showed their first-degree relatives of DM from their mother`s side than their father`s side 9.85% (n=10). Interestingly, 98.0% (n=100) of women were not taking insulin. Very few 2.0 % (n=2) eat healthily, more than 88.2% (n=90) reported that physical activities were "not' for them, and most commented: "eating fries with bread or vetkoek is what we can afford'. Only less than 8.8% (n=9) of women kept checking their glucose levels; the majority, 91.2% (n=93), were not even monitoring their glucose levels. Women taking antenatal supplements were less 41.2% (n=42) than women not taking antenatal supplements 57.8% (n=59). All results are presented in Table 3-1b.

Table 3-1b Knowledge about diabetes mellitus (n=102)

Variables	FREQUENCIES	PERCENTAGES
	(n=102)	100%
Tested/diagnosed with DM previously	n or Median (IQR)	(%)
Yes	40	39.2%
No	57	55.9%
Do not remember	5	4.9%
Diabetes status		
Type 2 DM	1	1.0%
Awaiting results	1	1.0%
Not tested / diagnosed	62	60.8%
Previous diagnosis of GDM	25	24.5%
Do not remember	13	12.7%
History of GDM		
Yes	25	24.5%
No	62	60.8%
Not Applicable	15	14.7%
Maternal age diagnosis		
1-12 weeks	1	1.0%
13-28 weeks	6	5.9%
29-40 + weeks	8	7.8%

Not Applicable	78	76.5%
Normal blood sugar level	2	2.0%
Do not know	7	6.9%
Family history of DM		
Yes	42	41.2%
No	54	52.9%
Do not know	6	5.9%
Family history member		
Mother's side	27	26.5%
Father's side	10	9.8%
Not applicable to my family	54	52.9%
Brother	6	5.9%
Sister	3	2.9%
Mother`s side: child	1	1.0%
Do not know	1	1.0%
Insulin intake		
Yes	2	2.0%
No/Not applicable (n=93)	100	98.0%
Healthy meals		
Yes	2	2.0%
No=1; Not applicable (n=90)	91	89.2%
Not always	9	8.8%
Exercising		
No	12	11.8%
Not applicable	90	88.2%
Monitor glucose level		
No	9	8.8%
Not applicable	93	91.2%
ANC Supplements		
Yes	42	41.2%
No=59; NP (n=1)	59	57.8%

*NP - Not pregnant

3.6.3 Common Complications in Pregnancy

Majority, 45.1% (n=46) participants had vaginal delivery. Approximately 59.8% (n=61) participants delivered full term babies. However, most 35.3% (n=36) participants delivered their babies with weights between 2.6-3.5kg, while 10.8% (n=11) participants had baby's weights >4.5kg. Out of, 53.9% (n=55) participants that had previous babies, only 10.8% (n=11) participants that tested their baby's glucose level after delivery, 31.4% (n=32) had no previous babies, 29.4% (n=30) reported not applicable and 20.6% (n=21) did not know.

Table 3-1c Common complications in pregnancy (n=102)

Variables	FREQUENCIES	PERCENTAGES
	n=102	100%
	N	(%)
Delivery mode		
Caesarean section	24	23.5%
Vaginal delivery	46	45.1%
Not applicable	32	31.4%
Delivery mode status		
Pre-term	4	3.9%
Full-term	61	59.8%
Induced labour	3	2.9%
Late delivery	1	1.0%
Not applicable	33	32.4%
Baby`s weight in previous pregnancy		
<2.6 kg	10	9.8%
2.6 - 3.5kg	36	35.3%
3.6-4.0kg	8	7.8%
>4.0kg	11	10.8%
Do not remember	5	4.9%
Not applicable	32	31.4%
Baby`s blood test		
Yes	11	10.8%
No	8	7.8%
Do not know	21	20.6%
Not applicable	30	29.4%

No previous baby	32	31.4%

3.6.4 Clinic Attendance

The World Health Organization improved the schedule pregnant women from four to eight ANC visits. In our study, the majority, 63.0% (n=46) of pregnant women, had less than four visits. This was expected since the women were accepted for the study at their first gestational age. However, Table 3.1-A showed that in a total of n=73 pregnant women, 16.7% (n=17) started their clinic in the first trimester, 29.4% (n=30) second trimester, and 25.5% (n=26) in the third trimester. The majority, 77.5% (n=79) participants, had no challenges attending the clinic.

Table 3-1d Clinic attendance (n=102)

Variables	FREQUENCIES	PERCENTAGES
	N=102	100%
Clinic attendance	(n)	(%)
<4.0	46	45.1%
Four -Six	11	10.8%
Six- Seven	11	10.8%
> Seven	6	5.9%
Not pregnant	28	27.5%
Clinic attendance challenges		
Travelling time	1	1.0%
Unintended pregnancy	22	21.6%
Not applicable/no challenges	79	77.5%

3.6.5 Awareness of the Maternal Death Rate in KwaZulu -Natal

The majority, 56.9% (n=58) of participants strongly agreed that GDM may lead to maternal death. Approximately, 72.5% (n=74) fully agreed that it is important to control DM. Half 44.1% (n=45) of participants had no idea of the maternal death rate in KZN. Very limited, 1.0% (n=1) of women did not know about ANC as a preventative measure for maternal death rate, whereas the majority, 98.0% (n=100), knew. Out of n=102 participants, most, 90.2% (n=92) participants were unaware of GDM severe complications due to genetic involvement. Therefore, awareness of maternal complications, including GDM is needed as soon as possible among black SA women in eThekwini district, KwaZulu-Natal. Table 3-1e.

Table 3-1e Awareness of maternal death rate in KwaZulu-Natal (n=102)

Variables	FREQUENCIES	PERCENTAGES
	n=102	100%
	N	(%)
GDM leads to death		
Do not know	42	41.2%
Agree	2	2.0%
Strongly agree	58	56.9%
Importance of controlling DIP		
Do not know	24	23.5%
Agree	4	3.9%
Strongly agree	74	72.5%
KZN has the highest maternal death rate		
Do not know	45	44.1%
Agree	1	1.0%
Strongly agree	56	54.9%
Attending ANC to prevent maternal death.		
Do not know	1	1.0%
Agree	1	1.0%
Strongly agree	100	98.0%
Unawareness of GDM maternal complications in KZN		
Do not know/not sure	1	1.0%
Disagree	1	1.0%
Strongly disagree	4	3.9%
Agree	4	3.9%
Strongly agree	92	90.2%

3.6.6 The Comparison of prevalence risk factors between pregnant and non-pregnant women

The number of participants randomly recruited in the study was n=102. The total final sample size was 96 participants suitable for the study. The mean age of the study population was 27.7% ± 3.5 years. The comparison of sociodemographic factors between pregnant women and the

healthy control group was performed and presented as frequencies (n) and percentages (%). Table 3.2. demonstrated most women 24% (n=23) belonged to the age group 31-35 years. There were no significant differences for the mean age (31.4±12.6 years) between the two groups (p=0.712), parity 1-2 children (p=0.714), BMI (p=0.192), hypertension (p=0.665), previous history GDM (p=0.124), and family history of diabetes mellitus (first degree relative) (p=0.263). Women with gravidity 1-2 was significantly higher in pregnant 55.9% (n=39) than non-pregnant 53.8% (n=14, p=0.001). Antiretroviral (ARV) treatment showed increased among pregnant women 52.9% (n=37) than non-pregnant 30.8% (n=8, p=0.054). Approximately, 60% (n=42) pregnant women were not diagnosed with DM when compared with non-pregnant women 34.6% (n=9, p=0.032). Among those who were diagnosed, n=27 were pregnant women, and n= 16 were non-pregnant women.

Table 3-2 Comparison of prevalence of risk factors in pregnant and non-pregnant women

Variable	Pregnant (n=70)	Non-Pregnant (n=26)	Total group (n=96)	P-value
	FREQUENCY (PERCENTAGE)			
	n (%)	n (%)	n (%)	
Age (years)				0.712 Chi-square
15-20	15(21.4%)	5(19.2%)	20(20.8%)	
21-25	12(17.1%)	6(23.1%)	18(18.8%)	
26-30	13(18.6%)	7(26.9%)	20 (20.8%)	
31-35	19(27.1%)	4(15.4%)	23(24.0%)	
36+	11(15.7%)	4(15.4%)	15(15.6%)	
Parity				0.714 Chi-square
0	23 (32.9%)	9(34.6%)	32 (33.3%)	
1-2,	40 (57.1%)	13(50.0%)	53 (55.2%)	
3+	7(10.0%)	4(15.4%)	11 (11.5%)	
Gravidity				*< 0.001 Fisher's
0	0 (0%)	6 (23.1%)	6 (6.2%)	
1-2,	39 (55.7%)	14 (53.8%)	53(55.2%)	
3+	31 (44.3%)	6(23.1%)	37(38.5%)	

BMI (kg/m^2)				0.192 Chi-square
Normal	20(28.6%)	10(38.5%)	30 (31.2%)	
Overweight	24 (34.3%)	4(15.4%)	28(29.2%)	
Obese	26 (37.1%)	12(46.2%)	38 (39.6%)	
Hypertension				0.665 Fisher's
Normal	55 (78.6%)	20 (76.9%)	75(78.1%)	
Elevated	14 (20.0%)	4 (15.4%)	18(18.8%)	
Stage II	1(1.4%)	1(3.8%)	2 (2.1%)	
Missing	0(0%)	1(3.8%)	1(1.0%)	
ARV Treatment				*0.054 Chi-square
No	33 (47.1%)	18(69.2%)	51(53.1%)	
Yes	37 (52.9%)	8 (30.8%)	45 (46.9%)	
Pre-existing DM (T1DM/T2DM/GDM)				*0.032 Chi-square
No	42(60.0%)	9 (34.6%)	51 (53.1%)	
Yes	27(38.6%)	16 (61.5%)	43(44.8%)	
Missing	1(1.4%)	1(3.8%)	2(2.1%)	
Family History DM				0.263 Chi-square
No	41(58.6%)	11(42.3%)	52(54.2%)	
Yes	26 (37.1%)	12 (46.2%)	38 (39.6%)	
Missing	3 (4.3%)	3(11.5%)	6(6.2%)	

DM: Diabetes Mellitus; P-values based on non-missing cases only- Rank Sum Test, Chi-square test, Fisher's exact test **Note:** * Level statistical significance: P <0.05. BMI-Body Mass Index, ARV-Antiretroviral Therapy.

3.6.7 Identification of Prevalence of Gestational Diabetes Mellitus

Participants' self-reports (questionnaires) in three clinics (KwaDabeka CHC, KwaMashu CHC, and KEVIII Tertiary hospital) were named GDM or non-GDM. Pre-existing diabetes mellitus (T1DM or T2DM) were called pre-GDM. According to Anderson *et al*. (2017), this has been considered a diagnostic of GDM without confirmation with the standard 2-hour, 75g oral glucose test (Lawrence *et al.,* 2019). Participants were asked about their diabetes mellitus status before

and during pregnancy (Appendix H). Face-to-face personal interview was performed on pregnant women in their first to the third trimester of pregnancy in which women were asked (DK-01) "Have you ever been tested and diagnosed with diabetes mellitus before pregnancy? Expected answer 'yes' or 'no' or 'don't remember' if 'yes' type1, type2, GDM and coded as pre-GDM. Another question was from DK-03: "Have you ever been diagnosed with diabetes mellitus during pregnancy? 'Yes or 'no' or 'don't remember'. All women with history of GDM in their previous pregnancies were coded as GDM, if WHO 2013 diagnostic criteria were met for GDM diagnosis in their respective ANC clinics. In the current study, some pregnant women attending KEHVIII Hospital reported 'no' for diagnosis during pregnancy; this was because of Oral OGTT's outstanding results. According to self-reported data, other women (pregnant and non-pregnant) had 'no' as answers and showed a severe history of complications in pregnancy, including macrosomic infants. This group was coded as undiagnosed diabetes mellitus (Appendix K).

Pregnant and non-pregnant women who reported "yes" for a previous diagnosis of GDM were n=25, and "no" were n=62, and n=15 (not applicable) Table 3-1b. Participants (n=15) with inappropriate answers were excluded. In a total of n=70 pregnant women, approximately, 22.9% (n=16) had GDM and 77,1% (n=54) had no-GDM. Out of 17 non-pregnant women with no-GDM history, 9 never had babies. Table 3.3 represent the GDM prevalence of black SA women in eThekwini in pregnant women.

Table 3-3 Prevalence of GDM using self-report data (Jupp, 2006)

Diabetes in Pregnancy (DIP)	**Pregnant (n)**	**Prevalence (%)**	**Non- Pregnant (n)**	**Prevalence (%)**	**Total (n)**
GDM	16	22.9	9	52.9	25
Non-GDM	54	77.1	8	47.1	62
Total	70	100	17	100	87

*GDM-Gestational diabetes mellitus. *Out of 17 non-pregnant women, 8 had no babies and no GDM history.

3.6.8 The comparison of prevalence risk factors between women with GDM and the non-diabetic group in the study population.

In a total of 70 pregnant women at their gestational age visits 1-12 weeks; 13-28 weeks and 29-40+ weeks were screened for GDM using their past GDM history. Of 70 participants, 22.9% (n=16) had GDM. The remaining formed the non-diabetic group 77.1% (n=54). Table 3.4 shows the comparison of the prevalence of risk factors between GDM and non – GDM groups. The mean age (31.4 ±12.6 years), of GDM women, was significantly higher than non- diabetic group (26.5 ±6.7 years) (p=0.033). Multiparous women, and multigravida (1-2) showed significantly higher (54.0%; 52.9% respectively) prevalence of GDM compared to multiparous women and multigravida (3+ (12.6%; 42.5%, p=0.011 and p= 0.002 respectively), however, GDM women with parity 1-2 showed higher (64.0%) prevalence than non-diabetic group (50.0%, p=0.011). Pre-existing diabetes mellitus (pre-GDM) was significantly higher (66.1%) in non-diabetic group compared to GDM women (24.0%) (p= <0.01). The prevalence of GDM was higher (59.7%) in a non-diabetic group with FHD (first-degree relative) than in GDM women (32.0%) p<0.011). The correlation between BMI, hypertension, ARV treatment, and GDM was not observed among black SA women living in the eThekwini health district.

Table 3-4: The comparison of prevalence risk factors between women with GDM and no-GDM.

Variable	Non- GDM (n=62)	GDM (n=25)	Total group (n=87)	P-value	Associations
	FREQUENCY (PERCENTAGE)				
	n (%)	n (%)	n (%)		
Age (years)				*0.033 Fisher`s	Yes
15-20	16 (25.8%)	2 (8.0%)	18 (20.7%)		
21-25	13 (21.0%)	4 (16.0%)	17(19.5%)		
26-30	13 (21.0%)	3 (12.0%)	16 (18.4%)		
31-35	14(22.6%)	7(28.0%)	21 (24.1%)		
36+	6 (9.7%)	9 (36.0%)	15 (17.2%)		
Parity				*0.011 Chi-quare	Yes
Nullipara	26 (41.9%)	3 (12.0%)	29 (33.3%)		
1-2,	31 (50.0%)	16 (64.0%)	47 (54.0%)		

3+	5 (8.1%)	6 (24.0%)	11 (12.6%)		
Gravidity				*0.002 Fisher's	Yes
0	4 (6.5%)	0 (0%)	4 (4.6%)		
1-2,	39 (62.9%)	7 (28.0%)	46(52.9%)		
3+	19 (30.6%)	18(72.0%)	37(42.5%)		
BMI (kg/m^2)				0.284 Fisher's	No
Normal	21 (33.9%)	4(16.0%)	25 (28.7%)		
Underweight	1 (1.6%)	0 (0%)	1(1.1%)		
Overweight	19 (30.6%)	7 (28.0%)	26 (29.9%)		
Obese	20 (32.3%)	12(48.0%)	32 (36.8%)		
Missing	1(1.6%)	2 (8.0%)	3(3.4%)		
Hypertension				0.891 Fisher's	No
Normal	49 (79.0%)	19 (76.0%)	68 (78.2%)		
Elevated	10 (16.1%)	5 (20.0%)	15(17.2%)		
Stage II	2(3.2%)	1 (4.0%)	3(3.4%)		
Missing	1(1.6%)	0(0%)	1(1.1%)		
ARV treatment				0.563 Chi-square	No
No	34 (54.8%)	12 (48.0%)	46 (52.9%)		
Yes	28 (45.2%)	13 (52.0%)	41 (47.1%)		
Pre-existing DM (T1DM/ T2DM/GDM)				*<0.001 Chi-square	Yes
No	41(66.1%)	6 (24.0%)	47(54.0%)		
Yes	19 (30.6%)	19 (76.0%)	38(43.7%)		
Missing	2(3.2%)	0(0%)	2(2.3%)		
Family History DM				*0.010 Chi-square	Yes
No	37(59.7%)	8 (32.0%)	45 (51.7%)		
Yes	22 (35.5%)	17 (68.0%)	39 (44.8%)		
Missing	3 (4.8%)	0 (0%)	3 (3.4%)		

DM: Diabetes Mellitus; Note**:** *The statistical significance: P <0.05.

3.6.9 Multiple logistic regression analysis between risk factors and pregnancy.

Gestational diabetes mellitus was more prevalent in women aged ≥36 years (p=0.033), in multiparous women 1-2 (p=0.011), women with pre-existing diabetes mellitus (p<0.001), and with a positive FHD in first degree relative of pregnant women (p=0.010).

In the Fisher`s Exact test, no relationship was found between BMI plus mean SD (CV%) [(29.0 ±7.31 (25.2)], hypertension (p=0.891). Again, the Chi-square test did not show any correlation between women taking ARV treatment and the risk of GDM (p=0.563). A multivariate analysis using a multiple back step logistic regression model was performed to investigate whether the well-described risk factors age, BMI, parity, hypertension, ARV treatment, previous history with GDM, and family history of diabetes mellitus (first-degree relative) were associated with GDM in this prospective case-control study. There was no significant difference between age 21-25 (OR: 0.47, p=0.505) ; 26-30 years (OR: 0.57, p=0.606) ; 31-35 years (OR: 1.62, p=0.729); 36+ (OR: 1.00, p=0.998) ; parity 1-2 and 3+ children (OR: 0.81, p=0.808; OR: 0.33, p=0.422 respectively) hypertension elevated and stage II (OR:1.37, p=0.733; OR: 0.26, p=0.438 respectively); previous history with GDM yes or no (OR: 1.38, p=0.716; OR: 0.23, p=0.112) , and those who had family history of diabetes mellitus (FHD) (first-degree relative) (OR :0.46, p=0.251). Women on ARV treatment showed 3.34 -folds more likely to develop GDM, the odd ratio with 95% confidence interval, 1.10 - 11.310; p=0.04, when compared to women not on ARV treatment, whereas women with pre-existing diabetes mellitus were 69% less likely to develop GDM [0.31 (1- 0.31) 69%; p=0.014)] than women with no previous history of diabetes mellitus. Furthermore, those who were overweight showed 6.94 -times higher risk to GDM development (OR: 6.94; 1.35 - 54.81; p=0.033) compared with obese women (OR: 1.46, 95%CI: 0.43 - 5.20; p=0.546). Pre-existing DM (T1DM, T2DM, GDM) ARV treatment and overweight (≥25 kg/m^2) remained major independent risk factors for GDM (P<0.05).

Table 3-5 Multiple regression analysis between risk factors and pregnancy

Risk Factors	Odds Ratio (OR)	95% Confidence interval (CI)	P-Value	OR (95%CI, p-value Back-Step
Age (years)				
21-25	0.47	0. 05 - 4.49	0.505	-
26-30	0.57	0.07 - 4.93	0.606	-
31-35	1.62	0.11 – 27.90	0.729	-

36+	1.00	0.05 – 23.11	0.998	-
Parity				
1-2	0.81	0.13 – 4.47	0.808	-
3+	0.33	0.02 – 5.03	0.422	-
Blood Pressure				
Elevated	1.37	0.23 – 9.38	0.733	-
Stage II	0.26	0.01 – 10.99	0.438	-
ART Treatment				
Yes	3.01	0.64 – 15.60	0.170	3.34 (1.10 – 11.310; p= 0.04)
Pre-existing DM (T1DM/T2DM/GDM)				
Yes	0.19	0.04 – 0.75	0.023	0.23 (0.07 – 0.71; p= 0.014)
Family History DM				
Yes	0.46	0.12 – 1.72	0.251	-
BMI Status				
Overweight	8.19	1.17 – 92.94	0.053	6.94 (1.35 – 54.82; p= 0.033)
Obese	1.37	0.31 – 6.57	0.681	1.46 (0.43 – 5.20; p= 0.546)

DM: Diabetes Mellitus; Note: *The statistical significance: P <0.05.*Predicting the likelihood of Pregnant status = Yes, Input observation n=96, Regressed observation n-86, ART: Antiretroviral Therapy; BMI: Body Mass Index. OR below 1 calculated as (1- OR= percentage).

3.7 Discussion

This research represented a population community-based study in which risk factors associated with GDM were investigated for the first time in women with GDM history from previous pregnancies, living in KwaZulu-Natal, eThekwini health district. Women with pre-existing GDM are more at higher risk of developing T2DM than women who developed GDM for the first-time during pregnancy (American Diabetes Association (ADA), 2018; ADA, 2020). Screening every three years in women previously diagnosed with GDM in their past pregnancies may prevent any severe complications in pregnancy that might lead to maternal and foetal death (ADA, 2018; ADA, 2020). In this present study population, age, parity, ARV treatment, pre-existing DM,

overweight, and FHD were significantly associated with GDM. Anti-retroviral treatment, pre-existing DM, and overweight are independent risk factors in women developing GDM. Reasons such as socioeconomic status, genetics, and environmental factors may play a vital role in the development of GDM in black SA women in KwaZulu-Natal, eThekwini health district.

Gestational diabetes mellitus is a public health concern, and its prevalence rate is showing an increase from 5% to 33% globally (International Diabetes Federation, 2021). The IDF's latest report (2021) indicated that there is a variation in the prevalence of GDM in different countries and ethnic groups globally. United States (US) prevalence is estimated at 20.1%, Spain (32.8%), Italy (14.3%), India (29.3%), Bangladesh (18.5%), Brazil (5.4%), Thailand (10.3%), Qatar (24.0%), Nigeria (18.2%) and Tanzania (7.5%). African literature also showed a variation in GDM prevalence among women in Africa (Dias *et al*., 2019; Muche and Gete, 2019; Adam and Rheeder, 2019; Mamabolo *et al.,* 2007; Macaulay *et al.,* 2018). Researchers suggested that the variations of increased prevalence in GDM could be screening and diagnostic criteria for GDM and the debate is still on (Nguyen *et al*., 2018; Adam and Rheeder, 2017; Macaulay *et al.,* 2018; Corrado and Pintaudi, 2018; Huvinen *et al*., 2018; Xiao *et al*., 2018; Luewan *et al*., 2018; Chi *et al*., 2018) Table 3-6.

In 2013, new diagnostic criteria for GDM were implemented to improve the severe complications in pregnancy such as macrosomia, gestational hypertension, and preeclampsia (WHO, 2013). These criteria reduce the rate of GDM but raise the increased rate of adverse pregnancy and future metabolic outcomes (Chi *et al*., 2018). The result in Table 3-6 shows that there is still a variation in the screening and diagnosis of GDM in Africa and globally: Environmental factors, genetic polymorphism, screening, and diagnostic criteria could be a reason for these variations (Luewan *et al.,* 2018). In the current study, prevalence rate was identified in women with previous diagnosis of GDM assuming one of the criteria methods was met (WHO 1999 or 2013). Our finding was driven by small number of cases (25/87), and further studies are required to validate our results. Therefore, our finding should be interpreted with caution.

Table 3-6: Comparison of prevalence in screening and diagnostic methods for GDM globally

Author	Country	Risks screen	WHO 1999	WHO 2013
Corrado *et al.,* 2018; Ferraiolo *et al.,* 2018	Italy	11%	22.9%	12.7%
Hong *et al.,* 2020	South Korea		29.8%	16.0%
Gilder *et al.,* 2014	Burma		6.6 %	10%
Chi *et al.,* 2018	Singapore		18.7%	13.0%
Luewan *et al*., 2018	Thailand		32%	
Sesmilo *et al*., 2020	Spain			16.8%
Al Subhi *et al*, 2021	Muscat		26.4%	48.5%
Olagbuji *et al*., 2015	SSA and Nigeria		3.8%	8.1%
Njete *et al*., 2018	Tanzania			19.5%
Adam and Rheeder, 2017	South Africa	25.8%		
Mamabolo *et al*., 2007	South Africa		8.8%	
Macaulay *et al*., 2018	South Africa			9.1%
Current study	South Africa	28.7%		

*SSA-Sub-Saharan-Africa

Being overweight is a significant risk factor for women developing GDM (Lee *et al.,* 2018; Bian *et al.,* 2020; Zhang *et al.,* 2021). Body Mass Index (BMI) is a helping tool for screening and estimating children, adolescents, young adults, and women of childbearing age (National Institute of Diabetes and Digestive and Kidney Diseases, 2017). However, the BMI range between ≥25 – 29.9 kg/m^2 is associated with overweight (WHO, 1999). The great concern is that the prevalence of overweight has tripled from 1975 to 2016. In 2016, women aged ≥18 years indicated a higher (40%) prevalence of overweight than obese women (15%), and a considerable increase of 4% to >18% was noted among children and adolescents aged between 5 to 19 years old globally (WHO Factsheets, 2021). Again, young girls are more overweight and obese than boys in the SA population, KwaZulu -Natal (Negash *et al.,* 2017; Otitoola *et al*., 2020).

The present study showed that BMI was 29.0±7.31 (25.2). However, the obese women showed a significantly higher proportion of 48% (n=12) than overweight 28.0% (n=7) women, but the odds ratio of developing GDM was 6.94 higher among overweight pregnant women (OR 6.94, 95%CI: 1.35-5.48; p=0.033) than obese mothers. There was no significant difference in age >20 years old and overweight women (p>0.05). In a meta-analysis of 84 studies, Lee *et al*. (2018) found that

BMI ≥25 kg/m^2 (OR: 3.27, 95%CI: 2.81 - 3.80; P<0.05) is an independent risk factor for GDM risk in the Asian population. Zhang *et al*. (2021) found similar results of BMI ≥25 kg/m^2 in Chinese women with GDM. Bian *et al. (*2020), showed that high BMI ≥25 kg/m^2 is a marker for GDM in women with a previous history of GDM. Our results presented a pilot study with a sample size of (n=25/87). Although, they look consistent with other kinds of literature, but should be treated with reserve.

Age is an independent risk factor for GDM risk. The current study found that age ≥36 years is associated with GDM. Moreover, no significant difference was observed between age≥ 20 years and risk factors in pregnant women living eThekwini district, KwaZulu-Natal (OR 0.47, 95%CI: 0.05-4.49; p=0.505). African literature showed that maternal ages ≥25 and ≥35 years are independent risk factors in women developing GDM (Macaulay *et al*., 2018; Ewenighi *et al.*, 2013; Njete *et al.*, 2018; Zhang *et al*., 2021). Yong *et al*. (2020) discovered that age ≥35 years is an independent risk factor for GDM risk among Asian women. Therefore, consider age before planning for pregnancy. Due to small sample size (25/87), our results should be treated with reserve.

Pregnant women with more than one child are more at risk of developing GDM (Schwartz *et al*., 2015; Zhang *et al*., 2020). Our study discovered that mothers with one to two children are more at risk of developing GDM. Still, no significant difference was demonstrated between risk factors and pregnant women with parity 1-2, and 3+(OR, 0.81; 95%Cl: 0.13-4.47) p=0. 808. Again, non-GDM had a significantly higher (n=31) proportion than GDM women (n=16). In the US, Schwartz *et al*. (2015) indicated that multiparous non-Hispanic black women with GDM had a higher recurrence rate (48%) compared with non-Hispanic White women (39%). Zhang *et al*. (2020) showed that multiparous is an independent risk factor for GDM risk in Asian pregnant women (OR, 0.752; 95%Cl: 0.698-0.810) among Asians. Ewenighi *et al.* (2013) found no association between GDM and parity (P=0.953) in Nigerian women. Therefore, women must seek health professional advice or counselling before they add more children to the family.

Pre-existing diabetes mellitus is an independent risk factor for GDM (Lee *et al*., 2018; Li *et al.*, 2020; Xiang *et al.*, 2018). Approximately 10.6 % of diabetes mellitus are detected before pregnancy, however, 9.1 % are considered T1DM or T2DM during pregnancy (IDF, diabetes Atlas, 2021 10th edition). Of concern about 9.8 million (46.3%) of them are below 30 years old

and live in low -and -income countries where the ANC is limited . The present study reported a prevalence of 1% in women with pre-existing T2DM and 28.7% for GDM. In Canada, Feig *et al. (*2010) found a prevalence rate of 1.9% (pre) and 9.3% for GDM in women with pre-existing DM. Wahabi *et al.* (2017), showed an estimation of 4.3 % (pre) and 24.2% (GDM) in Saudi Arabian women. In Tanzania, Njete *et al.* (2018) found a prevalence of 3% in women who had pre-existing DM (T1DM, T2DM) and GDM was reported at 19.5%. The current study indicated - consistency with other findings. However, the data was limited for statistical power, suggesting further studies with a larger sample size to validate these findings.

Furthermore, some studies have shown that women with pre-existing T1DM, T2DM and GDM are at higher risk of developing GDM during pregnancy (Li *et al.,* 2020, Lee *et al.,* 2018; Xiang *et al.,* 2018). The present study discovered that pre-existing DM is a significant independent risk factor for GDM, but less likely to cause GDM in black South African women living in KwaZulu-Natal (OR: 0.23, 95%CI: 1 - 0.31) 69%; p=0.014). Similarly, Lee *et al. (*2018) also discovered that pre-existing DM is a predictor of GDM in Asian women (OR:8.42, 95%CI: 5.35-13.23; P<0.05). Li *et al.*(2020), showed that pre-existing GDM is a dependent risk factor (associated with GDM but not a predictor for women developing a GDM) for Chinese women. Therefore, pregnant, and non-pregnant women must consider their diabetes status before planning their next pregnancy. Due to the sample size, our results should be treated with reserve.

Antiretroviral treatment for HIV -infected women has been reported as an important risk factor for women predisposed to GDM (Biadgo *et al.*, 2019; Marti *et al.,* 2007). South Africa is the most prevalent country, of HIV/AIDS. The recent data from UNAIDS Factsheet, (2020) showed that the disease has increased from 3 200 000 – 6 100 000 among South African women aged ≥15 years. The prevalent rate is higher (27.7%) in females than in males (13.5%) aged between 15-49 years old. Highly Active Antiretroviral Therapy was implemented to suppress the viral load, improve CD4 counts, reduce the mortality rate in HIV-infected people, and prevent mother-to-child transmission (MTCT). Studies have shown that certain drugs for HIV infection may predispose women to T2DM and GDM in HIV-infected pregnant mothers (Timmerman *et al*., 2005; Gonzalez-Tome *et al*., 2008). The prevalence of GDM in pregnant women living with HIV infection is estimated at 3.58% (USA), 7.1% (Asia), 5.83% (Europe), 7.0% (Spain), and 3.19% (Africa).

The present study found that ARV treatment is a significant independent risk factor for GDM in black South African women living with HIV infection (OR:3.34, 95%CI: 1.10-11.310; P=0.004). A cohort study by Marti *et al.* (2007), showed that the class of ARV treatment -Protease Inhibitor (PI), is a predictor for GDM in HIV-infected women than in the healthy group. Similarly, Gonzalez-Tome *et al*. (2008) reported the same results in Spanish pregnant women with HIV infection. In a recent study, Biadgo *et al. (*2019) reported PI findings as a marker for GDM development in pregnant women with HIV infection (OR:0.31, 95%CI: 1-0.31; 69%, P=0.02). Hitti *et al*. (2007) failed to demonstrate PI and GDM risk among HIV -I infected pregnant women. Soepnel *et al*. (2017) found no association between PI and GDM in pregnant women infected with HIV (OR:0.80, 95%CI: 0.47 -1.37). Adam and Rheeder, (2017) reported the same findings in South African women living with HIV infection. Similarly, Mmasa *et al*. (2021) showed no link between ART treatment and GDM among pregnant women living with HIV infection in Botswana. Our results are in line with Gonzalez -Tome *et al*. (2008) on Spanish pregnant women. Again, Biadgo *et al*. (2019), confirm our results that ART treatment contributes to the development of GDM in pregnant women with HIV infection. Therefore, more studies are required in KwaZulu-Natal to investigate specifically the class of ART treatments and the development of GDM. These results might help medical practitioners look at changing the medical treatment in pregnant and non-pregnant mothers living in KwaZulu-Natal, eThekwini. Although the current study is a pilot (n=25/87), it was comparable with other previous studies therefore, studies with a larger sample size are advised to generalize the findings of this study.

3.8 Conclusion

Our prospective case-control study, reports GDM prevalence at 28.7% using self-report data (questionnaire), in women residing eThekwini, KwaZulu-Natal, South Africa. This increased prevalence highlights the importance of selecting the proper screening and diagnostic criteria for GDM, carry out prevalence studies in different areas of South Africa including other races, will help us get the exact prevalence of GDM within the country. However, pre-existing diabetes mellitus, body mass index ≥25 kg/$m^{2,}$ and ART treatment were found to be the most significant independent risk factors for GDM. Preventing further complications in pregnancy among women in the eThekwini community, proper maternal and child health training has become an urgent matter. This was a pilot study: therefore, results are treated with reserve.

3.9 Reference

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CHAPTER 4: THE ASSOCIATION OF SNP GENOTYPES WITH GDM AND ITS RISK FACTORS IN THE STUDY POPULATION

Manuscript 2

This manuscript will be written in a submission format according to the authors guidelines for the Journal and submitted to the journal. The manuscript has an introduction of gestational diabetes mellitus, detailed material and method section followed by the results and conclusions. This paper is more focuses on the genetic polymorphisms and the associations with GDM and obesity.

Abstract

Introduction: The associations between genetic variants of the Melatonin Receptor 1B (*MTNR$_1$B*) gene, Peroxisome Proliferator-Activated Receptor-Alpha (*PPARα)* gene, Transcription factor 7-like 2 (*TCF$_7$L$_2$*) gene, and gestational diabetes mellitus (GDM), and obesity have been demonstrated, mainly in Caucasian, Asian and less in the Black African population. This study aimed to investigate the possible associations between *MTNR$_1$B* (rs1387153), PPAR*α* (rs4253778), and *TCF$_7$L$_2$* (rs12255372) polymorphisms , GDM and obesity in black South African women, from eThekwini district, KwaZulu-Natal.

Method: A case-control study was performed on pregnant and non-pregnant women aged between 15 – 45 years old, attending clinics from KwaDabeka CHC, KwaMashu CHC, and King Edward VIII Tertiary Hospital. Sociodemographic data (Appendix : G&H) including BMI and previous pregnancy history was collected from participants ANC Cards. In a total of 87 women, sixty-two women with non-GDM were used as a control group and twenty-five women as part of the GDM (experimental group). Blood samples between 2-4ml were collected from each participant into a vacutainer EDTA tube (BD Diagnostic, SA) for molecular analysis. The blood samples were collected for DNA extraction for the genetic polymorphisms' investigation and GDM. The BMI was measured as kg/m^2 and the following genetic variants: *MTNR$_1$B* (rs1387153), *PPARα* (rs4253778), *TCF$_7$L$_2$* (rs12255372) were genotyped for each participant using the PCR-RFLP technique. Sanger sequencing was done at Central Analytical Facility (CAF). Stellenbosch University, South Africa. Categorical variables were analysed using the Chi-square test or Fisher`s exact test, where appropriate. A result of p-value <0.05 was considered statistically significant.

Results: In this study genetic polymorphisms in genes: *MTNR$_1$B* (rs1387153), PPARα *(rs*4253778) and TCF$_7$*L$_2$* (rs12255372) were not associated with GDM in black SA women living in eThekwini, KZN ($p > 0.05$). Variant rs1387153 genotype CC and CT indicated a significantly higher proportion , 50% 928/56) in non-GDM pregnant women ($p<0.05$). For rs4253778, genotype CC showed a significantly higher proportion, 72.9% (43/59) in both diabetic and non-diabetic pregnant women ($p< 0.01$), and genotype GG was only observed in the non-diabetic pregnant women. The homozygous G allele of *TCF$_7$L$_2$* rs12255372 was most frequently observed in both pregnant women with and without GDM.

Conclusion: Based on the current results, the association between the three SNPs and GDM was not observed. This could be a small sample size that was used in this study. Therefore, larger sample size is recommended for the validation of this results.

4.1 Introduction

Gestational diabetes mellitus (GDM) is a type of hyperglycaemia, commonly 'known' as non-communicable disease, induced by pregnancy but resolved after delivery. Although prevalence differs with race/ethnicity, approximately 16.7% of pregnant women aged 20-49 years, representing 21.1 million births annually are affected by hyperglycaemia in pregnancy (Adugna *et al*., 2020; IDF, diabetes Atlas, 2021 10th edition; WHO, 2013). The latest IDF report (2021) has indicated an increased prevalence of GDM in Africa, with South Africa estimated at 11%. Studies have shown that pregnant mothers with a history of DM such as T1DM, T2DM and GDM, maternal overweight/obesity, advanced maternal age, ethnicity, FHD (T2DM), history of macrosomic babies increased the risk of developing GDM (Kuti *et al.*, 2011; John *et al.*, 2019; Macaulay *et al.*, 2018;). Women with a previous history of GDM are more at risk of developing T2DM in the next 5-50 years after delivery (Li *et al.*, 2020; Diaz-Santana *et al.*, 2022). Female and male offspring of GDM mothers are associated with severe complication in pregnancy including, birth weight larger than gestational age (≥4.5kg), labour difficulties associated with shoulder dystocia, severe haemorrhage, C-section, as well as T2DM, overweight/ obese, metabolic syndrome, neonatal hypoglycaemia including congenital disabilities such as ADHD, recurring seizures, and female infants develop GDM in their future life (Kampmann *et al.*, 2015; Plows *et al.*, 2018; Thepampan *et al.*, 2021; Control *et al*., 2011; MacIntyre *et al.*, 2019; Kamana *et al.*,2015; Martinez-Cruz *et al.*, 2021).

The cause of GDM is not clear but genetic polymorphisms such as *TCF_7L_2* rs12255372 (T-allele), *MTNR1B* rs1387153 (T-allele), *PPARα* rs4253778 (C-allele), maternal obesity (BMI ≥30kg/m^2) or overweight (BMI ≥25kg/m^2), and environmental factors may predispose women to develop GDM. Polymorphisms of these genes can modulate pancreatic β-cell dysfunction, insulin resistance, insulin secretion and obesity by signalling in different pathways (Lyssenko *et al*., 2009; Kwak *et al*., 2012; Shaat *et al.,* 2007; Muoio *et al.,* 2002).
The genetic profile of GDM overlaps with T2DM as both are expressed in the pancreatic β-cell islets function and are involved in glucose homeostasis. Therefore, genetic variants for candidate genes like *TCF_7L_2* rs122553272 (T allele) for GDM risk can predispose humans to T2DM because they share genetic profile.

Again, many candidate genes interact with environment to trigger the GDM condition. Currently, the genetic testing is not yet been implemented. Certain studies have found that pregnancy serves as a state in some women who are already genetically predisposed to develop diabetes mellitus (Reece *et al*., 2009). For instance, researchers found that genetic variants in the *TCF_7L_2* gene (rs7903146, rs4506565, and rs12255372) were associated with an increased risk of GDM among pregnant women (Shaat *et al.,* 2007; Cauchi *et al*., 2007; Chandak *et al*., 2006). Identifying genetic variations associated with human diseases, assists health professionals finding ways of preventing and treating the disease.

Genetic variants previously shown in large studies associated with a high risk of T2DM were also demonstrated in the association of increased GDM among Caucasians and Asians. In the African population, the investigation was more of a shared genetic locus and T2DM. For example, SNP rs7903146 (T-allele) was associated with GDM among Indian women (Lenin *et al.,* 2018); the same SNP was associated with T2DM in the Northern African general population (Lasram *et al.,* 2014) but not associated with T2DM in general Moroccan population (Benrahma *et al.,* 2014). Insulin-like growth factor 2 mRNA binding protein 2 genes (*IGF_2BP_2)*, SNP rs4402960 showed an association (p=0.046) with a risk of GDM among Danish Caucasian women (Lauenborg *et al*., 2009). There was no association found in Chinese women with GDM (Liu *et al.,* 2020). The same SNP rs44202960 was associated with T2DM in the Egyptian (El-Lebedy *et al.,* 2015), Tunisian (Lasram *et al.,* 2015), and Moroccan (Benrahma *et al.,* 2014) general population.

In addition, other studies also showed that the same genetic variants predispose to T2DM and GDM can be associated with higher BMI ≥25kg/m^2, and ≥30kg/m^2. Several genetic variants were investigated in different ethnic/race groups. However, most of the investigations for GDM associations were performed on Caucasians and Asians (Shintaro *et al*., 2008; Kwak *et al*., 2012; Stuebe *et al.*, 2014; Frayling *et al.*, 2007). Moreover, some studies demonstrated that not all loci associated with T2DM had shown association with GDM. For example, in a case-control study by Popova *et al*., 2017, genetic variants rs10830963 and rs1387153 were associated with GDM in Caucasian Russian women. Similarly, Huopio *et al.*, (2013) found the same results in Caucasian Finnish women. Stuebe *et al*. (2014) found no association of rs10830963 with GDM and T2DM in African American women. Heshmat *et al*. (2014) found the association of rs10830963 SNP with T2DM and obesity among the Egyptian population.

Therefore, investigating genetic variants associated with GDM, and overweight/obesity in black South African pregnant and non-pregnant women may be helpful in undiagnosed women with diabetes mellitus before and during pregnancy. Furthermore, once they know their genetic status, women can find a way of preventing and treating the disease before it causes any severe complications in pregnancy that may lead to maternal and neonatal death. This study aims at investigating association of genetic polymorphism rs12255372 of *TCF_7L_2* gene, rs1387153 of *MTNRIB* gene, and rs4253778 of *PPARα* gene with GDM and additional risk factors in Black women from KwaZulu-Natal province, South Africa.

4.2. Material and Methods

4.2.1 Sample Collection

In this case-control study, we used 87 women included seventy pregnant and seventeen non-pregnant attending their clinic at KEVIII tertiary hospital ANC, KwaDabeka CHC, and KwaMashu CHC in Durban, SA. Twenty-five had been diagnosed previously with GDM (experimental group) and sixty – two did not have GDM (healthy control). Women were selected based on their interest, completed questionnaire, and a signed written informed consent form. Inclusion criteria were black South African women aged between 15-45 years, residing in eThekwini district, KwaZulu-Natal, and having pre-existing diabetes mellitus (type 1 or 2 and GDM). Women suffering from HIV and AIDS, or other metabolic syndrome, were also included in the study. The exclusion criteria were non-black SA women. The study was approved by UKZN, the research ethics committee, and the DoH. The BREC number was BE 378/19 and NHRD Ref no: KZ_201909-040.

4.2.2 Anthropometric and clinical data on study participants

Anthropometric measurements were obtained from the Antenatal Care (ANC) registry such as patients` gestational age, biological age, hypertension (systolic/diastolic), weight (kg), and height (cm). The ANC data from the registry was collected to measure the participant's maternal health impact. Exploratory data was captured on the system for the following information extracted from the participants: knowledge and family history about diabetes mellitus, common complications in pregnancy, clinic attendance, and participants' awareness of maternal death rate in KwaZulu-Natal. Whole blood samples (2-4ml) were routinely collected from pregnant women into vacutainer EDTA tubes (BD Diagnostic, SA) for molecular analysis. All blood samples collected in the study approved by ANC Clinics were transported on ice to the Genetics Research Laboratory in the Department of Physiology, UKZN, and kept frozen at -20°C until DNA Extraction. All DNA extractions were performed at Genetic Research Laboratory in the same Department.

4.2.3 DNA Extraction

DNA was extracted from whole blood (2-4ml) collected in EDTA tubes using Gen Elute Mammalian, genomic DNA miniprep Kit, GN350 (Sigma-Aldrich), following manufacturer`s instructions. Genomic DNA concentrations were measured using Thermo Scientific Nanodrop 2000 spectrophotometer (Inqaba, Biotec, Hartfield, Pretoria, South Africa), and the manufacturer`s instructions were followed. The absorbance was measured at a value of 260 and 280 nm wavelengths, respectively. DNA purified had an A260/A280 ratio between 1.6 and 1.9. All original whole blood samples were stored at -80°C (Lasec Freezer, South Africa), and extracted DNA samples were stored at -20 °C

4.2.4 SNP Genotyping

4.2.4.1 Selection of target SNPs and Primers

Three genes and one SNP in each gene were targeted for the present study. *MTNR$_1$B*(rs1387153), *PPARα* (rs4253778), and *TCF$_7$L$_2$* (rs12255372) were selected based on the previous reports that found the link with GDM and /or T2DM (Vlassi *et al.,* 2012; Luo *et al.,* 2013; Gu *et al.,* 2014; Yao *et al.,* 2015). The oligonucleotide sequences for amplifying the genetic variants used in the present study are shown in Table 4-1. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Table 4-1 Primer sequence information for 3 SNPs used for PCR-RFLP method.

Genetic Polymorphism	Primer sequence 5`- 3.'	Genomic location and Intron/Exon	Amplicon size	Author
MTNR1B rs1387153 C>G/C>T	**Forward** GCCTGTCGACTTGGGTTGGTGT **Reverse** CCCCTGGGCCTAAGAGCCTCC	*92940662 Exon_4	486bp	(Vlassi *et al.*, 2012)
PPARα rs4253778 G>C	**Forward** ACAATCACTCCTTAAATATGGTGG **Reverse** AAGTAGGGACAGACAGGACCAGTA	*26021203 Intron_7	267bp	(Luo *et al.*, 2013; Gu *et al.*, 2014)
TCF7L2 rs12255372 G>A/G>T	**Forward** ACGTTGGATGCAGAGGCCTGAGTAATTATC **Reverse** ACGTTGGATGTGCAAATCCAGCAGGTTAGC	*113049143 Exon_20	384bp	(Yao *et al.*, 2015)

*Genomic location obtained from GeneCards: - [(https://www.genecards.org/cgi- bin/carddisp.pl? gene) Assessed on 13.04.2021]

A 100µM stock of primers was reconstituted using nuclease-free water as instructed by the synthesis report (Metabion, Anatech, Planegg, Germany). From 100µM stock, 2.5µl of each primer was added to 47.5µl of Nuclease-free water to make a working stock of 5µM stored at -20°C for further use.

4.2.5 Restriction Fragment Length Polymorphism (RFLP)

PCR reactions were conducted in a total volume of 25µl, containing 9.5µl nuclease free water, 12.5µl Dream Taq1 Master -Mix (2X) (Promega), 0.2uM of primer concentration, forward 1µl, Primer reverse 1µl and 1µl of 50-100ng genomic DNA (qualified using a NanoDrop spectrophotometer): Thermal cycling conditions were as follows: -Initial denaturation for 3 minutes at 95°C, denaturation for 30 seconds at 95°C, annealing for 30 seconds at 52°C for *TCF7L2* (384bp), 61°C for *MTNR1B* (486bp) and 50°C for *PPARα* (267bp), extension for 1 minute at 72°C,

for 30 cycles, followed by the hold at 4°C. PCR products were evaluated by running 10µl of the amplicon on 2 % agarose gel containing 2.5µl of Ethidium Bromide (10mg/ml stock) in a 1X TAE buffer solution (Thermo Scientific, Lithuania, USA) for 1 hour at 60 volts. A 100 bp DNA ladder, 6x Orange DNA Loading Dye, and 1ml (Thermo Scientific, SA) were used for sizing.

4.2.6 Restriction Digestion of PCR Products

Restriction fragment length polymorphism (RFLP) analysis was conducted using the restriction enzymes given (Table 4-2). For the restriction reaction using *MTNR1B* and *TCF7L2* , 30 µl total volume was made by mixing: 10µl of PCR product + 2.0µl restriction enzyme + 2.0µl (10X) restriction enzyme`s buffer + 16 µl nuclease-free water and incubation at 37°C for 2hrs. For the restriction reaction using *PPARα* , 25.5µl total volume was made by mixing: 25µl of PCR product + 0.5µl restriction enzyme and incubation at 65°C for 2hrs. DNA fragments were resolved in 2% agarose gel visualized by Ethidium Bromide (EtBr) (10mg/ml stock) staining. The restriction sites and size of the DNA fragments are shown in Table 4- 2.

Table 4-2 Genotype restriction sites

Gene Name	SNP	Restriction enzyme and site	Restriction product size (Common allele)	Restriction product size (Alternative allele)
MTNR1B	rs1387153	HpyCH4V (5,000units/ml) 5′...TG▼CA...3′ 3′...AC▲GT...5′	83bp and 403bp - C/T allele. Homozygote (CC) or Heterozygote (C/T)	83bp, 95bp and 308bp- G allele (homozygote)
PPARα	rs4253778	Taq1 (1,000 units/ml) 5′...T▼CGA...3′ 3′...AGC▲T...5′	267bp- G allele - homozygote (GG)	51 and 216bp- GC allele, heterozygote
TCF7L2	rs12255372	M1uCI (10,000 units) 5′...▼AATT...3′ 3′...TTAA▲...5′	35,66,117,162bp- G/A allele	32,35,40,45,66 and 162bp -T allele (homozygote)

4.2.7 Sequencing of PCR Products

PCR products were also sequenced via the Sanger Sequencing method to confirm the allele present. Sequencing was done at Central Analytical Facility (CAF). Stellenbosch University, South Africa.

4.3 Statistical Analysis

SNPs were expressed as allelic frequency (q) and prevalence of genotypes (%). A Chi-square or Fisher`s test was applied to compare genotype and allele frequencies for statistical significance between diabetic patients and controls. The Hardy -Weinberg equilibrium (HWE) at individual loci was assessed using the Chi-square test.

4.4 Results

In the present study, three SNPs from genes *PPARα* (rs4253778), *MTNR$_1$B* (rs1387153), and *TCF$_7$L$_2$* (rs122553772) were analysed in African women with and without GDM from Durban, South Africa. The PCR results for each gene are depicted in Figure 4-1 below with *PPARα* product at 267 bp, *MTNR$_1$B* at 486 bp, and *TCF$_7$L$_2$* at 384bp. The genotype and allele frequencies of the *MTNR1B, TCF$_7$L$_2$,* and *PPARα* gene polymorphisms in the women who had GDM, and the healthy group are presented in Table 4-3.

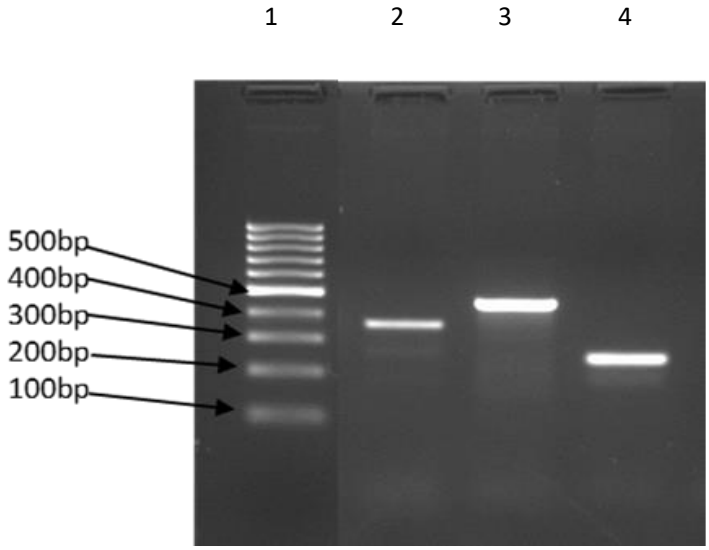

Figure 4-1: PCR Amplification of target genes. Lane 1: DNA Ladder (100bp); Lane 2:*TCF$_7$L$_2$* (384bp); Lane 3 *MTNR$_1$B* (486bp); Lane 4 *PPARα* (267bp).

RFLP – PCR Results

Three SNPs from genes *MTNR1B* (rs1387153), *PPARα* (rs4253778), and *TCF7L2* (rs12255372) were evaluated in this study by RFLP-PCR and direct sequencing of the PCR products. The results are shown below for each gene.

4.5.1 PPARα Gene

The PPARα amplicon was restricted with the *Taq1* restriction enzyme. The restricted reaction resulted in 50 and 216bp fragments depicting the presence of the homozygote C allele (Figure 4.2). Double bands on the gel at 216 and 267bp indicated a heterozygote allele (C/G) (Figure 4.2). An unrestricted product of 267bp was produced for the homozygote G allele. Results from PCR-RFLP methods were validated by DNA sequencing (Figure 4.3).

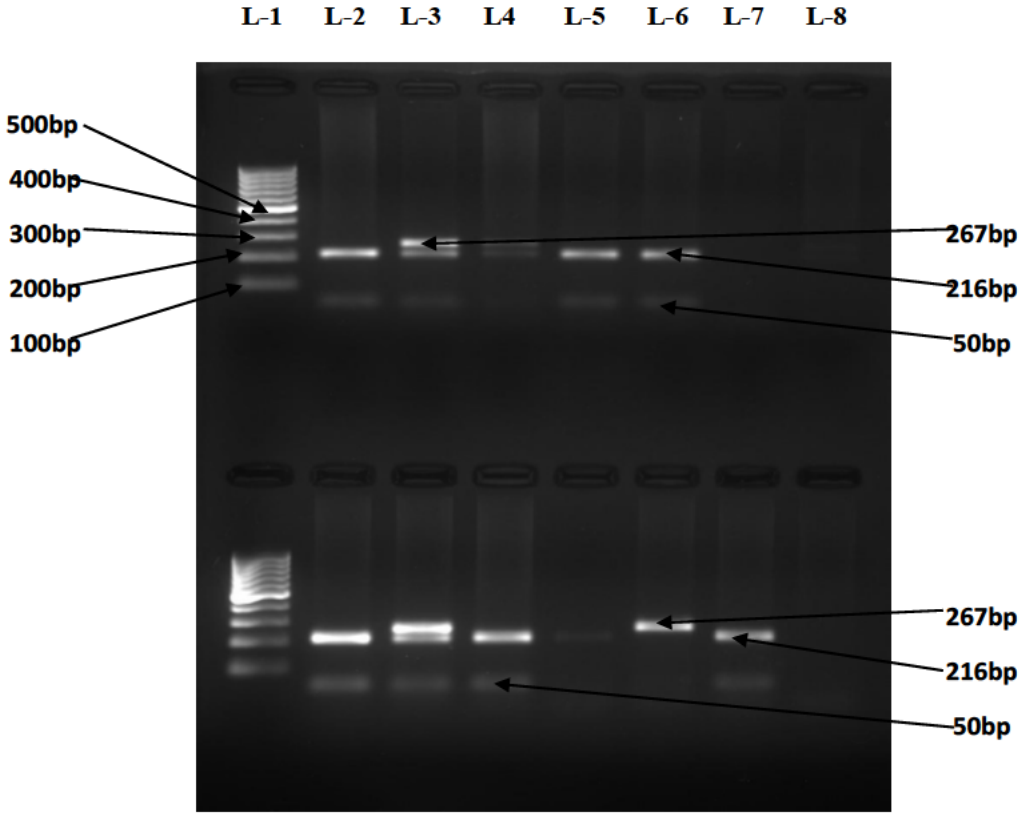

Figure 4-2a: A 2% agarose gel depicting genotype results of the *PPARα* (rs4253778) (G/C) polymorphism by RFLP-PCR assay. Lane 1:100bp DNA Ladder (Thermo Scientific, SA). Lane:2: A 216 DNA fragment indicated the presence of the C allele, Lane 3: the double band (216 and 267bp) revealed the presence of heterozygote (G/C) in the sample. Lower gel. Lane 6: A single band 267bp present homozygous G allele. Lane 8 (upper and lower gel): template control. *** L:** Lane

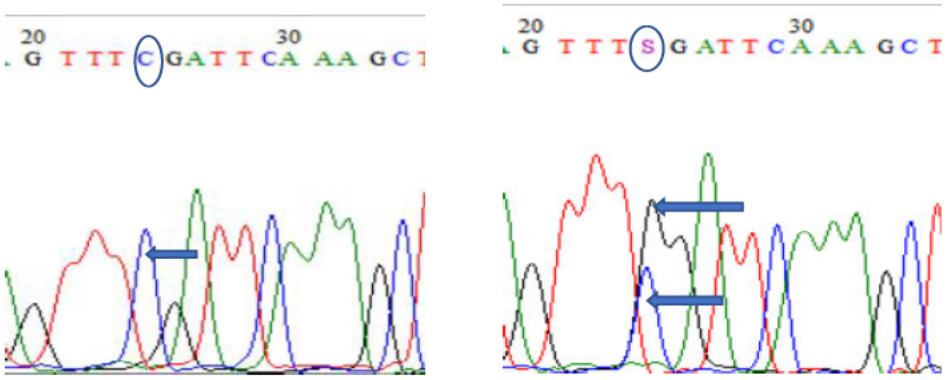

Figure 4-2b: Sanger sequencing results of *PPARα* (rs4253778) polymorphism. a. Presence of homozygous C allele. b.Presence of heterozygous (C/G) allele.

For a total of 25 women with gestational diabetes mellitus, following distribution of genotypes was observed. CC (64%), GC (36%), GG (0%), and among 60 women without gestational diabetes mellitus (control group) following distribution of genotypes was noted: CC (71.6 %), GC (23.3%), GG (5%). Allele frequencies of C and G alleles in pregnant women with diabetes mellitus were 0.826% for C, and 0.180% for G, and the control group C (0.833%) and G (0.167%). Fisher`s exact test showed no statistically significant association between SNP rs4253778 and diabetes during pregnancy (p=0.390). However, genotype CC showed a significantly higher proportion, 71.6% (43/60) in non-diabetic pregnant women (p<0.01) (Table 4-3), and genotype GG was only observed in the non-GDM women (n=3) (Figure 4-3). No significant association was observed between rs4253778 and BMI status (p=0.489) (Figure 4-4), but Genotype CC, and GG were significantly associated with BMI status in pregnant women with GDM and non-GDM (P<0.05). There was no significant deviation from the HWE observed in either diabetic pregnant (p>0.01) or control non-diabetic groups (p>0.01).

Table 4-3: Genotype and allele frequencies of genetic variants *PPARα* rs4253778, *MTNR1B* rs1387153 and *TCF7L2* rs12255372 in women with GDM and control group.

Genetic variants	Genotype and Alleles	GDM	Non-GDM	P-value
PPARα				
rs4253778	**Genotype**			
	CC	16 (27.1%)	43 (72.9%)	4.4e-04
	GC	9 (39.1%)	14 (60.9%)	0.297
	GG	0	3 (100%)	0.083
	Total	25	60	
	Frequency Allele			**Fisher`s exact**
	C	0.83%	0.83%	0.390
	G	0.18%	0.17%	
MTNR1B				
rs1387153 (C/T)	**Genotype**			
	CC	10 (26.3%)	28 (73.7%)	0.004
	CT	6 (25%)	18 (75%)	0.014
	TT	6 (37.5%)	10 (62.5%)	0.317
	Total	22	56	
	Frequency Allele			**Chi-square**
	C	0.59%	0.59%	0.647
	T	0.41%	0.41%	
TCF7L2				
rs12255372	**Genotype**			
	GG	24 (27%)	53 (59.6%)	3.13e-07
	Total	24	53	

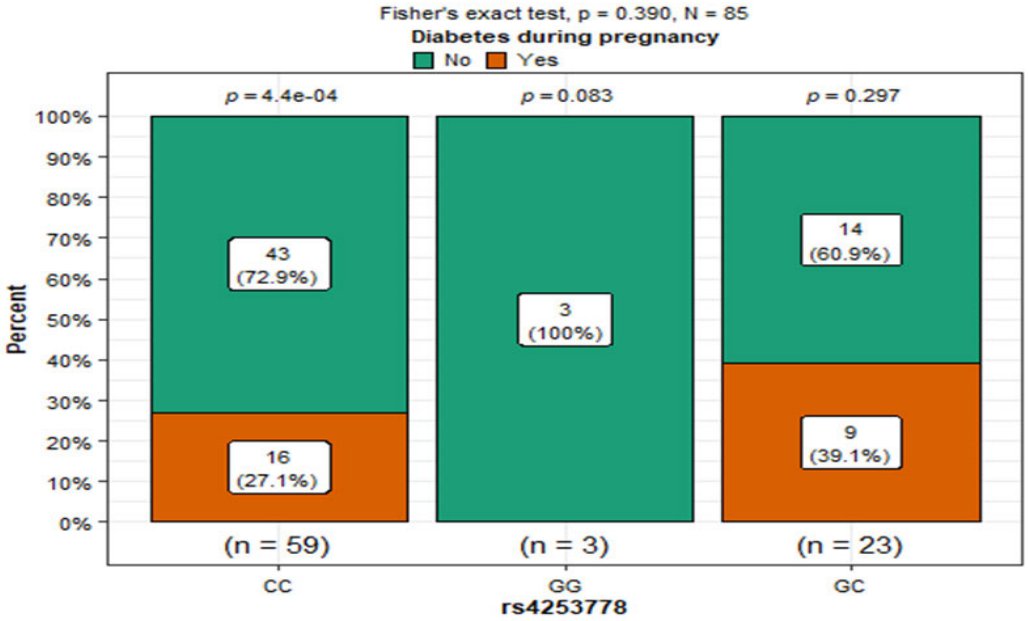

Figure 4-3: Illustrating the association between *PPARα* (rs4253778) genetic polymorphism and risk of GDM compared with the control group.

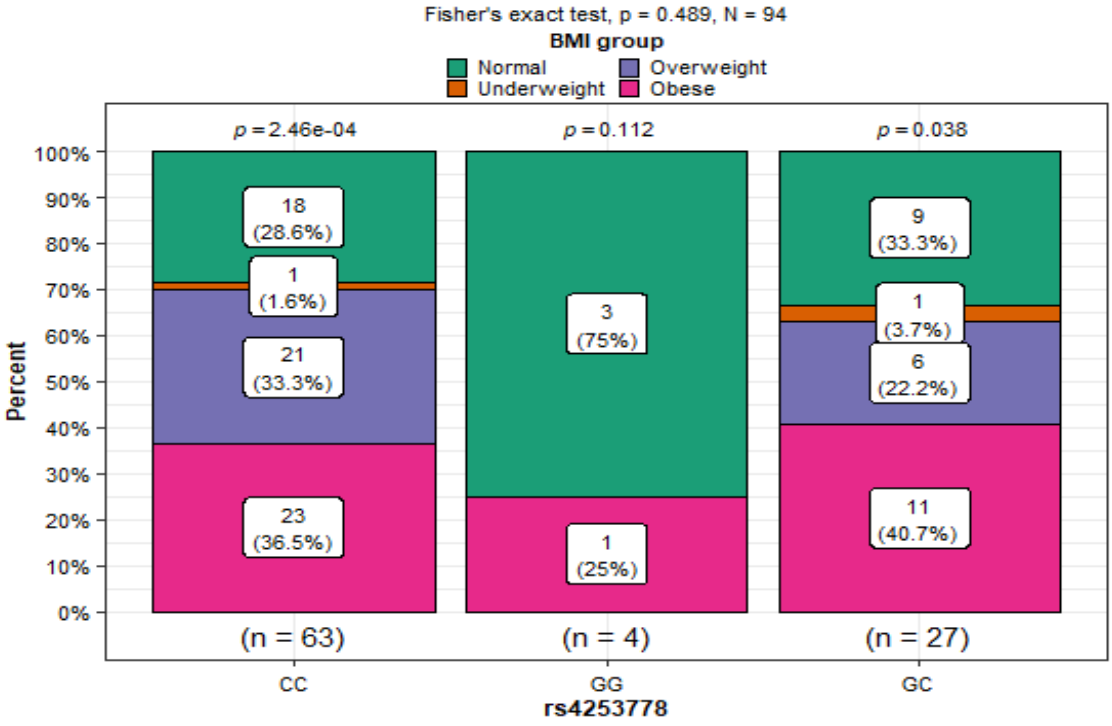

Figure 4-4: Comparison of the BMI in women with GDM according to the different genotypes of the genetic variant rs4253778 in the *PPARα* gene.

4.5.2 MTNR$_1$B Gene

The amplicon was restricted with the *HpyCH4V* restriction enzyme. Restriction reaction resulted in 83 and 403 bp fragments depicting the presence of the homozygote C or T allele or the heterozygote (Figure 4-5a).

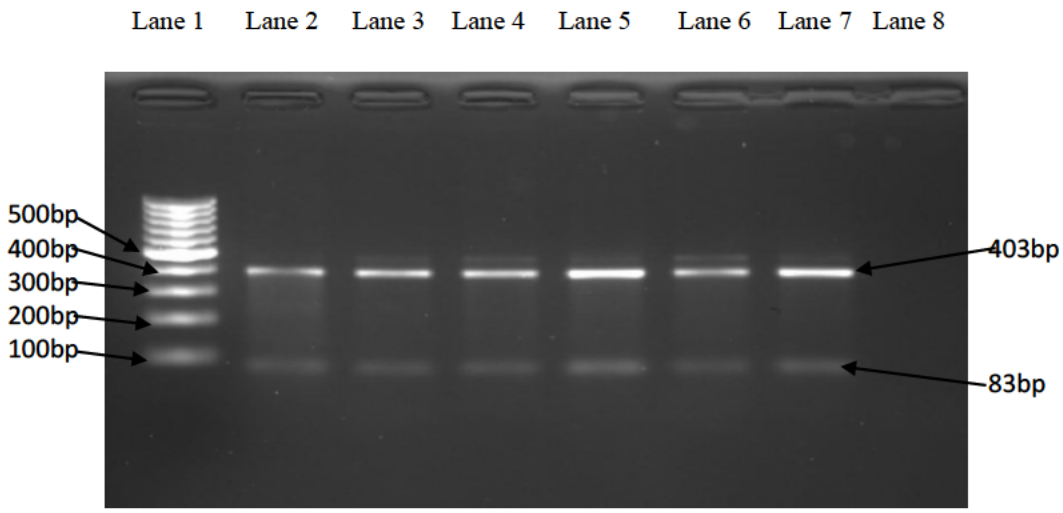

Figure 4-5a: A 2% agarose gel depicting genotyping results of the *MTNR$_1$B* (rs1387153) (C>G / C>T) polymorphism by RFLP-PCR assay. Lane 1:100 bp DNA Ladder (Thermo Scientific, SA). Lane 2 -7: A DNA fragments of 83 and 403 bp indicated homozygous C, T, or heterozygous CT. Lane 8: No template control.

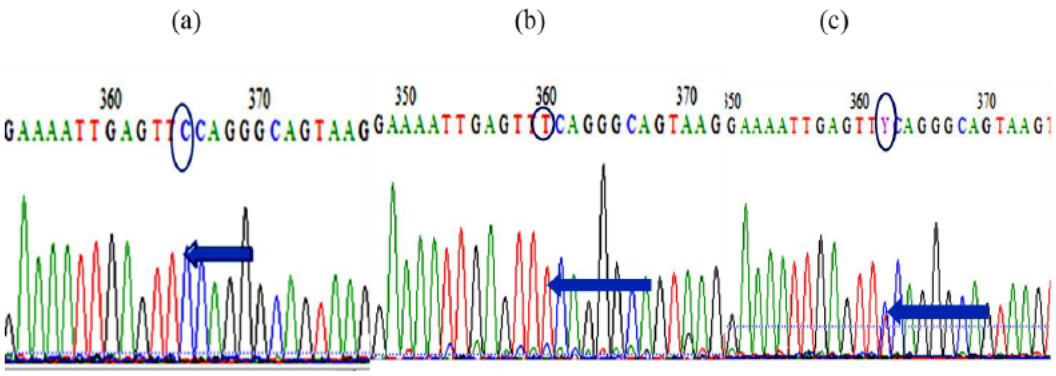

Figure4-5b: Sanger DNA sequences analysis of the *MTNR1B* (rs1387153) genetic polymorphism showing presence of a. CC (homozygote), b. TT (homozygote) and CT (heterozygote) genotype.

The presence of the G allele was not observed in any of the GDM or the control groups. RFLP - PCR could not differentiate between homozygous or heterozygous C or T alleles; hence DNA sequencing was done for all samples (Figure 4-5b). Of 22 pregnant women (3 samples failed to amplify) with diabetes mellitus, 45.5% displayed CC genotype, 27.3% showed TT, 27.3% showed CT, and in the control group (n=56) (4 samples failed to amplify), following distribution was observed CC (50%), TT (17.9%), CT (32.1%). Allele frequency distribution in pregnant women was C (0.591), T (0.409) and for the control group was C (0.589) and T (0.411) (Table 4-3).

A significant association between the SNP rs1387153 and diabetes during pregnancy was not observed, with the level of significance p >0.05. Furthermore, genotype CC and CT showed a significantly higher proportion, 50% (28/56) and 32.1% (18/56) respectively in non-diabetic pregnant women (p<0.05) (Figure 4-6). Significant association was not observed between rs1387153 and BMI status (p> 0.05), but obese women showed higher proportion 40.5% (17/42) of CC genotype than overweight and normal weight (Figure 4-7).

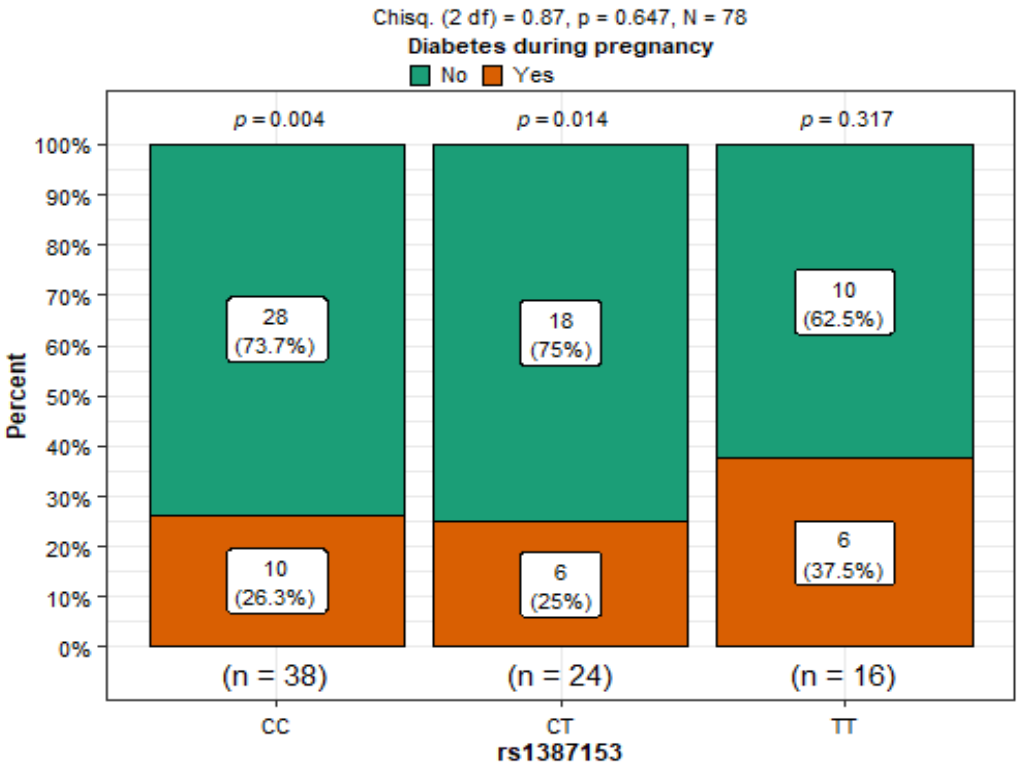

Figure 4-6: Illustration of the association between SNP genotype, pregnant and non-pregnant women with diabetes mellitus

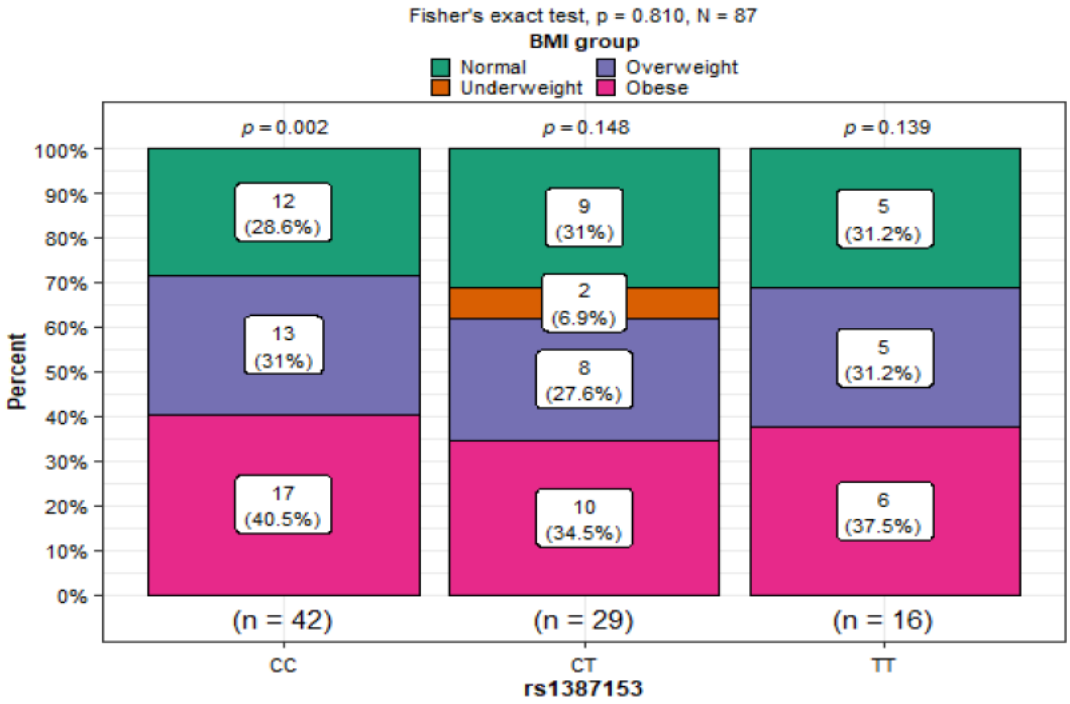

Figure 4-7: Comparison between SNP genotype (rs1387153) and women with obesity.

4.5.3 TCF7L2 Gene

The TCF7L2 amplicon was restricted with the *M1uCI* restriction enzyme. Restriction reaction resulted in 35, 66, 117, and 162bp fragments depicting the presence of the homozygote G allele (Figure 4-8a). T and A alleles were not observed in the studied population. DNA sequencing results are displayed in Figure 4-8b.

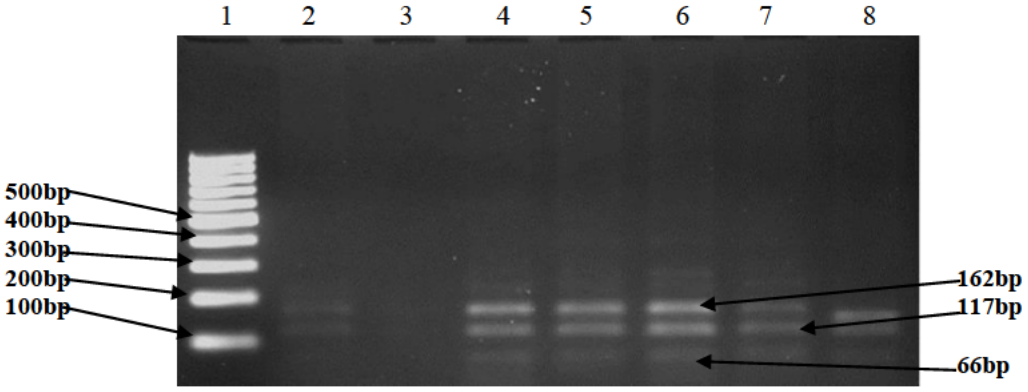

Figure 4-8a: A 2% agarose gel depicting genotyping results of the *TCF7L2* (rs12255372) polymorphism by RFLP-PCR assay. Lane 1: Molecule Weight (MW) – 100bp DNA Ladder (Thermo Scientific, SA). Lane 2, 4, 5, 6, and 7, triple band of the DNA fragments (66, 117, 162 bp) indicated the presence of homozygote G in the DNA samples. Lane 3: No template control.

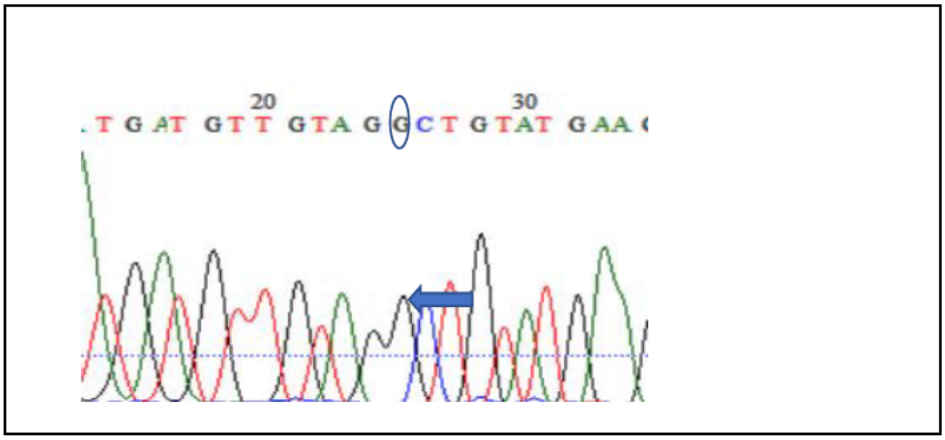

Figure 4-8b: Sanger sequencing results for *TCF7L2* (rs12255372) indicating the presence of the homozygote G allele.

Of 24 women with diabetes mellitus, and 53 women in the control group GG genotype (100%) was observed. The allele frequency for pregnant and non-pregnant women was 1% for the homozygous G allele.

Fisher`s exact test showed no statistically significant association between SNP rs12255372 and GDM ($p=0.269$) (Figure 4-9). Furthermore, the link between rs12255372 and BMI status was not seen (Figure 4-10), but the significant association was higher in obese pregnant women with GG genotype ($p<0.01$).

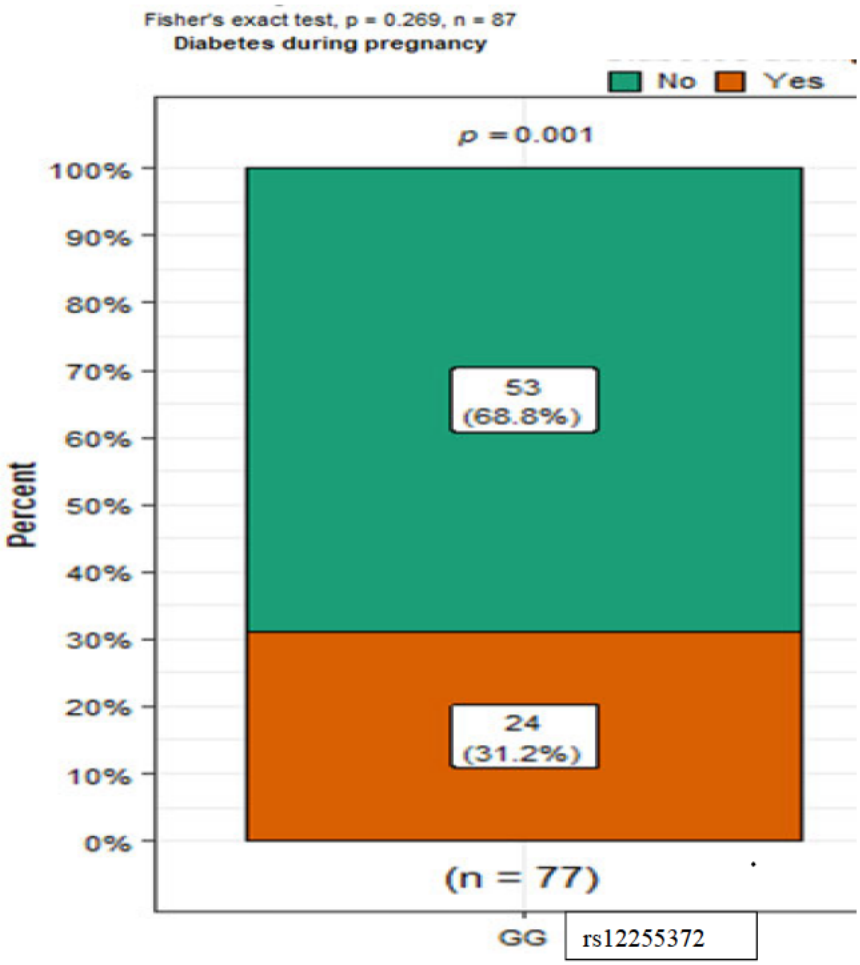

Figure: 4-9: Illustrating the SNP genotype GG of *TCF7L2* rs12255372 polymorphism and the association with GDM.

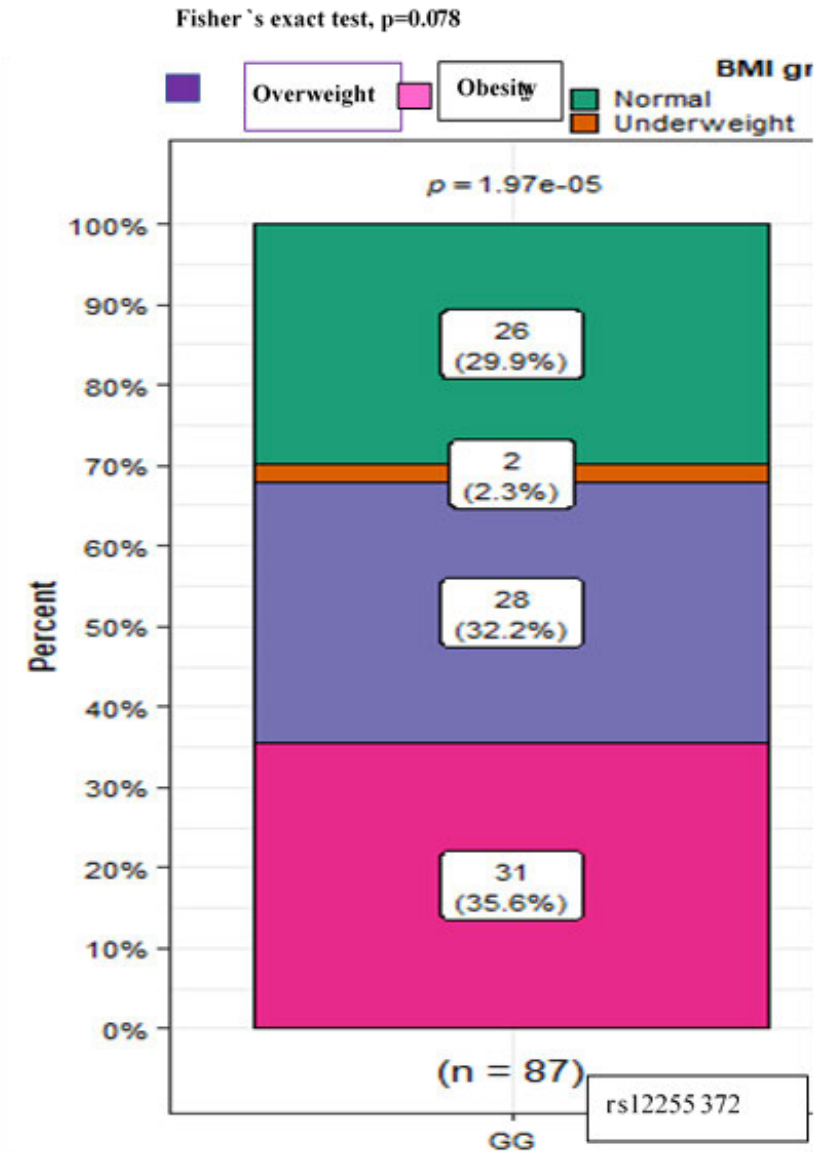

Figure 4-10: Comparison between SNP genotype (rs12255372) and pregnant women with obesity and non-obesity.

4.5.4 Collective comparison between genotypes in pregnant women with GDM and control Group.

On comparing the profile of all three SNPs, it was observed that genotype CC (rs4253778), CC (rs1387153), and GG (rs12255372) in combination were found to be significantly higher ($p<0.05$) in the control group of pregnant women (Figure 4-11) and obese subjects ($p<0.05$) (Figure 4-12).

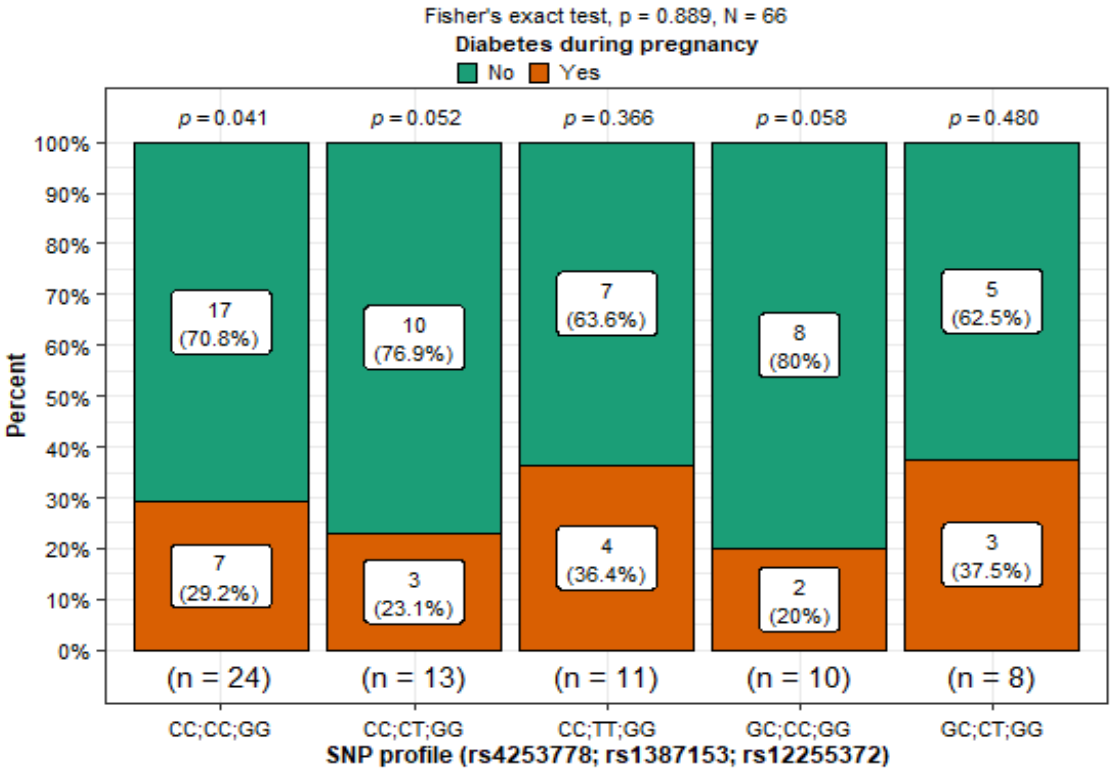

Figure 4-11: Genotype distribution in pregnant women with GDM and non-GDM.

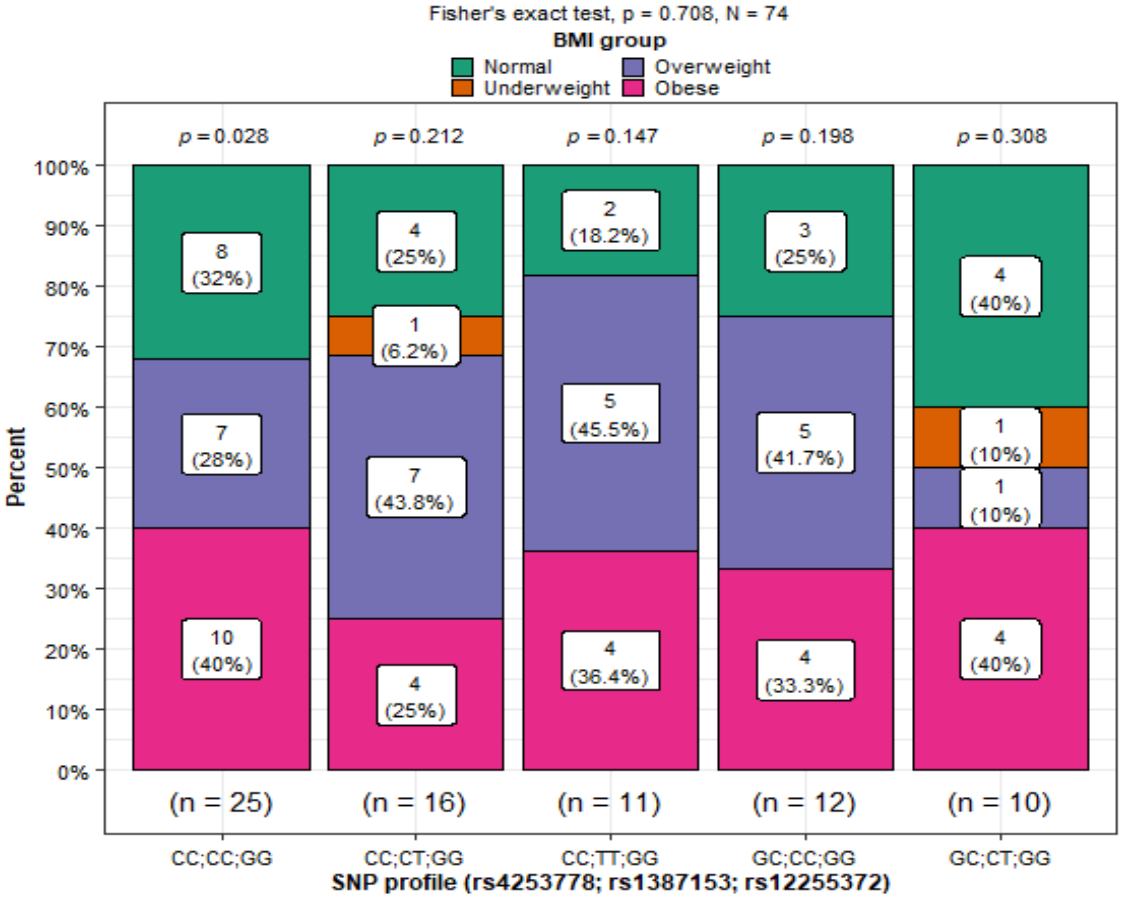

Figure 4-12: Genotype distribution of three SNPs rs4253778, rs1387153, and rs12255372, in pregnant women with obesity and non-obesity.

4.5 Discussion

Determining the genetic profile in women predisposed to developing GDM in the early gestational age of pregnancy has become urgent need.

In this present, we investigated a case-control study of black South African women with GDM and healthy controls, we investigated the associations of the *PPARα* rs4253778, *MTNR1B* rs1387153, *TCF7L2* rs12255372 polymorphisms with GDM and obesity/overweight.

4.6.1 PPARα rs4253778 polymorphism and (G>C) genotype

PPARα gene is located on chromosome 22q13.31, which encodes for a protein peroxisome proliferator-activated receptor α (*PPARα*).This protein consists of 468 amino acids .Genetic variations on *PPARα* -gene have been found to be associated with GDM, T2DM, and metabolic syndrome (obesity, insulin resistance, dyslipidaemia, and cardiovascular diseases) (Doney *et al*.,

2005; Flavell *et al.,* 2005; Jin *et al*., 2020; Yong *et al.,* 2008). Literature indicates that genetic variation of *PPARα* gene such as SNP rs4253778 (Intron 7 G>C), has been associated with GDM, obesity, and dyslipidaemia (Iyidir *et al.,* 2015; Ibanoglu *et al.,* 2019; Ruscica *et al.,* 2019; Mazzotti *et al.,* 2011). Furthermore, genetic polymorphisms such as *PPARα* rs4253778 C-allele has been reported to be associated with GDM. Kalabay *et al.* (2002) found that *PPARα* rs4253778 polymorphism was significantly associated with GDM risk among Asian pregnant women between the second and third trimester (20-40 gestational weeks). Iyidir *et al.* (2015) found similar results in Asian pregnant women in their second trimester (24-28 gestational weeks). Kralisch *et al*.(2017) showed no association between rs4253778 C-allele and GDM in pregnant women living in Asia. The latest results, by Jin *et al*. (2020) revealed that rs4253778 C-allele was associated with a risk of GDM in the first trimester of Asian pregnant women. The present study showed no association between *PPARα* rs4253778 C-allele variant and pregnant women with GDM in their first to third trimester (p=0.390). Luo *et al*.(2013) found similar results in Chinese Han population.

Our small sample size (25/87) and lower risk allele frequencies limited our power to determine the associations. Again, our analysis was limited to candidates with genetic polymorphisms that had been validated in large studies globally. Therefore, further studies with larger sample size are needed to validate our results.

The minor allele of *PPARα* G>C rs4253778 polymorphism increase the risk of DM and the level of Fetuin-A (Ruscica *et al.,* 2019). Fetuin-A is a hepatic glycoprotein that contributes to the development of obesity, insulin resistance, T2DM, adipocyte dysfunction, lipid profile, and metabolic syndrome (Reinehr *et* 2008; Stefan *et al*., 2006; Stefan *et al*., 2008; Wassel *et al.,* 2008; Rametta *et al.,* 2014; Zachariah *et al*., 2017). Studies have shown that Fetuin-A is significantly associated with severe complication in pregnancy, such as obesity, macrosomia, FGR, GDM, and lipid profile (Ibanoghu *et al*., 2017; Miroshnik *et al.,* 2021; Marcus *et al*., 2017; Iyidir *et al*., 2015, Wang *et al*., 2018). Iyidir *et al.* (2015) found that C-allele of gene *PPARα* rs4253778 polymorphism GG/GC/CC genotype was significantly associated with Fetuin-A.

The current study showed that CC and GC genotype of *PPARα* rs4253778 variant was significantly associated with obesity in pregnant women with and without GDM. This shows that Fetuin -A is an independent risk factor for women developing obesity. Again, high level of Fetuin

-A indicates an increased level of insulin resistance which may lead to severe complications in pregnancy (Jin *et al.,* 2020). Therefore, women in this study might have an increased level of Fetuin – A, but further study is needed with large sample size to confirm our results. Moreover, physical activities including exercises may reduce the risk of developing GDM and severe complications in the mother and child (Almaki *et al*., 2021; Liu *et al.,* 2008; Marcus *et al.,* 2014).

In a mixed Brazilian population including 423 females, C-allele at intron 7 of *PPARα* rs4253778 was significantly associated with dyslipidaemia and LDL cholesterol level (Mazzotti *et al*., 2011). In contrast, in the Chinese population, dyslipidaemia was linked with the G-allele of *PPARα* rs4253778 (Gu *et al.,* 2014). Genetic variant intron 7 G>C *PPARα* affects physical activities such as sports. In a female study, *PPARα* intron 7 rs4253778 CC genotype showed a negatively effect by increasing LDL level and glucose concentration after aerobic exercises (Maciejewska-Skrendo *et al.,* 2019). Furthermore, the same author found that C allele carriers of *PPARα* rs4253778 influenced human left ventricular growth. The CC homozygous allele had 3 times increased left ventricular mass than the heterozygote GC, and G allele homozygote. Moreover, lipid profiling in pregnant women with GDM and healthy control is suggested in the future study.

4.6.2 TCF_7L_2 rs12255372 polymorphism and (G>T) genotype

A transcription factor 7-like 2 (OMIM: 602228) is highly expressed in the pancreatic β-cells and adipose tissue disrupting the Wingless/Integrated (WnT) signalling pathway and leading to insulin secretion during pregnancy. *TCF_7L_2* associations were first detected in the Mexican American population, and Struan Grant later reported the specific gene in 2006. Since then, there has been an increased number of studies showing that *TCF_7L_2* polymorphisms are associated with GDM risk (Watanabe *et al*., 2007; Reyes-Lopez *et al*., 2014 ; Chang *et al*., 2017; Papadopolou *et al.,* 2011; Engwa *et al.,* 2020; Shalabi *et al.,* 2021). However, the results of the studies with GDM risk are limited in the African population. Therefore, we carried out a case-control study to determine the association between genetic profile of rs12255372 in GDM, non-GDM and obese women belonging to the black South African population, living in eThekwini district, KwaZulu-Natal.

Genetic polymorphism of *TCF_7L_2* rs12255372 homozygous T allele is the most common genetic risk investigated with GDM, T2DM, and obesity in different populations (Reyes-Lopez *et al*., 2014; Chang *et al.,* 2017; Diseko, 2018; Engwa *et al.,* 2020). It has been studied chiefly in Caucasians and Asians but less among the African people. Most studies performed in African

countries involved T2DM in the general population, meaning not in pregnant mothers. That has made it difficult to compare current results with other black women in other countries. Women accepted in this study were black South African women living eThekwini district, KwaZulu-Natal with and without a history of GDM from previous pregnancies. Women with prior history of GDM were regarded as having "GDM". Regarding *TCF7L2* rs12255372 polymorphism, all pregnant women, both GDM and the control group, were homozygous G, and the frequency allele was 1%. The association between *TCF7L2* rs12255372 G allele and GDM was not observed using Fisher`s exact test (p=0.414). However, the GG genotype was more prominent among non-diabetic pregnant women (p<0.001). No significant association was noted between *TCF7L2* rs12255372 and BMI status (p=0.078), but GG genotype carriers were significantly associated with overweight and obesity (p<0.01).

In a large meta-analysis by Chang *et al.* (2017) the association between T allele rs7903146, rs12255372, and rs7901695 polymorphisms and the risk of GDM was observed. However, the association was demonstrated among Caucasians and other ethnic groups but not the Asian population. In Mexican American (US) women with a previous history of GDM, Watanabe *et al*. (2007) found a significant association between a genetic variant *TCF7L2* rs12255372 T allele and the risk of GDM and BMI to alter insulin secretion. In another study, in the Mexican population, Reyes-Lopez *et al*. (2019) discovered that women previously diagnosed with GDM showed elevated GLP-1 indicating some defects in insulin secretion. Additionally, low β- cell function, high pre-pregnancy obesity, and rs12255372 T allele polymorphism were found as the main predictor of GDM development (Reyes-Lopez *et al.*, 2014). Francaite-Daugeliene *et al*. (2021) found no association between genetic polymorphism of *TCF7L2* rs12255372 (T-allele) and risk of GDM among Lithuanian women previously diagnosed with GDM, but the link was only demonstrated with metabolic parameters (obesity, lipids). Shalabi *et al.* (2021) showed a significantly higher risk of GT/TT genotype in rs12255372 SNP of *TCF7L2* gene among Egyptian women with GDM than in a non-diabetic group. Papadopolou *et al.* (2011) found an association between rs12255372 and GDM risk where the T allele was shown as a risk factor for T2DM. African literature showed that *TCF7L2* rs12255372 TT genotype is associated with T2DM when compared to GG genotype, indicating a higher frequency (0.44) of homozygous T allele compared with homozygous G (0.17) in Cameroon general population (Nanfa *et al.*, 2015), Lesotho (Diseko, 2018), Nigeria (Engwa *et al*., 2020). Munoz *et al*. (2006) found that polymorphism *TCF7L2* rs12255372 homozygous T allele reduces insulin secretion among non-diabetic women in African

and European Americans. El-Lebedy and Ashmawy, (2016) showed that homozygous T allele carriers have a 3.58-fold higher risk of developing T2DM than the GG genotype in the general Egyptian population. Yao *et al*. (2015) found no difference in the T -allele between T2DM male patients and the control group in females suggesting that the T allele of rs12255372 was a risk factor for T2DM in male subjects.

Our results showed that the GG genotype of *TCF_7L_2* rs12255372 had a higher frequency in non-diabetic pregnant women. In contrast, in a Nigerian study, Engwa *et al*. (2020) found that the GG genotype was more frequent in the non-diabetic general population. Bodhini *et al.* (2007) found similar results in the Asian Indian population as in Nigeria (Engwa *et al.,* 2020). Shalabi *et al. (*2021) found a significantly higher risk of GT/TT genotype in rs12255372 SNP of *TCF_7L_2* gene among Egyptian women with GDM compared with a non-diabetic group. Although the comparison was between T2DM and the general population versus GDM and pregnant women, the results were considered comparable with other kinds of literature (Engwa *et al.,* 2020; Bodhini *et al.,* 2007) because of the common genetic profile between GDM and T2DM (Cho *et al.,* 2009). This also shows that the GG genotype of *TCF_7L_2*rs12255372 is common and non-significant to black people. Maybe we need to investigate another common variant of *TCF_7L_2* with large sample size. Moreover, our study had small sample size and the results were compared with large studies. Therefore, the current results should be treated with caution.

4.6.3 $MTNR_1B$ rs1387153 polymorphism and (C/T) genotype

Melatonin receptor 1B (*$MTNR_1B$*) human gene is located on chromosome 11q14.3, encodes MT2 protein, receptor for melatonin, a primary hormone secreted in the pineal gland. This gene product is an integral membrane protein, a G-protein coupled receptor, that is expressed in the pancreatic β-cells to regulate insulin secretion and blood glucose homeostasis (Lyssenko *et al.,* 2009, Kim *et al.,* 2011). Literature has shown that *$MTNR_1B$* polymorphisms are associated with GDM risk and obesity in different ethnic groups (Kim *et al.,* 2011; Ding *et al.,* 2018; Alharbi *et al*., 2019;). Korean studies demonstrated that women with GDM may develop T2DM because of similar genetic backgrounds (Cho *et al.,* 2009; Kwak *et al*., 2012).

In a large case-control study of 112 SNPs, Ding *et al.* (2018) found that polymorphism of *$MTNR_1B$* rs1387153(T-allele) is associated with GDM and obesity in pregnant women living in the USA and Denmark. The current study showed no association between *$MTNR_1B$* rs1387153 and obesity

in women with GDM. Alharbi *et al.* (2019) found that rs1387153 T allele was associated with GDM development but not with obesity in Saudi women. After the investigation of five *MTNR1B* genetic polymorphisms (rs10830963, rs1387153, rs2166706, rs1447352, and rs4753426), Jia *et al*. (2020) showed that rs1387153 (T-allele) was not significantly associated with GDM and obesity in Southern Chinese pregnant women, whereas in the same single nucleotide polymorphisms investigation (rs10830963, rs1387153, rs2166706, rs1447352, and rs4753426), Shen *et al.* (2020) found a significant association between rs1387153T allele and pregnant women with GDM risk and obesity in two independent Chinese population. This shows that genetic mutation can be affected by environmental factors. A repeat study is suggested with a larger sample size to validate these results.

In addition, pre-pregnancy obesity and fasting plasma glucose predisposes pregnant women to develop GDM (Kim *et al.,* 2011). In Russia, Popova *et al*. (2021) found that the *MTNR1B* rs1387153 variant was associated with pre-pregnancy obesity in women developing GDM risk, and in Finnish pregnant women (Huopio *et al.,*2013), further noticed that *MTNR$_1$B* rs1387153 polymorphisms are significantly associated with increased fasting glucose levels in the early gestational week. In Greek women, the *MTNR$_1$B* rs1387153 variant was not associated with a higher level of glucose (Vlassi *et al.,* 2012). Among Korean women, rs1387153 polymorphisms was associated with glucose homeostasis of β-cell function and fasting glucose levels (Kim *et al.,* 2011).

The risk alleles frequencies *MTNR$_1$B* rs1387153 polymorphism may differ globally in women with GDM and non-GDM. Our population study found that C-allele carriers for rs1387153 polymorphism, were not associated with GDM risk, but the CC genotype of rs1387153 polymorphism was associated with obesity/overweight (p=0.002). In pregnant women with GDM, no, statistically significant difference was observed between the proportions of GDM within the group of pregnant women with TT genotype (p=0.317). In Saudi Arabia, Alharbi *et al*. (2019) found that *MTNR$_1$B* rs1387153 TT genotype was associated with increased blood glucose level after delivery in pregnant women with GDM (p<0.18). Although our study showed consistency with Vlassi *et al.* (2012) in the Greek population., the study had limited statistical power with a relatively small sample size. Therefore, a repeat is suggested with a larger sample size.

4.6 Conclusion

The present study targeted the black South African women from the Province of KwaZulu-Natal. To the best of my knowledge, no previous genetic study comparing association between genetic polymorphism and GDM has been conducted on this population group. Though no significant association was observed with the target SNPs, the results of the study does indicate towards association of the genetic polymorphisms with obesity in the test subjects. The sample size of the women with GDM was a major limiting factor. Hence, future studies should target the same population with a larger sample size. Additionally, further studies are required to validate these results to see if maternal genotype can be used to identify women at risk, and plan for the treatment.

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CHAPTER 5: DISCUSSION

5.1 Synthesis

Gestational diabetes mellitus (GDM) is a short -term complication in pregnancy regarded as "silent killer" to mothers and infants during pregnancy (Armod *et al*., 2017; Lagese *et al*., 2016; Maternal Mortality, WHO Factsheet, February 2023). Despite the improvements done in communicable diseases such as H1V and TB, non-communicable diseases, including hypertension as part of heart -related diseases, diabetes mellitus, and cancer are still major health concern. Although GDM may disappear after delivery it reappear in the next pregnancy to women with pre-existing diabetes mellitus (Feig *et al.*, 2010; Wahabi *et al*., 2017; Njete *et al.*, 2018). Women with pre-existing DM develop GDM in early pregnancy (John *et al*., 2020; Sesmilo *et al.*, 2020) . GDM is characterised by environmental factors, maternal obesity and genetics elevating insulin demand to the pregnant mother. Maternal well-being and the placenta determine the foetal development. Obese pregnant women are associated with GDM and T2DM after delivery. Women with pre-existing GDM increase the risk of foetal macrosomia and CNS birth defects (Anderson *et al*., 2005; Correa *et al*., 2015; Gallo *et al*., 2017). Delivering a foetus ≥4kg with shoulder dystocia is directly linked with direct obstetric causes such as haemorrhage, and hypertensive disorders (Said and Manji, 2016). However, haemorrhage is a leading cause of maternal death in Africa (Legesse *et al.*, 2017; Manyeh *et al.*, 2018; Lancaster *et al.*, 2020). Approximately, 11% cases of maternal death causes, remained unknown in the middle-aged South African women (Tlou *et al.*, 2018). All women with a history of GDM are treated as high risk, and screening is advised every three years (American Diabetes Association, 2018; ADA, 2020) . If pre-existing DM is detected treatment must be considered to prevent further complications . This pilot current study aimed at identifying the prevalence rate of pre-existing GDM and associated risk factors in women previously diagnosed with GDM. Furthermore, this study intended to investigate the associations between the maternal genotype and GDM in obese pregnant women.

Gestational diabetes mellitus is any degree of glucose intolerance primary detected at any time either early or later during pregnancy (World Health Organization, 2013). This explanation includes undiagnosed women with T2DM in an early gestational week. Diabetes mellitus in pregnancy (DIP) is a type of diabetes that is diagnosed at any time in pregnant women with pre-existing diabetes mellitus either T1DM or T2DM during pregnancy or current pregnant women

previously diagnosed with GDM for the first time met "WHO diagnostic criteria". The actual GDM is usually diagnosed for the first time between 24 – 28 weeks during pregnancy, whereas DIP is detected at any time during pregnancy (WHO, 2013).

The diabetes in pregnancy was reported as GDM in this study and the prevalence rate was estimated at 28.7% (n=25/87). The results were identified using self-report data collected in black women living eThekwini district, KwaZulu-Natal. KEHVIII Tertiary Hospital in Durban is considered a referral for critical cases in pregnant women. Although, the majority (n=49) of women were attending in the Township communities than in Urban (n=38), but the current data indicated that GDM risk prevalence was higher (n=16) in pregnant women attending at KEVIII Tertiary Hospital (urban) than in Township community (n=9). Moreover, most women were diagnosed in their last trimester 7.8% (n=8) than first and second trimester and the worst part was those women who did not know or remember their diabetes status during their previous pregnancies (n=13).

Our result was higher (28.7%) compared with black South African women in larger studies previously conducted in South Africa: Limpopo province (8.8%), Gauteng (9.1% ; 25.8%) and among Indian women in KwaZulu- Natal (KEVIII) (23%) (Mamabolo *et al.*, 2007; Macaulay *et al.*, 2018; Adam and Rheeder, 2017; Notelovitz, 1969 respectively).
When the same diagnostic criteria were applied, GDM prevalence amongst black women showed higher 28.7% prevalence than that reported in a cohort of New Zealand white women (6.2%) (Lawrence *et al.*, 2019). The difference could be a sampling size, ethnic group, and geographic area. New Zealand had a cohort of 6822 pregnant women and the current study had 70 pregnant women residing in eThekwini KwaZulu- Natal. Therefore, the hypothesis was rejected. Again, further studies are recommended with a larger sample size to increase the strength and power for a better understanding of this pilot study result. Hence, the interpretation of the current results should be treated with reserve and caution-However, these results may pave a way to further understand why women above the age ≥36 years, who had more than one child been at high risk of GDM. In addition, pre-existing DM, using of ARV treatment, and BMI were predictors in pregnant women developing GDM.

Pre-existing diabetes mellitus

Women with pre-existing DM are at high risk of developing GDM (Xiang *et al.,* 2018). Furthermore, women with GDM history are at risk of T2DM between 5-10 years after delivery than women without GDM history (Armod *et al.,* 2017; Anderson *et al*., 2005; Correa *et al*., 2008; Li *et al.,* 2020). Their children are more at risk of being obese /overweight, T2DM in their childhood or adolescents' life. Women with a history of pre-existing DM (T1DM or T2DM or GDM) have implications for pre-eclampsia, pre-term labour, C-section, stillbirth, macrosomic infants in the early gestational age (<12 weeks) (Diabetes Care, 2016; CDC, 2018). In this case-control study, about 28.7% (n=25) of pregnant women indicated pre-existing GDM

Women with pre-existing DM usually develop GDM in the early gestational age. This study showed that women diagnosed in the first trimester were lesser 1% (n=1) than women diagnosed in the third trimester 7.8% (n=8). However, women had miscarriages were higher 21.6% (n=22) followed by women who delivered macrosomic neonates 10.8% (n=11), neonatal death 3.9% (n=4), and stillbirth/birth defect 2.0% (n=2). Our findings reported that pre-existing DM is a marker in women developing GDM. Lee *et al*. (2018) found similar results in Asian women, even in their subsequent investigation (Li *et al*., 2020). Therefore, this current study is in line to report the risk factors of GDM in black South African women.

ARV treatment

The development of gestational diabetes mellitus has also been associated with the use of Highly Active Antiretroviral Therapy (HAART). Highly active antiretroviral therapy is indicated to induce insulin resistance by causing dysregulation of human placental hormones such as oestrogen, hPL, hPGH, in pregnant women with HIV infection (Jao *et al.,* 2013). Highly active antiretroviral therapy which is used for preventing mother-to -child transmission (PMTCT) has been demonstrated to alteration of maternal homeostatic profile (Gonzalez-Tome *et al.,* 2008; Biadgo *et al.,* 2019). Proteus inhibitor is known to increase hepatic glucose, and stavudine increases the level of visceral fat with subcutaneous fat increasing the level of insulin resistance which leads to impaired glucose tolerance and diabetes mellitus (Carr *et al*., 1998; Lenhard *et al*., 2000). The current study showed that women taking ART treatment were lesser 46.1% (n=47) than women not on ART treatment 53.9% (n=55). The results indicated an improvement in black SA women living with HIV and AIDS. However, UNAIDS's latest report (2020), showed that South Africa is remaining as the most prevalent country in HIV and AIDS. The disease is

increasing (3 200 000 -6 100 000) in women aged ≥ 15 years, and the prevalence rate is higher 27.7% in females compared with men 13.5% aged between 15-49 years old. Another study investigating specific ARV treatment is suggested with a larger sample size of women living eThekwini district, KwaZulu-Natal.

Previous investigations on ARV treatment were specific in other countries including Africa. Biadgo *et al.* (2019) found that PI is a significant independent risk factor for GDM among pregnant women infected with HIV in Asia, Europe, and Africa. Marti *et al.* (2007) reported the same results, and similarly, Gonzalez-Tome *et al.* (2008) had the same findings in Spanish pregnant women with HIV infection. Soepnel *et al.* (2017) found no significant difference between PI and GDM in pregnant women infected with HIV. Similarly, Hitti *et al.* (2007) failed to demonstrate PI and GDM among pregnant women infected with HIV-1. Mmasa *et al.* (2021) found no link between ART treatment and GDM among pregnant women living in Botswana. Adam and Rheeder, (2017) reported the same findings in South African women living in Gauteng. The current study showed consistency with other kinds of literature, but ART treatment was not specific to PI. Therefore, we reject the hypothesis.

BMI (≥25 kg/m^2)- Overweight

Overweight and obesity are the most common findings in women of childbearing age. South African studies are showing that black girls are more overweight and obese than boys, and the prevalence is increasing (Craig *et al.*, 2013; Negash *et al.*, 2017). Globally, the report showed that women aged ≥18 years old had a higher (40%) prevalence of overweight than obese women (15%), and a huge increase of 4% to >18% was observed among children and adolescents aged between 5-19 years old (WHO Factsheet, 2021). In our study, 89.2%(n=91) of black women indicated unhealthy eating. This was a comment reported at the time of an interview: 'Eating vetkoek and fries is what we can afford. This kind of meal is cheaper, easy to find, and every street has it.' The majority 88.2% (n=90) reported that exercises are not for them. This was another great concern in a meal that is full of cooking oil.

The present study showed that overweight in pregnant women was not age-related ($p>0.05$), but overweight was an independent risk factor for GDM in pregnant women living eThekwini district, KwaZulu-Natal. Moreover, a significant proportion was higher in obesity 48% (n=12) than overweight 28%(n=7) of women with GDM. Bian *et al.* (2020) found that high BMI ≥25kg/m^2

was associated with GDM in women previously diagnosed with GDM. Lee *et al. (*2018) showed that BMI ≥25kg/m ^{2} is an independent risk factor for GDM risk in the Asian population. Zhang *et al.* (2021) found similar results of BMI ≥25kg/m^2 in Chinese pregnant women with GDM risk. Our study has consisted of the literature. The hypothesis was rejected.

Li *et al.* (2020) found that overweight BMI ≥25kg/m^2 and obesity (BMI ≥30kg/m^2) before pregnancy and GWG between 15 -20 gestational weeks are associated with an increased risk of GDM in women aged ≥ 30 years old. This could be due to an increased insulin demand caused by weight gained because of hormonal changes in the early gestational age. Yoger *et al.* (2009) suggested that overweight and obese people are at a 10 times more increased risk of developing T2DM compared to GDM women. Other literature says women who are overweight and obese are 2-fold higher risk of developing GDM and delivering a macrosomic newborn (Black *et al.*, 2013; Yong *et al.*, 2020). Moreover, macrosomic newborns are at higher risk of developing T2DM, and becoming more overweight or obese later in life (Ntenda and Kazambwe, 2019; Yeshaw *et al.*, 2020; Feng *et al.*, 2019; Miao *et al.*, 2017). Overweight mothers with offspring overweight higher than normal mothers were observed in women who developed GDM (Pirkola *et al.*, 2010). Lieshout *et al.* (2011) found a link between maternal obesity, and ADHD children, adolescents with eating disorders, and adults with psychotic conditions, but not with foetal macrosomia. Physical activities including exercises were detected as a way of reducing the high risk of GDM in women living in Gauteng -Soweto (Khan *et al*., 2016).

The objective of the study here, was to determine whether genetic polymorphisms associated with T2DM are associated with GDM in obese women. We conducted a GDM investigation on 25 of 87 (28.7%) black women in SA. There was no significant association between SNP rs4253778, rs1387153, rs12255372 and the risk of GDM and obesity (p>0.05), but there was a significant association between SNP rs4253778 CC genotype, and GC genotype, rs1387153 CC genotype, rs12255372 GG genotype and the risk of developing GDM and obesity in pregnant and non-pregnant women (<0.05).

The risk alleles of GDM and obesity were not significantly associated with genetic variants of *PPARα* rs4253778, rs1387153 of *MTNR$_1$B* gene, rs12255372 of *TCF$_7$L$_2$* gene in pregnant and non-pregnant women (p>0.05). However, the genetic polymorphism of the PPARα rs4253778 CC genotype showed significantly higher in non-GDM pregnant women, and GG genotype was only observed in non-diabetic pregnant women. Furthermore, *MTNR$_1$B* rs1387153 polymorphism

CC and CT were significantly higher in non-diabetic pregnant women. Moreover, the GG genotype of *TCF7L2* rs12255372 polymorphism was observed in pregnant and non-pregnant women who participated in the study.

In the combined SNP genotypes. SNP profiles of rs4253778, rs1387153, and rs12255372 had a significantly higher frequency of CC, CC, and GG genotypes in pregnant women with non-GDM. Moreover, CC and GG genotype carriers may have a risk of developing obesity during pregnancy.

5.2 Challenges and limitations of the study

The study was originally planned to be conducted at the Municipality clinics and the Department of Health in the eThekwini region. However, the study approval was only received at the DoH. The clinics approved by DoH were as follows: KwaMashu, Inanda, and Phoenix CHCs in the Northern region, KwaDabeka CHC in the Western region, Folweni Clinic in the Southern region, and King Edward VIII Tertiary Hospital in the Central region of eThekwini Health District. The time waited for the Municipality's response caused a delay in the commencement of the data collection. The minimum number of targeted samples to be collected was 200. Furthermore, data collected had to be stopped due to the Global pandemic outbreak in mid-March 2020. Samples n=103 were only collected in the following clinics: KwaMashu CHC, KwaDabeka CHC, and KEVIII Tertiary Hospital. The clinics were operating from Mondays to Fridays. Mondays and Tuesdays were specifically for first-time ANC visits. Wednesdays were for the returns. Wednesdays and Thursdays were days for collecting data in KEVIII Tertiary Hospital. If it were not for the Global pandemic, I would have collected at least 200 and more sample sizes to make this study more represented and generalized for the whole population of women with gestational diabetes in the community health centres approved by the district and King Edward VIII Tertiary Hospital. The researcher checked for completeness of the questionnaire after the interview.

The arrangements were made to collect patients' records for screening and diagnoses of GDM on the patients' ANC registry. The researcher planned to collect all GDM records at the end of the data collection in all clinics approved by the district. Unfortunately, that did not happen due to the global pandemic in March 2020. To overcome the problem, the researcher used a self-data report collected on women with or without a previous diagnosis of GDM from previous pregnancies without the confirmation of a 2-hour OGTT as part of the standard procedure.

5.3 Conclusion

The gestational diabetes mellitus prevalence rate was 28.7%. Age, parity, FHD, and pre-existing DM were associated with GDM, but ARV treatment, overweight and pre-existing DM were predictors for GDM. This study also found evidence that the association between SNPs and the risk of GDM was not observed. Maternal genotyping in obese women may identify the risk of GDM.

The information gathered in this study affirms that obesity and HIV/AIDS still exist among black SA women residing in the eThekwini district, KwaZulu-Natal, and since no interaction was observed in the current data due to sample size therefore, the possibility of them developing GDM in their early gestational week in their next pregnancies is high. This is alarming as the fertility rate indicates that the youngest pregnant woman is less than 10 years old (Stats, SA 2020). Therefore, prevention of further complications in pregnancy, and treatments are considered urgently with larger sample size studies to validate these results.

5.4 Recommendations

1. More data collection is needed for this study to make a better generalization of the population of women living eThekwini district, KwaZulu-Natal. The next data collection needs to be conducted in the Municipality clinic regions, Inanda, Phoenix, Folweni clinics, and other provinces within the South African continent.
2. Findings are to be presented at DoH and in the approved ANC clinics eThekwini health district as requested.
3. Awareness and education regarding obesity in pregnant and non-pregnant women should be considered. All radio stations, schools, and clinics must be used as a form of platform for communication, preferably IsiZulu as a language of their choice.
4. UKZN to collaborate with the Ntshembo (Hope) project that is already existing as a way of addressing the improvement of obesity among younger generations in the black SA community (Draper *et al*., 2014). Suggesting mothers attend the meetings with their daughters.

5.5 Recommendations for future studies

1. A study investigating the lifestyle of single pregnant women living in eThekwini district, KwaZulu-Natal.

2. More studies to investigate GDM risk in South African women selecting the same genes to validate the current data.
3. The role of epigenetic transformation in GDM pathogenesis demonstrates the interaction between environmental and genetic factors that predisposed women to GDM risk.
4. Large study to investigate the prevalence of GDM in black women living in KwaZulu - Natal looking at other mechanisms like lipid profile.
5. The mechanism by which the mother could develop GDM includes the foetal genome (imprinted genes).
6. Investigating GDM and obesity in HIV-infected women on PI and other classes of ARV treatment in black women living in KwaZulu-Natal.
7. Investigating the presence of Fetuin-A in black women of KwaZulu-Natal.
8. Follow -up study on mothers who delivered macrosomic babies

5.6 Reference

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APPENDICES

Appendix A: Approval letter – UKZN BREC Ref no: BE378/19

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

09 September 2022

Ms AN Moloi 218087155
School of Laboratory Medicine and Medical sciences
College of Health Sciences
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Dear Ms Moloi

Protocol: Associations between foetal imprinted genes and incidences of gestational diabetes mellitus (GDM) in pregnant women of eThekwini Municipality
Degree: MMedSc
BREC Ref No: BE378/19
New Title: Investigating Genetic Predisposition to Gestational Diabetes Mellitus Among Black Women Residing eThekwini, KwaZulu-Natal, South Africa.

We wish to advise you that your response to BREC letter dated 29 August 2022 has been **noted** by a subcommittee of the Biomedical Research Ethics Committee. Your letter dated 25 August 2022 submitting an application for amendments listed below for the above study has now been **approved** by a subcommittee of the Biomedical Research Ethics Committee:

Amendments noted and approved:

- Change of supervisor from Dr Hlengiwe Prosperity Mbongwa, to Dr. Khethelo R. Xulu.
- Change of title to the above new title.

The committee will be notified of the above at its next meeting to be held on 11 October 2022.

Yours sincerely

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Appendix B: Approval letter - Department of Health, NHRD Ref no: KZ_201909_040

health
Department:
Health
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DIRECTORATE:
Health Research & Knowledge Management

NHRD Ref No.: KZ_201909_040

Dear Ms AN Moloi
UKZN

Approval of research

1. The research proposal titled **'Associations between foetal imprinted genes and incidences of gestational diabetes mellitus among pregnant women in eThekwini Health District in KZN'** was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at Inanda, KwaDabeka, KwaMashu and Phoenix Community Health Centre and; Folweni Clinic.

2. You are requested to take note of the following:
   a. Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.
   b. Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.
   c. Provide an interim progress report and final report (electronic and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 31/10/19

Fighting Disease, Fighting Poverty, Giving Hope

Appendix C: Permission to conduct a Research at eThekwini District Facilities

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE: CORPORATE SERVICES

83 King Cetshwayo Highway
Mayville, Durban, 4001
Tel: 031 240 5455 Email:
www.kznhealth.gov.za

ETHEKWINI HEALTH DISTRICT OFFICE

26th June 2019

Dear Angie Moloi

Re: Permission To Conduct Research at eThekwini District Facilities.

This letter serves to confirm that your application to conduct the research study titled, "**Associations between foetal imprinted genes and incidences of gestational diabetes mellitus (GDM) among pregnant women in eThekwini Municipality.**" in the eThekwini district at the following health care facilities has been recommended:

1. Phoenix CHC
2. Inanda CHC
3. KwaMashu CHC
4. Folweni Clinic
5. KwaDabeka CHC

Kindly upload this letter together with your application as required to the Health Research and Knowledge Unit for the KZN Department of Health for Approval.

Please also note the following:

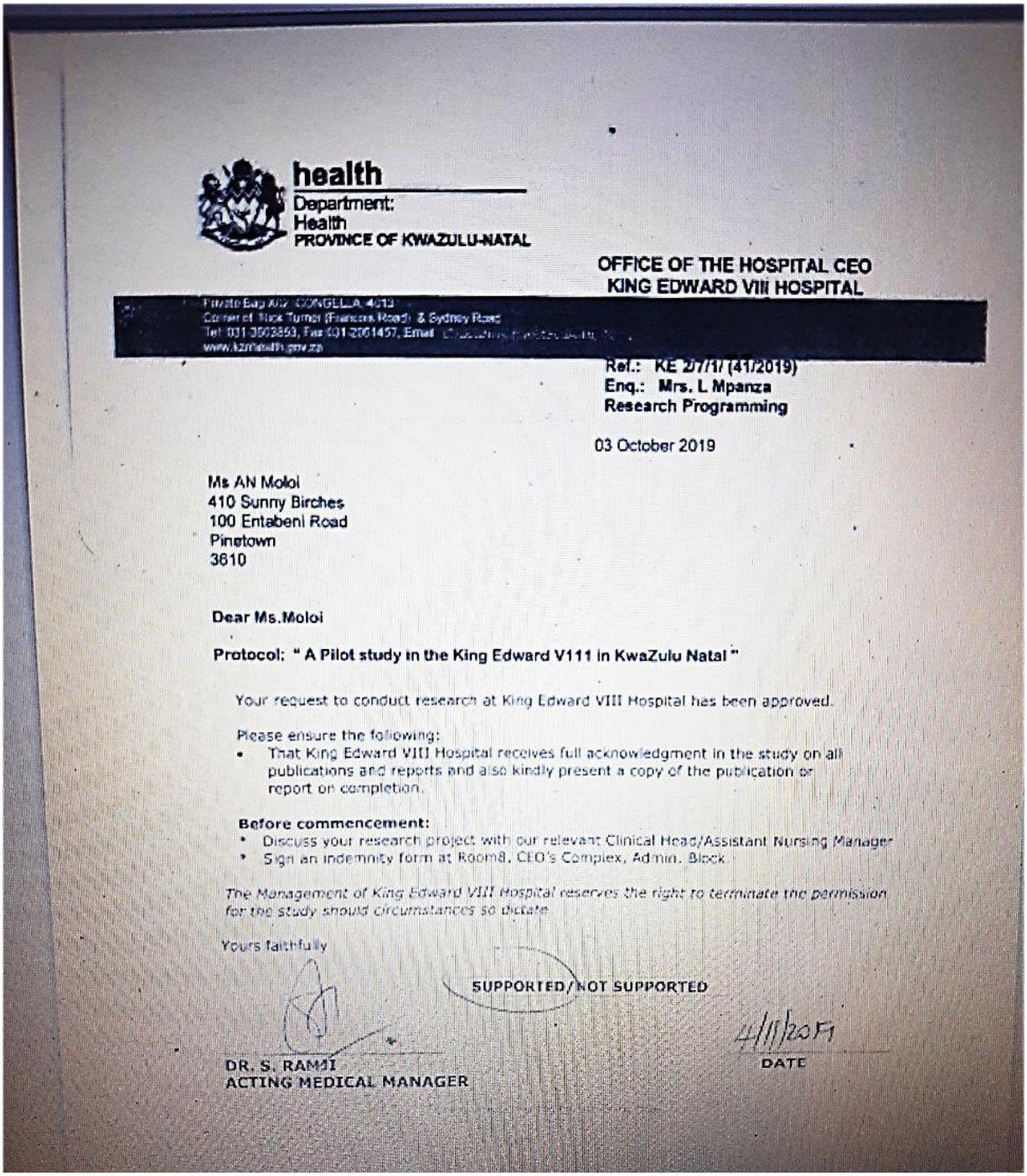
1. This research project should only commence after final approval by the KwaZulu-Natal Health Research and Knowledge Unit, and full ethical approval, has been granted.
2. That you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
3. All research activities must be conducted in a manner that does not interrupt clinical care at the health care facility.
4. Ensure that this office is informed before you commence your research
5. The District Office/Facility will not provide any resources for this research
6. All logistical details must be arranged with the CEO/medical manager /operational manager of the facility.
7. You will be expected to provide feedback on your findings to the District Office/Facility

Yours sincerely

Dr N Green(District Research Coordinator)
Pp Ms. T. P. Msimango
Chief Director (Acting)
eThekwini Health District

Fighting Disease, Fighting Poverty, Giving Hope

Appendix D: Permission to conduct Research at King Edward VIII Tertiary Hospital



health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII HOSPITAL

Private Bag X07, CONGELLA, 4013
Corner of Rose Turner (Francois Road) & Sydney Road
Tel: 031-3903893, Fax: 031-2061457, Email:
www.kzndoh.gov.za

Ref.: KE 27/1/ (41/2019)
Enq.: Mrs. L Mpanza
Research Programming

03 October 2019

Ms AN Moloi
410 Sunny Birches
100 Entabeni Road
Pinetown
3610

Dear Ms.Moloi

Protocol: " A Pilot study in the King Edward V111 in KwaZulu Natal "

Your request to conduct research at King Edward VIII Hospital has been approved.

Please ensure the following:

- That King Edward VIII Hospital receives full acknowledgment in the study on all publications and reports and also kindly present a copy of the publication or report on completion.

Before commencement:

- Discuss your research project with our relevant Clinical Head/Assistant Nursing Manager
- Sign an indemnity form at Room8, CEO's Complex, Admin. Block.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

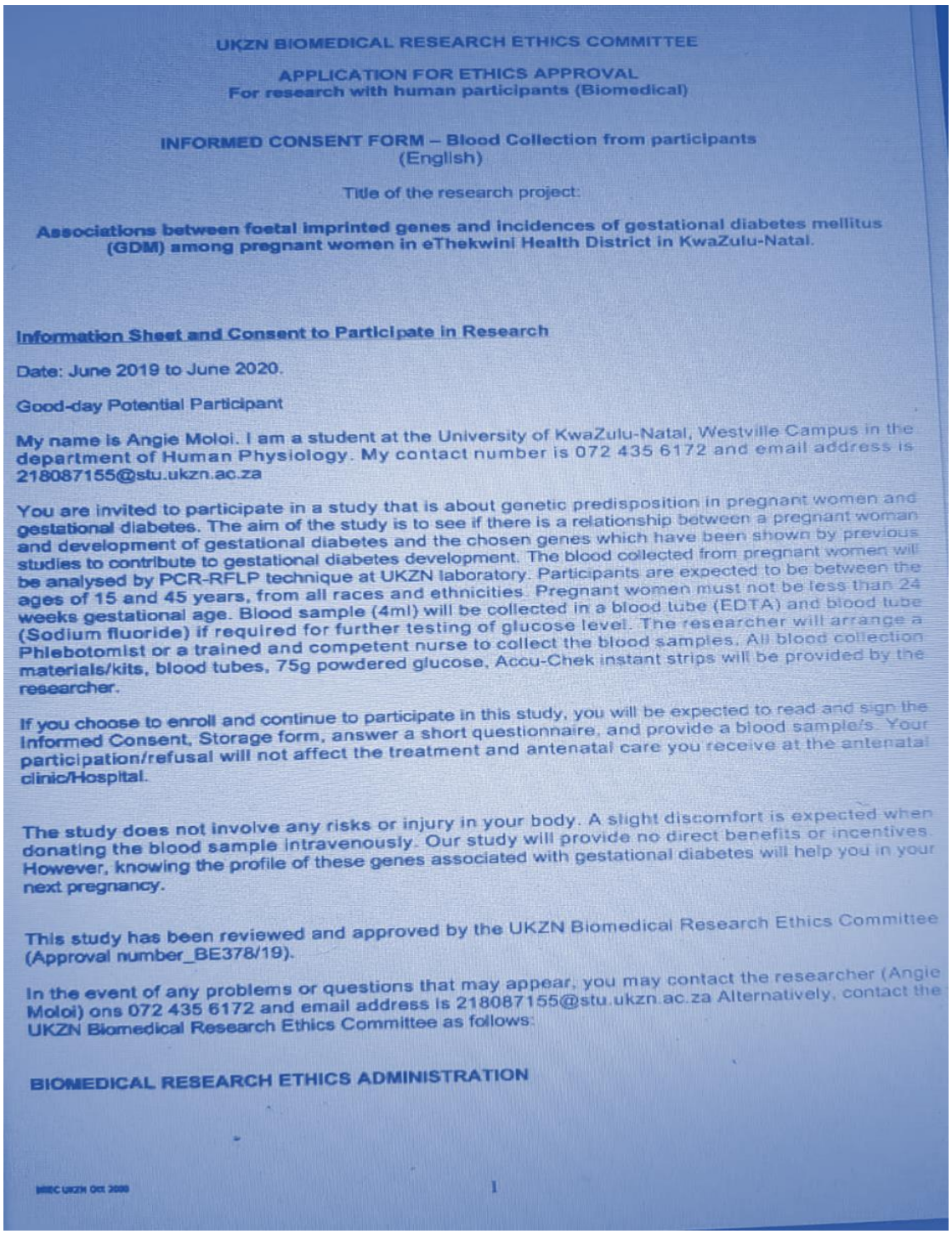
Yours faithfully

SUPPORTED/NOT SUPPORTED

4/11/2019
DATE

DR. S. RAMJI
ACTING MEDICAL MANAGER

Appendix E: Written Informed Consent (English)



UKZN BIOMEDICAL RESEARCH ETHICS COMMITTEE

APPLICATION FOR ETHICS APPROVAL
For research with human participants (Biomedical)

INFORMED CONSENT FORM – Blood Collection from participants
(English)

Title of the research project:

Associations between foetal imprinted genes and incidences of gestational diabetes mellitus (GDM) among pregnant women in eThekwini Health District in KwaZulu-Natal.

Information Sheet and Consent to Participate in Research

Date: June 2019 to June 2020.

Good-day Potential Participant

My name is Angie Moloi. I am a student at the University of KwaZulu-Natal, Westville Campus in the department of Human Physiology. My contact number is 072 435 6172 and email address is 218087155@stu.ukzn.ac.za

You are invited to participate in a study that is about genetic predisposition in pregnant women and gestational diabetes. The aim of the study is to see if there is a relationship between a pregnant woman and development of gestational diabetes and the chosen genes which have been shown by previous studies to contribute to gestational diabetes development. The blood collected from pregnant women will be analysed by PCR-RFLP technique at UKZN laboratory. Participants are expected to be between the ages of 15 and 45 years, from all races and ethnicities. Pregnant women must not be less than 24 weeks gestational age. Blood sample (4ml) will be collected in a blood tube (EDTA) and blood tube (Sodium fluoride) if required for further testing of glucose level. The researcher will arrange a Phlebotomist or a trained and competent nurse to collect the blood samples. All blood collection materials/kits, blood tubes, 75g powdered glucose, Accu-Chek instant strips will be provided by the researcher.

If you choose to enroll and continue to participate in this study, you will be expected to read and sign the Informed Consent, Storage form, answer a short questionnaire, and provide a blood sample/s. Your participation/refusal will not affect the treatment and antenatal care you receive at the antenatal clinic/Hospital.

The study does not involve any risks or injury in your body. A slight discomfort is expected when donating the blood sample intravenously. Our study will provide no direct benefits or incentives. However, knowing the profile of these genes associated with gestational diabetes will help you in your next pregnancy.

This study has been reviewed and approved by the UKZN Biomedical Research Ethics Committee (Approval number_BE378/19).

In the event of any problems or questions that may appear, you may contact the researcher (Angie Moloi) ons 072 435 6172 and email address is 218087155@stu.ukzn.ac.za Alternatively, contact the UKZN Biomedical Research Ethics Committee as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

BREC UKZN Oct 2008

1

Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

You are not forced to participate in this study, you may withdraw at any time. You will not be penalized or lose anything with your withdrawal. If you wish to withdraw, you may contact the researcher and you will be withdrawn immediately.

Your blood sample will be labelled according to a number system created for this study together with age and ethnicity. Your name will not be written on the tube of your blood sample. All your information will be kept confidential.

Samples will be stored at the Human Physiology Department at University of KwaZulu-Natal Westville campus until the end of the study. All results that will be obtained will be kept in the computer and the files will be password protected.

All blood samples and results that will be obtained will be destroyed accordingly at the end of the research study.

CONSENT

This is for prospective participants to this study between the ages of 15 to 45 years.

Agreement to participate in the study.

I..... (participant`s full name) ID number.....
have been informed about the study ***entitled associations between foetal imprinted genes and incidences of gestational diabetes mellitus (GDM) among pregnant women in eThekwini Health District in KwaZulu-Natal*** by Dr Hlengiwe P. Mbongwa.

(Please tick √ the box if agree and cross X if disagree)

☐ I understand the purpose and procedures of the study.

☐ I have been given an opportunity to ask questions about the study and have been answered to my satisfaction.

☐ I declare that I understand that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

☐ I have been informed that no compensation is available, but it is the responsibility of the researcher to provide any medical treatment if an injury occurs to me because of study-related procedures.

☐ If I have any further questions/concerns or queries related to this study, I understand that I may contact the researcher, Angie Moloi at 072 435 6172 and 218087155@stu.ukzn.ac.za

☐ If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

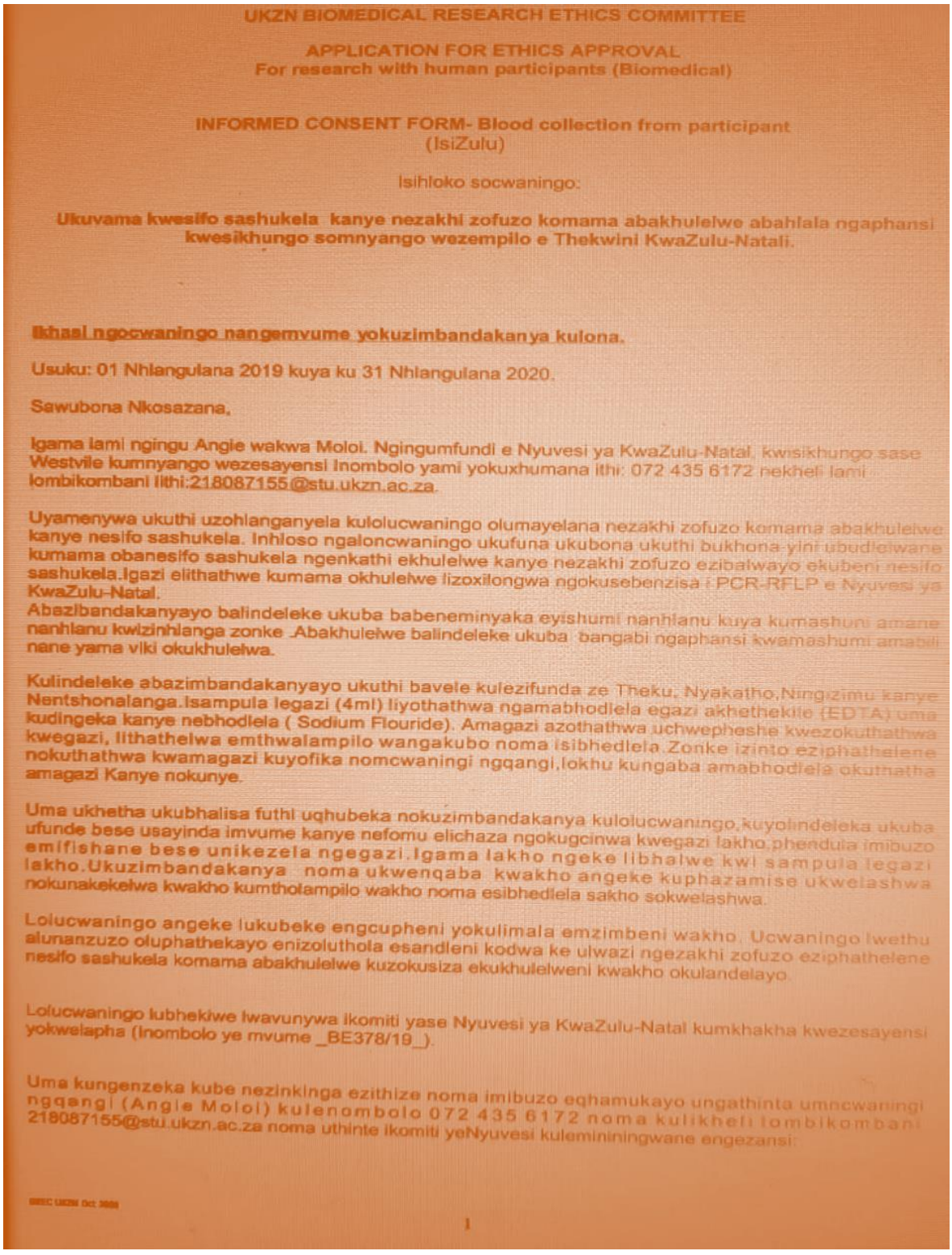
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Signature of Participant **Date**

Signature of Witness (Where applicable) **Date**

Signature of Translator (Where applicable) **Date**

Appendix F: Written Informed Consent (IsiZulu)



UKZN BIOMEDICAL RESEARCH ETHICS COMMITTEE

APPLICATION FOR ETHICS APPROVAL
For research with human participants (Biomedical)

INFORMED CONSENT FORM- Blood collection from participant
(IsiZulu)

Isihloko socwaningo:

Ukuvama kwesifo sashukela kanye nezakhi zofuzo komama abakhulelwe abahlala ngaphansi kwesikhungo somnyango wezempilo e Thekwini KwaZulu-Natali.

Ikhasi ngocwaningo nangemvume yokuzimbandakanya kulona.

Usuku: 01 Nhlangulana 2019 kuya ku 31 Nhlangulana 2020.

Sawubona Nkosazana,

Igama lami ngingu Angie wakwa Moloi. Ngingumfundi e Nyuvesi ya KwaZulu-Natal, kwisikhungo sase Westvile kumnyango wezesayensi Inombolo yami yokuxhumana ithi: 072 435 6172 nekheli lami lombikombani ithi:218087155@stu.ukzn.ac.za.

Uyamenywa ukuthi uzohlanganyela kulolucwaningo olumayelana nezakhi zofuzo komama abakhulelwe kanye nesifo sashukela. Inhloso ngaloncwaningo ukufuna ukubona ukuthi bukhona yini ubudlelwane kumama obanesifo sashukela ngenkathi ekhulelwe kanye nezakhi zofuzo ezibalwayo ekubeni nesifo sashukela.Igazi elithathwe kumama okhulelwe lizoxilongwa ngokusebenzisa i PCR-RFLP e Nyuvesi ya KwaZulu-Natal.
Abazibandakanyayo balindeleke ukuba babeneminyaka eyishumi nanhlanu kuya kumashumi amane nanhlanu kwizinhlanga zonke .Abakhulelwe balindeleke ukuba bangabi ngaphansi kwamashumi amabili nane yama viki okukhulelwa.

Kulindeleke abazimbandakanyayo ukuthi bavele kulezifunda ze Theku, Nyakatho,Ningizimu kanye Nentshonalanga.Isampula legazi (4ml) liyothathwa ngamabhodlela egazi akhethekile (EDTA) uma kudingeka kanye nebhodlela (Sodium Flouride). Amagazi azothathwa uchwepheshe kwezokuthathwa kwegazi, lithathelwa emthwalampilo wangakubo noma isibhedlela.Zonke izinto eziphathelene nokuthathwa kwamagazi kuyofika nomcwaningi ngqangi,lokhu kungaba amabhodlela okuthatha amagazi Kanye nokunye.

Uma ukhetha ukubhalisa futhi uqhubeka nokuzimbandakanya kulolucwaningo,kuyolindeleka ukuba ufunde bese usayinda imvume kanye nefomu elichaza ngokugcinwa kwegazi lakho,phendula imibuzo emifishane bese unikezela ngegazi .Igama lakho ngeke libhalwe kwi sampula legazi lakho.Ukuzimbandakanya noma ukwenqaba kwakho angeke kuphazamise ukwelashwa nokunakekelwa kwakho kumtholampilo wakho noma esibhedlela sakho sokwelashwa.

Lolucwaningo angeke lukubeke engcupheni yokulimala emzimbeni wakho. Ucwaningo lwethu alunanzuzo oluphathekayo enizoluthola esandleni kodwa ke ulwazi ngezakhi zofuzo eziphathelene nesifo sashukela komama abakhulelwe kuzokusiza ekukhulelweni kwakho okulandelayo.

Lolucwaningo lubhekiwe lwavunywa ikomiti yase Nyuvesi ya KwaZulu-Natal kumkhakha kwezesayensi yokwelapha (Inombolo ye mvume _BE378/19_).

Uma kungenzeka kube nezinkinga ezithize noma imibuzo eqhamukayo ungathinta umncwaningi ngqangi (Angie Moloi) kulenombolo 072 435 6172 noma kulikheli lombikombani 218087155@stu.ukzn.ac.za noma uthinte ikomiti yeNyuvesi kulemininingwane engezansi:

BREC UKZN Oct 2018

1

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Awuphoqiwe ukuba uhlanganyele kulolucwaningo ungayeka noma yinini.Ukuyeka kwakho angeke uhlawuliswe.Uma usufisa ukuyeka ungathintana nomncwaningingqangi bese uyekiswa ngokushesha.

Isampula lakho lizobhalwa ngendlela yesampula ezobonisa umniniyo kuphela okuzotshengisa ubuli bakho,iminyaka nohlanga. Igama lakho angeke limataniswe nesampula lakho legazi .Imininingwan yakho yogcinwa iyimfihlo.

Amasampula azogcinwa kuphela kusukela ngo 2019 kuya ku 2020 eMnyango wezempilo ephathelene nesayensi yokusebenza kwezitho zomzimba kumuntu,eNyuvesi ya KwaZulu-Natal esikhungweni sase Westville.Yonke imiphumela eyotholakala iyogcinwa kwi khomputha bese ivikelwa nge phasiwedi. Amasampula egazi nemiphumela ezotholakala kuyolahlwa ngendlela efanelekile ekuphelen kocwaningo.

IMVUME

Lapha ngabazimbandakanyayo kwiminyaka ephakathi kweshumi nanhlanu kuya kumashumi amane nanhlanu yeminyaka nabafunayo ukuzimbandakanya kucwaningo.

Isivumelwano sokuzimbandakanya nocwaningo.

Mina..... (Igama nesibongo) Inombolo kamazisi.....
Ngazisiwe ngu Dokotela Hlengiwe P.Mnobgwa mayelana nocwaningo oluphathelene nokuvama phakathi kwesifo sashukela komama abakhulelwe kanye nezakhi zofuzo komama abahlala e Thekwin ngaphansi komnyango wezempilo.

(Ngicela ubeke loluphawu ebhokisini uma uvuma √ noma X uma ungavumi)

☐ Ngiyayiqonda inhloso nenqubo ngalolucwaningo.

☐ Nginikezwe ithuba lokubuza imibuzo mayelana nalolucwaningo ngaphenduleka ngokugculisayo.

☐ Ngiyavuma ukuthi angiphoqwanga ukuzimbandanganya kulolucwaningo ngingayeka noma kunini futhi ukuyeka kwami angeke kuvimbele usizo ebengigaludinga emtholampilo noma esibhedlela.

☐ Ngitsheliwe ukuthi angeke ngikhokhelwe ngalolucwaningo, kodwa uma kungenzeka nanoma yini ngenkathi ngithathwa igazi ,izindleko zokwelashwa ziyobhekana nomncwaningi.

☐ Uma nginemibuzo ephathelene nalolucwaningo ngingathintana nomncwaningingqangi Angie Moloi kulenombolo 072 435 6172 kanye nombikombani 218087155@stu.ukzn.ac.za

☐ Uma nginanoma imiphi imibuzo mayelana namalungelo ami njengomhlanganyeli, imibuzo

ngocwaningo, noma imibuzo ngomcwaningi ,ngingathinta inkomiti yase Nyuvesi kulemininingwane engezansi:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Isignesha yomhlanganyeli **Usuku**

Isignesha yofakazi (Uma ekhona) **Usuku**

Isignesha yomhumushi (Uma ekhona) **Usuku**

Appendix G: Research Questionnaire (English)

RESEARCH QUESTIONNAIRE

ASSOCIATION BETWEEN FEOTAL IMPRINTED GENES AND INCIDENCES OF GESTATIONAL DIABETES MELLITUS (GDM) AMONG PREGNANT WOMEN IN ETHEKWINI HEALTH DISTRICT OF KWAZULU-NATAL

The study you are participating in is about imprinted/ autosomal genes in pregnant, non-pregnant women and Gestational diabetes. The aim of the study is to investigate the genes susceptible to Gestational Diabetes Mellitus during pregnancy.

The survey will take 5 to 10 minutes to complete.□

BI: BASIC INFORMATION *(Please tick [√] or circle the appropriate box)*

BI-01: Date	BI-02: Participant Code	BI-03: Pregnant[Q1]		BI-04: Clinic Name [Q2]
DD / MM / YYYY		Yes [1]	No [2]	Phoenix CHC (A1) [1] Inanda CHC (A2) [2] KwaMashu CHC (A3) [3] Folweni Clinic (B1) [4] KwaDabeka CHC (C1) [5] King Edward VIII ANC (PS1) [6]

BI-05: Sub- District [Q3]
eThekwini Central [1] North [2] South [3] West [4]

BI-06: Clinic Location [Q4]			
Urban [1]	Township [2]	Rural [3]	Informal settlement [4]

DM: DEMOGRAPHICS *(Please tick [√] or circle the appropriate box)*

DM-01	What is your age? [Q5]	15- 20 [1] 21- 25 [2] 26 -30 [3] 31- 35 [4] 36+ [5]	DM-02	What is your race? [Q6]	African [1]	White [2]	Indian [3]	Coloured [4]	Other [5]

DM-03	What is your marital status? [Q7]	Single [1]	Married [2]	Living together [3]	Separated [4]	Divorced [5]	Widowed [3]

DM-04	Parity (number of children) [Q8]	0 -0 [1] 1-2 [2] 3+ [3]	DM-05	Gravidity (number of pregnancies) [Q9]	0-0 [1] 1 -2 [2] 3+ [3]

DM-06	Gestational age at first visit? [Q10]	1 - 12 weeks [1]	13 - 28 weeks [2]	29 - 40+ weeks [3]	Non-pregnant [4]

DM-07: BMI [Q11]
Weigh (KG) / Height (M)

DM-08: Systolic (upper) and Diastolic (lower) pressure in mmHg [Q12]				
< 120 & < 80 Normal [1]	120-129 & < 80 Elevated [2]	130-139 & 80-89 Hypertension stage 1 [3]	> 140 &/or >90 Hypertension stage 2 [4]	> 180 & Hypertensi

Below 18.5 - underweight [1] 18.5 - 24.9 - Normal [2] 25.0 - 29.9 Overweight [3] 30.0+ Obese [4]	BMI		DM-9	Taking ARV treatment for HIV and AIDS? [Q13]	Yes [1]	No [2]

DM-10: Employment Status[Q14]		
Yes [1]	No [2]	Never [3]

DM-11: Previous pregnancy history[Q15]				
Miscarriage [1]	Stillbirth [2]	Neonatal death [3]	Birth defects [4]	None of the abov

KNOWLEDGE ABOUT DIABETES MELLITUS *(Please tick [√] or circle the appropriate box)*

DK-01: Have you ever tested and diagnosed for sugar diabetes previously? [Q16]		
Yes [1]	No [2]	Do not remember [3]

DK-02: What type of diabetes you were previously diagnosed		
Type 1 Diabetes [1]	Type 2 Diabetes [2]	Gestational Dia [3]
Above detectable limit [4]	Waiting for the results [5]	Not tested
Normal blood sugar level [7]	Do not remember [8]	

DK-03: Have you ever been diagnosed with diabetes during pregnancy? [Q18]		
Yes [1]	No [2]	Do not remember [3]
Not - Applicable [4]		

DK-04: At what stage in pregnancy were you diagnosed of [Q19]		
1 - 12 weeks [1]	13 - 28 weeks [2]	28 - 40 weel
Not Applicable [4]	Normal blood sugar [5]	Do not knov

DK-05	Is there any member of your family or generation who has /had diabetes? [Q20]	Yes [1]		No [2]		Don`t know [3]
If Yes', Tick only' that applies						
[Q21]	Mother's side	Father's side	Not applicable to my family	Do not know.	My sister	My brother	My child
DK-05a	1	2	3	4	5	6	7

DK-6	Lifestyle of pregnant and non-pregnant women	Yes	No	Not always	Not Applicabl
DK-06a	Are you taking Insulin to manage your diabetes during pregnancy? [Q22]	1	2	3	4
DK-06b	Are you eating 6 healthy meals a day? [Q23]	1	2	3	4
DK-06c	Are you exercising for at least 20 minutes after each meal? [Q24]	1	2	3	4
DK-06d	Do you often check /measure your glucose level throughout the day? [Q25]	1	2	3	4
DK-07	Are you taking antenatal multivitamins? [Q26]	1	2	3	4

PC: COMMON COMPLICATIONS IN PREGNANCY *(Please tick [√] or circle the appropriate box)*

PC-01	Do you have a baby(ies) delivered from previous pregnancy(ies)? [Q27]	Yes [1]	No [2]

If yes,

PC-02	What type of delivery did you have? [Q28]	Caesarean section [1]	Vaginal delivery [2]	Never been pregnant [3]	PC-02a	Pre-term delivery [1]	Full term [2]	Induced labour [3]	Late delivery [4]	Never been pr [5]

PC-03: What was your previous baby's weight? [Q29]			
≤ 2.5 kg [1]	2.5 - 3.5 kg [2]	3.6 - 4.0 kg [3]	≥ 4.0 kg [4]
I do not know [5]	I do not remember [6]	Never been pregnant [7]	

PC-04: Was your baby tested for glucose level afte		
Yes [1]	No [2]	Don`t rem [3]
I do not know [4]	Glucose level was not tested [5]	Never been pr

DK:

CA: CLINIC ATTENDANCE *(Please tick [√] or circle the appropriate box)*

AC-01	Please rate your antenatal clinic attendance. [Q31]	≤ 3 visits Poor [1]	4 - 6 visits Good [2]	6 - 7 visits Very good [3]	≥ 8 visits Excellent [4]	Not Preg

AC-02	What are the challenges that are preventing you from attending the clinic? [Q32]	Community/ cultural beliefs [1]	Privacy [2]	Inadequate knowledge of ANC [3]	Traveling time [4]	Unintended pregnancy [5]	Attitudes of health personnel [6]	Long wa
		No reasons that can prevent me [8]	Not Pregnant [9]					

MD: AWARENESS ABOUT MATERNAL DEATH RATE IN KWAZULU-NATAL *(Please tick [√] or circle t appropriate box)*

	Read below, understand and mark appropriately.	Strongly agree	Agree	Strongly disagree	Disagree	Do No know
MD-01	Gestational diabetes is a serious condition that can lead to maternal death worldwide? [Q33]	5	4	3	2	1
MD-02	It is important to control your diabetes while pregnant as this can prevent complications to you and your baby. [Q34]	5	4	3	2	1
MD-03	KwaZulu-Natal is one of the highest provinces with a higher maternal death rate in South Africa? [Q35]	5	4	3	2	1
MD-04	Attending the antenatal clinic accordingly can avoid any problems that might lead to maternal mortality? [Q36]	5	4	3	2	1
MD-05	I do not know the negative effect caused by the abnormalities of imprinting / autosomal genes and the gestational diabetes during pregnancy? [Q37]	5	4	3	2	1

Thank you for your time and cooperation!!!

Appendix H: Research Questionnaire (IsiZulu)

IMIBUZO YOCWANINGO

UCWANINGO NGEZAKHI ZOFUZO LUKASHUKELA KOMAMA ABAKHULELWE NABANGAKHULELWE ABAHLALA NGAPHANSI KWESIKHUNGO SOMNYANGO WEZEMPILO ETHEKWINI KWAZULU-NATALI.

Ucwaningo lolu ozohlanganyela kulo lumayelana nezakhi zofuzo komama abakhulelwe kanye nesifo sashukela esiqalayo kumama okhulelwe.Inhloso ngalolucwaningo ukufuna ukubona ukuthi bukhona yini ubudlelwane phakathi komama okhulelwe kanye nezakhi zofuzo ezibalwayo ekubeni nomthelela kulesifo.

Lokhu kuhlanganyela ngeke kuthathe ngaphezu kwemizuzu eyisihlanu kuya kweyishumi ukuze uqede.□

BI: BASIC INFORMATION *(Uyacelwa ukufaka loluphawu[√] noma uzungeze ibhokisi elifanele)*

BI-01: Usuku	BI-02: Inombolo ka Mbandakanyi	BI-03: Ukhulelwe ?		BI-04: Igama lomtholampilo
DD / MM / YYYY		Yes [1]	No [2]	Phoenix CHC (A1) [1] Inanda CHC (A2) [2] KwaMashu CHC (A3) [3] Folweni Clinic (B1) [4] KwaDabeka CHC (C1) [5] King Edward VIII ANC (PS1 [6]

BI-05: Isifunda somtholampilo	BI-06: Indawo yomtholampilo			
eThekwini Central [1] North [2] South [3] West [4]	Edolobheni [1]	Elokishini [2]	Emakhaya [3]	Emijondolo [4]

DM: DEMOGRAPHICS *Uyacelwa ukufaka loluphawu [√] noma uzungeze ibhokisi elifanele)*

DM-01	Mingaki iminyaka yakho?	15-22 [1] 21-25 [2] 26-30 [3] 31-35 [4] 36-40 [5] 41-45 [6]	DM-02	Luthini uhlanga lwakho?	Mpisholo[1]	Mhlophe[2]	Indiya[3]	Khaladi[4]	Nokunye[5]

DM-03	Sithini isimo sakho kwezomshado ?	Yedwana [1]	Shadile [2]	Hlalandawonye [3]	Mfelokazi [4]	Hlukene [5]	Hlukanisile [6]

DM-04	Unabantwana abangaki	0 -0 [1] 1-2 [2] 3-4 [3] 5-6 [4] 7-8 [5] 9-10 [6]	DM-05	Unamasu amangaki (ukhulelwe kangaki)	0 -0 [1] 1 -2 [2] 3-4 [3] 5-6 [4] 7-8 [5] 9-10 [6]

DM-06	Isikhathi obususikhulelwe ngenkathi ufika okuqala?	1 - 12 weeks [1]	13 - 28 weeks [2]	29 - 40 weeks [3]	Akuqondene n [4]

DM-07: Ubukhulu bomzimba wakho [1]	DM-08: Umfutho wegazi				
Isisindo somzimba (KG) / Ubude (M) Below 18.5 underweight [1] 18.5 -24.9 Normal [2] 25.0 -29.9 Overweight [3]	< 120 & <80 Normal [1]	120-129 & < 80 Elevated [2]	130-139 & 80-89 Hypertension Stage 1 [3]	>140 &/ or >90 Hypertension Stage 2 [4]	> 180 &/or > 120 Hypertension Crisis

DM-07	Angisikhumbuli isisindo sami (KG)	[2]	DM-09	Ingabe uthatha umshanguzo wengculaza nesandulela sayo ?	Yebo [1]	Cha [2

DM-10: Ukuqashwa			DM-11: Umlando wokukhulelwa kwakho ngaphambilini			
Yebo	Cha	Angikaze	Ukuphuphunyelwa yisisu [1]	Ingane yazalwa isishonile [2]	Ingane eshone iqeda ukubelethwa.[3]	Umntwana obelethw ekhubazekile [4]
[1]	[2]	[3]	Akukho kulokhu okubaliwe :[5]			

DK: KNOWLEDGE ABOUT DIABETES MELLITUS *(Uyacelwa ukufaka loluphawu [√] noma uzungeze ebhokisini elifanele)*

DK-01: Wake wahlolwa ngaphambilini maqondana nesifo sashukela?		
Yebo [1]	Cha [2]	Angikhumbuli [3]

DK-02: Yiluphi uhlobo olwatholakala ngaphambilin		
Uhlobo lokuqala [1]	Uhlobo lwesibili [2]	Uhlobo lwabakhu [3]
Uhlobo lukashukela olungaphezu kwenani elivumelekile [4]	Ngilinde imiphumela yokuhlolwa[5]	Aluhlolwanga
Kwatholakala ushukela olingene [7]	Akuqondene nami [8]	

DK-03: Wake wahlolwa isifo sashukela ngenkathi ukhulelwe ?		
Yebo [1]	Cha [2]	Angikhumbuli [3]
Akuqondene nami [4]		

DK-04: Wawunezinyanga ezingaki ukhulelwe ngenkathi uhlolwa		
1 - 12 weeks [1]	13 - 28 weeks [2]	28 - 40 wee
Akuqondene nami [4]	Kwatholakala ushukela olingene [5]	Angikhumb

DK-05	Ingabe ukhona emndenini wakho noma esizukulwaneni oke waba nesifo sashukela?			Yebo [1]	Cha [2]	Angazi [3]
		Uma impendulo kungu yebo, Uyacelwa ukubeka uphawu lokuphendula ebhokisini [√]				
	Ngasohlangothini lukamama?	Ngasohlangothi ni lukababa?	Akuqondene nomlando womndeni	Angazi	Dadewe thu	Mfowethu	Ingane yan
DK-05a	1	2	3	4	5	6	7

DK-06	Ukuzinakekela komama abakhulelwe nabangakhulelwe.	Yebo	Cha	Hayi njalo	Akuqonden nami.
DK-06a	Ingabe uthatha insulini ukunakekela ushukela wakho ?	1	2	3	4
DK-06b	Ingabe udla ukudla okunempilo okungenani kasithupha ngelanga?	1	2	3	4
DK-06c	Ingabe welula umzimba emva kokudla imizuzu engamashumi amabili noma ngaphezulu?	1	2	3	4
DK-06d	Ingabe ubheka ushukela wakho ngokuhamba kosuku?	1	2	3	4
DK-07	Ingabe uyawathatha yini amaphilisi andisa umsoco, enzelwe abakhulelwe?	1	2	3	4

PC: COMMON COMPLICATIONS IN PREGNANCY *(Uyacelwa ukufaka loluphawu [√] noma uzunge ibhokisi)*

PC-01	Ingabe unaye umntwana noma abantwana obathole ekukhulelweni kwakho okudlule?	Yebo [1]	Cha [2]

Uma impendulo ithi yebo,

PC-02	Iyiphi indlela owabeletha ngayo?	Wahlinzwa [1]	Wabeletha ngendlela ejwayelekile [2]	PC-02a	Wabeletha inyanga yakho ingakafiki [1]	Wabeletha ngenyanga yakho[2]	Wasuselwa [3]	Wabeletha iny isidlulile [4]

CA: CLINIC ATTENDANCE *(Uyacelwa ukufaka loluphawu [√]noma uzungeze ibhokisi elifanele)*

AC-01	Ngicela izikhathi zakho zokuhamba umtholampilo?	≤ 3 visits [1]	4 - 6 visits [2]	6 - 7 visits [3]
		≥ 8 visits [4]	Akuqondene nami [5]	

AC-02	Yiziphi izizathu ezikuvimbela ukuba ungayi emtholampilo ?	Izinkolelo ngamasiko [1]	Ukungabikho kwezimfihlo [2]	Ukungabi nolwazi olwanele mayelana nomtholampilo wabakhulelwe. [3]	Isikhathi osihambayo emtholampilo
	Ukukhulelwa okungahleliwe [5]	Izindlela abahlengikazi abaziphatha ngayo [6]	Ukulinda isikhathi eside. [7]	Azikho izizathu ezingangivimbela. [8]	Akuqondene nar

MD: AWARENESS ABOUT MATERNAL DEATH RATE IN KWAZULU-NATAL *(Uyacelwa ukufak loluphawu [√] noma uzungeze ibhokisi elifanele)*

	Uyakwazi lokhu:	Ngiyavuma kakhulu	Ngiyavuma	Angivumi kakhulu	Angivumi	Anginalwazi/ Angiqinisekang
MD-01	Isifo sikashukela komama abakhulelwe singabangela ukufa emhlabeni wonke?	5	4	3	2	1
MD-02	Kubalulekile ukunakekela ushukela wakho ngenkathi ukhulelwe ukuvikela izinkinga ezingavela kuwe noma umntwana?	5	4	3	2	1
MD-03	IKwaZulu-Natali ingenye yezifundazwe ezinezinga eliphezulu ekufeni komama abakhulelwe Kanye nabantwana babo?	5	4	3	2	1
MD-04	Ukuhamba umtholampilo ngokufanelekile kungagwema ezinye zezinkinga ezingaholela ekufeni?	5	4	3	2	1
MD-05	Anginalo ulwazi mayelana nezinkinga ezingabangelwa izakhi zofuzo kanye nesifo sashukela	5	4	3	2	1

Sibonge kakhulu ngokuzinikezela ngesikhathi sakho nangokubambisana!

Appendix I: SNP Genotype Restriction Sites (MTNR1B, PPARα and TCF7L2)

1. MTNR1B GENE

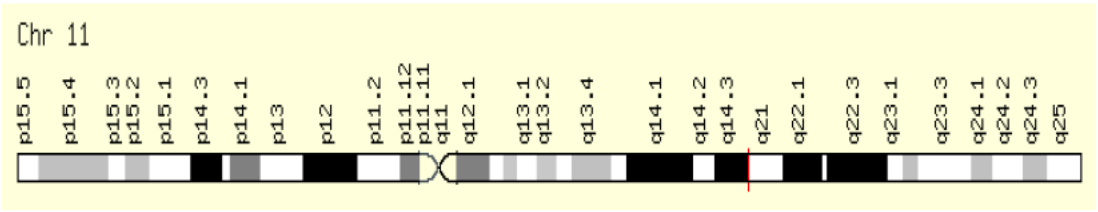
Gene Name: Melatonin receptor 1B (*MTNR1B*-Human),

Function: Expressed on the pancreatic B-cells for the insulin secretion.

Gene location: Melatonin receptor 1B (human), is found in the single intron in the long arm chromosome 11q 21-q22 exon 1 and 2, encodes MT2 protein, carries 362 amino acids melatonin receptor. This gene is expressed in the retina, brain, pancreatic islets, placenta, and central nervous cells.

Genomic location site: Gene Cards -

(NC_000011.10 (92969651- 92986241).



GeneCards/bands showing gene location on *MTNR1B* gene. [* Genomic location obtained from GeneCards: www.genecards.org/cgi-bin/cardisp-pl? – Assessed on 13.04.2021].

Amplicon size: 486bp

Restriction: a) Enzyme – HPYyCH4V b) Site$_1$ – TGCA (cut at G allele), Site$_2$ - CCWGG (cut at C allele) snp rs1387153: C>G / C>T .

Restriction product size: 83bp; 403 bp (C- allele), 83 bp, 95bp and 308 bp (snp - G)

Sequences 5' - 3'

cCtgtctGACTTGGGTTGGTGTtctcacccactcacagcagctgctccaggcactagtgagatgtgccacttactgaagcctgcacacctta
tgtgtgtttagttatcacaataacccacagcgtcattatgacacctctttcatgaataaggctgtggaagctcacaaaagttactccatttgcccaagaccaac
agagccactctggcaaagccaagattaactggcttccagatctgctctcttcctatgaaaataaggtaacacatggaaaatgcttgtaacttgtaaaactctg
tattgacaatggtattaccattctcagtggtccttactctctctagagctcacaactttgtgttgcttttacagataagaaaattgagttCcagggcagtaagtta
atttgctagcattccacagacagagttgggtttggaaccaggtctgtgcttctcaaaatGGAGGCTCTTAGGCCCAGGGG

- Forward and Reverse Primers

- Restriction site

- Location of the SNP

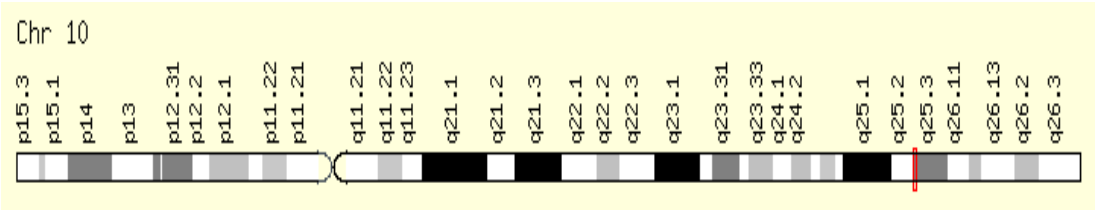
2. TCF7L2 GENE

Gene name: Transcription factor like 7 -like 2 (*TCF7L2*, Human),

Function: Expressed in pancreatic Beta cells disrupting the WnT signalling pathway leading to insulin secretion during pregnancy.

Location: Chromosome 10q25.2-q25.3

Genomic location: Gene Cards



GeneCards/ Showing genetic location of *TCF7L2* gene. [* Genomic location obtained from GeneCards: www.genecards.org/cgi-bin/cardisp-pl? – Retrieved on 13.04.2021]

Amplicon size: 384bp

Restriction: a) Enzyme - MIuCI b) Site$_1$ – AATT (cut at T allele), Site$_2$ - TARCCA (cut at A allele), rs12255372 $_{(snp)}$: G>A / G>T

Restriction product size: 43bp and 307bp.

Restriction product sizes: 35bp, 43bp, 63bp, 77, and 162bp (T- Allele demonstrable)

Restriction product sizes:35bp, 63bp, 120bp and 162bp (G-Allele detected)

Sequences 5'-3'

GGACTTGATTGTTGATTATGGGCaatagatacattttaagaatgatgttgtaggctgtatgaagtcatttgatgattgttttgttaatggctt
gcaggtcagattttcatctttttaaattaattatcatagaaggagaaaacaactgatttcagaattgtcccttgaggtgtactggaaactaaggcgtgagggac
tcataggggtctggcttggaaagtgtattgctatgtccagtttacacaggatgtgcaaatccagcaggttagctgagctgcccaggaatatccaggcaaga
atgaccatattctgataattactcaggcctctgcctcatctccgctgcCccccgcccccCTGACTCTCTTCTGAGTGCCAA

in sequencing result, one a of GAAT is missing. Both 69 and 68 are same. have G allele

Forward and Reverse Primers

Location of the SNP

Restriction sites

3. PPARα GENE

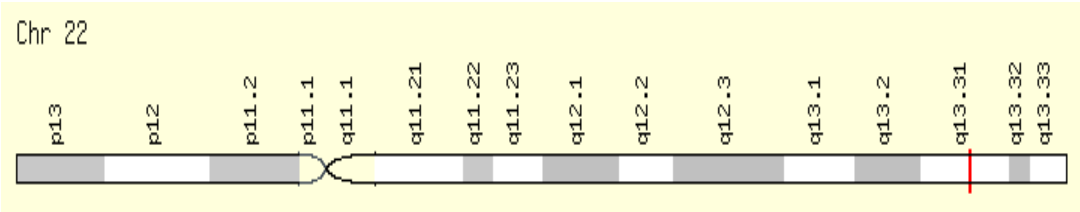
Gene name: Peroxisome proliferator activated receptor gamma (PPARA, Human),

Function: Expressed in the adipose tissues to cause obesity and type2 diabetes mellitus.

Location: Chromosome -22p13.31

Genomic location: Gene Cards

PPARα gene is specifically located on chromosome 22p13.31 (OMIM: 170998) and has 468 amino acids in, the DNA sequence (NC_000022.11).



GeneCards/ Showing genetic location of *PPARα* gene. [* Genomic location obtained from GeneCards: www.genecards.org/cgi-bin/cardisp-pl? – Assessed on 13.04.2021].

Amplicon size: 267bp

Restriction: a) Enzyme - Taq1 b) Site – TCGA (cut at C allele), rs4253778: G>C/G>T

Restriction product size:50bp and 217bp (no snp)

Sequences 5' - 3'

ACAATCACTCCTTAAATATGGTGGaacacttgaagcttgatatctagtttggattcaaaagcttcatttcccatattatgcaaaactggt
ggttgtgatctccagaatgtactgttcctcctactagctctaatttttctccctgacaggtggtcatcaggtaaatcacaagtgaaaaggccgcaccataaggt
gtacttagggcactattgccgcctagtagtatgaatatttaggaaagagTACTGGTCCTGTCTGTCCCTACTT

Forward and Reverse Primers

Location of the SNP

Appendix J: SNP Genotype Results of MTNR1B, PPARα, and TCF7L2 Genes

PPARA SNP rs 4253778 GENOTYPE RESULTS					
No.	**Amplification: Yes/ No**	**Amplification size**	**Genotypes/Alleles**	**Group**	**GDM/No-GDM**
1	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
2	Yes	216bp-sb	C	Pregnant	No-GDM
3	Yes	50bp; 267bp-db	GC	Pregnant	No-GDM
4	Yes	267bp-db	GC	Pregnant	No-GDM
5	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
6	Yes	217bp-sb	C	Pregnant	No-GDM
7	Yes	50bp; 217bp-sb	C	Pregnant	GDM *
8	Yes	267bp-db	GC	Pregnant	No-GDM
9	No	No band	No allele	Control	No-GDM
10	Yes	216bp-sb	C	Pregnant	No-GDM
11	Yes	216bp-sb	C	Pregnant	No-GDM
12	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
13	Yes	267bp-sb	G	Pregnant	No-GDM
14	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
15	Yes	50bp;217bp-sb	C	Pregnant	No-GDM
16	Yes	50bp ;267bp-db	GC	Pregnant	No-GDM
17	Yes	50; 217bp-sb	C	Pregnant	No-GDM
18	Yes	267bp-db	GC	Pregnant	No-GDM
19	Yes	No band	No allele	Pregnant	No-GDM
20	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
21	No	No band	No allele	Control	No-GDM
22	Yes	217bp-sb	C	Control	No-GDM
23	Yes	217bp-sb	C	Control	No-GDM
24	Yes	267bp-sb	G	Control	No-GDM
25	Yes	217bp-sb	C	Control	GDM *
26	Yes	217bp-sb	C	Control	GDM*
27	Yes	50bp; 217bp -sb	C	Pregnant	No-GDM
28	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
29	Yes	50bp; 217bp-sb	C	Control	No-GDM
30	Yes	50bp; 217bp-sb	C	Control	GDM*

31	Yes	50bp; 217bp-sb	C	Control	No-GDM
32	Yes	50bp; 217bp -sb	C	Control	No-GDM
33	Yes	50bp; 267bp-db	GC	Pregnant	No-GDM
34	Yes	217bp-sb	C	Pregnant	No-GDM
35	Yes	267bp-sb	G	Pregnant	No-GDM
36	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
37	Yes	217bp-sb	C	Pregnant	No-GDM
38	Yes	267bp -db.	GC	Pregnant	No-GDM
39	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
40	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
41	Yes	50bp; 217bp -sb	C	Pregnant	GDM*
42	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
43	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
44	Yes	50bp; 217bp-sb	C	Pregnant	GDM*
45	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
46	Yes	267bp -db.	GC	Pregnant	GDM*
47	Yes	50bp; 217bp -sb	C	Pregnant	GDM*
48	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
49	Yes	50bp; 217bp-sb	C	Pregnant	GDM*
50	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
51	Yes	267bp-db	GC	Pregnant	GDM*
52	No	Missing	Missing	Control	No-GDM
53	Yes	50bp; 217bp-sb	C	Pregnant	No-GDM
54	Yes	50; 267bp-db	GC	Pregnant	No-GDM
55	Yes	267bp -db.	GC	Pregnant	No-GDM
56	Yes	50bp;217bp-sb	C	Pregnant	No-GDM
57	Yes	50bp; 217bp-sb	C	Control	No-GDM
58	Yes	267bp-db	GC	Control	GDM*
59	Yes	217bp-sb	C	Control	GDM*
60	Yes	267bp-db	GC	Control	GDM*
61	Yes	217bp-sb	C	Pregnant	No-GDM
62	Yes	267bp-db	GC	Pregnant	No-GDM
63	Yes	217bp-sb	C	Pregnant	No-GDM
64	Yes	217bp-sb	C	Pregnant	No-GDM
65	Yes	217bp-sb	C	Pregnant	No-GDM
66	Yes	267bp-db	GC	Pregnant	No-GDM

67	Yes	267bp-db	GC	Pregnant	No-GDM
68	Yes	267bp-db	GC	Control	No-GDM
69	Yes	267bp-db	GC	Control	No-GDM
70	Yes	217bp-sb	C	Control	No-GDM
71	Yes	267bp-sb	GC	Control	No-GDM
72	Yes	217bp-sb	C	Control	No-GDM
73	Yes	267bp-db	GC	Control	No-GDM
74	Yes	217bp-sb	C	Pregnant	GDM*
75	Yes	217bp-sb	C	Pregnant	GDM*
76	Yes	267bp-sb	G	Pregnant	No-GDM
77	Yes	217bp-sb	C	Pregnant	No-GDM
78	Yes	217bp-sb	C	Pregnant	GDM*
79	Yes	267bp-db	GC	Pregnant	GDM*
80	Yes	217bp-sb	C	Pregnant	GDM*
81	Yes	217bp-sb	C	Pregnant	No-GDM
82	Yes	217bp-sb	C	Pregnant	No-GDM
83	Yes	217bp-sb	C	Pregnant	GDM*
84	Yes	217bp-sb	C	Pregnant	No-GDM
85	Yes	217bp-sb	C	Control	GDM*
86	Yes	217bp-sb	C	Control	GDM*
87	Yes	267bp-db	GC	Control	GDM*
88	Yes	217bp-sb	C	Pregnant	No-GDM
89	Yes	217bp-sb	C	Pregnant	No-GDM
90	Yes	217bp-sb	C	Pregnant	No-GDM
91	Yes	267bp-db	GC	Control	No-GDM
92	Yes	217bp-sb	C	Control	No-GDM
93	Yes	267bp-db	GC	Control	No-GDM
94	Yes	217bp-sb	C	Pregnant	GDM*
95	Yes	267bp-db	GC	Pregnant	GDM*
96	Yes	217bp-sb	C	Pregnant	No-GDM
97	Yes	267bp-db	GC	Pregnant	No-GDM
98	Yes	217bp-sb	C	Pregnant	No-GDM
99	Yes	267bp-db	GC	Pregnant	GDM*
100	Yes	267bp-db	GC	Pregnant	No-GDM
101	Yes	217bp-sb	C	Control	No-GDM
102	Yes	217bp-sb	C	Pregnant	No-GDM

103	Yes	217bp-sb	C	Control	GDM*

*This information was retrieved from self-reported data (Questionnaire) in the current study. In a total of 103 women, 24.3% (n=25) had pre-GDM (previously diagnosed with GDM). This included current pregnant women 15.5 % (n=16), and non-pregnant women 8.7% (n= 9), non-diabetic control 75.7% (n= 78). Out of 73 pregnant women, 21.9% (n=16) had GDM from previous pregnancies and 78% (n=57) had no GDM previous diagnosis from self-reported data. According to self-reported data, GDM prevalence is estimated at 21.9%.

MTNR1B SNP rs1387153 GENOTYPE RESULTS					
No.	**Amplification**	**Amplification size**	**Genotypes/Alleles**	**Group**	**GDM/No-GDM**
1	Yes	No band	No allele	Pregnant	No-GDM
2	Yes	No band	No allele	Pregnant	No-GDM
3	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
4	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
5	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
6	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
7	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
8	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
9	No	No band	No allele	Control	No-GDM
10	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
11	No	No band	No allele	Pregnant	No-GDM
12	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
13	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
14	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
15	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
16	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
17	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
18	Yes	83bp; 403bp-db	CC	Pregnant	No-GDM
19	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
20	No	No band	No allele	Pregnant	No-GDM
21	Yes	403bp-db	CC	Control	No-GDM
22	No	No band	No allele	Control	No-GDM
23	Yes	83bp; 403bp-sb	C	Control	No-GDM
24	Yes	83bp; 403bp-sb	C	Control	No-GDM
25	Yes	83bp; 403bp-db	CC	Control	GDM*
26	Yes	83bp; 403bp-sb	C	Control	GDM*
27	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
28	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
29	Yes	83bp; 403bp-sb	C	Control	No-GDM
30	Yes	83bp; 403bp-sb	C	Control	GDM*
31	Yes	83bp; 403bp-sb	C	Control	No-GDM
32	Yes	83bp; 403bp-sb	C	Control	No-GDM
33	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM

34	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
35	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
36	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
37	Yes	83bp; 403bp-db	CC	Pregnant	No-GDM
38	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
39	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
40	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
41	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
42	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
43	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
44	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
45	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
46	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
47	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
48	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
49	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
50	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
51	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
52	No	Missing	Missing	Control	No-GDM
53	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
54	Yes	83bp; 403bp-sb	C	Pregnant	No pre-GDM
55	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
56	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
57	Yes	83bp; 403bp-sb	C	Control	No-GDM
58	Yes	83bp; 403bp-sb	C	Control	GDM*
59	Yes	83bp; 403bp-sb	C	Control	GDM*
60	Yes	83bp; 403bp-sb	C	Control	GDM*
61	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
62	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
63	Yes	403bp-sb	C	Pregnant	No -GDM
64	Yes	83bp; 403bp-db	CC	Pregnant	No-GDM
65	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
66	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
67	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
68	Yes	83bp; 403bp-sb	C	Control	No-GDM
69	Yes	83bp; 403bp-sb	C	Control	No-GDM
70	Yes	83bp; 403bp-sb	C	Control	No-GDM
71	Yes	83bp; 403bp-sb	C	Control	No-GDM
72	No	No band	No allele	Control	No-GDM
73	No	No band	No allele	Control	No-GDM

74	No	No band	No allele	Pregnant	GDM*
75	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
76	Yes	403bp-sb	C	Pregnant	No-GDM
77	No	No band	No allele	Pregnant	No-GDM
78	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
79	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
80	No	No band	No allele	Pregnant	GDM*
81	Yes	403bp-sb	C	Pregnant	No-GDM
82	Yes	403bp-sb	C	Pregnant	No-GDM
83	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
84	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
85	Yes	83bp; 403bp-sb	C	Control	GDM*
86	Yes	83bp; 403bp-sb	C	Control	GDM*
87	Yes	83bp; 403bp-db	CC	Control	GDM*
88	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
89	Yes	83bp; 403bp-db	C	Pregnant	No-GDM
90	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
91	Yes	403bp-sb	C	Control	No-GDM
92	Yes	403bp-sb	C	Control	No-GDM
93	Yes	83bp; 403bp-sb	C	Control	No-GDM
94	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
95	Yes	83bp; 403bp-sb	C	Pregnant	GDM*
96	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
97	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
98	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
99	No	No band	No allele	Pregnant	GDM*
100	Yes	403bp-sb	C	Pregnant	No-GDM
101	Yes	83bp; 403bp-sb	C	Control	No-GDM
102	Yes	83bp; 403bp-sb	C	Pregnant	No-GDM
103	Yes	403bp-db	CC	Control	GDM*

TCF7L2 rs12255372 GENOTYPE RESULTS					
No.	**Amplification**	**Amplification size**	**Genotype /Allele**	**Group**	**GDM/ No - GDM**
1	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
2	No	No band	No allele	Pregnant	No-GDM
3	Yes	120bp; 162bp; 307bp-sb	G	Pregnant	No-GDM
4	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
5	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
6	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
7	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	GDM*
8	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
9	No	No band	No allele	Control	No-GDM
10	No	No band	No allele	Pregnant	No-GDM
11	No	120bp; 162bp -sb	G	Pregnant	No-GDM
12	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
13	No	No band	No allele	Pregnant	No-GDM
14	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
15	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
16	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
17	Yes	120bp; 162bp; 307bp-sb	G	Pregnant	No-GDM
18	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
19	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
20	No	No band	No allele	Pregnant	No-GDM
21	Yes	120bp; 162bp-sb	G	Control	No-GDM
22	No	No band	No allele	Control	No-GDM
23	Yes	120bp; 162bp-sb	G	Control	No-GDM
24	Yes	63bp; 77bp; 120bp; 162bp-sb	G	Control	No-GDM
25	Yes	63bp; 77bp; 120bp; 162bp-sb	G	Control	GDM*
26	Yes	63bp; 77bp; 120bp; 162bp-sb	G	Control	GDM*
27	Yes	63bp; 77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM

28	Yes	77bp; 120bp -db.	GG	Pregnant	No-GDM
29	Yes	77bp; 120bp; 162bp-sb	G	Control	No-GDM
30	Yes	120bp; 162bp-sb	G	Control	GDM*
31	Yes	77bp; 120bp; 162bp-sb	G	Control	No-GDM
32	Yes	77bp; 120bp; 162bp-sb	G	Control	No-GDM
33	No	No band	No allele	Pregnant	No-GDM
34	No	No band	No allele	Pregnant	No-GDM
35	Yes	120bp; 162bp -sb	G	Pregnant	No-GDM
36	No	No band	No allele	Pregnant	No-GDM
37	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
38	Yes	63bp; 120bp -db.	GG	Pregnant	No-GDM
39	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
40	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
41	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	GDM*
42	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
43	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
44	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	GDM*
45	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
46	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	GDM*
47	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	GDM*
48	Yes	63bp; 77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
49	Yes	120bp; 162bp-sb	G	Pregnant	GDM*
50	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
51	Yes	120bp; 162bp-sb	G	Pregnant	GDM*
52	No	Missing	Missing	Control	No-GDM
53	Yes	77bp; 120bp; 162bp; 307bp-sb	G	Pregnant	No-GDM
54	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
55	Yes	120bp; 162bp; 307bp-sb	G	Pregnant	No-GDM
56	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
57	Yes	77bp; 120bp; 162bp-sb	G	Control	No-GDM
58	Yes	120bp; 162bp; 307bp-sb	G	Control	GDM*
59	Yes	120bp; 162bp; 307bp-sb	G	Control	GDM*
60	Yes	120bp; 162bp; 307bp-sb	G	Control	GDM*
61	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
62	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
63	Yes	63bp; 120bp; 162bp	G	Pregnant	No-GDM

64	No	No band	No allele	Pregnant	No-GDM
65	Yes	77bp; 120bp; 162bp; 307bp-sb	G	Pregnant	No-GDM
66	Yes	120bp; 162bp; 307bp-sb	G	Pregnant	No-GDM
67	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
68	Yes	120bp; 162bp-sb	G	Control	No-GDM
69	Yes	120bp; 162bp-sb	G	Control	No-GDM
70	Yes	120bp; 162bp; 307bp-sb	G	Control	No-GDM
71	Yes	63bp; 120bp; 162bp-sb	G	Control	No-GDM
72	Yes	63bp; 120bp; 162bp-sb	G	Control	No-GDM
73	Yes	63bp; 120bp; 162bp-sb	G	Control	No-GDM
74	Yes	63bp; 120bp; 162bp-sb	G	Pregnant	GDM*
75	Yes	120bp; 162bp-sb	G	Pregnant	GDM*
76	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
77	Yes	120bp-db	GG	Pregnant	No-GDM
78	Yes	120bp; 162bp-sb	G	Pregnant	GDM*
79	Yes	120bp; 162bp-sb	G	Pregnant	GDM*
80	Yes	120bp; 162bp-sb	G	Pregnant	GDM*
81	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
82	Yes	63bp; 120bp-db	GG	Pregnant	No-GDM
83	Yes	63bp; 120bp; 162bp-sb	G	Pregnant	GDM*
84	Yes	63bp; 120bp;162bp-sb	G	Pregnant	No-GDM
85	Yes	63bp; 120bp;162bp-sb	G	Control	GDM*
86	Yes	63bp; 120bp;162bp-sb	G	Control	GDM*
87	Yes	63bp; 120bp;162bp-sb	G	Control	GDM*
88	Yes	63bp; 120bp;162bp-sb	G	Pregnant	No-GDM
89	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
90	Yes	63bp; 120bp;162bp-sb	G	Pregnant	No-GDM
91	Yes	120bp; 162bp -sb	G	Control	No-GDM
92	Yes	120bp -db.	GG	Control	No-GDM
93	Yes	120bp; 162bp -sb	G	Control	No-GDM
94	Yes	162bp-sb	G	Pregnant	GDM*
95	No	No band	No allele	Pregnant	GDM*
96	Yes	77bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
97	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
98	Yes	63bp; 120bp; 162bp	G	Pregnant	No-GDM
99	Yes	120bp; 162bp-sb	G	Pregnant	GDM*

100	Yes	63bp; 120bp; 162bp-sb	G	Pregnant	No-GDM
101	Yes	120bp;162bp -sb	G	Control	No-GDM
102	Yes	120bp; 162bp-sb	G	Pregnant	No-GDM
103	Yes	120bp; 162bp -sb	G	Control	GDM*

Appendix K: Undiagnosed and outstanding results of gestational diabetes mellitus

Table 4-4: Illustrating the undiagnosed and outstanding results of gestational diabetes mellitus

Participant Pregnant (P/N)	Age (years)	Parity	Gravida	History of complications in pregnancy	PPARα	MTNR1B	TCF7L2
6 – P*	36	01	02	FHD, CS, macrosomia	C	C	GG
18 – P*	19	00	02	Overweight (>25kg/m^2)	GC	CC	GG
19 – P*	33	02	04	Miscarriages, F. macrosomia	-	C	GG
27- P*	25	00	01	Overweight (>25kg/m^2)	C	C	GG
28 – P*	29	02	03	Overweight, pre-term birth, F. macrosomia.	C	C	GG
31-N	27	02	02	CS, macrosomia	C	C	GG
32-N	27	01	02	CS, miscarriage, infant low birth weight.	C	C	GG
35-P*	31	00	03	ARV treatment, miscarriages	G	C	GG
42-P*	32	01	03	ARV treatment, CS, FHD, neonatal death, macrosomia	C	C	GG
45-P*	29	00	02	ARV treatment, FHD, pre-term birth, stillbirth, infant low birth weight.	C	C	GG

52- N	37	00	02	FHD, induction of labour, miscarriage, stillbirth	Missing	Missing	Missing
55-P*	34	02	03	ARV treatment, CS, FHD, F. macrosomia	GC	C	GG
56- P*	20	00	02	Miscarriages	C	C	GG
64-P*	27	01	02	ARV treatment, CS, foetal macrosomia	C	CC	-
65- P*	26	01	02	ARV treatment, macrosomia, obese (>30kg/m^2	C	C	GG
66-P*	17	00	01	Overweight (>25kg/m^2)	GC	C	GG
67-P	24	01	03	ARV treatment, miscarriage, induction of labour.	GC	C	GG
70- N	24	01	01	FHD, pre-term birth, low infant birth weight.	C	C	GG
71-N	29	02	03	FHD, CS, miscarriage, Foetal macrosomia.	GC	C	GG
77-P*	25	00	03	ARV treatment, miscarriages	C	-	GG
84-P	34	03	05	ARV treatment, FHD, miscarriage, overweight.	C	C	GG

89-P*	32	01	02	Obese, low infant birth weight	C	C	GG
90-P*	25	00	01	Overweight (>25kg/m^2)	C	C	GG
93-N	41	00	02	ARV treatment, miscarriages	GC	C	GG
100-P	25	01	02	FHD, macrosomia	GC	C	GG
102-P*	32	02	03	ARV treatment, CS, low infant birth weight, obesity.	C	C	GG

*Weight was not part of the history. F: Foetal, ARV: Anti-retroviral, CS: Caesarean section, FHD: Family history of diabetes mellitus, P: Pregnant, N: Non-pregnant. KEHVIII - six critical cases had "no" as answers due to outstanding results for75g oral 2-hour OGTT. KwaDabeka CHC – five severe cases not yet diagnosed, and KwaMashu CHC -fifteen cases showed GDM risk factors, and needed urgent attention (ten pregnant women + five non-pregnant women). Standard 2-hour OGTT can only be done at referral (KEHVIII) tertiary hospital.