PATHOLOGICAL STUDY OF STILLBIRTH PLACENTAS AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM TANZANIA

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By,

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A Dissertation submitted in partial fulfillment of the requirement for the Degree of Master of Medicine in Anatomical Pathology of

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CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences of the dissertation entitled **"*Pathological Study of Stillbirth placentas at Muhimbili National Hospital, Dar Es Salaam Tanzania"*** in fulfillment of the requirements for the degree of Master of Medicine in (Anatomical Pathology) of Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Atuganile E Malango**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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DEDICATION

To the Almighty God

To my lovely husband Curthbert

To my lovely daughters Grette and Gayle

To my beloved parents and in laws

To my brothers and sisters

I dedicate this work

ABSTRACT.

Background: World Health Organization (WHO) defines stillbirth as fetal death occurred at gestational age (GA) of equal to or greater than 28 weeks of gestation and/or a birth weight of 1000grams or more. It is a devastating experience for the family and causal identification helps the parents in mourning process, to determine recurrence risk and aids in counseling regarding future pregnancy. The unknown causes of stillbirths range from 3.8-57.4% depending on system used to classify stillbirths. Systems which have pathological examination of the placenta may identify causes of stillbirths in more than 90% of cases, therefore pathological examination of placenta in stillbirths may contribute to significant reduction of unknown cause of stillbirths. To date, clinical assessment is the only evaluation used to find cause of stillbirths at Muhimbili National Hospital (MNH). To our knowledge, this is the first study to be conducted at MNH to evaluate placenta pathologies in stillbirths.

Objective: To perform pathological study of placenta to describe placenta pathologies with risk to cause stillbirth at MNH, from June 2018 to December 2018.

Methodology: Hospital based, descriptive cross-sectional study, 80 singleton stillbirths placentas delivered from June - December 2018 at MNH labor ward were macroscopically and microscopically examined. Neutral buffered formalin (NBF) 10% was used as a fixative. Placentas were placed immediately (within 30minutes) in a container filled with 10% NBF in a ratio of 1:10 and fixed for 8-12 hours. Grossing of the placenta was according to surgical pathology grossing manual by Tidiane. Placenta pathologies with risk to cause stillbirths were defined according to Amsterdam Placental Workshop Group Consensus. The data were analyzed by using SPSS version 20 computer software and results were summarized in frequencies and proportions.

Results: The findings from this descriptive cross sectional study showed that 32 (40%) of stillbirths had unknown clinical diagnosis and 11 (13.8%) of mothers of stillbirths had previous history of adverse pregnancy outcome. Majority of the stillbirth placentas 71 (91%) had either one or combined pathologies with the risk to cause fetal death. Majority of placenta pathologies were combined 45 (56.3%). Maternal vascular malperfusion was the commonest

pathology 45 (56.25%) among all and it was significantly associated with preterm stillbirths (<37 weeks of gestation).

Conclusion: Pathological examination of placenta in stillbirths gives information about intrauterine life, in our study 40% of stillbirths had unknown clinical diagnosis, with detailed pathological examination of placentas 91.3% had either combined or isolated pathologies with risk to cause stillbirths. Pathological examination of placenta in stillbirths and clinical evaluation when used together can significantly reduce the proportion of unknown cause of stillbirths.

Recommendations: Pathological examination of placenta should be done in all cases of stillbirths at MNH, especially those with unknown clinical diagnosis. Further studies with the use of proper controls of live births should be done to establish strong causal relationship of placenta pathologies and fetal death. Universal guideline on reporting placenta pathologies should be adopted in order to improve reproducibility of results.

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ABBREVIATION

AGA	Appropriate for Gestational Age
GA	Gestational age
HPF	High power field
LGA	Large for Gestational Age
MFI/MPFD	Maternal floor infarction/Massive perivillous fibrin deposits
MNH	Muhimbili National Hospital.
MUHAS	Muhimbili University of Health and Allied Sciences
MVM	Maternal Vascular Malperfusion
NBF	Neutral Buffered Formalin.
n	Number of cases
N	Sample size
SCRN	Stillbirth Collaborative Research Network
SGA	Small for Gestational Age
VUE	Villitis of Unknown Etiology

OPERATIONAL DEFINITIONS

Stillbirth is defined as fetus born at gestational age of equal or more than 28weeks and/or birthweight of 1000grams, with no signs of life and scored 0/0 at 1 and 5 minutes on Apgar score (1).

Adverse pregnancy outcome is the pregnancy that did not result in a live birth (this included both abortions and stillbirths), whereby abortion is regarded as fetal loss before 28 weeks of gestation (2).

CHAPTER ONE

INTRODUCTION

1.0 Background

The World health organization (WHO) defines stillbirths according to the 10th edition of the International Classification of Diseases ICD-10 as fetal death at gestational age equal to or greater than 28 weeks or weighing more than 1000g at any gestational age (1). Additionally, the Stillbirth Collaborative Research Network (SCRN) described a stillbirth as a newborn with no signs of life whose Apgar score is 0/0 at 1 minute and 5 minutes (3).

Studies have shown that, gestational age threshold is a better predictor of maturity and hence viability than birth weight because fetuses at risk of stillbirth may have preceding fetal growth restriction. Moreover it was found that chance of survival of the fetus of equal or greater than 28weeks of gestation is approaching 100% if other causes of death are avoided (4).

In 2015, the global stillbirth rate was 18.4 per 1000 births, 25.5% reduction compared to a rate of 24.7 per 1000 births in 2000. Stillbirth rate varies greatly from place to place. It ranges from less than two per 1000 births in Western Europe to 32 per 1000 in South Asia and Sub-Saharan Africa. The estimated rate of stillbirths remains high in sub-Saharan Africa and south Asia as 98 % of global stillbirths occur in these regions, with most of deaths (two-third) occur prior to labor(ante partum) (5,6). In United State rate of 1 in 160 pregnancies was higher compared to other developed countries (7).

The stillbirth rate in Tanzania is incorporated in perinatal deaths which include pregnancy loss that occurs after 7 months of gestation and early neonatal deaths (deaths of live births within the first 7 days of life). The Tanzania Demographic Health Survey of 2015/2016 reported Perinatal Mortality Rate (PMR) of 39 deaths per 1000 pregnancies. That survey also found that the perinatal mortality rate is highest among the youngest mothers less than 20 years of age and older mothers 40-49 years old, and it was higher in urban than in rural areas which was 47 deaths against 37 deaths per 1000 pregnancies respectively (8).

Hospital based studies done in Western and Northern Tanzania found that PMR was 38/1000

and 58/1000 respectively (9). Kidanto et al, in a study to assess qualitative perinatal audit at Muhimbili National Hospital(MNH) found PMR of 92 per 1000, among these, stillbirth rate was 64/1000 which was commonest in youngest mothers, similar to what was reported at Kenyatta Hospital with stillbirth rate of 59.6/1000 (6,10,11).

Stillbirth is a devastating and stressful experience to the parents, caregivers and it is of public health concern which is under-researched (12). The WHO places it as fifth leading cause of death preceded by ischemic heart disease, stroke, chronic obstructive pulmonary disease and lower respiratory infections (7).

Identifying the cause of stillbirth is of medical, epidemiological and social importance. It helps the parents in their process of mourning, to ascertain risk of recurrence and so aids in counseling regarding future pregnancy outcomes (12).

It is important to understand the underlying cause of death and the events followed resulting in stillbirth as it is usually difficult to pinpoint a single cause of death (13). Although some conditions certainly cause fetal death, usually it is only the association of the condition to fetal death which is established especially when multiple conditions occur together (14).

Stillbirth can be broadly classified as antepartum fetal death which occurred before labor or intrapartum which occur during labor, this categorization is aided by clinical presentation and degree of maceration. Macerated fetus presents with peeling off of skin on touch, umbilical cord become brown, discolored dermis to grey brown as well as disarticulation of calvarial bones all these are the signs of autolysis, but the placenta remains viable after fetal death (4).

Causes of stillbirths remain unexplained in 25-60% even after several diagnostic methods are used which leads to difficulties in targeted interventions. (12,15–17)

Apart from the setback of classification system used being a factor of these unknown cause of stillbirth, importantly lack of placental and postmortem examination play a role (18). This has been proved to be true as recent classification systems of stillbirth which stress on placental examination results reduce the numbers of unexplained stillbirths (15).

Worldwide, there is no uniform guideline for the evidence based workup of fetal death therefore local protocols are formulated depending on experience. Currently, there are no tests and examination that are acceptable to determine the cause of death but several studies supported that fetal autopsy and placental pathology were valuable (12,19).

Some of the studies reported that reliable way of evaluating these causes of stillbirth include autopsy, placental examination and chromosomal analysis (15).

These examinations are usually not done in our setting probably due to the difficult on getting consent to do autopsy on the fetus and expenses of doing chromosomal examinations. Roberts reported that in limited resources setting there are few to none perinatal pathologist, most pathologist do not have experience in performing fetal autopsy and most of the time it is uncomfortable experience (4). Therefore clinical evaluation and placental examination remain the tools of choice in stillbirth.

As important as it is elsewhere in the world placenta examination is usually not performed and not emphasized as it was once written by Ballantyne ,"a diseased fetus without placenta examination is an imperfect specimen, and a description of a fetal malady, unless accompanied by a notice of placental condition is incomplete" (20).

The above description is valid because placenta connects the mother and the fetus, it has several functions such as for the development of the fetus, hormonal secretions, neurotransmitter secretion as well as prevention of toxins, infections to cross from the mother to the fetus, some called placenta as a black box which provide information of the intrauterine life (21).

There are several placental pathologies and umbilical cord lesions linked to stillbirth such as coiling of the umbilical cord, abnormal insertion of the umbilical cord, abnormal implantation site of the placenta, infections and vascular abnormalities (17).

Several studies have shown limitations in examining placenta pathology in stillbirth, include lack of universal agreed protocol for placental examination, controls used are not appropriately selected as well as insufficient sample size (22).

As it has been stressed above, ideally all placenta of stillbirth should be submitted for pathologist to examine, the pathologist should have adequate information given in the request form with regard to age of the mother, body mass index, any maternal conditions presents such as HIV infection, diabetes mellitus, hypertension as well as history of previous pregnancies. Furthermore gestational age of this index pregnancy, birth weight of the infant, mode of delivery and any event which occurred during delivery and if fetus is not sent for autopsy then it is important to document whether it was fresh or macerated stillbirth (17).

Placenta should either be submitted as soon as possible to the laboratory or refrigerated at 4 degree of Celsius or fixed in adequate formalin. Advantage of formalin fixation is that the placenta become less infectious though some color changes and sometimes unsuitable for further examination of the bacteria (23).

Pathologist is needed to give thorough macroscopic and microscopic description of the umbilical cord, membranes, placental disc and parenchyma (17).

At MNH there is no routine examination of placenta as part of management in stillbirth and placental examination can be performed in cases where consent is denied for fetal autopsy. Therefore the aim of this study was to characterize placenta pathologies in stillbirth and show its usefulness in this institutional setting.

1.1 Literature Review

1.1.1 Clinical characteristics associated with stillbirths.

To have a proper guide of pathological assessment of placenta in stillbirth thorough clinical information should be provided which include history of previous pregnancies, underlying maternal conditions, gestational age, birth weight, mode of delivery and condition of stillbirth whether fresh or macerated. In a systematic literature review done in low and middle income countries it was found that extremes of age(≥35 years, or <20 years), parity(1 or ≥5), extremes of birth weight (≤1500 grams or ≥4000grams) were significantly associated with stillbirths (24). Similar findings of young age of mothers of stillbirths (< 20 years) was reported by Kidanto et al (10). Low level of education have been associated with pregnancy in young women pregnancy which are mostly unwanted (25).

Most stillbirths are preterm that is delivered at gestational age of equal or below to 37 weeks, this finding was seen in Rwanda, Nigeria, India and United States, Kidanto et al in Tanzania found that 43.5% of stillbirths were below 37weeks (11,26–33). Bonette et al, by using the gestational-related optimal weight software found that 93 fetuses (70.5%) had appropriate weight for gestational age while 39 fetuses (29.5%) showed a weight below the 10th customized percentile (34). Multigravidity is also reported commonly in stillbirths as seen in a study done in India (28). Majority of stillbirths in a significant proportion are macerated (26,35).

Maternal conditions have been associated with increased risk of stillbirths; these include hypertension, pre-eclampsia and eclampsia, severe anemia and diabetes mellitus, recurrences of stillbirth with or without these maternal conditions was reported to be about 9.2% (36) . Aminu et, al found that maternal conditions with increased risk of stillbirths ranged from 8-50% commonly reported were syphilis, hypertensive disorders in pregnancy, diabetes mellitus and HIV infection (24). Divya et al, found that pre-eclampsia , eclampsia, abruptio placenta, gestational hypertension, severe oligohydramnios and severe anemia to be associated with stillbirths (28).

Lethal congenital anomalies of the fetus associated with stillbirth reported in different magnitude, it ranges from 2.2% - 33.3% these included those anomalies which can be detected macroscopically and be identified by clinicians or nurses during delivery and those which require fetal autopsy to be done such as respiratory, gastrointestinal and cardiovascular anomalies (24). Sharma et al found that 8.8% causes of stillbirth were contributed by congenital malformation, these malformation were hydrocephalus and anencephaly both involving central nervous system (36). A slightly high rate of 10.3% was reported in other study which included fetal death due to congenital malformations and chromosomal abnormalities (37).

Obstetric conditions such as rupture of uterus, cord prolapsed, prolonged and obstructed labor observed to cause fresh stillbirths, Kidanto et al reported decline in fresh stillbirths in Tanzania due to proper management during delivery and other programs such as Helping Babies Breath (HHB) (11,29). Cord prolapse was seen in 0.9% of cases of stillbirths (38). These non-placental causes of stillbirths which are due to fetal and maternal factors are found more in gestational age of less than 37 weeks (39).

1.1.4 Unexplained cause of stillbirth

The systematic literature review done by Aminu et al, found that the rate of unexplained cause of stillbirth varied from 3.8 - 57.4% depending on classification system which were; Extended Wiggleworth, Aberdeen and other used local guidelines (24). Bonetti et al by using ReCoDe classification system found that unexplained causes of stillbirth accounted for 20.16%, a lower rate of 5.6% was reported by Sharma et al (36) and 12.3% was reported by Bring et al (37) in both of these cases local guidelines which included pathological examination of the placentas were adopted. Unknown cause of stillbirths was found to occur more in young gestational age (39). At Muhimbili National Hospital, it was found that the rate of unknown cause of intrapartum stillbirths alone being 12.8% (10).

Vergan et al, in a study to identify cause of stillbirth by comparing four classification systems found that the classification system which has included thorough placenta examination had lower number of unexplained cause of stillbirth, and therefore made a conclusion that a system which classify cause of stillbirth without including pathological examination of the placenta

was inadequate (40)**.** With detailed placenta examination the cause of stillbirth may rise to more than 90% (17). .

1.1.2 Placenta pathologies in stillbirths

Umbilical cord pathologies

In general three quarter of stillbirth placentas have combined pathologies (41). Umbilical cord abnormalities are many ranging from those with no clinical significance such as false knots to developmental lesions (insertion, length, coiling) and accidents (entanglement or prolapse) which are associated with adverse fetal outcomes most importantly stillbirths (42). These lesions cause stillbirths and other adverse fetal outcome by diminishing blood flow in umbilical vessels and subsequent fetal hypoxia or circulatory compromise (43).

Gurusamy et al and Chang et al, found that the common umbilical cord lesions were due to abnormal coiling (41,44). Hypercoiling of umbilical cord occurred in 22%, hypocoiled umbilical cords occurred in 11% and long cord in 11% (41), although in this study there was no documentation of definition criteria of these umbilical cord lesions. The associated factors of these umbilical cord lesions include maternal factors (preeclampsia, oligohydramnios and gestational diabetes), histological placental features of maternal vascular malperfusion, fetal vascular malperfusion and inflammation (44).

Among abnormal umbilical cord insertions associated with stillbirths which are furcate, marginal and velamentous insertion, furcate insertion is the rarest occurring in 0.1% of cases. Marginal insertion occurs in 7% of stillbirths, though Igualada et al found that it occurred in 23% of stillbirths, velamentous insertion was found to occur in approximately 5% of stillbirths (22,39).

Single umbilical cord artery has been found to occur in 7.7% of stillbirths (22), 13 to 56% of cases are associated with chromosomal defects or other non-chromosomal fetal congenital anomalies such as esophageal atresia, central nervous system defects, vertebral defects, cardiac defects, imperforate anus and unilateral renal agenesis (47).

Placental parenchyma and membranes

Literatures show that the frequency of placenta parenchyma causes of stillbirth ranges from 7% to 65% (6,24,48). However, it is assumed that these histopathological findings could be present in a placenta in an uncomplicated pregnancy, but their grades are higher in stillbirth (32). Pinar et al, added that in most cases there was heterogeneity of these lesions (22).

There are several placental lesions with risk of recurrence which include rare lesion such as chronic histiocytic intervillositis and maternal floor infarction/massive perivillous fibrin deposition which have recurrence rate of 75-90% and 40-60% respectively, others are more common lesions such as vilitis of unknown etiology, maternal vascular malperfusion and acute chorioamnionitis which have recurrence rate of 25-50%, 10-25% and 10-25% respectively (49).

Maternal Vascular Malperfusion

Maternal vascular malperfusion lesions occur due to hypoxic ischemic damage to the placenta and leads to adverse outcomes of the pregnancy. The pathogenesis being deficiency of trophoblastic invasion and spiral arterioles remodeling which occur in early pregnancy. These lesions were originally explained as specific for pre-eclampsia and eclampsia however currently they have been described in diseases such as systemic lupus erythematosus, abuptio placenta, gestational diabetes, preterm labor, premature rupture of membranes and antiphospholipid antibody syndrome (50) .

Maternal vascular malperfusion pathology is an umbrella of several lesions which occur in the placenta parenchyma and umbilical cord these are acute atherosis, decidual arteriopathy, infarction, accelerated villous maturation, distal villous hypoplasia and increased immature intermediate trophoblast (51).

Among the maternal vascular lesions the common ones are acute atherosis and decidual arteriopathy, the least ones are distal villous hypoplasia and accelerated villous maturation (52). Maternal vascular malperfusion have been associated with small placental weight and

narrow umbilical cord, the reported frequency of these lesions is between 46% to 70.7% of stillbirths mostly in association with other lesions, isolated maternal vascular pathology occur in 11% of cases (41,52,53).

There are reasons which make challenges in detection of maternal vascular malperfusion lesions which occur on the vessels as they occur in deeper decidual or myometrial segments of spiral arteries than in superficial segment, delivered placentas retain only a thin layer of decidua which is superficial and more likely to be transformed than the underlying deep decidual and myometrial segments. The lesions on the vessels also occur focal therefore it will depend on sampling of specimen during grossing and intensity of search on microscopic examination (51). Gelbaya et al and Ogweyo et al, reported that low dose aspirin given during pregnancy improves maternal vascular perfusion and therefore can be given in mothers with previous history of stillbirths due to maternal vascular malperfusion (54,55).

Fetal Vascular Malperfusion

Fetal vascular malperfusion relates to the lesions which affect fetal vascular system (56). They occur due to obstruction of blood flow as in cord accidents, hypercoiling of umbilical cord, umbilical artery thromboses or vascular damage as in meconium associated myonecrosis and inflammatory pathology such as acute chorioamnionitis, heart failure, long umbilical cords or rarely in inherited or acquired coagulopathy (57).

Fetal vascular malperfusion in stillbirths range from 48% to 51% (41,52), these lesions also occur as postmortem changes in stillbirth and therefore should be differentiated from ante mortem one. That occur as postmortem changes are globally distributed in a section when examining microscopically while those which occur as ante mortem cases are spatial/temporal distributed (57).

Massive Perivillous Fibrin Deposition/ Maternal Floor Infarction

Maternal floor infarction (MFI) and massive perivillous fibrin deposition (MPFD) are rare and idiopathic placental disorders which overlap in both clinical and pathological presentations

and therefore there is uncertainty in literatures whether they are distinct entities or different manifestations of common underlying pathophysiology and therefore used interchangeably by some pathologists; MFI is not true vascular infarction and was a misnomer that originates from the German word “Gitterinfarct”. The differential diagnosis of these conditions includes infarctions which are well circumscribed and show crowding of villi while perivillous fibrin lesions tend to have serpiginious appearance and maintain intervillous space (58–60).

There are several hypothetical etiologies which include autoimmunity, infection, cytotoxicity and failure of maternal tolerance to the fetus, they are also associated with intrauterine growth restriction, intrauterine fetal death and recurrence (60,61).

These lesions occur in less than in 1% of all pregnancies with recurrence rate of 12-78%, it has several complications worst of all being stillbirth with reported rate of 13-50% (62). A slightly higher rate of 1.2% was reported by Korteweg et al, in a study to evaluate diverse placenta pathologies in fetal death, with no association with gestational age (39).

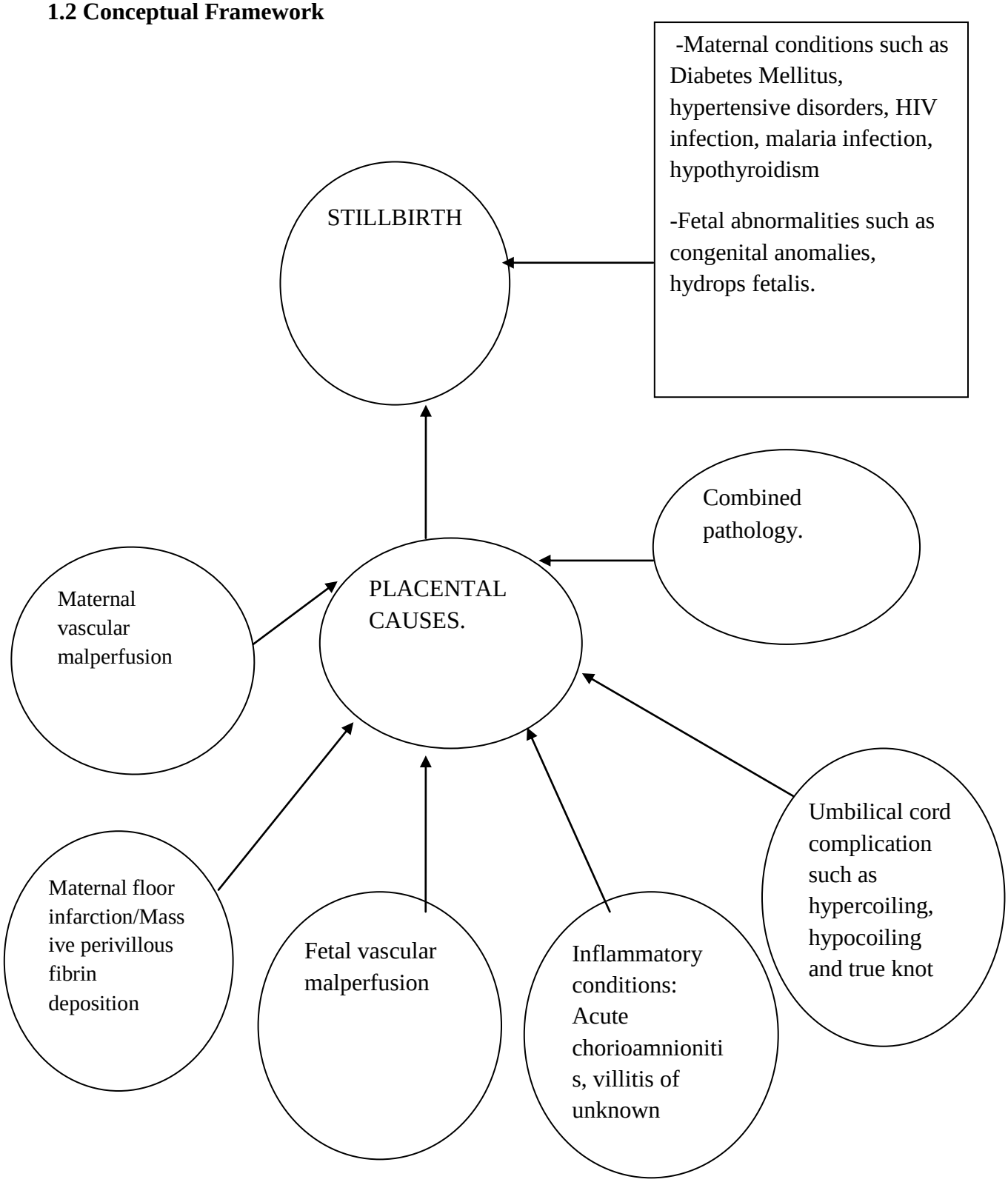
Treatment with heparin, aspirin and intraveneous immunglobulins have been found successful and results in healthy pregnancies in cases with previous history of MFI/MPFD (59).

Inflammatory Conditions

Acute chorioamnionitis is the major inflammatory lesion, characterized by the presence of neutrophils derived from maternal circulation, fetal circulation or both (56). Chronic inflammatory lesions are called chronic villitis which in more than 99% is thought to be due to maternal alloimmune response and termed as villitis of unknown etiology (56). Acute chorioamnionitis and villitis of unknown etiology occurred in 19% and 16% respectively (41,52). The organisms which cause acute chorioamnionitis are Listeria monocytogens, Campylobacter fetus, Chlamydia psittaci, Francisella tularensis, group B streptococcus and Coccidioides immitis (63).

Maternal conditions, fetal abnormalities and placenta pathologies have been associated with the risk to cause stillbirth. To the best of our knowledge, in Tanzania no documented data on placenta pathologies with the risk to cause stillbirth has been published before.

1.2 Conceptual Framework



1.3 Problem Statement

The current stillbirth rate at MNH is 64/1000 births with clinical assessment only being the method of evaluation used to determine the cause of death (10). Of intrapartum stillbirths, 12.8% showed no cause of death clinically (11), furthermore no published figures of proportion of unknown clinical diagnosis of antepartum stillbirths were found for Tanzanian population. Various guidelines and publications have recommended the use of clinical assessment, fetal autopsy, placenta examination and karyotyping analysis should be performed in assessing cause of stillbirths (15,19). However karyotyping is currently unavailable at MNH and lack of consent to perform fetal autopsy are major obstacles in fulfilling these guidelines. Placental examination is an additional tool to clinical assessment to establish the cause of death in stillbirth.

1.4 Rationale

Pathological examination of the placenta aids in explaining the pathophysiology behind the cause of stillbirth and therefore may reduce the proportion of unknown cause of stillbirth.

This project is of public health importance because it aims to reduce the perinatal mortality rate which is among the eight Millennium Development Goals. Moreover the project is of clinical importance as it is helpful for the purpose of counseling of future pregnancies, risk of recurrence and aids in the process of mourning for the family. Additionally, it is important for policy makers to know specific areas of focus to reduce stillbirths in the population and for medico legal purposes.

The project builds a base for other studies on placental pathology in stillbirths and possibly to introduce the use of placental examinations in cases of stillbirth which can be adopted for routine workup of stillbirth at MNH.

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1.5 Research Questions

What are the clinical characteristics related to stillbirths at MNH?

What are the umbilical cord pathologies with risk to cause stillbirth found in stillbirth placentas at MNH?

What are the placenta parenchyma pathologies with risk to cause stillbirth found in stillbirth placentas at MNH?

1.6 Objectives

1.6.1 Broad Objective

To determine clinical characteristics related to stillbirths and to describe the pathological changes of the placenta and umbilical cord which may be the risk factors for stillbirth at MNH, from June 2018 to December 2018

1.6.2 Specific Objectives

- To determine the clinical characteristics related to stillbirths at MNH.
- To determine the frequency of unknown category of clinical diagnoses of stillbirths at MNH.
- To describe stillbirth-related pathological features of placentas at MNH.
- To determine the association between stillbirth-related placenta pathologies and gestational age.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Study design

Hospital based, descriptive cross sectional study.

2.2 Study area/ setting

The study was conducted at Muhimbili National Hospital, the largest teaching and referral hospital in Tanzania located in Ilala District in Dar es Salaam region. The hospital has 1,500 bed facility, attending 1,000 to 1,200 outpatients per day, admitting 1,000 to 1,200 inpatients per week. The patients population are coming from Dar es Salaam and within coastal regions and a small proportion from more distant regions.The study was conducted at labour ward where there are 20-30 deliveries per day with an average of 15- 20 stillbirths per month. The samples of placenta of stillbirths were collected and fixed by placing in a container containing 1.25l of 10% NBF (Neutral buffered formalin) and sent immediately to the Central Pathology Laboratory (CPL) histopathology unit.

2.3 Study population

The population of this study included all fetuses delivered vaginally born with no signs of life, and declared dead at gestational age of equal to or more than 28weeks, delivered between June 2018 and December 2018.

2.3 Eligibility criteria

2.3.1 Inclusion Criteria

Placentas from the fetuses who were born with no sign of life at gestational age of 28 weeks or more, or with birthweight of equal to or more than 1000gram.

2.3.2 Exclusion Criteria

Placentas, umbilical cord and membrane of stillbirths which were absent or incomplete for evaluation.

2.4 Study period

The study was conducted from June 2018 to December 2018 (from Monday to Sunday)

2.5 Sample size estimation

The sample size for this study was estimated to be 79. This sample size was adopted from a study done at Kenyatta Hospital in Kenya with a stillbirth rate of 59.6/1000 which is 5.96%; they found that among 102 study population, 22.5% had significant placental pathologies which explained cause of death (6). The stillbirth rate at Muhimbili National Hospital (MNH) according to Kidanto et al was found to be 64/1000 which is 6.4%. Based on these findings, since the population size of 102 was used in a population with a stillbirth rate of 59.6/1000 at Kenyatta Hospital, then population size with accordance to the stillbirth rate of 64/1000 % at MNH was calculated to be 110.

Therefore sample size for this study was approximated to be;

Sample size formula: $n = [DEFF*Np\ (1-p)]/\ [(d^2/Z^2_{1-\alpha/2}*(N-1) + p*(1-p)]$

Population size(for finite population correction factor)(*N*):	110
Hypothesized % frequency of outcome factor in the population (*p*):	22.5%
Confidence limits as % of 100(absolute +/- %)(*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

The sample size for 95% confidence level was calculated to be 79.

OpenEpi formula for calculating sample size for a proportion was used

2.6 Sampling Technique

Convenience sampling was used in order to maximize chance of obtaining cases for a short period. Therefore any case of stillbirth which occured in the labor ward at Muhimbili National Hospital during the study period was included in the study.

2.7 Sample collection, data collection and Laboratory methods

The study had principal investigator and 3 research assistants who were the nurses from labor ward at MNH who had different work shift. Once a mother who delivered a singleton stillbirth by sponateneous vaginal delivery was identified, she was given counselling and explained the purpose of the study followed by signing consent form. Identification number was given and filled on three areas these were the consent form, a structured questionnaire adopted from previous surveys and reviewed articles and on a 2.5l container which was used to keep placenta. The three assistants were taught to do quick examination of placenta, membranes and attached umbilical cord grossly for completeness, within 30 minutes from delivery these placentas were supposed to to kept in a container of 2.5l with a labelled identification number filled with 1.25l of 10% neutral buffered formalin, the placenta , membranes and umbilical cord had to be totally immeresed in formalin. The clinical data were collected by principal investigator which included age of the mother, gestational age of index pregnancy, birth weight of the fetus, condition of the fetus whether fresh or macerated, maternal condition, previous history of stillbirth or abortion or preterm delivery and clinical diagnosis which was given by clinician as the cause of stillbirth. Five containers of 2.5l filled with 1.25l of NBF were submitted after every 2days in labor ward for placenta collection. Sample and data collection were done from Monday to Sunday, by the principal investigator and three trained research assistants.

2.7.1 Grossing procedure and microtomy

After 8 -12 hrs of fixation, placenta, umbilical cord and fetal membranes were removed from the 10% NBF, washed then weighed Grossing was proceed acccording to the surgical pathology grossing manual by Tidiane (64). Placental membranes were examined first, for color and translucency. The umbilical cord length, diameter, insertion, coiling, and knot were assesed, placenta disc was examined for retroplacental clots. Then extraplacental membranes and the umbilical cord were trimmed from placental disc, two representative sections from umbilical cord one from fetal surface and the other from maternal surface, two sections of membrane rolls near the rupture were taken. Shape of placenta disc and three dimensional

measurements were taken these included greatest linear dimension as length, perpendicular dimension as width and maximum depth. The placenta was examined for completeness presence of 15 or more cotyledons, maternal surface was examined for accumulation of blood and blood clots. Then cross-sectional cuts at intervals of no greater than 1cm were made, color and appearance were examined grossly, red color suggestive of recent lesion and tan white color for an older lesion. Size of the lesion, if well circumscribed or irregular and estimated percent of the placental volume occupied by all lesions and whether central or periphery located. Three representative sections from placental disc (fetal surface, maternal surface and periphery) and section from any abnormal lesion were taken (56). Then specimens were processed using automated processing machine followed by sectioning at 3-4 micrometers using SAKURA microtome. The sections were harvested from waterbath by frosted slides which were labelled with the identification number previously given.

2.7.2 Hematoxylin and Eosin stain.

The sections were dewaxed on the hotplate and xylene, followed by hydration using descending order of alcohol. Routine H and E stain was done followed by dehydration in ascending order of alcohol,clearing by xylene and mounting by DPX.

Microscopic examination were done by the investigator and later reviewed by two senior Pathologists. An OLYMPUS SC30 camera mounted was used to take pictures and visualize them by using OLYMPUS soft imaging solution GMBH software in OSIS USB camera input mode.

2.7.3 Results Interpretation

There is no international uniform diagnostic guideline for stillbirth; therefore placental lesions with increased risk of stillbirths were defined according to Amsterdam Placental Workshop Group Consensus Statement (65) .

Long umbilical cord was defined as umbilical cord of length of equal to or more than 70 cm.

Abnormal cord coiling was defined as coiling index of 0.1 coils/cm or less for hypocoiling and 0.3 coils/cm or more for hypercoiling.

Marginal insertion was defined as umbilical cord insertion in less than 1cm from the nearest margin.

Furcate insertion was defined by the cord separates into two or more vessels just before insertion to the placenta and these vessels are not protected by wharton's jelly.

Velamentous insertion was defined as umbilical cord vessels run through the membranes, branching while in the membrane and lack protective wharton's jelly.

Single umbilical artery was defined as two vessel cord which has one artery and one vein, in some instance hypoplastic artery can be present.

Acute chorioamnionitis was defined by the presence of polymorphonuclear inflammatory cells in more than 20% of fetal-maternal (choriodecidual space) or into fetal tissue which can be in chorioamnionitic membranes, amnionitic fluid or umbilical cord.

Chronic villitis was defined by the presence of mononuclear inflammatory cells in the villi and was graded as low grade if less than 10villi were involved and as high grade if more than 10 were involved. Chronic intervillositis was defined by the presence of prominent mononuclear inflammatory cells in the intervillous space.

Maternal vascular malperfusion as an umbrella which included;

i. Decidual arteriopathy was defined as persistence of muscularized basal plate and mural hypertrophy.
ii. Acute atherosis was defined as fibrinoid necrosis of the vessel wall and foamy macrophages embedded within fibrinoid necrosis.
iii. Infarction was defined as decrease of intervillous space with collapsing villi which appear smudgy and fade away (ghost appearance) surrounded by fibrinoid necrosis.
iv. Accelerated villous maturation was defined by the presence of syncitial knots in more than 30% of the villi examined in the lower power in 75% of the section.
v. Distal villous hypoplasia defined by thin and elongated villi with decreased vascular branching in more than 30% of section of placenta parenchyma excluding the 25% of the placental parenchyma just beneath the chorionic plate.

vi. Increase of immature trophoblast with eosinophilic or vacuolated cytoplasm by at least 10-20 cells within superficial decidua.

vii. Narrow cords due to diminished Wharton's jelly was defined as maximum cord diameter <0.8cm either focally or involving a longer segment.

viii. Absolute too low less than 10th percentile of placenta weight.

ix. Abruptio placenta was defined as presence of retroplacenta hematoma with or without infarction grossly, on microscopic examination villous infarction with hemorrhage this should be supported by clinical history suggestive of it.

Maternal Floor Infarction (MFI) /Massive perivillous fibrin deposit (MPFD) was defined as gross finding of more than 25% areas of pale and firm placenta, histologically MFI presents with basal villi of the entire maternal floor encased by perivillous fibrin of equal to more than 3mm thickness on at least one slide. MPFD identified by perivillous fibrinoid material extending from the maternal surface to fetal surface encasing 50% of the villi or more on at least one slide.

Fetal thrombotic vasculopathy was defined by the presence of either avascular villi or villous stromal karyorrhexis (VSK) which was defined by the presence of fragmented erythrocytes or karyorrhectic debris in terminal villi.

Lethal congenital anomalies were defined as defect incompatible with life or those anomalies which can be treated but cause death (66).

Placental malaria was defined as accumulation of parasitized and non-parasitized erythrocytes in the maternal villous spaces, leukocyte accumulation (villitis), malarial pigment accumulation in phagocytes, its deposition in villous spaces and villi, and widespread fibrinoid necrosis of villi and basement membrane thickening (67,68).

Birth weight of the fetus was grouped in percentile according to WHO growth charts for estimated fetal weight for male and female in relation to their gestational age (69) and grouped as small for gestational age for fetus with birth weight less than 10^{th} percentile of estimated fetal weight, appropriate for gestational age for fetus with birth weight between 10^{th} -90^{th}

percentile of estimated fetal weight and large for gestational age for fetuses with birth weight more than 90th percentile of estimated fetal weight (70).

2.8 Data management and analysis

Data entry and cleaning were done by research principle investigator, the data which were feed included clinical data, macroscopic findings and conclusion of placenta examination, Data analysis was done using SPSS version 20 computer software, these data were summarized into proportions and frequencies tables. The associations of $p < 0.2$ was analyzed using logistic regression analysis.

2.9 Ethical clearance

Ethical review and approval was obtained from MUHAS ethical clearance committee and permission to conduct the study was obtained from the Executive Director of MNH through Head of Histopathology unit and Head of department Obstetrics and gynecology, so as to be allowed to use patient's information and other materials.

2.10 Ethical consideration

Participants were informed on the objectives of the study, and no name appeared on the questionnaire. Before taking any information willingness of respondents was required and written informed consent form was provided. The informed consent document was written both in English and Swahili, though the informed consent was provided in Swahili unless otherwise. There were no costs for the participation in the study or financial compensation to participants. Contact address of the principal investigator was given to mothers of stillbirth infants in case of any needed clarification or concern about the study.

CHAPTER THREE

3.0 RESULTS.

From June to December 2018 total number of deliveries at Muhimbili National Hospital was 4779. Among these 4502 were live births and 277 were stillbirths. This makes the incidence stillbirth rate of 58 per 1000 births. Among all the stillbirths, 80 stillbirths who met inclusion criteria were recruited in this study.

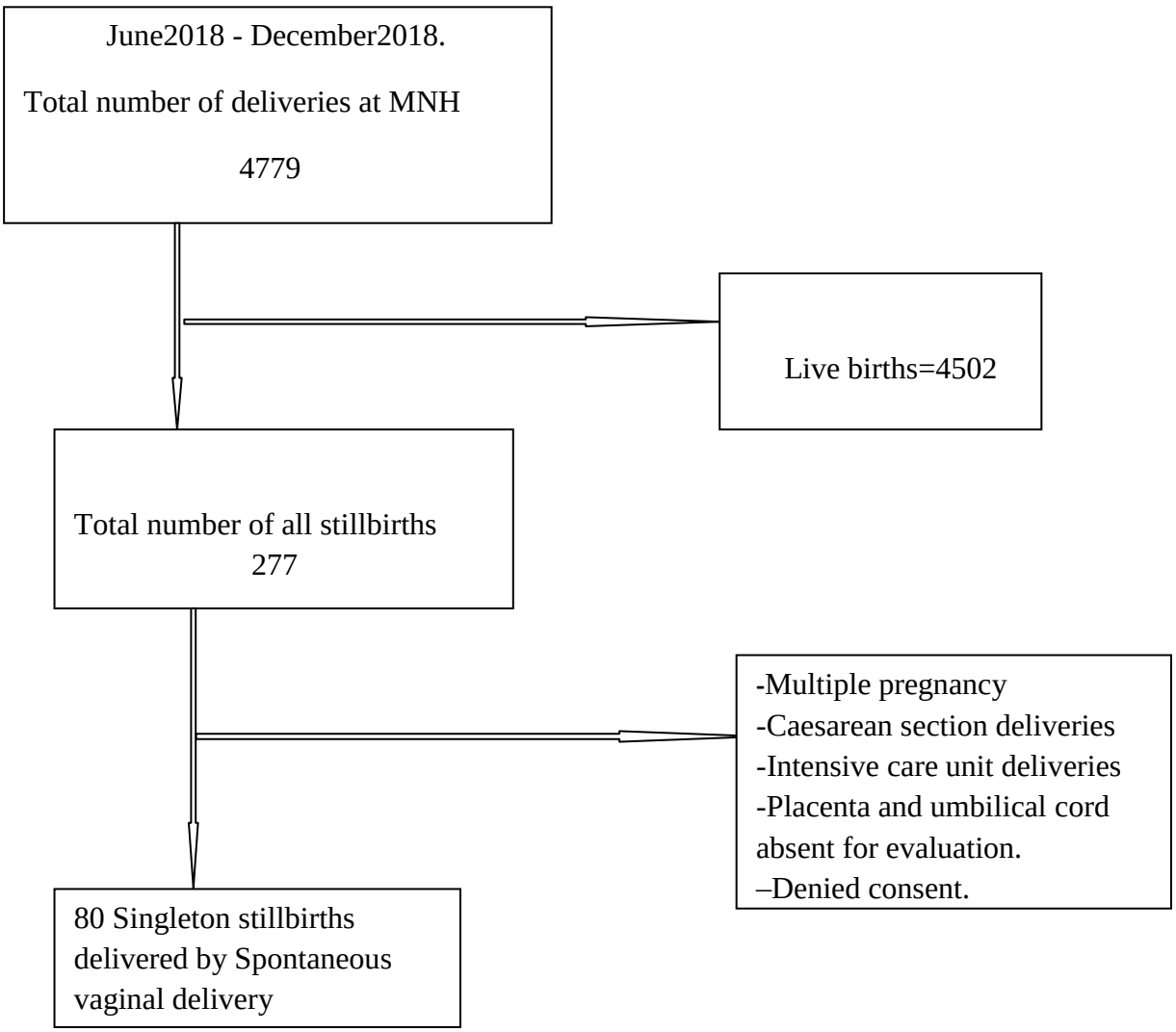


Figure 1: Cases sorting flow chart

3.1 Clinical characteristics of stillbirths

Age of mothers of stillbirths ranged from 18-41 years with a mean age of 27.5 years ± 5.6SD, most of whom 64 (80%) fell in the age group of 20-34 years. More than half of the mothers 44 (55%) were multigravida. All mothers had at least two antenatal clinic visits, most of them 43 (53.8%) attended three visits. Previous history of adverse pregnancy outcome was seen in 11(13.8%) mother of stillbirths. Out of 80 stillbirths, 74 (92.5%) were preterm (≤37weeks of gestation) and the ratio of male to female was 1:1. The mean birth weight was 1753grams ± SD 806.39 with a range of 630-4620grams, nevertheless 50 (62.5%) were small for gestational age. Most fetuses were found to be macerated 64 (80%). See table 1 below.

Table 1: Clinical characteristics of stillbirths at MNH

Clinical characteristics	Frequency N=80 n (%)
Age of the mother(years)	
19 and below	3(3.8%)
20-34	64(80%)
35 and above	13(16.3%)
History of adverse pregnancy outcome(stillbirth and abortion)	
Yes	11(13.8%)
No	69(86.3%)
No of pregnancies	
No previous pregnancy	36(45%)
0ne to three	40(50%)
Four or more	4(5%)
Number of antenatal care visits	
Two visits	6(7.5%)
Three visits	43(53.8%)
Four visits	27(33.8)
Five visits	4 (5%)
Gestational age at delivery(weeks)	
37 or less	74(92.5%)
38-42	6(7.5%)
Weight of the fetus(grams)	
1500 and below	11(13.8%)
1501- 4000	66(82.5%)
4001 and above	3(3.8%)
Fetal birth weight percentile	
Less than 10^{th} percentile (SGA)*	50(62.5%)
10^{th} -90^{th} percentile (APA)*	24(5%)
Above 90^{th} percentile (LGA)*	6(7.5%)
Sex of the fetus	
Male	40(50%)
Female	40(50%)
Condition of the fetus.	
Fresh	16(20%)
Macerated	64(80%)

*SGA (Small for Gestational Age), LGA (Large for gestational age) APA (Appropriate for Gestational age).

Clinical diagnosis of stillbirths

More than half 41 (51.3%) of fetal deaths were due to maternal causes, 3 (3.8%) due to fetal causes and 4 (5%) due to other causes. A significant proportion of stillbirths 32 (40%) had unknown clinical diagnosis as shown in table 2.

The commonest maternal causes of stillbirths were preeclampsia 13 (16.3%) and abruption placenta 12 (15%). Fetal causes were due to congenital anomalies; these were two cases of anencephaly and one case of omphalocele. Other factors were three cases of cord prolapse and one case of cord around the neck. See table 3.

Although there was no significant association of clinical diagnosis and gestational age, majority of maternal causes 36 (87.8%) and unknown causes 24 (75%) occurred at gestational age of equal to or less than 37 weeks. All stillbirths due to fetal and other causes were preterm as shown in table 4 below.

Table 2: General clinical diagnosis of stillbirths

General clinical diagnosis	Frequency(N)
Maternal causes	41(51.3%)
Fetal causes	3(3.8%)
Other causes*	4(5%)
Unknown causes	32(40%)

*Others factors were cord around the neck and cord prolapse.

Table 3: Clinical diagnosis of stillbirths

Clinical diagnosis	Frequency (N=80)
Known Diagnosis	48(60%)
Pre-eclampsia	13(16.3%)
Abruptio placenta	12(15%)
Severe pre-eclampsia	8(10%)
Lethal congenital anomalies *	3(3.8%)
Cord prolapse	3(3.8%)
Pregnancy induced hypertension	2(2.5%)
Gestational diabetes mellitus	2(2.5%)
Eclampsia	2(2.5%)
Hydrops fetalis	1(1.3%)
Severe oligohydramnios	1(1.3%)
Cord around the neck	1(1.3%)
Unknown Diagnosis	32(40%)

*Lethal congenital anomalies were two cases of anencephaly and one case of omphalocele.

Table 4: Clinical diagnosis by gestational age (in weeks)

	Gestational age (weeks)			
Clinical diagnosis	≤37	>37	Total (N)	*p*-Value
Maternal causes	36 (87.8%)	5(12.2%)	41(100%)	0.203
Fetal causes	3(100%)	0(0%)	3(100%)	
Other causes	4(100%)	0(0%)	4(100%)	
Unknown causes	24(75%)	8(25%)	32(100%)	

3.1.2 Frequency of unknown clinical diagnosis

The pie chart below shows that 48 (60%) of stillbirths had known clinical diagnosis as seen in table2 above, while 40% of stillbirths had unknown clinical diagnosis.

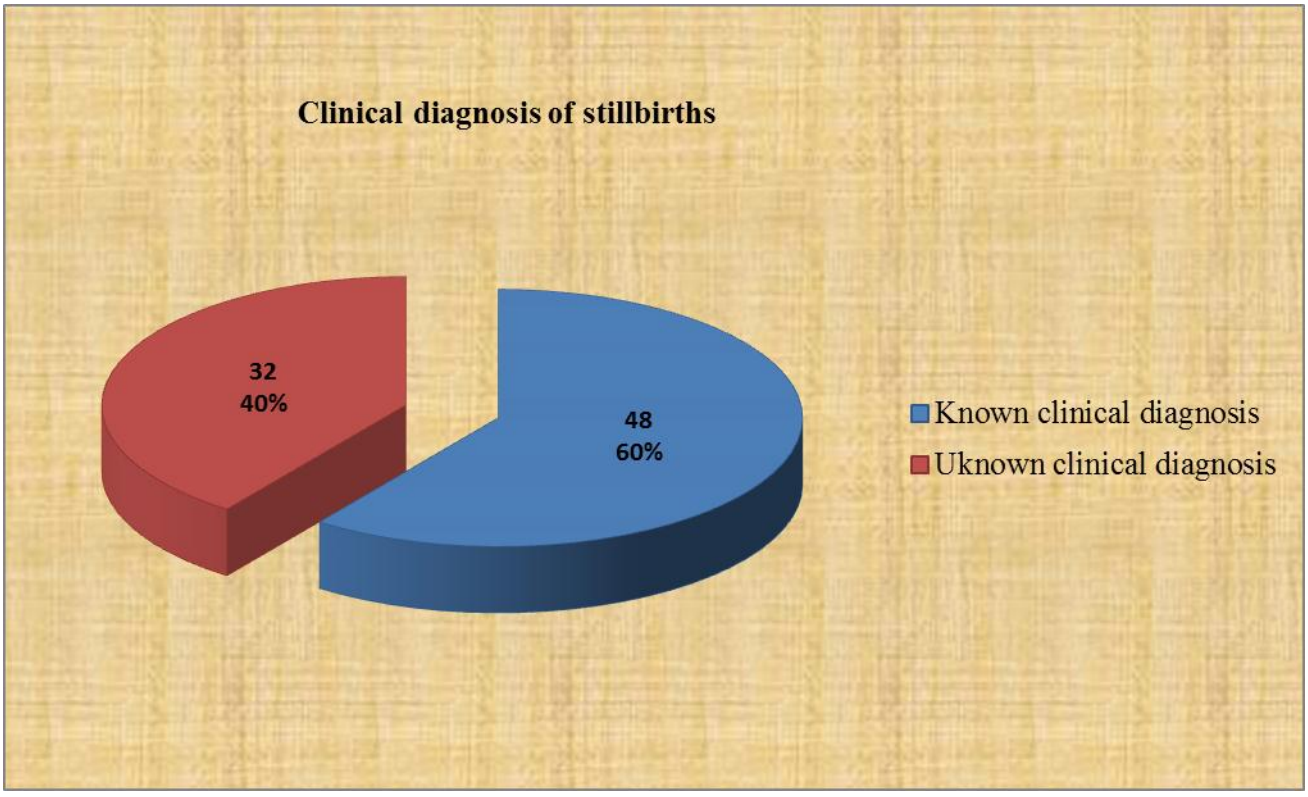


Figure 2: Clinical diagnosis of stillbirths

3.1.3 Gross and microscopic pathologies of placentas in Stillbirths

Out of 80 examined placentas, 45 (56.3%) had combined morphological pathologies with risk to cause stillbirth. Isolated placenta parenchyma and isolated umbilical cord pathologies were seen in 26 (32.5%) and 2 (2.5%) respectively. In general 73 (91.3%) of the stillbirths' placentas had pathologies with risk to cause stillbirths while 7 (8.8%) of placentas had no pathology found as shown in figure 3 below.

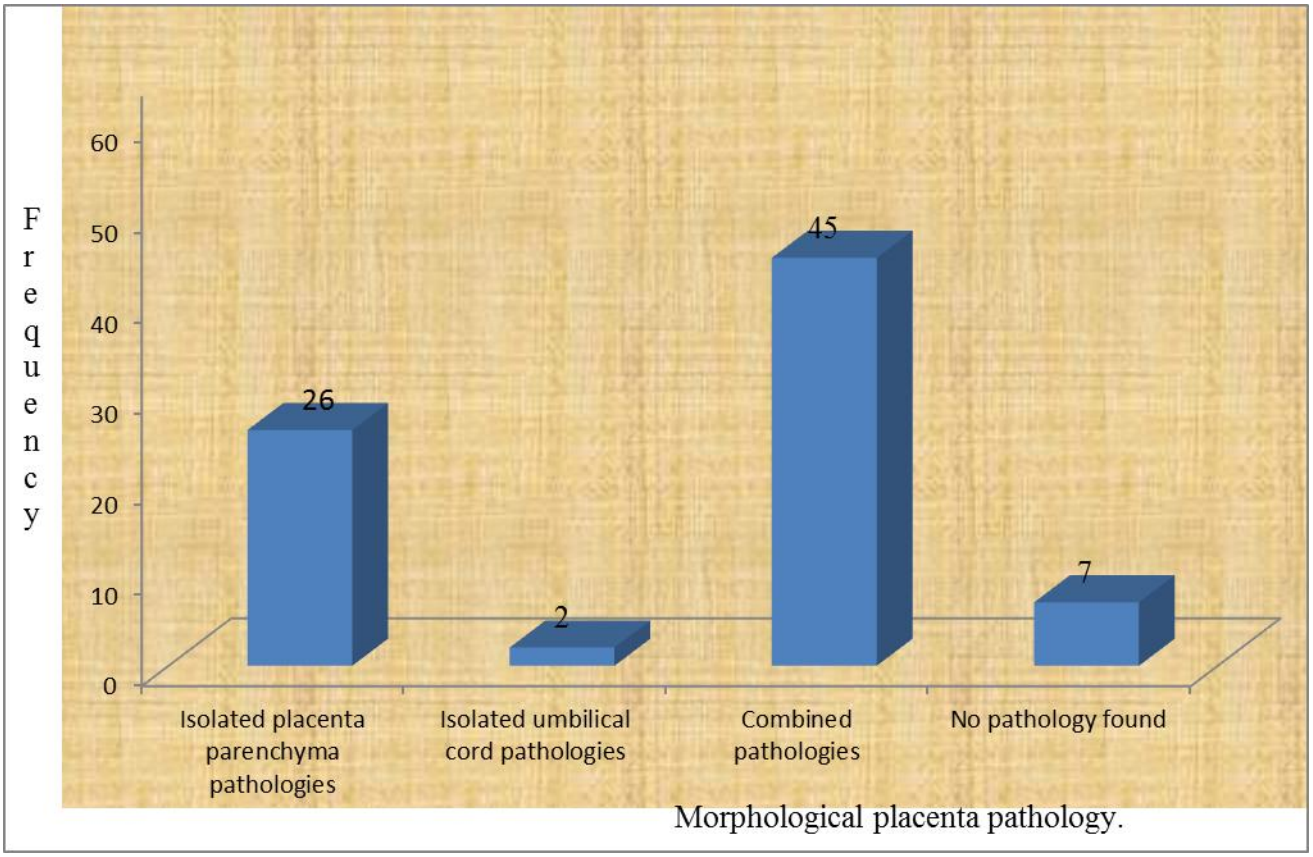


Figure 3: Overall morphological placenta pathologies in stillbirth

The commonest placenta parenchymal pathology was maternal vascular malperfusion 45 (56.25%), followed by acute chorioamnionitis 32 (40%). Other lesions were villitis of unknown etiology 6 (7.5%), fetal vascular malperfusion 6 (7.5%), massive perivillous fibrin deposition/maternal floor infarction 4 (5%) and placental malaria 2 (2.5%).

Abnormalities of coiling of umbilical cord were the commonest among umbilical cord pathologies accounted for 11 (13.8%) for hypercoiling and 8 (10%) for hypocoiling. Other abnormalities were single umbilical cord artery, velamentous insertion each occurred in 3 (3.8%) cases, furcate insertion was the least common abnormality seen in 1 (1.3%) of all stillbirths. See table 5 below.

Table 5: Morphological pathologies of the placenta with risk to cause stillbirth

Morphological placental pathologies	Frequency (N=80) n(%)
Placenta parenchymal pathologies	
Maternal Vascular Malperfusion	45(56.25%)
Acute Chorioamnionitis	32(40%)
Villitis of Unknown Etiology	6(7.5%)
Fetal Vascular Malperfusion	6(7.5%)
Massive Perivillous Fibrin deposition/Maternal floor infarction	4(5%)
Malaria	2(2.5%)
Umbilical Cord Pathologies	
Hypercoiling of umbilical cord	11(13.8%)
Hypocoiling of umbilical cord	8(10%)
Single Umbilical Artery*	3(3.8%)
Velamentous insertion	3(3.8%)
Furcate insertion	1(1.3%)

* One case of single umbilical cord artery occurred together with omphalocele.

Characteristic lesions of maternal vascular malperfusion (MVM) seen in this study are listed in figure 4 below. Infarction was the commonest lesion found in 22 (32%) followed by abruption placenta 14 (20%) which requires clinical history to support diagnosis. Others were decidual arteriopathy 11 (16%), accelerated villous maturation 9 (13%), distal villous hypoplasia 5(17%), acute atherosis 4 (6%) and chorangiosis 4 (6%).

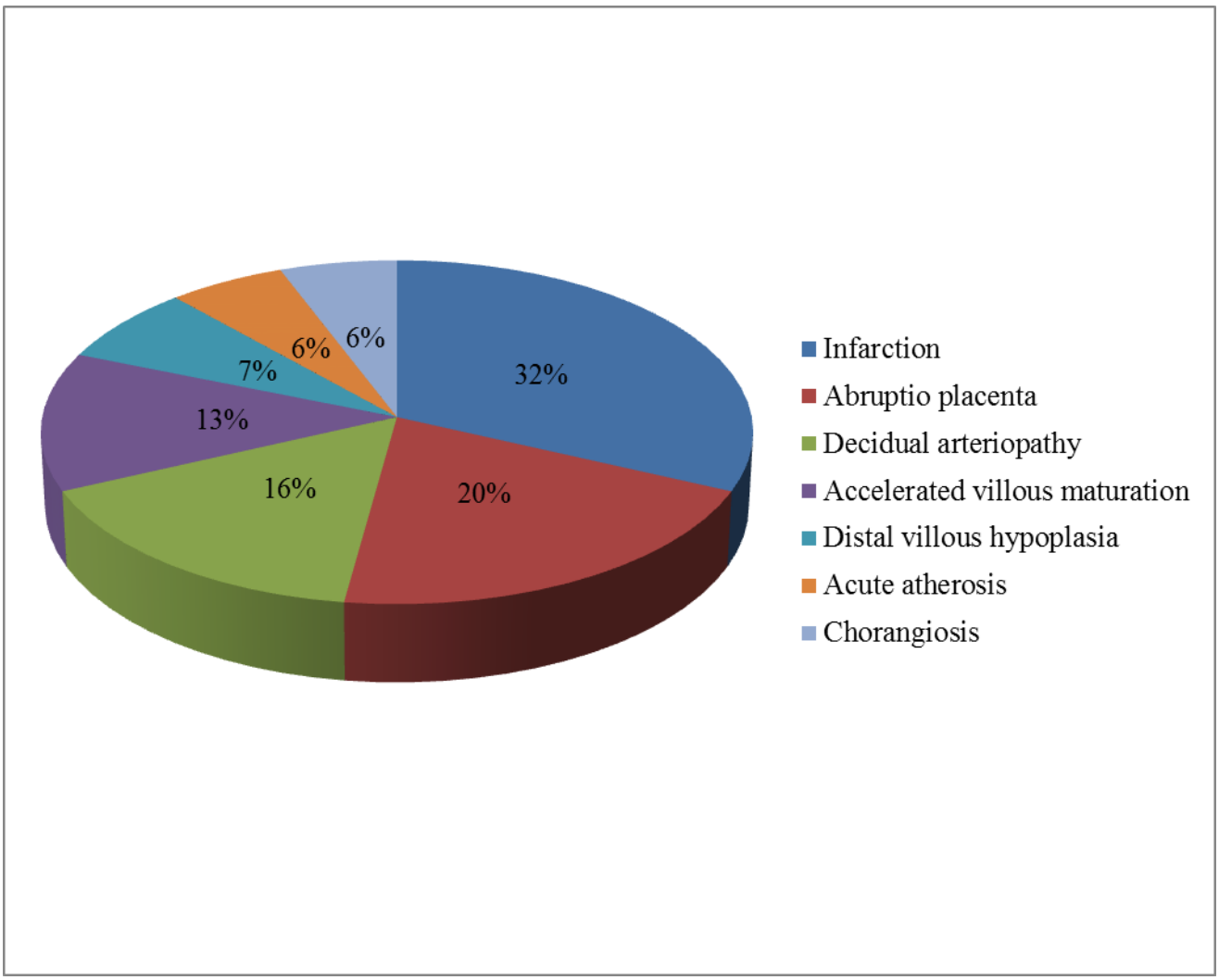


Figure 4: Characteristic lesions of maternal vascular malperfusion

Among several placenta pathologies with risk to cause stillbirths shown in table 5 above, some have risk of recurrence as shown in table 6 below. Maternal vascular malperfusion was the commonest followed by acute chorioamnionitis.

Table 6: Placenta pathologies with risk of recurrence

Placenta pathologies with risk of recurrence	Frequency n (%)
Maternal Vascular Malperfusion	45(56.25%)
Acute Chorioamnionitis	32(40%)
Villitis of Unknown Etiology	6(7.5%)
Massive Perivillous Fibrin deposition/Maternal floor infarction	4(5%)

In the group of placenta pathologies with risk of recurrence, 35 (77.8%) of maternal vascular malperfusion had clinical diagnosis and this was statistically significant. Other pathologies with risk of recurrence in at least half of cases they had unknown clinical diagnosis as seen in table 7 below.

Table 7: Association of placenta pathologies with risk of recurrence and clinical diagnosis of stillbirth

Placenta pathologies		**Clinical diagnosis**			
		Known	**Unknown**	**Total**	***p*-Value**
Acute chorioamnionitis	Yes	15 (46.9%)	17(53.1%)	32(100%)	0.05
	No	33(68.8%)	15(31.2%)	48(100%)	
Maternal Vascular malperfusion	Yes	35(77.8%)	10(22.2%)	45(100%)	0.001
	No	13(37.1%)	22(62.9%)	35(100%)	
Maternal floor infarction/MPFD	Yes	2(50%)	2(50%)	4(100%)	0.678
	No	46(60.5%)	30(39.5%)	76(100%)	
Villitis of unknown etiology	Yes	3(50%)	3(50%)	6(100%)	0.607
	No	45(60.8%)	29(39.2%)	74(100%)	

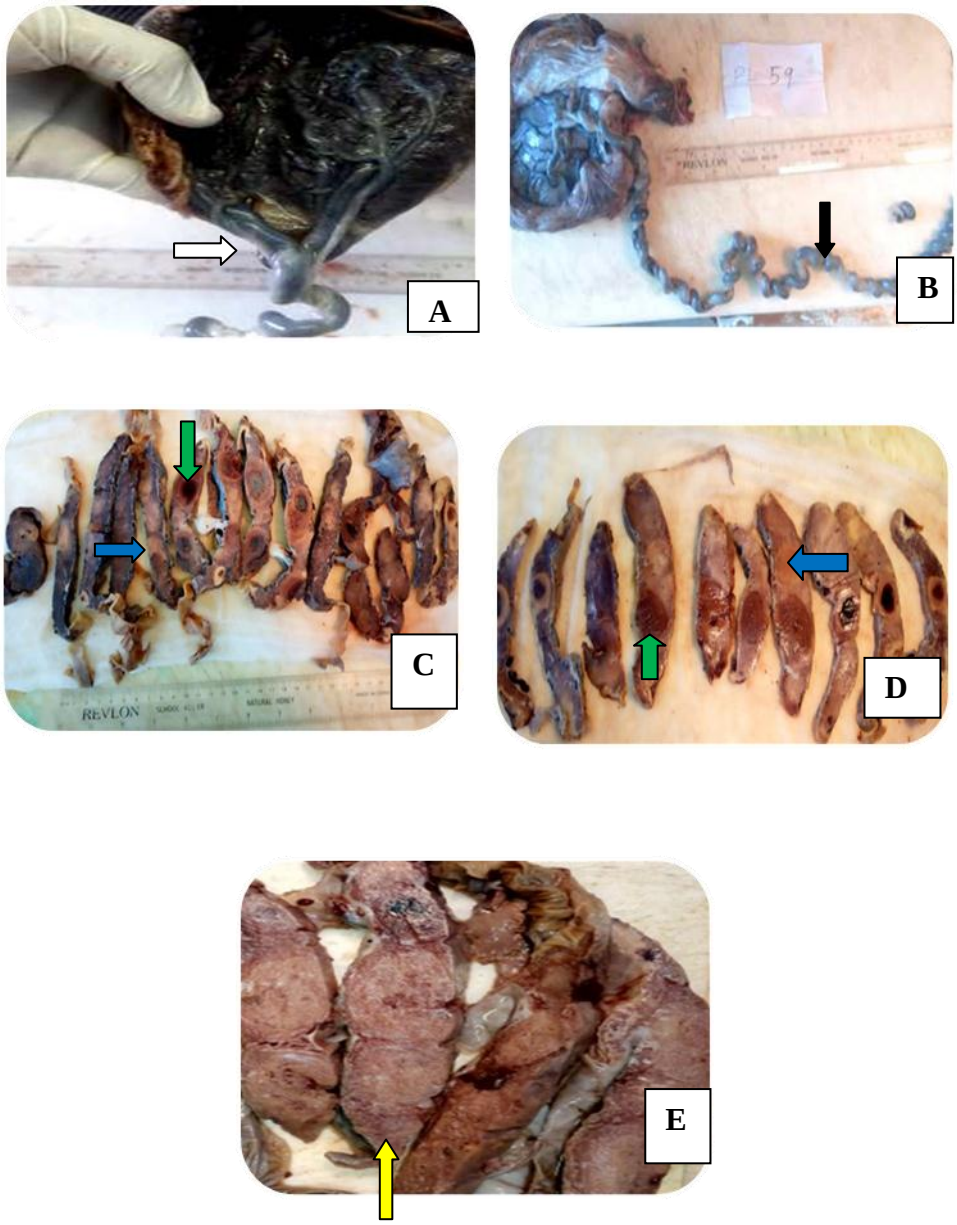


Figure 5: Gross appearance of placenta pathologies

Figure 5 above shows different gross pathologies of the placenta with risk to cause fetal death. A: Furcate umbilical cord insertion B: Hypercoiling of umbilical cord. Figure C and D: Show areas of infarction, green arrows show new infarct and blue arrows show old infarct. E: This shows normal placenta parenchyma.

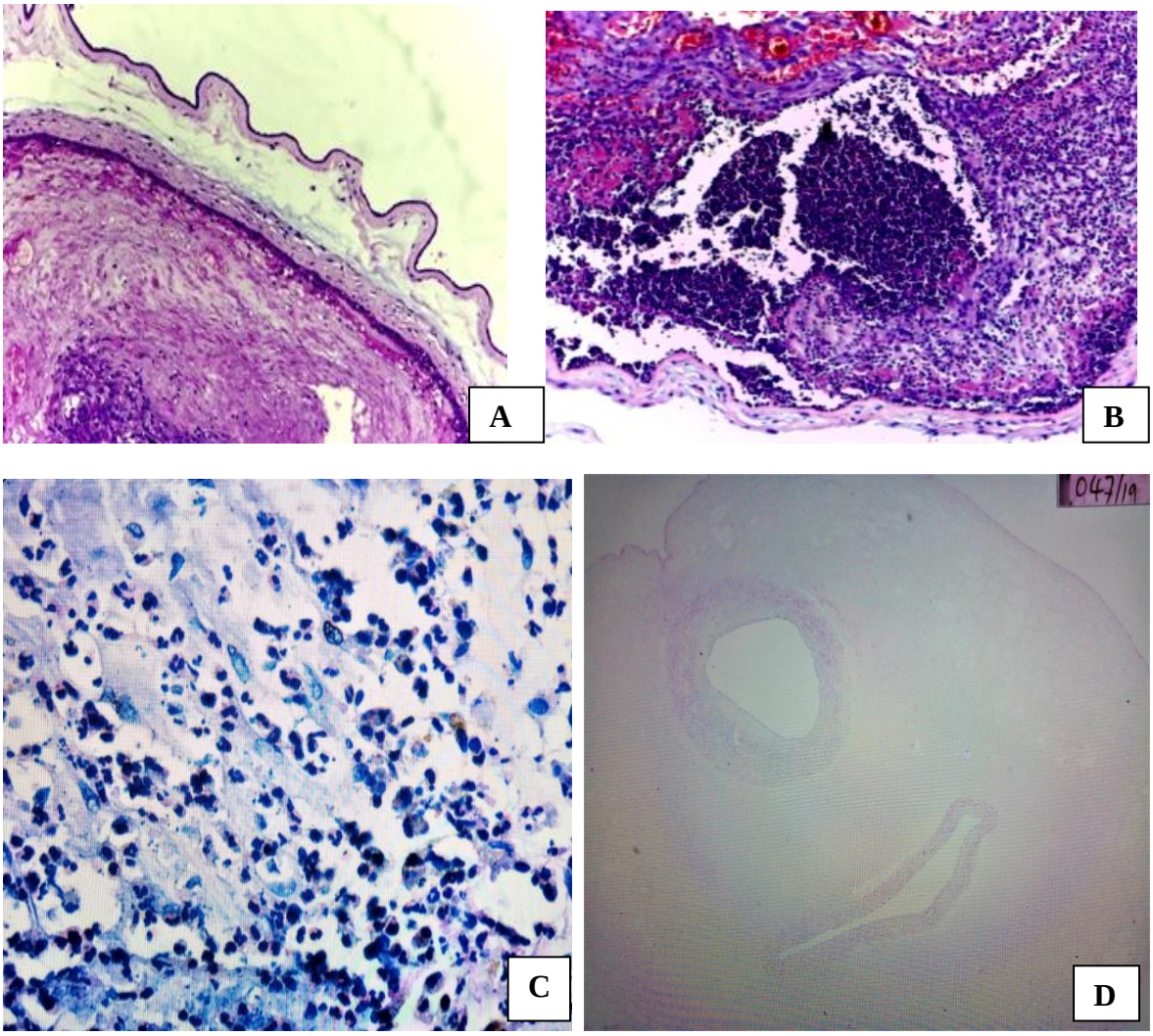


Figure 6: Photomicrographs of histomorphological pathologies of placenta with risk to cause fetal death.

A: Section of normal placenta membrane devoid of polymorphonuclear inflammatory cells. Original magnification 4x hpf. B and C: Show acute chorioamnionitis, brisk polymorphonuclear inflammatory cells are observed in the membranes and decidua. Original magnification;10x hpf. D: Section of umbilical cord shows single umbilical cord artery

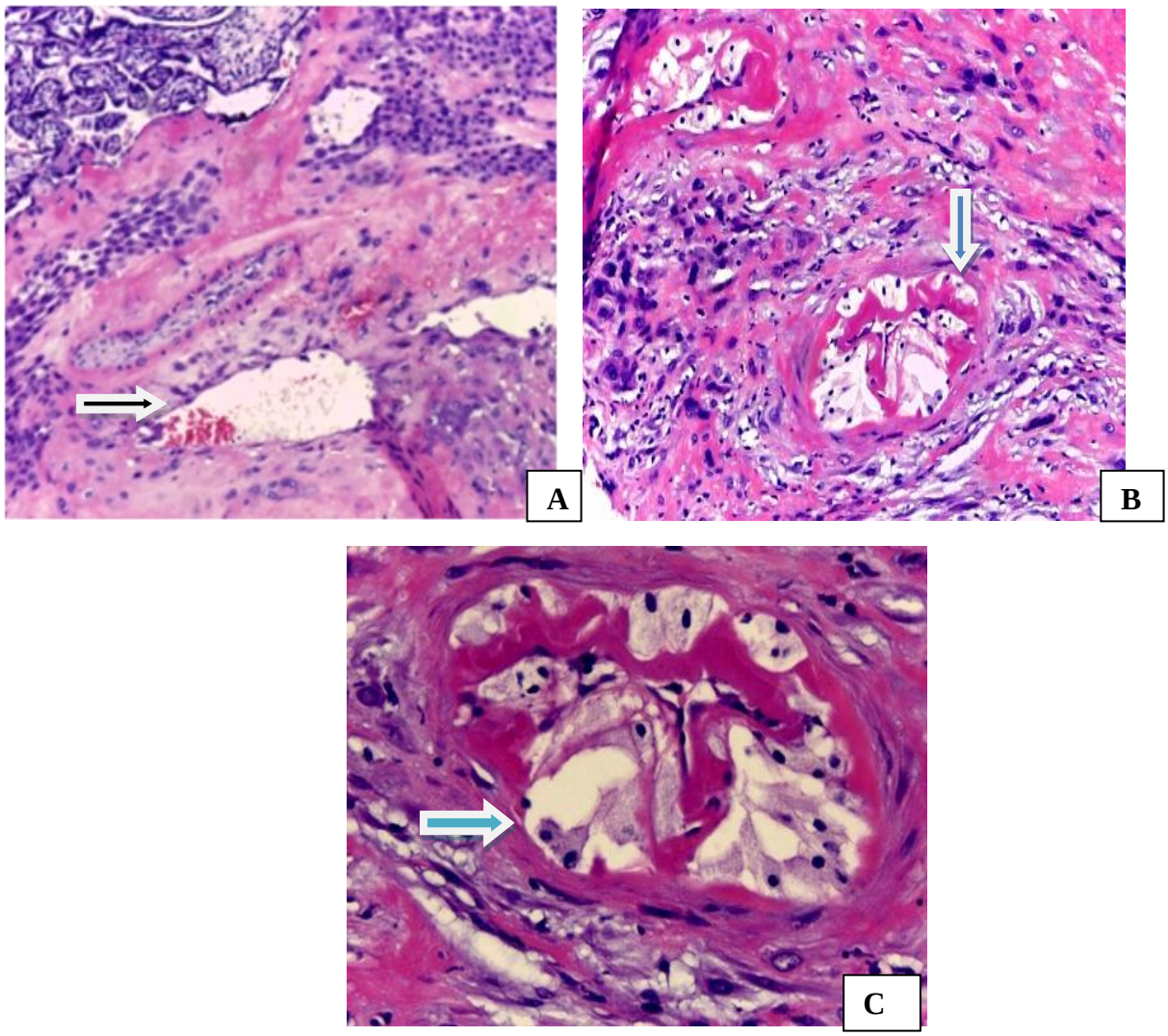


Figure 7: Photomicrographs of histomorphological pathologies of placenta with risk to cause fetal death

A: The black arrow shows transformed decidual plate vessels due to invasion by trophoblasts. Original magnification ; 4x hpf. B: The blue arrow shows vessel with acute atherosis due to deficiency of trophoblast invasion, these vessels show fibrinoid necrosis of the wall and foamy macrophages embedded within fibrinoid necrosis. Original magnification : 10x hpf. C: The high power view of the vessel in figure C above showing acute atherosis. Original magnification ; 40x hpf.

* hpf: High power field

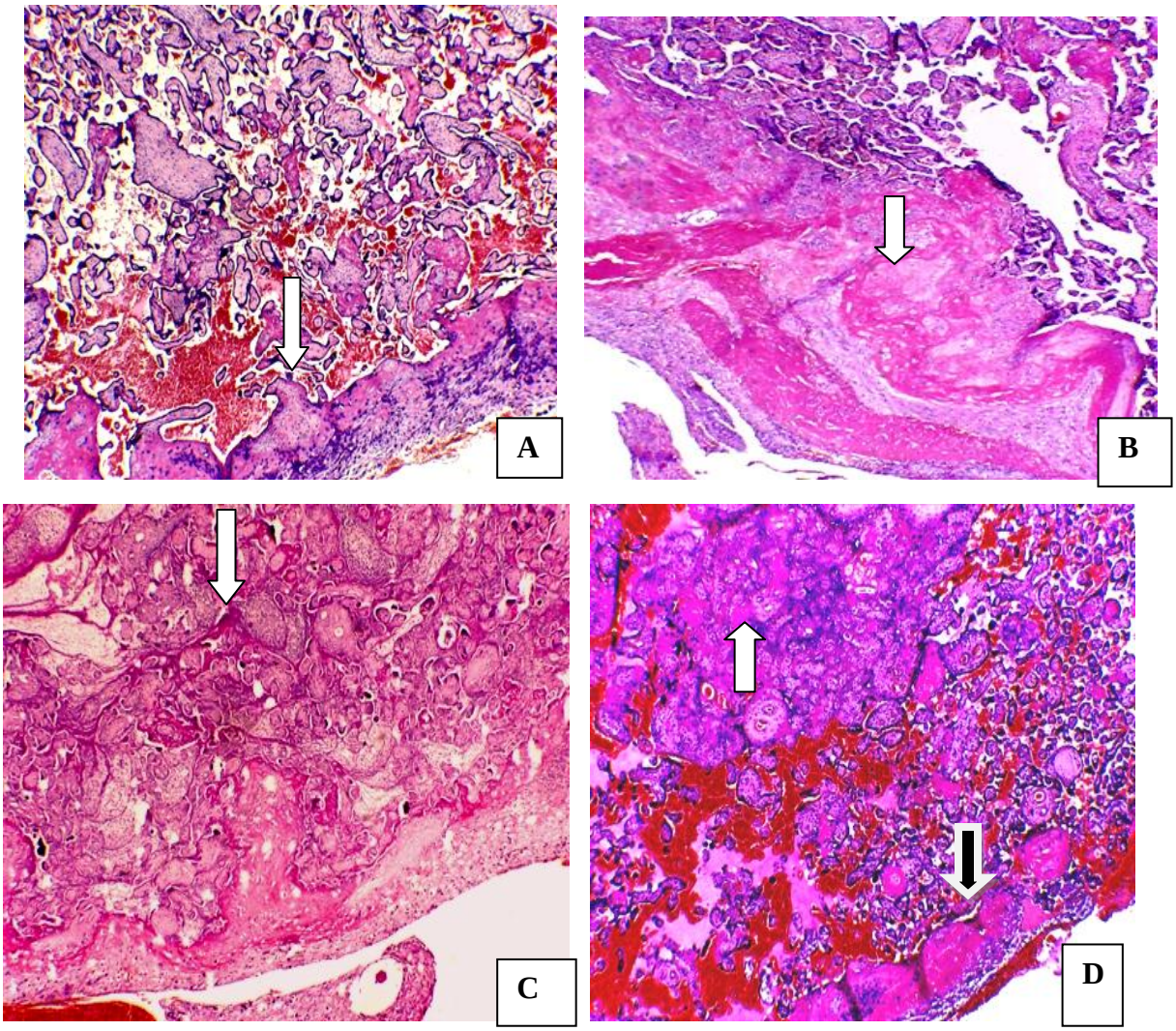


Figure 8: Photomicrographs of histomorphological pathologies of placenta with risk to cause fetal death

A: Normal appearing basal villi of the maternal floor of the placenta shown by an arrow. B: Placenta infarction of term placenta involving focal area of the maternal floor villi (whitearrow) Original magnification: 10x hpf C: Maternal floor infarction whereby basal villi of the entire maternal floor were encased by perivillous fibrin of equal to or more than 3mm thickness this is supported by 25% or more gross involvement of the placenta. Original magnification: 4x hpf. D: Infarcted placenta showing well circumscribed perivillous and intravillous fibrin deposit of the villi(white arrow), sparing maternal floor (black arrow). Original magnification : 4x hpf .

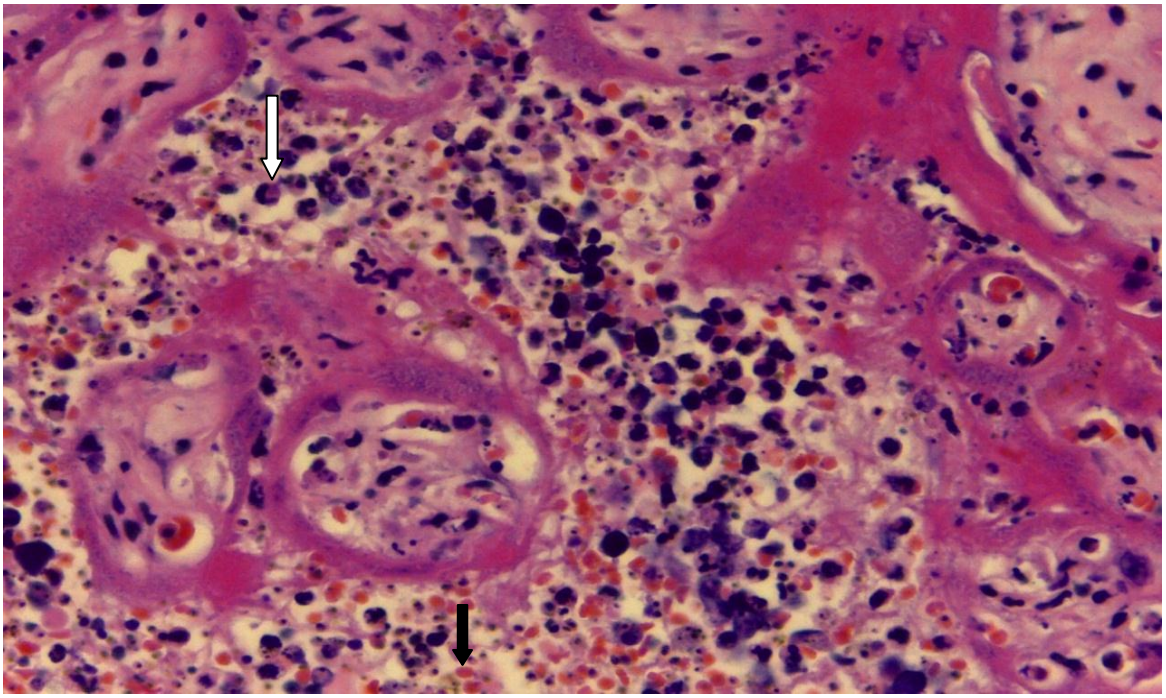


Figure 9: Photomicrographs of histomorphological pathologies of placenta with risk to cause fetal death

The section shows parasitized erythrocyte (white arrow), the black arrow shows malaria brown pigments. Original magnification: 40x hpf

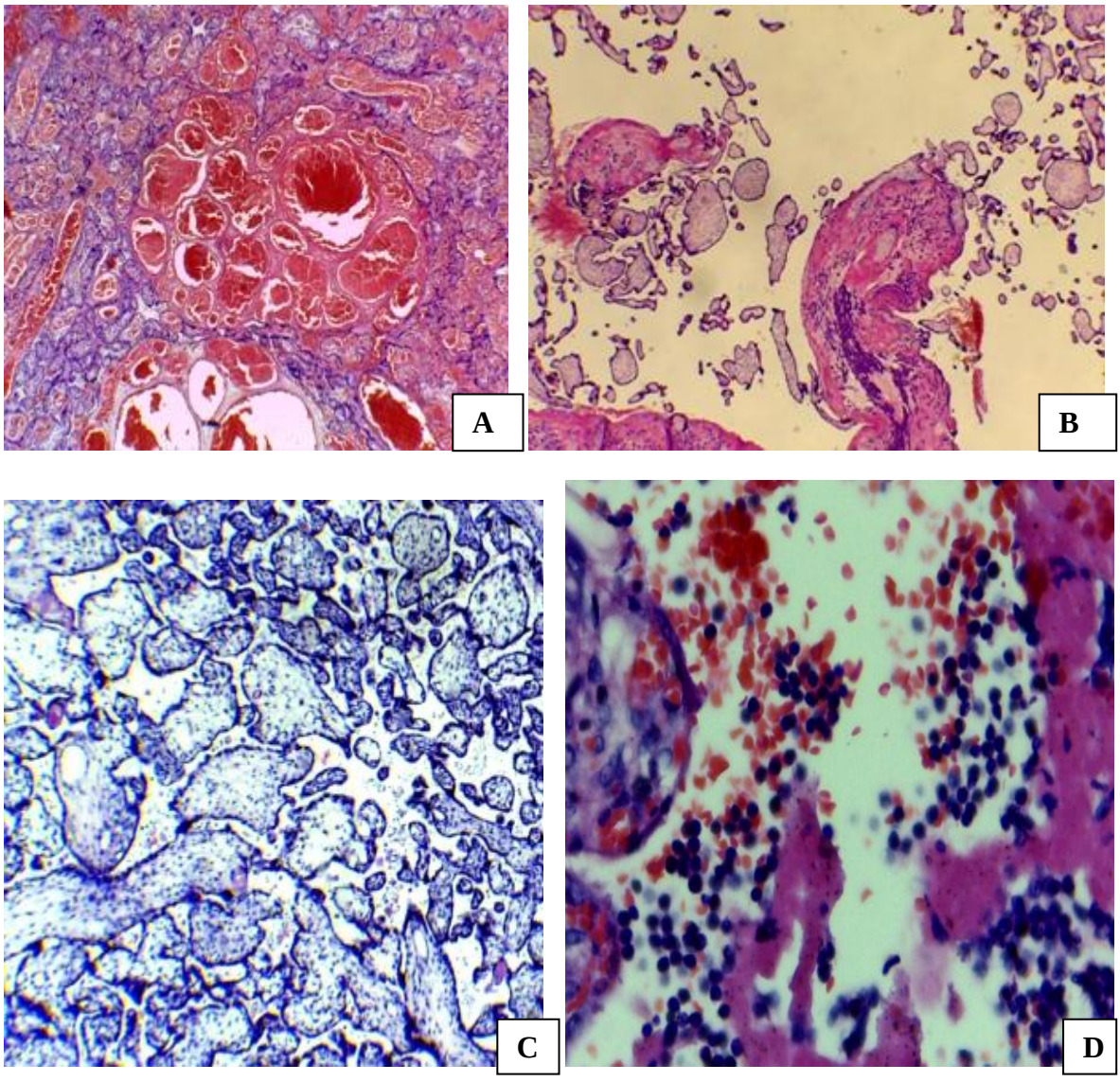


Figure 10: Photomicrographs of histomorphological pathologies of placenta with risk to cause fetal death.

A: Histological features of chorangioma whereby there was proliferation of vessels and vascular congestion.

B: Showed distal hypoplasia whereby the distal villi become few and elongated.

C: Showed delayed maturation of the villi, they were large with reticular stroma.

D: Lymphocytic infiltrate in villi and intervillous space in villitis of unknown etiology.

3.1.4 Association of placenta pathologies and gestational age.

Table 8 below shows that 91% of stillbirths with maternal vascular malperfusion had gestational age of equal to or less than 37 weeks, the association of this pathology and gestational age was statistically significant.

Table 8: Association of Microscopic Placenta Pathologies and Gestational Age.

		Gestational age(weeks)			
Placenta pathologies		≤37	37- 42	Total	*p*-Value
Acute chorioamnionitis	Yes	25 (78.1%)	7(21.9%)	32(100%)	
	No	42(85.7%)	6(12.2%)	49(100%)	0.13
Fetal vascular malperfusion	Yes	4 (80%)	1 (20%)	5(100%)	
	No	63(84%)	12 (16%)	75(100%)	0.41
Maternal Vascular malperfusion	**Yes**	**41(91%)**	**4(9%)**	**45(100%)**	
	No	**26(74.3%)**	**9(25.7%)**	**35(100%)**	**0.02**
Maternal floor infarction/MPFD	Yes	3(75%)	1(25%)	4(100%)	
	No	64(84.2%)	12(15.8%)	76(100%)	0.31
Villitis of unknown etiology	Yes	5(83.3%)	1(16.7%)	6(100%)	
	No	62(83.8%)	12(16.2%)	74(100%)	0.499

CHAPTER FOUR

4.0 DISCUSSION

Stillbirth is devastating and stressful experience to parents and caregivers, identifying the cause aids in the process of mourning, to determine risk of recurrence and counseling regarding future pregnancy (12). The magnitude of unexplained cause of stillbirths ranges from 3.8-57.4% depending on classification system used. The systems which involve pathological examination of placenta have lower proportion of unknown cause of stillbirths (24). At MNH clinical evaluation is the only method used with no published figures of proportion of unknown clinical diagnosis. This descriptive cross-sectional study showed most stillbirths were preterm, macerated and small for gestational age. It also showed that 40% of cases of stillbirths had unknown clinical diagnosis. Furthermore majority of the placentas of stillbirths 73 (91.3%) had one or combined pathologies with the risk to cause stillbirths. Maternal vascular malperfusion was the commonest placental pathology, it has been reported to be associated with risk of recurrence and it was significantly associated with low gestational age (≤37 weeks of gestation).

A total number of deliveries during the study period were 4779, 277 were stillbirths among these 80 were recruited in the study. An incidence stillbirth rate of 58 per 1000 was found, similar rate was observed in Northern Tanzania (9). This stillbirth rate is lower than what was observed by Kidanto et al at MNH on the assessment of qualitative perinatal audit whereby stillbirth rate was 68 per 1000 (10). The decline in stillbirth rate could be due decline in intrapartum stillbirths, programs such as help baby breathe reduce intrapartum stillbirths (11). Reduction in intrapartum stillbirths can also explain the reason why majority of stillbirths 64 (80%) in our study were macerated. This high rate of macerated stillbirths was also reported in other regions of sub-Saharan Africa and South Asia (48).

Perinatal audit done at MNH found that most of stillbirths occur in younger mothers, aged less than 20 years in contrast to this current study 64 (80%) of mothers of stillbirths aged between 20-35 years with the mean age of 27.5 years (9). This could be due to the fact that the

more the women advance in formal education the lower the frequency of pregnancy at younger age (25). Most mothers in this study had at least secondary education 72 (90%); the levels of education of the stillbirths' mothers were not documented in the perinatal audit study. Moreover 74 (92.5%) of stillbirths in our study were preterm comparable to the findings in Tanzania, Rwanda, India and United States (11,26,28,32). Stillbirths are strongly interrelated to preterm births (33).

Pre-eclampsia, severe pre-eclampsia, eclampsia, pregnancy induced hypertension, abruption placenta, severe oligohydramnios and Diabetes mellitus were maternal factors which cause stillbirth reported in this study, these findings agree with the findings in other studies (23,26,32). Maternal factors were seen in 41 (51.3%) of stillbirths, similar to the findings by Aminu et al, where a range of 8-50% was reported (24).

Lethal congenital anomalies in the current study occured in 3 (3.8%) cases of stillbirths, commonly affecting central nervous system. There were two cases of anencephally and one case omphalocele, comparable findings were reported by Grover et al, who found that most of the the lethal anomalies were affecting central nervous system (71). Other studies reported a range of 2.2% -33.3% of lethal congenital anomalies (24). High proportion of lethal congenital anomalies were seen in studies which fetal autopsies were performed. In our study fetal autopsies were not performed and this gives room for further studies.

In 32 (40%) of 80 cases of stillbirths had no diagnosis reached to explain the cause of fetal death after clinical evaluation alone. This is within a range of 3.8% to 57.4% reported in systematic literature review by Aminu et al, the difference in proportion of unknown diagnosis of stillbirths from one study to another was due to classification system used. Extended Wiggleworth, Aberdeen and locally adopted classification system were used, classification system which had incorperated pathological examination of placenta had lower number of unknown cause of stillbirths (24). A study done by Bonetti et al, by using another classification system(ReCoDe) which placenta examination is part of it found that

20.16% of stillbirths had unknown diagnosis (34). Lower proportion of 5.6% of unknown cause of stillbirths was reported by Sharma et al, whereby a local guideline which involved examination of placenta was adopted. Unlike in our study the proportion of unknown clinical diagnosis was found to be higher because placental examinations are not routinely performed at MNH.

Combined placenta pathologies were the most common lesions in our study seen in 45 (56.25%), the heterogeneity of placenta pathologies was also reported by Pinar et al (22), concord to other studies which reported that most pathologies in stillbirths placentas are combined (41). Moreover in this study pathologies in placentas of stillbirths (combined , isolated umbilical cord and isolated placental parenchyma pathologies) were present in 73 (91.3%) of all stillbirths, this finding is supported by Kulkarni et al who reported that with detailed placenta examination in more than 90% cause of stillbirth may be identified (17). Vergan et al also concluded that systems which classify cause of stillbirth without including pathological examination of the placenta as inadequate (40).

Among the umbilical cord pathologies in this series, umbilical coiling abnormalities were common parallel to the report by Gurusamy et al and Chang et al (41,44). Other umbilical abnormalities were single umbilical cord artery abnormality which occurred in 3.8% a lower rate compared to 7.7% reported by others (22). It has been found that up to 56% of cases of single umbilical cord abnormalities are associated with chromosomal defects or non-chromosomal congenital defects (47). In our study one case of single umbilical cord artery (1.3%) was associated with congenital anomaly (omphalocele) although chromosomal abnormalities were not part of assessment in this study.

Abnormal umbilical cord insertions found in the current study included velamentous insertion which occurred in 3.8% this did not differ much from 5% reported by others. Furcate insertion was the rarest among umbilical cord abnormalities occurred in 1.3% accord with other studies (22,43). Tendency of umbilical abnormalities to occur together with placenta parenchyma pathologies was also reported in other studies whereby association with maternal vascular

malperfusion, fetal vascular malperfusion and inflammation was found (44). These umbilical cord lesions they diminish blood flow in the umbilical vessels which lead to hypoxia and thereafter fetal death (43).

Among placental parenchyma pathologies, maternal vascular malperfusion was the commonest occured in 45 (56.25%) , frequency of 46% was reported by Chang et al (41) and 70.7% by Pacora et al (52). The difference can partly be explained by challenges in diagnosing maternal vascular malperfusion lesions due to the fact that they occur deeper in decidual while delivered placenta has only thin layer of decidua which is superficial and more likely to be transformed than deeper decidua. These lesions also occur only focally which require extensive and careful search (51). In this study the characteristic lesions of maternal vascular malperfusion in descending order were infarction, abruptio placenta, decidual arteriopathy, accelerated villous maturation, distal villous hypoplasia, acute atherosis and chorangiosis these lesions were also reported by other studies (51).

Most of stillbirths with maternal vascular malperfusion pathology in their placentas had known clinical diagnosis. This could be due to the fact that MVM has been described to be caused by several maternal diseases such as pre-eclampsia, eclampsia, abruptio placenta and gestational diabetes mellitus (50).

Recurrence rate of maternal vascular malperfusion ranges from 10-25% (49), hence counseling regarding future pregnancies is important. MVM leads to fetal death by causing hypoxic damage to the placenta, deficiency of spiral arterioles remodeling and therefore responsiveness to vasoconstrictors has been found to be its pathogenesis (50). Trial of low dose aspirin has been found successful as it causes vasodilatation and increase of blood perfusion and therefore improve maternal vascular perfusion (54,55).

Acute chorioamnionitis and villitis of unknown etiology (VUE) were the inflammatory conditions found in our study. Acute chorioamnionitis occurred in 51.3% a frequency near to the observation seen by others (24), a lower rate of 19% was seen by Chang et al (41) which is attributed by differences in definitions of this lesion. Organisms such as listeria

monocytogens, group B streptococcus and chlamysia psittaci have been found to cause acute choriamnionitis (63), although no further staining methods to identify microorganism were done in our study due to financial constrains.

Villitis of uknown etiology occurred in 7.5%, a slightly higher frequency of 19% was seen by others (52). These differences could be due to usage of different guidelines in defining placental pathologies as there is no universal guidelines for definitions of placenta pathologies (12). Etiology of VUE in more than 90% is unknown and postulated to be due to maternal alloimmune response (56).

Both of these inflammatory conditions have risk or recurrence, acute chorioamnionitis have a recurrence rate of 10-25% while villitis of unknown etiology have recurrence rate of 25-50% (49). Correct diagnosis of these lesions is important for estimation of of risk of recurrence and therefore counselling to the family regarding future pregnancies.

Greater disparity from other studies was observed in the proportion of fetal vascular malperfusion (FVM). In our study it occurred in 6 (7.5%) while other studies report a rate of 48% -51% (41,52). FVM mimics postmortem changes, global distribution favors postmortem changes rather than antemortem changes (57). Therefore care should be taken to distinguish the antemortem and postmortem changes as there is tendency to over diagnose this lesion.

Maternal floor infarction/massive perivillous fibrin deposition (MFI/MPFD) is the rare pathology which occur in 1% of all pregnancy but with a high recurrence rate of up to 78% (62). Four out of 80 (5%) stillbirths placentas had this pathology in our study, the etiologies could be autoimmune, cytotoxicity, infections and failure of maternal tolerance to the fetus (60,61). Mothers of these stillbirths can benefit from intravenous immunoglobulin, heparin, aspirin and pravastatin to reduce risk of recurrence of this pathology in future pregnancies (59).

Although most of placenta pathologies found in this study occurred at gestational age of less or equal to 37 weeks, only maternal vascular malperfusion was significantly associated with preterm stillbirth, comparable to the findings by Wright et al (53). Proper diagnosis of

placental pathologies is mandatory as some of them have risk of recurrence which ranges from 10-75% (49), thus aids in counseling and close follow up of future pregnancies.

Study limitation.

1. This study was a prospective study conducted in a period of six months, due to time factor and financial constraints larger sample size of more than 80 placentas of stillbirths could not be collected; convenience sampling was used in order to maximize sample size.
2. Due to failure to do further special staining for microbiological analysis pose some difficulties to confirm the diagnosis of infectious agent. Moreover failure to do chromosomal analysis made difficulties to confirm the diagnosis of genetic related disease.
3. Due to time factor proper controls of live births placentas could not be studied, this would have helped to establish causal association between stillbirths' placentas and pathologies, hence increase the power of the study.
4. Lack of universal agreed protocols on interpreting placenta lesions made difficulties in comparing results with other studies.
5.

Strength of the study

Muhimbili National Hospital received referrals from different parts of Tanzania therefore this was better study location to study and describe pathological lesions of placenta associated with stillbirths. Since it was a prospective study principal investigator made sure that fixation protocols of placenta and umbilical cords were adhered in terms of time and type of fixative used (10% neutral buffered formalin). Grossing techniques were followed, extensive review of literatures and guidelines were used to make detailed interpretation of both macroscopic and microscopic findings of placenta and umbilical cords.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION+

5.1 Conclusion.

Pathological examination of placenta in stillbirths is important as it gives information about intrauterine life, in this study clinical assessment alone was not able to make a diagnosis in up to 40% of stillbirths. With detailed pathological examination of placenta more than 90% of placentas were found to have pathological lesions. In cases of multiple conditions which occur together, the association of these conditions to fetal death may also be established. Pathological examination of placenta and clinical evaluation when used together can significantly reduce the proportion of unknown cause of stillbirths.

5.2 Recommendations.

1. We recommend that pathological examination of placenta should be performed in all cases of stillbirths at MNH especially those with unknown clinical diagnosis as useful details may be found to estabilsh cause of fetal death.
2. Further case control studies of stillbirths should be done in order to elucidate strong causal relationship of placenta pathologies to the stillbirths.
3. Universal guidelines on reporting placenta pathologies should be adopted in order to improve reproducibility of results.

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APPENDICES

Appendix I: Consent Form (English Version)

ENGLISH VERSION CONSENT FORM FOR PARTICIPATION IN THE STUDY

Consent to volunteer in a study Titled: Pathological study of placenta in stillbirths at Muhimbili National Hospital Dar es Salaam Tanzania.

Introduction

My name is Atuganile E Malango a Resident pursue Master of Medicine in Anatomical pathology at Muhimbili University of Health and Allied Science. My Dissertation project is aimed at performing pathological study of placenta in stillbirths at MNH

Purpose of the study

The purpose of this study is to assess the role of pathological examination of placenta in determining cause of stillbirth which has been suggested by several studies and guidelines to be one of the assessments in stillbirth. This will reduces unknown causes of stillbirths and to explain the pathophysiology of clinical diagnosis reached.

Description of Research activities

If you agree to participate clinical information will be taken, including age, GA, clinical diagnosis and previous history of stillbirth if any, then to examine grossly placenta, umbilical cord and membrane. This will be followed by taking placenta, umbilical cord and membrane to the laboratory for microscopic examination.

Risks

No risks involved with the regard of the study.

Confidentiality

Information which will be gathered will be accessible to the research team only, however the knowledge which will be obtained from this study will be shared with other health professionals and the results will be published.

Who to contact

If you have any questions or problem concerning this research, please contact any of the following

1. PROF.JAMES KITINYA
   P O BOX 65001
   MUHAS – DAR ES SALAAM
   MOBILE PHONE 255 688 435 002

2. DR.PAUL NG'WALALI
   P O BOX 65001
   MUHAS –DAR ES SALAAM
   MOBILE PHONE 255 782 250 061

3. ATUGANILE MALANGO
   P O BOX 65001
   MUHAS – DAR ES SALAAM
   MOBILE PHONE 255 717 179 797

Consent

I have read the foregoing information/ it has been read to me and I understood the explanation of the study. I accept to participate in the study.

Signature or thumb print of the participant and
date……

Give thanks for participation

Name and signature of the person who fill the questionnaire ……

Appendix II: Consent Form (Swahili Version)

Utangulizi.

Naitwa Atuganile E Malango ni mwanafunzi wa udhamili katika chuo cha sayansi na tiba Muhimbili. Utafiti wangu unahusiana na uchunguzi wa kipatholojia wa kondo la nyuma la uzazi kwa mtoto aliyezaliwa akiwa amefariki.

Dhumuni la utafiti.

Dhumuni la utafiti huu ni kujua kwa umuhimu wa uchunguzi wa kipatholojia wa kondo la nyuma la uzazi, kwani tafiti mbalimbali yanapendekeza uchunguzi wa kipatholojia wa kondo la nyuma la uzazi ufanyike kama moja ya huduma ya uchunguzi wa mtoto aliyezaliwa akiwa amefariki.Hii itapunguza viwango vya kutogundua kisababishi cha kifo hicho na hivyo itasaidia katika kutoa huduma husika.

Maelezo kuhusu shughuli za utafiti huu

Endapo utakubali utafiti huu ufanyike, historia fupi kuhusiana tutachukua ambayo itahusisha umri wako,umri wa ujauzito, historia ya nyuma ya kujifungua mtoto aliyefariki kama ipo. Kondo la nyumanla uzazi nalo litachunguzwa kwa macho na baadae kupelekwa maabara kwa utafiti zaidi kwa kutumia darubini.

Mambo hatarishi

Utafiti huu hausababishi hatari yoyote kwa mshiriki

Siri

Taarifa yoyote itakayokusanywa hapa itakuwa inaweza kupatikana kwa watafiti tu, ila manufaa yatakayotokana na utafiti huu yatapelekwa kwa watu wote wahuduma wa afya na majibu yanaweza kuchapishwa kwenye majarida mbalimbali.

Mawasiliano

Kama una swali au tatizo lolote kuhusu utafiti huu waweza kuwasiliana na

1. PROF.JAMES KITINYA
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   MOBILE PHONE 255 782 250 061

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   P O BOX 65001
   MUHAS – DAR ES SALAAM
   MOBILE PHONE 255 717 179 797

Ridhaa

Nimesoma/nimesomewa taarifa hapo juu na nimeelewa malezo ya utafiti huu. Ninakubali kushiriki kwenye utafiti huu.

Sahihi ya Mshiriki na tarehe ……..

Dole gumba kwa mshiriki asiyeweza kusaini

Tarehe ……

Kumshukuru Mgonjwa

Jina na sahihi ya aliyemuhoji mgonjwa……..

Appendix III: Data Collection Sheet (Questionnaire)

PART A.

1. A. Patient MNH ID No

   B. Study ID No.

2. Age of the mother ……..

3. Number of antenatal care visits during this pregnancy …….

4. Previous history of adverse pregnancy outcome

   I) Stillbirth A) Yes B) No

   ii) Abortion A) Yes B) No

   iii) Preterm delivery A) Yes B) No

5. Gestational age of delivery of this pregnancy ……

6. Sex of the fetus A.)Male (B) Female

7. Condition of the fetus A) Fresh (B) Macerated.

8. Gross lethal congenital anomaly of the fetus A)Yes B)No

If yes name it…….

6. Clinical diagnosis A) Known (B) Unknown

If known, clinical diagnosis is ……..

PART B.

8 Gross anomalies on the umbilical cord

A) Yes B)No

If yes name it…….

9 Gross anomalies on the membranes

A) Yes B)No

If yes name it…….

10 Gross anomalies on the Placenta

A) Yes B) No

If yes name it…….

11 Microscopic features of placenta, umbilical cord and membrane

A) Description of microscopic features of the Placenta

B) Description of microscopic features of the umbilical cord

C) Description of microscopic features of the membranes.

12 Final Conclusion of placenta examination ……..

Appendix IV: Grossing of the Placenta

The routine processing for single gestation placenta is as follows.

A.

1. To record standard measurements which are dimensions of the disk and length of the umbilical cord.
2. Membranes are examined for color and transparency. Normally they are shiny, translucent and thin .The distance from the point of rupture to the edge of the placenta is recorded.
3. Umbilical cord is examined; normal has two arteries and a vein. An area at least 5 cm above the chorionic plate should be examined as the arteries normally fuse near the insertion.
4. The location of the insertion of the umbilical cord, which may be central, eccentric, marginal, velamentous or furcate, is noted. In a partially membranous (velamentous) cord, the cord vessels separate and run within the membrane for a distance. The lengths of the velamentous vessels are measured. In a case of furcate insertion, the cord vessels spread out over the placental disk within membranes.
5. If a true knot occurs in an umbilical cord, the presence, tightness, and differences in cord color and diameter on either side of knot are noted. False knots appear as bulbous expansions of the cord and are formed by ectatic vessels and a reduction in the amount of Wharton's jelly present. Any thrombosis, congestion, thinning, or necrosis of the cord is noted.
6. The insertion of the membranes into the placental disk is examined, and the percent of each type of insertion is estimated; normally they insert at the edge, which is known as marginal insertion. In marginal insertion, they insert inside the edge; and in circumvallate insertion, they insert inside the edge and a prominent ridge is created as the membrane folds back upon itself.

B. **Cotyledons:**

The placenta is weighed after the umbilical cord and membranes are cut off. The placenta is serially sectioned into 0.5 cm sections with a large sharp knife. The parenchyma is examined carefully for infarcts, which appear as firm white-yellow areas, and for intervillous thrombi, significant areas of hemorrhage, and any other unusual findings.

Representative sections are submitted as follows;

Block ___ Umbilical cord (fetal end, mid-section, maternal end)

Block ___ Membrane roll (2 sections)

Block ___ Cotyledon sections (include full thickness chorionic plate to decimal plate)

Appendix V: Haematoxylin and eosin

1. Retrieve blocks and make tissue sections using microtome [3-5 microns]

2. Pick the section on a slide from water floatation and place a hot plate 60^0C for 50 minutes.

3. Deparaffinise sections, hydrate through graded alcohol to water

4. Remove fixation pigments if necessary

5. Stain in alum haematoxylin for a suitable time.

6. Wash well in running tap water until section 'blue' for 5 minutes

7. Differentiate in 1% acid alcohol (1% HCL in 70% alcohol) for 5-10 seconds.

8. Wash well in tap water until sections are again 'blue' (10-15 minutes)

9. Stain in 1% eosin Y for 10 minutes.

10. Wash in running water for 1-5 minutes

11. Dehydrate through alcohols (70, 95, 100%), clear and mount

Appendix VI: Ethical Clearance

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Ref. No. DA.287/298/01A/ 13th June, 2018

Dr. Atuganile Malango
MMed. Anatomical Pathology
MUHAS.

RE: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED: "GROSS AND HISTOMORPHOLOGICAL STUDY OF PLACENTA IN STILLBIRTH AT MUHIMBILI NATIONAL HOSPITAL"

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has, on behalf of the Senate, approved ethical clearance for the above-mentioned study. Hence you may proceed with the planned study.

The ethical clearance is valid for one year only, from 12th June, 2018 to 11th June, 2019. In case you do not complete data analysis and dissertation report writing by 11th June, 2019, you will have to apply for renewal of ethical clearance prior to the expiry date.

Dr. Emmanuel Balandya
ACTING: DIRECTOR OF POSTGRADUATE STUDIES

cc: Director of Research and Publications
cc: Dean, School of Medicine