

**PLACENTAL VILLOUS CHANGES AMONG HYPERTENSIVE AND NORMOTENSIVE
PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL**

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF DEGREE IN
MASTERS OF MEDICINE, HUMAN PATHOLOGY, AT THE UNIVERSITY OF NAIROBI

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H58/80881/2015**

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STUDENT’S DECLARATION

A declaration is hereby made that this dissertation entitled “Placental Villous Changes among Normotensive and Hypertensive Pregnant Women at Kenyatta National Hospital” is my original work and has not been presented for examination in any other University or Institution.

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DEDICATION

I dedicate this book to my nephew Kyle Hannington Wangulu and the rest of my family. You have been my source of inspiration and a blessing to me in the course of my studies.

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LIST OF ABBREVIATIONS

AM: Amniochorionic membrane

ATI-AA: Angiotensin II type I receptor Agonistic Antibody

COL: Collagen fiber

CTGF: Connective tissue growth factor

CTLs: Cytotoxic T lymphocytes

DNA: Deoxyribonucleic Acid

DVH: Distal villous hypoplasia

EMA: Epithelial membrane antigen

FFPE: Formalin-fixed paraffin-embedded

FN: Fibronectin

H&E: Haematoxylin and Eosin

hCG: Human chorionic gonadotropin

hPL: Human placental lactogen

IHC: Immunohistochemistry

IUGR: Intrauterine uterine growth retardation

KNH: Kenyatta National Hospital.

PLAP: Placental like alkaline phosphatase

RAAS: Renin angiotensin aldosterone system

RDS: Respiratory distress syndrome

SMA: Smooth muscle actin

SPI: Pregnancy-specific β 1-glycoprotein

SPSS: Statistical program for social scientists' software package

TNF: Tumor necrosis factor

UoN: University of Nairobi

VUE: Villitis of unknown etiology

DEFINITION OF TERMS AS USED IN THE STUDY

Eclampsia: the occurrence of convulsions in the presence signs of pre-eclampsia (hypertension and proteinuria) during pregnancy or labor or within 7-days of delivery. The etiology of convulsions should not be due to epilepsy or other convulsive disorders.

HELLP syndrome: Is a syndrome that complicates pre-eclampsia and is characterized by hemolysis, elevated liver enzyme levels and a low platelet count.

Histomorphology: the study of structure and form including the histology and cytology of the tissues of the body as observed under light microscopy.

Hypertension: Sustained elevation in blood pressure; (≥ 140 mmHg systolic or ≥ 90 mm Hg). The blood pressure should be correctly taken i.e. in the upper arm using the correct sized cuff.

Intrauterine uterine growth retardation (IUGR): refers to less than 10 percent of predicted fetal weight for gestational age

Preeclampsia: A new onset of sustained elevated blood pressure and proteinuria (at least 1+ on dipstick or ≥ 300 mg in a 24-hour urine collection) first occurring after 20 weeks of gestation.

Villous Histomorphology: the study of structure and form including the histology and cytology of human placental chorionic villi as observed under light microscopy

ABSTRACT

Background

Hypertensive pregnancy disease is a major cause of maternal morbidity and mortality in Africa, complicates 2.73% of pregnancies in Kenya and is one of the major causes of maternal deaths in Kenyan public health facilities. Hypertensive disease in pregnancy, particularly preeclampsia, has been associated with placental villous histomorphological changes. Few studies have been done elsewhere to demonstrate these changes and have found different and conflicting findings. Additionally, there are still few requests for placental histopathological evaluation by clinicians in Kenyatta National Hospital and in other hospital settings probably due to a perceived low clinical utility. This study hypothesized that established associations between placental villous histopathology and clinical variables such as maternal blood pressure status and neonatal outcomes adds to the available evidence and help sensitize clinicians on the need for histological examination of placentae.

Study Objective

The main objective of this study was to determine placental villous changes among hypertensive and normotensive pregnant women who delivered at Kenyatta National Hospital.

Materials and Methods

This was a laboratory based retrospective cross-sectional analytical study design carried out at the University of Nairobi (UoN) Histopathology Laboratory. The study made use of archived placental tissue blocks obtained from hypertensive pregnant women and their normotensive counterparts who delivered at Kenyatta National Hospital between July and December 2015. All available specimens ($n=138$) were retrieved, processed and analyzed. Data was entered and analyzed using SPSS version 20. Proportions of villous histomorphological findings were compared between hypertensive and normotensive groups. A *Chi* square test of association was used to determine association between the villous histomorphological findings and

hypertensive pregnancy disease. Association between villous histomorphological findings and the different clinical groups of hypertension in pregnancy was determined using Fisher's exact test. Villous histomorphological findings was associated with neonatal outcomes using *Chi* square test.

Results

Placentae from women with hypertensive pregnancy disease at Kenyatta National Hospital had significantly higher rates of accelerated villous maturity, distal villous hypoplasia, stromal fibrosis, decidual arteriopathy, villous infarction and an increased area (>25%) of intervillous fibrin deposition ($p<0.01$). The Pre-eclampsia-eclampsia clinical group had placentae characterized by lesions associated with placental ischemia supporting evidence that placental hypoperfusion could be characteristic of pre-eclampsia-eclampsia rather than gestational hypertension. Villous histomorphological findings associated with poor neonatal outcomes include accelerated maturation, villous hypoplasia, villous infarction, stromal fibrosis and decidual arteriopathy.

Recommendation

Sensitization of clinicians in Kenyatta National Hospital on the clinical utility and need for placental histopathological examination in the following clinical scenarios among others: intrapartum management of hypertensive pregnant women, postmortem evaluation of unexplained maternal mortality where hypertensive pregnancy disease needs to be ruled out and evaluation of poor neonatal outcomes for possible aetiology. Future studies should use multiple and variably selected representative sections of the placental disc with equal sampling from all the hypertensive disease groups to enable better comparisons.

1.0 INTRODUCTION

Hypertension in pregnancy complicates about 10% of pregnancies worldwide; 2-12% and 5-6% pregnancies in the United States and the United Kingdom respectively (1). It is a major cause of maternal mortality in Africa (2), complicates 2.73% of pregnancies in Kenya (3) and is one of the main causes of maternal deaths in Kenyan public health facilities (3).

Hypertensive disorders in pregnancy have been classified by the National High Blood Pressure Education Program (4) as Chronic Hypertension, Pre-eclampsia-Eclampsia, Pre-eclampsia Superimposed on Chronic Hypertension and Gestational Hypertension. This is based on the timing of onset of high blood pressure and appearance of proteinuria among other clinical findings. Hypertension in pregnancy complicates with premature deliveries, low birth weights, still births, neonatal deaths and long-term neonatal neurological sequelae (5,6). Maternal complications include risk of recurrence in subsequent pregnancies, an increased cardiovascular disease risk, placenta abruptio (6), renal failure, coagulopathy, cardiac arrest, cerebrovascular accidents, respiratory failure and liver failure (7).

Histopathological examination of the placenta provides an accurate record of infant's prenatal experiences. Structural and functional derangement of the placenta helps establish adequacy of the maternal environment and provides information on expected fetal outcomes. Despite this utility of placental histopathology examination, there are still few requests for placental histopathological evaluation by clinicians in Kenyatta National Hospital and in other hospital settings(7) within the country, probably due to perceived low clinical utility.

Hypertensive disease in pregnancy, particularly preeclampsia, has been associated with placental villous histomorphological changes (8–10). Few studies have been done to demonstrate these changes and have found different findings (2,7,9,11–17). None of the studies included other hypertensive groups in pregnancy such as chronic hypertension in pregnancy and gestational hypertension and therefore probable villous histomorphological changes remain unknown. In addition, no studies have been done in Kenya to demonstrate variations or

changes in villous histomorphology in normotensive and hypertensive pregnant women. Studies elsewhere have evaluated some of the villous histopathology features with different findings about their association with immediate neonatal outcomes (7). No studies have been done locally to determine this association. This study hypothesizes that established associations between placental villous histopathology and clinical variables such as maternal blood pressure status and neonatal outcomes will add to the available evidence and help sensitize clinicians on the need for histology examination of placentae in Kenyatta National Hospital and other hospital settings.

1.1 Literature Review

1.1.1 Histology of Mature (Third Trimester) Placental Tissue

Placental tissue consists of a chorionic plate (fetal component) and decidua (maternal component) that are held together by chorionic villi as illustrated in figure 1.

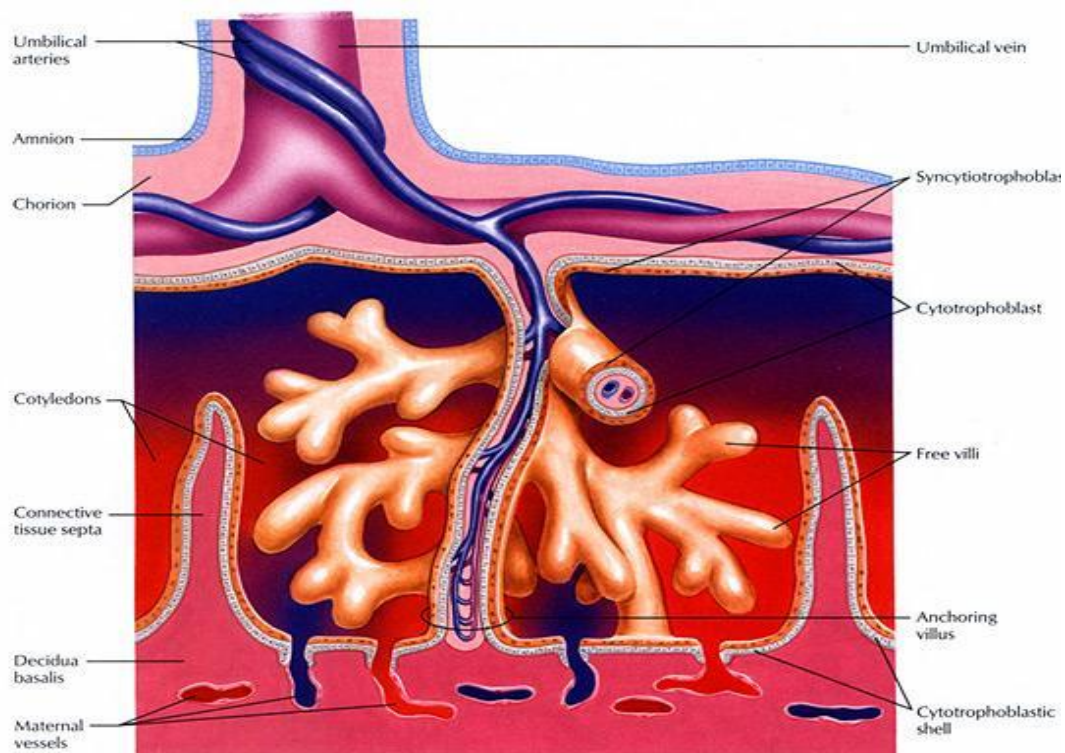


Figure 1. Layers of placental tissue in the third trimester. Sourced from Atlas of Non Tumor Pathology-Placental Pathology (18).

The amniochorionic membrane forms the outer limits of the sac that encloses the fetus whereas innermost layer is the amniotic membrane (Figure 2). The amniotic membrane consists of a cuboidal epithelium that overlies a thick basement membrane and a stromal layer.

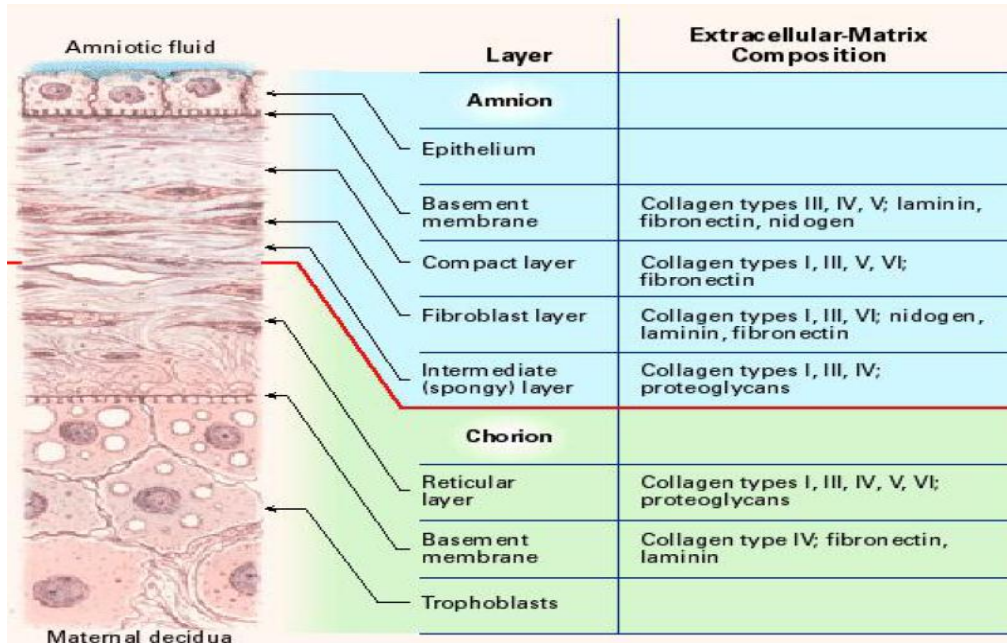


Figure 2. Histology of the amniochorionic membrane. Sourced from the European Cells & Materials Journal (19).

The chorionic membrane lies deep to the amnion and is composed of an inner layer of vascularized connective tissue (reticular layer) consisting of sparsely cellular collagen layer and an outer layer of trophoblasts overlying a basement membrane. The surface of the chorionic plate consists of outgrowths of villi (Figure 1). The villi are sectioned in many different planes, and their attachment to the chorionic plate may not be evident (Figure 3). The decidual layer is composed of decidualized maternal cells, fibrin, vessels and areas of hemorrhage.

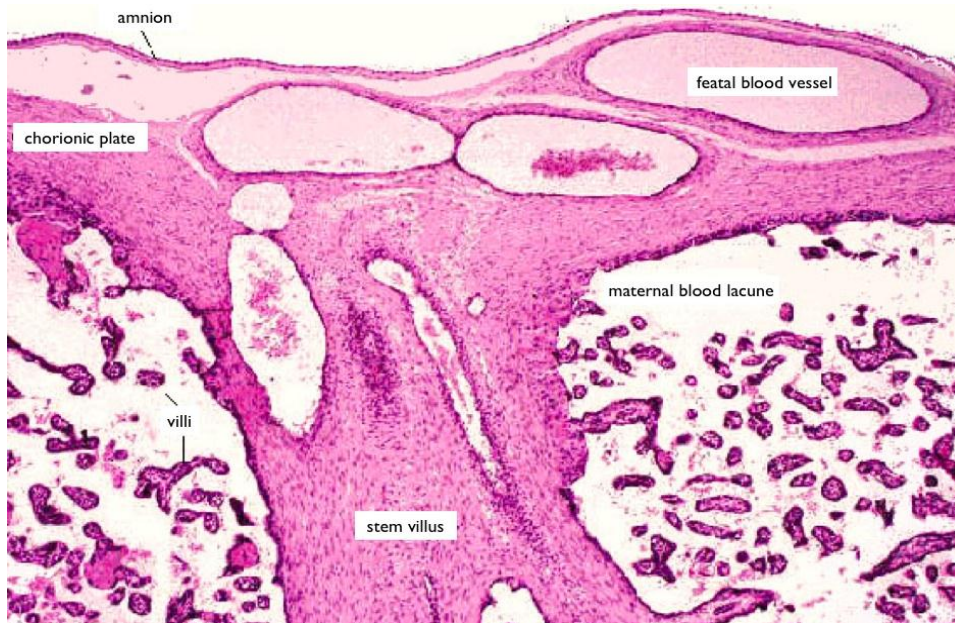


Figure 3. H & E stained section of placental tissue showing the amniochorionic membrane and villi. Sourced from Virchows Archiv: European Journal of Pathology (17).

1.1.2 Placental Villous Histology

During fetal development, the human villous placenta develops after 10 weeks' gestation within a pool of maternal blood. It develops into a tree-like structure gradually branching and become elaborate to form terminal villi. The terminal villi is composed of fetal capillaries interspersed with stroma that underlies trophoblast cells. Villous trophoblasts are epithelial cells of fetal origin and include an outer layer of multinucleated non-replicating syncytium (the syncytiotrophoblast) and underlying immature, replicating, mononuclear cytotrophoblasts (Figure 4). The syncytiotrophoblast is constantly renewed by loss of aged nuclei and cytoplasm in structures called syncytial knots and is replenished by fusion of underlying cytotrophoblasts.

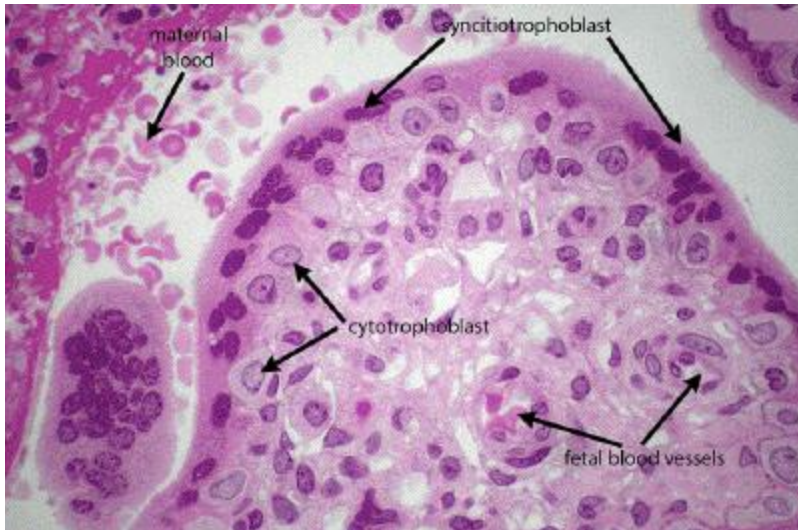


Figure 4: H & E stained section of placental tissue showing terminal villus consisting of multilayered trophoblastic cells (cytotrophoblast and syncytiotrophoblast) and fetal blood vessels . Sourced from Virchows Archiv: European Journal of Pathology (17).

During the first trimester, the villous trophoblast has an outer syncytiotrophoblast layer and an inner cytotrophoblast layer. In term placenta, the cytotrophoblast is discreet and the syncytiotrophoblast is clumped in some areas forming syncytial knots. The syncytiotrophoblast is composed of multinucleated giant cells with abundant acidophilic cytoplasm that is strongly immunoreactive for human chorionic gonadotropin (hCG). Other reactive immunohistochemistry markers include keratin, placental-like alkaline phosphatase (PLAP), human placental lactogen (hPL), inhibin and pregnancy-specific β 1-glycoprotein (SP1). Negative immunohistochemistry markers include epithelial membrane antigen (EMA), CD146 (Mel-CAM) and HNK1 (CD57). The cytotrophoblast is the progenitor of syncytiotrophoblast and appears microscopically as mononuclear cells with clear cytoplasm and a distinct cell membrane. It is only immunoreactive to keratin. The syncytiotrophoblast forms a feto-maternal immunological barrier and direct contact with maternal blood facilitates exchange of nutrients and gases. Early in embryonic development, syncytiotrophoblasts

produce proteolytic enzymes that break down extracellular matrix facilitating invasion into endometrial wall. The cells also secrete Human Chorionic Gonadotropin (hCG) hormone that maintains the corpus luteum and human placental lactogen which has anti-insulin like metabolic function.

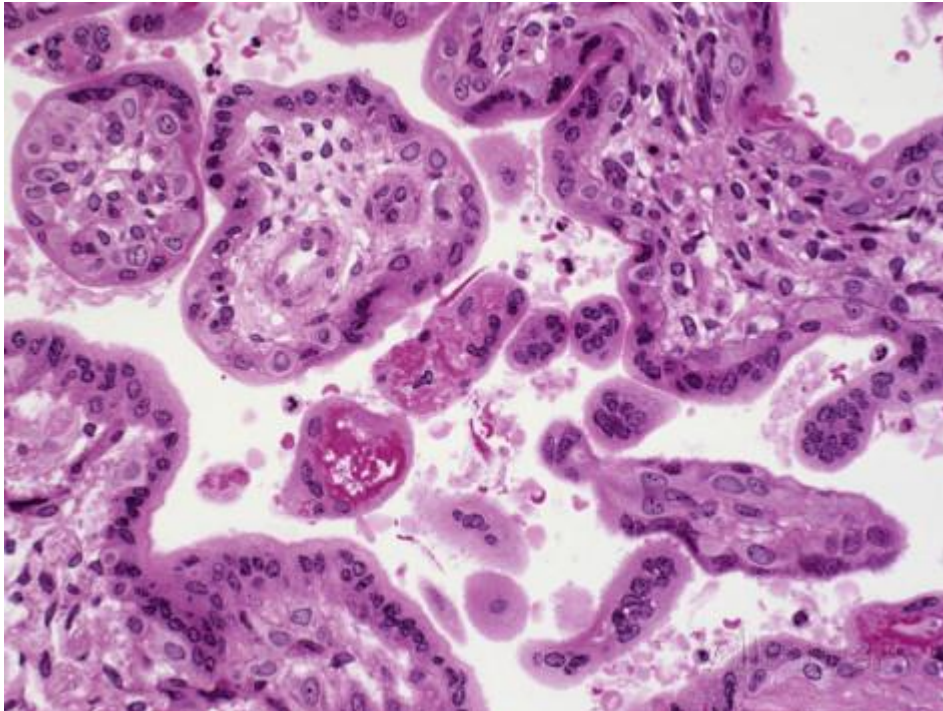


Figure 5. H&E section of mature placenta at term with villi covered by syncytiotrophoblasts. Sourced from Virchows Archiv: European Journal of Pathology (11).

1.1.3 Hypertension in Pregnancy

Hypertension in pregnancy is defined by a sustained elevation in blood pressure; (≥ 140 mmHg systolic or ≥ 90 mm Hg). The blood pressure should be correctly taken i.e. in the upper arm using the correct sized cuff. The pregnant woman should have rested for several minutes and a different reading confirmed at least twenty-minutes later or on a separate occasion. Hypertension in pregnancy is classified by the National High Blood Pressure Education Program(4) as illustrated in Table 1 based on the timing of onset of high blood pressure and appearance of proteinuria.

Table 1. Classification of Hypertension in Pregnancy (4).

Classification	Diagnosis
Chronic hypertension	(i) Increased blood pressure before 20 weeks gestation or existing prior to pregnancy. (ii) persistent hypertension after 12 weeks postpartum
Preeclampsia-eclampsia	(i) Onset of hypertension after 20 weeks gestation (ii) Proteinuria of 300 mg/24 hr or more
Preeclampsia superimposed upon existing hypertension	(i) Onset of proteinuria on pre-existing hypertension
Gestational hypertension	(i) Transient hypertension after 20 weeks gestation (ii) Return to normal blood pressure after delivery (iii) Absence of proteinuria

Pre-eclampsia and eclampsia are both forms of hypertensive disorders in pregnancy. Severe Preeclampsia is defined by features listed in Table 2 in addition to sustained elevation of blood pressure and proteinuria. Eclampsia is characterized by episodes of convulsions in the presence features of pre-eclampsia during pregnancy or labor or within 7-days postpartum. The etiology of convulsions should not be due to epilepsy or other convulsive disorders.

Table 2. Findings in Severe Pre-eclampsia (4).

Severe Pre-eclampsia	<ol style="list-style-type: none">1. Blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic2. Urine protein excretion of greater than five grams in a 24-hour collection3. Neurologic disturbances (visual changes, headache, seizures, coma)4. Pulmonary edema5. Hepatic dysfunction (elevated liver transaminases or epigastric pain)6. Renal compromise (oliguria or elevated serum creatinine concentration)7. Creatinine ≥ 1.2 is considered abnormal in women without a history of renal disease)8. Thrombocytopenia9. Placental abruption, fetal growth restriction, or oligohydramnios
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1.1.4 Epidemiology of Hypertensive Pregnancy Disease

About 76,000 pregnant women die each year from hypertensive disease in pregnancy (1). It complicates 10% of pregnancies worldwide; 2-12% and 5-6% pregnancies in the United States and the United Kingdom respectively (20). Hypertension in pregnancy is a major cause of maternal mortality in Africa accounting for 9.1% of maternal deaths (20). In Kenya, hypertensive pregnancy disease complicates 2.73% of pregnancies and accounts for 19% of maternal deaths in Kenyan public health facilities (21).

1.1.5 Aetiopathogenesis of Hypertensive Disease in Pregnancy

Chronic hypertension is defined by sustained elevation of blood pressure before pregnancy (with or without use of antihypertensive medications), before 20 weeks of gestation in pregnant women or persistence of hypertension for more than 12 weeks postpartum. Risk factors for

chronic hypertension include increase in age and obesity (6). Gestational hypertension is characterized by onset of hypertension after mid-pregnancy (Table 1). Pre-eclampsia refers to new onset hypertension after mid-pregnancy (Table 1) accompanied by proteinuria. The pathophysiology of pre-eclampsia remains unclear but several theories have been adopted and have been classified as: placental, renal, immunologic, dietary and genetic (4).

The placental theory is derived from that fact that delivery of the placenta results in resolution of clinical symptoms. During placental development, vascularization involves a balance between of antiangiogenic and proangiogenic factors. An imbalance in these factors results in abnormal placental vascularization with consequent hypertension and proteinuria.

According to the renal theory, despite a reduction in renin, angiotensin, aldosterone system (RAAS) activity and plasma levels of renin as compared to uncomplicated pregnancy, there is still an unexpected finding of elevated blood pressure. Loss of angiotensin II resistance as expected in normal pregnancy has been attributed to the angiotensin II type 1 receptor agonistic antibody (AT1-AA) that increase the renin, angiotensin, aldosterone system (RAAS) leading to elevated blood pressure. Activation of AT1 receptors has also been shown to increase factors such as sFlt1, Plasminogen activator inhibitor-1, sEng, reactive oxygen species, NADPH oxidase and tissue factor that lead to endothelial cell dysfunction. This results in vascular damage which has been associated with pre-eclamptic state.

The immune theory postulates that immunologic difference between couples inherited by the fetus generates an immune response in pregnant women. Persistent maternal antibodies to fetal human leucocyte antigens (HLA) of paternal origin are thought to mediate inflammatory events in pre-eclampsia. The cytokine release induced by inflammation resulting in endothelial injury is one of the main mechanisms underlying the pathogenesis of preeclampsia.

There's evidence that diet is contributory to development of hypertension in pregnancy.

Diet is also a risk factor for cardiovascular disease in both pregnant and non-pregnant state.

Serum levels of nutrient elements; hypocalcemia, hypomagnesemia, hypertriglyceridemia, increased fatty acids and reduced levels of vitamin D and zinc have been linked to an increased risk of pre-eclampsia in the pregnancy state. Furthermore, increased inflammation and oxidative stress is a key feature in pre-eclampsia and has been associated with decreased vitamins E and C, iron, zinc and increased polyunsaturated fatty acids.

Genetics also plays a role in the development of pre-eclampsia. Family history of preeclampsia among two first degree relatives bestows a 2 to 4-fold risk of preeclampsia to a woman. Several polymorphisms in the players in the placental development angiogenic imbalance mainly sFlt1 and VEGF have been found in pre-eclamptic women. However, no causal mutations have been identified for PGF, sFlt1, and sEng so far. A higher incidence of preeclampsia has been shown in women who delivered trisomy 13 fetuses. As concerns paternal genotype, males who have fathered preeclamptic pregnancies have an increased risk of fathering a pre-eclamptic pregnancy with a different woman. In addition, there is higher risk of fathering a pre-eclamptic pregnancy by men born from a preeclamptic pregnancy.

1.1.6 Feto-Maternal Complications of Hypertensive Disease in Pregnancy

Chronic hypertension in pregnancy has been associated with preterm and small for gestational age births (6). There is an even higher risk for both among those with pre-eclampsia (5). Other fetal complications of hypertension in pregnancy include still births, neonatal deaths and long-term neonatal neurological sequelae (5).

Pregnant women with chronic hypertension can develop superimposed preeclampsia (6). Preeclamptic ones have risk of recurrence in subsequent pregnancies and an increased cardiovascular disease risk. Severe maternal morbidity associated with preeclampsia and eclampsia includes renal failure, stroke, cardiac arrest, respiratory failure, coagulopathy and liver failure (5). Placenta Abruptio, premature separation of the placenta from the underlying myometrium, is also a common complication of hypertension in pregnancy and is a known

cause of antenatal hemorrhage (6).

1.1.7 Utility of Placental Histopathology Examination

The placenta is an accurate record of infant's prenatal experience. Structural and functional derangement of the placenta may be the only yardstick to measure adequacy of the fetal environment. Findings from placental examination provide information on expected fetal outcomes (7). This enables prior counselling on anticipated developmental complications and planning on medical follow up.

Few studies have been done elsewhere on placental microscopic findings in hypertensive pregnancies with different findings reported (7). A number of microscopic abnormalities in the villi like basement membrane thickening, decreased villous vascularity, stromal fibrosis, syncytial knot formation, cytotrophoblastic proliferation and villous fibrinoid necrosis have been reported (8–10). No studies have been done locally to narrow down on the chorionic villous which is the main placental functional unit.

1.1.8 Villous Histomorphology in Hypertensive Pregnancy State

This study is a continuation of an age matched case control study {cases (n=94) being women with hypertensive disease in pregnancy delivered at the KNH and the controls (n=94) being normotensive women delivered during the same period} by Ogutu *et al.* Their study assessed morphological differences in placental pathology between pregnant women with hypertension and their normotensive counterparts who delivered in KNH labour ward in between July and December 2015. Specifically, they compared the gross and histological changes in the placentae between the normotensive and hypertensive groups. Additionally, they also correlated the gross anatomical and histological changes with foetal outcomes.

In their study, gestation at delivery (36 weeks Vs 38.9 weeks), mode of delivery (C-section Vs Vaginal), fetal weight (600g smaller) and NBU admission (four times more due to poor APGAR score, prematurity, respiratory distress syndrome and asphyxia) significantly varied between

the hypertensive and normotensive groups respectively. Gross placental morphological findings included a shorter cord and lower (800g less) placental weight for the hypertensive group. Presence of infarction and areas of calcification were also significantly dominant in the hypertensive group. Statistically significant findings on microscopy were the presence of sub chorionic fibrin, syncytial knots and hyalinised spots within villi. With preliminary findings of some villous histomorphological changes (syncytial knots and hyalinised spots), there is need to comprehensively evaluate the chorionic villi among the two groups to include other unreported areas such as maturity, stromal changes, vascular changes, presence of inflammation and fibrin deposition. This study made use of a new tool, the Amsterdam Criteria (22), that provides a defined clinicopathological correlation between these findings and fetomaternal outcomes.

1.1.8.1 Villous Maturity in Hypertensive Pregnancy Disease

Uteroplacental hypoxia that is associated with pregnancy-induced hypertension results in different patterns of villous maldevelopment (7). Different and conflicting findings have been reported including delayed villous maturity (23), distal villous hypoplasia (15) and accelerated maturity (24). It will be important to establish the pattern of villous maturity in the local population in this study.

Delayed villous maturation (DVM) shows villi of increased villous diameter with cellular stroma and increased extracellular matrix. Capillaries are centrally placed with reduced vasculosyncytial membranes. Villous trophoblast is hypercellular with persistent cytotrophoblast. Diagnostic for DVM is more than 30% immature villi within the basal two-thirds of the placental parenchyma on microscopy. Delayed villous maturation is clinically associated with intrauterine hypoxia, fetal growth restriction and fetal death (23).

Distal villous hypoplasia (DVH) is defined as paucity of villi accompanied by an extended intervillous space. Villi are thin, appear elongated and have increased syncytial knots. At low-

power microscopy, these features are seen in the lower two-thirds and involve at least 30% of 1 full-thickness parenchymal slide. DVH has been associated with maternal vascular malperfusion, clinical pre-eclampsia, fetal growth retardation, poor neurodevelopmental outcome and adult cardiovascular health (15).

Accelerated villous maturation refers to presence of small or short hypermature villi for gestational period, usually accompanied by an increase in syncytial knots. Microscopically, they appear as a diffuse pattern of term-appearing villi with increased syncytial knots and intervillous fibrin alternating with areas of villous paucity. Accelerated villous maturation is commonly seen in placental insufficiency (24), which includes preeclampsia (7). These features are seen as a placental reaction to decreased materno-placental perfusion that is associated with pregnancy induced hypertension (24). The resulting placental ischemia results in accelerated villus branching and formation of large and numerous syncytial knots (25) as an adaptive response.

1.1.8.2 Villous Stromal Changes in Hypertensive Pregnancy Disease

Villous stroma forms supportive matrix for the trophoblastic cells and a filtration barrier between maternal and fetal circulations. Cells within the stroma include macrophages (Hofbauer cells), few mast cells and plasma cells (26).

Villous stromal fibrosis has been associated with hypertensive disease in pregnancy (16). This has been explained by placental hypoxia in pregnancy induced hypertension that upregulates expression of fibrosis related factors that include smooth muscle actin (SMA), collagen fiber (COL), fibronectin (FN) and connective tissue growth factor (CTGF) (16).

Villous edema refers to accumulation of fluid in the villous interstitium. This causes disruption and fluid replacement of intravillous cellular structure. It has been seen in pre-eclamptic placentas and has been attributed to functional inadequacy of the fetal circulation (7). Hydropic change of the edematous villi may decrease competence of the intervillous space and thus

constraint maternal flow through the placenta. It has been shown to result in adverse fetal outcomes including fetal ischemia (7).

1.1.8.3 Villous Inflammation and Fibrin Deposition in Hypertensive Pregnancy Disease

Term placentas exhibit perivillous fibrin or fibrinoid deposition. It is thought to develop following damage to syncytiotrophoblast resulting in clotting in the intervillous space and closure

of the trophoblastic defect by a fibrinoid plug. Microscopically, it appears as pink and lamellar in structure at times encasing villi. Proportion of fibrinoid deposition increases with advancing pregnancy but a diffuse increase may reflect chronic intervillous perfusion defects as seen in preeclampsia (7,12,27). Increased fibrin may impair gas exchange and lead to growth restriction, oligohydramnios, premature birth, neurologic impairment or fetal death (7,12).

Chronic villitis refers to a lymphohistiocytic infiltrate involving the chorionic villi which can be due to infectious agents or Villitis of Unknown Etiology (VUE). Placental ischemia and hypoxic injury in hypertensive pregnancy disease with resultant inflammatory response has been suggested as a cause of chronic villitis observed in pre-eclampsia (12,27).

1.1.8.4 Villous Vascular Changes in Hypertensive Pregnancy Disease

Chorangiosis is defined as 10 or more capillaries in each of 10 villi in 10 fields inspected with a X10 objective in three different non-infarcted areas of the placenta. This increase in villous capillaries is an adaptation to hypoxia in hypertensive pregnancy disease (28). Chorangiosis has been associated with perinatal mortality, pregnancy disorders and perinatal circumstances that suggest chronic hypoxia (28,29).

During placental development, placental trophoblasts infiltrate decidual arterial beds and superficial myometrium at the implantation site. They destroy arteriole walls and replace them with fibrinoid transforming them into enlarged, tortuous and rigid channels not capable of a vasoconstrictive response to local vasoactive mediators. In preeclampsia, this invasion is

altered and referred to as lack of physiologic conversion. Instead, there is fatty infiltration and lipid-laden myogenic foam cells (atherosis), decidual vascular thrombosis and fibrinoid necrosis of the media (25).

Placental infarcts represent dead villous tissue because of poor maternal circulation. Microscopically, early infarcts appear as congestion of villous capillaries, intravillous hemorrhage followed by villous agglutination or collapse of the intervillous space. Trophoblast and villous stromal cells lose their staining characteristics in late infarcts and appear as ghost villi. They are

completely collapsed, interspersed only with a thin layer of fibrinoid material. Placental hypoxic injury in hypertensive pregnancy disease explain the villous infarcts (7,12,27).

1.1.9 Villous Histomorphological Findings among Hypertensive Pregnancy Disease Groups

The clinical groups of hypertensive disorders include Chronic Hypertension, Pre-eclampsia-Eclampsia, Gestational Hypertension and Pre-eclampsia Superimposed on Chronic Hypertension. The pathophysiology of preeclampsia is multifactorial (4). Aberrations in extravillous trophoblast insertion into maternal wall vasculature during placental development results in uteroplacental hypoperfusion (7,12,27). Consequent release of antiangiogenic factors into the maternal circulation causes endothelial damage, hypertension and other attendant clinical features; hematologic, cardiac, neurologic, renal, pulmonary, and hepatic dysfunction (7). Triggers for abnormal extravillous trophoblast insertion and subsequent events remains unknown. Eclampsia is considered the convulsive manifestation of preeclampsia at the worse end of the severity spectrum (4).

Gestational hypertension is considered a transient condition where the final diagnosis is determined upon re-evaluation at 12 weeks postpartum (4,30). There is characteristic return to normotensive state. Persistence beyond 12 weeks is considered to be chronic hypertension. It

remains unclear whether gestational hypertension and pre-eclampsia are independent conditions with a similar phenotype (hypertension) or if gestational hypertension is an early mild stage of preeclampsia (30). Gestational hypertension can complicate with proteinuria therefore developing into preeclampsia (30).

Chronic hypertension in pregnancy refers to sustained elevated blood pressure before pregnancy, before 20 weeks of gestation, use of antihypertensive medications before pregnancy, or persistence of hypertension for more than 12 weeks after delivery (4,6). Secondary causes of chronic hypertension have been defined but the primary causes are not well understood. It is postulated that genetic and environmental factors interact to alter both renal and cardiovascular physiology (6). Like gestational hypertension, chronic hypertension can complicate with superimposed preeclampsia (4,6).

Chronic hypertension, pre-eclampsia-eclampsia and gestational hypertension are regarded as distinct entities with respect to their epidemiology including risk factors and recurrence rates, pathogenesis, hemodynamic characteristics and severity of maternal and neonatal adverse outcomes (31). Chronic hypertension and gestational hypertension can complicate with pre-eclampsia and subsequently develop HELLP syndrome (4,6,31). It was important to try and reconcile these two apparently conflicting observations.

In this regard, there is long-standing controversy of whether chronic, gestational hypertension and pre-eclampsia are independent disorders or a mild pre-onset form of pre-eclampsia. Determining the difference between villous histomorphological findings among the hypertensive pregnancy disease groups (pre-eclampsia eclampsia, chronic hypertensive disease and gestational hypertension) may provide insight onto whether the different groups have a common pathological end point; an altered villous histomorphology. This could possibly explain progression from a mild altered villous histomorphology (chronic hypertensive disease and gestational hypertension) to a more severe alteration (pre-eclampsia).

Correa *et al* (32) established that women with pre-eclampsia had placentae characterized by increased syncytial knots and differences in fibrin deposit pattern and size as compared to their gestational hypertension counterparts. In a different study (33), placentae from women with pre-eclampsia had a higher incidence of villous infarction and decidual vasculopathy. These studies did not include the chronic hypertension group which too can progress to pre-eclampsia and did not evaluate other placental villous 'syndromes' like villous maturation, villous stromal changes, villous inflammation and other vascular changes like chorangiosis. No studies have been done locally to evaluate these differences.

1.1.10 Villous Histomorphology Findings and Neonatal Outcomes.

Histopathology examination of the placenta can help diagnose a wide array of lesions and help predict and explain adverse neonatal outcomes. The role of the placental examination in fetal mortality, neonatal morbidity including neurological sequelae is becoming increasingly clear as suggested by several studies where placental lesions were one of the causes of fetal demise (31). For instance, placental features of maternal vascular underperfusion has been listed as the main (34 -38%) cause of fetal death (34). Features of maternal vascular underperfusion are a main feature in hypertensive pregnancy disease placentae (28,29).

Different studies have found an association between placental abnormalities and neonatal conditions like birth asphyxia (31), necrotizing enterocolitis (35), fetal cardiac abnormalities (36), neonatal respiratory distress syndrome (37). Studies elsewhere have evaluated some of the villous histopathology features; villitis, chorangiosis, maternal vascular malperfusion and villous edema with conflicting findings about their association with immediate neonatal outcomes (31). No studies have done locally to determine this association.

Histopathology examination of the placentae from normotensive and hypertensive pregnant women remains unrequested for by clinicians including pediatricians. This is despite current recommendations for placental examination in maternal conditions like hypertensive

pregnancy disease(38). This study hypothesizes that placental villous histological examination provides a possible explanation and therefore useful information in predicting immediate neonatal outcomes.

In other studies, neonatal mortality and morbidity shortly after birth has been determined by several clinical variables. These include gestational age at delivery, pregnancy outcome (live birth versus fresh and still births), birth weight, Apgar score, neonatal intensive care unit (ICU) or new born unit (NBU) admission, 72 hours' neonatal outcome among others (31). The study used well defined and reproducible chorionic villous lesions as defined by Amsterdam criteria (22) to determine their relationship with such clinical variables of neonatal outcomes.

1.1.11 Conceptual Framework

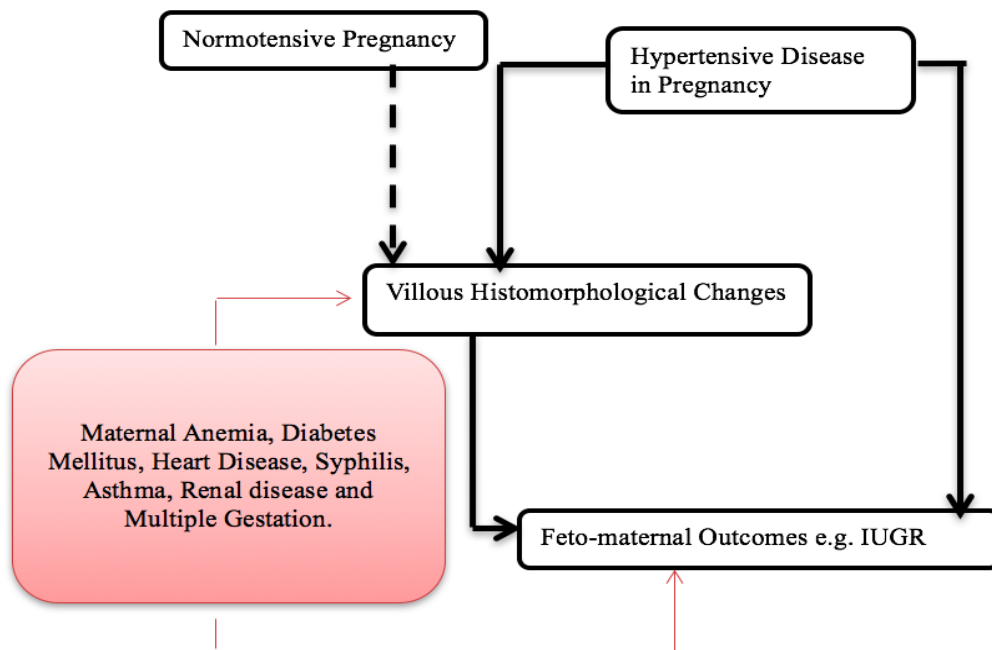


Figure 6. An illustration of the interaction between variables (hypertensive status, villous histomorphology), confounders (maternal anemia, multiple gestation, and diabetes in pregnancy, heart disease, syphilis, asthma and renal disease) and feto-maternal outcomes.

In this study, the independent variable was the blood pressure status of the pregnant woman classified as either normotensive or hypertensive and its effect on the placental villous morphology was determined. The dependent (outcome) variables in this study were villous histomorphological changes (presence or absence of distal villous hypoplasia, thickening of villous basement membranes, villous stromal fibrosis, villitis, increased or decreased villous vascularity, presence of vascular wall thickening, perivasculitis, thrombosis and lumen obliteration). Possible confounders causing villous histomorphological changes such as maternal anemia, multiple gestation, and diabetes in pregnancy, heart disease, syphilis, asthma and renal disease were excluded from the study. The various villous histomorphological changes were postulated to result in the adverse fetomaternal outcomes such as intra uterine growth restriction (IUGR), fetal death and newborn unit admission.

2.0 STUDY RATIONALE

2.1 Problem Statement

Hypertension complicates 2.73% of pregnancies and accounts for 19% of maternal deaths in Kenyan public health facilities. Hypertensive disease in pregnancy, particularly preeclampsia, has been shown to result in histomorphologic changes within the villi. Histopathological examination of the placenta provides a record of the infant's prenatal experiences and helps predict neonatal outcomes yet it remains unrequested for by clinicians elsewhere and in Kenyatta National Hospital. Few requests for placental histopathological evaluation by clinicians in Kenyatta National Hospital and in other hospital settings within the country are probably due to a perceived low clinical utility.

There is paucity of data given the few studies that have been done in other countries on villous histomorphology in pre-eclamptic women. Different and conflicting findings have been reported from these studies. Findings on other hypertensive groups (chronic hypertension and gestational hypertension) remain unknown. There are no local studies on villous histomorphological findings and their association of maternal blood pressure status and neonatal outcomes. There is an unresolved clinical question on whether gestational hypertension, chronic hypertension and pre-eclampsia-eclampsia are distinct entities or a continuum of increasing severity. Findings from this study provide additive evidence of presence or absence of a common pathology endpoint, alteration of villous histomorphology, for the different hypertensive groups.

In the preceding study by Ogutu *et al* at Kenyatta National Hospital, preliminary findings of few evaluated descriptive villous histomorphological changes (syncytial knots and hyalinised spots) among hypertensive pregnant women suggested a need to comprehensively evaluate the chorionic villi. This study used a new tool (Amsterdam Criteria) that provides a defined clinicopathological correlation between these findings and fetomaternal outcomes.

2.2 Justification

This study adds to the body of knowledge on placental changes in hypertensive diseases in pregnancy, and provides local data on changes in villous histomorphology associated with these disorders. It has also provided information on the difference in these changes among hypertensive groups and possible association with neonatal outcomes. This study has contributed to help resolve the clinical question on whether gestational hypertension, chronic hypertension and pre-eclampsia-eclampsia are distinct entities or a continuum of increasing severity. In this regard, findings from this study provide additive evidence of presence or absence of a common pathology end point, alteration of villous histomorphology, for the different hypertensive groups.

This study confirmed that placental villous histological examination could provide histopathology evidence of hypertensive pregnancy disease and assist in predicting neonatal outcomes. This has added to the available evidence to help sensitize clinicians on the need for histological examination of placentae in Kenyatta National Hospital and other hospital settings.

2.3 Research Questions

1. What are the placental villous changes among normotensive and hypertensive pregnant women who delivered at Kenyatta National Hospital?
2. Is there an association between placental villous changes and hypertensive disease among pregnant women who delivered at Kenyatta National Hospital?
3. Is there difference in villous histomorphological findings between the hypertensive pregnancy disease groups among women who delivered at Kenyatta National Hospital?
4. Is there association between villous histomorphology findings and neonatal outcomes among normotensive and hypertensive pregnant women who delivered at Kenyatta National Hospital?

2.4 Objectives of the Study

2.4.1 Broad Objective

To determine placental villous changes among hypertensive and normotensive pregnant women who delivered at Kenyatta National Hospital.

2.4.2 Primary Specific Objectives

1. To describe placental villous histomorphology among normotensive and hypertensive pregnant women who delivered at Kenyatta National Hospital.
2. To determine association between placental villous histomorphology and hypertensive disease in pregnant women who delivered at Kenyatta National Hospital.
3. To determine difference in villous histomorphological findings between the hypertensive pregnancy disease groups among women who delivered at Kenyatta National Hospital.
4. To determine association between villous histomorphology findings and neonatal outcomes among normotensive and hypertensive pregnant women who delivered at Kenyatta National Hospital.

3.0 METHODOLOGY

3.1 Study Design

A retrospective cross-sectional analytical study design was adopted for this study. This enabled determination of incidence of placental villous variables in hypertensive and normotensive pregnant women.

3.2 Study Setting

The study was carried out at the University of Nairobi (UoN) Histopathology laboratory located at Kenyatta National Hospital. The laboratory has capacity to carry out special staining techniques requested for by pathologists.

3.3 Study Population

The study population comprised of hypertensive and normotensive women who delivered at Kenyatta National Hospital between July and December 2015 and had their placental tissues archived.

3.3.1 Inclusion Criteria

1. Case files of pregnant women who delivered at KNH in their third trimester (>28 weeks gestation) between July and December, 2015; and their placental tissue obtained and archived at KNH Histopathology Laboratory in the preceding study.
2. Case files of normotensive (sustained blood pressure obtained at least 6 hours apart; <140/90mmHg at the point of diagnosis) and hypertensive pregnant women (sustained blood pressure obtained at least 6 hours apart; > 140/90mmHg at the point of diagnosis).

3.3.2 Exclusion Criteria

1. Placental tissue from participants with conditions that are associated with placental ischemia or placental changes: anemia, multiple gestation, diabetes mellitus in

pregnancy, heart disease, syphilis, asthma and renal disease.

2. Placental tissue from participants with no accompanying or incompletely filled proformas.
3. Poorly prepared or poorly labelled placental tissue specimens.

3.4 Sampling Procedure

3.4.1 Sample Size calculation

This study compared villous histomorphology in placenta specimens obtained from pregnant women who delivered at KNH with hypertensive pregnancy disease and those of their normotensive counterparts. Sample size was calculated using a formula for comparing two proportions (39) as follows:

$$n = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2 P_{av}(1-P_{av})}{(P_0 - P_1)^2}$$

n is the sample size required in each group

$Z_{1-\alpha/2}$ refers to the level of significance or confidence interval = 1.96 for 95% CI

$Z_{1-\beta}$ refers to the power of obtaining difference between the two groups = 0.84 for 80% power

P_0 – Villous vascularity (11%) and stromal fibrosis (20%) in normotensive women (7) { $P_0 = 0.11$ and 0.20 respectively}.

P_1 – Increased villous vascularity (32%) and stromal fibrosis (73%) in hypertensive pregnant women (7) { $P_1 = 0.32$ and 0.73 } respectively.

P_{av} – Average proportion of the two groups = 21.5% and 46.5% ($P_{av} = 0.22$ and 0.47) respectively.

n per group = 60 for villous vascularity and n per group = 17 for syncytial knot index.

Substituting into the formula: A minimum sample size (n) of **60** placental specimens in each group (total of **120** specimens) was required to establish the risk of placental villous histomorphological changes associated with hypertension in pregnancy with 80% power. However, all available specimen samples were retrieved, processed and analyzed.

3.4.2 Samples Selection

Proformas from the previous study having participants' age, clinical summary and assigned laboratory numbers were retrieved. The pre-assigned laboratory numbers were used to retrieve formalin preserved paraffin-embedded placental tissue blocks ($n=188$) from normotensive pregnant women ($n=94$) and those with hypertension ($n=94$) who delivered in Kenyatta National Hospital between July and December 2015. These had been archived following a previous study that sought to assess the morphological differences in placental pathology between pregnant women with hypertension and their normotensive counterparts who delivered in KNH between July and December 2015. Corresponding data on neonatal outcomes was obtained from the accompanying proformas from the previous study having participants biodata and clinical summary which included gestational date at delivery, birth weight, birth outcome, APGAR score at 5 minutes, need for resuscitation, NBU (New Born Unit Admission) and 72 hour outcome.

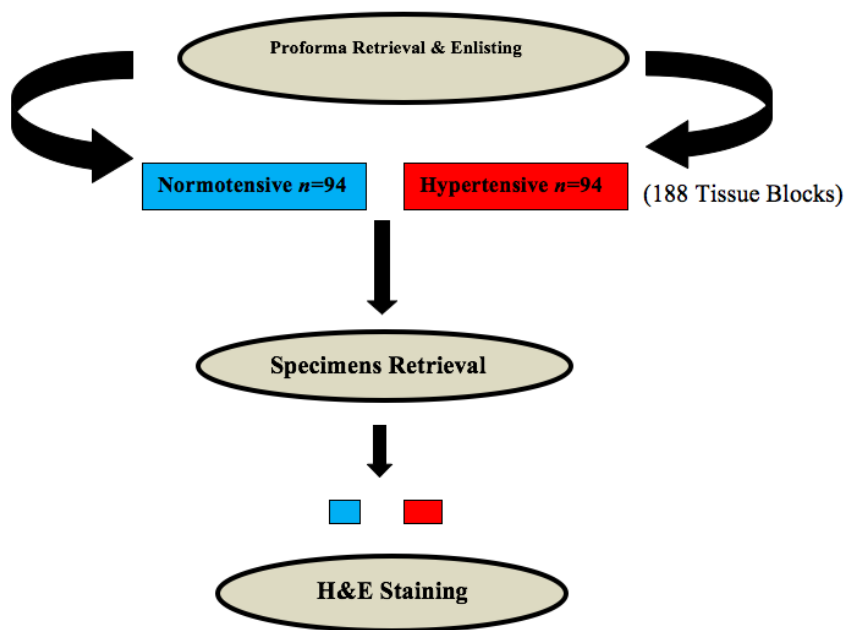


Figure 7. Schematic representation of sampling procedure and specimen processing.

3.4.3 Laboratory Methods

All the formalin preserved placental tissue blocks from the previous study were retrieved. These were single full thickness placental tissue sections from each participant. 5- μ m thin sections were stained with haematoxylin and eosin to enable villous histomorphological assessment. Microscopic screening of H&E sections was done by the principal investigator and later reported together with a qualified anatomic pathologist.

3.4.4 Quality Assurance

Pre-analytical aspects of quality assurance encompassed all the processes involved in generating the histological section from the archived tissue blocks. The analytical part concerned the interpretation of the slide and making an accurate diagnosis. The post-analytical part involved

generation and transmission of the histopathology report, storage/disposal of slides and proper retention of test results.

3.4.4.1 Pre-Analytical Stage

This involved all processes involved up to the submission of stained slides for analysis. The following was observed:

- a. Proper Specimen (placental tissue blocks) identification and retrieval was done and tallied against available clinical data. Specimen identification (Laboratory number) and relevant clinical data was carefully entered into a standard document to avoid mix-up and transcription errors.
- b. Tissue (placental tissue blocks) processing steps were documented in a simple language understandable by involved technical staff. The standard operating procedure (SOP) was available at the work station(s) to be followed by all involved technical staff.
- c. The microtome was of good quality (routinely serviced with periodic calibration) to ensure consistency of section thickness.
- d. Care was taken not to induce tissue artifacts due to sectioning, staining and mounting. The stains were kept covered and daily filtering done before use. Contamination of slides was avoided by using standard staining rack.
- e. Controls for routine H & E staining were used. One tissue block with a good mixture of hematoxyphilic and eosinophilic tissue (cervix) was used to make a H &E control slide. The control slide was stained before the routine batch of slides to ascertain recommended staining character. This was done daily and compared with that of the previous day to ensure consistency of staining. A record of the staining character was maintained.

- f. The reagents were stored in a refrigerator at the recommended temperature by the manufacturer. Correct temperature recording of water floatation bath and slide warming table was also done on a daily basis.
- g. Slide labeling was legible with the corresponding laboratory identity number and other sub-identifiers.

3.4.4.2 Analytical Stage

Subjectivity of histopathology reports confers a relative (compared to other disciplines of laboratory medicine) difficulty in quality assessment during specimen analysis. Despite this, error detection and avoidance was done to improve accuracy and precision using the following:

- a) Use of standard reporting/data collection tool for reporting villous histomorphology
- b) Hierarchical form of reporting was applied where the researcher screened the histological sections and then reported them with a qualified anatomic pathologist.
- c) Blinded re-reporting of all cases was also done together with a different qualified anatomic pathologist.
- d) A third reviewer (qualified anatomic pathologist) was consulted on discrepant reports.
- e) Random blinded reviews were also applied. In this regard, 10% of randomly selected histological sections were re-examined by an independent anatomic pathologist.

3.4.4.3 Post-Analytical Stage

Post-analytical aspects of quality assurance involved harmonized report generation (from all the pathologists) using a standard data collection tool without transcription errors. Secure storage of reported material as well as reported data and safe disposal of specimens was done.

3.5 Data Management

3.5.1 Data Collection

Specimen analysis results were recorded in a structured questionnaire by the principal investigator. Corresponding data on neonatal outcomes was obtained from the accompanying proformas from the previous study having participants' clinical summary which included gestational date at delivery, birth weight, birth outcome, APGAR score at 5 minutes, need for resuscitation, NBU (New Born Unit Admission) and 72 hour outcome.

3.5.2 Data Analysis

Data was entered, cleaned, coded and analyzed using statistical program for social scientists (SPSS) software package version 20 (IBM SPSS Inc., IL, USA). Descriptive statistics were used to summarize the various villous histomorphological findings in both normotensive and hypertensive groups. For categorical data, frequencies and proportions were reported and distributions presented using charts. *Chi* square test was used to determine the association between villous histomorphology findings and hypertensive pregnancy disease. Association between histomorphology findings and the different clinical categories of hypertension in pregnancy was determined using Fisher's exact test. Villous histomorphology findings was associated with neonatal outcomes using *Chi* square test.

Chi square test statistic and corresponding *p*-values were reported. All statistical tests were performed at 5% level of significance. Photomicrographs representing some of the histomorphological findings were also reported.

3.5.3 Data Confidentiality and Storage

Unique identity numbers were used for each specimen for anonymity. Filled questionnaires were kept under lock and key after the study. The data was also stored and backed up in electronic computer devices with only access to the researcher by use of password. Data

collected was kept for a minimum period of 5 years.

3.6 Ethical Consideration

3.6.1 Informed Consent

This study recognizes a preceding study done by Dr. Flavian Ogutu *et al* where all the formalin preserved paraffin embedded tissue blocks to be used in this study were retrieved with her permission. Informed consent was sought by Dr. Ogutu from participants in the preceding study which had been approved by the Kenyatta National Hospital/ University of Nairobi Ethical and Research Committee (KNH/UON-ERC).

3.6.2 Ethical Approval

Ethical approval for the study protocol was sought from Kenyatta National Hospital/ University of Nairobi Ethical and Research Committee (KNH/UON-ERC) before the study was conducted. Permission was also sought from the head of UoN Histopathology laboratory as a site for specimen processing and analysis.

4.0 RESULTS

All age matched formalin preserved placental tissue blocks with completely filled proformas from the previous study were retrieved ($n=149$). The remainder ($n=39$) were excluded because of either missing proformas, no laboratory numbers, incomplete clinical information, missing tissue blocks, multiple pregnancy cases or had no age matched pairs. 6 processed slides were unevaluable due to poor tissue processing. A total of 143 cases were examined under light microscopy and histomorphology findings reported (Figure 8).

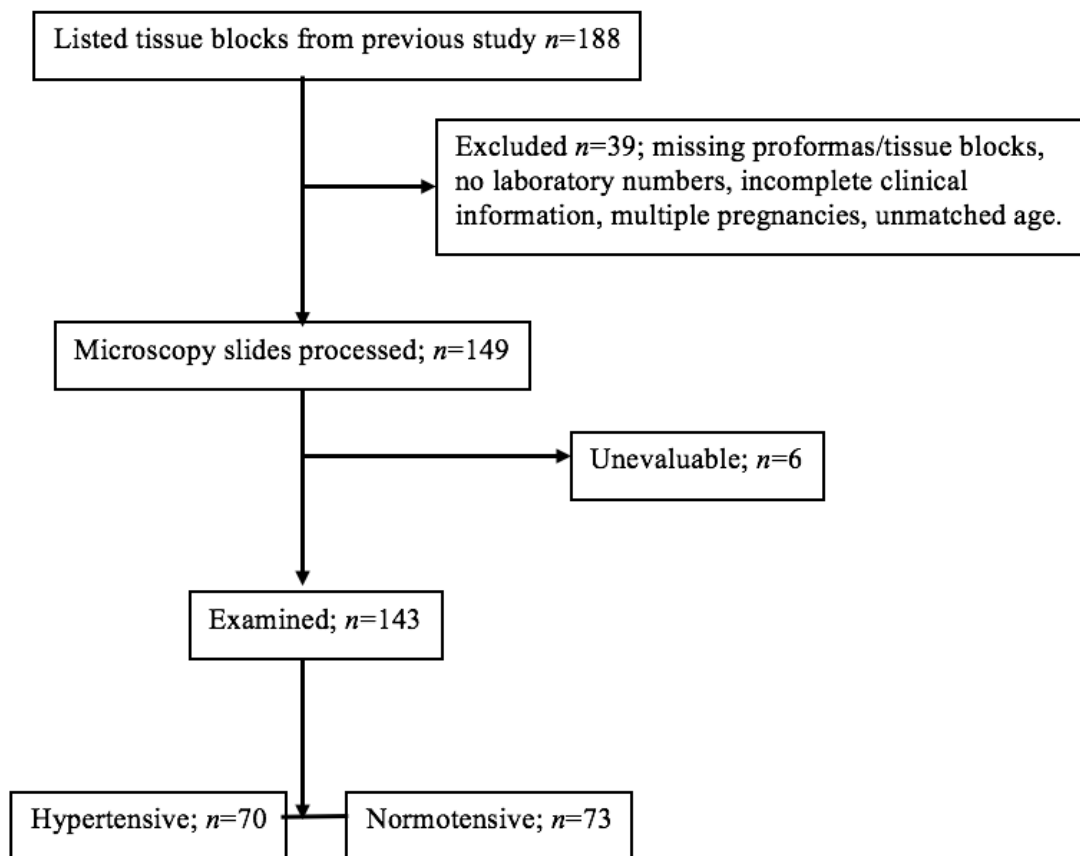


Figure 8. Flow chart showing stepwise flow of study events from specimen retrieval, preparation to microscopy analysis

4.1 Population Characteristics

A total of 73 (51%) placentae were from normotensive pregnant women and the rest, 70 (49%), from their hypertensive counterparts. Distribution per clinical category for the hypertensive group consisted of: 12 (17%) gestational hypertension, 1(2%) chronic hypertension, 54 (77%) pre-eclampsia and 3 (4%) with eclampsia as shown in figure 9 below.

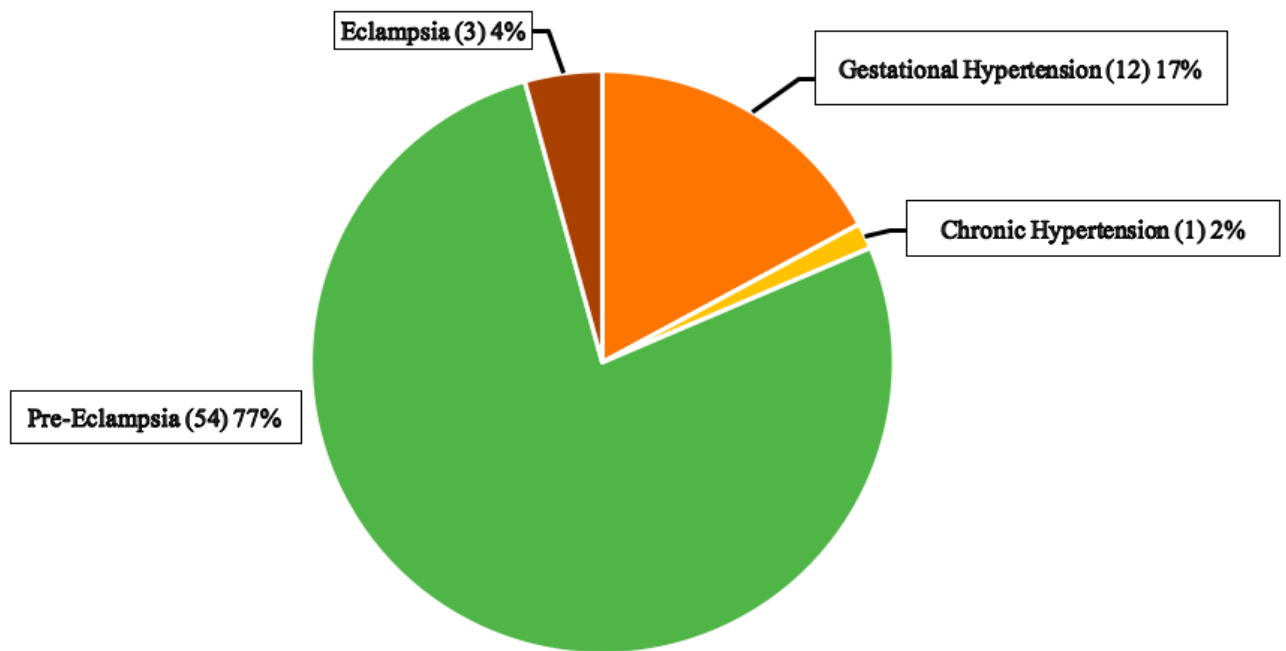


Figure 9. Distribution per clinical category for the hypertensive group

The pregnant women were 26 ($SD=5.5$) years old on average with a mean(SD) gestation of 37.7(3.2) weeks. The two groups (hypertensive and normotensive) were age-matched in cohorts and therefore no significant age difference was observed between the two groups (Table 3).

Table 3.Age of Participants

Variable	Frequency n (%)	Hypertensive n(%)	Normotensive n(%)	<i>p</i> value
Mean age (SD)	26.2 (5.5)	26.2 (5.3)	26.1 (5.6)	0.888
15-19	10 (7.6)	4 (6.9)	6 (8.2)	0.955
20-24	49 (37.4)	26 (36.2)	28 (38.1)	
25-29	35 (26.7)	19 (27.6)	19 (26.0)	
30-34	25 (19.1)	15 (20.7)	13 (17.8)	
35-39	11 (8.4)	6 (8.6)	6 (8.2)	
40-44	1 (0.8)	0	1 (1.4)	

Participants with hypertensive pregnancy disease were more likely to have premature deliveries ($p=0.014$). Their mean gestation was 35.9 ($SD=3.4$) weeks. A total of 131 (91.6%) participants had live births. Still births (fresh still births and macerated still births) were more likely to be observed in the hypertensive group ($p<0.001$). This is shown in Table 4 below.

Table 4. Participants' Pregnancy Outcomes.

Variable	All participants n(%)	Hypertensive n(%)	Normotensive n(%)	<i>p</i> value
Mean gestation weeks (<i>SD</i>)	37.7 (3.2)	35.9 (3.4)	38.8 (2.5)	0.014
Birth status				<0.001
Live	131 (91.6)	46 (79.3)	73 (100.0)	
FSB	4 (2.8)	4 (6.9)	0	
MSB	8 (5.6)	8 (13.8)	0	

4.2 Villous Histomorphology Among Normotensive and Hypertensive Pregnant Women

Hematoxylin and Eosin (H & E) stained sections from the selected tissue blocks were examined under light microscopy and villous histomorphological changes were reported (Table 5). When compared to their normotensive counterparts, the hypertensive group had significantly higher rates of accelerated villous maturity (44.8% versus 2.7%; $p<0.01$) and distal villous hypoplasia (46.6% versus 4.1%; $p<0.01$). Representative photomicrographs are shown in figure 10.

Table 5. Placental Villous Histomorphology Findings Among Participants

Variable	Hypertensive n (%)	Normotensive n (%)	OR (95% CI)	p-value
Delayed villous maturity				
Present	6 (15.4)	17 (25.0)	0.6 (0.2-1.5)	0.244
Absent	33 (84.6)	51 (75.0)	1.0	
Accelerated villous maturity				
Present	26 (44.8)	2 (2.7)	28.8 (6.5-129.0)	<0.001
Absent	32 (55.2)	71 (97.3)	1.0	
Distal villous hypoplasia				
Yes	27 (46.6)	3 (4.1)	20.3 (5.7-72.0)	<0.001
No	31 (53.4)	70 (95.9)	1.0	
Villous edema				
Yes	6 (10.3)	3 (4.1)	2.7 (0.6-11.3)	0.183
No	52 (89.7)	70 (95.9)	1.0	
Villous infarction				
Yes	7 (12.1)	2 (2.7)	4.9 (1.0-24.4)	0.036
No	51 (87.9)	71 (97.3)	1.0	
Villous stromal fibrosis				
Present	13 (22.4)	4 (5.5)	5.0 (1.5-16.3)	0.004
Absent	45 (77.6)	69 (94.5)	1.0	
Presence of Villitis				
Present	2 (3.4)	1 (1.4)	2.6 (0.2-29.1)	0.584
Absent	56 (96.6)	72 (98.6)	1.0	
Presence of Intervillositis				
Present	3 (5.2)	1 (1.4)	3.9 (0.4-38.8)	0.321
Absent	55 (94.8)	72 (98.6)	1.0	
Percentage of Fibrin Deposition				
<25%	39 (67.2)	63 (86.3)	1.0	0.009
≥25%	19 (32.8)	10 (13.7)	3.1 (1.3-7.3)	
Villous vascularity				
Increased	8 (13.8)	9 (12.3)	1.1 (0.4-3.2)	0.804
Not increased	50 (86.2)	64 (87.7)	1.0	
Decidual arteriopathy				
Present	8 (20.0)	2 (3.9)	6.3 (1.2-31.3)	0.014
Absent	32 (80.0)	50 (96.1)	1.0	

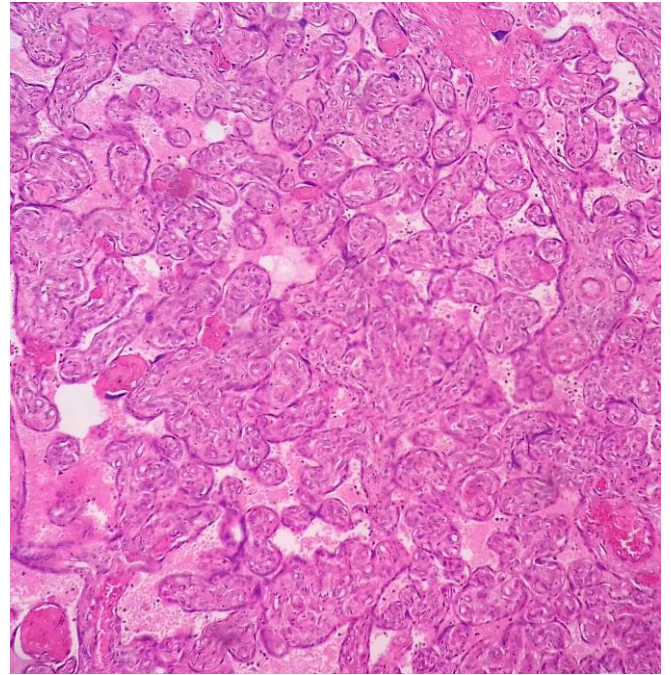
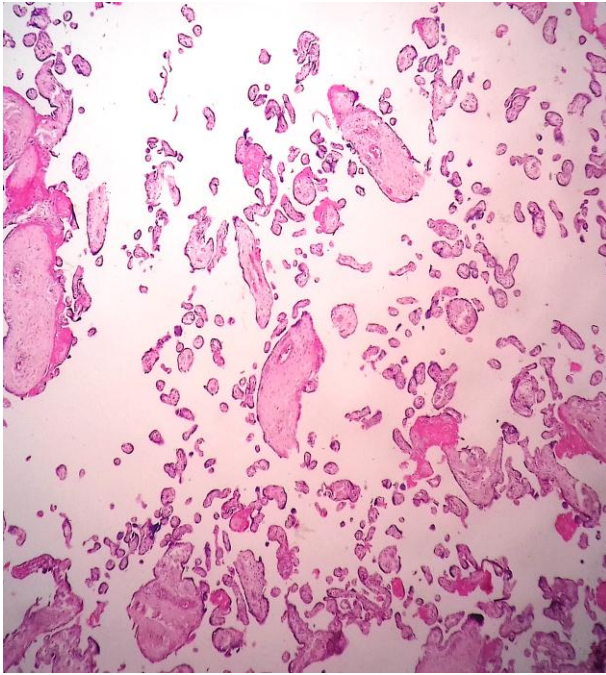


Figure 10. H& E sections (left) showing chorionic villi from a hypertensive pregnant woman at 29 weeks' gestation. It shows sparse villi (distal villous hypoplasia) and is hypermature for age (accelerated maturity). Delayed villous maturity in a 37-week gestation is shown on the right. The micrograph shows hypercrowded villi with centrally placed blood vessels.

Stromal changes; villous edema and fibrosis (figure 11) were observed in both groups. The hypertensive group had higher rates for both villous edema (10.3% versus 4.1%; $p=0.183$) and stromal fibrosis (22.4% versus 5.5%; $p<0.01$).

When compared to their normotensive counterparts, the hypertensive group had higher rates of villous infarction (12.1% versus 2.7%; $p=0.036$) and decidual arteriopathy (20% versus 3.9%; $p=0.014$). Representative photomicrographs are shown in figure 12.

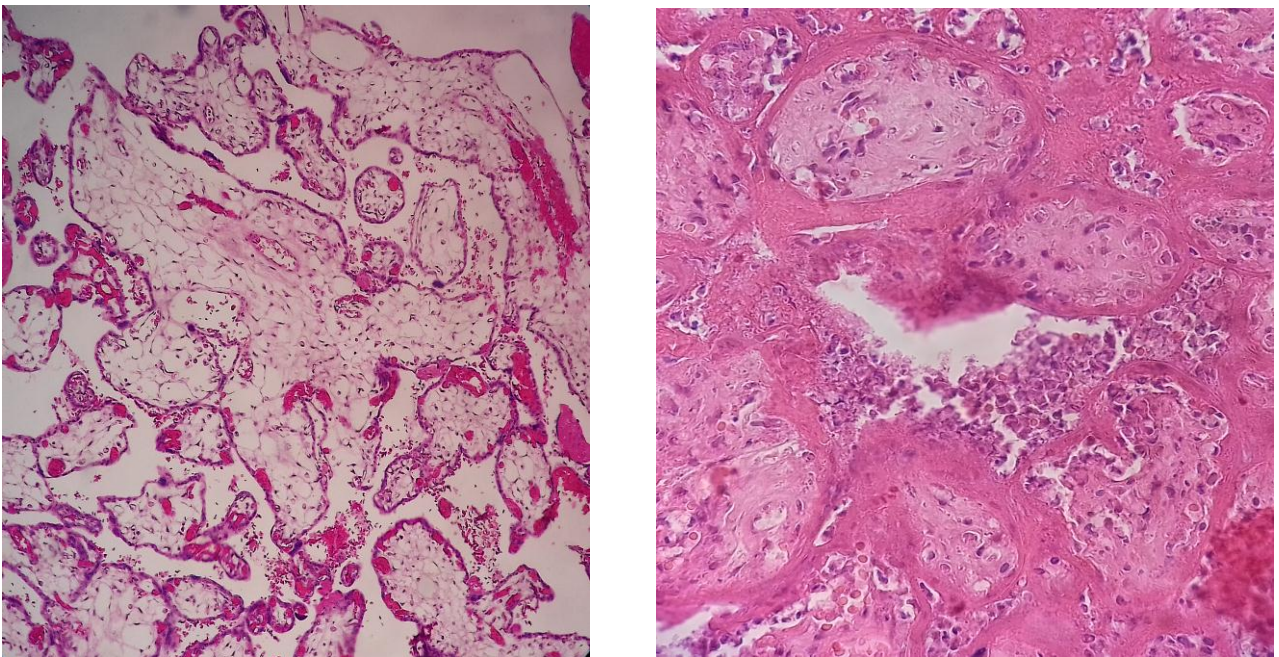


Figure 11. H & E stained sections showing villous edema (left) with enlarged and hydropic villi. The right micrograph shows villous stromal fibrosis; the stroma is replaced by hyalinized collagen and few or no blood vessels can be seen.

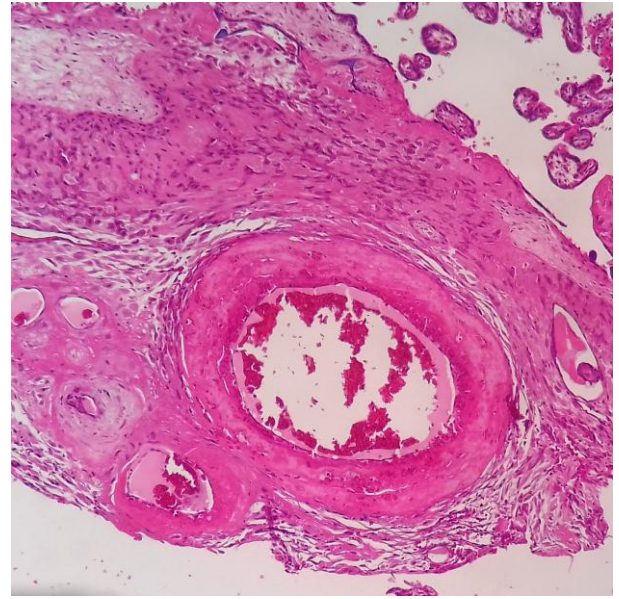
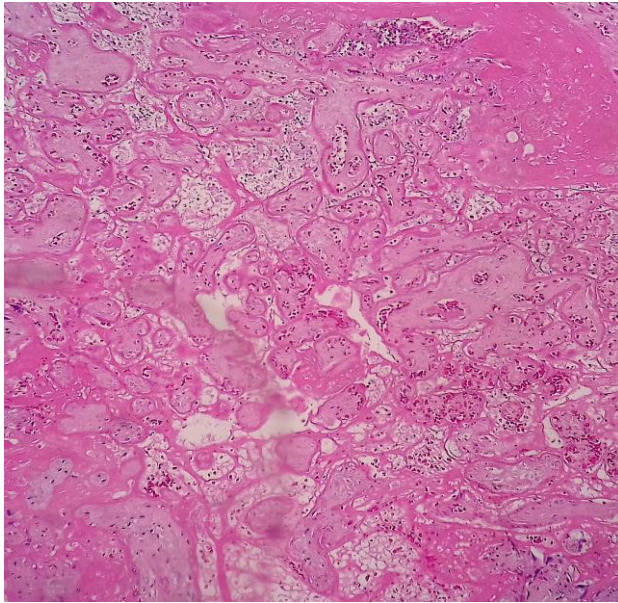


Figure 12. H & E stained section showing infarcted chorionic villi (left); ghost villi with remnant karyorrhectic nuclei and features of decidual arteriopathy (right); thickened and hyalinized vessel wall due to fibrin deposition.

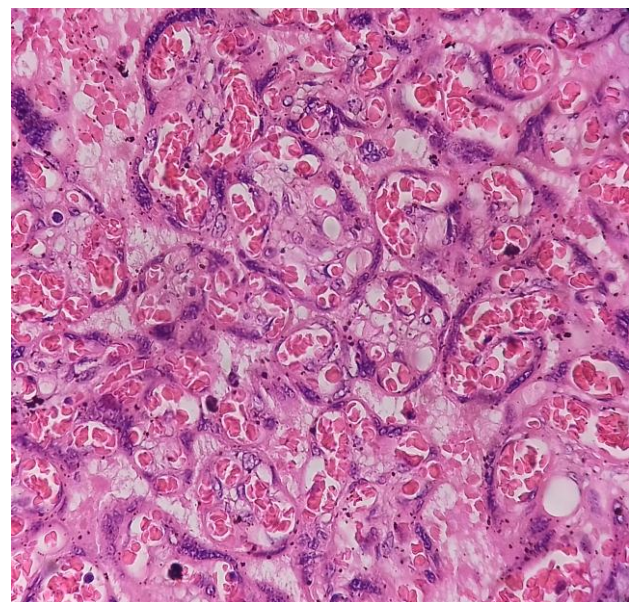
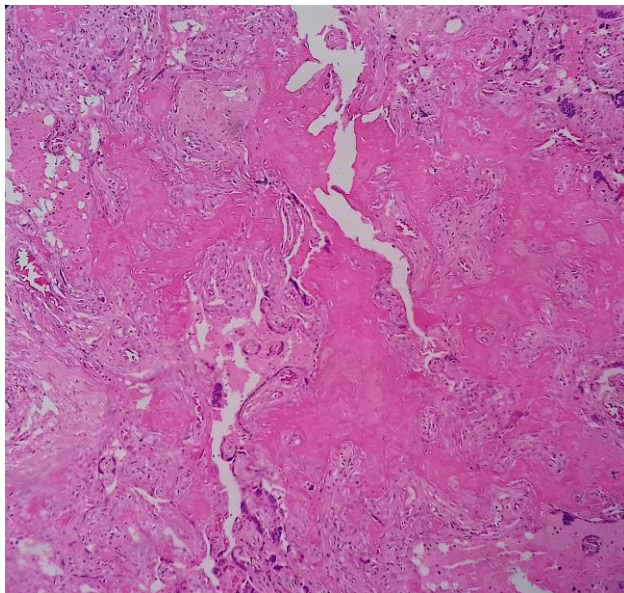


Figure 13. H & E sections showing fibrin deposition (left); deposition of amorphous eosinophilic material on in between and within villi. The right micrograph shows chorangiomas; increased number of blood vessels within villi.

As regards placental inflammation, 3.4% and 5.2% of the hypertensive cases had chronic villitis and Intervillositis (Figure 14) respectively. Fibrin deposition was seen in all the cases. However hypertensive group had significantly higher rates (32.8% verses 10% $p<0.01$) of more than 25% microscopic field of fibrin deposition.

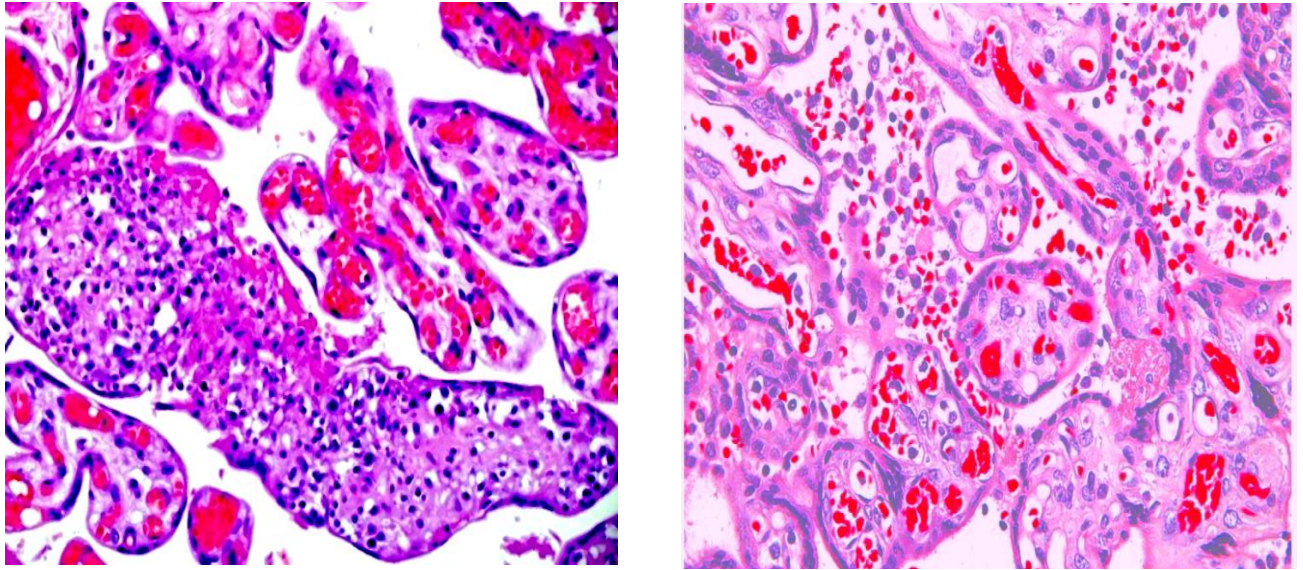


Figure 14. Right H & E micrograph shows lymphoplasmacytic infiltrate within villi (chronic villitis). Left H & E section showing chronic intervillitis; intervillous space infiltrated by chronic inflammatory cells; lymphocytes, plasma cells and histiocytes.

4.3 Villous Histomorphological Findings Associated with Hypertensive Pregnancy Disease.

Proportions of villous histomorphological findings were compared between hypertensive and normotensive groups. A χ^2 test of association with CI = 95% and $p = 0.05$ as criterion for significance was used to determine association between the villous histomorphological findings and hypertensive pregnancy disease.

Table 6. Villous Histomorphology findings associated with hypertensive pregnancy disease

Variable	OR (95% CI)	p value
Delayed villous maturity	0.6 (0.2-1.5)	0.244
Accelerated villous maturity	28.8 (6.5-129.0)	<0.001
Presence of distal villous hypoplasia	20.3 (5.7-72.0)	<0.001
Presence of villous edema	2.7 (0.6-11.3)	0.183
Presence of villous stromal fibrosis	5.0 (1.5-16.3)	0.004
Presence of villitis	2.6 (0.2-29.1)	0.584
Presence of Intervillositis	3.9 (0.4-38.8)	0.321
Intervillous fibrin deposition area; >25%	3.1(1.3-7.3)	0.009
Presence of villous infarction	4.9 (1.0-24.4)	0.036
Chorangiosis	1.1 (0.4-3.2)	0.804
Decidual arteriopathy	6.3 (1.2-31.3)	0.014

Placentae from women with hypertensive pregnancy disease had significantly higher rates of accelerated villous maturity ($p<0.01$), distal villous hypoplasia ($p<0.01$), villous infarction ($p=0.036$), stromal fibrosis ($p<0.01$), decidual arteriopathy ($p=0.014$) and increased area (>25%) of Intervillous fibrin deposition ($p=0.014$). All this is shown in Table 6 above.

4.4 Difference in Villous Histomorphological Findings Between Hypertensive Pregnancy Disease Groups

The hypertensive group consisted of: 12 (17%) gestational hypertension, 1(17%) chronic hypertension, 54 (77%) pre-eclampsia and 3 (4%) with eclampsia. Comparisons were made to determine the difference in incidence of villous histomorphology lesions between the two main clinical groups; pre-eclampsia-eclampsia and gestational hypertension. There were significantly higher rates of accelerated maturity ($p<0.01$) and villous hypoplasia ($p<0.01$) among the pre-eclampsia-eclampsia group. The pre-eclampsia-eclampsia group was also

characterized by a trend towards higher rates of villous infarction (11.3% vs 0%; $p=0.6$) and decidual arteriopathy (22.2% vs 0%; $p=0.3$). All this is shown in Table 7 below.

Table 7. Villous Histomorphological Findings Among Hypertensive Pregnancy Disease Groups

Variable		Pre-Ecl/Ecl. n(%)	Gest HTN n (%)	Chronic HTN n(%)	<i>p</i> –value
Accelerated maturity (Yes/No)	Yes	25 (47.2)	0	0	0.002
	No	28 (52.8)	12 (100.0)	1 (100.0)	
Villous Hypoplasia(Yes or No)	Yes	26 (49.1)	0	0	0.001
	No	27 (50.9)	12 (100.0)	1 (100.0)	
Villous Edema(Yes/ No)	Yes	6 (11.3)	0	0	0.621
	No	47 (88.7)	12 (100.0)	1 (100.0)	
Stroma Fibrosis(Yes/No)	Yes	13 (24.5)	2 (16.7)	0	0.781
	No	40 (75.5)	10 (83.3)	1 (100.0)	
Villitis(Yes or No)	Yes	2 (3.8)	0	0	1.000
	No	51 (96.2)	12 (100.0)	1 (100.0)	
Intervillositis(Yes or No)	Yes	3 (5.7)	0	0	1.000
	No	50 (94.3)	12 (100.0)	1 (100.0)	
Fibrin Deposition(<25% or >25%)	<25%	36 (67.9)	10 (83.3)	1 (100.0)	0.632
	>25%	17 (32.1)	2 (16.7)	0	
Chorangiosis(Yes or No)	Yes	7 (13.2)	3 (25.0)	0	0.471
	No	46 (86.8)	9 (75.0)	1 (100.0)	
Villous Infarction(Yes or No)	Yes	6 (11.3)	0	0	0.621
	No	47 (88.7)	12 (100.0)	1 (100.0)	
Decidual (Yes or No)Arteriopathy	Yes	8 (22.2)	0	0	0.308
	No	28 (77.8)	11 (100.0)	1 (100.0)	

4.5 Association Between Villous Histomorphological Findings and Neonatal Outcomes

Incidences of placental villous histomorphology findings were compared for the different neonatal outcomes; Gestation by date, birth outcome, APGAR score at 5 minutes, need for resuscitation, New Born Unit (NBU) admission and 72-hour outcome. A chi square correlation was done to determine association between the villous histomorphology findings and neonatal outcomes. Statistically significant ($p<0.05$) variables were reported.

Placentae with accelerated maturation were associated with preterm delivery ($p<0.001$), still births ($p<0.01$), NBU admission ($p<0.01$), unfavorable 72-hour outcome ($p<0.01$) Those with villous hypoplasia were associated with preterm delivery ($p<0.01$), still births ($p<0.01$), APGAR score of less than 7 ($p<0.01$), NBU admission ($p<0.01$) and unfavorable 72-hour

outcome ($p<0.01$). Presence of villous edema was associated with still births ($p<0.01$) and unfavorable 72-hour outcome ($p<0.01$). All this is shown in table 8 and 9.

Table 8. Association Between Villous Histomorphological Findings and Neonatal Outcomes; Gestation, Birth Outcome and APGAR Score at 5 minutes.

Variable	Gestation			Birth Outcome			APGAR at 5"		
	<37 n(%)	>37 n(%)	<i>p</i> value	Alive n(%)	MSB or FSB n(%)	<i>p</i> value	7-10 n(%)	<7 n(%)	<i>p</i> value
Accelerated Maturity	21 (56.8)	6 (5.9)	<0.001	20 (15.5)	7 (70)	<0.001	2 (40)	18 (14.5)	0.171
Villous Hypoplasia	22 (59.5)	7 (6.9)	<0.001	22 (17.1)	7 (70.0)	0.001	3 (60)	19 (15.3)	0.035
Villous Edema	4 (10.8)	6 (5.9)	0.456	6 (4.7)	4 (40)	0.002	1 (20)	5 (4)	0.215
Stroma Fibrosis	8 (21.6)	11 (10.8)	0.100	13 (10.1)	6 (60)	<0.001	0	13 (10.5)	1.000
Villous Infarction	3 (8.10)	5 (4.9)	0.439	4 (3.1)	4 (40)	0.001	0	4 (3.2)	1.000
Decidual Arteriopathy	5 (23.8)	5 (6.3)	0.032	8 (8.5)	2 (33.3)	0.109	1 (25)	7 (7.8)	0.304

Placentae with villous infarction ($p<0.01$) and stromal fibrosis ($p<0.01$) were independently associated with fetal death at birth (MSB or FSB) ($p<0.01$). Those with decidual arteriopathy were associated with preterm delivery ($p<0.01$) and NBU admission ($p<0.01$). All this shown in Table 8 above and Table 9 below.

Table 9. Association Between Villous Histomorphological Findings and Neonatal Outcomes; Need for Resuscitation, New Born Unit (NBU) Admission and 72-hour Outcome.

Variable	Need for Resuscitation			NBU Adm.			72 hrs Outcome		
	Yes n(%)	No n(%)	<i>p</i> value	Yes n(%)	No n(%)	<i>p</i> value	Discharged Alive n(%)	Admitted or Died n(%)	<i>p</i> value
Accelerated maturity	5 (27.8)	15 (13.5)	0.156	9 (42.9)	11 (10.2)	0.001	11 (10.0)	16 (55.2)	<0.001
Villous Hypoplasia	6 (33.3)	16 (14.4)	0.083	9 (42.9)	13 (12.0)	0.002	13 (11.8)	16 (55.2)	<0.001
Villous Edema	1 (5.6)	5 (4.5)	1.000	2 (9.5)	4 (3.7)	0.252	4 (3.6)	6 (20.7)	0.006
Decidual Arteriopathy	3 (33.3)	5 (5.9)	0.027	4 (36.4)	4 (4.8)	0.006	4 (4.8)	6 (37.5)	0.001

5.0 DISCUSSION

5.1 Villous Histomorphology in Hypertensive Pregnancy Disease.

Evidence suggests impaired physiological conversion in hypertensive pregnancy disease (14,40) where extravillous trophoblast invasion is limited to the decidual portion of uterine spiral arteries with resulting reduced vascular diameters than in normal pregnancies (14,40). Some utero-placental arteries undergo decidual arteriopathy; fatty infiltration and lipid-laden myogenic foam cells (atherosis), decidual vascular thrombosis and fibrinoid necrosis of the media (25,40). Similar to other studies, significantly higher rates of decidual arteriopathy was observed in placentae from hypertensive pregnant women (14,25,40).

Decidual arteriopathy, with attendant reduced vascular diameters, (25,40) and indirect constriction of fetal stem arteries (41) are thought to cause uteroplacental hypoperfusion and hypoxia in hypertensive pregnancy disease (40). This can result in proliferation of villous capillaries as an adaptation to chronic oxygen deficiency (28,29). Increased villous vascularity (chorangiosis), has been associated with hypertensive pregnancy related uteroplacental hypoperfusion (13,28,29) which is contrary to findings in this study. The finding of chorangiosis in normotensive placentae could be explained by other probable causes like pre uterine hypoxia from hypoxemic states and fetal normoblastemia (13). In other literature, the pathogenesis and clinical significance of chorangiosis remains uncertain although it should be noted in pathology reports (28).

Uteroplacental hypoxia associated with pregnancy-induced hypertension results in different patterns of altered villous development mainly, accelerated villous maturity and distal villous hypoplasia (DVH) (7,12). These features are seen as a placental reaction to decreased materno-placental perfusion that is associated with pregnancy induced hypertension (24). The ensuing placental ischemia results in increased villus branching and formation of large and numerous syncytial knots which is characteristic of accelerated villous maturation.

Inadequacy of compensatory mechanisms to uteroplacental hypoperfusion results in villous infarction. In this study, significantly higher rates of villous infarction observed in the hypertensive group could be as a result of deficient intervillous (maternal) circulation due to inadequacy of compensatory mechanisms to uteroplacental hypoperfusion. Significantly higher incidence of villous stromal fibrosis was also observed in the hypertensive group similar to other studies (16,42). This could possibly be due to placental hypoxia in pregnancy induced hypertension that upregulates expression of fibrosis related factors including smooth muscle actin (SMA), collagen fiber (COL), fibronectin (FN) and connective tissue growth factor (CTGF) (16).

Villous edema in pre-eclamptic placentas has been attributed to functional insufficiency of the fetal circulation (7). This probably explains why the hypertensive group in this study had a higher incidence of villous edema. Perivillous fibrin or fibrinoid deposition is thought to develop following damage to syncytiotrophoblast with subsequent clotting in the intervillous space and closure of the trophoblastic defect by a fibrinoid plug. This study involved third trimester placentae where findings of fibrin deposition is not uncommon (7). Fibrin deposition was seen in all the cases in this study. However, more than 25% microscopic field of fibrin deposition was observed significantly among the hypertensive group. According to other studies (12,27), a diffuse increase in fibrin deposition possibly reflects chronic intervillous perfusion defects seen in preeclampsia.

5.2 Villous Histomorphology Among Different Hypertensive Pregnancy Disease Groups

In agreement with recent epidemiological findings in sub-Saharan Africa (5,43), this study found chronic hypertension and gestational hypertension to be less common than pre-eclampsia. The hypertensive group consisted of: 12 (17%) gestational hypertension, 1(17%) chronic hypertension, 54 (77%) pre-eclampsia and 3 (4%) with eclampsia. Comparisons were made to determine the difference in incidence of villous histomorphology lesions between the

two main clinical groups; pre-eclampsia-eclampsia and gestational hypertension. Women with pre-eclampsia-eclampsia had placentae characterized by significantly higher rates of accelerated maturity and villous hypoplasia. Similar to Maloney *et al* (33), they were also characterized by a trend towards higher rates of villous infarction and decidual arteriopathy. Given that these lesions; accelerated maturity, villous hypoplasia, villous infarction and decidual arteriopathy are associated with placental ischemia (9), the findings are consistent with placental ischemia being characteristic to pre-eclampsia-eclampsia. This supports available evidence that pre-eclampsia-eclampsia and gestational hypertension are different disease entities given their distinct epidemiologic, pathogenesis and hemodynamic characteristics (31).

Correa *et al* found a higher number of syncytial knots among pre-eclamptic placentae(32). A higher number of syncytial knots is a characteristic feature of accelerated maturity (22) which is consistent with findings in this study. They also found a significant difference in quantity of fibrin deposits between the preeclampsia-eclampsia and gestational hypertensive groups which is contrary to findings from this study. Fibrinoid deposition is thought to represent clotting in the intervillous space following damage to syncytiotrophoblast with subsequent clotting in the intervillous space. It is a non-specific finding with variable findings in pregnancy state according to some studies (7). Maloney *et al* (33) found no statistically significant difference in pathologic lesions present between the different clinical types of hypertensive pregnancy disease. However, placentae from women with pre-eclampsia were characterized by a higher incidence of decidual vasculopathy (47% vs. 33%; $p = 0.08$) and villous infarction (50% vs. 38%; $p = 0.1$), in keeping with placental ischemia as a key feature in pre-eclampsia.

5.3 Villous Histomorphology Findings and Neonatal Outcomes

Villous histomorphological findings were correlated with specific neonatal outcomes; Gestational age at delivery, birth outcome, APGAR score at 5 minutes, need for resuscitation,

New Born Unit (NBU) admission and 72-hour outcome. Decidual arteriopathy is a known cause of uteroplacental hypoperfusion in hypertensive pregnancy disease that complicates with placental ischemic lesions and adverse fetal outcomes (14,40). The reduced vascular caliber compromises maternal blood flow therefore altering nutritional and gaseous exchange that is vital for fetal survival (44). This possibly explains why placentae with decidual arteriopathy were associated with preterm delivery and NBU admission in this study.

Loss of villous capillaries due to villous stromal fibrosis and infarction further compromises fetomaternal exchange in placental ischemic states (45). Similar to findings in this study; stromal fibrosis and infarction, which cause avascular villi, have been associated with fetal demise (16,22,45). Other adverse fetal outcomes include fetal growth restriction, thromboembolic events/necrosis of multiple fetal organs and neurological complications.

Accelerated maturation and villous hypoplasia arise due to uteroplacental hypoperfusion (12,24) and present a smaller or reduced trophoblastic surface area in direct contact with maternal blood for nutritional and gaseous exchange. This deprives the fetus of nutrients required for fetoplacental growth resulting in poor neonatal outcomes (44). This possibly explains why in this study, they were associated with preterm deliveries, still births, NBU admission, still births, APGAR score of less than 7 and mortality or morbidity 72 hours after delivery.

Villous edema has been associated with neonatal complications such as fetal death, respiratory and central nervous system (CNS) morbidity (46). Apart from causing vascular compression, edema between villous capillaries and trophoblastic covering forms a barrier to gas exchange between the mother and fetus. This impairs placental function and could explain the association with still births and mortality/morbidity 72 hours after delivery in this study. This finding was however not significantly associated with hypertensive disease in pregnancy

5.4 Conclusion

1. Placentae from women with hypertensive pregnancy disease at Kenyatta National Hospital had significantly higher rates of accelerated villous maturity, distal villous hypoplasia, stromal fibrosis, decidual arteriopathy, villous infarction and an increased area (>25%) of intervillous fibrin deposition.

2. Pre-eclampsia-eclampsia group had placentae characterized by lesions associated with placental ischemia; significantly higher rates of accelerated maturity, villous hypoplasia and a trend towards higher rates of villous infarction and decidual arteriopathy. This supports available evidence of placental hypoperfusion as characteristic of pre-eclampsia-eclampsia rather than gestational hypertension.

3. Villous histomorphological findings associated with poor neonatal outcomes include accelerated maturation, villous hypoplasia, villous infarction, stromal fibrosis and decidual arteriopathy.

5.5 Study Limitation

Study specimens were formalin preserved paraffin-embedded placental tissue blocks retrieved from an archival collection. These were single full thickness placental tissue sections from each participant. Findings from this study were therefore limited to the number and area represented by the available tissue sections. The study was also limited to the clinical groups of hypertensive pregnancy disease that had been randomly selected in the preceding study.

5.6 Recommendation

1. Clinicians in Kenyatta National Hospital to be sensitized on the clinical utility and need to submit placental histopathology examination requests for placentae in the following requisite clinical scenarios:
 - a) Intrapartum management of hypertensive pregnant women.
 - b) Postmortem evaluation of unexplained perinatal mortality with unclear antenatal

history especially where hypertensive pregnancy needs to be ruled out.

c) Evaluation of poor neonatal outcomes for possible aetiology.

2. In future studies, multiple and variably selected representative sections of the placental disc should be used with equal sampling from all the hypertensive disease groups to enable comparisons.

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APPENDIX 1: SECTIONING OF TISSUE BLOCKS

1. Release the brake and rotate the hand wheel until the handle is at 1 o'clock position and re-apply the brake.
2. Push the quick release lever of the cassette clamp backward, insert the cassette clamp backward, insert the cassette, release the lever and check that the cassette is firmly clamped.
3. Use the vertical and horizontal tilt controls to orientate the specimen correctly with the knife edge and lock the orientation head.
4. Release the brake and turn the coarse advance knob clockwise and anticlockwise to bring the tissue block closer or away from the cutting edge.
5. Trim the block using the coarse advance knob until the full face is attained.
6. Set the section thickness with thickness control knob.
7. Turn the hand wheel to cut the sections.
8. Pick the sections and float in warm water to remove the creases.
9. Fish the sections and mount on clean microscope slides. Label the slides with a lead pencil or diamond pencil.
10. Put the slides in a hot air oven at 56 degrees Celsius for 1hour. Remove the slides and stain.

APPENDIX II: HAEMATOXYLIN AND EOSIN STAIN PREPARATION

Reagents

1. Eosin 1% aqueous solution; Eosin 10g distilled water- 1litres
2. Harris-haematoxylin solution; Haematoxylin-5g, Ethyl alcohol-50ml, Ammonium aluminium -100g, Distilled water-1 litre, Mercuric oxide red 2.5g
3. Scotts tap water; Na hydrogen carbonate-3.5gMgSo4 -20g, Distilled water-1 litre, Acid alcohol
(0.5% HCl in 70% alcohol)

Procedure for staining

1. Dissolve the ammonium aluminium in distilled water heat, stirring frequently.
2. Dissolve the haematoxylin in the alcohol and add to aluminium solution.
3. Bring to the boil while stirring.
4. Mix and allow cooling.
5. Filter into a glass stain bottle and the solution is ready for use.
6. De-wax sections with two changes of xylene.
7. Re-hydrate sections with two changes of absolute alcohol and wash in running tap water.
8. Stain with haematoxylin solution for up to 5 minutes.
9. Wash in running tap water.
10. Differentiate in acid alcohol for approximately 5 minutes.
11. Wash in running tap water.
12. Blue in Scotts tap water for few seconds.
13. Wash in running tap water.
14. Stain with eosin for approximately for 5 minutes.
15. Wash in running tap water.
16. Dehydrate, clear and mount section.

APPENDIX III: DATA COLLECTION TOOL

SECTION A: Laboratory & Clinical Information

1	Lab No.	
2	Date of Specimen Collection.	
3	Age of Participant (months)	
4	Blood Pressure Status	
5	Placental Weight	
6	Placental Size	
7	Gestation by Date (weeks)	
8	Birth Status	
9	Birth Weight (Grams)	

SECTION B: Villous Microscopy Findings

1. Delayed villous maturity (only for 36 or more weeks' gestation)

(Enlarged distal villi with excessive stroma, hypercellular villous trophoblast, central blood vessels, paucity of vasculosyncytial membranes)

Not Applicable Absent Present

2. Accelerated villous maturity

Present Absent

(In preterm; small/short hypermature villi for gestational period. Diffuse pattern of term-appearing villi with increased syncytial knots and intervillous fibrin, usually alternating with areas of villous paucity)

3. Presence of distal villous hypoplasia

Yes No

(Paucity of villi in relation to the surrounding stem villi. The villi are thin and relatively elongated-appearing (lack of branching), and syncytial knots are increased).

4. a) Presence of villous edema

Yes No

b) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

5. a) Presence of villous infarction

Yes No

b) If present, indicate whether early or late

Early Late

c) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

6. a) Thickening of villous basement membrane

Present Absent

b) If present, indicate percentage of villi affected as
A (< 25%) **B (25-50%)** **C (>50% -75%)** **D (>75%)}**

7. a) Presence of villous stromal fibrosis Present Absent

b) If present, indicate percentage of villi affected as
A (< 25%) **B (25-50%)** **C (>50% -75%)** **D (>75%)}**

SECTION C: Inflammation and Fibrin deposition

1. a) Presence of Villitis Present Absent
 b) If present, indicate percentage of villi affected as
A (< 25%) **B (25-50%)** **C (>50% -75%)** **D (>75%)}**

2. a) Presence of Intervillositis Present Absent
 b) If present, indicate the proportion of Intervillous area affected as
A (< 25%) **B (25-50%)** **C (>50% -75%)** **D (>75%)}**

3. a) Presence of Fibrin deposition Present Absent
 b) if present, indicate the pattern and proportion of villi/Intervillous area affected)
 Intravillous Intervillous
A (< 25%) **B (25-50%)** **C (>50% -75%)** **D (>75%)}**

SECTION D: Villous Vascular Findings

1. Villous vascularity Increased Not increased
(Altshuler criteria: More than 10 capillaries in at least 10 terminal villi in 10 or more non infarcted areas in at least 3 low power fields of the placenta)

2. a) Decidual arteriopathy Present Absent Cannot be evaluated
 b) If present, specify
 Muscular wall hypertrophy Fibrinoid Necrosis Atheromatous changes

3. a) Fetal vascular malperfusion (Fetal Thrombotic Vasculopathy)
 Present Absent
 b) If present, indicate whether global or segmental. Global Segmental

SECTION E: FETAL OUTCOMES

1. Gestation at delivery _____
2. What was the mode of delivery?
 vaginal delivery
 caesarean section
3. Sex of the foetus
 Male
 Female
4. Outcome of the foetus
 Fresh stillbirth
 Macerated stillbirth
 Live
5. What was the Apgar score of the foetus at 5 minutes?
 <3
 3-7
 >7
6. Fetal weight in grams _____
7. Was resuscitation required?
 Yes
 No
8. Admission to NBU?
 Yes
 No
9. If yes what was the indication for admission to NBU
 Prematurity
 Respiratory Distress Syndrome
 Poor apgar score/ asphyxia
 Congenital anomalies
 Other(specify)
10. At 72 hours what is the outcome of the fetus?
 Alive still admitted
 Alive discharged home.
 Deceased
 Other

Signature of Consultant /Student:

Date of Report:

APPENDIX IV: ETHICAL CLEARANCE



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11th April, 2019

Dr. Wangulu Collins
Reg. No. H58/80881/2015
Dept. of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Wangulu

Research proposal: Placental Villous changes among Hypertensive and Normotensive Pregnant women at Kenyatta National Hospital (P743/12/2017)

Further to our communication Ref. No. KNH-ERC/A/112 dated 12th March 2018, the KNH-UoN ERC hereby acknowledge receipt of proposal version 2 dated April 2019.

The same was discussed and **approved** during the regular KNH-UON ERC monthly meeting held on 10th April 2019.

Yours sincerely,

PROF. A.N. GUANTAI
CHAIRPERSON, KNH- UoN ERC

cc. The Principal, College of Health Sciences, UoN
The Director, Clinical Services, KNH
The Dean, School of Medicine, UON
The Chair, Dept. of Human Pathology, UON
Supervisors: Dr. Edwin O. Walong, Dr. Ann K. Barasa, Dr. Patricia O. Okiro

Protect to discover

APPENDIX IV: PLAGIARISM CHECK

PLACENTAL VILLOUS CHANGES AMONG HYPERTENSIVE AND NORMOTENSIVE PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL

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