UNIVERSITY OF NAIROBI
COLLEGEOF HEALTH SCIENCES
SCHOOL OF MEDICINE
DEPARTMENT OF OBSTETRICS & GYNAECOLOGY

To the Chair,
Department of Obstetrics & Gynecology

RE: <u>RE-EVALUATION OF DISSERTATIONS</u>

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Study title: **PLACENTAL HISTOLOGY AND PERINATAL OUTCOMES IN WOMEN WHO DELIVERED AT TERM WITH AND WITHOUT GESTATIONAL DIABETES MELLITUS AT BUNGOMA COUNTY REFERAL HOSPITAL IN 2017**

I have re-evaluated the above student's dissertation and do confirm that the comments given to the student have been addressed to my satisfaction.

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PLACENTAL HISTOLOGY AND PERINATAL OUTCOMES IN WOMEN WHO DELIVERED AT TERM WITH AND WITHOUT GESTATIONAL DIABETES MELLITUS AT BUNGOMA COUNTY REFERAL HOSPITAL IN 2017

A Thesis submitted in partial fulfilment of the requirements for the Degree of Master of Medicine in Obstetrics and Gynaecology of the University of Nairobi

BY

LUTUKAYI STEPHEN MARUMBU

2022

DECLARATION

I declare that this thesis is my original work and has not been presented elsewhere for the award of a degree.

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DECLARATION OF ORIGINALITY FORM

This form must be completed and signed for all works submitted to the University for examination.

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DECLARATION

I understand what Plagiarism is, and I am aware of the University's policy in this regard. I do declare that this thesis is my original work and has not been submitted elsewhere for examination, an award of a degree or publication. Where I have used other people's work or my own work, this has been appropriately acknowledged and referenced following the University of Nairobi's requirements. I have not sought or used the services of any professional agencies to produce this work. I have not allowed, and shall not allow anyone to copy my work to pass it off as his/her own work. Lastly, I understand that any false claim in respect of this work shall result in disciplinary action following University Plagiarism Policy.

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CERTIFICATE OF AUTHENTICATION

This is to certify that this thesis is the original work of Dr. Lutukayi Marumbu Stephen, Master of Medicine Student in the Department of Obstetrics and Gynaecology, College of Health sciences, registration number: H58/11706/2018, University of Nairobi. This work was supervised and guided by Prof Omondi Ogutu, Prof M Obimbo, and Dr. Walong. It has not been presented elsewhere for the award of a degree.

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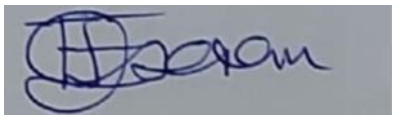
Signed. 

Date: 1st December 2021

DEDICATION

I dedicate this work to all women who lose their unborn babies to diabetes.

ACKNOWLEDGEMENT

This dissertation has been made possible by the great support of a big team to whom am greatly indebted.

Thanks to the Almighty God who make all things complete in His own time, bringing to accomplishment this work.

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Abbreviations

APWG- Amsterdam placental workshop group

BCRH- Bungoma county referral hospital.

BMI - Body mass index

CHD- Congenital heart disease

DM - Diabetes mellitus

GDM- Gestational diabetes mellitus

HIP- Hyperglycaemia in pregnancy

IDF- International diabetes federation

KNH-Kenyatta national hospital

NCD-Non communicable diseases

NTD- Neural tube defects.

SDG-Sustainable development goals

VQ –Vascularization quotient

VUE- Villitis of unknown aetiology

DEFINITION OF OPERATIONAL TERMS

1. **Atherosis;** is the incomplete remodelling of spiral arteries leading to necrosis of decidual vessels.
2. **Broad consent**; Consent that is given once by the patient to cover unforeseen future studies and incidental findings are not mandatorily reported to the subjects.
3. **Cases**; Placentae from women with Gestational diabetes mellitus (GDM)
4. **Controls** ; Placenta from women without GDM
5. **Dynamic consent**; This is when a patient is notified every time a new study is utilizes the bio-bank material. Here the subject is able to continuously consent for studies and be notifies of any significant findings. This consent utilizes electronic media.
6. **Foetal vascular malperfusion;** reduced chorionic villi perfusion secondary to capillary vasculopathy
7. **GDM;** (Gestational diabetes mellitus) Glucose intolerance of varying degrees with first recognition in pregnancy.
8. **Histomorphometry;** is the measurement of shape and form of tissues to deduce its wellbeing.
9. **Maternal vascular malperfusion;** reduced perfusion of the decidua by sinusoids/ spiral arteries.
10. **Placental morphology**; The study of placental structure and form.
11. **Placentome**; is the functional unit of the placenta.
12. **Secondary data analysis**; It is the analysis of data that was collected for a different study.
13. **Vascular quotient (VQ)**; The ratio of the cross-sectional surface area of blood vessels to that of the chronic villi in which the vessels are.

ABSTRACT

Background: Gestational Diabetes Mellitus (GDM) is a crucial underdiagnosed and poorly managed disease in our set up. It is a significant cause of perinatal morbidity, mortality, and poses health risks to the future child. The adverse outcomes are primarily linked to placental changes that alter perfusion and diffusional efficiency. These placental histomorphometric changes are associated with GDM. The study sought to study histomorphometric features following the Amsterdam Placental Workshop Group recommendations to generate local data that could be used for risk stratification so as to tailor ANC follow up for GDM women.

Research question; what are the differences in placental histology and perinatal outcomes in women with and without GDM delivering at term at BCRH in 2017.

Objectives: To compare the differences in the placental histological and perinatal outcomes in women with and without GDM delivering at term at BCRH in 2017.

Materials and methods: This was a nested case control study; as computed by formulae a sample size of 19 GDM and 57 non GDM placentae as cases and controls respectively. My study was nested in a larger on-going study which seeks to develop a neonatal infection diagnostic tool by use placental tissue. The samples were drawn from Bungoma County Referral Hospital and analysis done at the Basic, Clinical and Translational (BCT) research laboratory located in the Department of Human Anatomy, University of Nairobi. Formalin-fixed paraffin-embedded placental tissues were obtained from the placental repository; selected according to the study's inclusion and exclusion criteria then processed for microscopy. The general histology organisation was analysed by light microscopy after staining. Histomorphometry including diameter and cross sectional surface area of the terminal villi were conducted using Image J analysis. Perinatal outcome was assessed by birth weights. The data was then analysed using SPSS version 26 into means, standard deviation, and P values. A P value of <0.05 was deemed significant.

Results: The socio-demographic features of the two groups were largely comparable, with most women being multiparous married and high school leavers. The mean age for the cases was 27 years compared to the control which was 28 years.

Neonatal weights were significantly higher in the cases 3666g compared to 2789g in the cases (p-value of 0.001), further the neonatal placental weight ratio was significantly increased in cases 1:6.91 compared to 1:5.32(p-value of 0.001).

Histologically we observed more oedema in the cases 53% compared to 2% in the controls, increased cytotrophoblast in the cases 33% to none in the controls, villus infarcts were increased in the cases 40% compared to nil in the controls, significant peri villus fibrinoid deposition in the controls 27% to 4% in the cases (P Value of < 0.03). The cases had more chorangiosis 27% compared to 2 % in the controls, furthermore 33% of the cases had vessels centrally placed in the villus than controls 0% Histomorphometrically the cases had significantly larger terminal villus, mean size of 145.18μM (±2SD) in the cases compared to 69.58 μM in the controls (P value of< 0.001), with significantly lowed vascularization quotient of 0.20 compared to 0.40 in the controls(P value of<0.001). These findings were all in keeping with maternal vascular malperfusion (MVM) an Amsterdam Placental Workshop Group (APWG) pattern.

Conclusion

Our study showed that maternal-vascular malperfusion (MVM) occurred more frequently in the GDM group than in the non-GDM, with heavier neonates in the GDM group compared to the non-GDM.

Recommendation

We recommend tight antenatal glycaemic control to prevent MVM. Further we recommend routine immediate postnatal placental sampling and microscopy for all women with GDM; this will enable subsequent pregnancy risk stratification in women with histologically confirmed MVM.

Future work in this area could focus on clinical, sonographic and molecular correlates to the pathognomic lesions with the view of being able to prenatally diagnose and mitigate.

CHAPTER ONE

1 INTRODUCTION

1.1 Background

Gestational Diabetes Mellitus (GDM) is any degree of glucose intolerance with first recognition in pregnancy. It constitutes 84% of hyperglycaemia in pregnancy (HIP) (1)(2)The highest global prevalence is 24.5% in North Africa and lowest at 2 % in Europe; in sub-Saharan Africa is at 8.9% while in western Kenya is at 2.9% (3)(4). One in every six live births is affected by some form of hyperglycaemia, according to FIGO.(5)It is a critical under-diagnosed; under-reported and poorly managed cause of increased pregnancy loses; perinatal morbidity and mortality and by extension pose health risks to the future child. Signs of neonatal morbidity are first picked as low APGAR scores a measure of physiological maturity and wellness (6)(7) A two-fold increase in congenital anomalies and macrosomia is seen in 30% of the cases with related shoulder dystocia and associated nerve injuries, abruption is also frequently seen in GDM(8) These changes include a highly branched immature, oedematous placentome and maternal decidual vasculopathy; these placentae physiologically performs below par.

While a lot of placental histology work has been done globally to understand the placental changes and its impact to the growing foetus and new born, adverse perinatal outcomes persist. This global trend has found its way to our set up leading to great socioeconomic cost. This is being driven by changes in diet and adoption sedentary lifestyle. While local histological data is of immense significance in the fight against GDM and its impact, there is glaring scarcity of this local data. In the 2016 Amsterdam Placental Workshop Group (APWG) publication that is the recommended placental study protocol, it was noted that that there are lesions that showed high recurrence rates in subsequent pregnancies. This can be used to achieve risk stratification for the purposes of tailored antenatal care (ANC) (8)

This study set out to compare placental histology and associated perinatal outcomes in women with and without GDM living in western Kenya employing the APWG recommendations to

investigate for lesions of clinical value. A combination of histologic and histomorphometric techniques will be employed to map out these lesions of interest.

There is a concerted effort to flatten the curve of morbidity and mortality emanating from adverse perinatal outcomes related to GDM; both the 3rd SDG and FIGO's have made pronouncements to this effect. (5) A lot of GDM related placental histo-morphology studies have been undertaken but there is no local data on the histology of the placenta, we propose to study these changes and associated perinatal outcomes while employing the APWG recommendations to generate local data. We conducted our investigation on bio-bank placenta specimens. The bio-bank was created by an extensive on-going primary placenta study titled, "Rapid and multiplex diagnostics for maternal infections"; as one of its objectives. It also aims to develop test kits for diagnosing asymptomatic neonatal infection by use of placental samples. It is in this that our study will be nested. Placentae for this study were collected in Bungoma County Referral Hospital (BCRH). The women recruited were sure about the date of their last menstrual period (LMP) and confirmed via obstetric ultra sound at 16 weeks, GDM was diagnosed via OGTT at 25 weeks of gestation.

1.1 Literature review

1.1.1 General introduction: GDM and Placenta

Gestational diabetic Mellitus (GDM) is a degree of glucose intolerance with first recognition in pregnancy. (1) The highest global prevalence is 24.5% in North Africa and the lowest at 2 % in Europe while in Sub-Saharan Africa it is at 8.9% and 2.9% in Western Kenya.(3)(4) In 2019 according to IDF 20.4 million representing 15.8% of all live births globally were affected by HIP of which 83.6% were GDM; in Africa, 3.5 M live births representing 9.6% were affected by GDM (2). It is a critical and underdiagnosed cause of increased perinatal morbidity and mortality. These include a two-fold increase in congenital anomalies linked to the release of free radicals; i.e. neural tube defect (NTD) and congenital heart disease (CHD); macrosomia is seen in 30% of the cases with related shoulder dystocia and associated nerve injuries contributing to adverse perinatal outcomes. (18) Hypoglycaemia and hyperbilirubinaemia in neonatal life critically affect the onset of life. All these adversely affect the future child. When the pregnancies do not go to term GDM is a notorious culprit, with a 6.9% increase in prematurity rates. Prematurity and pregnancy loss are primarily linked to foetal hypoxia from placental histology changes; this affects efficient work of supply and evacuation of waste to and from the foetus via the placenta.

The placenta is mainly a vascular organ, is vulnerable and sustains a lot of hyperglycaemic injuries. Two recognizable placental lesion patterns associated with GDM are noted, maternal vascular malperfusion (MVM) and foetal vascular malperfusion. (8) In general the underlying impact is a reduction in villi surface area to volume ratio and increment in the distance between foetal and maternal circulations. For optimal placental function, the integrity of the delicate and precise histoarchitecture is essential; thus proper placentation requires optimal conditions that include euglycaemia, right oxygen tension and proper nutritional supply; thus changes in the placental histology occur in the absence of the said conditions. (15)

1.1.2 Microscopic features of Chorion, Decidua and Villi in GDM

The placenta has three tissue compartments, a chorionic plate, a basal plate made up of decidua and an intervening intervillous space where villi make contact with maternal blood in the lacuna. The chorionic plate is made up of the amnion that is a single layer of cuboidal cells; extra-embryonic mesoblast, cytotrophoblast and syncytiotrophoblast. In it, the umbilical artery divides and ends up in the chorionic villi inside the lacuna where they spend their life immersed in maternal blood. Five chorionic villi subtypes exist; this include; mesenchymal villi, immature intermediate villi, stem villi, mature intermediate villi, and terminal villi; further the villi can be classified according to function that includes anchoring the placenta to the decidua. (12)(10) A section through a term villi, from out in, is made of a layer of microvilli followed by a layer of syncytiotrophoblast that have thin areas of substance exchange and thick areas of bio metabolism. A thin spread layer of scanty cytotrophoblast (compared to immature villi) is seen beyond which is a basal lamina that surrounds connective tissue that suspends fibroblast, foetal capillaries and Hofbauer cells. (14)

The decidua is divided into; from superficial to deep stratum compactum, stratum spongiosum and unaltered boundary layer that is attached to the muscular layer of the uterus, it is through the decidua that syncytiotrophoblast implants. (12) This invasion is under the influence of the metabolic environment, including those of nutritional and glycaemic status physically limited by the nitabuch layer . (15) The decidual stroma is made up of epithelioid cells; these are enlarged polygonal endometrial stromal cells, granular leukocytes, uterine natural killer cells and peri-cellular matrix with overall increased vascularity. Maternal vessels are widened and are less tortuous compared to spiral arteries that they are modelled from. (12) Across the decidua, there are areas of fibrinoid deposition in response to syncytiotrophoblast damage. An area of fibrinoid deposition concentration named the Rohr layer marks the point where the trophoblasts meet the stratum compactum. In contrast, the nitabuch layer is the junction between the stratum spongiosum and compactum and is the limit of physiological invasion. (12) Marginal infarcts on term placentae are a common feature without much significance. (7)

Dysglycemia in the preconception period and the first trimester is associated with adverse perinatal outcomes via its impact on the placenta while correction of hyperglycaemia does not necessarily alter placental lesions but just reduces the severity of the lesions In GDM, in the placenta, there is villous maturation defects that is characterised by the presence of macrophages, oedema, increased number of capillaries and increased amount of cytotrophoblast for gestational age in villi this leads to foeto-placental malperfusion.(13) Despite the increase in villus capillaries, most are immature, centrally located in the villus and leaky thus leading to oedema, as well as increased syncytial knotting. (14) Poor placental tissue perfusion as reported by other studies is linked to decidual vasculopathy (DV) a failure of proper spiral remodelling. DV is characterised by villous infarcts, perivillous fibrin, fibrin deposition, increased syncytial knots and in some cases it could involve hypertrophic decidual vasculopathy.(15)(16)

These lesions that result from placental hyperglycaemic injury and its adaptive mechanisms have clearly been established by studies over the years from across the globe. (15).

Proper placentation that is optimal surface area to volume ratio of the placenta and the shortest distance between the two circulations is influenced by the foetal genetic pool (17) and microenvironment factors in which placentation occur. These factors include oxygen tension, immunological competence, maternal nutritional supply, and maternal chronic illnesses and infections e.g. Hypertension, Diabetes Mellitus, Malaria, HIV and many more. (15)

Villi core histomorphometric picture in GDM is that of thick, sparsely populated terminal villi per unit area; further, despite increased angiogenesis, there is reduced vascularisation quotient by up to 50% as the vessels have narrow bores, overall the placenta is larger and thicker. These villi are metrically changed to respond to hypoxia created by initial hyperglycaemic injury as there is reduced surface area to volume ratio for exchanges, and the two circulations are set far apart. (18)(19)(20)(21)

1.1.3 Perinatal outcomes

The placental morphological and histological changes noted in GDM that leads to reduced substance exchange capacity are associated with adverse perinatal outcomes, namely macrosomia with associated birth trauma. Poor APGAR scores ranging from low to zero implying mortality have been reported. These mortalities have been reported to occur just before delivery or during delivery to produce macerated and fresh still births. Conflicting reports pertaining to perinatal mortality related to GDM have been documented, majority report increased perinatal mortality in GDM women.(22)(23) The 5-minute scores have been documented to be four-fold more likely to be below 8 in GDM. GDM neonates tend to be larger for gestational age (LGA) this is associated with birth trauma, hypoglycaemia, childhood obesity and metabolic syndrome. These significantly contribute to neonatal and early childhood illness and mortality.(7) Further, other studies have also reported congenital malformations and IUGR.

1.1.4 The State of Research and way forward

GDM related histomorphometric and morphological changes have been shown to mould term villi to appear immature oedematous and poorly perfused, there is decidual vasculopathy leading to poor placental perfusion this creates foetal hypoxia and related adverse perinatal and early childhood morbidity. A concerted effort is on-going to reduce adverse outcomes related to GDM; as per SDG 3, it targets to eliminate preventable deaths of new-borns and children under-five by 2030. In line with SDG 3, a 2018 FIGO working group meeting on HIP in Brazil put its weight behind the effort to contain GDM. It declared “That we resolve to address this challenge and to convert it into an opportunity for improved health outcome for mothers and their newly born babies and stem the rising curve of non-communicable diseases (NCD) and improve future population health.” This revitalised the focus on GDM to improve pregnancy outcomes.

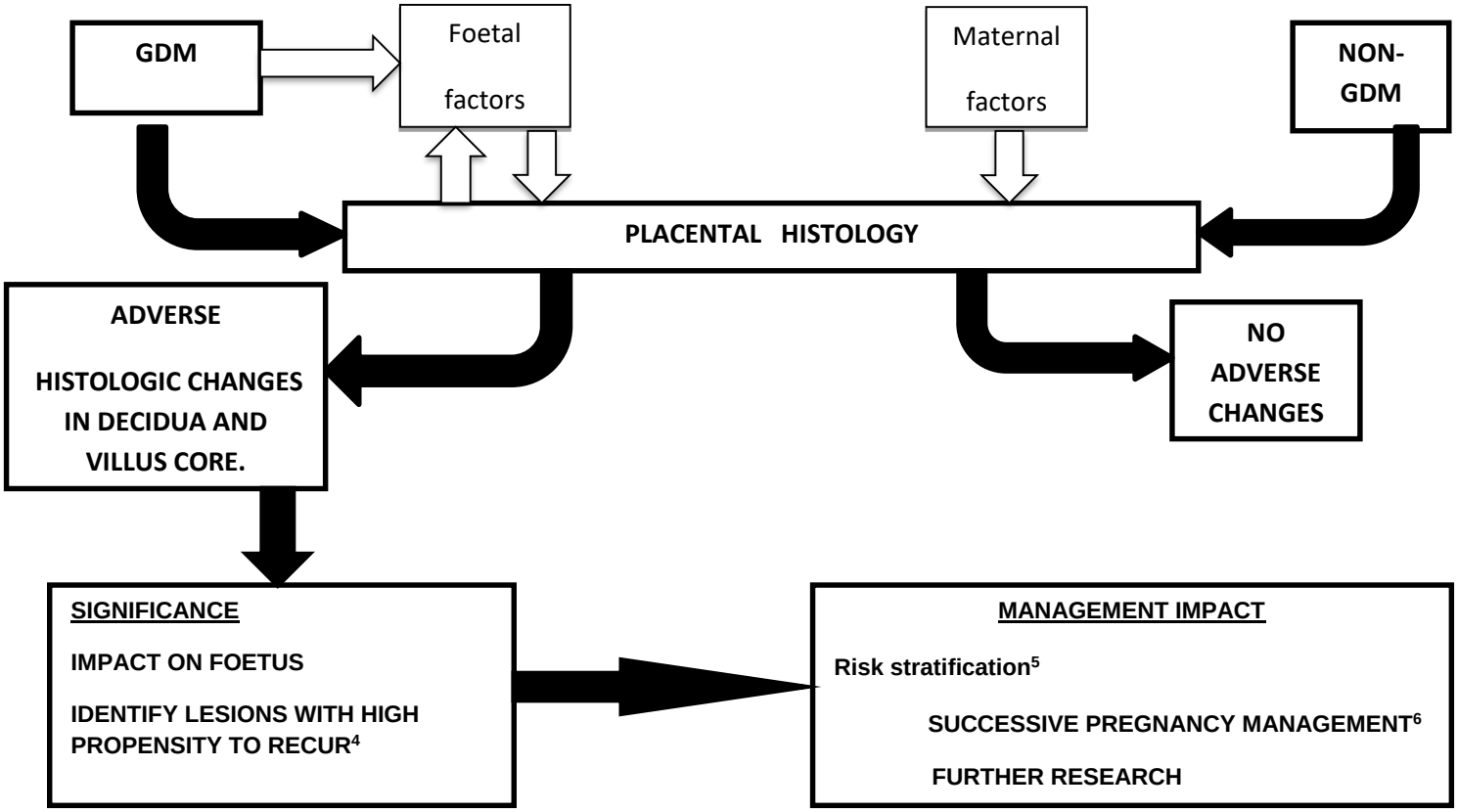
The use of clinical methods to triage these women for care has been the main stay of management, these includes past medical history evaluation and routine one off random blood sugar assessment in the first trimester, while this has played a role in picking this women for treatment a more accurate objective way of triaging this patients is needed. (24)

The Amsterdam Placental Workshop Group (APWG) was set up and met in 2015 to formulate and publish agreed upon protocols for placental studies, these included how to take samples definition of lesions, working terminologies for lesions and diagnosis and overall importance of these lesions and diagnosis. This led to their 2016 publication that was recommended to be the standard placental study protocol. This publication contained significant standout information; it reported that there are lesions that showed high recurrence rates in subsequent pregnancies.

Locally a glaring scarcity of placental histologic studies exists; this data could prove handy in laying a foundation for future studies in this area. We conducted a study combining histologic and histomorphometry techniques as well as investigated the perinatal outcomes. This approach allows the assessment of GDM impact on the maternal side and objective metric assessment of the foetal villi response. The generated local data on these lesions will help in risk stratification and help local authorities put in place strategies to drive up ANC attendance. In furtherance the generated data will be in recommended APWG terminologies, this findings will be added to other similar studies and form a repository of study that are searchable using the APWG terms for purposes of comparison and reference. To achieve the above a combination of light microscopy techniques including microphotography for further IMAGEJ assisted metric assessment were employed on bio banked specimen.

This bio-bank was created as one of the objectives of the primary study; these samples were collected for an on-going placenta primary study titled, "Rapid and Multiplex Diagnosis of Maternal infections". It targets the development of test kits for diagnosis of asymptomatic neonatal infection by use of placental samples.

1.2 CONCEPTUAL FRAMEWORK



1. Maternal factors including; socio-demographic data, chronic illness and medication e.g. Age, parity, body mass index, hypertension, anaemia, anaemia, malnutrition, connective tissue diseases, steroids use, anti-thyroid hormone medications among others.
2. Foetal factors genetic pool, progressive foetal weight, foetal demands.
3. Sampling factors include sample collection and processing techniques and timing.
4. Maternal floor infarction and maternal malperfusion
5. Risk stratification
6. Preconception DM screening, Early ANC GDM screening, regular antenatal care, frequent foetal surveillance, aspirin, early delivery and others.

NB: Bold arrows and boxes depict the main study channel

.1.3 Problem Statement

1 in 6 live births [approximately 20.4 million] is affected by hyperglycaemia in pregnancy (HIP), 84% of which are GDM. The prevalence of HIP across the world stands at 15.8%; in Sub Sahara Africa it is at 8.7% while in Western Kenya is at 2.9%. GDM continues to be associated with adverse perinatal outcomes via its effect on the chorionic villi and decidual vasculopathy. A lot has been done leading to the understanding of the pathology behind GDM and the Placenta; this has drastically reduced the adverse perinatal outcomes associated with GDM. Despite this progress, undesired perinatal outcomes persist, pushing for further studies, more so in placental elements that are yet to be explored with associated perinatal outcomes.

1.5 Justification

There have been numerous studies done on GDM epidemiology, screening, clinical pathology, immunology and its impact on placental morphology. Recently the APWG published study protocols for placental histology studies. Among the new recommendations are terminologies and the significance of certain lesions that were identified. The group identified lesions with high recurrence rates in subsequent pregnancies. One of those lesions was maternal vascular malperfusion that was noted to recur in 10 to 25% of pregnancies. There is scarcity of local studies combining histologic and histomorphometric techniques to assess the impact of GDM on the placenta to assess for lesions deemed significant by APWG for their high recurrence rates. This critical information can be used for in risk stratification more so in my study set up that has recorded poor ANC attendance. This will be important in risk stratification for purposes of driving up ANC attendance. Secondly it will generate data with recommended terminologies for purposes of future reference.

1.6 Research question

What are the differences in placental histology and perinatal outcomes in women with and without GDM delivering at term at BCRH in 2017.

Hypothesis

1.7 Null hypothesis

There are no differences in placental histomorphometry and perinatal outcomes in women with and without GDM delivering at term at BCRH in 2017.

1.8 Objectives

1.8.1 Broad objective

To compare the differences in the placental histological and perinatal outcomes in women with and without GDM delivering at term at BCRH in 2017

1.8.2 Specific objectives

Among women with and without GDM delivering at term at BCRH:

1. To compare the histology of the chorion and villus core.
2. To compare the perinatal outcomes in the two groups.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Study design

This was an unmatched; nested case control employing both clinical and laboratory techniques. This design was chosen as it covers past exposures, asses outcomes as in my study and picks up rear outcomes. This study compared the histology differences in GDM placentae vs. non-GDM placentae. The cases were placentae from women with GDM while the controls were placentae from women without GDM. The cases had been diagnosed by the primary study.

2.2 Study site and setting

We conducted the study at the Basic, Clinical and Translational (BCT) research laboratory located in the Department of Human Anatomy, University of Nairobi. The BCT laboratory is in the Department of Human Anatomy. The laboratory focuses on Placentae [Human and Murine], endometrial and the biology of other reproductive structures. It has a capacity of 10 scientists. We used bio-banked specimens that were collected from the Bungoma County Referral Hospital (BCRH). These samples and patient clinical and non-clinical information were from an on-going placenta study titled Rapid and multiplex diagnosis of maternal infections, this study aims to create early rapid neonatal infection diagnostic tool by use of placental samples, the study also had an objective of creating a bio bank hence collected placentae from women with a variety of conditions in pregnancy including GDM a condition of interest to us. The Ethical Review Committee number is MKU/ERC/0543(Appendix 5 attached) and authorization letter to use bio back (Appendix 6 attached). Bungoma County Referral Hospital formerly Bungoma District Hospital is situated about 400km North West of Nairobi with a 216-bed capacity; 40-bed maternity handling approximately 600 deliveries a month. Its main catchment area encompasses the immediate town and the wider rural area surrounding the town namely Mayanja, Kibabii, Kanduyi, Siritanyi Bwema, Mlaa, Sinoko, Bumula Musikoma, Sang'alo, Sikata, Bukembe, Kabuchai among others.

2.3 Study population

Bio-banked placenta specimens of women with and without GDM who delivered at BCRH were studied. This diagnosis was made by the primary study by use of OGTT at 25 weeks. The study specimens were grouped into two; cases GDM and controls Non-GDM. The cases were placentae from women with GDM who delivered at 37 weeks plus 0 days and 40 weeks plus 6 days. The treatment status and glycaemic levels that were not recorded by the primary study won't be considered, On the other hand, the controls were placentae of women without GDM who delivered babies between 37weeks plus 0 days and 40 weeks plus 6 days.

2.4 Inclusion criteria

Bio-banked placenta specimen of women;

1. Aged between 18 and 40 years
2. With GDM and without GDM
3. Who delivered single live babies at 37 completed weeks to 40 weeks plus 6 days.
4. Women who were sure of the date of the first day of the last normal menstrual period and corroborated by an obstetrics ultrasound scan before 16 weeks.

2.5 Exclusion criteria

Bio-banked placenta of women with medical and obstetric complications namely:

1. Chorioamionitis
2. Chronic hypertension,
3. Preeclampsia,
4. HIV
5. Malaria
6. Cardiovascular diseases
7. Malnutrition.

This was to reduce cofounders as the lesions of interest occur in these conditions as well; secondly the samples we used had already been sorted to only have GDM.

2.6 Sample size determination and sampling procedures

2.6.1 Sample size

Jaykaran Charan and Tamoghna Biswas in their 2013 review of sample size calculation formulae recommended the formula below for cohort studies with quantitative variables. The paper was published in the Indian Journal of Psychological Medicine. (32) Initial sample size calculation arrived at a sample 27 and a 10% mark-up arriving at 31 placentae per arm giving a total of 62 samples, but at the point of recruitment from the bio-bank only 19 cases samples were realized necessitating recalculation of the sample size.

The following assumptions from a similar study *Effects of Gestational Diabetes Mellitus on Umbilical Cord Morphology*: (26) was considered during the calculation, that study found umbilical cord diameter (cm) to be 1.303 ± 0.1884 and 1.163 ± 0.1815 in GDM and controls respectively. This was significant with a p-value of 0.0001.

Sample size = $\frac{r+1}{r}\frac{SD^2(Z_\beta+Z_{\alpha/2})^2}{d^2}$ $\frac{3+1}{3}\times\frac{0.1851^2(1.96+0.84)^2}{0.14^2}$ =19

$Z_{\alpha/2}$ Standard normal variate for a level of significance. At 5% (P <0.05) it's 1.96

Z_β Standard normal variate for power. At 80% power, it is 0.84

r Ratio of controls to cases which is 3

SD Standard deviation used 0.1851 from a similar study

d Expected mean difference between cases and controls is 0.14 from a similar study

This gave a sample size of 19 and 57 study subjects for GDM and controls respectively.

2.6.2 Sampling procedures

Bio-banked placentae specimens were picked out to form the cases and controls groups; GDM and non GDM, respectively. The selected samples had been handled as per the rigorous bio bank SOPs to ensure suitability to the study. This included well labelled with no obvious gross damages. Each group comprised of one bio-banked specimen (placenta blocks) from 19 cases and 57 controls study subjects. The data collection sheet for the selected study placentae was used to identify a block for each of the study placenta. The placenta blocks were analysed as described later in this section. Clinical data included age, gestational age and GDM status were analysed.

Placental samples preserved by fixation in wax blocks were obtained from the bio-bank, five-micrometre serial sections of the blocks cut using Leitz Wetzlar sledge microtome, floated in warm water then mounted on a glass slide and dried in a hot air oven for 40 degrees centigrade overnight. The sections were then stained with hematoxylin and eosin to demonstrate the histomorphometry and Masson's trichome to highlight the connective tissue. This specimen was examined under a Leica Automated Systems light microscope connected to a computer and monitor.

The investigators were be blinded to the GDM status. The general histological organisation of the chorion and villus core of the placenta was recorded. The following features were described as per APWG guidelines and altshuler criteria; villous maturation, vascularity, amount of cytotrophoblast, syncytial knotting, macrophages and fibrinoid deposition.

For morphometric analysis a placental slide was subjected to microscopy at 400X. Digital images of the tissue sections were captured using a Canon colour camera interfaced with an Olympus BX41 microscope. The images were scanned at a resolution of 1600 X 1200 pixels using Olympus software and stored. Only terminal villus showing complete outline on a microscope field were measured. The images were used as raw data to quantify the villus architecture using image analysis software Image J and to measure the surface area of the terminal villi (μm^2), the cumulative surface area on villi cross sectional (μm^2) then a

vascularization quotient were calculated from the two surface areas. The ImageJ application was downloaded from the internet onto a desk top, once installed and opened, a given image was lifted and dropped on the application; the image was calibrated using the image on board scale and both max villus diameter and surface areas measurements were taken using ImageJ tools. For the max diameter a random villus was selected and measured using the measuring tool. For surface area, thresholding was used to measure the area of the randomly selected terminal villus with all round visible margins.

2.7 Quality assurance

Internal quality assurance

Tissue samples from undamaged placenta only were collected, processed and kept in the bio bank as per internationally accepted standards. During the analysis stage, the specimens were processed further and analysed according to the predetermined standard operating procedures and the Principal researcher underwent a 2 week on bench practical mentorship sessions on basic preparation, observation and analytical methods and analysed the samples in the Department of Human Anatomy at the University of Nairobi. The findings were counter checked by one of my supervisors; a PhD holder in Anatomy and well versed with placenta research. Analysed data was entered into a dedicated data collection form that is always be in the secure hands of a designated custodian.

External quality assurance

10% of the results were counter checked by an independent pathologist.

2.8 Ethical considerations

We sought ethical review and approval from the KNH/UoN Ethical and Scientific Review Committee (P95/02/2021) for purposes of utilizing study population information as well as bio banked placental samples at the Basic Clinical and Translational Laboratory at Chiromo Campus

that is the custodian of the samples and not owners. The bio-bank did not ask for any monetary returns for use of the samples as per international practice in the general EU practise.(27)(28) Permission to access the facilities in the lab was sought from the concerned authorities and laboratory SOPs were observed. Further; authority to use the bio-bank was given by the primary study vide the authorization letter ref in appendix 6. Placental tissues were handled according to the provisions of use of human tissues as provided for by the human tissue and anatomy act cap 249 and 252 of the laws of Kenya. Anonymity was highly maintained. Ethical approval for the primary study was obtained from the Mount Kenya University Ethical Review Committee (see appendix 5)

Table 1:Data Variables

Specific Objectives	Exposure Variable	Outcome Variable	Source Of Data
To analyse the microscopic structural organisation of the chorion and villous core structure.	General structural organization of the chorion, and villus core 1.Number of Macrophages per hpf 2.Oedema, 3. Increased amount of cytotrophoblast for gestational age in villi 4. syncytial knotting 5.Villus infarcts. 6.Perivillus fibrin. 7.Fibrin deposition. 8.Number of foetal capillaries in the villous stromal core and location	Histological differences among GDM and non GDM	Microscopic observation of the 5µM placental tissue slices. As per APWG Classification. [Normal (2-6 capillaries), high (more than 10 capillaries) or low (0-1 capillaries) based on] Classification of Altshuler. Capillary location within the villi Central and peripheral.
To describe the Histomorphometry of the terminal villi.	Histomorphometry of villi cross-section 1.The diameter of the terminal villi and the S.A The cumulative S.A of the blood vessels and VQ	Histomorphometric Differences among GDM vs. non GDM	Images of 5µM placenta tissue processed for histomorphometric analysis
To tabulate perinatal outcomes	birth weights and APGAR scores	Birth weights differences among GDM and non GDM	Birth weight Entries

2.9. Data collection and management

The principal researcher recorded the data on histology and morphometric features into dedicated data entry forms (appendix 4). Further, photos of the histological features were taken. Raw data was entered into excel sheets. All data was kept securely in a password protected folders in an external hard disk; this has been in the custody of the Principal researcher with cloud back up.

2.10 Statistical Analysis

In these analyses, the two subgroups of GDM and Non-GDM placenta were compared for histological findings, histomorphometry and associated perinatal outcomes. The data obtained was analysed using SPSS, version 26. Means, standard deviations, and frequency tables were compiled as descriptive data. Placental characteristics of the GDM and Non-GDM groups and associated perinatal outcomes were compared and analysed using t-tests/Mann-Whitney u test and chi-square/Fisher's Exact test. A p-value of <0.05 showed statistical significance for all statistical tests carried out.

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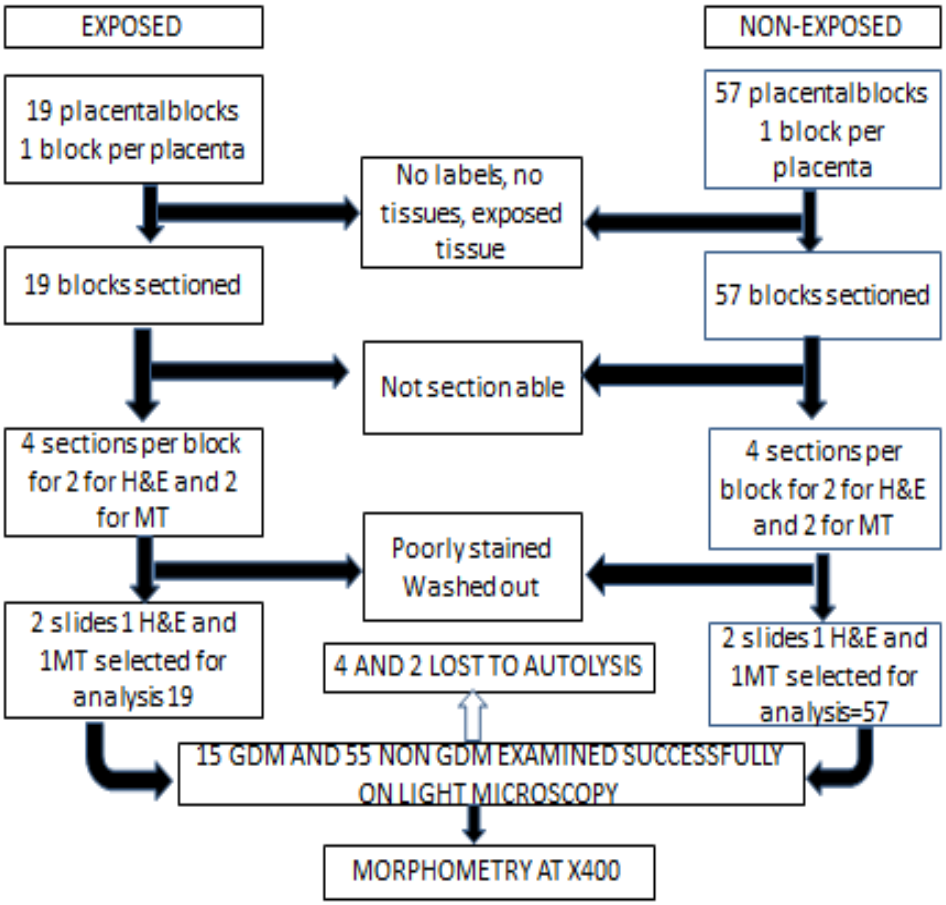
To achieve specific objective 1: Both numerical and categorical data of macroscopic features of the placenta features of delayed villous maturation and features of decidual vasculopathy were analysed. To compare histomorphometric differences between placentae of GDM women and non GDM women, descriptive statistics will first be conducted. The means, standard deviations will be computed for numerical variables while frequency tables with "n" and "%" were computed for data that is categorical. To determine the association between histological/ histomorphometric findings and GDM status, Student's t-test and Chi-square tests will be used for numerical and categorical data, respectively.

To achieve specific objective 2: The data obtained on foetal outcomes was tabulated in terms of the GDM status of the study subject. Then mean and standard deviation was computed as descriptive statistics. To compare the associated perinatal outcomes between the cases and controls student's t-test /chi-square was used.

STUDY FLOW CHAT



3.0 Results

A total of 70 Placenta were studied 15 Cases and 55 controls. This was from a total sample of 76 (19 cases and 57 controls), 4 samples cases (Gestational Diabetes Mellitus) and 2 controls of selected processed samples could not be used as they did not meet the quality assurance.

Socio-demographic characteristics

Most of the patients were multiparous married women with high school level education. None of the socio-demographic and clinical characteristics were found to be significantly different between the 2 groups (Table 2)

Table 2: Socio-demographic and Clinical characteristics of patients

		GDM N=15		Control N=55		P-value	Crude OR (95%CI)
		n/mean	%/SD	n/mean	%/SD		
Age		27	4.48	28	4.04	0.527	-
	18-25	5	33%	12	22%	0.328	-
	25-30	5	33%	30	54%		
	>30	5	34%	13	24%		
marital status	Single	2	13%	10	18%	1	Ref
	Married	13	87%	45	82%		1.44(0.28,7.44)
level of education	Primary	3	20%	10	18%	0.572	-
	High School	10	67%	30	55%		
	College	2	13%	15	27%		
HB level		11.52	0.84	11.69	1.25	0.931	-
	<10g/Dl	0	0%	2	4%	1	-
	>10g/Dl	15	100%	53	96%		
Parity	Primi-para	1	7%	4	7%	1	Ref
	Multipara	14	93%	51	93%		1.10(0.11,10.63)

Histologic features including oedema, amount of cytotrophoblasts, syncytial knotting, villous infarcts, fibrin deposition, peri villous Fibrin, no of foetal capillaries were found to be significantly different in regards to the two groups.

Table 3: Changes of the Placental villi, decidua, and chorion

		GDM N=15		Control N=55		P-value	Crude OR (95%CI)
		n/mean	%/SD	n/mean	%/SD		
Macrophages	Significant Focal	1	7%	0	0%	0.114	-
	Significant Diffused	1	7%	1	2%		
	Non-Significant	13	86%	54	98%		
Oedema	Significant Diffused	8	53%	1	2%	***<0.001***	61.71(6.68,569.91)
	Non-Significant	7	47%	54	98%		Ref
Increased amount of cytotrophoblast	Significant Diffused	5	33%	0	0%	***<0.001***	-
	Non-Significant	10	67%	55	100%		
syncytial knotting	Significant Diffused	11	73%	54	98%	***0.006***	0.05(0.01,0.50)
	Non-Significant	4	27%	1	2%		Ref
Villous infarcts	Significant Focal	6	40%	0	0%	***<0.001***	-
	Non-Significant	9	60%	55	100%		
Fibrin Deposition	Significant Focal	1	7%	13	24%	0.054	-
	Significant Diffused	6	40%	30	54%		
	Non-Significant	8	53%	12	22%		
Peri villous Fibrin	Significant Focal	0	0%	2	4%	***0.03***	-
	Significant	4	27%	2	4%		

	Diffused						
	Non-Significant	11	73%	51	92%		
No of fetal capillaries	High	4	27%	1	2%	***0.002***	-
	Normal	11	73%	42	76%		
	Low	0	0%	12	22%		
location of capillaries	Central	5	33%	0	0%	***<0.001***	-
	Peripheral	10	67%	55	100%		

Terminal villous diameter, terminal villi cross sectional area was increased while the VQ was reduced.

Table 4:Morphometric Changes of Villous structure

		GDM N=15		Control N=55		P-value	Crude OR (95%CI)
		n/mean	%/SD	n/mean	%/SD		
Terminal villus max diameter		145.18	70.22	69.58	12.60	***<0.001***	-
Terminal villus cross sectional area		23,761.96	23,631.81	3,235.72	1,158.99	***<0.001***	-
Terminal villus blood vessel cross sectional area		4,388.64	3,602.06	1,282.01	456.49	***<0.001***	-
Vascularisation quotient (VQ)		0.20	0.10	0.40	0.12	***<0.001***	-
	≥0.40	14	93%	17	31%	***<0.001***	31.29(3.80,257.56)
	<0.40	1	7%	38	69%		Ref

Table 5: Perinatal Outcomes. The birth weights were significantly higher in the GDM group as compared to controls. Most of the babies in the GDM group (73%) were between 3.5 kgs and 4 kgs. The neonatal placental ratio was increased.

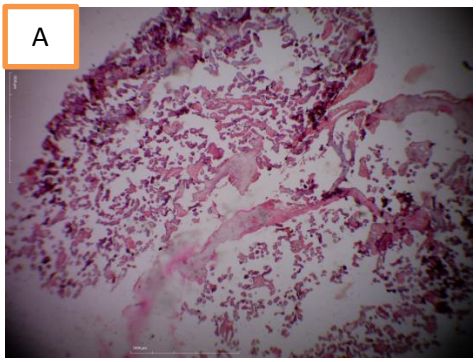
		GDM N=15		Control N=55		P-value
		n/mean	%/SD	n/mean	%/SD	
Birth weight		3,666	195.48	2,789.64	478.79	***<0.001***
	3.5 kgs - 4 kgs	11	73%	2	4%	***<0.001***
	3kgs - 3.5 kgs	4	27%	19	34%	
	2.5 kgs - 3 kgs	0	0%	18	33%	
	< 2.5 kgs	0	0%	16	29%	
Neonatal placental Ratio		6.91	0.88	5.32	0.77	***<0.001***
	<=5	0	0%	20	36%	***0.004***
	>5	15	100%	35	64%	

Table 6: APWG Diagnoses; 86% of diagnoses were MVM , 7% infections and 7% were normal placenta in the cases compared to 15% MVM, 65% normal and 11% infections in the controls

		GDM N=15		Control N=55		P-value
		N	%	N	%	
Diagnosis	MVM	13	86%	8	15%	***<0.001***
	FVM	0	0%	5	9%	
	Normal	1	7%	36	65%	
	Infections	1	7%	6	11%	

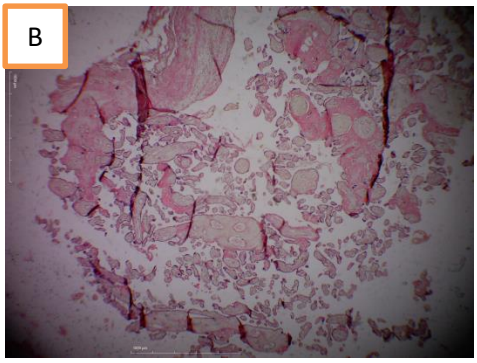
Sample micrographs

A



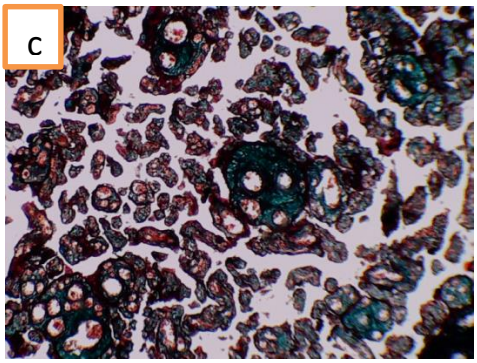
Micrograph A in H&E at x400 showing villi hypoplasia

B



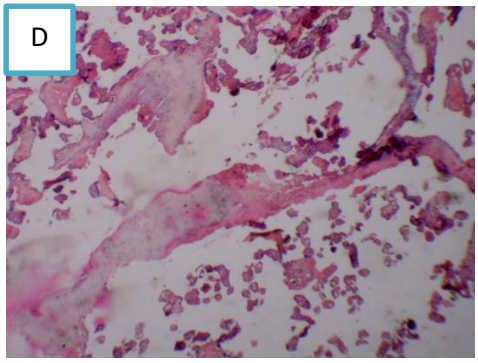
Micrograph B in H&E at x400 showing significant but diffuse villous oedema.

C



Micrograph C (zoomed in from x400) in MT showing centrally located vasculature.

D



Micrograph D in H&E at x400 showing significant but diffuse perivillous fibrinoid deposition

3.1 DISCUSSION

Most of the patients were multiparous married women with high school level education. The mean age of the GDM group was 27 years while the non GDM was 28years. None of the socio-demographic and clinical characteristics were found to be significantly different between the 2 groups.

We observed oedematous terminal villi in 53% of the cases compared to 2% of the controls, this agrees with works by Iva Carrasco who noted increased villous oedema is noted in GDM villi that have immature vasculature that leak out, this increases diffusional distances and hence negatively impacts on the efficiency of the villi, this is termed delayed villous maturation an APWG entity.(8) (29) (39) Increased amounts of cytotrophoblast was noted in the cases 33% compared to none in the controls, this compares with findings by Magee TR where she noted reduced apoptotic activity in GDM reducing the fusion of cytotrophoblast necessary for maturation of the villi leaving immature villi with thicker layer of cytotrophoblast, further Belkacemi demonstrated reduction in apoptotic index in GDM vs non GDM 0.05±0.01 compared to 0.17±0.04 with a $p<0.04$. (29)(30)(31) Syncytial knotting was significantly increased in the controls as compared to the cases, this relates to the delayed villous maturation noted in the cases group, this contrasts with other studies that have reported increased knotting in GDM associated with cytotrophoblast hyperglycaemic injuries and attempted repair .Villous infarcts were found to be significantly increased in cases 40% compared to none in controls this agrees with works by Memon S.(32) This is associated with complete decidual vascular occlusion as per APWG.

We also noted increased vascularity in 27% of cases compared to 2 % further this were centrally placed arteries in 33% of GDM compared to none in the controls (8) The end result of the above noted changes was a reduced vascularisation quotient of 0.20 compared to 0.40 in the controls that is within expected ranges 0.41 to 0.50; the above phenomenon draws a picture of delayed villus maturation.(33)

We also found largely heavier neonates at birth in the cases compared to the controls this largely agrees with other studies, they are thought to be heavier because of accelerated growth driven by

abundance of glucose in neonatal circulation, though the babies were heavy they were not as extreme as those noted by Kamana KC et al.(34) This could be attributed to glycaemic control a factor that I did not asses in my study, further we assessed neonatal placental ratios in this two groups and noted increased ratio in the cases 1:6.91 as compared to 1:5.32 which was significant for the cases, the increased ratio are associated with smaller placenta in comparison to the neonate this is associated with villus hypoplasia. The hypoplasia is associated with poor maternal perfusion that hinders production of molecular drivers of placental growth. These factors include placental growth factor PIGF a member of VEGF is poorly expressed in vasculopathy associated with GDM. Further leptin an energy regulatory hormone in the body that is abundant in the placenta is thought to be at the centre of this pathogenesis (35)(36)

The above histologic findings constitute an MVM diagnosis that was noted in 86% of the cases compared to none in the controls. MVM as applied replaces the society for paediatric pathology term maternal placental under perfusion as recommended by the APWG. The new term was so chosen as it encompasses other aspects of perfusion not captured in the older terminology this include high pressure turbulent flow that predispose to thrombi formation and arterial occlusion. Redline et al in their 2016 consensus publications noted that MVM showed recurrence rates of 20 to 25% in the subsequent pregnancies this becomes very useful in risk stratification of ANC women. In our finding we noted areas of partial MVM associated with partial occlusion of arterial blockage while in 2 cases we observed areas of infarction associated with total vascular blockage. (8)(9)

MVM has also been described in hypertensive diseases in pregnancy, this is thought to be the main mechanism by which systemic elevation in blood pressure starts as well as the linkage between GDM and preeclampsia as noted by (37)Linda M Ernst in her work on MVM. MVM is thought to result from vasculopathy associated with GDM mediated by atherosis from deposition of form cell in the arterial walls; this reduces the calibre of sinusoids resulting in high pressure low capacitance vessels according to(38) Maria Portelli in her work on GDM and bio chemical deregulation. Atherosis from hyperglycaemia is a known mechanistic way of reducing arterial

calibre producing high pressure turbulent blood flow towards the lacuna; this interferes with the dynamics of the Virchow triad producing thrombi leading to arterial occlusion. MVM then encompasses the whole spectrum of disordered perfusion from reduced high pressure perfusion to no perfusion.(39)

The high recurrence rates associated with MVM can be leveraged on to achieve risk stratification this enables tailored ANC follow up to achieve the best outcomes.

3.2 Conclusion

Our study showed that maternal-vascular malperfusion (MVM) occurred more frequently in the GDM group than in the non-GDM with associated heavier neonates. MVM, a constellation of histologic changes seen above can be prevented through tight glycaemic control.

3.3 STUDY STRENGTHS AND LIMITATIONS

The lack data on APGAR scores and glycaemic control limited the full extent of the occurrence of some lesions and hence the altered findings. My study utilized bio banked placental samples this greatly reduced the cost of undertaking this study as well as retain the samples that could be used for further research based on finding of my research or otherwise.

3.4 RECOMMENDATIONs

We recommend routine immediate postnatal placental sampling and microscopy for all women with GDM, this will enable subsequent pregnancy risk stratification in women with histologically confirmed MVM.

Future work in this area could focus on clinical, sonographic and molecular correlates to the pathognomic lesions with the view of being able to prenatally diagnose and mitigate.

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APPENDIX

Appendix 1: SOP placenta sample collection and preparation

Placenta sample collection and preparation for placental bank

Section 1: Describes the general collection of placental biopsies

1. Informed authorized consent
2. Harvest placental biopsies immediately after birth as described below
3. Harvest a full thickness placental biopsy 2cm by 2cm from six sites of the placenta
    a. 2 central biopsies from the sides of the cord insertion
    b. 4 peripheral biopsies taken at 12, 3, 6 and 9 O'clock positions of the disk
4. A membrane roll from the smooth chorion
5. 2 cord biopsies, one 1cm from the cord insertion and the other from the cut end of the cord
6. Wash the biopsies thoroughly in PBS until there is no more obvious blood
7. Cut the biopsy samples into three smaller samples A, B and C and prepare them as described in section 2 below.

Section 2: Describes processes of fixing the biopsies

Preparation of A: for fixed frozen section

1. After washing in PBS, fix the cut biopsy in 3% PFA for 12 hours
2. Transfer the biopsy and orientate into labeled biopsy cryomold containing OCT. The tissue should be covered with OCT
3. Freeze in dry ice/ -80^{o} freezer
4. Cover in a labeled foil and store at -80^{o} (freezer/dry ice)

Preparation of B: for electron microscopy

1. After washing in PBS, trim the tissue and separate it into two segments (the basal and chorion portions) each measuring 0.3cm by 0.3cm
2. Fix the biopsies in 3% glutaraldehyde at 2-8o for 12 hours
3. Ship for post-fixation in Osmium tetraoxide

Preparation of C: for paraffin blocks

1. After PBS wash, fix the biopsy samples in 10% formal saline solution
2. Transfer to the lab within 24 hours put them in 70% ethanol
3. Store at 4^{o} for a maximum of 7 days
4. Start preparing blocks through standard procedure

In our case we need to ship in batches every three days for further processing

Appendix 2: Client information and consent form

This is the consent form that was used to recruit participants at BCRH for another placental study that is on-going. Secondary data analysis will be conducted for the data obtained from the primary study.

CLIENT INFORMATION AND CONSENT FORM
Study title

Rapid and Multiplex Diagnosis of Maternal Infections

Study no.....

Date __/__/__

Investigator : Dr Jesse Gitaka

Telephone contact: 0722425613

RESEARCHERS' STATEMENT

We are asking you to participate in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether you should be in this study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear to you. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called 'informed consent.' We will give you a copy of this form for your records.

INTRODUCTION

Rapid and multiplex detection during pregnancy of bacteria that cause still births, preterm deliveries and neonatal infections can enable prompt treatment improving outcomes. There is increasing evidence that bacterial infections that are mostly subclinical contribute significantly to the inflammatory processes that underlie still births and preterm labour and jeopardise the new-born. This study aims at reducing neonatal mortality rate.

PURPOSE AND BENEFITS

We would like to come up with a novel diagnostic tool that will detect bacterial infections simultaneously in mothers. There will be additional benefits to you as a participant in this study. There will be treating of those infected and information obtained would contribute to overall improvement of neonate's health and well –being nationally.

Procedure

Once you have agreed to participate in the study, you will sign this consent form to allow us to include information obtained from you in our data. Your personal details will not be included in this questionnaire so

as to protect your privacy. We will take a small portions of your delivered placenta for the purpose of this study. We will also look at your antenatal record to obtain more information which will remain confidential. You will continue to receive appropriate management while at the hospital. We also guarantee your safety during your participation in this study. If you agree to let the researchers collect specimens, the following will happen:

- There will be no mutilation of the placenta
- Measurements will be taken with the organ intact and only small blocks will be extracted for histology
- The tissue blocks will be stored in a placental biorepository for further and future research.

Confidentiality

All the information obtained from you will be treated with utmost confidentiality. Your name will not appear on the questionnaire. A study number will be used instead.

You may choose to withdraw from the study or refuse to answer questions at any point of this study. Your decision will not affect your care at while at the hospital.

Subject's statement

I, the undersigned have been explained to and have understood the above and willingly accept to participate in the research study. I understand that participation in the study does not entail financial benefit. I have been assured that any information obtained will be treated with utmost confidentiality and my treatment will not be compromised if i decline to participate in or withdraw from the study.

I have had a chance to ask questions and if other questions arise, I can ask the researcher.

No coercion has been used to influence my decision to participate in the study whose nature, benefits and risks have been explained to me by Dr/Mr./Mrs./Ms.....

Signature/ Left thumbprint **Signature of the witness**

_____ _____

(Participant) *(Witness)*

Appendix 3: Certificate of Informed Consent

The above information has been read and explained to me. I also had the opportunity to ask questions regarding the study and I have been answered satisfactorily. I consent voluntarily to participate in this study.

Participant Name: _____ (PRINT)

Signature of Participant _____

Or

Thumb print of participant

Date _____

Statement by the principal investigator/research assistant taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the study protocol.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that this consent has been given voluntarily without any coercion.

Name of the person taking the consent _____ (PRINT)

Signature _____ Date _____

For any questions or concerns about the study contact

Dr Jesse Gitaka on 0722425613.

P.o.BOX 342-01000, Kenya.

For any questions pertaining to rights of as a research participant ,contact the secretary

Ethical review committee

P. O. Box 342-01000 Thika;

tel :0725809429.email: research@mku.ac.ke

Appendix 3: Clinical Data Form

CLINICAL DATA FORM

Rapid and Multiplex Diagnosis of Maternal Bacterial Infections

Participant Study Number: ____|____|____

Study group: ____|____

MATERNAL PROFILE

Participant Number			
ANC NUMBER			
Study Site (Health Centre Name)			
Inclusion/exclusion criteria *Patient must meet all criteria to eligible for the study	Met all ☐1.	Not met* ☐2.	
Date of Informed Consent	D D M M M Y Y Y Y		
Date of Birth	D D M M M Y Y Y Y	Or estimated age _____	
Gravida			
Parity			
Estimated Gestational Age _____weeks			
Date of Enrolment	D D M M M Y Y Y Y		
Marital status	☐1. S	☐2. M	
Education	☐1.Primary	☐2.Secondary Sch	☐9. University

Address			
Telephone			
Occupation			
Next of kin			RELATIONSHIP: _____
Next of Kin's contact/phone			

MEDICAL HISTORY

Malnutrition_____	**Diabetes_____**	**Preeclampsia_____**
HIV_____	**Malaria_____**	
Family History: Twins Y or N		

PHYSICAL EXAMINATION (First Visit)

General_____

CVS_____ Resp._____

Breasts_____

Abdomen_____

Vaginal Examination_____

Discharge/GUD_____

Weight in kgs_____ Gestation in weeks_____

Antenatal Profile

Hb_____

Blood Group_____

Rhesus_____

Serology(VDRL/RPR)_____

TB Screening

HIV:

Reactive ☐

Non reactive ☐

Not tested ☐

Urinalysis_____

Bs for Mps_____

Neonatal outcome:

Live
YES

NO

If NO; tick appropriately
Fresh stillbirth _____

Macerated stillbirth _____

APGAR score

Neonatal weight

_____ **grams**

Appendix 4: Data Collection Sheet

Data Collection Sheet

Study Number: _____

Sex: F

Maternal age ☐ years

Gestation in weeks ☐

HIV status Positive ☐ Negative ☐

CD4 count ☐

HIV viral load ☐

ART Treatment Yes ☐ No ☐

Route of delivery: Vaginal ☐ Caesarean section ☐

Areas of infarction Yes ☐ No ☐

Areas of thrombosis: Yes ☐ No ☐

Site of cord insertion: Central ☐ Eccentric ☐ Marginal ☐ Velamentous ☐

Cord diameter: mm

Cord length:_____cm

Shape of the placenta: Discoid ☐ Annular ☐ Circular ☐ horseshoe ☐

Color of the membranes and chorionic plate

Maroon ☐ Green-brown ☐ Yellow-gray ☐

Areas of calcification Yes ☐ No ☐

Cord colour; White ☐ dark brown ☐ black green ☐

Number of vessels in the cord; one ☐ two ☐ three ☐ more than three ☐

Umbilical cord hemorrhages. Yes ☐ No ☐

Weight of the placenta (gms) ☐

Diameter of the placenta (cms) in three dimensions; Greatest ☐ Major ☐ Minor ☐

Thickness of the placenta (cms); Greatest ☐ Minor ☐

Histomorphology Placenta

Central sections 1 and 2

1_____

2 _____

Peripheral sections 1, 2, 3, 4, 5, 6

1_____

2_____

3_____

4_____

5_____

6_____

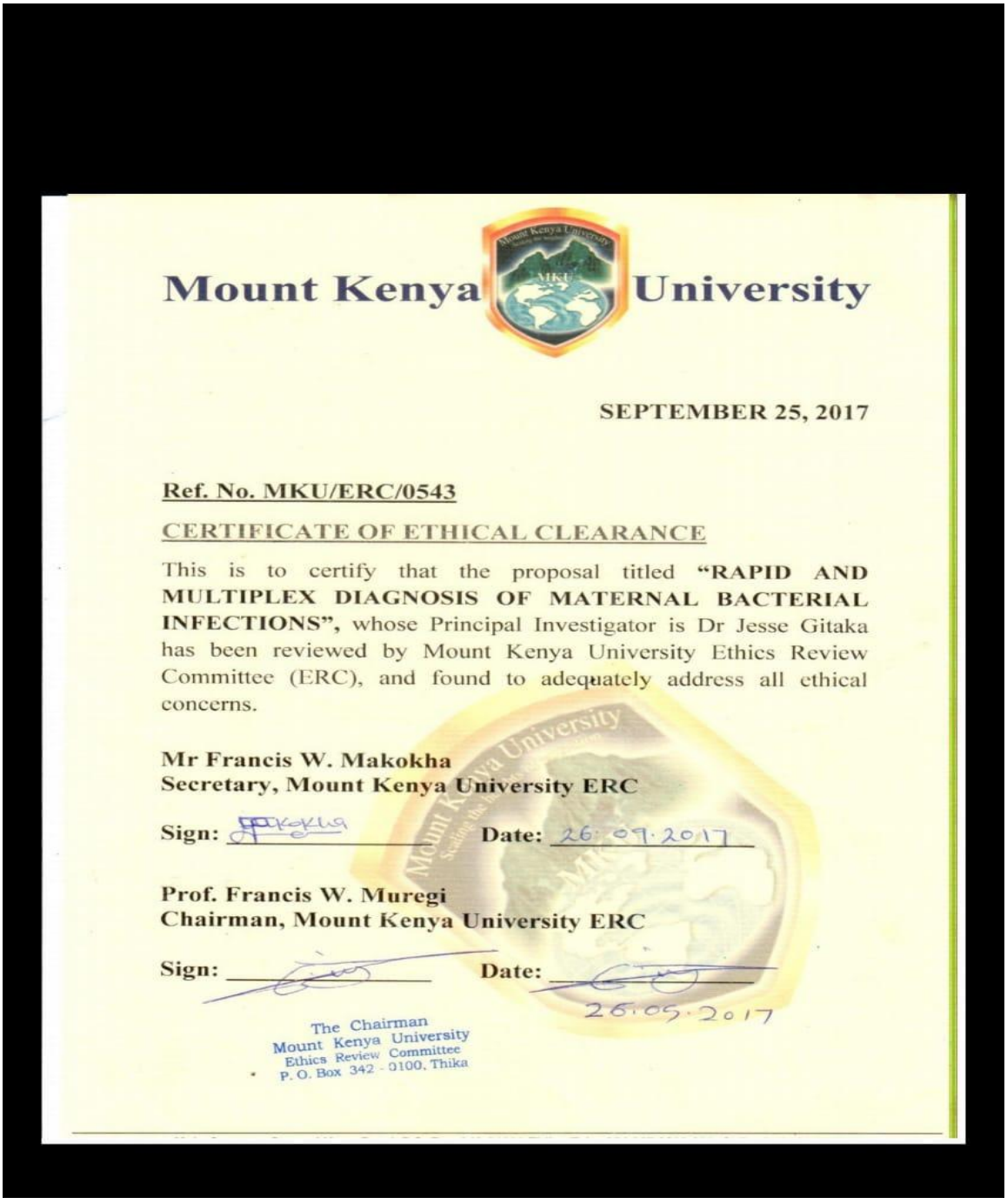
Histomorphology, umbilical cord taken in two sections

1 _____

2 _____

Results of Giemsa staining

Appendix 5: Ethics Approval Certificate



Mount Kenya University

SEPTEMBER 25, 2017

Ref. No. MKU/ERC/0543

CERTIFICATE OF ETHICAL CLEARANCE

This is to certify that the proposal titled **"RAPID AND MULTIPLEX DIAGNOSIS OF MATERNAL BACTERIAL INFECTIONS",** whose Principal Investigator is Dr Jesse Gitaka has been reviewed by Mount Kenya University Ethics Review Committee (ERC), and found to adequately address all ethical concerns.

Mr Francis W. Makokha
Secretary, Mount Kenya University ERC

Sign: _____ **Date:** 26.09.2017

Prof. Francis W. Muregi
Chairman, Mount Kenya University ERC

Sign: _____ **Date:** 26.09.2017

The Chairman
Mount Kenya University
Ethics Review Committee
P. O. Box 342 - 0100, Thika

Appendix 6 Authorization letter to use bio bank

Mount Kenya University

TO:
KNH-UoN ERC
Email: uonknh_erc@uonbi.ac

RE: CONSENT TO THE USE OF BIOBANKED PLACENTA SPECIMENS ACQUIRED FOR *"RAPID AND MULTIPLEX DIAGNOSIS OF MATERNAL BACTERIAL INFECTION"* PROJECT (REFERENCE NUMBER: MKU/ERC/0543)

We make reference to the above matter.

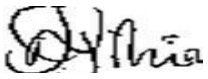
I , **Dr. Jesse Gitaka** , the Principal Investigator of the above named study do give my consent to the use of the Biobanked Placenta Specimen to the following investigators in the University of Nairobi Obstetrics and Gynecology Department:-

INVESTIGATOR'S NAME	**COURSE**
Dr. Consolata Wangeci Kihagi;	Comparison of placental microbiome in women with undernutrition and those with normal nutritional state at Bungoma County Referral Hospital.
Dr. Yusuf Adam Khalil;	Placental histological changes in preterm births with placental malaria and HIV coinfection.
Dr. Everett Lamulungi;	Structural differences in placentas of women with malaria-preeclampsia comorbidity in healthy pregnancies
Dr. John Kamau Mwangi;	The vaginal microbiome of women with preterm births versus women with term births who attended ANC at Thika Level 5 County Referral Hospital between January 2019 and March 2019
Dr. Stephen Lutukayi Marumbu	Comparison of placental morphology and perinatal outcomes in women with and without GDM among low

	income rural population in Kenya.
Dr. Maero Diogracious Moses	Comparison of placental structure in pregnant women with undernutrition and those with normal nutrition delivering at Bungoma County Referral Hospital.

Kindly accord them the necessary assistance
Thank you in Advance.

Yours Faithfully;



……
Dr. Jesse Gitaka, MD, MTM, PhD