COMPARISON OF PLACENTAL STRUCTURE IN PREGNANT WOMEN WITH UNDERNUTRITION AND THOSE WITH NORMAL NUTRITION DELIVERED AT BUNGOMA COUNTY REFERRAL HOSPITAL BETWEEN JANUARY 2018 AND DECEMBER 2019

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I do declare that this research is to be undertaken in part fulfilment of the Master of Medicine in Obstetrics and Gynaecology from the University of Nairobi and is my original work and has not been undertaken and presented for a degree in any other university.

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This is to certify that this dissertation is the original work of Dr. Maero M.O. Deogracious an M. Med student in the Department of Obstetrics and Gynaecology, College of Health Sciences, University of Nairobi, under the guidance and supervision of Prof. E. Cheserem, Prof. Moses Obimbo, and Prof Omondi Ogutu. This is to confirm that this dissertation has not been presented in the University for the award of any other degree.

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Table of Contents

LIST OF ABBREVIATIONS

ACOG - American college of obstetricians and gynaecologists

APGAR - Appearance Pulse Grimace Activity Respiration

BCRH - Bungoma County Referral Hospital

BMI- Body Mass Index

ERC- Ethical review committee

FGR – Foetal Growth Retardation

G.A- Gestational Age

KNH - Kenyatta National Hospital

MUAC- Mid Upper Arm Circumference

MUN- Maternal Undernutrition

UON - University of Nairobi

WHO - World Health Organization

OPERATIONAL DEFINITIONS OF TERMS

- **Undernutrition:** Lack of adequate nutrition, induced by lack of sufficient food intake and or by not consuming sufficient food containing elements that are essential for ideal growth and health.
- **Gravid:** Pregnancy
- **Perinatal:** Relating to the time, usually a number of weeks, immediately before and 7 days after birth.
- **Neonatal:** Relating to new-born in the first one month after birth
- **Post-partum:** The period following childbirth
- **Anaemia in pregnancy:** Defined as Hb < 11 g/dl   Ref source WHO
- **Neonatal Haematocrit:** Range of normal between 45-65% of the ratio of red blood cells to the total volume

LIST OF TABLES AND FIGURES

ABSTRACT

Background: The prevalence of maternal, fetal and newborn morbidity and mortality associated with undernutrition is high in the lower and middle income countries. A significant number of the pregnant population in Africa is suffering from malnutrition, above 10% of the standard acceptable malnutrition rate. Pregnancy solely depends on the health and nutrition status of the pregnant woman. Poor access to food due to poverty, poor infrastructure and political instability leads pregnant women to poor health and associated complications such as foetal growth restriction due to the associated factors such as fetal anaemia. Even though the relation between undernutrition in the pregnant woman and developing fetus has been extensively correlated, its effect on the placenta has not been widely studied, more so in our setting. Determining the effect of undernutrition on the placenta requires an extensive study to improve both the pregnant woman's health and health of the developing fetus.

Objective: To compare placental structure in pregnant women with undernutrition and those with normal nutrition who were delivered at Bungoma county Referral hospital.

Methodology: This was a comparative cross-sectional laboratory-based study. The study was done at the Basic, Clinical and Translational (BCT) research laboratory located in the Department of Human Anatomy, University of Nairobi. We utilized placenta samples from the placenta biorepository at the Basic, Clinical and Translational (BCT) research laboratory located in the Department of Human Anatomy, University of Nairobi.

A gross appearance including obvious colour change, shape, diameter, weight, umbilical cord insertion thrombosis and infarction of the placentae of women who meet the inclusion criteria were noted. Sectioned blocks of placentae collected from 6 different regions in the placentae were analysed histologically for changes in the structural appearance of terminal villous and basal plate. A morphometric analysis of the placental terminal villi was done, and the results compared to findings in normal placentae.

Data management and analysis

A team of three comprising of the PI, an anatomist and a pathologist examined the placental blocks to minimize on bias. They were blinded to the disease status of the placenta and captured images of the placenta was stored for future reference. All infection control protocols were adhered to throughout the study procedure. This included aseptic technique for all invasive medical procedures, hand hygiene and use of personal protective equipment for all medical procedures.

Clinical, Histomorphologic and Histomorphometric data was coded and entered into SPSS software (Version 25.0, Chicago Illinois) for statistical analysis. The scale variables were expressed in means ± standard deviation. Normality of the data was assessed using the Shapiro-Wilk test and visual inspection of the histograms, box plots and normal Q-Q plots generated from the data. The data displayed skewed distributions. Thus, non-parametric tests were employed. Mann Whitney U tests were carried out to assess for significant differences in the numerical variables between the controls and cases. Chi square tests were used to assess for significant associations between the categorical data and the nutritional status. A p value of ≤ 0.05 was considered significant. Photomicrographs were used to demonstrate the histological findings. Tables and graphs were used to illustrate the measurements from the control and case groups.

Utility of the Study: A study on the structural placental changes would help identify causes of poor neonatal outcome caused by undernutrition and guide on management of future pregnancies with undernutrition by assisting in the future development of markers which will identify foetuses at risk of poor outcomes due to malnutrition and allow for prompt intervention.

This study findings will contribute to the local knowledge base on maternal and child health, and on the importance of balanced nutrition in pregnant women, and the foetus.

1.0 INTRODUCTION

Nutritious and balanced diet is essential for human growth and health but most importantly for pregnant women. Expectant mothers as well as breast feeding mothers require enough nutrients for the proper development and growth of the foetus. The energy and nutrients should be sufficient to cater for the mother and the foetus and go further to lactation period. (1) (2)

It has been postulated that the placenta is the human bank of knowledge-Chief Executive officer - in understanding many of the foetal pathologies. Various studies have directly pointed to the placenta as the gate way to the insult directed to the foetus either directly or indirectly. According to some studies, small gestational age babies are at risk later in life of developing some cardiovascular diseases, diabetes and hypertension, due to the alteration in placental growth. The effect of undernutrition to pregnant women and developing foetus has been well studied but only a few have studied the placenta as the link between the two. This study seeks to investigate the macroscopic pathological placental changes in placentas of pregnant women with undernutrition.

This study also seeks to evaluate the microscopic changes in the placenta of the same women with undernutrition. L Belkacemi et al in a study titled “Maternal Undernutrition Influences Placental-Foetal Development” described that undernutrition induces changes to the placental surface, the width of the surface, and the makeup of the cells of the various gestational ages that impact the ability of placental transport. Noticeably, they result in human foetal growth restriction (FGR), following diminished placental surface area and lower trophoblast volume density (4,5). In guinea pigs subjected to undernourishment, these structural alterations in the placenta are found compared with control diets (3). Such placental structural alternation can be observed in guinea pigs that has been exposed to undernutrition than compared to a control experiment using controlled diet.

Numerous animal experiments have also shown the relation between maternal malnutrition and abnormalities in development of various organs including the placenta. Undernutrition especially in early gestation has been associated with abnormalities in embryogenesis leading to congenital malformation and incorrect placentation resulting in fetal growth restriction (4).

The placenta is an important interface between the mother and the growing foetus both as a conduit for nutrients and oxygen, in this regard a healthy functioning placenta is paramount for development of a healthy foetus. Many studies done both recently and, in the past, have linked the status of the placenta with that of the mother. The weight of placenta is correlated to the intake of varied diet during pregnancy in mammalian. However, undernutrition effects is definite, the mass of the placenta can still be affected by the duration, timing and aetiology of nutritional restrictions (3) (5–7). This study aims to evaluate the effects of undernutrition, both macroscopic and microscopic, to the human placenta and seek answers to the question, what are the structural changes in placenta of women with undernutrition in pregnancy at Bungoma county referral hospital.

Childhood survival as well as that of the pregnant mother can be affected by malnutrition thus leading to adverse consequences in their life. The major risk factor for transgressional obesity has been discovered to be parental obesity for a long time. The cause of a good percentage of maternal death can be traced back to different kinds of complication during pregnancy or immediately after birth. Such complication includes unsafe abortion, pregnancy and child birth malnutrition, and obstetric complication like excess bleeding, obstructed labor and more (8–10). Poor pregnancy outcome is mostly contributed to by malnutrition which can lead to postpartum haemorrhage, premature birth, low birth weight and obstructed labour. (11, 12) Taking these findings into account, it has been shown that complex patterns of fetal and placental development have a significantly increased risk of developing increased levels of identified risk factors for heart disease during middle adulthood (13).

This study sought to compare placentas of healthy mothers with those of women with undernutrition.

2.0 LITERATURE REVIEW

In placental-foetal development and growth, an adequate parental nutrient supply does have a critical function. During pregnancy, maternal inadequate feeding contributes to placental diseases and foetal growth restriction (FGR) and low-birth-weight new-borns. The constraint of intrauterine growth is related to increased maternal morbidity and mortality. The adverse effects of maternal feeding have usually concentrated on the decreased availability of maternal nutrition to the foetus.

During pregnancy, the placenta usually undergoes both physiological and anatomical changes regulated by several factors including hormonal factors to increase and maximize its potential function and meet the demand of the developing uterus. Any insult to the placenta including maternal undernutrition will seriously interfere with the growth of the placenta and in turn the developing foetus.

A pregnant or lactating woman is considered to be underweight when her mid upper arm circumference (MUAC) reading is less than 23.5cm or has a body mass index (BMI) below 18.5. A woman at pre-conception is considered underweight when the MUAC reading is less than 21cm (14).

2.1 Background

During pregnancy a woman needs good nutritional status for a healthy outcome. This includes adequate proportional intake of both micronutrient and macronutrient. The micronutrient are inclusive of folic acid, iron, iodine and calcium while macronutrients include proteins, carbohydrates and fats (14, 15). The nutritional status of a woman before conception is a key determinant of the pregnancy outcome and the health of the new-born. Adolescent girls and women need to attain appropriate nutritional status in order to prepare them to meet the future needs of pregnancy for both the mother and unborn child (14).

Women at risk of malnutrition and its complications are those who were obese at the time of conception, who gain too little or too much weight, whose height is less than 145cm, those with MUAC less than 23.5 cm, pregnant adolescents (younger than 18 years of age), those with short birth intervals (less than 1 year), with too-early pregnancies below 18 years of age, with too-late pregnancies above 40 years of age, with a history of low birth weight infants (less than 2500gm), who are HIV positive/ have AIDS, those living in poor socio-economic situations and those in emergency situations like famine, wars, civil strife and other hazards both manmade and natural (10, 14, 16).

Maternal undernutrition is highly prevalent in resource-poor settings, generally ranging from 10% to 19%, but reaching up to more than 20% in some areas, such as in Sub-Saharan Africa, South-Central and South-Eastern Asia, and Yemen (15).

Maternal general condition has been shown to have a direct or indirect effect to the general condition of the placenta. Placentas of women with chronic conditions after delivery show pathological signs of the disease. (3, 17–19) In a study looking at prevalence of placental pathology in low-birth-weight infants by Kleebka et al., a descriptive study of 114 placentas from infants weighing between 500 and 2,499 grams delivered between June 2002 and June 2004 at Srinagarind Hospital, in Thailand, multiple causes of foetal growth restriction were reported, one of which was placental abnormalities such as infarction (19). In order to minimize pregnancy complications and to avoid potential diseases in postnatal life, a healthy maternal diet has a significant role. (5) The Barker Theory indicates that adult infectious conditions are linked to foetal programming, which will have a lasting impact on the structure and physiology of the human body by any stimulation or insult throughout foetal development. (5, 9, 20, 21)

There are heightened nutrient needs during pregnancy and lactation. Without an increase in energy and other nutrient intake to meet the increased needs during this time, the woman's body uses its own reserves, leaving her weakened and vulnerable to pregnancy-related complications. (14) Globally about 468 million women aged 15 to 49 years are thought to be anaemic, at least half because of iron deficiency. The highest proportions of these anaemic women live in Africa (48% to 57%) (22)

Maternal undernutrition does not only start during pregnancy. A 2010 guideline on maternal nutrition in Uganda noted that many girls are undernourished at birth, stunted during childhood, become pregnant during adolescence, are underfed as well as overworked during pregnancy and lactation, and, consequently, give birth to low birth weight babies. It is these children who eventually become stunted women, perpetuating the intergeneration cycle of malnutrition among women. (14, 23)

Undernutrition weakens a woman's ability to survive childbirth and give birth to a healthy baby, translating into increased morbidity and mortality of mothers and their infants. (14) (20). Two results of pregnancy which are closely associated with poor maternal nutrition are peri-natal deaths and low birth-weight infants. (23) (20, 24, 25)

Anaemia and iron deficiency, which are associated with a lower physical capacity and increased susceptibility to infections, need to be tackled before women become pregnant in order to reduce the risks of poor maternal health and low birth weight babies. (14, 16, 22, 23)

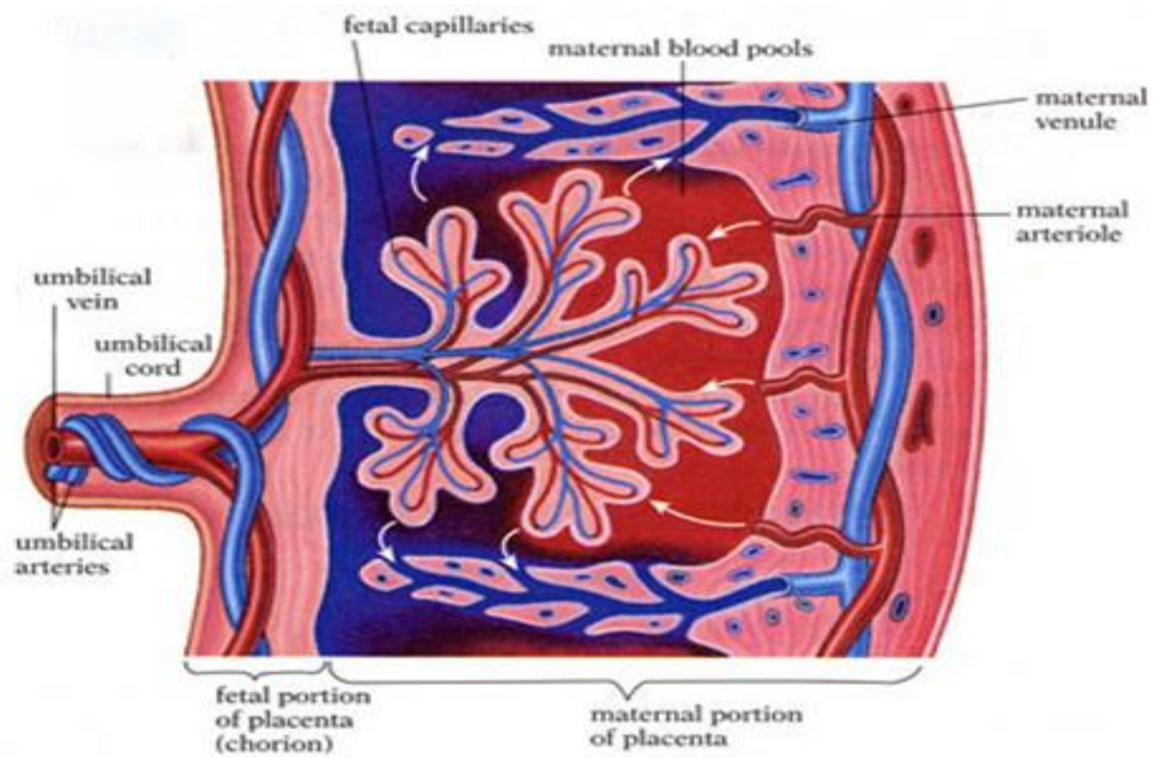
One of the obstacles to the provision of improved maternal nutrition health services is lack of comprehensive reference nutrition recommendations for health service providers to use in providing nutrition counselling to women on how to meet their nutritional requirements through dietary and behavioural changes. (22) This calls for a need of local data to support the same.

Even though few studies have shown an association of placental pathologies with undernutrition including thrombosis and infarction of the placenta, there is need for more studies to strongly correlate this phenomenon. Yet, no local data is available to support the same and therefore creating a need for local interrogation of the same.

2.2 Placental Structure and Function

The normal term placenta is a circular to discoid shaped structure with approximate diameter of 22 cm, a central thickness of 2.5 cm, and weight of 470 g. It consists of foetal surface, maternal surface and intervening placental parenchyma. The foetal surface is made of chorion covered by amnion while the maternal surface is made of a basal plate which has flat grooves and deep clefts subdividing it into placental lobes (See Figure 1 below).

Figure 1: Placental Lobes



Placental Lobes (Bernischke et al.,2000) (26)

The placenta is a vital and highly effective organ with several important functions including acting as an interface and coordinating signals between mother and foetus to cater for foetal demand for supply of nutrients and gases. It also acts as an important conduit for excretion of waste products from the foetus to the mother. Disruption of the function and structure of the placenta inclusive of weight, morphology, vascular development, and transport of important substrates will contribute to altered nutrient supply.

2.3 Macroscopic and Microscopic changes of the Placenta in Undernutrition

Undernutrition has been casually linked to placental structural changes like shape, weight, infarcts, and colour that lead to detrimental foetal complications. In a study done by L. Belkacemi, et al, (3) they found placentas of sheep with undernutrition being heavier than those of healthy sheep. In their research they found that there is increased placental weight: foetal weight ratio increased malnutrition during early to mid-pregnancy in sheep by without alteration at term. This means when nutrient deficiency correlates with the time when foetal nutrient demand is maximum, MUN irrevocably affects ovarian weight. This indicates that when nutrient deficiency correlates with the period when foetal nutritional demand is maximum, MUN irrevocably influences placental weight.(3)

Study done by K. Godfrey, et al., in 1995 at Southampton general hospital in the UK, a cross sectional study on maternal nutrition in relation to foetal and placental growth did experimental studies in sheep that linked maternal undernutrition in mid pregnancy with profound detrimental effects on placental and effectively to foetal growth. They observed that if subjected to a further duration of malnutrition around pregnancy, sheep approaching pregnancy with reduced nutrient stores experienced pronounced impairment of foetal and placental growth and then had duration of dietary restriction while pregnant women experienced decidual hypertrophy including epithelial degradation and responsive changes. These same findings were reported in a study of rodents and ruminants with undernutrition by Zhang et al., in a descriptive study done in Australia, 2015, titled placental adaptations in growth restriction (13) (27, 28)

It was also found that MUN creates placentas that have a decreased villus contact surface and have a reduced trophoblast mass and volume which contributes in individual FGR. (6) (20,24) Such modifications in the placenta have also been confirmed in the guinea pig that is exposed to MUN compared to healthy ones. Nutrition-deprived sheep displayed placentas with exchange surface that reduced by up to 70 percent resulting in reduced labyrinth development, while membrane density increased by 40 percent in subsequent pregnancies.

The effect of maternal nutritious wellbeing to the placenta was also evidenced in studies where placentas of healthy mothers were studied for any pathology and none was reported. As a matter of fact, nutritional supplementation was observed to have an increase in placental weight, foetal weight and a net reduction in foetal mortality. (13)

Based on the findings, the same results are expected in this analysis for variations in the exchange surface region, barrier density, variations in placental cell structure, mass and endothelial influences placental transport ability and thus influencing the foetal development.

Many of these studies have been done on animal models and only few on the human placenta. Recent research suggests that placental hypertrophy can also occur in humans in response to undernutrition pointed out some of these observations. They claim one of the very few variables known to be associated with high placental weight is severe maternal anaemia. They studied obstetric data of women born in Oxford in order to assess if maternal anaemia could be associated with placental hypertrophy. For each g/dl drop in maternal minimum haemoglobin during pregnancy, they observed that placental weight steadily increased by 18.0 g. (1, 13, 29, 30) the results were inconclusive, and this calls for a need for more data to give a strong association.

2.4 Placental Adaptation to Undernutrition

Contrary to the findings that link maternal undernutrition to adverse placental structural changes, some findings report the reverse. Data have linked an adaptive response of the placenta in terms of increase in size and weight to increase its potential and therefore cushioning the developing foetus. (3) New research has postulated that placental overgrowth typically compensates for early pregnancy undernourishment in order to retain normal foetal weight in earlier pregnancy, but such mitigation is inadequate to preserve later gestational foetal growth. (31)

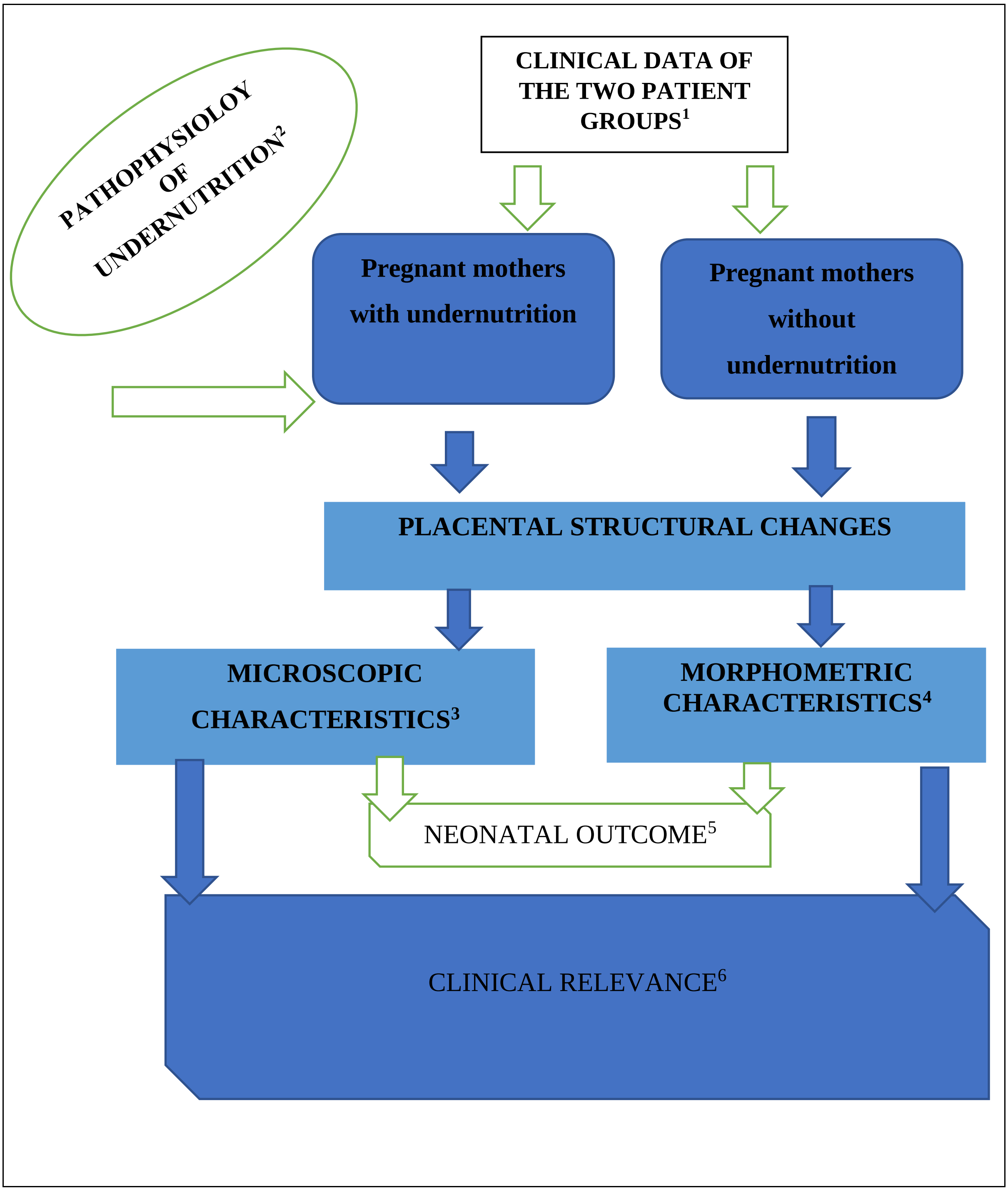
The effect of undernutrition has been shown to be more during the second half of pregnancy as compared to the first half squarely because foetal growth increases exponentially in the later gestation and therefore in favour of other factors like gestation being import confounding factors rather than undernutrition as a standalone cause of the structural changes. (31)

Instead of malnutrition, some individuals have claimed that deficiency of a specified nutrient portion is the insult that causes the shift in placenta and foetus growth trajectories. A crucial component in the nutritional alteration of placental weight has indeed been confirmed to be particular protein insufficiency: foetal weight ratios and that protein restriction in the diet of 9% vs. 18% casein in the rat through gestation developed heavier placentas and decreased foetal growth in late gestation. (2, 28, 32)

Cohesively, these data indicate that a placenta adaptive reaction increases the transition of proteins from maternal to foetus, enhance the stability of the use of substrates, or both when nutritional supply is reduced.

3.0 CONCEPTUAL FRAMEWORK

Figure 2: Conceptual Framework



1.**Clinical data include**: Age, Weight, Height, BMI, MUAC, 24 hours prior diet history, GA, Comorbidities.
2. **Pathophysiology of undernutrition include:** Insufficient intake of calories and nutrients (primary cause) and decreased food intake, nutritional deficiencies, poor use of nutrients, and loss of nutrients due to chronic infection and other clinical conditions (Secondary causes)
3**. Microscopic characteristics include**: Villous degeneration, Syncytial knotting, Vascularity, Adhesion of red blood cells to terminal villi, Deciduitis and Villitis, Syncytiotrophoblast delamination, amnion, chorion, and placenta overall structure
4. **Morphometric characteristics include**: Diameter of the villous terminal, surface area of the villous terminal and number of foetal capillaries in the villous stromal centre
5. **Neonatal outcome include**: Apgar score and foetal weight
6. **Clinical relevance include**: Identify markers of poor neonatal outcome, Management of future pregnancies, Lay ground for further research

4.0 JUSTIFICATION

The placenta is the most available tissue, yet it is the organ that is the least extensively examined. There are many explanations why knowledge of placental pathology can help in understanding infants who have defects or neonatal disorders more materially than infants from mothers who have undernourishment. A careful analysis of the placenta at birth will provide a thorough perspective into foetal life and its future complications than is possible from the patient's background notes. (28) The significance of a better understanding of the relationship between maternal nutrition, placental development and harmful foetal consequences has been illustrated by recent research.(13) (19).

A cross-sectional study by Gelebo et al., 2021 on undernutrition among pregnant women in rural communities in Southern Ethiopia found that, there is an immense occurrence of maternal and infant problems, as well as a correlation of mortality with anaemia and malnutrition (1). Pre-existing diseases, such as malnutrition and anaemia, are more prevalent and responsible for 28 percent of the most important causes of mortality and morbidity. There is no local evidence that examines the impact of deprivation on the composition of the placenta. Maternal undernutrition has been proven to directly and indirectly affect the Fetus. Its effect to the placenta has not been thoroughly investigated though.

A study on the structural placental changes would help identify causes of poor neonatal outcome caused by undernutrition and guide on management of future pregnancies with undernutrition by assisting in the future development of markers which will indicate foetuses at risk of poor outcomes due to malnutrition and allow for prompt intervention.

This study will contribute to the local knowledge base on maternal and child health, and on the importance of balanced nutrition in pregnant women, and in turn to the developing baby.

4.1 Research Question

Are there differences in the placental structure of pregnant women with undernutrition compared to those with normal nutrition delivered at Bungoma County Hospital between Jan 2018 to Dec 2019?

4.2 Study Objectives

4.2.1 Broad Objective

To compare placental structure in pregnant women with undernutrition and those with normal nutrition delivered at Bungoma county Referral hospital between Jan 2018 to Dec 2019.

4.2.2 Specific Objectives `

Among patients with normal and undernutrition who delivered at Bungoma Hospital

1. To compare clinical data of the two patient groups
2. To describe the histomorphological structure of the basal plate, villous and the chorionic plate.
3. To describe the histomorphometry of the terminal villous

5.0 RESEARCH METHODOLOGY

5.1 Study Design

This was a case control laboratory-based study. In this study, the exposure (Undernutrition or absence of it) and outcome (the structure of the placenta) were measured at the same time. Although placental samples were collected in a retrospective timing, the exposure and outcome were measured simultaneously. Further, other clinical data and factors that may predispose the mothers to alterations in placental structure were also collected retrospectively.

5.2 Study Location

The study was done at the Basic, Clinical and Translational (BCT) research laboratory located in the Department of Human Anatomy, University of Nairobi. The BCT laboratory is a new laboratory in the Department of Human Anatomy run by one of my mentors on this study. The laboratory focusses on Human and Murine placenta, endometrial and other reproductive structures biology. It has a capacity of 10 scientists. The placenta specimens were obtained from Bungoma County Referral Hospital in Bungoma County, Kenya. Formerly Bungoma District Hospital is situated about 400km west of Nairobi with a 216-bed capacity. Its main catchment area is the immediate town and the larger rural area surrounding the town.

5.3 Study Population

Placenta samples from the placenta biorepository at the Basic, Clinical and Translational (BCT) research laboratory located in the Department of Human Anatomy, University of Nairobi were utilised. The placentae were obtained from pregnant women with undernutrition and those with normal nutrition who delivered at Bungoma County Referral Hospital between Jan 2018 and Dec 2019. The diagnosis of undernutrition was made in those pregnant women who had a MUAC of less than 22, a BMI of less than 19.8 and a 24 hour prior to labour diet history that is suggestive of inappropriate and inadequate nutrition. The Age, Weight, Height, BMI, MUAC, GA and history of Comorbidities were captured and recorded.

The placenta specimens that were collected for this purpose met the following

Inclusion criteria:

Bio-banked placentae specimen of women;

1. Age above 18 years
2. A singleton pregnancy
3. Complete medical record
4. Bio-banked specimen for which consent was obtained for their use

Exclusion criteria included:

Bio-banked placentae specimen of women;

1. Age below 18 years
2. Concomitant medical conditions in pregnancy
3. Multiple gestation
4. Bio-banked specimens that were not adequately processed e.g., not fully embedded in wax and difficultly in sectioning because of brittleness
5. Blocks without labels

5.4 Sample Size

In this study we shall compare the morphology of placenta of women with undernutrition and those with normal nutrition. We shall employ the Kelsey et al 1996 formula: To calculate the sample size, we use a local study by Obimbo et al (26) titled, "Placental structure in preterm birth among HIV-positive versus HIV-negative women in Kenya." One of the study's finding was that syncytiotrophoblast delamination was significantly different in HIV-positive versus HIV-negative placentae (10/22 (46%) vs. 2/22 (9%); P = 0.006). This was for a subset of preterm placentae (n = 22/group). We used the percentages as the proportion for the exposed and non-exposed respectively to calculate the sample size.

$$n = \frac{(Z\alpha + Z\beta)^2 p(1-p)(r+1)}{r(p1-p2)}$$

Two-sided significant level 1-alpha =95 (0.05)

Power (1-beta) % of detecting difference =80 (0.8)

Ratio r of sample size unexposed/exposed =1

Proportion of disease in population 1 P1 (cases) =0.46

Proportion of disease in population 2 P2 (control) =0.09

Sample size with above formula is 22 placentae

With a 10% increment=24 placentae for each arm and a total of 48 placentae

5.5 Study Procedures

5.5.1 Recruitment, Consenting and Data Collection

There was no recruitment in this study. 48 placentae specimens, 24 from pregnancies with undernutrition and 24 from those with normal nutrition in keeping with the inclusion/exclusion criteria was used.

The placental samples for histology obtained through a standard protocol used by the Anatomical Pathology studies, membrane roll, and 6 sections of parenchyma were picked out for examination from the placental lab. They were then processed as described below.

5.5.2 Clinical Data

Maternal and peripartum clinical data for the recruited placentae samples were collected including participant number, ANC number, Age, gestational age, MUAC, height, weight, diet history, medical history and neonatal outcome inclusive of appearance, pulse, grimace, pulse and respiration (Apgar) score and foetal weight.

5.5.3 Light Microscopy

For microscopic examination six biopsies (numbered) were collected as follows; two central blocks from either side of the cord insertion and four from the periphery at 3, 6, 9 and 12. For this study, two bio-banked placenta samples were analysed, one central and one peripheral. Those latter blocks were established as follows; 10% formalin was fixed with neutral buffering, then membranes were separated and formed into rolls. In increasing alcohol concentrations (from 70 percent to 100 percent) one hour per concentration, the rolls and placental biopsies were then dehydrated. They then cleared for 2 hours with trichloroethane and infiltrated for 12 hours with liquid paraffin. The sample blocks were finally be embedded for 12 hours in molten paraffin (wax). By using Leitz Wetzlar sled microtome for inspection, five-micrometre serial parts of the blocks were cut, submerged in warm water, put on a glass slide and then dried at room temperature in a 40 degrees Celsius hot air oven. The parts were then stained with haematoxylin and eosin to show histomorphometry, Masson's trichome to highlight the components of the connective tissue, and finally to stain the collagen fibres with picrosirus red. These specimens were then studied underneath a light microscope from Leica Automated Systems which are connected to a monitor and computer.

5.5.4 Morphometry of the Placental Villi

The morphometry of the terminal villi was studied. The terminal villi were identified using these criteria; a) flattened trophoblastic lining, b) lack of muscularized arterioles and c) large foetal capillary occupying more than 50% of the cross-sectional area.

These features were analysed at a magnification of x400 using Fiji image J Software for morphometric measurements of the terminal villi while STEPanizer © stereology tool, Version 1.0. was used to obtain surface densities.

5.5.4.1 Number of Capillaries per Terminal Villi

The images taken at x400 magnification were opened in Fiji ImageJ software whereby 5 terminal villi were identified. The number of capillaries in each terminal villus was counted and recorded. The number of capillaries per terminal villus was taken as the average of the 5 values recorded.

5.5.4.2 Diameter and Perimeter of the Terminal Villi

The images taken at x400 magnification were opened in Fiji ImageJ software. For each of the 5 terminal villi selected above, the diameter and perimeter in µm were taken. The perimeter was taken using the Freehand Line Tool that was used to trace a line around the periphery of each terminal villus. The length of this line was then recorded. The diameter was taken by taking the average of the diameters taken using the Straight-Line Tool at the 2 widest points of each villus. The average diameter and perimeter of the terminal villi was taken as an average of the 5 values recorded. The determination of the diameter and perimeter is shown in *Figure 1* below.

Figure 1: Estimation of the diameter (dotted lines) and perimeter (yellow continuous line) of a terminal villus

5.5.4.3 Surface Density of the Terminal Villi

Surface densities were evaluated using 64 test lines on STEPanizer © stereology tool, Version 1.0 applying the formula:

$Sv = 2 \times I/LT$

Where Sv: surface density, I: number of intersections between test lines and surface of interest (peripheral villi), LT: total length of the test system (Tschanz et al., 2011). This is illustrated in *Figure 2* below:

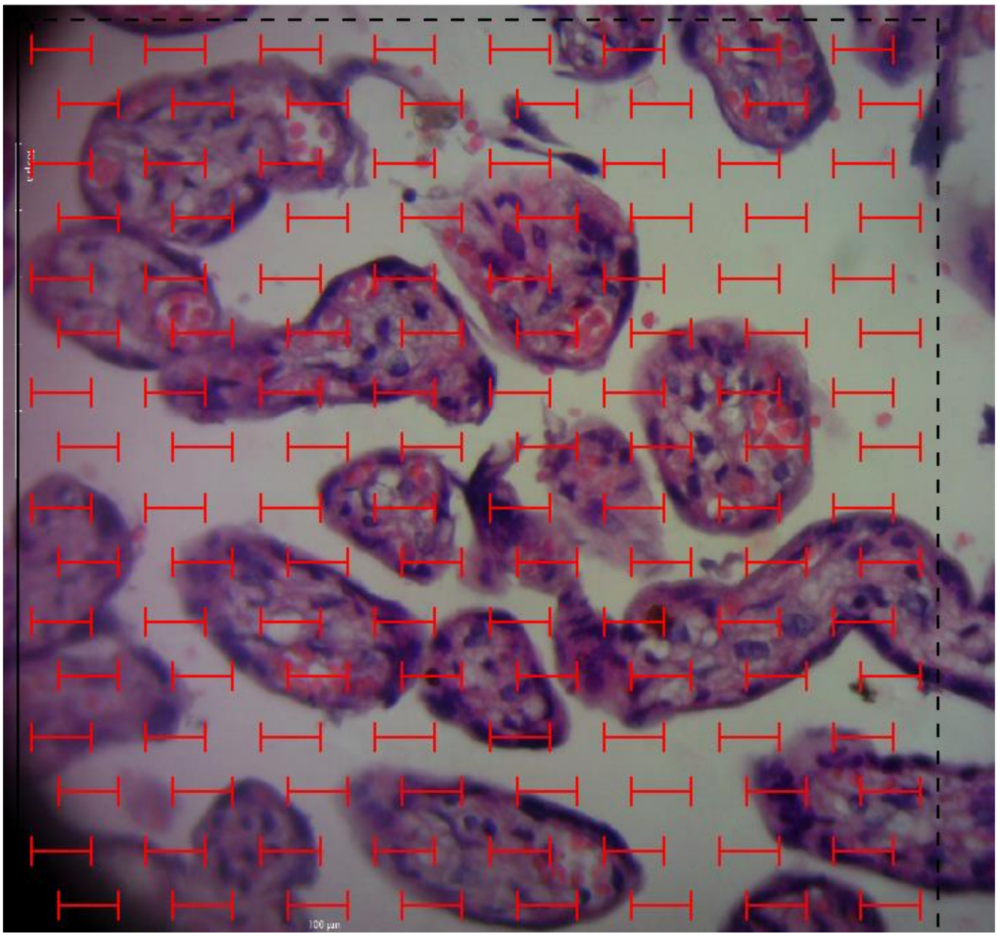


Figure 2: Estimation of surface density of the terminal villi

5.6 Data Variables

SPECIFIC OBJECTIVES	EXPOSURE VARIABLE	OUTCOME VARIABLE	SOURCE OF DATA
Clinical data	Age Weight Height BMI Gestational age Comorbidities 24hr prior diet history	1.Mother with undernutrition 2.Healthy mothers	Available data base
To describe changes of the Basal plate, villous, and chorionic plate	Delayed Villous Maturity Accelerated Villous Maturity Distal Villous Hypoplasia Villous edema Villous Necrosis Syncytial Knots Increased or decreased syncytial knots for gestational age Thickening of Villous Membrane	1.Mother with undernutrition 2.Healthy mothers	Microscopic examination of the serial 5-µm slices

	Villous stromal fibrosis Villitis Intervillositis Fibrin deposition Fetal Inflammatory Response		
To describe the morphometric changes of the terminal villous	Morphometric features. The diameter and perimeter of the terminal villous The surface density of terminal villous Number of foetal capillaries in the terminal villous	1.Mother with undernutrition 2.Healthy mothers	Placenta blocks prepared for morphometric analysis

5.7 Data Collection, Management and Analysis

5.7. 1 Data Collection

The equipment used was obtained from the Basic, Clinical and Translational (BCT) research laboratory located at the Department of Human Anatomy, University of Nairobi. The data on histomorphometry and morphometric features was recorded by the investigator on specifically designed data entry forms. Additionally, photos of the histological feature were taken and stored. Numerical data was collected in excel sheets. All data were stored in an external hard disk in a password protected folders that was in the custody of the Principal investigator. Back up storage of data was arranged in another external hard disk.

5.7.2 Data Management/ Laboratory Procedures

5.7.2.1 Quality Control

A team comprised of three comprising of the PI, an anatomist and a pathologist examined the placental blocks to minimize on bias. They were blinded to the disease status of the placenta. Captured images of the placenta were stored for future reference.

5.7.2.2 Infection Control

All infection control protocols were adhered to throughout the study procedure. This included aseptic technique for all invasive medical procedures, hand hygiene and use of personal protective equipment for all medical procedures.

5.8 Data Analysis

Clinical, histomorphology and Histomorphometric data was coded and entered into SPSS software (Version 25.0, Chicago Illinois) for statistical analysis. The scale variables were expressed in means ± standard deviation. Normality of the data was assessed using the Shapiro-Wilk test and visual inspection of the histograms, box plots and normal Q-Q plots generated from the data. The data displayed skewed distributions. Thus, non-parametric tests were employed. Mann Whitney U tests were carried out to assess for significant differences in the numerical variables between the controls and cases. Chi square tests were used to assess for significant associations between the categorical data and the nutritional status. A p value of ≤ 0.05 was considered significant. Photomicrographs were used to demonstrate the histological findings. Tables were used to illustrate the measurements from the control and case groups.

5.9 Ethical considerations

a. Ethical Review

The Ethical approval for the primary collection of the samples were granted by the Mount Kenya University, Ethical and review Committee to Dr. Jesse Gitaka and Dr. Obimbo Moses, the PI and CO-investigator respectively. Permission to analyse the samples was sought from the Kenyatta National Hospital/University of Nairobi Ethical Review Committee (KNH/UoN ERC).

b. Human subject involvement and characteristics of the study population

While the bio-banked placenta samples are not Human subjects, they represented the study population in this research. The placenta specimen for this study were collected for another placenta study and transported to the Basic Clinical and Translational Laboratory at Chiromo Campus. This study population represent the Catchment areas of Bungoma County and Referral Hospital (BCRH) who are drawn from Bungoma, Kakamega, Busia and Trans-Nzoia Counties.

c. Potential risks

Since it was a sample-based analysis there was no risks to the patients

d. Confidentiality

Information collected was handled with Belmont’s principles of confidentiality (Respect for persons, Beneficence and Justice).

e. Training

The PI underwent 2-week practical sessions at the histology lab in the Department of Human Anatomy at Chiromo Campus. These sessions were directly supervised by the Chairman of the Human Anatomy Department. The sessions provided the PI with basic skills in tissue preparation for histopathology evaluation, sectioning specimen, staining specimens, mounting on slides and basic analysis of tissues under the microscope to identify the basic histological organization of placenta.

6.0 RESULTS

6.1 Clinical Data

A total of 48 placentae were studied. Placentae from 24 pregnancies with normal nutrition and 24 with undernutrition were employed in the study. The mean maternal age was 26 ± 3.593 years and 26 ± 3.113 years respectively.

Table 1: Clinical Data

	Pregnancies with undernutrition N=24		Pregnancies with normal nutrition N=24		
	n/M	***(%)/SD***	***n/M***	***(%)/SD***	***p-value***
Age	26	3.113	26	3.593	0.663
15-24	7	29%	10	42%	0.365
25-64	17	71%	14	58%	
Parity	1.63	0.924	1.25	0.897	0.142
Nullipara	2	8%	4	17%	0.305
Primipara	10	42%	13	54%	
Multipara	12	50%	7	29%	
Marital status					0.505
Single	7	29%	5	21%	
Married	17	71%	19	79%	
Level of education					**0.001**
Primary School	17	71%	5	21%	
High School	7	29%	13	54%	
College	0	0%	6	25%	
Placental Shape					0.140
Ovoid	7	29%	12	50%	
Circular	17	71%	12	50%	
Colour of Membranes					0.05
Maroon	19	79%	24	100%	

Grey	5	21%	0	0%	
Placental Infarction/ Thrombosis					0.763
Yes	9	38%	8	33%	
No	15	62%	16	67%	
Gestational Age (weeks)	37	2.177	39	1.100	**<0.001**
Placental Weight (gms)	488	25.144	533	30.571	**<0.001**
Neonatal Weight (gms)	2916	230.272	3133	248.758	**0.005**
Cord length (cm)	46	5.123	53	4.684	**<0.001**
Cord diameter (cm)	1.2	0.25	1.9	0.19	**<0.001**
MUAC (cm)	20.99	1.142	-	-	**-**
BMI	17.99	0.639	-	-	-

The numbers in bold are statistically significant

The table above summarizes the clinical characteristics of patients with Count (n) & percentage (%) for categorical variables and Mean (M) & standard deviation (SD) for the numeric variables.

Statistically significant differences between the exposed and non-exposed were noted in the level of education, gestational age, placental and neonatal weights and the cord lengths and diameters.

The exposed generally had lower levels of education when compared to the non-exposed (p=0.001). The exposed also had lower placental (p<0.001) and neonatal weights (p=0.005) when compared to the non-exposed. The exposed tended to deliver before the controls as they had a significantly lower gestational age at delivery (p=<0.001). Mothers with undernutrition also displayed smaller cord lengths (p<0.001) and diameters (p<0.001) when compared to those who were adequately nourished

6.2 Histomorphology

Table 2: Histomorphological Changes in the Parenchyma of Placenta

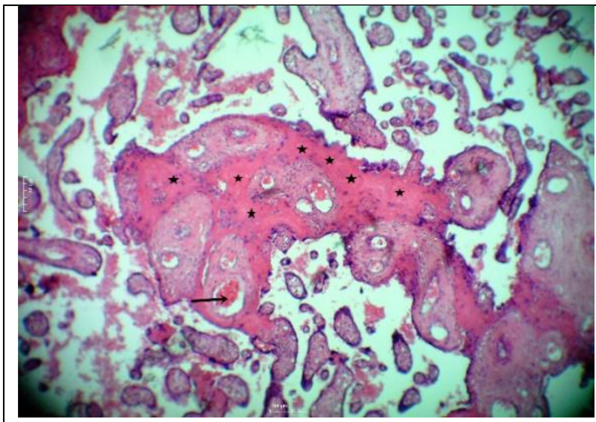
Histomorphological Characteristic	**Pregnancies with undernutrition N=24**		**Pregnancies with normal nutrition N=24**		
	n	***(%)***	***n***	***(%)***	***p-value***
Delayed Villous Maturity					1.000
Present	2	8%	1	4%	
Absent	22	92%	23	96%	
Accelerated Villous Maturity					0.525
Present	8	33%	6	25%	
Absent	16	67%	18	75%	
Distal Villous Hypoplasia					0.104
Present	9	38%	4	17%	
Absent	15	62%	20	83%	
Villous Edema					1.000
Present	1	4%	2	8%	
Absent	23	96%	22	92%	
Villous Necrosis					0.666

Present	4	17%	2	8%	
Absent	20	83%	22	92%	
Syncytial Knots					**<0.001**
Present	9	38%	24	100%	
Absent	15	62%	0	0%	
Thickening of Villous Membrane					0.666
Present	2	8%	4	17%	
Absent	22	92%	20	83%	
Villous stromal fibrosis					0.330
Present	5	21%	8	33%	
Absent	19	79%	16	67%	
Villitis					**0.022**
Present	18	75%	0	0%	
Absent	6	25%	24	100%	
Intervillositis					**0.022**
Present	18	75%	0	0%	
Absent	6	25%	24	100%	
Fibrin deposition					**0.003**
Present	13	54%	22	92%	
Absent	11	46%	2	8%	

Foetal Inflammatory Response					-
Present	-	-	2	8%	
Absent	-	-	22	92%	
Massive histiolytic inter-villous					-
Present	-	-	0	0%	
Absent	-	-	24	100%	
Villous Vascularity					-
Decreased	-	-	11	46%	
Normal	-	-	12	50%	
Increased	-	-	1	4%	
Basal Vessel Wall abnormality					-
Present	-	-	5	21%	
Absent	-	-	19	79%	
Foetal Thrombotic Vasculopathy					-
Present	-	-	4	17%	
Absent	-	-	20	83%	
Numbers in bold are statistically significant					

Table 2 summarizes the histological changes of the placenta. Statistically significant differences were noted in the presence of syncytial knotting between the 2 groups with the placentae from the mothers with normal nutrition being more likely to present with syncytial knotting ($p<0.001$). Villitis and intervillositis also displayed statistically significant results with the placentae from mothers with undernutrition presenting more commonly than the non-exposed group ($p = 0.022$). Fibrin deposition occurred in both groups but was more common in the exposed when compared to the non-exposed ($p = 0.003$).

Figure 3. Histomorphologic Features of the Placentae



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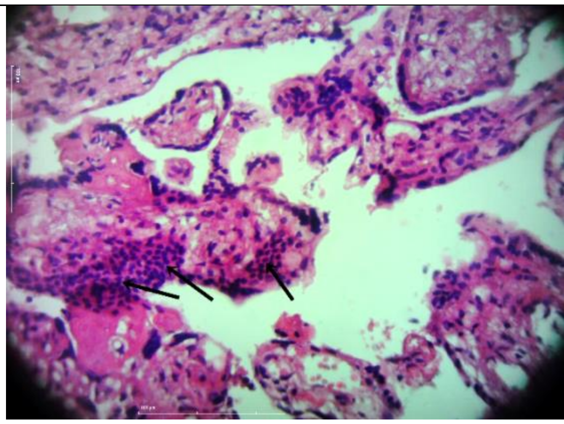

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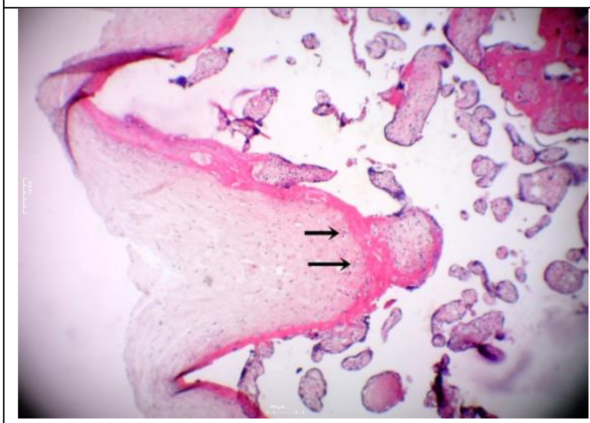

C

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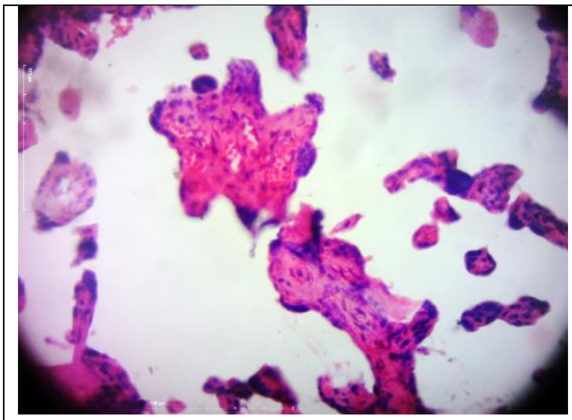
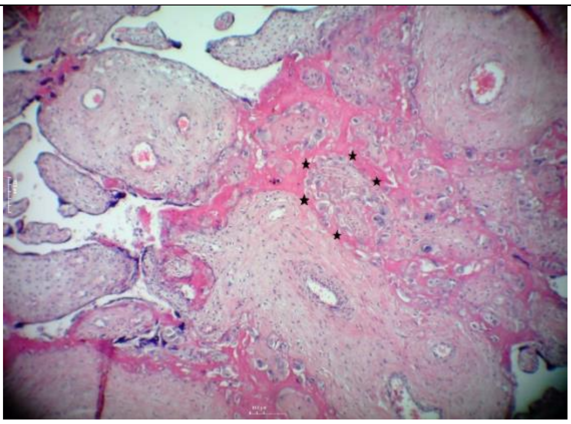
 E	 F

LEGEND FOR FIGURE 3: Histomorphologic Features of Placentae of women with undernutrition

FIGURE 3A- Photomicrograph of the placenta exhibiting fibrin deposition as illustrated by the black stars. Stain: Hematoxylin and Eosin, Magnification: X 100.

FIGURE 3B- Photomicrograph of the placenta exhibiting intervillous chronic inflammation as illustrated by the black arrows. Stain: Hematoxylin and Eosin, Magnification: X 400.

FIGURE 3C- Photomicrograph of the placenta exhibiting subchorionitis as illustrated by the black arrows. Stain: Hematoxylin and Eosin, Magnification: X 100.

FIGURE 3D- Photomicrograph of the placenta exhibiting chronic chorioamnionitis as illustrated by the black arrows. Stain: Hematoxylin and Eosin, Magnification: X 400.

FIGURE 3E- Photomicrograph of the placenta exhibiting distal villous hypoplasia. Stain: Hematoxylin and Eosin, Magnification: X 400.

FIGURE 3F- Photomicrograph of the placenta exhibiting infarcts as illustrated by the black stars. Stain: Hematoxylin and Eosin, Magnification: X 100.

6.3 Histomorphometry

Table 3: Histomorphometric Changes in the Terminal Villi of Placenta

Parameter	**Pregnancies with undernutrition N=24**		**Pregnancies with normal nutrition N=24**		
	M	***SD***	***M***	***SD***	***P- value***
No of capillaries/ villous	3.22	1.366	2.6	1.189	0.112
Diameter of terminal villi (µm)	57.4	35.617	43.1	10.760	**0.035**
Perimeter of terminal villi (µm)	186.2	81.267	143.4	35.325	**0.013**
Surface Density	0.037	0.010	0.040	0.009	0.314
Numbers in bold are statistically significant					

Table 3 summarizes the Histomorphometric features of the placenta. The number of capillaries per villous and the surface density of the terminal villi did not show statistically significant differences between the exposed and non-exposed. However, the diameter and the perimeter of the terminal villi showed statistically significant differences and both were greater in the exposed than in the non-exposed (p = 0.035 and p = 0.013 respectively).

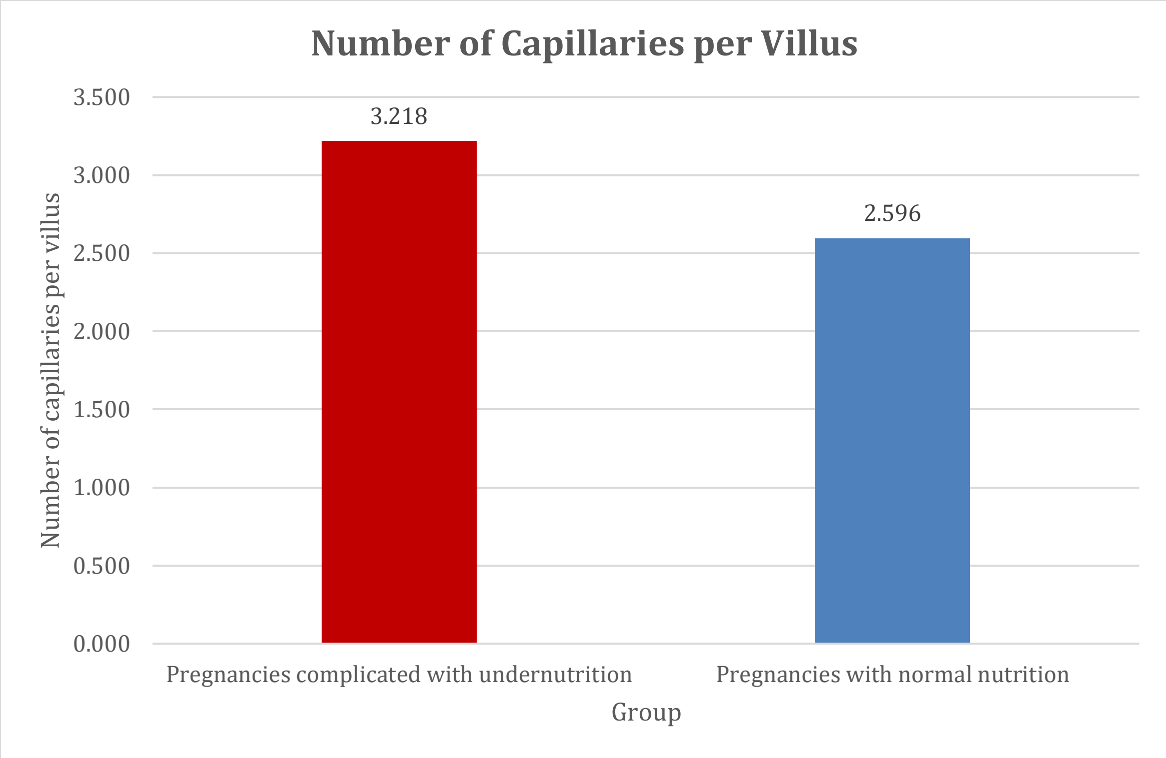


Figure 4: Bar graph displaying the average number of capillaries per villus in the exposed and non-exposed

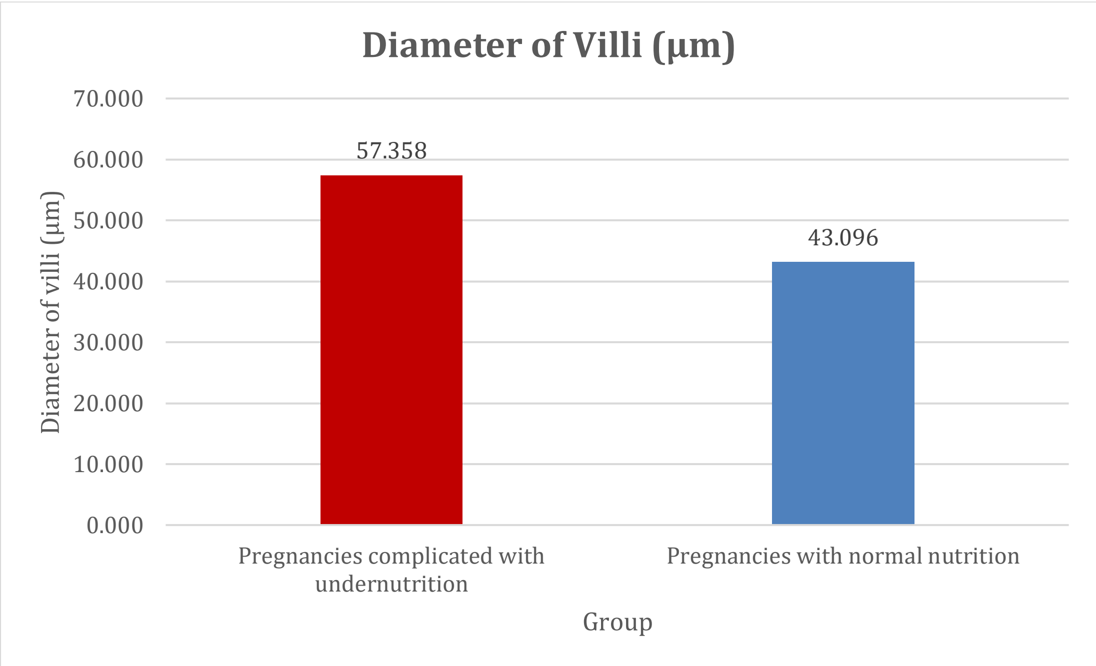


Figure 5: Bar graph displaying the average diameter of villi (µm) in the exposed and non-exposed

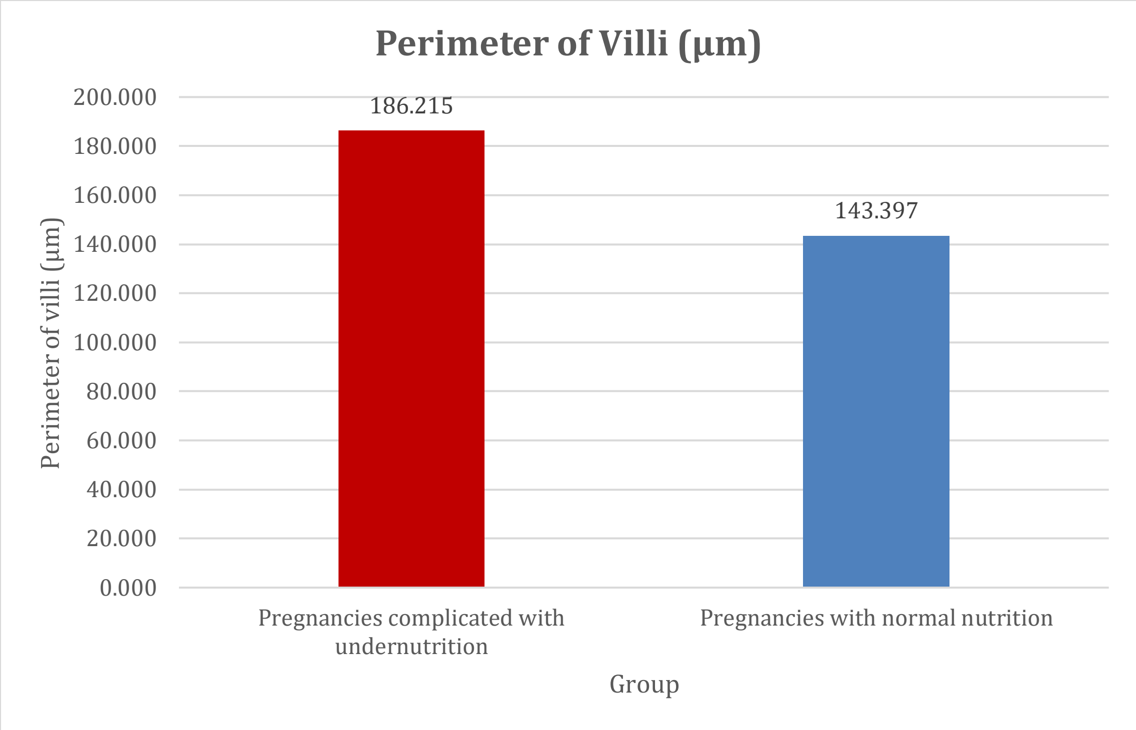


Figure 6: Bar graph displaying the average perimeter of villi (µm) in the exposed and non-exposed

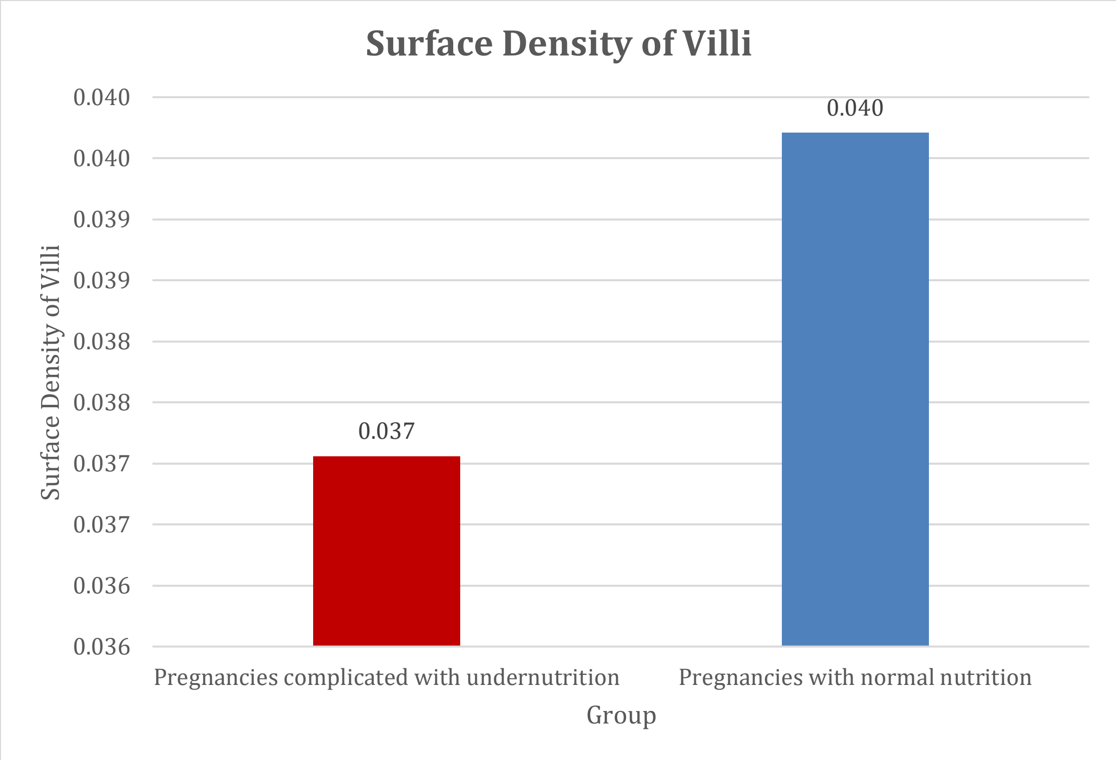


Figure 7: Bar graph displaying the average surface density of the villi in the exposed and non-exposed

7.0 DISCUSSION

Clinical Data of Study Participants

Pregnant women exhibiting undernutrition were found to generally have lower levels of education, lower placental and neonatal weights and also tended to deliver at earlier gestations than the non-exposed. Mothers with undernutrition also displayed smaller cord lengths and diameters when compared to those who were adequately nourished.

Low levels of education were noted in pregnant women exhibiting undernutrition. 0% of women with undernutrition had attended college education, 29% secondary education and significantly only 21% had attended primary education unlike women with normal nutrition where 25% had attended college, 54% secondary education and significantly 71% attending primary education. This finding corroborates those in a study by Gelebo et al., 2021, a cross sectional study on the prevalence of undernutrition and the associated factors among pregnant women in Ethiopia, where 75.7% of women largely affected by undernutrition had no education, 20.3% had primary education and only 4% had higher education. Low levels of education influence health seeking behaviour and have been found to delay health care acquisition, thus posing a barrier to optimal antenatal care (33–37). A study by Muyunda et al., 2016 (34), a descriptive study on the higher educational attainment associated with optimal antenatal care visits among childbearing women in Zambia found that educational attainment-associated differentials are linked with antenatal care attendance and therefore, women from poor socioeconomic and education backgrounds remain ignorant regarding nutritional requirements during pregnancy thus posing the developing foetus to risks associated with malnutrition. (2,3)

Suboptimal maternal nutrition was also associated with low placental weight and correspondingly low birth weight. Women with undernutrition had placental weights averaging 488 grams and neonatal birth weights averaging 2961grams while the non-exposed group had placental weights averaging at 533 grams and neonatal weights averaging 3133 grams. These findings were clinically not significant. Even though the values were lower than the non-exposed group, they were still within the normal ranges of 442-632grams for placental weight and 2000grams to 4000grams basing on the 2016 WHO guideline on good maternal nutrition. (1) These findings were similar with P.M. Coan et al., 2010 in a cross-sectional study titled adaptations in placental phenotype support fetal growth during undernutrition of pregnant mice where they argued that placental overgrowth usually compensates for undernutrition in early gestation to maintain normal foetal weight. This may explain why changes in placental and neonatal weight were not clinically significant. Contrary findings were shown by Bishwajit et al., 2019 in a prospective multicentre study titled Maternal BMI and nutritional status in early pregnancy and its impact on neonatal outcomes at birth in Bangladesh Bishwajit where 11.6% of neonates delivered were under weight (<2500grams). This may be attributed to the pivotal role of the placenta, being the gateway through which gases, nutrients, and wastes are exchanged between the maternal-foetal circulations, in the determination of the pattern of foetal growth as well as the birth weight (38–40). The size, morphology, and nutrient transfer capacity of the placenta determines the growth trajectory of the foetus. When the placenta malfunctions, it is unable to supply an adequate amount of oxygen, nutrients, and other factors to the growing foetus, and without this vital support, the foetus cannot grow and thrive to its full potential (41, 42). Placental insufficiency, as a result of maternal undernutrition, can lead to intrauterine growth restriction, a condition in which the foetus in utero fails to achieve its genetic full potential for growth and size (39, 43). This predisposes it to a high risk of neonatal morbidity (41). Mothers with low BMI and MUAC have lower placental weight and concurrently lower neonatal weights than their counterparts. (4)

Mothers with undernutrition also displayed smaller cord lengths and diameters when compared to the non-exposed. Gill et al., 1993 reported similar findings in a study comparing growth restricted foetuses with appropriate for gestational age group where they noted that the cross-sectional area of the umbilical cord is reduced in the former implying that thin umbilical cords are associated with foetal growth impairment (50, 51), placental disruption due to difficulty in nutrients transfer from the placenta to the baby, hypoxic ischemic encephalopathy, umbilical cord rupture, intrauterine growth restriction, operative interference, intrapartum complications, increased foetal heart rate abnormalities, and more chances of birth asphyxia among others (47–49). Short cords have also been associated with foetal akinesis or maldevelopment of the central nervous system and is a significant early marker of developmental abnormalities.

Contrary findings were reported by Raio et al. (1999) in a study done in New York, USA looking at the relationship between foetal growth restriction and maternal undernutrition in sheep where they found no difference in the range or distribution of cord lengths, cord diameters and placental surface area between conceptuses of the well-fed and nutrient-restricted sheep.

These studies are not conclusive and further studies need to be done.

Histomorphology of the Placenta

The general structural organization of the placenta consisting of chorionic plate, the parenchyma and the basal plate was similar to what previous studies have described (52). However, statistically significant differences were noted in the presence of syncytial knotting ($p<0.001$), villitis and intervillositis and fibrin deposition between the exposed and non-exposed ($p=0.022$).

In this study, 100% of mothers with normal nutrition presented with syncytial knotting and only 38% of those with undernutrition. Syncytial knots are multinucleated aggregates of syncytial nuclei, displaying dense aggregates of heterochromatin, at the surface of terminal villi in the placenta (53). They are consistently present, increasing with increasing gestational age, and can be used to evaluate villous maturity (54, 55). Their subtypes include syncytial sprouts, true knots, false knots and syncytial bridges (56). Thus, a decrease in syncytial knotting may indicate that the placentae in malnourished mothers had a higher transcriptional activity even at a later gestational age which may be an expression of structural placental insufficiency (57, 58).

Villitis and intervillositis also displayed statistically significant results. 75% of mothers with undernutrition had placental villitis and intervillositis while the non-exposed group had 0 %. This finding corroborates with that of Mukherjee et al., 1999, India, on the effect of malnutrition on the placenta. They noted the extent and degree of infarction, degeneration, calcification, fibrinoid necrosis of villi, intervillous fibrin deposition, villous fibrosis, syncytial knotting of villi and proliferation of Langhan's cell of the villi remarkably increased in placentas of women with undernutrition, at 67%, as compared to the non-exposed. Villitis, inflammation of chorionic villi, and intervillositis, inflammation of the intervillous space, inhibit the smooth exchange of gases and nutrients between the mother and foetus (59). The inflammatory response in placental villitis is characterized by the invasion of foetal villi by maternal T cells and associated with focal destruction of the syncytiotrophoblast (60). Villitis and intervillositis have been suggested to lead to hypoxic-ischemic encephalopathy, hydrops fetalis, intrauterine growth restriction and respiratory problems (61). It therefore appears that placentas of malnourished mothers become underdeveloped having pathological changes greater in extent and degree than the non-exposed group resulting in inadequate supply of nutrients from mother to foetus.

Fibrin deposition occurred in both groups but was more on the non-exposed group, 92% compared to the 54% in the exposed. This finding corroborates with that of Mukherjee et al., 1999 in a cross-sectional study in India on the effect of undernutrition on the placenta. Greater fibrin deposition was noted in the placentas of mothers who were non-exposed. A small amount of fibrinoid deposition within placenta is considered a normal ageing process as a result of eddying within the intervillous space (62). Fibrin deposition can be used as a marker of normalcy vis a vis a pathological process. This can explain why an extensive amount of fibrin deposition was found in the non-exposed group.

Extensive fibrinoid necrosis however, suggests an immune attack on trophoblastic cell and may be a result of thrombosis of maternal blood. Portions of chorionic villi get entrapped by fibrin obliterating the intervillous space causing atrophy of villous structure. These villi thus remain incapable of participating in any transfer activity (63). Impaired substance exchange is associated with perinatal morbidity, mortality, and can recur in subsequent pregnancies (64). Moderate-to-severe fibrin deposition has been associated with morphometric modifications of placenta and with an increased risk of severe adverse neonatal outcome (65). Further studies should be planned to explore this.

Histomorphometry of the Placenta

The diameter of terminal villi averaged 57.4 and perimeter of terminal villi 186.2 micrometres for the exposed. These were statistically significantly different compared to the non-exposed witch averaged at 43.1 and 143.4 micrometres respectively. However, the number of capillaries per villous and the surface density of the terminal villi did not show statistically significant differences between the two groups. Similar findings were noted by Mukherjee et al., 1990 in a cross-sectional study in India on the effect of undernutrition on the placenta where diameter of terminal villi averaged 63.2 micrometres and perimeter of terminal villi 173.8 micrometres.

Decreased villi perimeter in placenta of mothers with under nutrition may have occurred due to new branching of intermediate villi into multiple terminal villi. This is similar to findings by Mukherjee et al.,1990 where they noted degenerative changes of the placental villi and thus decreased villi surface perimeter. This is a compensatory process following poor placental development and dysfunction as evidence by a smaller villi surface area. The resultant smaller villi invariably have smaller perimeters. Accelerated villous branching with formation of syncytial knots and resultant hypervascularity of the villus are indicative of reduced placental perfusion and foreshadow poor obstetric outcomes (66, 67).

The placenta of mothers with under nutrition had reduced villi surface density. Mukherjee et al., 1990 noted degenerative changes of the placental villi and thus decreased villi surface density. This finding implies a likelihood of placental insufficiency that may result in poor foetal outcomes such as intrauterine growth restriction because of limited maternal-foetal interaction. A similar observation has also been noted in placentas of those infected with HIV and malaria (67, 68)

Terminal villi capillary hypervascularity was observed in placenta of mothers with under nutrition. Presence of new capillary sprouting demonstrates a chronic hypoxic picture and has been encountered in other conditions such as maternal anaemia, in pregnancies at high altitude, preeclampsia/eclampsia, diabetes mellitus, drug ingestion, and urinary tract infection, placental abnormalities, certain infectious diseases such as rubella virus, cytomegalovirus, HIV and syphilis (67, 69–71). Rutland et al., 2006 n a study of mouse in University of Nottingham, UK had similar findings. They noted a decrease in length of labyrinthine vessels indicating hypoxic events.

This implies that the effect of under nutrition in placenta may cause chronic placental hypoxia necessitating new capillary formation.

CONCLUSION

These results suggests that undernutrition is heavily associated with smaller cord lengths and diameters, villitis and intervillositis, greater terminal villi, decreased syncytial knotting, increased fibrin deposition and lower placental weights all associated with reduced placental perfusion which can potentially imperil the developing foetus.

The findings of this study necessitate closer monitoring and prompt intervention in pregnant women with undernutrition to avoid adverse impairment of placentas and in turn adverse foetal outcomes.

RECOMMENDATIONS

1. Good and closer monitoring and prompt intervention of pregnant women with undernutrition to avoid adverse impairment of placentas and in turn adverse foetal outcomes.

2. Further investigation on the effect of undernutrition to the neonate later in postnatal life
3. A study looking at biomarkers that can be used for early diagnosis and thus early intervention.

8.0 STUDY LIMITATIONS

The study focuses on pathological changes on the placenta caused by undernutrition and no attention to over nutrition which is also common among the Kenyan community. This can be addressed by other future prospective studies. This study, also, did not follow up the babies in the neonatal period to identify the implications of under nutrition at this age group. The study over looks confounding factors that the participant may not have revealed, and which may also have pathological effects to the placenta. We however using our exclusion criteria ruled out conditions that can affect the placenta structure including hypertension, diabetes, HIV and malaria.

9.0 DISSEMINATION OF RESEARCH FINDINGS

Dissemination of the results will be as follows:

- A report will be sent to the department of obstetrics and gynaecology as well as Bungoma County department of health
- Publication of papers in general national and international journals
- Presentation of papers at conferences both internationally and nationally

STUDY TIMELINE

	2020		**2021**				
	September-November	**November-December**	**December-January**	**January - February**	**February-March**	**March-April**	**April-December**
Proposal Development							
Proposal Presentation							
Ethics Committee Review							
Data Collection							
Data Analysis							
Results Presentation							
Publication							

BUDGET

	Item	Amount (Kshs)
Proposal Development	Printing: log books	10,000
	Envelopes	2,000
	Proposal copies	5,000
	KNH-UoN ERC	2,000
Data Collection	Training research assistants	8,000
	Research Assistants x 2 pax	20,000
	Stationary	5000
	Laboratory costs	10,000
	Internet	5,000
	Airtime	5,000
	Transport/Meetings	10,000
Equipment	Clean gloves, Sterile gloves, Stain fixatives, collection bottles and preservatives	10,000
	External hard drive/ Flash disc	10,000
Data Analysis	Statistician	30,000
Thesis write up	Printing drafts	5,000
	Printing thesis	6,000
	Contingency	5,000
	TOTAL	**148,000**

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DUMMY TABLES

Table 1: maternal demographic data

Participant no.		**Date :**		
Name (initials)				
Age				
Parity				
LMN				
EDD				
GBD				
MUAC				
WEIGHT				
HEIGHT				
BMI				
VDRL	Positive Negative Unknown			
HIV	Positive Negative Unknown			

Hepatitis B	Positive Negative Unknown			
Any other diseases	Yes No	Which one?		

Table 2: Neonatal outcome data

Variable		**Date:**	**Participant no.**
Sex	Male Female		
Gestational age			
APGAR score	At 1 min At 5 min At 10 min		

Birth weight	
Need for neonatal resuscitation	
Neonatal ward admission	

Table 3: Placental pathological features

Variables		**Date:**	**Participant no.**
Macroscopic features	**1. Placental;** ● **Weight** ● **Thickness**		

	• Density • Volume • Shape 2. Cord coiling.		Yes No		
Microscopic features	**Differences between villous and inter-villous spaces** **Extent of fibrosis** **Endovascular:** **1.Trophoblastic depth** **2.Extension and** **3.Morphometry** **Mitotic activity**				

APPENDICES

APPENDIX I: LETTER TO ERC

Dr Maero Deogracious

P. O. Box 1162,

Mumias.

Date:

The Chairperson,

Ethics, Research and Standards Committee,

Kenyatta National Hospital and University of Nairobi,

P.O. Box 20723,

NAIROBI.

Dear Sir,

<u>RE: SUBMISSION OF MASTER'S DEGREE RESEARCH PROPOSAL FOR APPROVAL</u>

I wish to submit my research proposal for approval by your committee. I am currently a 3rd year student pursuing a master's degree in Obstetrics and Gynaecology at the University of Nairobi, College of Health Sciences.

Yours Sincerely,

APPENDIX II: ETHICAL REVIEW COMMITTEE APPROVAL

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: Makokha **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 III: AUTHORIZATION LETTER

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 Deogracious 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

APPENDIX IV: CLIENT INFORMATION AND CONSENT FORM

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

APPENDIX V: DATA COLLECTION TOOL

CLINICAL DATA FORM

Rapid and Multiplex Diagnosis of Maternal Bacterial Infections

Participant Study Number: | | | |

Study group: | | |

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

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			
Date of Birth			Or estimated age ____
Gravida			
Parity			
Estimated Gestational Age __________weeks			
Date of Enrolment			
Marital status	☐1. S	☐2. M	
Education	☐1.Primary	☐2 Secondary Sch	☐9. University

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

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		

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

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 VI: 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

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

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 ______________________________

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

ASSESSMENT OF UNDER NUTRITION IN PREGNANCY

PARAMETER		LIST
List the food items taken in the last 24hrs	Breakfast	
	Snack	
	Lunch	
	Snack	
	Dinner	
MUAC using UNICEF recommended tape	<22cm	Undernutrition
BMI	<19.8cm	

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

Sylvia