

**COMPARISON OF PLACENTA STRUCTURE IN PRETERM DELIVERIES  
BETWEEN HIV INFECTED AND UNINFECTED WOMEN IN KENYA**

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

**BY  
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## **Dedication**

*Dedicated to my wife Esther Nabifwo*

*My son*

*Manuel Obimbo*

*My daughter*

*Lisa-Marie Obimbo*

*My parents*

*My late Mum Respah Minayo*

*Dad Joash Madadi, Mum Joyce Nafula*

*To my sisters*

*Kageha, Tsindori, Nanjala*

*To my teachers, friends and colleagues*

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**List of abbreviations**

ART – Antiretroviral therapy

CD4 - Cluster of differentiation 4

CTB - Cytotrophoblast

HIV – Human Immunodeficiency virus

HPF – High power field

ICAM - Intracellular cell adhesion molecules

KNH – Kenyatta national hospital

MDG – Millennium development goals

MTCT - Maternal to child transmission

PMTCT – Prevention of maternal to child transmission

PTB – Preterm birth

RNA – ribonucleic acid

STB – Syncytiotrophoblast

TV – Television

VCAM - Vascular cell adhesion molecules

WHO – World Health Organization

## **Abstract**

### **Background:**

Preterm birth (PTB) is a major cause of infant morbidity and mortality in developing countries. The underlying structural mechanisms by which PTB occur are largely unknown. Whereas the association between maternal HIV infection and adverse obstetric outcomes is known, there is a paucity of data that investigate the structural changes in the placenta amongst HIV infected versus uninfected women. This data could be useful in interpreting whether specific lesions are related to increased risk of pre or perinatal HIV transmission besides providing a further understanding of biology of preterm placenta in vertical transmission of HIV and PTB.

### **Objective:**

To compare the structural features of the placenta delivered by HIV infected and uninfected women with preterm births.

### **Material and methods:**

This was a descriptive cross-sectional laboratory based study of placenta delivered by mothers at Kenyatta National and Pumwani maternity hospitals. Forty-three and thirty-eight preterm placentas from HIV seronegative and seropositive women respectively who delivered between 28 and 37 weeks of gestation were analyzed. The samples were obtained after relevant ethical and administrative approvals. Ten placentas each from HIV positive and negative mothers with term deliveries were also studied for comparative purposes. Placentas were examined grossly for obvious color change, shape, diameter, weight, umbilical cord insertion thrombosis and infarction. Samples were then processed for paraffin wax embedding at *Lancet Laboratories* and studied in a blinded fashion for light microscopy, morphometry and stereology at the *University*

*of California San Francisco*. Observations were recorded in preformatted data sheets and photomicrographs of various placental sections taken. Clinical data included age, gestational age, recent CD4 count, HIV viral load, status of antiretroviral therapy (ART). This information was then correlated with the placental findings and analyzed using SPSS version 20.

### **Results:**

Preterm HIV seropositive placenta were significantly associated with thrombosis, infarction, anomaly in cord insertion and unusual colour of the membranes ( $p < 0.001$ ,  $p = 0.041$ ,  $p = 0.022$ ,  $p = 0.43$  respectively). Comparatively only infarction and anomaly in cord insertion were significantly associated with term HIV seropositive placenta ( $p = 0.028$ ,  $p = 0.022$  respectively). Preterm placenta from HIV seronegative women appeared to be significantly thicker ( $p = 0.010$ ) than those from matched HIV seropositive placenta. There was, however, no significant difference in cord diameter, cord length, weight and great diameter of the placenta between the two preterm groups. Microscopically, preterm placentas were in general associated with immature villi, syncytial knotting, villitis and deciduitis. Main findings associated with preterm HIV seropositive placenta included massive fibrinoid deposition with villi degeneration, syncytiotrophoblast delamination, increased red cell adhesion to the terminal villi and increased neoangiogenesis. Compared to term and preterm HIV seronegative placenta, HIV preterm seropositive placentas showed significantly smaller villous area ( $p < 0.05$ ) being also fewer per unit area, increased vascularity and diminished perimeter.

### **Conclusion:**

These results imply that HIV is associated with salient morphological changes in preterm placentas that could potentially exacerbate preterm birth. The unique villous architectural

changes may signify hypoxia effects in placentas related to HIV infected and/or ART use putting the pregnancy at greater risk of PTB. Further research to explore potential mechanisms will help elucidate these pathways, and could lead to interventions to decrease the risk of PTB.

## **INTRODUCTION AND LITERATURE REVIEW**

### **INTRODUCTION**

Preterm birth, a major cause of infant morbidity, mortality and early childhood chronic diseases affects about 10% of pregnancies worldwide (Gouyon et al., 2012). It is estimated that about 60% of babies born too soon take place in parts of Africa and Asia. The mechanism by which preterm labour occurs is poorly understood. Various workers have attempted to explain, although inconclusively, the mechanism of its occurrence (Kraumer et al., 2012). Whereas traditionally, preterm deliveries have been looked at as a single entity occurring between week 22 and 37 of gestation, recently some workers have alluded to different causes of preterm birth that also vary with gestational age (French et al., 1999, Wadha et al., 2001, Hecht et al., 2008, Himes and Simhan 2008) with both systemic and intrauterine infection being responsible for early preterm labor and psychological or physical stress, placental thrombosis, infarcts, intrauterine vascular lesions and cervical incompetence as possibly responsible for late preterm labour and deliveries.

Normal weight of placenta varies between 150–170 grams to 500–600 grams at 20 weeks and term respectively (Thompson et al., 2007). Placenta is made of fetal and maternal components, the structural unit being the stem villus. The stem villous subdivides into intermediate villi that then form the terminal villi (Castellucci & Kaufmann, 2006, Castellucci et al., 2000). The anchoring villi provide stability to the placenta while the floating villi interact with the intervillous space for feto-maternal



exchange. The renewal of syncytiotrophoblast just like epithelia is maintained by fusion with underlying cytotrophoblasts villous (Kufmann & Stegner, 1972).

In complicated pregnancies, placental structure is altered. In preeclampsia for example, cytotrophoblast invasion is limited to the superficial decidua layer and there is poor invasion of spiral arterioles as a result of abnormalities in adhesion molecules in the cytotrophoblasts. This implies that the trophoblast cells fail to differentiate with an effect on histological and ultrastructural features of the placenta (Kadyrov et al., 2006). In diabetes, studies suggest placentas are grossly large with disposition of aberrant villous maturation (Mayhew and Sisley, 1998, Benirschke et al., 2006). HIV infection is a known cause of unfavorable pregnancy outcomes (Temmerman et al. 1990). However, the effect of HIV infection on placenta of preterm deliveries is poorly understood. Whereas some studies have indicated alteration in both gross and histopathologic transformation, other reports suggest minimal to no changes (Jauniaux et al., 1988, Backe et al., 1994, Temmerman et al., 1994). Further, the placental morphological changes associated with antiretroviral treatment (ART) or non-treatment is inadequately addressed in literature.

Whereas the association is known between HIV infection and poor pregnancy outcomes, there is insufficient data to investigate the structural changes in the placenta amongst HIV infected versus uninfected women. This data would be useful in comparing HIV associated placental changes versus those in other complicated pregnancies in the context of on-going work in placenta pathologies complicated with medical conditions in pregnancy. Further, this data may be useful in interpreting the

relationship between specific lesions and the risk of PTB and therefore provide a further understanding to biology of preterm placenta in vertical transmission of HIV and PTB.

## **LITERATURE REVIEW**

The role of placenta in growth and development of a healthy fetus cannot be overstated. There is a strict regulation and coordination of function of the placenta are to maximize its function between the maternal and fetal circulatory systems. Alteration of this arrangement may lead to adverse pregnancy outcome. The gamut of HIV pathological lesions encountered in the placenta has not been well documented in the literature.

### **1.1.1 Structure of placenta**

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 fetal surface, maternal surface and intervening placental parenchyma. The fetal surface is made of chorion covered by amnion while the maternal surface is made basal plate which has flat grooves and deep clefts subdividing it into placental lobes (Bernischke et al., 2000).

The parenchyma of the placenta consists mainly of a villous tree that is in close contact with maternal blood. The fetal vessels that gets into the placenta via the chorionic plate branch extensively and are covered by outer trophoblast layer and inner mesenchyme layer from the extraembryonic mesoderm, making up the chorionic villous tree. In the first and second half trimester, trophoblast undergoes key changes to adapt to pregnancy. Towards the end of pregnancy in the later second half of the second

trimester and third trimester, extensive angiogenesis and vascularization occur. This is illustrated by growth and remodeling of new vessels and firming of the existing vascular beds. There is interaction of fetal blood vessels and maternal intervillous space to augment the exchange of oxygen and nutrients at the fetomaternal interface, thus the structure of placenta in humans adapts it to its function (Demmir et al, 1995, Castelucci et al., 2000).

### **1.1.2. Placenta in complicated pregnancies**

In complicated pregnancies associated with unfavorable outcomes, placentas have been shown to be grossly smaller, have non-central umbilical cord insertion, have large areas of infarction, have non-discoid shapes and have aberrant fetal blood vessel arborization (Heazel, 2010). In diabetes, for example, various placental abnormalities such including hematoma formation, presence of infarction, visible calcification and deposition of fibrinoid material are quite obvious and may obliterate the efficiency of materno-fetal interaction. With alteration of placental structure in diabetes mellitus, its function is also affected and could be associated with increased rates of unwanted fetal and maternal morbidity including miscarriage (Leach et al., 2009). Pregnancy complicated with malaria on the other hand has placenta demonstrates accumulation of parasites in the intervillous space, leucocytic and macrophage infiltration, fibrinoid material accumulation and alteration of cytotrophoblastic structure including thickening of the trophoblastic basement membrane (Matteelli et al., 1997).

Microscopically, a placenta in the 2<sup>nd</sup> and 3<sup>rd</sup> trimester of pregnancy is made up of basal and chorionic plates, placental parenchyma that houses chorionic villi, intervillous spaces and cell islands. The chorionic plate is lined by cuboidal to low

columnar cells and extraembryonic mesoderm. Septation of the placenta comes from infolding at the level of the basal plate and there their absence may be indicative of maternal floor infarction (Kaufmann et al., 1996).

### **1.1.3. Placenta in preterm birth**

The incidences of preterm deliveries are on the rise worldwide and are worse in the developing countries. It accounts for the majority of attendant newborn complications and more than 50% of long-term infant morbidities (Goldenberg et al., 2008). Complications of prematurity include impaired cognitive and motor functions, risk of chronic lung disease, necrotizing enterocolitis and neurological impairment. Further evidence suggests that early preterm birth is associated with the infection and severe inflammation in the placenta and amniotic fluid (Goldenberg et al., 2008, Jones et al., 2009).

It is generally acknowledged that placenta of spontaneous preterm birth are highlighted by features of acute inflammation including villitis, chorioamnionitis and funisitis. In addition, disorders that involve blood vessel adaptations in the decidua may play a role in poisoning of preterm labor (Arias et al., 1993). Moreover, it has been shown that there is ischemia and infarction has been demonstrated to occur at a much higher rates in patients with PTB than those who get to deliver at term (Germain et al., 1999).

### **1.1.4. Human Immunodeficiency Virus and placenta**

The prevalence of Human Immunodeficiency Virus (HIV) in Kenya ranks amongst the highest in the world (UNGASS, 2010). HIV has been associated with poor

pregnancy outcomes (Temmerman et al. 1990). The spectrum of HIV-induced placental pathologies is not well understood. There are conflicting reports indicating the timing of HIV during maternal fetal transmission. Whereas most studies show bulk of HIV transmission occurring intrapartum, informed by previous regimens initiation at 34 weeks and even later at 28 weeks, some reports, however, indicate that some of HIV transmission to the fetus may occur at or near term (Douglas et al., 1992, Minkoff et al., 1995, Bryson 1996).

Factors that have been suggested to facilitate intrauterine transmission via a transplacental route include placental transfer via coreceptors and cytokines, cell free versus cell associated virus, ill in the placental barrier and transport across the cell. The exact sequelae of HIV infection of the placenta are also controversial. Some studies indicate features of placental membranes including inflammation, funisitis, chorioamnionitis, villitis, decreased or increased placental weight while others report minimal to no changes (Jauniaux et al., 1988, Ryder et al, 1989, Chandwani et al., 1991, Gichangi et al., 1993, Backe et al., 1994, Temmerman et al., 1994).

A strong positive correlation exists between HIV RNA levels, maternal HIV viral load and subsequent HIV transfer via transplacental route. Hence, treatment with antiretrovirals (ART), which strongly reduces the viral load, decreases the chances of HIV transmission to the fetus (Newelle and Thome, 2004, Volmink et al., 2007). The placental morphological changes associated with ART treatment or non-treatment are, however, not sufficiently addressed in the literature.

It is acknowledged that microscopic examination of placentae in HIV-infected and uninfected women do not always reveal pathological changes. Indeed, it has been

said that some may be histologically normal or demonstrate minimal alterations and therefore use of immunohistochemical studies may help demonstrate molecular alterations in the proinflammatory molecules such as intracellular cell adhesion molecules (ICAM) in villous stroma and basement membrane of the syncytiotrophoblast (Bogliolo, 2006)

Comparing the gross, microscopic and morphometric structure of placentas in HIV infected and uninfected patients with preterm births would help shed more light on the existing controversies regarding structure of preterm placenta in the face of HIV infection, the role of placental structure in MTCT of HIV and PTB. Further, information obtained from this study may help lay a better foundation to develop interventions to decrease the burden of PTB.

## **1.2 Statement of the problem and rationale**

Kenya has one of the highest prevalence of HIV and PTB with geographical variation (WHO, 2006, UNGASS, 2010, KDHS, 2014). The mechanisms underlying preterm births are not well understood despite it being amongst the leading causes of infant disease in Africa (Gouyon et al., 2012). Until recently, there was very little effort put towards tackling the threat posed by preterm birth worldwide and specifically in Kenya with imminent danger to the sustainable development goals enshrined in the Post 2015 framework agenda.

As much as is believed that placenta may play a role against mother to child transmission of HIV, the effect the latter has on preterm placenta are not absolutely understood and the available data are conflicting. For example, while it is known that most of the mother to child transmission (MTCT) of HIV occurs during the pregnancy

period, some data show a decline in both intra and peripartum transmission with use of ART; but the underlying structural basis is not known. Meanwhile, other studies have shown a rise in the rates of prenatal intrauterine transmission of HIV suggesting a potential alteration in placental structure and integrity. It is not known whether placental morphology in preterm births is similar in both HIV seronegative and seropositive patients.

### **1.3 Study justification**

There have been studies that have related histostructure of term placenta in relation with HIV. However, to date, the structural changes of the preterm placenta associated with HIV and without has not been adequately investigated especially in patients of African descent. In view of this, we aimed at evaluating the association of structural changes on the placenta and HIV status amongst women with preterm birth. This data will be useful in comparing HIV associated placental changes versus those in other complicated pregnancies in the context of on-going work in placenta pathologies complicated with medical conditions in pregnancy. Further, this data may be useful in defining how specific lesions in HIV infection could increase the risk of perinatal transmission of HIV and preterm birth and therefore provide a further understanding to biology of preterm placenta in vertical transmission of HIV and PTB. This may also help in design and implementation of intervention measures in addition to bringing more understanding to the subject of preterm labour.

### **1.4 Hypothesis**

Null (H<sub>0</sub>)

There is no difference in villitis of placenta delivered by HIV seropositive and seronegative mothers who have preterm birth.

**OBJECTIVES:**

**Broad objective:**

To compare the structural features of the placenta delivered by HIV infected and uninfected women with preterm births

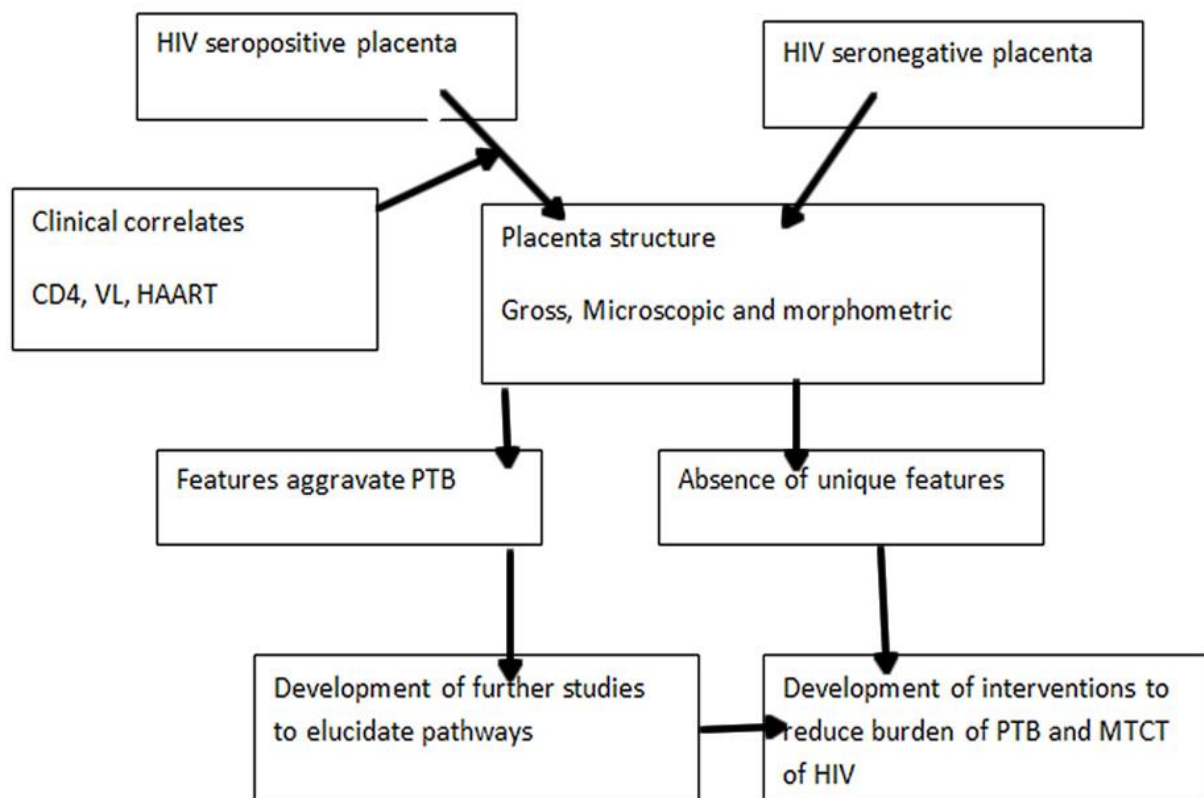
**Specific objectives**

To compare the following features between placenta delivered by HIV seropositive and seronegative mothers:

- i. Macroscopic
- ii. Morphometric
- iii. Microscopic



## 1.5 Conceptual framework



## **METHODS**

### **2.1. Study population**

Only placenta from mothers of African descent ages between 18 and 40 years with singleton live births were taken in this study. The placental samples meeting inclusion criteria were collected by simple random sampling. The estimation of gestational age was done through calculation using last normal menstrual period and confirmed via an early obstetric ultrasound. PTBs were defined as delivery at less thirty seven weeks and term births were defined as delivery above thirty seven weeks. In all patients, we excluded those with medical or obstetrical complications during pregnancy. Age matched placenta nearest to the week and varied from 28 to  $<36^{0/7}$  weeks.

#### **Inclusion criteria**

Mothers of African descent age between 18 and 40 years

Those defined to have preterm birth

No obvious comorbid condition

Those who consented to participate in the study

Mothers who are either HIV seropositive or negative

#### **Exclusion criteria**

Mothers with recurrent pregnant losses

Mothers with multiple gestations

## **2.2 Study site**

This study was undertaken at Kenyatta National Hospital and Pumwani maternity hospital. Kenyatta National Hospital (KNH) is the national referral hospital with bed capacity of 1800. The hospital has an active labour ward unit carrying out approximately 1000-2000 deliveries monthly. Pumwani maternity hospital is a county referral maternity hospital in Eastlands area of Nairobi county and run by Nairobi county government and has 1500 to 3000 deliveries per month during September to November. Gross measurements and photographs were undertaken at the site of placental collection, others were done from the laboratory.

The harvested sample specimens (described below) were prepared at the University of Nairobi, Human Anatomy Department and Lancet Laboratories, where initial slides were prepared for microscopy. Representative blocks were picked from each of the harvested sections and further processed for microscopy and morphometry at the University of California San Francisco (UCSF) and also at the University of Nairobi. The lead investigator was personally involved in the processing and evaluating of the slides at UCSF Centre for Reproductive Sciences laboratory for a total period of six weeks. This was made possible as a result of mentorship program advanced by UCSF to help build skills and capacity in the areas of placental biology, advanced microscopy, morphometry and stereology in the principal investigator via the International mentored scientist program in which the lead investigator was a grant beneficiary.

Figure 1: The study sites; Kenyatta National Hospital and Pumwani Maternity



### 2.3 Study design

Descriptive cross-sectional laboratory based study. In this study, the exposure (HIV infection or absence of it) and outcome (the structure of the placenta) were measured at the same time. Although, placental samples were collected in a prospective timing, the exposure and outcome were measured simultaneously. Furthermore, the outcome measures had no effect on the measure of exposure and thus eliminating recall bias.

### 2.4 Sample size

A previous study, Vermaak et al., 2012, estimated that only 19.4% of HIV seronegative women demonstrated features of villitis on the placenta. Assuming a 2-sided chi-squared test and a significance level of 0.05 and applying formula by Kelsey et al., 1996.

$$n = \frac{1.96^2 p(1-p)(r+1)}{r(p_1 - p_2)}$$

Two sided significance level (1-alpha) – 95

Power (1-beta, % chance of detecting difference) – 80

Ratio (r) of sample size, unexposed/exposed – 1

Percent of unexposed (p2) with outcome – 19.4

Percent of exposed (p1) with outcome – 51.4

Applying this formula, sample size of 36 women in each arm sufficed. We had 95% power to detect a 40.1% difference in villitis amongst the HIV seropositive and seronegative women. Presuming 10% placental damage, our sampling target was 80 placentas (40 per arm). The allowed Type I error probability linked with this test of the null hypothesis was 0.05.

## **2.5 Methods**

### **Recruitment, consenting and data collection**

The total sample size was 81 placentas, 38 were HIV-seropositive (the study group) and 43 HIV seronegative (the control group, obeying inclusion/exclusion criteria). We paired the samples in both groups by gestational age (which varied from 28 to 37 weeks) and route of delivery. We collected and studied 9 and 11 placentas each from HIV infected and uninfected women respectively. The structure of term placenta in both HIV seropositive and seronegative states has been studied and the samples in this study were purely for comparison purposes.

Mothers who met the inclusion criteria were identified by the primary nurses while in latent phase of labour, they got a full explanation concerning the study by the

lead researcher or his trained research assistants, then requested to consent and participate in the study. They were assured of their confidentiality. For those that chose not to take part in the study, their treatment was not affected in any way. The spouses of these patients were not involved. Upon consenting, the patients were told that only a small portion of their placenta (as explained in subsequent protocol) was going to be taken for a study both within the country and abroad. The rest of the placentas were disposed in accordance to KNH standard protocol for placenta disposal.

The placentas were obtained aseptically immediately after delivery either via emergency or elective caesarean section or spontaneous vaginal delivery at the maternity unit of the Kenyatta National Hospital and Pumwani maternity hospital. The placenta tissue was fixed in 10% buffered formalin. The lead researcher macroscopically examined all the placentas and took photographs of interest. The collections of samples for Histology was done through a standard protocol used by the Anatomical Pathology studies, membrane roll, and 6 sections of parenchyma. They were then processed as described below.

Maternal clinical data that was collected included age, gestational age, use of alcohol/smoking, mode of delivery, degree of immunosuppression via CD4 count, HIV viral load of the mother and antiretroviral therapy. All this were obtained from the patients' medical records.

The delinked data were obtained by the principal investigator and trained research assistants from the medical records after sufficient explanation to the patient and obtaining informed consent.

## **2.6 Macroscopic and morphometric features**

Immediately after delivery the placentas were examined for obvious gross pathology. Gross examination of the placenta yielded information on the impact of maternal disorders on the fetus or the cause of preterm delivery. This technique observed: areas of infarction or thrombosis, site of cord insertion, shape of placenta and appearance of membranes in colour. The weight of the placenta was taken using a Digital Weighing Scale with Dual Display-Camry Scale (CE certified) [Figure 2] and recorded with respect to gestation. Reference values have been developed by Thompson et al., 2007.

Figure 2: Digital weighing machine



The dimensions of all the umbilical cord segments were taken. The umbilical cord segment closer to the insertion site to the placental disc was designated proximal end and the opposite segment distal end. The length of umbilical left attached to the newborn was approximately 10 cm. The proximal part of the cord was measured using a flexible tape measure and added to the length of the distal segment.

The cord diameter was determined at three cord regions using a digital vernier caliper and average computed. The greatest diameter of the placenta was measure using the flexible tape and recorded in centimetres. Placental thickness was estimated by a sharp calibrated rod pierced through the placental parenchyma. Shape of placenta was observed and recorded in respect to five different shapes; discoid, annular, circular, horse shoe or star shaped. Placental membranes colour were typed either as maroon, green-brown or yellow-gray. The entire protocol is contained in the placenta pathology reports that the Fisher lab routinely generates for cases involving obstetrical complications at University of California San Francisco (UCSF).

## **2.7 Light Microscopy**

Six sections were sampled from each placenta, two from the centre and four from the periphery of the placenta in a clockwise pattern. They were prepared for light microscopy. The specimens were fixed in 10% formaldehyde solution and then dehydrated in increasing concentrations of 70% to absolute alcohol each for one hour. They were prepared for paraffin wax embedding by clearing in Trichloroethane (TCE) for two hours and infiltrated with wax for 12 hours. They were then embedded in fresh molten wax for 12 hours overnight. The blocks were serialized and coded and the team



was blind to the HIV status of the samples for the purposes of unbiased interpretation during microscopy. Five micrometer thin serial sections were cut using a Leitz Wetzlar sledge microtome, floated in warm water and thereafter mounted and then dried in a hot air oven at 40° C overnight. The sections were stained with Masson's Trichrome and Picro-sirius red that defined any changes in the connective tissue distribution of the placenta especially at the collagenous stroma and perivascular zones and delineate smooth muscle composition in the placenta. Hematoxylin and Eosin (H&E) was useful in demonstrating general histomorphology and cellular architecture in the placenta including decidual arteriopathy, amniotic epithelial reactive changes, amniotic epithelial erosion, chronic multifocal erosion, pigment deposition, acute inflammatory cells and micro-organisms that can occasionally be seen on routine H&E staining.

Slides were examined under a Leica Automated Systems Light microscope connected to a computer screen. Observations were made to elucidate the following: general structural organization of the membranes, chorionic plate and fibrinoid material. Signs of vasculitis were noted. The entire spectrum of alterations that were recorded is described in a recent publication from the Fisher group (Hrnotka et al., 2013).

## **2.8 Morphometry of the placental villi**

We studied the morphometry of the terminal villi since most of the HIV associated changes result in villitis. We identified the terminal villi using these criterion; a) flattened trophoblastic lining , b) lack of muscularized arterioles and c) large fetal capillary occupying more than 50% of the cross sectional area. A total of five slides per placenta for a total of five placentas per each group were assessed in three main areas:

number of capillaries in the terminal villus, surface area (size) of terminal villi and perimeter measurement of terminal villi, both the mean perimeter of individual villi and the total perimeter measurement in ten high power field (hpf).

The slides were examined and digital images captured from ten randomly selected points at  $\times 400$  magnification using Canon colour camera interfaced with an Olympus BX41 microscope. We used the intermediate area of the placenta with complete microscopic visualization of the terminal villi and devoid of infarction, fibrinoid deposits, distorted villi. Images that were captured were used to quantify the villous architecture, area, perimeter and numbers as normal, high or low based on Altshuler et al., (1984) using the image analysis software FIJI-Image J analyzer via ImageJ plugin for morphological data mining – TrackEM2 (Version 1.46, NIH, Maryland, USA).

### **2.9a Statistical Analysis**

In these analyses the two subpopulations of HIV seropositive and seronegative placenta were compared for gross anatomical features, histological findings and morphometry. Numerical data involving placental villi population count, weight and morphometry were analyzed using SPSS, version 20 (Chicago, Illinois, USA). Means, standard deviations, and frequency tables were compiled using descriptive data. Features of the HIV seropositive versus HIV seronegative groups were compared using chi square test. For the ordinal evaluation, like villi number vis a vis HIV status, one way analysis of variance (ANOVA) was used. Correlations between number of the terminal villous, perimeter and numbers of capillaries between the preterm and term clinical groups were assessed using Kendall's rank correlation coefficient. The independent-

samples t-test was used to compare the means between HIV status groups on the same continuous. P value of  $<0.05$  was considered statistically significant for the hypothesis.

### **2.9b Ethical considerations**

We sought Ethical approval from Kenyatta National Hospital/University of Nairobi Ethics and Research committee. Authority to conduct the study was obtained from KNH and administrative office of Pumwani maternity hospital, permission from the respective maternity wards, verbal consents were obtained from all the patients who were willing to donate their placenta for this study and allow for use of their clinical data.

The patients were given full explanation of the importance of the research. Upon completing a gross morphology examination and harvesting appropriate sections of the placenta for microscopy, the placentas were handled according to the hospital policy of disposal. The harvested specimens were handled according to the provision for use of Human tissue as provided in the Human Tissue and Anatomy act cap 249 and 252 of the laws of Kenya. Material transfer agreement was signed between the University of Nairobi and University of California San Francisco and authority to transfer material to USA obtained from Kenyatta National Hospital/University of Nairobi Ethics and Research committee. Donation of the placentas was anonymous.

# RESULTS

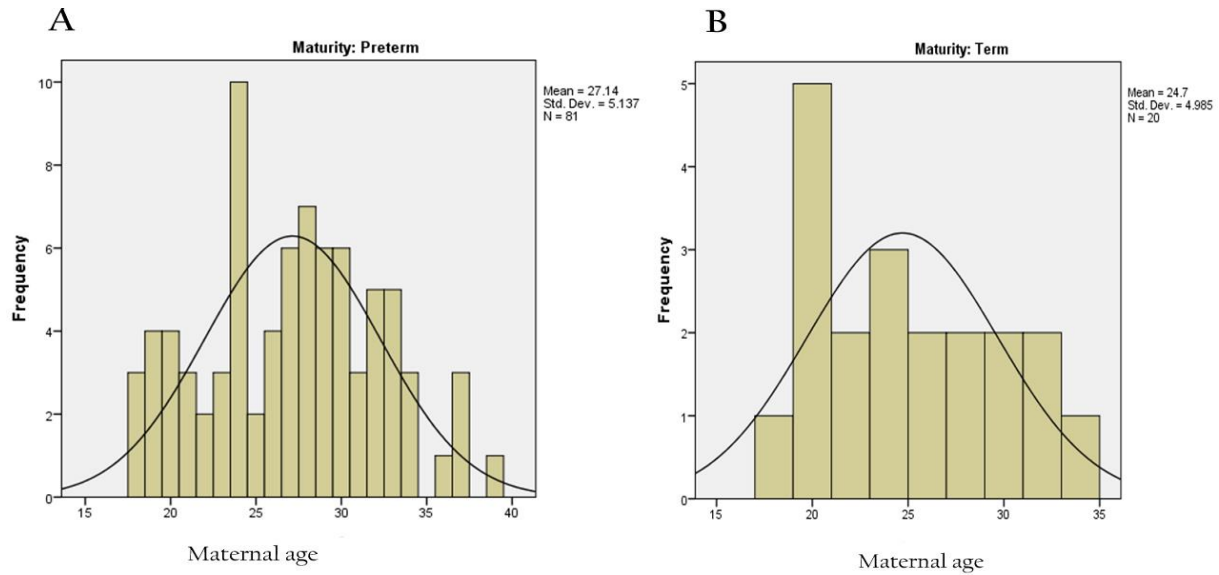
## **3.1 General characteristics of the sample population**

A total of 101 women were included in this study. Eighty one women had preterm delivery and 20 women had delivery at term. The general demographic characteristics of this population in terms of maternal age, gestational age and route of delivery are outlined below. All patients in this study that were HIV infected were on highly antiretroviral treatment but the type and duration of treatment was not investigated. The CD4 and viral load count was established in a few patients who were HIV infected (18 and 6 of the 38 respectively) but this data was not sufficient to enable analysis.

### **3.1.1 Distribution of maternal age**

The maternal age for preterm delivery group ranged between 18 and 39 years. The mean age was 27.14 years with median of 27 years (Figure 3 A). For the term deliveries, the women had aged 18 to 34 years. The mean age was 24.7 years and median of 24 years (Figure 3B).

**FIGURE 3: Histogram showing maternal age characteristics**

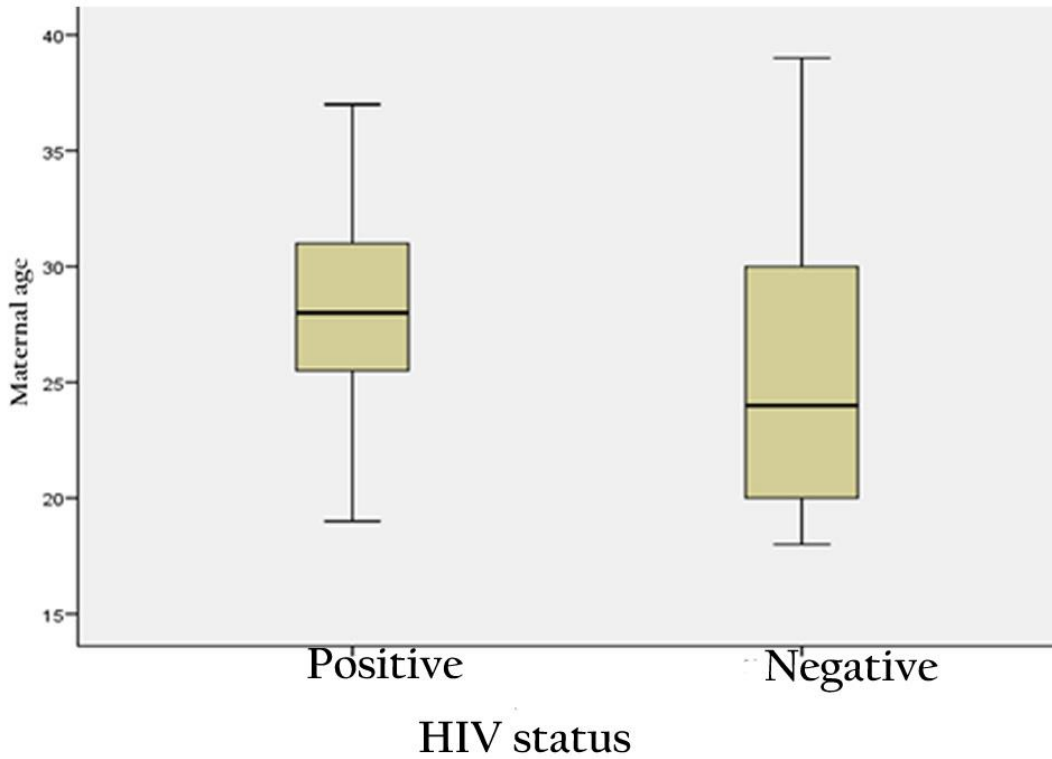


A: Distribution of maternal age in preterm birth

B: Distribution of maternal age in term birth

The HIV seropositive women had a higher average age (28 years) compared to those that were HIV uninfected (24 years) (Figure 4). There was a statistically significant difference in the ages of the women with preterm birth versus HIV status ( $p < 0.001$ ).

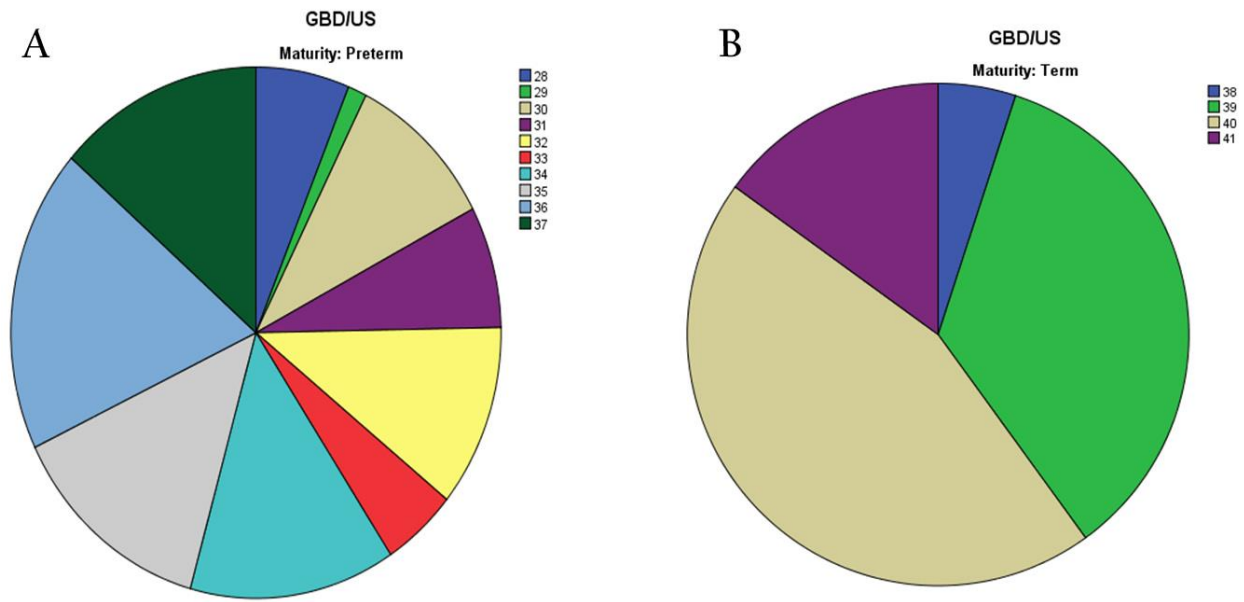
**FIGURE 4: Maternal age versus HIV status**



### 3.1.2 Distribution of gestational age

Gestational age was determined by last normal menstrual period corroborated by an early obstetric ultrasound dating. The mean gestational age for the preterm delivery group was 33.5 weeks with median of 34 weeks. On the other hand the mean gestational age for the term group was 39.7 weeks with median age of 40 weeks (Figure 5 A and B).

**FIGURE 5: Gestational age distribution**



A: Distribution of gestational age in the preterm birth

B: Distribution of gestational age in term delivery

### 3.1.3 Distribution of sample population based on route of delivery

Majority of women with preterm birth delivered via vaginal route 62% (n=50) and 38% (n=31) had cesarean birth. In the group with term delivery, there was equal frequency (50%) of delivery via vaginal and cesarean route. When HIV status was considered as a factor, route of delivery seemed to be significantly influenced by the HIV status in term pregnancy ( $p=0.025$ ) but not in preterm (Table 1).

Table 1: Showing distribution of route of delivery with HIV status

Route of delivery	Preterm			Term		
	Reactive n (%)	Non-Reactive n (%)	P value	Reactive n (%)	Non-Reactive n (%)	P value
<b>Vaginal</b>	20 (24.7)	30(37)	0.113	2(10)	8(40)	<b>0.025</b>
<b>Cesarean</b>	18(22.3)	13(16)		7(35)	3(15)	

### 3.2 General features of preterm and term placenta

The placentas consisted of both fetal and maternal parts. The fetal aspect was composed of the chorionic plate that was covered by the amnion. The amnion was only weakly attached to the chorion and the two could easily be separated. The umbilical cord inserted in a marginal position into the chorionic plate of most preterm placenta and mostly central in term placenta. The maternal surface was composed of the basal plate which represented an artificial surface that emerged from the separation of the placenta from the uterine wall during delivery. It was composed of lobes, fibrinoid material and blood clots. Chorionic and basal plates fused at the placental margins forming the smooth chorion. The smooth chorion was composed of three layers: the amnion, the chorion with a layer of extravillous trophoblast and the decidua capsularis. The placenta gross findings were described in terms of shape, site of cord insertion, colour of membranes, thrombosis and infarction.



### 3.2.1 The Shape of placentas

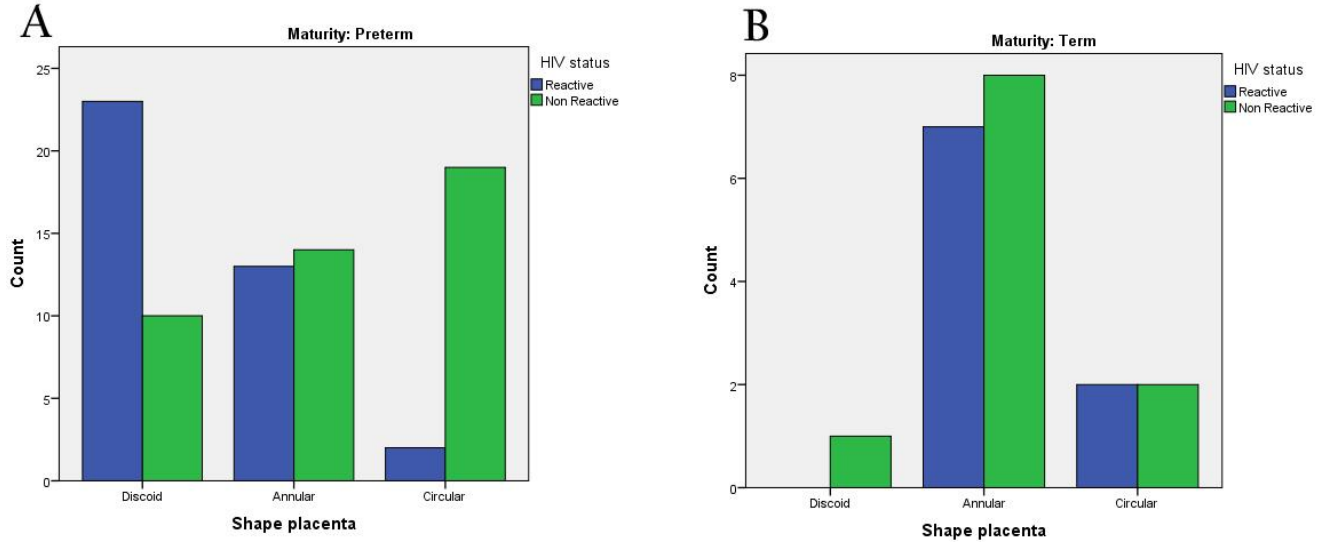
The placentas were classified into several well-defined geometrical patterns (Discoid, Annular, Circular, horseshoe and star shape). Majority of the preterm placenta were discoid in shape (Table 2) while the majority of the term placenta were annular in shape. The differences in shape seemed to be influenced by HIV status of the patient (Figure 6). Accordingly, most of the HIV seropositive placentas in preterm birth were discoid in shape (n=23, 28.4%) and circular in shape in HIV seronegative cases (n=19, 23.5%). Horseshoe and star shaped placenta were not identified in this study.

Table 2: Distribution of different shapes of placenta

Shape of placenta	PRETERM		TERM	
	HIV- n (%)	HIV+ n (%)	HIV- n (%)	HIV+ n (%)
<b>Discoid</b>	<b>23 (28.4)</b>	10 (12.3)	0 (0)	1 (5)
<b>Annular</b>	13 (16)	14 (17.3)	7 (35)	8 (40)
<b>Circular</b>	2 (2.5)	<b>19 (23.5)</b>	2 (10)	2 (10)
<b>Horse shoe</b>	0 (0)	0 (0)	0 (0)	0 (0)
<b>Star shaped</b>	0 (0)	0 (0)	0 (0)	0 (0)

The distribution of the different shapes is further annotated in the figures 6 A and B below

**Figure 6: Bar chart showing distribution of different placental shapes**



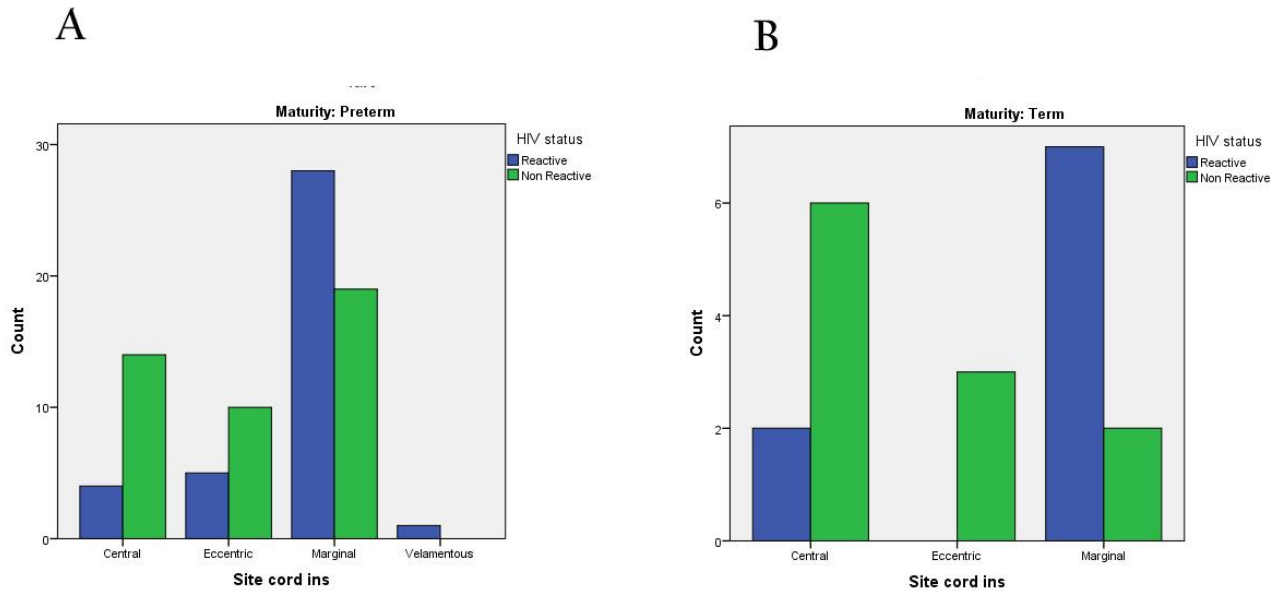
A: Distribution of different placental shapes in preterm birth

B: Distribution of different placental shapes in term delivery

### 3.2.2 The site of cord insertion

Out of the 81 preterm placental specimens, more than 58% had marginal insertion of the umbilical cord. Only 40.7% had what would be considered normal cord insertion (eccentric or central cord insertion). One placenta had velamentous cord insertion. In term delivery, the site of cord insertion was mostly central in HIV seronegative group and marginal in HIV seropositive group (Figure 7 A and B).

**Figure 7: Bar chart showing distribution of region of cord insertion on the placenta**



A: Distribution of site of cord insertion in preterm birth

B: Distribution of site of cord insertion in term delivery

### 3.2.3 Colour of placental membranes

The colour and translucency of the peripheral membranes were quite variable. The colours of membranes were typed as maroon, green-brown or yellow-grey. In preterm birth, HIV seropositive membranes had significant green-brown colouration compared to the seronegative placenta (Table 3). There was no significance in terms of differences in colour of membranes in HIV seropositive and seronegative term placentas.

Table 3: Distribution of different colours of placental membranes

Colour of membranes	PRETERM		TERM			
	HIV+ n (%)	HIV- n (%)	p=0.043	HIV+ n (%)	HIV- n (%)	p=0.35
<b>Maroon</b>	17 (21)	30 (37)		8 (40)	9 (45)	
<b>Green-brown</b>	19 (23.5)	10 (12.3)		1 (5)	2 (10)	
<b>Yellow-grey</b>	2 (2.5)	3 (3.7)		0 (0)	0(0)	

### 3.2.4 Thrombosis and infarction

A total of 49 placentas with preterm birth had thrombosis. Of these, 31 were HIV seropositive and 18 were HIV seronegative. The difference between these two groups was statistically significant ( $p < 0.001$ ) [Table 4]. At term, only 2 placentas from HIV seropositive patients demonstrated thrombosis. Infarction was significantly associated with placentas of preterm birth ( $p = 0.041$ ). Thrombosis was mostly subchorionic (Figure 8A) and involved almost the entire mass of the placenta. Infarction on the other had was at times focal and sometimes involved the entire spectrum of the placenta (Figure 8B).

Table 4: Distribution of thrombosis and infarction in preterm and term placenta

		Preterm		Term	
		HIV+ (%)	n (%)	HIV - (%)	n (%)
<b>Thrombosis</b>	Yes	31 (38.3)	18 (22.2)	2 (10)	0 (0)
	No	7 (8.6)	25 (30.9)	7 (35)	11 (55)
				$p \leq 0.00$	$p = 0.09$
<b>Infarction</b>	Yes	17 (21)	10 (12.3)	6 (30)	2 (10)
	No	21 (25.9)	33 (40.7)	3 (15)	9 (45)
				$p = 0.04$	$p = 0.02$
				1	8

**Figure 8 A and B. Macrographs showing preterm placenta with areas of infarction and thrombosis**

A: Preterm placenta at 35 weeks showing multiple infarcts, marked by the asterisk

B: Placenta at 34 weeks with areas of thrombosis. Notice subchorionic thrombosis, white arrows.

**FIGURE 8: Macrographs showing preterm placenta with areas of infarction and thrombosis**



### **3.3 Morphometry of the preterm and term placenta**

The placenta exhibited differences in morphometry in preterm and term birth. Description of morphometry was based on cord length, cord diameter, weight and great diameter of the placenta. The mean umbilical cord diameter from the preterm sero-reactive patient was 9.66mm while that from seronegative patients was 10.53mm. This difference was not statistically significant. Cord length varied from 38 cm to 66 cm for the preterm placenta and 48 cm to 66 cm for the term placenta.

The mean cord length from HIV infected preterm placenta was 54.74 cm and that from uninfected group was 54.37cm. Weight showed wide variability and seemed to increase with gestational age. The variation in weight in preterm placenta was between 356 grams and 650 grams, and in term was between 598 grams and 646 grams. Mean weight in preterm HIV infected placenta was 509.55 grams and that from HIV uninfected placenta was 524.12 grams. This difference was, however, not statistically significant. The mean weight of term placenta was 618 grams and there was no difference in the two groups of HIV infected and uninfected. The mean great diameter of the preterm placenta in HIV infected group was 15.21 cm and that of HIV uninfected was 16.00 cm. There was, however, significant difference in thickness of the between the two groups with mean thickness from infected and uninfected group being 1.974 cm and 2.226 cm respectively ( $p=0.010$ ). The differences in the morphometric parameters are summarized in tables 5 and 6 below.



**Table 5: Analyses of continuous outcome variable versus HIV status in preterm placenta.**

		N	Mean	SD	SEM	p VALUE
Cord diameter	Reactive	38	9.66	1.438	.233	0.054
	Non reactive	43	10.53	2.404	.367	
Cord length	Reactive	38	54.74	4.791	.777	0.784
	Non reactive	43	54.37	6.915	1.054	
Placental weight	Reactive	38	509.55	73.921	11.992	0.439
	Non reactive	43	524.12	92.148	14.052	
Placental greatest diameter	Reactive	38	15.21	1.234	.200	0.091
	Non reactive	43	16.00	2.591	.395	
Placenta thickness	Reactive	38	1.974	.2825	.0458	<b>0.010</b>
	Non reactive	43	2.226	.5287	.0806	

The table below shows the differences in morphometry in term placenta of HIV infected and uninfected placenta.

**Table 6: Analyses of continuous outcome variables versus HIV status in term placenta**

		<b>N</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>p VALUE</b>
<b>Cord diameter</b>	Reactive	9	12.11	1.453	.484	0.313
	Non reactive	11	11.45	1.368	.413	
<b>Cord length</b>	Reactive	9	57.89	4.755	1.585	0.850
	Non reactive	11	57.55	3.236	.976	
<b>Placental weight</b>	Reactive	9	614.22	14.948	4.983	0.198
	Non reactive	11	622.82	13.775	4.153	
<b>Placental greatest diameter</b>	Reactive	9	17.22	1.093	.364	0.166
	Non reactive	11	17.82	.751	.226	
<b>Placenta thickness</b>	Reactive	9	2.711	.2472	.0824	<b>0.057</b>
	Non reactive	11	2.936	.2461	.0742	

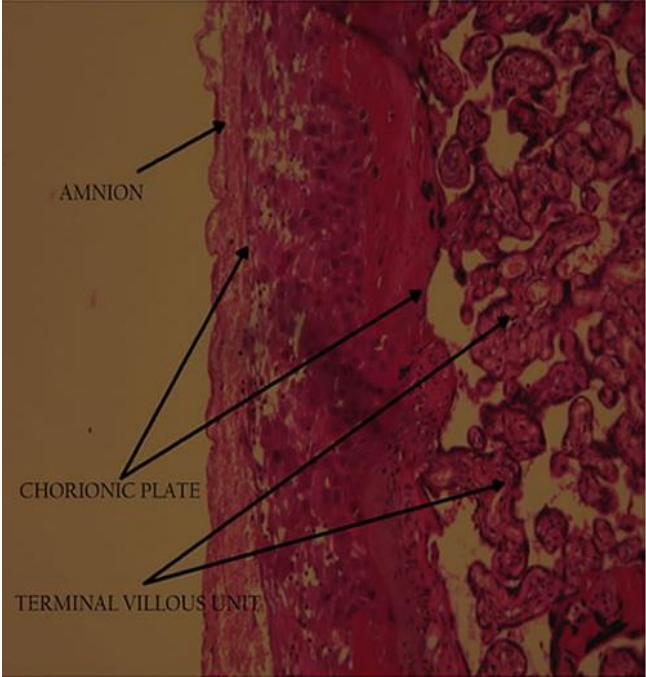
### **3.4 Microscopic organization of the preterm and term placenta**

The normal structure of placenta consisted of the chorionic plate, the villi unit and the basal plate. The chorionic plate consisted of the fused amnion and chorion. The amnion was lined by the simple cuboidal epithelium. The connective tissue of the amnion and chorion were fused and could not be separately distinguished microscopically (Figure 9 A). The chorionic plate had branches of the umbilical arteries and veins which extended into the villi. Emerging from the chorionic plate were stem villi which branched into smaller villi called terminal villi. Between the villi were the intervillous spaces that were filled with maternal blood and sometimes fibrinoid material (Figure 9B). Some stem villi extended the entire length of the placenta from the chorionic plate to the basal plate. The basal plate, sometimes known as, the decidua basalis consisted of part of the uterus to which the villi anchored. It contained decidual cells along with connective tissue elements (9C and D).

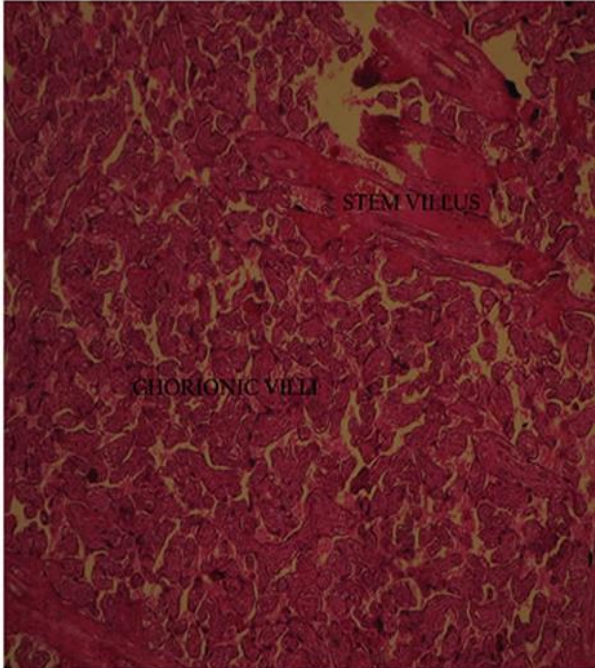
**Figure 9 A – D: Photomicrograph showing the general histostructure of placenta at 37 weeks:**

- A: General structure of Chorionic plate from central portion of placenta. Hematoxylin & Eosin stain, X100.
- B: General structure of chorionic villi from central portion of placenta. Notice the prominent stem villus. Hematoxylin & Eosin stain, X100.
- C: General structure of the decidual plate from central portion of placenta. Hematoxylin & Eosin stain, X100.
- D: Showing the decidua and the stem villous from the central placenta section. Note the big size of the stem villous. Hematoxylin & Eosin stain, X100

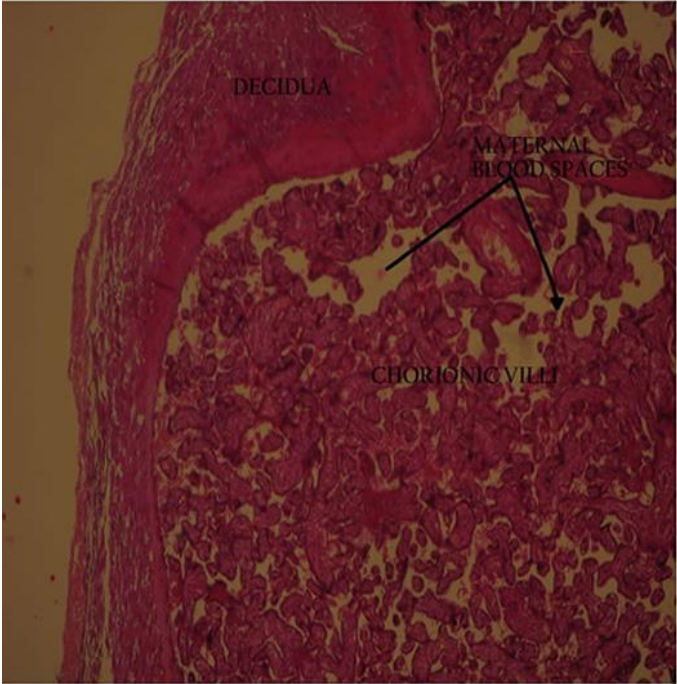
**Figure 9 A – D: Photomicrograph showing the general histostructure of placenta**



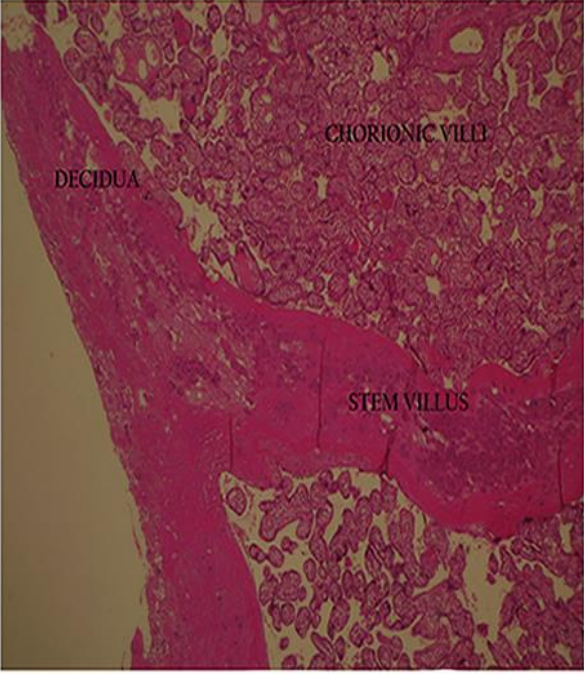
**A**



**B**



**C**



**D**

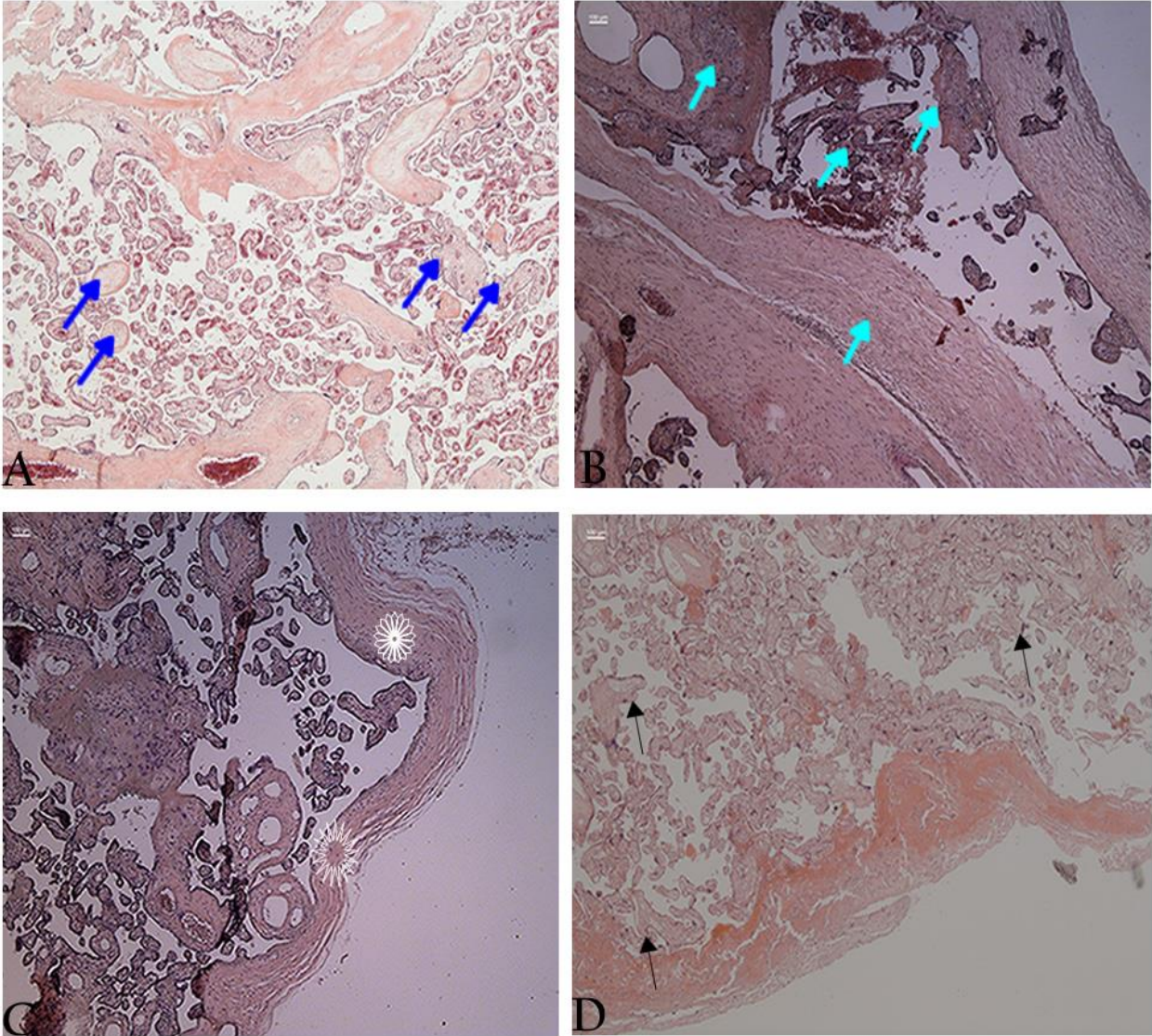
Light microscopic findings that were common to preterm placenta included immature villi to intermediate mature villi, syncytial knotting, villitis and in some aspects deciduitis. Syncytial knotting was also seen in term villous structures. Specific histological changes that were associated with HIV infected placenta included fibrinoid deposition with villi degeneration, syncytiotrophoblast delamination, increased red blood cell adhesion to the terminal villi and increased neo-angiogenesis.

The immature villi to intermediate mature villi were characterized by thick trophoblastic cover over the stem villi with intervening reticular stroma. There was also significant presence of connective tissue in the fetal perivascular regions and spectrum of scattered terminal villi (Figure 10 A). Villitis and deciduitis were common findings in both HIV infected and uninfected preterm placenta. It was characterized by infiltration of chronic inflammatory cells mainly lymphocytes and macrophages in the villi and the decidua (Figure 10 B and C). Syncytial knotting was a common feature and increased with advancement of gestation (Figure 10 D).

**Figure 10 A – D: Photomicrographs representing the general structure of preterm placenta**

- A: Shows a maturation spectrum of placental villi, note the immature and intermediate mature villi (blue arrows) harvested from a 32 week placenta. The immature villi are almost avascular. Hematoxylin and Eosin stain, X100
- B: Shows inflammatory cells within villi and chorion (light green arrows) harvested from a 32 week placenta. Hematoxylin and Eosin stain, X100.
- C: Shows inflammatory cells within the decidua (white asterisk) harvested from a 32 week placenta. Hematoxylin and Eosin stain, X100.
- D: Shows syncytial knotting in placenta from 34 week delivery (black arrows). Hematoxylin and Eosin stain, X100.

**Figure 10 A – D: Photomicrographs representing the general structure of preterm placenta**





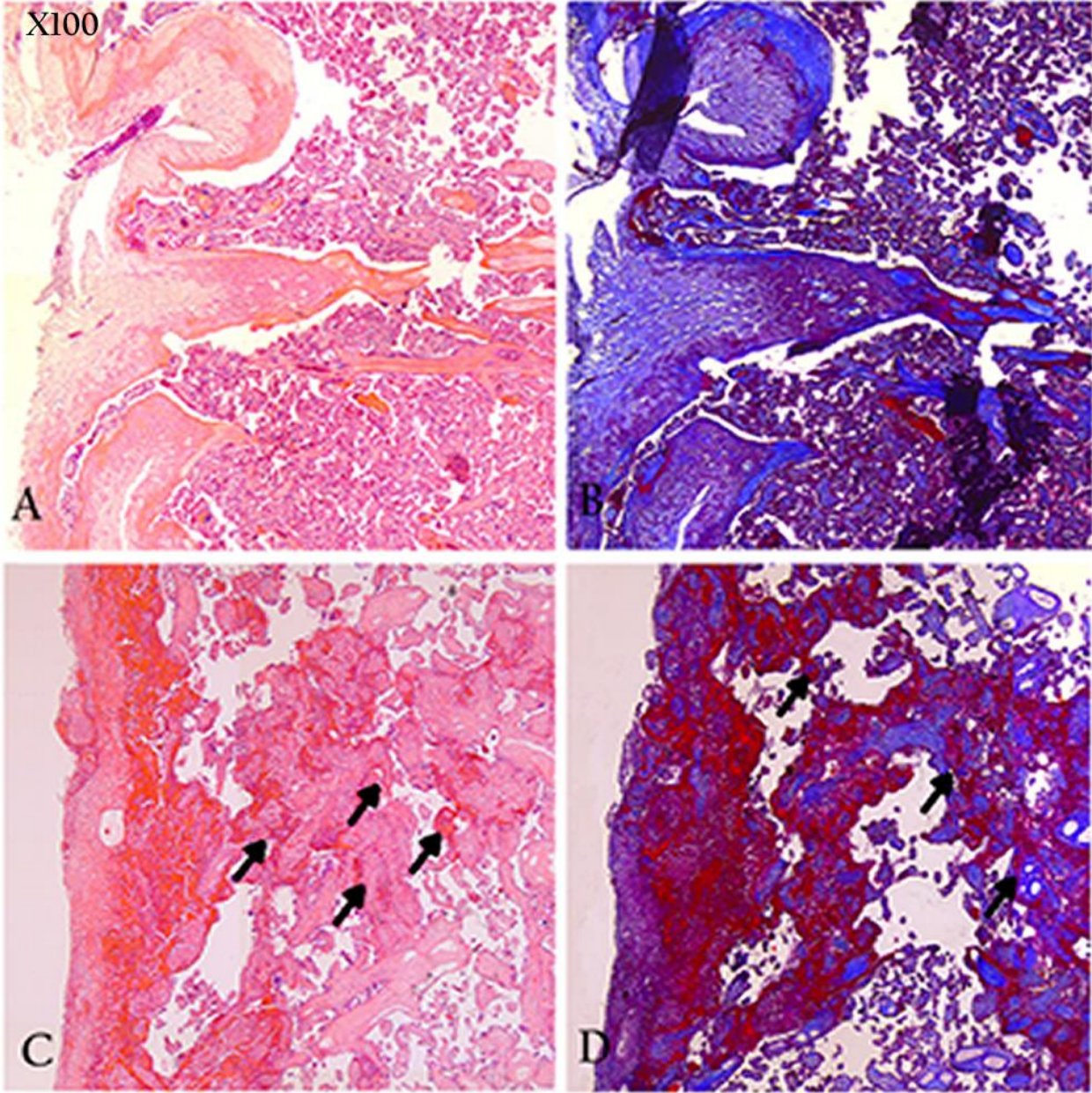
Specific lesions that were associated with HIV infected placenta included fibrinoid deposition with villi degeneration, syncytiotrophoblast delamination, increased red blood cell adhesion to the terminal villi and increased neo-angiogenesis. Massive fibrin deposition that was noted in this study (Figure 11 C and D) was characterized by extensive perivillous deposits of eosinophilic fibrinoid material almost obliterating the normal anatomy of the villous structure as seen in Figure 11 A and B. This resulted into villi degeneration and atrophy. A significant portion of the placenta was involved making this change significant.

In some cases of HIV infected placenta, syncytiotrophoblast delamination was seen as syncytial breaks and appearance of peeling that led to denudation of the villi. In a few cases it involved obliteration of villous integrity (Figure 12 A). As it is evident in Figure 12 B, maternal red blood cells were identified adhering to the syncytiotrophoblast of the placental villus. This was only seen in HIV infected placenta. The adherence of the red blood cells seemed to affect both the stem and the terminal villi structures. Also consistently seen in HIV seropositive preterm placenta were numerous new capillaries in the terminal villous structure. The capillaries seemed to arborize freely within the villous unit forming apparent anastomatic channels with varying sizes of the capillaries (Figure 12 C and D).

**Figure 11 A – D. Photomicrographs showing the structure of preterm placentas at 34 weeks gestation.**

- A: Placenta from HIV seronegative mother at 34 weeks gestation, note the normal anatomy of the villi and intervillous space. Hematoxylin and Eosin stain, X100
- B: Placenta from HIV seronegative mother at 34 weeks gestation, note the normal anatomy of the villi and intervillous space. Masson Trichrome stain, X100
- C: Placenta from HIV seropositive mother at 34 weeks gestation. Note the massive accumulation of fibrinoid material in the intervillous space with terminal villi degeneration and atrophy (arrows). Hematoxylin and Eosin stain, X100
- D: Placenta from HIV seropositive mother at 34 weeks gestation. Note the massive accumulation of fibrinoid material in the intervillous space with terminal villi degeneration and atrophy (arrows). Masson Trichrome stain, X100

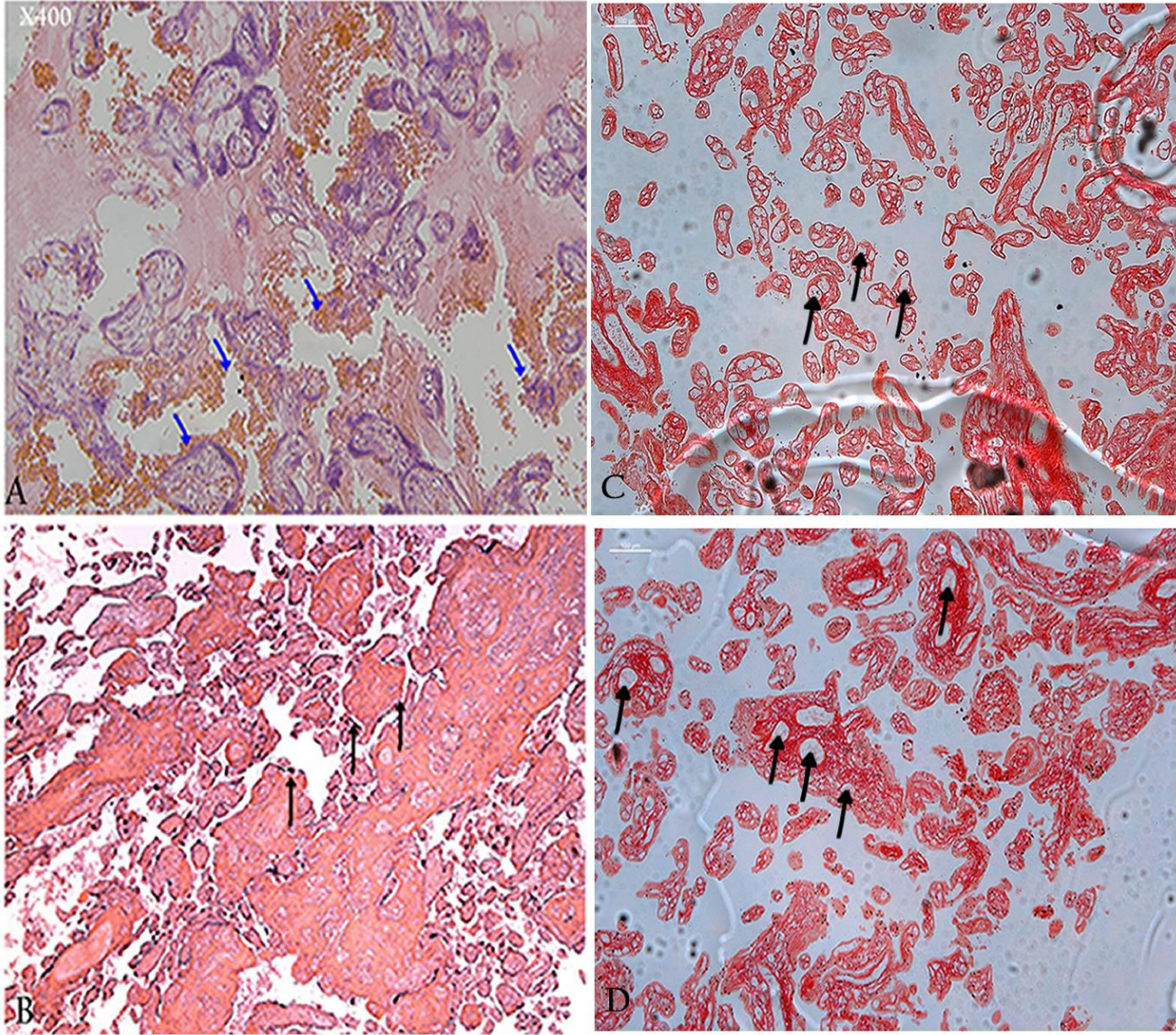
**Figure 11 A – D. Photomicrographs showing the structure of preterm placentas at 34 weeks gestation.**



**Figure 12 A – D. Photomicrographs showing different pathologies involving HIV seropositive preterm placentas.**

- A: Showing HIV seropositive placenta at 36 weeks, Notice the adherence of erythrocytes around the terminal villi in A (blue arrows). Hematoxylin and Eosin stain, X400
- B: Showing HIV seropositive placenta at 36 weeks, Notice the syncytiotrophoblast delamination (black arrows) along with multiple syncytial knots. Hematoxylin and Eosin stain, X100
- C: Showing HIV seropositive placenta at 35 weeks, Note the numerous capillary network in the terminal villi unit of the placenta (arrows). Picrosirius stain, X100
- D: Showing HIV seropositive placenta at 35 weeks, Note the numerous capillary network at a higher magnification showing different arborization patterns (arrows). Picrosirius stain, X 400

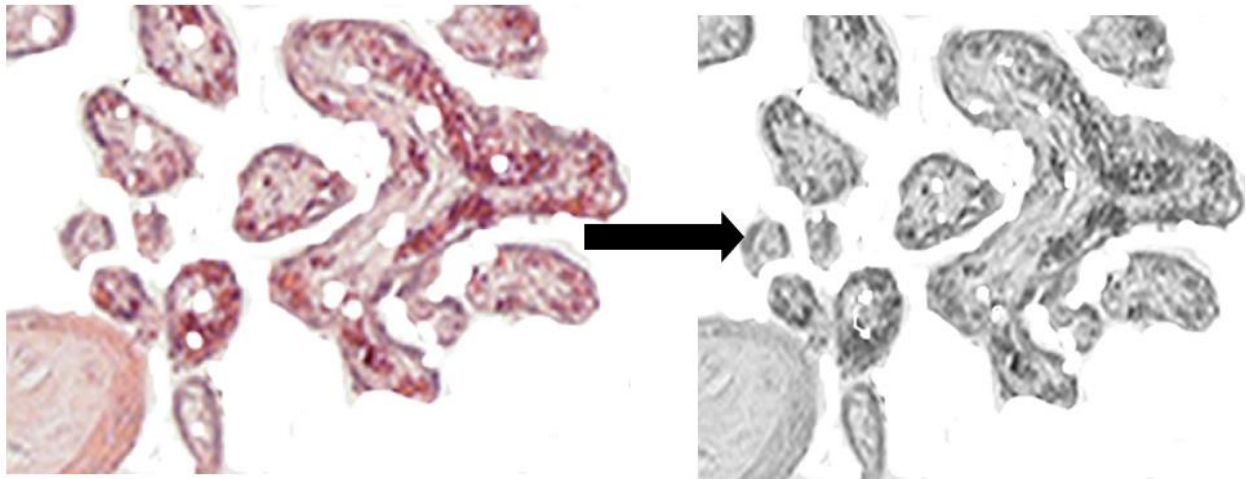
**Figure 12 A – D. Photomicrographs showing different pathologies involving HIV seropositive preterm placentas.**



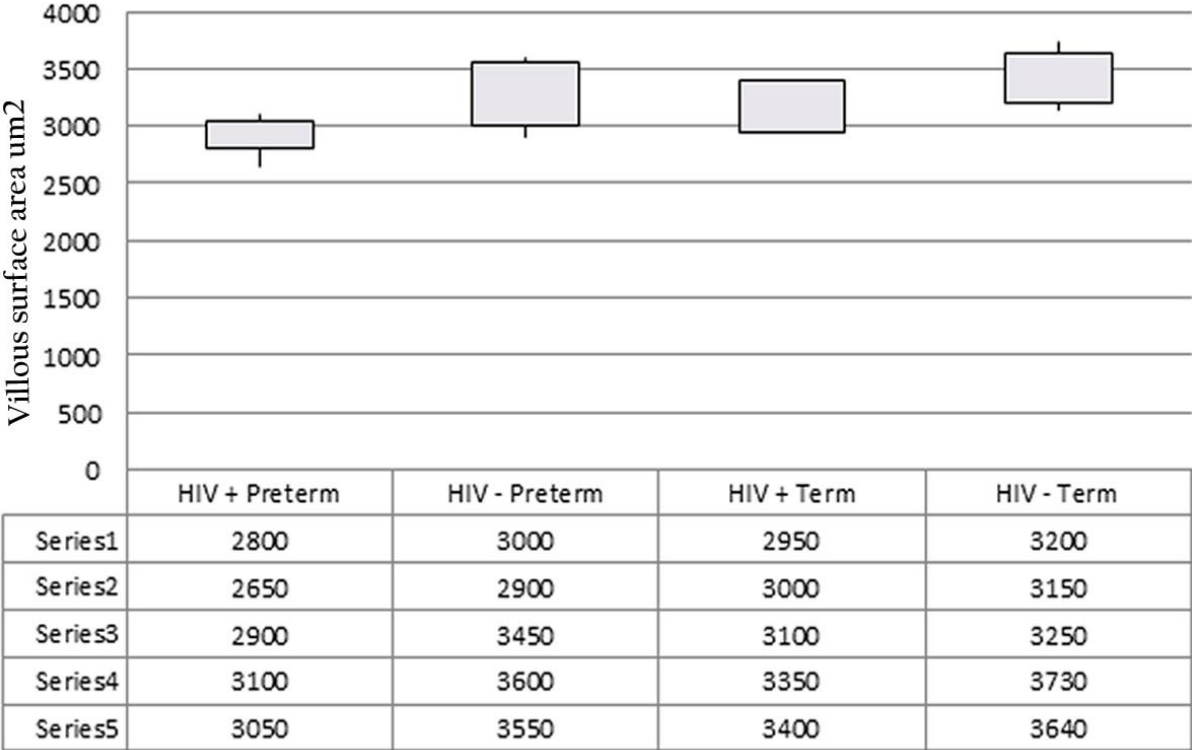
### 3.5 Villous morphometric features

Analysis of the villous structure was accomplished via modification of the images through macromedia fireworks (Figure 13) and dimensions obtained from Image J analyzer. Compared to term and preterm HIV seronegative placenta, HIV preterm seropositive placentas showed significantly smaller villous area. The surface area of the villous structure ranged from 2650 to 3150  $\mu\text{m}^2$  in HIV preterm placenta and 2900 to 3450  $\mu\text{m}^2$  in preterm HIV seronegative placenta ( $p < 0.05$ ) [Figure 14]. The term placenta had generally larger villous surface area than the preterm.

**Figure 13: Showing modified image obtained from high power field for Image J analysis**



**Figure 14: Surface area of villous structure from preterm and term placenta**



The HIV seropositive preterm placenta had increased villi vascularity but diminished villous perimeter ( $p < 0.005$ ) [Table 7].

**Table 7: Showing comparison of villous vascularity and perimeter of placenta**

	Preterm		Term	
	HIV +	HIV -	HIV +	HIV -
Villous vascularity (Based on Altshuler rule of 10s)	Increased	Normal	Normal	Normal
Mean villous perimeter (in $\mu\text{m}$ )	186	244	230	256



## **DISCUSSION AND CONCLUSION**

Placental samples used in this study were obtained from women possessing similar demographic characteristics as placenta from other women studied with medical complications in pregnancy and involving the placenta (Egbor et al., 2006, Zigic et al., 2010, Almasry & Elfayomy, 2012). The general structure of the placenta in our study was similar to what has been described in previous studies (Castellucci et al., 2000, Castellucci & Kaufmann, 2006). The unique findings of this study included marginal insertion of the cord, infarction and thrombosis, syncytiotrophoblast delamination, massive perivillous deposition with villi degeneration, erythrocyte adhesion to terminal villi and decreased villi surface area of the HIV positive preterm placenta.

### **4.1 General characteristics of the sample population**

The general demographics of the sample population is discussed in respect to maternal age, gestational age and route of delivery. In this study, all the patients that were HIV sero-positive were on highly active antiretroviral therapy (HAART). This observation is in keeping with current ministry of health of Kenya recommendation of administration of triple combination antiretroviral therapy to all pregnant HIV-infected women regardless of the CD4 count and continued for life (Option B+) [TITT, 2015]. It also buttresses the recommendation that ARV treatment is key to reduction of mother to child transmission of HIV and helps preserve the option of breastfeeding for these mothers (WHO, 2015). It was also observed that monitoring of CD4 and viral load count, vital components in evaluating treatment response, was a challenge in this sample population. Only a number of patients had values of CD4 and viral load count included in their medical records. CD4 count and human immunodeficiency virus load

tests have long been a part of the routine monitoring of HIV infection, but in resource-limited settings the ideal strategies for employing these tests have not been sufficiently investigated (Ford et al., 2012). World Health Organization (WHO) guidelines updated in 2013 recommend CD4 testing at the time of HIV diagnosis, with initiation of antiretroviral treatment (ART) depending on whether a country has adopted option A or B or B+ (WHO, 2015). In this study, the absence of records could have been due to inadvertent clerical error to enter data in the medical records or patients did not have the assays done in the prenatal period and may have been referrals from other facilities. Data concerning maternal clinical attendance and duration of ART was not assessed in the current study.

#### **4.1.1 Distribution of maternal age**

The age range of women who had preterm and term deliveries was from 18 to 39 years with median of 27 and 24 years respectively. This happens to be the optimal age of reproduction with a substantial decline in fertility in late 30's and early 40's (Anderson et al., 2003, Nelson et al., 2012). In this study, it appears that women in the reproductive cohort were slightly safer from biological risks, such as an increased likelihood of medical conditions like hypertension and diabetes that would normally occur with advanced maternal age (La Grew et al., 1996). In addition, this observation may also imply that these women were more likely to have had planned pregnancies than what happens for older women aged 35 and older or teenagers who have higher rates of unintended pregnancy than do women in their 20s and early 30s (Brown & Eisenberg, 1995). The higher maternal age for HIV infected group compared to HIV uninfected could imply higher risk of poor fetal outcome. Both HIV status and advanced

maternal age have been investigated separately for their influence on infant outcomes and concluded that both are associated with adverse fetal growth outcomes, including low birth weight and preterm birth (Brocklehurst & French, 1998, Townsend et al., 2007, Awoleke, 2012). However, the impact of the co-occurrence of these factors in relation to birth outcomes remains relatively understudied.

#### **4.1.2 Distribution of gestational age**

The mean gestational age for preterm birth was 33.5 weeks. This is a pattern observed in most preterm birth with late preterm birth beyond 32 weeks being most common (Lawn et al., 2010, Liu et al., 2012, Howson et al., 2013). Infants born before 32 weeks of gestation have little chance of survival. In contrast, the survival rate of infant born at 32 weeks and above as it happens in developed countries has an increased chance of survival. This implies that most infants born of these pregnancies were likely to have had a higher chance of survival (Tucker & McGuire, 2004).

#### **4.1.3 Mode of delivery**

Majority of preterm deliveries were vaginal as opposed to cesarean deliveries. The optimal mode of delivery for women who present with preterm labour remains controversial. Whereas some authors have argued that primary caesarean section may increase the risk of neonatal mortality and morbidity, such as pulmonary hypoplasia, necrotizing enterocolitis or sepsis for the neonate (Cohen, 1985, Malloy, 2009), others have maintained that cesarean section reduces neonatal mortality rate in preterm birth or very low weight (less than 1500 g) babies with breech presentation compared with vaginal delivery (Hannah, 2004). Furthermore, the lower segment may not have formed well in preterm labour and therefore an incision made, whether lower segment or

vertical, increases chances of postpartum bleeding, increased chances of placenta praevia or abruption in subsequent pregnancies and future need for a repeat cesarean section (Shah, 1990, Yang 2007).

In the current study, the reason to perform a vaginal birth or cesarean section was mainly informed by the fetal status, with fetuses that had non-reassuring stress or non-stress test subjected to cesarean delivery. Most term deliveries that were HIV seropositive delivered via cesarean section most likely due to a coincidental coexisting of non-reassuring fetal state with maternal HIV infection. It is most unlikely that the indications were due to prevention of mother to child transmission (PMTCT) as all the women in this study were on treatment for HIV.

## **4.2 General features of the placenta**

The general structural organization of the placenta consisting of chorionic plate, the parenchyma and the basal plate with margins made of the smooth chorion is similar to what other workers have described (Hertig et al., 1956, Hamilton and Boyd, 1960).

### **4.2.1 The shape of preterm and term placenta**

There were different shapes of placenta described in this study. Majority of the placenta were discoid in shape in preterm delivery and annular in term delivery. Most human placentas are represented as a round disk, with the umbilical cord inserting at the center of the chorionic plate. In clinical practice, however, true circular shape of the chorionic disk rarely exists. There are various factors that are thought to determine placental shape including where it is implanted in the uterus, regional variations in the decidua, determining areas of atrophy, variations in maternal vascular supply, with

placental infarcts resulting in altered shape and perhaps even the manner in which original implantation occurred (Benirschke, 2002).

We observed discoid placenta, a common shape in rodents and primates. In this form of shape, the feto-maternal interaction and blood content exchange is limited to the circular portion of the placenta. The term 'discoid' placenta in actual sense has been used classically to describe the normal shape of placenta (Columbus, 1559). Just like the circular shape, the discoid shape gives placenta ability to perform its function. We observed a number of annular placenta predominant in the HIV positive term placenta. In literature, however, annular placenta is a very rare shape and occurs hand in hand with placental vascular abnormalities. It is said that it increased the risk of antepartum and or postpartum bleeding and at times need for surgical intervention (Steemers et al., 1995). In substantive pathology, unique features of abnormal shapes of placenta and their association with reduced placental efficiency and its impact on fetal development has been observed (Thompson et al., 1969).

#### **4.2.2 The site of cord insertion**

Most of placenta in preterm birth had marginal cord insertion. Only about 41% of the placenta had either centric or eccentric insertion of the cord or what would be considered as normal insertion. The observation of predominantly marginal type of insertion is in contradiction with most other studies which have observed predominantly normal insertion (Finburg et al., 1998, Donald et al., 1998, Sepulveda et al, 2003, Sepulveda et al., 2009). This type of cord insertion has been associated with poor fetal outcome including preterm birth, intrauterine growth retardation and

possibility of developing velamentous cord (Brody & Frenkel, 1953, Kouyoumdjian, 1980, Paavonen et al., 1984).

It has been postulated that the variations in the site of insertion of umbilical cord result from the process known as trophotropism in which the chorionic frondosum or the early placenta migrates with a growing pregnancy to ensure a good blood supply from a well vascularized zone of the uterine wall (Monie, 1965, Robinson et al., 1983). Indeed abnormal umbilical cord insertion has been clinically linked to placental insufficiency (Viero et al., 2004). In a condition termed as chorionic regression, the placenta is characteristically small with eccentric or marginal insertion and is likely to have impairment of placental function (Whittle et al., 2006). It is therefore plausible to conclude that in our study, marginal cord insertion might have played a role in orchestrating preterm birth.

#### **4.2.3 Colour of placental membranes**

Colour and translucency of placental membranes differed from maroon, green-brown to yellow grey. Maroon indicates the normal colour of placental membrane, green-brown is suspicious of meconium staining and yellow-gray is suspicious for chorioamnionitis. Majority of preterm membranes were maroon in colour (58%). Green brown colouration was mostly noted in preterm HIV seropositive placenta than it was for HIV seronegative placenta. Literature alluding to this association is limited but our findings imply possibility of higher chances of fetal distress in pregnancies from HIV seropositive women with preterm labour. It could also mean inefficient placental delivery system that may contribute to fetal distress. There was almost equal numbers of women from HIV infected and uninfected women with preterm birth who had features

of chorioamnionitis evident on placental membranes. The infection could be contiguous or through a concomitant sexually transmitted infection (STI) during pregnancy. Presence of placental infection has a 2-fold increased risk of spontaneous preterm delivery (Polk et al., 1989).

This finding is biologically plausible as other studies in pregnant HIV negative women report that chorioamnionitis and acquisition of STIs during pregnancy is associated with increased risk for PTB (Gravett et al., 1986, Cotch et al., 1997, Gomez et al., 2008)

#### **4.2.4 Thrombosis and Infarction**

Thrombosis was a significant feature of preterm birth placenta and was predominantly seen in HIV infected placenta. Increased thrombosis has been cited in previous studies (Kraus et al., 1999, Verni et al., 2000, Leistra-Leistra et al., 2004) and has been associated with increased fetal thrombotic vasculopathy (Ariel et al., 2004, Redline, 2005). It has been suggested that there may be involvement of hemostatic pathway in the pathophysiology of thrombosis.

Thrombosis is primarily initiated and perpetuated by local activation of the extrinsic coagulation pathway. There is growing evidence that HIV may upregulate the extrinsic coagulation pathway via activation of the innate immune system through a series of events (Funderburg et al., 2010). First, pathological processes including bleeding or inflammation caused by HIV infection results in activation of coagulation cascade in the maternal circulation, secondly, thrombin generation happens as a result of depletion of anticoagulation proteins (Lange et al., 2003). Thrombin is a key enzyme in blood coagulation that plays a role in fibrin generation, platelet aggregation, and

tissue repair (Cunha-Bang et al., 2013). The processes leading to thrombin generation can be operationally described as the initiation, propagation and termination phase (Zetterberg et al., 2013) leading ultimately to formation of thrombosis. Presence of thrombosis in these placentas may imply its potential contribution to occurrence of preterm prelabour rupture of membranes (PPROM), preterm birth and potentially other obstetrical syndromes such as intrauterine growth restriction (IUGR), pre-eclampsia and or fetal demise.

Infarction was significantly associated with both preterm and term placenta of HIV infected women. A finding of a higher risk of maternal placental infarction and associated syndromes has been described in previous studies (Jauniaux et al., 1988, Chandwani et al., 1991, Suy et al. 2006,) but is in contradiction with other recent reports (Boer et al., 2007, Haeri et al., 2009, Ryan et al., 2015) among women with HIV. The etiology of placental infarcts is not well established but has been associated with depositon of fibrinoid material in the intervillous spaces. The occurrence of high rates of placental infarcts amongst women with HIV could provide insight into the cause of the higher-than-expected rates of adverse neonatal outcomes in this sub population.

### **4.3 Morphometric features of preterm and term placenta**

In this study, there were no significant differences in diameter and length of umbilical cord, weight and mean great diameter of the preterm placenta between HIV infected and uninfected groups. The absolute figures were however higher in HIV uninfected placenta. On the contrary, a significant difference was noted in thickness of the placenta with HIV uninfected placenta being significantly thicker ( $p=0.001$ ).



The findings in this study indicate appropriate cord diameters for the gestational ages (Pinnar & Iyugun, 2015), meaning that HIV did not particularly have a significant adverse effects on the morphology of umbilical cord. Studies comparing growth restricted fetuses with appropriate for gestational age group have shown that the cross-sectional area of the umbilical cord is reduced in the former implying that thin umbilical cords are associated with fetal growth impairment (Prabhcharan et al., 1993, Raio et al., 1999). A greater diameter umbilical cord diameter has been associated with pregnancies complicated by gestational diabetes and aneuploidies (Ghezzi et al., 2002, Cromi et al., 2007).

Umbilical cord length varied from 38 cm to 66cm. Mean length for HIV infected placenta was 54.74 cm and that from HIV uninfected placentas was 54.37cm. The mean umbilical cord length in this study is comparable to mean umbilical cord length of other authors (Blanc, 1972, Harld & Elston, 1978, Mishra et al., 1987). A typical cord length ranges from 30 cm to 90 cm (Krakowiak et al., 2004) averaging 50-60cm at time of birth. Imperatively, all the cords in this study were of normal caliber. Cord length may have appreciable variation, with extremes ranging from having no cord commonly termed as achordia to lengths of up to 300 cm (Browne, 1925, Malpas, 1964). Cords beyond 100 cm are known as long cords while those below 30 cm are said to be short. Short umbilical cords may be associated with adverse perinatal outcomes such as fetal growth restriction, congenital malformations, ante or intrapartum distress and increased risk of fetal death. Long cords on the other hand are associated with cord accidents such as true or false knots (Krakowiak et al., 2004).

Weight of preterm placenta ranged from 356 grams to 650 grams with term placenta ranging from 598 grams to 650 grams. The weight range observed in this study falls within the developed normal percentiles for age matched gestational ages for both the preterm and term placenta (Thompson et al., 2007). HIV status seemed not to have a significant effect on the placental weight in this study, though the mean weights of the two differed by 15 grams in favour of the HIV uninfected placenta. This is in contrast to other observations which have shown HIV infected placenta to be either grossly smaller and weighing less than those from HIV uninfected placenta or much heavier than normal placenta (Jauniex et al., 1988, Gichangi et al., 1993).

Placental weight determines birth weight and pattern of fetal growth in third trimester (Voldner et al., 2008). From our findings, it is plausible to suggest that HIV seropositive infants would have a lower birth weight than their counterparts from HIV seronegative mothers, but their weights accordingly would not be significantly different. The dimensions of great diameter of placenta were in keeping with placental weight. The dimensions were also within the acceptable range of preterm placenta (Kaufmann, 1985).

There was a significant difference in the mean thickness of preterm placenta between HIV infected and uninfected placenta. This finding is at variance with what has been reported by D'Costa et al., who found no difference in placental dimensions with HIV status (D'Costa et al., 2007) but similar to the work by Lopez et al., (2013). In a previous study, Nyberg and Finberg reported that the placental thickness goes hand in hand with gestational age and can be a good predictor of the of gestational age (Nyberg & Finberg, 1990). PT of less than 2.5 cm at term is associated with IUGR while PT of

greater than 4cm is associated with gestational diabetes, intra uterine infections and hydrops foetalis (Kunlmann & Warsof 1996, La Torre et al., 1979). Certain infections such as cytomegalovirus are associated with significant increase in PT (Tongsong et al., 1999). It seems that HIV has a regressive effect on thickness of placenta.

#### **4.4 Microscopic structure of preterm and term placenta**

The general microscopic structure of placenta composed of chorionic plate, villi unit and the basal plate has been described in literature (Steven, 1985, Bernischke et al., 2006 Castellucci et al., 2006).

The light microscopic features of placenta that were common to both HIV infected and uninfected preterm placenta included immature to intermediate mature villi, syncytial knotting, villitis and deciduitis. Immature intermediate villi as in previous studies were bulbous with a reticular stroma with a discontinuous cytotrophoblast layer. Immature intermediate villi are considered the growth centers of the villous trees (Castellucci et al., 2006) and could be the main sites of fetomaternal exchange in the first and second trimesters prior to full differentiation of the terminal villi. The intermediate mature villi were long and slender and gave rise to the terminal villi which have a high degree of fetal vascularization and form a paramount part for fetal-maternal exchange (Castellucci et al., 2000).

Syncytial knotting was observed in preterm placenta and increased with gestational age. Syncytial knots also known as Tenney-Parker bodies are aggregates of syncytial nuclei at the surface of terminal villi. Syncytial knots are consistently present, increasing with increasing gestational age and are used as a surrogate marker for villous maturity (Boyd & Hamilton, 1970). Increased syncytial knots as observed in this study

may be an expression of structural placental insufficiency that probably led to PTB (Werner & Bender, 1977) and the resulted from fetal inadequate perfusion of placental villi (Fox, 1965).

Villitis and deciduitis were seen in both arms of preterm but with increased frequency in HIV infected placenta. Villitis has been reported in previous studies (Nagamatsu & Schust 2010, Veemark et al., 2012) and is commonly reported in HIV infected placenta with a low CD4 count. Most cases of villitis in placenta are not known and are therefore called villitis of unknown etiology (VUE) [Russell, 1980]. In cases where the cause can be identified, the most common infectious agents are viral. Subsequently, the identified infective agent may be confined to the placenta or may spread into both maternal or fetal bloodstream through the villous capillaries and infect the fetus.

The method by which the infection gains entry into blood can be contiguous via cell to cell infection or inoculation of infected maternal cells or free pathogen into the maternal/fetal circulation (Kaplan, 1993), hence integrity of villous epithelium and Hofbauer cells are important in checking the transplacental route of the infection transfer (Schwartz et al., 1992). In pregnancy, the placenta is surrounded by specialized endometrial stromal cells called the decidua. Decidua basalis is at the base of the placenta and includes the maternal spiral arteries and venous sinuses (Lunghi et al., 2007). Decidua parietalis or capsularis is present on the maternal surface of the free membranes. The cause of deciduitis is almost invariably as a result of ascending infection (Khong et al., 2000) and may result in decidual necrosis and abruption with a number of lymphocytes present within the decidua. Chronic decidual inflammation may

be associated with infection or maternal immune response (Khong et al., 2000). Presence of deciduitis and villitis in this series indicates a possibility of undesirable fetal outcomes such as IUGR and fetal inflammatory response syndrome.

Specific lesions that were related to HIV included fibrinoid deposition with villi degeneration, syncytiotrophoblast delamination, increased red cell adhesion and increased neo-angiogenesis. A small amount of fibrinoid deposition may be found within placenta as a normal ageing process. It is formed as a result of eddying within the intervillous space (Fox and Elston, 1978). Extensive fibrinoid necrosis as seen in this study, however, suggests an immune attack on trophoblastic cell with concomitant villi degeneration. Perivillous fibrinoid deposition has also been observed in other studies (Mallik et al., 1968, Mirchandani et al., 1978). Perivillous fibrin deposition in intervillous space is a result of thrombosis of maternal blood. As a result, portions of chorionic villi get entrapped by fibrin obliterating the intervillous space, causing atrophy of villous structure and may be associated with cytotrophoblast proliferation. These villi are not infarcted but are incapable of participating in any transfer activity (Naeye, 1985) implying a possibility of poor obstetric outcome including PTB. The underlying mechanism perivillous fibrinoid deposit largely in HIV infected placenta remains poorly understood but could be related to placental infarction and thrombosis discussed above through HIV induced immunological pathway and activation of extrinsic clotting pathway via HIV prompted thrombin generation and modification of endothelial function (Bendon & Hommel 1996, Fox 1997, Funderburg et al., 2010).

Syncytiotrophoblast (STB) delamination was also seen mainly involving HIV seropositive preterm placenta. The syncytiotrophoblast is a continuous, normally

uninterrupted single layer that extends over the surfaces of all villous trees as well as over parts of the inner surfaces of chorionic and basal plates. It thus lines the intervillous space (Wang & Schneider, 1987, Tedde et al., 1988). Syncytiotrophoblast forms a formidable barrier to infection by virtue of multiple unique cell biological properties including lack of specific microbial receptors, lack of E-cadherin molecules and absence of intercellular junctional complexes amongst other factors (Leiser & Kaufmann 1994, Robbins et al., 2010, Robbins et al., 2012). Destruction of the syncytiotrophoblast could be via HIV induced cytokine mediated inflammatory response leading to delamination (Bacsi et al., 1999). The other pathway could be through the interaction of placental macrophages that are able to infect STBs via tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6). The STB are in turn also capable of infecting the placental macrophages through the same pathway. This interactive behavior is what could lead to STB delamination. Destruction of STB increases the chance of MTCT of HIV by removing the barrier effect (Toth et al., 1995, Bacsi et al., 2001).

Erythrocyte adhesion to the walls of terminal villi was noted in preterm placenta of HIV infected women. Red cell adhesion on the placental STB surface may be initiated by either circulating endotoxin or STB destruction or apoptosis and this could be a response mechanism in syncytial repair (Levy & Nelson, 2000). This observation is said to be associated with villitis (Vern et al., 2000). As a matter of fact, erythrocyte adhesion leads to formation of immune complexes and may further exacerbate syncytiotrophoblast damage and lead to massive fibrinoid deposition with villi degeneration and more adhesion hence causing a vicious cycle that ultimately destroys

the placenta (Xiao et al., 1997). In essence it is possible to suggest that in these patients, anticoagulant therapy may have some remedial effect on the maternal coagulopathy and in improving chances of good fetal outcome.

Terminal villi capillary hypervascularity and increased neoangiogenesis was observed in HIV infected preterm placenta. Villous hypervascularity, in which individual terminal villi contain an excessive number of vessels defined by Altshuler who considered that this abnormality could be diagnosed when microscopy with  $\times 10$  objective showed 10 villi, each with 10 or more fetal vessels, in 10 or more non-infarcted areas of the placenta (Altshuler, 1984). In this study we noted increased neoangiogenesis. Presence of new capillary sprouting demonstrates a chronic hypoxic picture and has been encountered in other conditions such as maternal anemia, 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 and syphilis are known to infect and induce proliferation of the endothelial cells (Kadyrov et al., 1998, Ogino & Redline 2000, La Ossa et al., 2001). This implies that effect of HIV in placenta may foreshadow chronic placental hypoxia necessitating new capillary formation.

#### **4.5 Villous morphometric features**

The villous morphometric parameters included villous surface area, villous perimeter and fetal capillary in the terminal villi. The aspect of villous hypervascularity in HIV infected placenta is discussed above. HIV infected preterm placenta had significantly reduced villi surface area. This implies that the small villi are likely to cause placental insufficiency resulting in poor fetal outcome such as IUGR because of reduced

surface area of maternal fetal interaction. This observation has also been seen in placenta infected with malaria (Souza et al., 2013). As noted above, reduced oxygenation via reduced blood flow and therefore reduction in the delivery of hormones associated with growth factors such as placental growth factor or insulin-like growth factor which may subdue the development of the villi tree.

In the present study, we also noted reduced villi mean perimeter in HIV infected preterm placenta. Decreased villi perimeter in HIV cases may have occurred because of new branching of the intermediate villi into multiple terminal villi in order to compensate for the placental poor development and dysfunction as evidence by a smaller villi surface area. The resultant smaller villi invariably will have smaller perimeters. Further, accelerated villous branching with formation of syncytial knots and resultant hypervascularity of the villus are indicative of reduced placental perfusion (Mayhew, 2002, Roberts et al., 2008) and foreshadows poor obstetric outcome.

In summary, the observations of the present study have revealed the following features of HIV infected preterm human placenta hitherto not described. It:

- a)** Is predominantly discoid in shape with marginal umbilical cord insertion.
- b)** Colour of the membranes compared to age matched HIV uninfected placenta is not significantly different.
- c)** Demonstrates significant thrombosis and infarction, features with impact to its microscopic organization.
- d)** Has weight, cord length and diameter, great diameter not significantly different from HIV uninfected placenta. Placental thickness is significantly smaller.



- e)** Demonstrates significant villitis, perivillous fibrinoid deposition, syncytiotrophoblast delamination, erythrocyte adhesion to terminal villi and capillary hypervascularity of the villi.
- f)** Has terminal villi that have smaller surface area and reduced perimeter.

These observations have been interpreted and discussed in detail in the thesis using appropriate references.

## **Conclusions:**

These results imply that HIV is associated with salient morphological changes in preterm placentas that could potentially exacerbate preterm birth. The unique villous architectural changes may signify hypoxia effects in placentas related to HIV infected and/or ART use putting the pregnancy at greater risk of PTB. Further research to explore potential mechanisms will help elucidate these pathways, and could lead to interventions to decrease the risk of PTB.

## **Recommendations and suggestions for further studies**

This study has laid ground for further studies that may focus on the aspects of:

- a) Correlation between viral load suppression and histostructure of placenta
- b) Structure of various placental cells through confocal imaging and in situ hybridization
- c) Analysis of placental histomorphometry in HIV co-infection with malaria.
- d) Electron microscopic HIV infected preterm placenta.

This will enhance understanding of placental biology in preterm birth and mother to child transmission of HIV. We recommend that treatment with HAART should strongly be adhered to forestall the likely effects of HIV infection in the placenta that can potentially lead to poor obstetrics outcome.

## **Study limitations and delimitations**

### **Limitations:**

There might have been some form of shrinkage of placenta upon its collection due to time it took to obtain consent; this may have affected morphometry results.

Lack of a uniform system of nomenclature and criteria for the diagnosis and scaling of placental lesions. Some good samples may be missed by the research team due to double study sites.

### **Delimitation:**

To overcome these problems preliminary comparison between these and freshly collected specimens were done to estimate the correction factor. Secondly used the expertise developed by Fishers lab to standardize the description of placental lesions as accurately as possible. We requested the midwives and staff in the concerned units to notify the researchers in case of appropriate candidates for the study.

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## Appendix 1 – 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

Shape of the placenta: Discoid       Annular       Circular       horseshoe

Color of the membranes and chorionic plate



Maroon  Green-brown  Yellow-gray

Areas of calcification Yes  No

Cord colour; White  dark brown  black green

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

Umbilical cord hemorrhages. Yes  No

Weight of the placenta (gms)

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

Thickness of the placenta (cms); Greatest  Minor

### Histomorphology Placenta

#### Central sections 1 and 2

1 \_\_\_\_\_

2 \_\_\_\_\_

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

1 \_\_\_\_\_

2 \_\_\_\_\_

3 \_\_\_\_\_

4 \_\_\_\_\_

5 \_\_\_\_\_

6 \_\_\_\_\_

Histomorphology, umbilical cord taken in two sections

1 \_\_\_\_\_

2 \_\_\_\_\_

Results of Giemsa staining

\_\_\_\_\_

## **APPENDIX 2: CLIENT INFORMATION AND CONSENT FORM**

Study title

COMPARISON OF PLACENTA STRUCTURE IN PRETERM DELIVERIES BETWEEN  
HIV-1 INFECTED AND UNINFECTED WOMEN IN KENYA

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Kenyatta National Hospital, Consultant Obstetrician and Gynaecologist.

Researcher's statement

This is a humble request for you to participate in this research study. I would like to give you information concerning this study. This information will help you make an informed decision on whether you would like to participate in this study or not. Please read the form carefully. Feel free to ask questions concerning any aspect of this study

and your role, the possible risks and benefits, your rights as a volunteer and anything else about the research. Your information will remain confidential.

#### Purpose and benefit

Kenya has one of the highest prevalence of HIV and PTB. The mechanisms underlying preterm births are not well understood despite it being a leading cause of infant morbidity and mortality in sub Saharan Africa. It is not known whether placental morphology in preterm births is similar in both HIV seronegative and seropositive patients. Such data may be useful in design and implementation of intervention measures in addition to bringing more understanding to the subject of preterm labour.

#### Procedure

Once you have agreed to participate in the study, you will sign this consent form to allow us to include information obtained from you in our data. Your personal details will not be included in this questionnaire so as to protect your privacy. We will take portions of your delivered placenta for the purpose of this study. We will also look at your antenatal record to obtain more information which will remain confidential. You will continue to receive appropriate management while at the hospital. We also guarantee your safety during your participation in this study. If you agree to let the researchers collect specimens, the following will happen:

- There will be no mutilation of the placenta
- Measurements will be taken with the organ intact and only small blocks will be extracted for histology

- The tissue blocks will be studied locally and sample blocks transported for further analysis at the University of California San Francisco, USA by the principal investigator himself. After analysis, the blocks will be transported back by the principal investigator and then be disposed in accordance with the Kenyatta National hospital policy.

### Confidentiality

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

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

### Subject's statement

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

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

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

Signature/ Left thumbprint

Signature of the witness

---

---

(Participant)

(Witness)

I, the investigator having explained in detail the purpose of the study; hereby submit that confidentiality of the data recorded shall be maintained and that no details will be revealed, apart from those related to the study.

Signature

Date

---

For further information or clarification, please feel free to contact the KNH/UoN Ethics and Review committee using the below addresses:

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## **KIAMBATISHO 2: TAARIFA YA MTEJA NA FOMU YA IDHINI**

UTAFITI

KULINGANISHA MUUNDO WA KONDO KATIKA WANAWAKE WANAOJIFUNGUA  
KABLA YA UMRI BAINA YA HIV – 1 WALIOAMBUKIZWA NA WASIOAMBUKIZWA  
HAPA KENYA

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Wasimamizi

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Dk John Ong'ech

MB.Ch.B, MMED (Obs / Gyn), Mkurugenzi Msaidizi, Idara ya Afya ya Uzazi, Kenyatta  
National Hospital, Mshauri Mtaalam wa Gynaecologist.

Taarifa ya mtafiti

Hili ni ombi nyenyekevu kwa wewe kushiriki katika utafiti huu. Napenda kukupa taarifa  
kuhusu utafiti huu. Habari hii itakuwa kukusaidia kufanya maamuzi sahihi juu ya kama  
ungependa kushiriki katika utafiti huu au la. Tafadhali soma fomu hii kwa makini.  
Jisikie huru kuuliza maswali kuhusu kipengele chochote cha utafiti huu na jukumu lako,



hatari na faida, haki yako kama utajitolea na kitu kingine chochote kuhusu utafiti huu.  
Maelezo yako yatabaki siri.

Madhumuni na faida

Kenya ina moja ya kiwango cha maambukizi ya juu ya HIV na na watoto wanaozaliwa kabla ya umri. Taratibu za msingi vizazi usiofika umri si vizuri licha ya kuwa ni sababu kubwa ya maradhi na vifo vya watoto wachanga katika Afrika Kusini mwa Sahara. Ni haijulikani kama muundo wa kondo katika vizazi hivi ni sawa katika walio na wasioambukizwa ugonjwa wa HIV. Takwimu hizo zinaweza kuwa na manufaa katika kubuni na utekelezaji wa hatua ya kuingilia katika Mbali na kuleta kuelewa zaidi kwa somo la uzazi usi wa umri.

Utaratibu

Mara ukikubali kushiriki katika utafiti huu , tia saina hii fomuya ridhaa ya kuturuhusu kuhusisha taarifa zilizopatikana kutoka katika takwimu zako. Maelezo yako binafsi hazitajumuishwa dodoso hii ili kulinda faragha yako. Tutachukua sehemu ndogo ya kondo yako kwa lengo la utafiti huu. Tutaangalia rekodi yako ya kliniki ili kupata taarifa zaidi ambayo itabaki siri. Utaendelea kupokea usimamizi mwafaka katika hospitali. Tutahakikisha usalama wako wakati wa kushiriki katika utafiti huu. Ikiwa utakubali watafiti kukusanya vielelezo, yafuatayo yatajiri:

- Hakutakuwa ukeketaji wa kondo
- Vipimo vidogo vitachukuliwa kwa histologia

□ Vitalu tishu zitakaguliwa ndani ya nchi na sampuli kidogo zitasafirishwa kwa uchambuzi zaidi katika Chuo Kikuu cha California San Francisco na mpelelezi mkuu mwenyewe. Baada ya uchambuzi, vitalu vitasafirishwa Kenya na mpelelezi mkuu na kisha kutupa kwa mujibu wa sera za Kenyatta National hospitali.

Usiri

Taarifa zote zitatibiwa na usiri mkubwa. Majina yako hayataonekana kwenye dodoso. Simu utafiti zitatumika badala yake.

Unaweza kuchagua kujiondoa katika utafiti au kukataa kujibu maswali wakati wowote hatua ya utafiti huu. Uamuzi wako hautaathiri huduma kwako katika hospitali.

Taarifa somo

Mimi aliyetia sahihi hapa chini nimeelezwa kwa kina na nimeelewa na kwa hiari kukubali kushiriki katika utafiti. Nimeelewa kwamba kushiriki katika utafiti huu haina faida ya kifedha. Nimehakikishiwa kwamba taarifa zote zitatibiwa na usiri mkubwa na matibabu yangu hayataathirika kama nitakataa kushiriki katika au kujiondoa katika utafiti.

Nilikuwa na nafasi ya kuuliza maswali na kama maswali mengine yatatokea, naweza kuomba mtafiti.

Sijalazimishwa wala kushawishiwa, uamuzi wangu wa kushiriki katika utafiti huu ambao asili, faida na hatari nimeelezwa na Dk / Bi / Bibi .....  
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Sahihi / kidole gumba kushoto

Sahihi ya shahidi

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(Mshiriki)

(Shahidi)

Mimi mpelelezi nimeelezea kwa undani madhumuni ya utafiti kwa mshiriki. Nahakikisha kwamba usiri wa kumbukumbu unapaswa kudumishwa na kwamba hakuna maelezo ya kufafanuliwa ila yale kuhusiana na utafiti.

Sahihi

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Tarehe

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Kwa habari zaidi au ufafanuzi, tafadhali jisikie huru kuwasiliana na kamati ya Maadili na uchambuzi ya KNH / UoN kutumia anwani hapa chini:

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