AUTOPSY BRAIN FINDINGS IN PREECLAMPSIA AND ECLAMPSIA AT KENYATTA NATIONAL HOSPITAL

MBAU LOISE WAITHERA

H58/7277/2017

A dissertation submitted in the Department of Human Pathology in partial fulfilment of the requirements for the award of the Master of Medicine in General Pathology at the University of Nairobi

2022

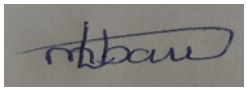
DECLARATION

I hereby declare that this is my original work under the guidance of my supervisors and has not been presented to the University of Nairobi or anywhere else for award of any degree or diploma.

Investigator: **Dr. Waithera Mbau**

H58/7277/2017

Email: waitherambau@gmail.com

Signature  Date 14/11/2022

SUPERVISOR'S DECLARATION

This dissertation is submitted with our approval.

Dr. EDWIN. O. WALONG

MBChB, MMED(PATH), FCPath ECSA

Lecturer UoN/ Consultant pathologist

School of Medicine, University of Nairobi

Signature  Date 14/11/2022

Dr. ALFRED OSOTI

MBChB, MMED, MPH, PhD

Senior Lecturer/ Consultant Obstetrician and Gynecologist, Epidemiologist

School of Medicine, University of Nairobi

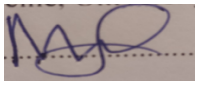
Signature  Date 14/11/2022

PROF. MWANDA O. WALTER

MBChB, MD

Professor, Department of Human Pathology

School of Medicine, University of Nairobi

Signature  Date 14/11/2022

DEDICATION

I dedicate this book to Ken, Jelani, and Jahari for their overwhelming support and for being a source of inspiration during the course of my studies.

ACKNOWLEDGMENT

I would like to thank God for His Grace throughout this entire period.

I would like to acknowledge and thank my entire family for their constant support throughout the course of this dissertation. I offer my sincere gratitude to the following people who made this study possible:

- My supervisors: Dr. Walong, Dr.Osoti and Professor Mwanda for their valuable assistance and guidance.
- Dr. Okemwa for reviewing histology slides.
- The KNH farewell funeral home staff especially Walter Mongare for his assistance in performing the postmortems.
- The UoN histopathology laboratory staff for the assistance in tissue processing.
- My classmates for their assistance and encouragement.
- My statistician, Wycliff Ayieko, for his help with data analysis.

LIST OF ABBREVATIONS

ACOG- American College of Obstetricians and Gynecologists

APPT- Activated partial prothrombin time

ALT- Alanine transaminase

AST- Aspartate transaminase

BMI- Body mass index

CNS- Central nervous system

CVR- Cerebral vascular resistance

DIC- Disseminated intravascular coagulopathy

H&E- Haematoxylin and eosin

HELLP- Haemolysis, elevated liver enzymes, low platelets

HI- Hypoxic ischemia

HTN- Hypertension

ISSHP- International society for the study of hypertension in pregnancy

KNH- Kenyatta National Hospital

LFTs- Liver function tests

PES- Pre-eclampsia with severe features

PRES- Posterior reversible encephalopathy syndrome

PT- Prothrombin time

SPSS- Statistical Package for the Social Sciences

T.BIL- Total bilirubin

TGF-β- Transformation growth factor beta

UEC- Urea electrolyte creatinine

UoN - University of Nairobi

WHO- World Health Organization

VEGF- Vascular endothelial growth factor

DEFINITION OF TERMS

Hypertension: Systolic blood pressure of 140mmHg or more or a diastolic blood pressure of 90mmHg or more

Macrovascular: Involving the large arteries of the brain

Microvascular: Involving the venules, capillaries, and arterioles with a diameter of less than 150 μm capillaries, and venules

Next of Kin: Persons authorizing the autopsy

Obstetric characteristic: This is pregnancy related clinical information including parity, singleton pregnancy, multiple pregnancy, and gestational age.

Parenchyma: The brain's cortical white matter, grey matter, and subcortical sites.

Preeclampsia: New episode of hypertension at or after 20 weeks' gestation with one or more of: proteinuria, renal insufficiency (creatinine ≥90 μmol/L), liver impairment (ALT or AST > 40 IU/L), neurological complications (e.g. eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent scotomata), haematological complications (thrombocytopenia, DIC, hemolysis) and uteroplacental dysfunction (fetal growth restriction)

TABLE OF CONTENT

Contents

LIST OF TABLES

LIST OF FIGURES

AUTOPSY BRAIN FINDINGS IN PREECLAMPSIA AND ECLAMPSIA AT KENYATTA NATIONAL HOSPITAL

ABSTRACT

Background

Preeclampsia and eclampsia are common pregnancy disorders and are the second most common cause of maternal mortality. They have diverse neuropathological presentations including stroke, intracranial haemorrhages, and vasculopathies which may result in maternal morbidity or mortality. An autopsy examination of the brain findings in pre-eclampsia and eclampsia at Kenyatta National Hospital will contribute to the understanding of the relationship between gross pathological changes and clinical neurological presentations.

Study objective: To describe the autopsy brain findings in preeclampsia and eclampsia at KHN

Study design: Descriptive cross-sectional autopsy study

Study area: Autopsies were done at the Kenyatta National Hospital farewell funeral home and tissue processing done at the University of Nairobi Anatomic Pathology Laboratory

Materials and methods

The study was conducted on decedents of pre-eclampsia and eclampsia who were identified on admission to the farewell home and informed consent sought from next of kin. Clinical and demographic information was obtained through interviews of the next of kin and abstraction of medical records. Autopsies were carried out using standard techniques. The brains were eviscerated, examined, and fixed by immersion in 15% neutral buffered formalin for two weeks and thereafter dissected. Tissue samples obtained from standard sites for histopathological evaluation were processed, sectioned, and stained with Hematoxylin and Eosin. Other sections

were stained with Masson's Trichrome (stain for differentiating between collagen and other cells). These were examined by the principal investigator and study pathologists.

Results: The study included 23 decedents out of whom 18 had eclampsia and 4 with preeclampsia with severe features. The gross brain findings included 12(52.2%) cases with features of raised intracranial pressure, 9(39.1%) cases with cerebral oedema, 8(34.8%) with haemorrhage and 1(4.3%) had liquefactive necrosis. There were 14(60.9%) cases with vascular findings on histology while 9(39.1%) cases had no vascular findings. The microscopic parenchymal findings included gliosis in 14(60.9%) cases, haemorrhage in 11(47.8%) cases and infarcts in 5(21.7%) cases.

Conclusion: The common histological parenchymal findings identified included neural changes due to hypoxic ischemia, cerebral oedema, haemorrhage, and old infarcts healing with gliosis while vascular changes identified were arteriosclerosis, thrombotic microangiopathy and vascular congestion. The laboratory parameters including liver function tests, activated partial thromboplastin time and prothrombin, and platelet count for majority of the decedents were deranged denoting a concurrent haemolysis, elevated liver enzymes, low platelet count syndrome. The relation of these lab parameters and the histology findings was not found to be statistically significant.

Recommendations:

1. Initial radiologic examination of severe cases should be done to exclude intracranial haemorrhage and assist in making appropriate management decisions.
2. There should be emphasize on the management of cerebral oedema to prevent further brain injury.
3. Further studies with use of immunohistochemistry to identify more lesions and studies to further correlate the histology findings with the clinical and laboratory findings should be done.

1.0 INTRODUCTION

Background

Pre-eclampsia and eclampsia are hypertensive disorders of pregnancy. Hypertensive disorders of pregnancy occur in up to 10% of pregnancies with pre-eclampsia accounting for 2-8% of these cases (1). The global prevalence of eclampsia is approximated to be around 0.3% (2).

Pre-eclampsia is clinically described as new hypertensive episodes occurring after 20 weeks gestation with associated renal, hepatic, proteinuria, neurovascular or haematological complications (3). Some of the neurologic symptoms are visual disturbances, persistent headaches, vomiting, eclampsia and cortical blindness (4). Eclampsia is a life-threatening complication presenting as seizures or altered state of consciousness occurring in the setting of pre-eclampsia (5).

An autopsy study done in Maputo observed cerebral oedema, hemorrhage, haemosiderin, parenchymal necrosis and small vessel thrombosis as the main lesions in the brain of persons who died due to preeclampsia and eclampsia (6).

There is paucity of local data on the brain changes in preeclampsia and eclampsia. An autopsy study of these changes will be an excellent source of information on the above thus aiding in the understanding of the prevention and management of neurological complications within our setting.

2.0 LITERATURE REVIEW

2.1 Introduction

Pre-eclampsia complicates between 2 and 8% of all pregnancies (7). It is a cause of perinatal and maternal morbidity and mortality accounting for about 50,000-60,000 maternal deaths (3) and over 500,000 fetal deaths annually (8), with a predominance in the low- and middle-income countries.

According to the Kenya Demographics and Health Survey 2014, maternal mortality rate is 362 per 100,000 live births. Hypertensive disorders in pregnancy account for 19% of these mortalities. The prevalence of preeclampsia in Kenya is about 5.6-6.6% with higher rates in the rural setting (9) while a multi-country survey by WHO reports a prevalence of 1.97% for pre-eclampsia and 0.32% for eclampsia (10).

Globally, the incidence of eclampsia is estimated as 16-69 cases per 10,000 births. In low- and middle-income countries, fatality rates from eclampsia are 3%-5%, in contrast to high income countries where the fatality is 1%, or less. (1) The incidence of eclampsia in KNH is 1.8 per 1000 deliveries with a mortality rate of 5% (11).

There are 2 subtypes of pre-eclampsia based on the time of onset, early-onset, and late-onset. This is based on clinical signs, with early onset appearing before 33 weeks and late-onset subtype appearing after 34 weeks. More than 80% of pre-eclamptic have the late onset subtype. Higher foetal and maternal morbidity and mortality rates are associated with the early-onset type (3).

Preeclampsia is classified into preeclampsia without and pre-eclampsia with severe features (PES). The severe features include blood pressure measurement greater than 160/110mmHg,

headache unresponsive to acetaminophen, pulmonary oedema, thrombocytopenia with a platelet count of 100,000cells/µl, renal insufficiency, impaired liver function and visual disturbances. (5) Neurological presentation of pre-eclampsia includes visual disturbances, headaches, tinnitus, brisk tendon reflexes and eclampsia. (6) Eclampsia presents seizures or altered state of consciousness in patients with pre-eclampsia which is not attributable to any other condition. (6,7)

2.2 Preeclampsia pathology

Preeclampsia is a multi- systemic syndrome with risk factors including chronic hypertension, nulliparity, history of previous preeclampsia, vascular disease such as diabetes, kidney disease, age ≥35 years, black race, BMI>30, assisted reproductive technology and multiple or molar pregnancy (5,12).

The aetiology of preeclampsia is poorly understood. It is hypothesized that aberrant placentation with defective cytotrophoblastic cells invasion of spiral arteries is the primary cause (3). In normal pregnancies, there is invasion of decidua tunica media of maternal spiral arteries by cytotrophoblast replacing the endothelium. This pseudo vascularization enables maternal spiral arteries transform to large low-resistance vessels allowing an increase in the flow of blood to the maternal-fetal interface.

Incomplete invasion of the spiral arteries causes placental hypoperfusion with systemic vasoactive compounds release causing endothelial damage, hypercoagulability, an abnormal inflammatory response, vasoconstriction, platelet dysfunction, and capillary leak. These cause organ dysfunction.

Additional factors such as oxidative stress, inflammation, genetic susceptibility and immune maladaptation, alteration of the renin–aldosterone–angiotensin II axis contribute to preeclampsia pathogenesis (12).

Pro-angiogenic factors Vascular endothelial growth factor (VEGF) and platelet growth factor (PlGF) and anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt1) expression is altered in PE (13). VEGF and P1GF are essential in placental angiogenesis. sFlt1 is secreted into the maternal circulation where it binds and antagonizes VEGF and PlGF (12,13). Endoglin, a receptor for TGF-β, is also increased in PE causing increased capillary permeability and inhibiting angiogenesis (13).

In women with preeclampsia, endothelial dysfunction and activation serum markers including von Willebrand antigen, endothelin, platelet derived growth factor, cellular fibronectin, soluble E-selectin, and soluble tissue factor are deranged. There is an increased sensitivity to norepinephrine and angiotensin II (14). There's impaired endothelium-dependent vasorelaxation in PE.

Placental hypoxia causes an overexpression of HIF-1α resulting in shallow trophoblast invasion of the spiral arteries (3). Oxidative stress stimulates cytokine, free radicals, oxidized lipids, and serum soluble vascular endothelial growth factor 1 release into the circulation of the mother leading to endothelial maladaptation.

Maladaptation by the immune system causes inadequate invasion by the cytotrophoblasts. There is extra-villous cytotrophoblast apoptosis induced by tumour necrosis factor alpha secretion (15). Pre-eclamptic women have reduced levels of HLA-E and HLA-G indicating the role of Human Leukocyte Antigen system in inadequate invasion of the spiral arteries (12).

In normal pregnancy, there's increased aldosterone and angiotensin circulating levels. In pre-eclampsia there's increased sensitivity to vasoactive peptide angiotensin 2 and other vasoconstrictors with suppression of RAAS. The local uteroplacental RAAS is however, upregulated in PE (13).

Having a first-degree relative with pre-eclampsia increases severe preeclampsia risk two- to fourfold, despite controlling for age, body mass index and smoking status. There is an increased risk of fathering a subsequent pregnancy complicated by preeclampsia with a different partner in men who have fathered a previous a pregnancy previously complicated by preeclampsia indicating the presence of a paternal component in the genetic predisposition (12).

Bellamy et al assessed women whose pregnancy had been complicated by preeclampsia and observed that they had an increased risk of ischemic heart disease, hypertension, stroke, and venous thromboembolism (7).

2.3 Cerebrovascular pathology

Central nervous system (CNS) manifestations including blurred vision, scotoma, cortical blindness, hyperreflexia, headaches, and generalized seizures are considered severe features of pre-eclampsia (6). Acute cerebral complications including eclampsia, edema, stroke, and brain herniation contribute significantly to maternal morbidity and mortality.

Decreased CVR in pre-eclampsia causing hyperperfusion can lead to vasogenic oedema and disruption of the BBB resulting in neurological symptoms. In some preeclamptic cases, there may be rise of arterial pressure above the autoregulatory range of cerebral blood flow. The increased intravascular pressures result in loss of hyperperfusion and autoregulation and leading to oedema, endothelial damage, and risk of brain injury through the inability of cerebral arterioles and arteries to provide vascular resistance. Decreased CVR that occurs in preeclampsia

may lead to exposure of the maternal brain to increased CPP secondary to a lack of the cerebral arteries hypertensive remodeling (16).

Rapid blood pressure rise can cause loss of autoregulatory capacity and blood-brain barrier (BBB) disruption thus causing vasogenic edema as the myogenic vasoconstriction of cerebral arteries and arterioles is overcome causing loss of vascular resistance. Posterior reversible encephalopathy syndrome ensues with a predilection for edema formation within the posterior cerebral cortex. A study done by Ingrid et al concluded that impaired maternal memory seemed worse after severe pre-eclampsia complicated pregnancies in comparison to those who had normotensive pregnancies (17).

Magnetic resonance imaging (MRI) and Computed tomography (CT) scans have identified various abnormalities in pre-eclampsia and eclampsia patients including focal infarctions, intracranial hemorrhages, cerebral edema, and posterior leukoencephalopathy. There are, however, no pathognomonic CT scan or MRI findings. CT scans are used for intracranial hemorrhage detection in patients who develop seizures, focal neurologic deficits, and severe and persistent headaches.

A study done by Schwartz et al found that the extent of brain edema on MRI on preeclampsia and eclampsia patients was correlated to the extent of endothelial damage and not with the hypertension level (18).

Posterior reversible encephalopathy syndrome, a clinico-radiological syndrome, is characterized by white matter vasogenic oedema predominantly in the parietal and occipital lobes. It presents as headaches, visual loss, seizures, and altered mental status. The lesions may also affect the frontal lobes, brainstem, and basal ganglia (22,23).

Autopsy studies show 60% of eclamptic women having intracranial haemorrhage grossly. Other changes seen on post-mortem include cortical petechial hemorrhages usually in the occipital lobes, subcortical edema, basal ganglia, or pons or intra-ventricular haemorrhage and hemorrhagic areas in the white matter (4).

In a study done by Amanda et al on 43 women with pre-eclampsia, they demonstrated Cerebral oedema in 27 of them. The severity of oedema of the oedema correlated with the duration of intermittent seizures. Intracranial haemorrhage was found in 4 of the 7 mortalities. Vasculopathy proportional to the number of seizures was also observed (19).

Hetch et al in an autopsy study done in Maputo observed cerebral oedema, hemorrhage, haemosiderin, parenchymal necrosis and small vessel thrombosis as the main lesions in the brain. These autopsy findings were, however, not correlated with the clinical findings (20).

2.3.1 Eclampsia

Eclampsia is seizures or altered state of consciousness not caused by other cerebral conditions in patients with preeclampsia. It is a life-threatening complication hypothesized to be caused by cerebral oedema or due to ischaemic brain injury resulting from cerebral blood flow dysregulation. These grand mal seizures may occur up to 48 hours postpartum. Approximately 0.5% of patients with preeclampsia without severe features and 2% to 3% of those with preeclampsia with severe features progress to eclampsia (2).

Miguil and Chekairi reported 23 deaths as a result of eclampsia. Of these, 61% had ischemia or cerebral hemorrhage, 50% had severely high blood pressures both systolic and diastolic. The mean platelet count was 105 000 cells/μ (21).

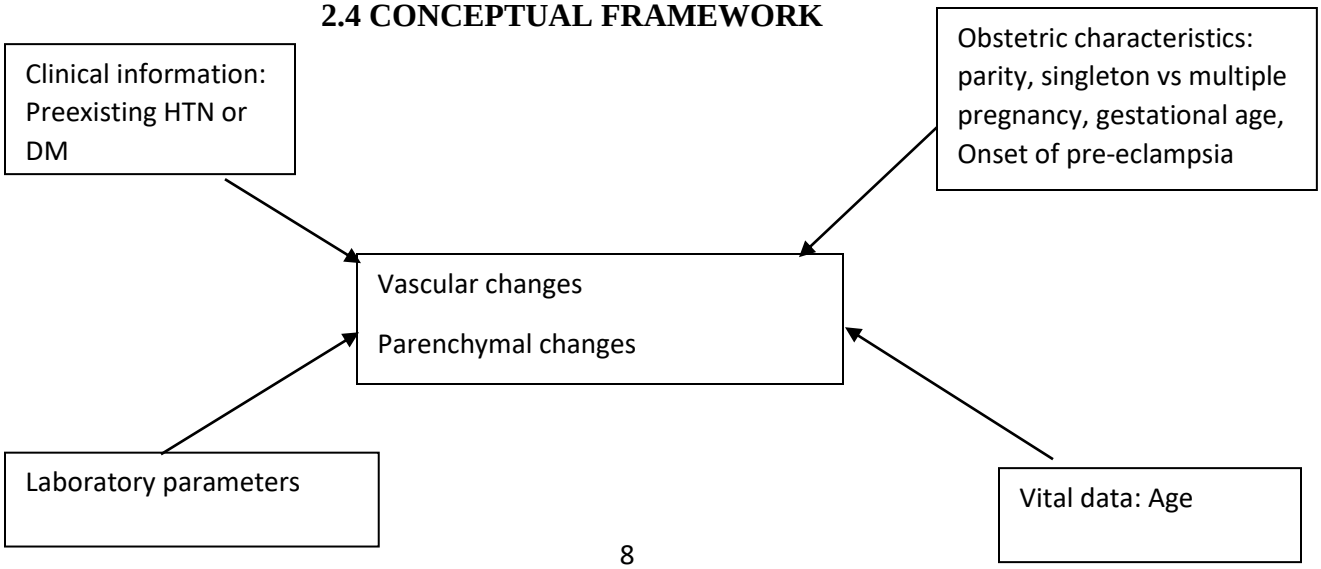
2.3.2 Stroke

The incidence of stroke during pregnancy is 10-34/100,000 deliveries. This incidence increases 4-5 times in pre-eclampsia compared to normotensive pregnant women with a 1.8-fold increased risk of stroke in their lifetime. Martin et al in a review of 28 women found that hemorrhagic stroke accounted for 89% of the stroke cases in preeclampsia. They also found that stroke was preceded by systolic blood pressure of approximately 155–160 mmHg and diastolic pressures of less than 105 mm Hg (22). Sharshar et al found eclampsia as the main cause of both intraparenchymal hemorrhage and non-hemorrhagic stroke, with intra-parenchymal haemorrhage having a poor prognosis (22).

2.3.3 Cerebral oedema

Localized cerebral edema is typically seen at the white-grey matter junction in the occipital lobe. Diffuse oedema may be seen in also be seen. The oedema is due to extravasation of fluids across the disrupted blood-brain barrier (23). An autopsy study done in Maputo, Mozambique by Hetch et al reported a prevalence of cerebral oedema of 68.4% in persons who died due to eclampsia (6).

2.4 CONCEPTUAL FRAMEWORK



2.5 JUSTIFICATION

Pre-eclampsia and eclampsia have long term effects on mothers including long-term cognitive changes, increased lifetime risk of a cerebral vascular accident, perceived lower quality of life and persistent white matter lesions. Cerebrovascular involvement is a direct cause of death, accounting for more than 40% of maternal mortalities during pregnancy (7). Endothelial dysfunction, blood pressure changes and thrombocytopenia confer a higher risk for cerebral complications in pre-eclampsia and eclampsia (8).

Autopsy studies have described various lesions in the brain associated with pre-eclampsia and eclampsia including cerebral oedema, haemorrhage, necrosis and thrombosis (6). There are limited studies with correlation of the lesions with clinical findings. Preeclampsia still accounts for a significant proportion of maternal mortalities, with decedents having had eclampsia. However, there is no published data from any study on the vascular and parenchymal brain changes in this setting.

A detailed postmortem evaluation of the brain findings in preeclampsia and eclampsia at Kenyatta National Hospital will enhance understanding of the burden of the neurological complications and identity the actual cause of these complications. This study is therefore designed to document these brain findings. Evaluation of brain features of PE and eclampsia on autopsy specimens will help identify therapeutic priority areas required for prevention of these mortalities.

2.6 RESEARCH QUESTION

What are the autopsy brain findings in preeclampsia and eclampsia?

2.6 OBJECTIVES

2.6.1 Broad objective

To describe the autopsy brain findings in preeclampsia and eclampsia at Kenyatta National Hospital.

2.6.2 Specific objectives

1. Describe the macrovascular changes of atherosclerosis, thrombosis, and aneurysms.
2. Describe the microvascular changes of congestion, thrombosis and thrombotic angiopathy associated with preeclampsia and eclampsia
3. Describe the brain parenchymal findings in preeclampsia and eclampsia
4. Evaluate sociodemographic, antemortem clinical and laboratory parameters correlates of brain autopsy findings.

3.0 METHODOLOGY

3.1 Study design

This was a descriptive cross-sectional study identifying the brain changes in deceased persons who received care at the KNH Obstetrics and Gynecology Unit and admitted at the KNH Mortuary with an antemortem diagnosis of pre-eclampsia or eclampsia.

3.2 Study site

The study was undertaken at the KNH farewell funeral home. The histological specimens were processed at the anatomic pathology laboratory.

3.3 Study duration

November 2020- April 2021

3.4 Selection criteria

- **Selection Criteria**

1. **Inclusion criteria**

- Deceased persons who received care at the KNH obstetrics and Gynecology Unit and were admitted at the KNH mortuary with an antemortem diagnosis of preeclampsia or eclampsia.

2. **Exclusion criteria**

- Lack of consent from the person authorizing the autopsy.
- Deceased persons whose clinical information was not accessible.

3.5 Sample size determination

Cochran's formula for sample size calculation was adopted. The prevalence of neurovascular changes in pre-eclampsia and eclampsia associated mortalities in Kenyatta National Hospital is unknown. To estimate the appropriate sample size, 50% of the assumed prevalence was used. Approximately 4 mortalities are recorded per month according to the KNH records. The total

number of pre-eclampsia and eclampsia mortalities recorded during the six months study period would be 24. Using the finite population correction for proportions, a representative sample was calculated.

$$n_0 = Z^2pq/d^2$$

$$n = \frac{n_0}{1+\frac{(n_0 - 1)}{N}}$$

Where:

n_0 = initial estimated sample study size

Z = standard normal deviate at 95% confidence interval (1.96)

p = estimated prevalence of neurovascular changes in pre-eclampsia/eclampsia associated mortalities

q = 1-p

d = Margin error taken as 0.05

N= Total population of pre-eclampsia/eclampsia mortalities recorded in six months in KNH (24)

$$n_0 = \frac{1.96^2*0.50\ (1-0.50)}{0.05^2}$$

$$= 384$$

$$n = \frac{384}{1+\frac{(384 - 1)}{24}}$$

= 23

3.6 Sampling method

Consecutive sampling of decedents who had died from pre-eclampsia and eclampsia was done.

3.7 Study procedure

3.7.1 Data accrual

Enrollment of study subjects was done from the KNH farewell funeral home. All decedents who died due to preeclampsia or eclampsia qualified for recruitment. Written informed consent from those who fulfilled the selection criteria was obtained from the deceased next of kin. Vital data and clinical history were obtained from the decedents' medical records and collaborated with information from the next of kin. This included maternal age, parity, whether it was a singleton or multiple pregnancy, gestational age, parity, personal and family history of pre-eclampsia, pre-existing hypertension, and laboratory parameters in the total blood count, liver function tests and U/E/Cs

3.7.2 Autopsy

Autopsies were carried out and the brains removed for fixation in 10% buffered formalin.

3.7.3 Procedure

Careful evisceration of the brain was performed. Dural sinuses were examined for thrombosis and histopathological sections obtained. Eviscerated whole brains were weighed, photographed, and immediately fixed in adequate quantities of 15% neutral buffered formal saline. After 2 weeks of fixation, the basal arteries of the brain (circle of Willis) were dissected, examined, photographed and serial sections submitted for histopathology. Serial coronal cortical sections were performed and photographed. Sagittal sections of the Cerebellum were performed, examined, and photographed. Serial dissections of the brainstem, pons and medulla were performed, examined, and photographed. Standard histopathological sections representing

cortical vascular watershed areas were obtained. Standard subcortical sections were obtained for histopathology.

Histopathological sections of the brain were processed, and 5 micrometer thin sections prepared. These were stained using hematoxylin and eosin. Additional 5 micrometer sections were prepared and stained with Luxol Fast Blue (stain for myelin) and Masson Trichrome (stain for differentiating between collagen and other cells). These were examined microscopically by the Principal Investigator and supervising pathologists and the histology findings input in the data collection tool.

3.8 Variables

Biodata, clinical information including parity, singleton or multiple, gestation age, onset of preeclampsia, previous preeclampsia, pre-existing hypertension or diabetes, and laboratory parameters.

3.9 Quality assurance

3.9.1 Pre-analytical

The principal investigators ensured correct identification of the decedent and there after assigned a unique study number. The brain specimens obtained were fixed in well labelled containers. The cassettes and slides were also clearly labelled. The stain used was obtained from a reputable distributor and the tissue processing and staining done following standard SOPs

3.9.2 Analytical

The principal investigator and a pathologist (the supervisor) reviewed the slides to determine the neuropathological changes. A second pathologist reviewed every 5^{th} slide for quality assurance.

3.9.3 Post-analytical

Data was immediately entered on a spread sheet to avoid any errors.

3.10 Ethical consideration

The proposal was submitted to KNH-UoN Ethics and Research Committee for approval prior to the study. The study was voluntary, written informed consent was sought and no cash incentive was given to the decedents next of kin. Each case was given a unique identifying number. Only the principal investigator had access to the patients' hospital files with privacy and confidentiality of the medical records strictly maintained. Tissue processing and histological examination costs were met by the study budget. The data from the study was archived in a computer that has data protection measures.

3.11 Data management

Data was collected from the decedents' hospital file and from the next of kin. Autopsy and histology findings were filled in a well-structured manner and stored securely. The data was fed in a Microsoft excel worksheet and verified for any errors. International Business Machine-SPSS Statistics version 21 application was used to analyze the data.

Demographic and clinical characteristics were analyzed and presented as frequency distributions and percentages for categorical variables and as means or medians for continuous variables.

Data was presented as bar graphs, pie charts and tables. Analysis of variance (ANOVA) was used to assess any association between the laboratory parameters and the vascular and parenchymal findings.

4.0 RESULTS

In the period of the study, 77 maternal mortalities were evaluated out of which 23 cases that met the inclusion criteria and were recruited for the study. There were 18 cases with a clinical diagnosis of eclampsia and 5 with an antemortem diagnosis of Preeclampsia with severe features. Twenty-two (95.7%) of the deceased persons were referred from other health facilities while 1(4.3%) was admitted from KNH.

Age distribution of cases

The age range was 16.0 to 40 years. The mean age was 27.8 years, while the median age was 28.0 years.

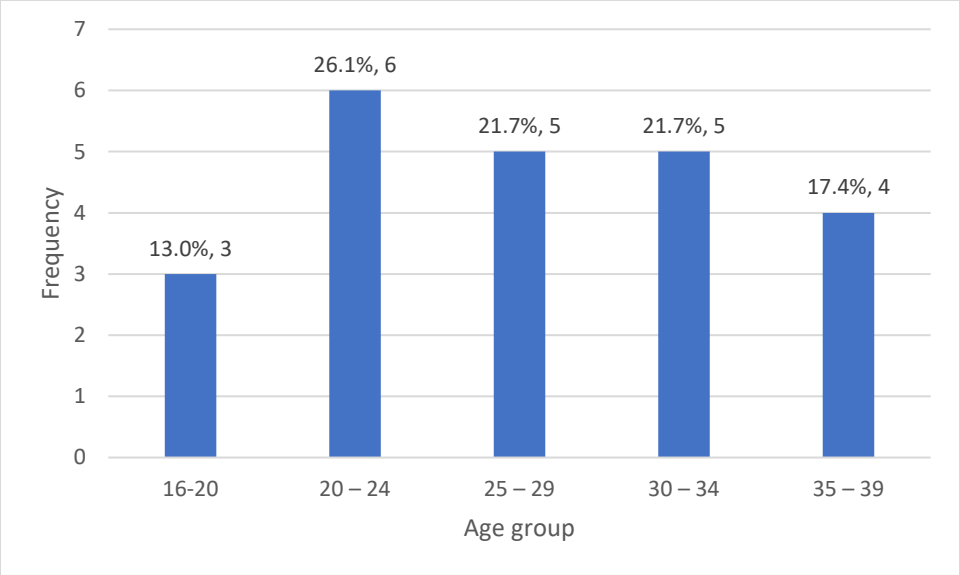


Figure 1: Age distribution of the decedents

Obstetric Characteristics

For the recruited cases, 8(34.7%) of them were primigravida, 14(60.9%) were multigravida while 1(4.3%) was a grand multigravida. Three (13%) presented in the 2nd trimester of pregnancy, 17(73.9%) presented in the third trimester while 3(13% presented in the postpartum period. 21(91.3%) had singleton pregnancies while 2(8.7%) had twin pregnancies.

Table 1: Obstetric characteristics

Gravid status	Frequency	Percentage
Primigravida	8	34.8
Multigravida	15	65.2
Trimester at presentation		
2nd Trimester	3	13.0
3rd Trimester	17	73.9
Postpartum	3	13.0
Singleton vs multiple		
Singleton	21	91.3
Multiple: twins	2	8.7

Medical history

There were 5(21.7%) cases with history of preeclampsia in the previous pregnancies and 18(73.9%) had no history preeclampsia in previous pregnancies. Twenty-two (91.3%) of them had no history of HTN/DM while 1(4.3%) was a known hypertensive patient

Table 2: Previous treatment for Hypertension or Diabetes

Preeclampsia	Frequency (n=23)	Percent
Yes	5	21.7
No	18	78.2
HTN/DM		
Yes	1	4.3
No	22	95.7

Mode of Delivery

The mode of delivery was via Cesarean section for 11(47.8%) of the cases and via vaginal delivery for 8(34.8%) of the cases.

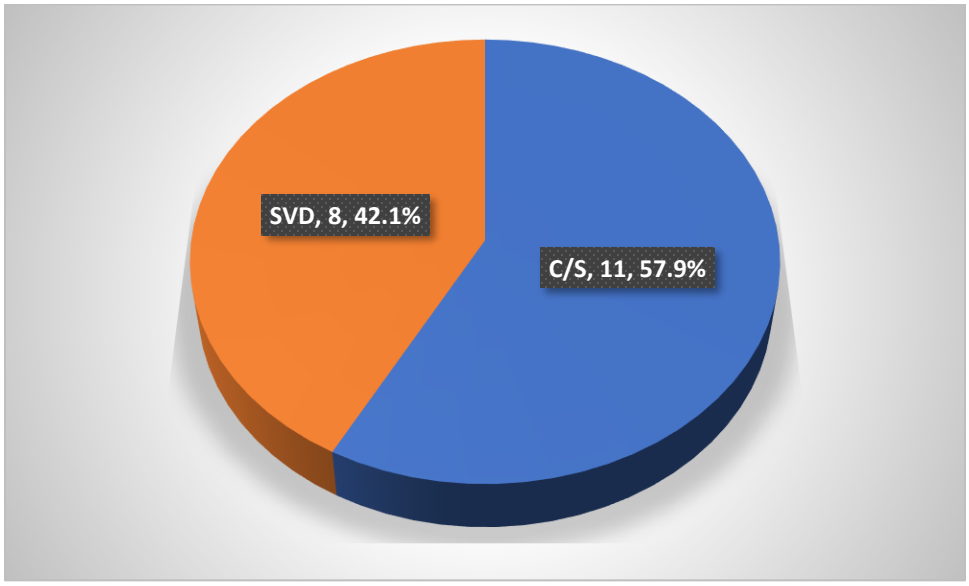


Figure 2: Mode of delivery

Pregnancy outcome

There were 4(17.4%) cases with fetuses in utero during postmortem. Eleven of them had live birth, 3(13%) had fresh stillbirths and 4(21.7%) had macerated stillbirths.

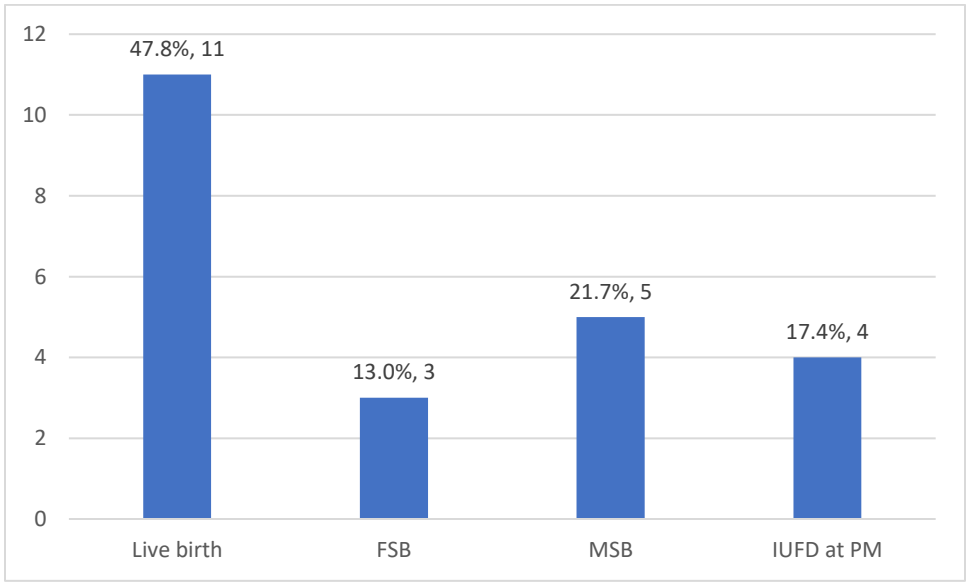


Figure 3: Pregnancy outcome

Antemortem findings

The cases recruited had varying clinical presentation according to the medical records. The neurological symptoms included convulsion in 18(78.3%) cases, confusion in 5(21.7%) cases, headaches in 6(26.1%), loss of consciousness in 2(8.7%) cases and blurred vision in 2(8.7%) of the cases. The renal signs and symptoms included oliguria in 3(13.0%) of the cases studied, anuria in 3(13.0%) of the cases and hematuria 2(8.7%) of the cases. The gastrointestinal symptoms were epigastric pain in 6(26.1%) of the cases and nausea and vomiting in 1(4.3%) case. Difficulty in breathing was reported in 9(39.1%) cases. Other symptoms reported included postpartum haemorrhage in 1(4.3%), epistaxis in 2(8.7%) cases, anasarca in 2(8.7%) cases and lower limb swelling in 4(17.4%) cases.

Table 3: Antemortem findings

	Frequency(n)	**Percent of cases (%)**
Neurological	21	91.3
GIT	6	26.1
Renal	7	30.4
Pulmonary	9	39.1
GUT	9	39.1
Other	7	30.4

Blood pressure recording at admission

There were 4(17.4%) cases that were normotensive at admission, 9(39.1%) had blood pressure recordings of between 140/90mmHg and 160/110mmhg while 10(43.5%) has blood pressure recordings of more than 160/110mmHg.

Table 4: Blood pressure recording at admission

	Frequency	Percent
Normotensive	4	17.4
140/90-160/110mmHg	9	39.1
>160/110mmHg	10	43.5

Laboratory test results

The laboratory test results were extracted from the decedents medical records. There were 21 cases with complete blood count results and 19 with PT and APPT results. All cases had liver function test and UEC results.

The haemoglobin levels ranged from 4.9g/dl to 17g/dl. The white cells count ranged from a leukopenia of $2.7x10^6$cell/L to a leucocytosis of $84x10^6$cells/L. The platelet count varied from a minimum count of $25x10^9$cell/L to a maximum count of $477x10^9$cells/L. All the PT and APPT values were deranged. The liver function tests ranged from normal to markedly deranged.

Table 5: Laboratory test results

Total blood count

	N	Median (IQR)	Min	Max
Haemoglobin(g/dl)	21	12.0 (10.1 – 14.4)	4.9	17.0
WBC count($x10^6$cell/L)	21	17.4 (14.7 – 26.9)	2.7	84.0
PLT count($x10^9$cell/L)	21	84.0 (40.0 – 118.0)	25.0	477.0

Coagulation test

PT (seconds)	19	17.9 (16.0 – 25.4)	13.1	120.0
APPT (seconds)	19	36.0 (31.4 – 46.4)	24.7	70.2

Liver Function Test

T.BIL (umol/l)	23	73.7 (25.9 – 111.6)	2.4	314.1
D.BIL (umol/l)	23	28.6 (14.0 – 52.0)	1.4	242.5
ALT (U/L)	23	150.0 (55.0 – 376.5)	5.0	1764.0
AST (U/L)	23	250.0 (47.0 – 778.5)	12.0	1284.0
Albumin (g/L)	23	29.0 (25.0 – 33.5)	16.0	45.0

U/E/Cs

Creatinine (umol/l)	23	179.0 (112.0 – 290.0)	92.0	541.0
Urea (mmol/l)	23	10.8 (5.9 – 18.5)	4.0	312.0
Na (mmol/l)	23	139.0 (133.0 – 140.5)	114.0	147.0
K (mmol/l)	23	4.7 (4.1 – 5.1)	2.7	6.1

Estimated Glomerular Filtration Rate

8(34.8%) had 2nd stage renal failure, 5(21.7%) had 3rd stage renal failure, 9(39.1%) had 4th stage renal failure while 1(4.3%) had 5th stage renal failure. The creatinine-based equation from the Modification of Diet in Renal Disease Study (MDRD) was used to estimate glomerular filtration rate (24).

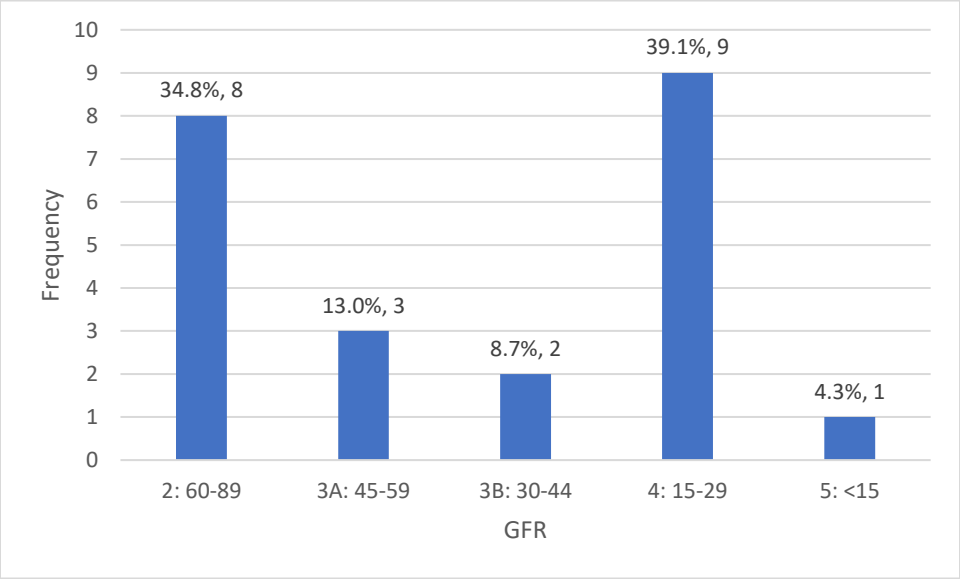


Figure 4: EGFR (ml/min/1.73m^2)

Correlation of APPT, PT and LFTs

Pearson correlation was used to determine the association of AAPT and PT with the LFTS. These correlations were not statistically significant, with exception of PT and T.BIL which was statistically significant.

Table 6: Association between APPT, PT and LFTs

AAPT	**Pearson correlation (r)**	**p-value**
T.BIL	0.283	0.240
D.BIL	0.348	0.144
ALT	0.140	0.567
AST	0.019	0.938
Albumin	-0.379	0.109
PT	**Pearson correlation (r)**	**p-value**
T.BIL	-0.446	**0.043**
D.BIL	-0.359	0.109
ALT	-0.184	0.425
AST	-0.278	0.223
Albumin	0.350	0.120

POSTMORTEM EXAMINATION RESULTS

These included findings upon extraction of the brain, during sectioning and on microscopic examination of the brain sections.

GROSS FINDINGS

The brain weights ranged from 945-1355grams with the average weight being 1214.8 grams.

Table 7: Brain weight (grams)

n	Mean±SD	Median (IQR)	Min	Max
23	1214.8±87.5	1210.0 (1175.0 – 1255.0)	945.0	1355.0

There were 12(52.2%) cases features of raised intracranial pressure including narrowed sulci, flat gyri, and displacement of the cingulate gyrus, medial temporal lobe, or cerebellar tonsils. Cerebral oedema was present in 9(39.1%) of the cases, 8(34.8%) had intracranial haemorrhage while 1(4.3%) had necrosis.

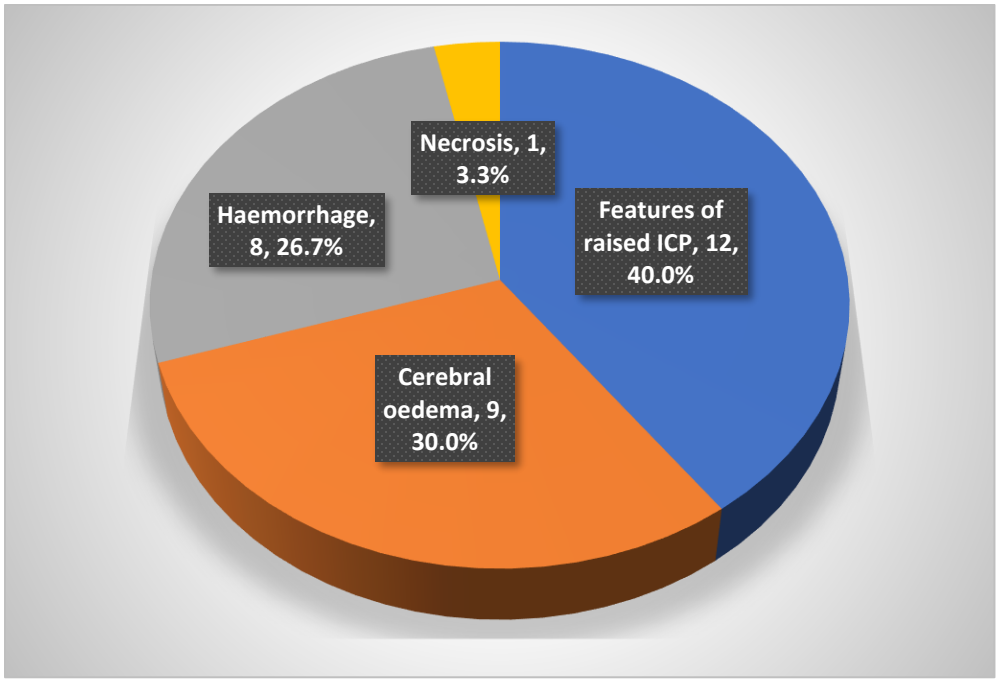


Figure 5: Gross findings

Gross findings

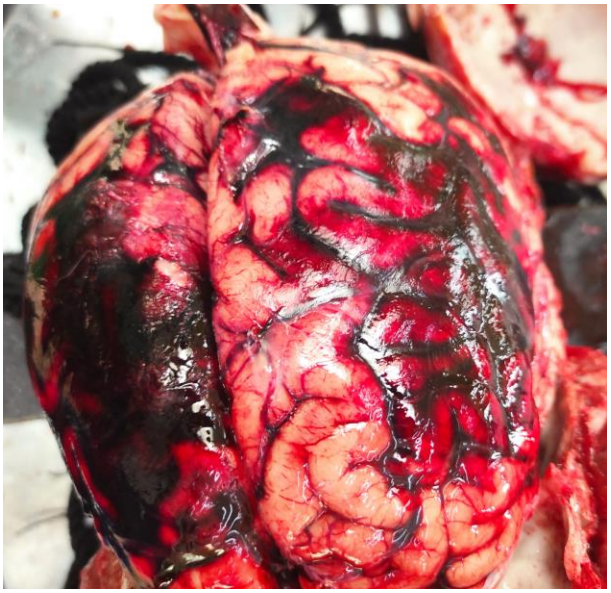


Figure 6: Subarachnoid haemorrhage

Figure 7: intraventricular and parenchymal

Figure 8: Pontine haemorrhage

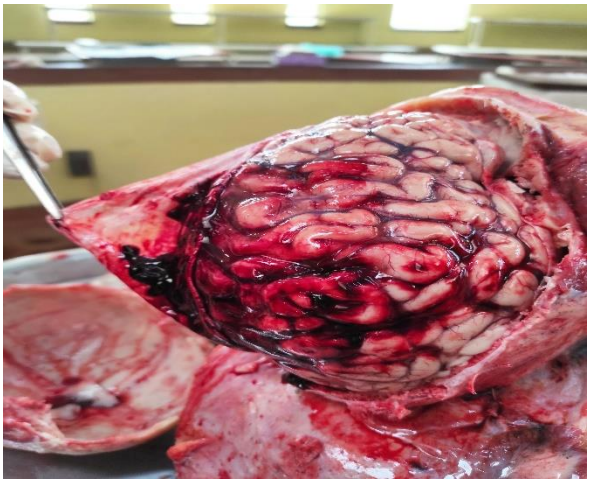


Figure 9:Subdural Haemorrhage

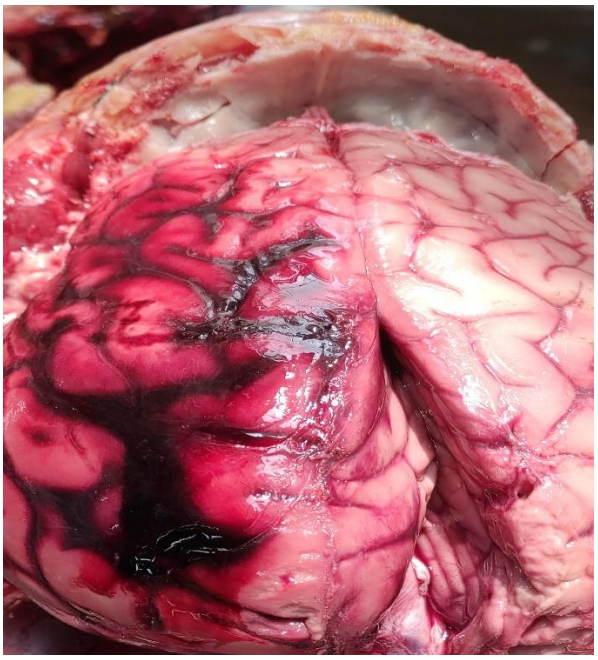


Figure 10:Brain with subarachnoid haemorrhage and features of raised ICP(Flat gryi & narrow sulci)

HISTOLOGY FINDINGS

The brain sections were examined microscopically to evaluate the parenchymal and vascular changes.

Vascular findings in the brain

The vascular findings evaluated in the study included macrovascular changes of atherosclerosis, thrombosis, and aneurysms and microvascular changes of congestion, thrombosis and thrombotic angiopathy. There were 14(60.9%) cases with vascular findings on histology while 9(39.1%) cases had no vascular findings.

Table 8: Vascular findings

	Frequency (n=23)	Percent
Yes	14	60.9
No	9	39.1

For the macrovascular changes 8 (34.8%) cases had atherosclerosis, there were no findings for thrombosis and aneurysms. The findings of the microvascular changes showed 2(8.7%) cases with thrombotic microangiopathy and 14(60.9%) of the cases having congestion, while no cases were found to have endotheliosis.

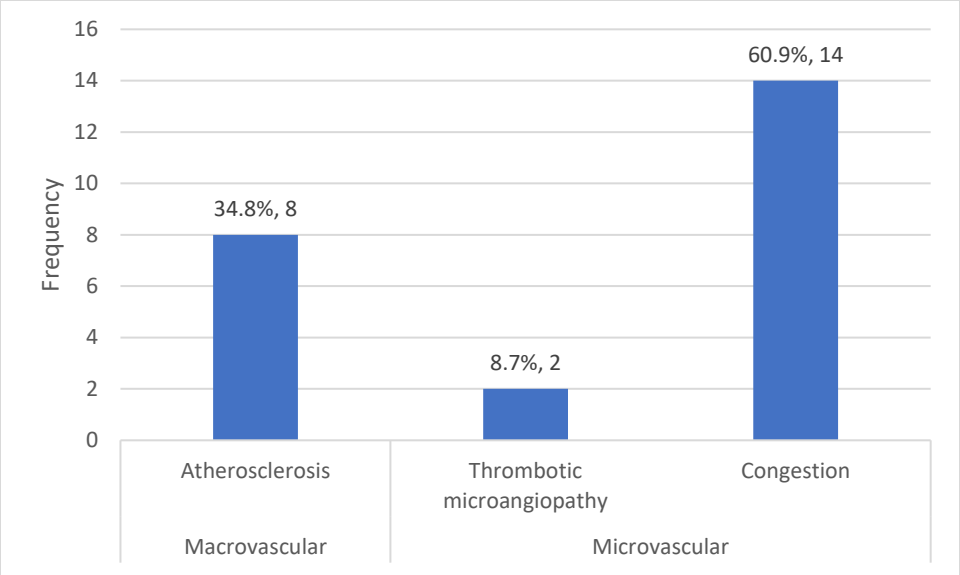


Figure 11: Microvascular findings

Age description of the vascular findings

The median age for atherosclerosis on histology was 26.5 year, 31 for thrombotic microangiopathy and 26.5years for vascular congestion

Table 9: Age description

	Atherosclerosis	**Thrombotic microangiopathy**	**Congestion**
Age, *median (IQR)*	26.5 (23.5 – 34.0)	31.0 (23.0 – 34.0)	26.5 (23.0 – 34.0)

Obstetric characteristics descriptive of the vascular findings

The atherosclerotic changes were seen in 3 primigravida, and 5 multigravidas, thrombotic microangiopathy was found in 2 multigravidas while vascular congestion was in 5 primigravids and 9 multigravidas.

For those with atherosclerosis, 2 presented in the 2nd trimester, 5 in the 3rd trimester and 1 during the postpartum period. Those with the findings of thrombotic microangiopathy presented in the third trimester while those with vascular congestion, 2 presented in the 2nd trimester, 9 in the 3rd trimester, and 3 in the postpartum period.

Table 10: Obstetric characteristics and vascular findings

	Atherosclerosis	Thrombotic microangiopathy	Congestion
Gravid status			
Primigravida	3	0	5
Multigravida	5	2	9
Trimester at presentation			
2nd Trimester	2	0	2
3rd Trimester	5	2	9
Postpartum	1	0	3

Laboratory test results correlates of vascular findings

Platelet count results

The median platelet count for the cases with atherosclerosis was 81x10^9cells/L, for thrombotic microangiopathy was 52 x10^9cells/L while that for those with congestion was 86 x10^9cells/L.

Table 11: Platelet count and vascular findings

	Atherosclerosis	Thrombotic microangiopathy	Congestion
	n=8	***n=2***	***n=13***
PLT(x109cells/L)	81.0 (52.0 – 117.0)	52.0 (34.0 – 70.0)	86.0 (46.0 – 116.0)

Coagulation test and vascular findings

The median PT values for cases with atherosclerosis was 22.5 seconds and 17.0 seconds for cases with congestion. The median APPT values for cases with atherosclerosis was 38.1 seconds, 36.4 seconds for the case with thrombotic microangiopathy and 34.0 seconds for cases with vascular congestion.

Table 12: coagulation test and vascular findings

	Atherosclerosis	**Thrombotic microangiopathy**	**Congestion**
	n=6	***n=0***	***n=10***
PT, (seconds) *median (IQR)*	22.5 (17.1 – 36.5)	-	17.0 (14.8 – 23.0)
	n=6	***n=1***	***n=10***
APPT, (seconds) *median (IQR)*	38.1 (32.0 – 55.6)	36.4 (-)	34.0 (33.0 – 46.5)

Liver function test results and vascular findings

All cases with atherosclerosis, thrombotic microangiopathy and congestion had elevated bilirubin levels, elevated transaminases levels and low albumin levels.

Table 13: Liver function tests and vascular findings

	median (IQR)		
	Atherosclerosis	**Thrombotic microangiopathy**	**Congestion**
	n=8	***n=2***	***n=14***
T.BIL (umol/l)	60.3 (31.4 – 189.7)	218.1 (173.1 – 263.1)	35.2 (29.8 – 116.3)
D.BIL (umol/l)	23.6 (14.1 – 125.0)	144.0 (45.5 – 242.5)	21.0 (15.1 – 45.5)
ALT (U/L)	188.5 (58.0 – 674.5)	249.5 (116.0 – 383.0)	125.0 (54.0 – 383.0)
AST (U/L)	281.0 (29.0 – 771.5)	394.5 (20.0 – 769.0)	138.0 (38.0 – 518.0)
Albumin (g/L)	26.0 (23.0 – 29.0)	27.5 (21.0 – 34.0)	27.5 (23.0 – 34.0)

Estimated Glomerular Filtration Rate

The median EGFR for the decedents with atherosclerosis was 22.5ml/min/1.73m^2, 35.7ml/min/1.73m^2 for thrombotic microangiopathy and 24.4ml/min/1.73m^2 for congestion.

Table 14: EGFR

	Atherosclerosis	**Thrombotic microangiopathy**	**Congestion**
	n=8	**n=2**	**n=14**
EGFR, (ml/min/1.73m^2) *median (IQR)*	22.5 (18.5 – 62.4)	35.7 (21.4 – 63.5)	24.4 (32.9 – 65.7)

BRAIN PARENCHYMAL FINDINGS

All deceased persons had parenchymal findings on histology. The parenchymal findings examined included neuronal degeneration, cerebral oedema, haemorrhage, necrosis, demyelination, and cortical infarcts.

All cases examined had neuronal degeneration and cerebral oedema. There were 14(60.9%) cases with gliosis, 11(47.8%) cases had haemorrhage while 5(21.7%) cases had infarcts.

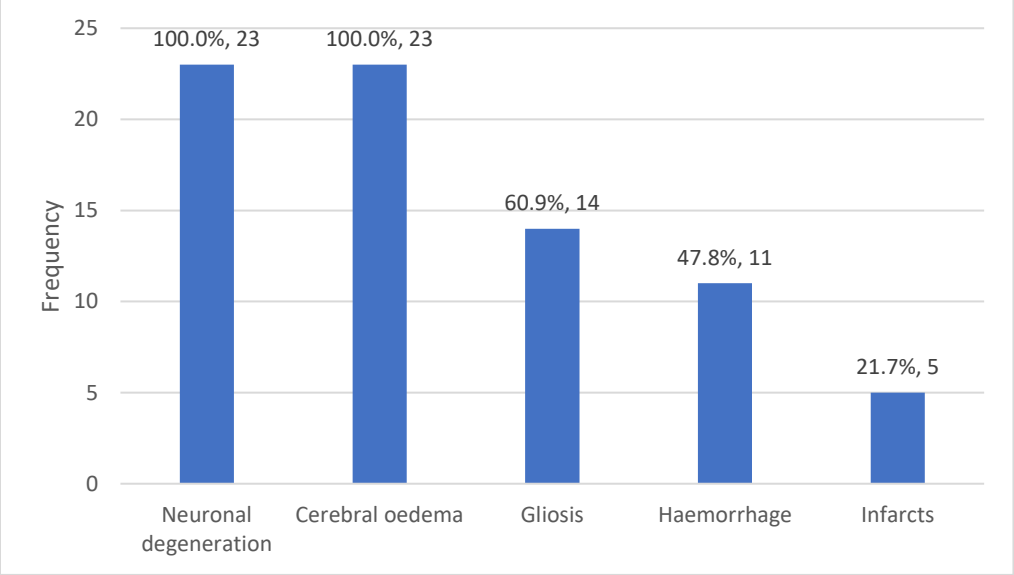


Figure 7: Brain Parenchymal histological findings

Haemorrhage

There were 11 cases in total that had haemorrhage, 8 had macroscopic haemorrhage while 3 had microscopic findings.

Table 15: Brain Haemorrhage findings

Macroscopic	**Frequency (n=11)**	**Percent of cases**
Subdural	1	9.1
Subarachnoid	5	45.5
Intraparenchymal	5	45.5
Pontine	4	36.4
Intraventricular	1	9.1
Microscopic		
Intraparenchymal	3	27.3
pontine	2	18.2

Age and Parenchymal findings

The median age for cases with gliosis was 26.5 years, 34 years for infarcts and 24.5 for cases with haemorrhage

Table 16: Age and Brain Parenchymal findings

	Gliosis	**Infarcts**	**Haemorrhage**
Age, *median (IQR)*	26.5 (23.0 – 30.0)	34.0 (28.0 – 37.0)	24.5 (21.5 – 34.0)

Obstetric characteristics and parenchymal findings

Gliosis was seen in 5 primigravid decedents and 9 multigravidas. Of these, 1 presented in the second trimester, 9 in the 3rd trimester and 3 in the postpartum period. Infarcts were found 1 primigravid decedent and 4 multigravidas, of whom 3 presented in the 3rd trimester and 2 in the postpartum period. Haemorrhage was found in 3 primigravid cases and in 8 multigravidas out of whom 1 presented in the 2nd trimester, 9 in the third trimester and 1 in the postpartum period.

Table 17: Obstetric characteristics versus parenchymal findings

	Gliosis	Infarcts	Haemorrhage
Gravid status			
Primigravida	5	1	3
Multigravida	9	4	8
Trimester of presentation			
2nd Trimester	1	0	1
3rd Trimester	10	3	9
Postpartum	3	2	1

Laboratory test results correlates of parenchymal findings

Total blood count and parenchymal findings

The median haemoglobin level was 11.8g/dl for the case with gliosis, 11.8g/dl for cases with infracts and 13.1g/dl for cases with haemorrhage. The median platelet count for the cases with gliosis was 115 $x10^9$cells/l, for infarcts was 78 $x10^9$cells/l with that of those with haemorrhage was 70 $x10^9$cells/l.

Correlation of the haemoglobin and platelet count with the parenchymal findings was not found to be statistically significant.

Table 18: Total blood count and parenchymal findings

	Gliosis	Infarcts	Haemorrhage
	n=13	***n=4***	***n=11***
HB(g/dl)	11.8 (10.1 – 14.4)	11.8 (8.4 – 13.0)	13.1 (10.6 – 14.5)
PLT ($x10^9$cells/l)	115.0 (58.0 – 149.0)	78.0 (48.5 – 117.5)	70.0 (37.0 – 103.5)

Coagulation test and parenchymal findings

The median PT values for cases with gliosis was 16.8seconds, 15.8seconds for cases with infarcts and 16.6 seconds for cases with haemorrhage. The median APPT values for cases with gliosis was 36.0 seconds, 27.4 seconds for cases with infarcts and 33.8 seconds for cases with haemorrhage. The correlation of haemorrhage with prothrombin time and APPT was not statistically significant.

Table 19: Coagulation test and parenchymal findings

	Gliosis	**Infarcts**	**Haemorrhage**
	n=11	***n=2***	***n=8***
PT, (seconds)	16.8 (15.4 – 21.0)	15.8 (14.8 – 16.8)	16.6 (16.0 – 19.0)
APPT, (seconds)	36.0 (29.1 – 38.1)	27.4 (24.7 – 30.0)	33.8 (29.7 – 42.6)

Liver function test and parenchymal findings

The median total bilirubin level for all cases was elevated above the upper limit of the reference range, the transaminases were elevated while the albumin levels were low.

Table 20: LFTs and parenchymal findings

	Gliosis	**Infarcts**	**Haemorrhage**
	n=14	***n=5***	***n=12***
T.BIL (umol/l)	33.2 (9.2 – 116.3)	29.8 (21.9 – 73.7)	58.7 (27.2 – 96.5)
D.BIL (umol/l)	21.0 (3.6 – 45.5)	21.3 (15.1 – 41.3)	24.6 (15.2 – 47.3)
ALT (U/L)	157.5 (51.0 – 802.0)	56.0 (54.0 – 116.0)	316.5 (125.0 – 465.0)
AST (U/L)	138.0 (56.0 – 792.0)	71.0 (35.0 – 121.0)	329.0 (45.5 – 846.5)
Albumin (g/L)	31.5 (27.0 – 35.0)	33.0 (21.0 – 38.0)	28.5 (24.0 – 33.0)

Estimated glomerular filtration rate

The median EGFR for cases with gliosis was 58.9, 24.7 for cases with infarcts and 53.8 for cases with haemorrhage.

Table 21: EGFR and parenchymal findings

	Gliosis	**Infarcts**	**Haemorrhage**
EGFR	***n=14***	***n=5***	***n=12***
	58.9 (24.0 – 78.7)	24.7 (21.4 – 61.5)	53.8 (21.7 – 64.6)

Additional findings

There were additional macrovascular findings of septic thrombi in 2 cases and lymphocytic meningeal infiltrate in 1 case. Additional microvascular changes included haemosiderin laden macrophages in Virchow Robbins space in 3 cases, perivascular haemorrhage in 5 cases, dystrophic calcification in 1 case and perivascular oedema in 3 cases.

Table 22: Additional findings

Macrovascular	**Frequency (n=3)**	**Percent of cases**
Septic thrombi	2	66.7
Lymphocytic meningeal infiltrate	1	33.3
Microvascular	**Frequency (n=10)**	**Percent of cases**
Haemosiderin laden macrophages in the Virchow Robbins space	3	30.0
Perivascular haemorrhage	5	50.0
Dystrophic calcification of mural surface vessels	1	10.0
Perivascular oedema	3	30.0

Histology Photomicrographs

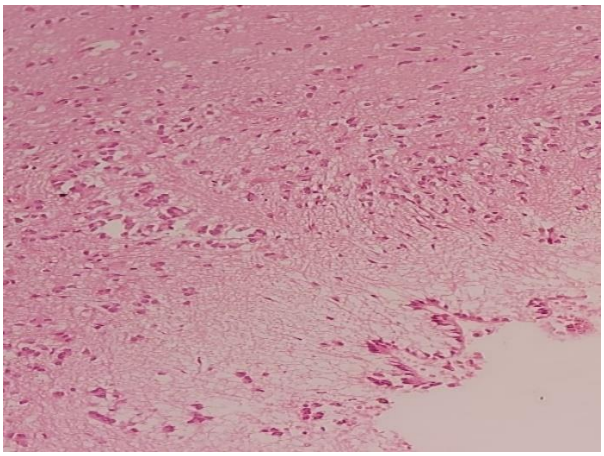


Figure 12:Subependymal oedema (x10 magnification)

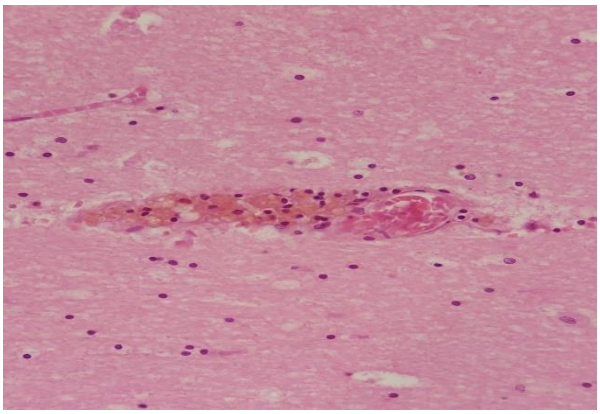


Figure 13: perivascular haemorrhage (x 40magnification)

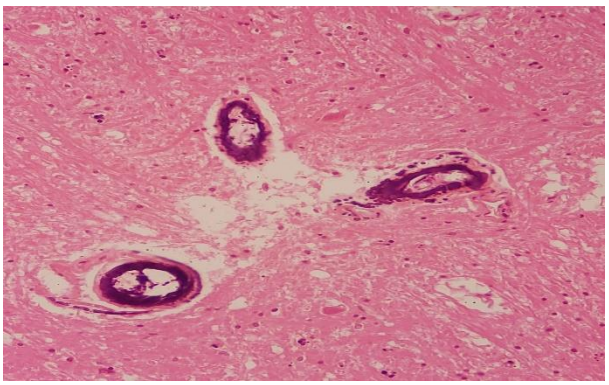


Figure 14:arteriosclerosis with dystrophic calcification (X40magnification)

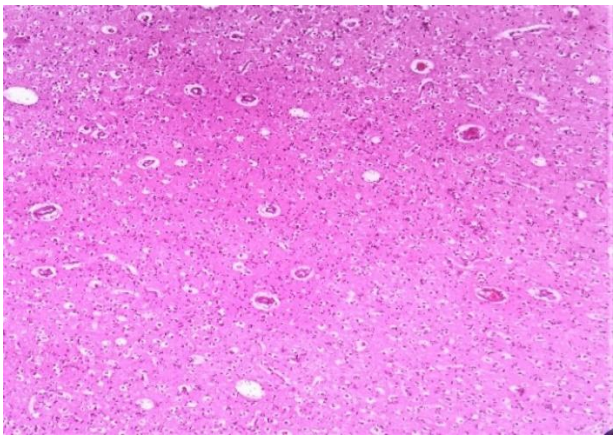


Figure 15:Gliosis (x10 magnification)

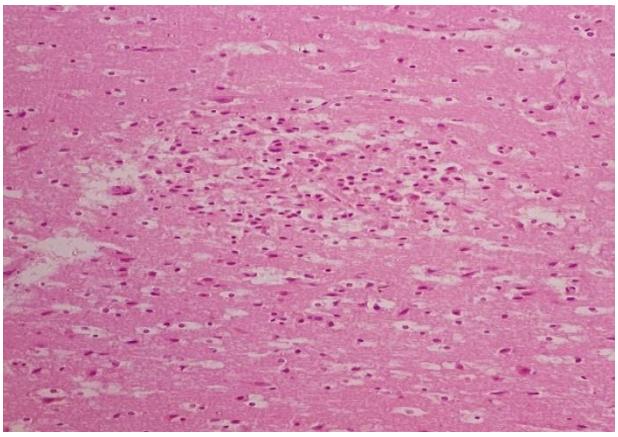


Figure 16:Old infarcts healing with glial nodules (x40 magnification)

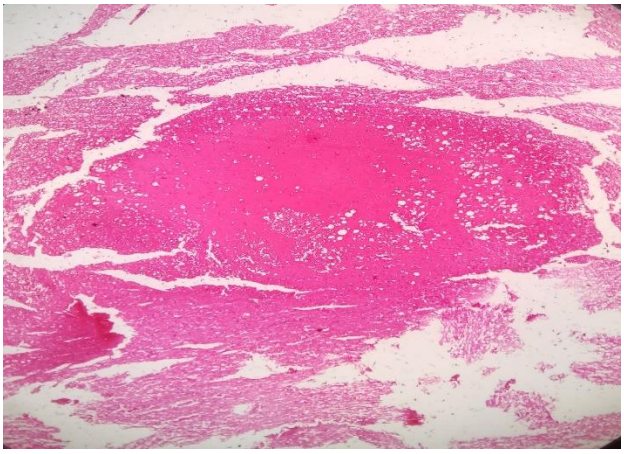


Figure 17:Pontine haemorrhage (x10 Magnification)

CHAPTER 5: DISCUSSION

Antemortem clinical and laboratory data

This autopsy study included 23 deceased persons, out of whom 18 had eclampsia and 4 had preeclampsia with severe features. During the study period, there were 77 maternal mortalities and Preeclampsia/Eclampsia accounted for about 35% of the mortalities. All the cases were of African descent, 8 of the 23 cases were nulliparous, 5 had history of preeclampsia in previous pregnancies, had multiple pregnancies and 1 was a chronic hypertensive. These findings are consistent with the known risk factors for preeclampsia/eclampsia (25).

Neurological symptoms were found in 91.3% of the cases ranging from convulsions in 18(78.3%) cases, altered mental status in 7(30.4%) cases, headaches in 6(26.1%), and blurred vision in 2(8.7%) of the cases. This symptomatology and neuroimaging findings of cortical oedema are consistent with the PRES syndrome (26). There were no neuroimaging findings documented for the study as no decedent had imaging done. However, cerebral oedema was observed on histology in 100% of the cases thus it can be inferred that PRES syndrome was prevalent.

Out of the 21 cases with platelet count results, 18 had thrombocytopenia of whom, 7 had thrombocytopenia of less than 50,000cell/µl. The low platelet count in HELLP syndrome is due to increased consumption. The platelets are activated, and adhere to damaged vascular endothelial cells, resulting in increased platelet turnover with shorter lifespan. Low platelets in the antemortem and postpartum period may be also seen in HUS and TTP (26). Severe thrombocytopenia is a risk factor for intracranial bleeds (16). Five of the 11 decedents who had ICH presented with severe thrombocytopenia.

Elevated transaminases were observed in 20 out of the 23 cases and 21 cases having elevated bilirubin levels which indicated ongoing hemolysis. These findings denote a concomitant HELLP syndrome which is characterized by low platelets less than 100,000cell/µl as seen in 14 of the cases, haemolysis, and elevated liver enzymes (28).

Parenchymal findings

Features of raise intracranial pressure was identified in 40% of the cases. The raised ICP was due to haemorrhage and/or cerebral oedema. Durets haemorrhages were identified in 4 cases, 3 of which had concomitant subarachnoid haemorrhages with all the cases having cerebral oedema. Durets haemorrhages are attributable to descending transtentorial herniation and have a poor prognosis (29).

There were 11 cases from the study with intracranial haemorrhages including subarachnoid, pontine, intraventricular, intracerebral haemorrhages and 1 case with a subdural haemorrhage. All the decedents who presented with ICH had an antemortem diagnosis of eclampsia.

Intracranial haemorrhage or stroke is the most common primary cause of death (26.4%) and the most common contributing factor to death (45%) in patients with preeclampsia/eclampsia (30).

The causes of the intracranial haemorrhage are multifactorial with disturbances of cerebral blood flow auto-regulation and subsequent cerebral hyperperfusion leading to vasodilation and brain edema; endothelial dysfunction, increased cerebral perfusion pressure and brain capillary permeability; microangiopathy, and vasospasm of brain vessels and thrombocytopenia as a predisposing factors (31).

Intracerebral haemorrhage occurs in 9 per 100,000 deliveries. Preeclampsia/ eclampsia accounts for 25–45% of those cases (32). The study identified 8 cases (34.8%) with intracerebral bleeds,

out of which 5 were identified grossly and 3 microscopically. Up to 40% of patients with eclampsia have been found to have intracerebral bleeds in autopsy series (33).

Pregnancy associated subdural hemorrhage is usually due to trauma or as a complication of epidural anesthesia during labor. There are reported cases of spontaneous subdural hematoma associated with preeclampsia (34). In this study, there was one case with subdural haemorrhage the cause of which was attributed to trauma due to presence of concomitant subgaleal haemorrhage.

Perivascular haemorrhage was found in 22.7% of the cases studied. This prevalence was lower than that found in an autopsy study done in Mozambique at 36.4% (20). The higher prevalence in the Mozambique study could be due to use of IHC in identification endothelial damage and intracerebral bleeds. Loss of cerebral autoregulation leads to cerebral vasculature wall permeation with plasma and undergoing fibrinoid necrosis. Extensive necrosis leads to perivascular haemorrhage (19).

Hypoxic-ischaemic brain damage was demonstrated histologically as neuronal changes in all the cases. The neuronal changes were pronounced in the hippocampal region of the temporal lobes and in the cerebellum. Neurons are sensitive to HI due to their vulnerability to excitotoxicity and high metabolic needs. The most sensitive neurons are the neurons of the hippocampal CA1 regions, the long-projection large neurons of the third and fifth neocortical layers, and the Purkinje neurons of the cerebellum (35).

There was gross necrosis in one of the cases identified as an area of softening despite adequate brain fixation. This is indicative of ischaemic infarction in which there's liquefactive necrosis. There were also old infarcts which had healed with gliosis. This could imply an underlying chronic hypertension on which the preeclampsia was superimposed.

Cerebral oedema was identified in all the cases studied ranging from focal to widespread diffuse oedema. This compares to that seen in MR imaging studies on 27 women which showed a prevalence of 93% (36). Cerebral oedema is both cytotoxic caused by ischemia and vasogenic edema due to disruption of the blood brain barrier associated with sudden or severe hypertension (23). Significant oedema can cause brain injury and precipitate seizures.

Vascular findings

Cerebral arteriosclerosis was identified in 8 cases with one of the cases having dystrophic calcification of the vasculature. This was done by examination of the internal carotid, middle meningeal, vertebral, basilar and vasculature within the brain parenchyma. The dystrophic calcification was in the intraparenchymal vasculature and was identified in a patient with preeclampsia superimposed on chronic hypertension.

Hypertension promotes formation of atherosclerotic plaques in cerebral arteries and arterioles, which may lead to arterial occlusions and ischemic injury and induces adaptive changes in cerebral arteries including hypertrophic remodeling of smooth muscle cells thus increasing the wall thickness. Hypertension also leads to vascular stiffening, a process that increases collagen content and rigidity of the vessel wall. These changes contribute to cerebral vascular dysregulation (37).

CONCLUSION

1. The common histological parenchymal findings include neural changes due to hypoxic ischemia, cerebral oedema, haemorrhage, and old infarcts healing with gliosis.
2. The vascular changes identified were arteriosclerosis, thrombotic microangiopathy and vascular congestion.

3. The laboratory parameters including LFTs, APPT and PT, and platelet count for majority of the decedents were deranged denoting a concurrent HELLP syndrome. The relation of these lab parameters and the histology findings was not found to be statistically significant.

RECOMMENDATION

1. Initial radiologic examination of severe cases should be done to exclude intracranial haemorrhage and assist in choosing the right management option.
2. There should be emphasize on the management of cerebral oedema to prevent further brain injury.
3. Further studies with use of immunohistochemistry to identify more lesions and studies to further correlate the histology findings with the clinical and laboratory findings should be done.

LIMITATION

The cases with microvascular thrombosis may have been missed in this study. There was widespread vascular congestion within the microvasculature hence the difficulty in identifying the microthrombi. This can be mitigated by use of special stain such as the fibrin stains in future studies.

Correlation of antemortem radiological findings and autopsy findings was not done as the cases had no imaging studies done.

Data collection tool

PROFOMA SHEET **SERIAL NUMBER**

Date of Autopsy

Study number

Autopsy number

IP number

Name of next of kin and contact information

Decedents details

Age _____ Date of birth_____

Weight _____

Parity _____

Gestation age _____

Single _____

Multiple gestation _____

Clinical information

Complaint: state _____Duration in days_____

Antemortem presenting symptoms _____

Time of onset of symptoms: 2nd trimester _____ 3rd trimester_____

History of:

Hypertension Yes _____ No_____ Unknown _____

Previous preeclampsia Yes _____ No_____ Unknown_____

Preeclampsia in family Yes_____ No_____ Unknown _____

Laboratory findings

Haemoglobin level _____

Platelet count _____

Liver function test _____

U/E/Cs _____

LIST OF REFERENCES

1. Duley L. The Global Impact of Pre-eclampsia and Eclampsia. Semin Perinatol [Internet]. 2009;33(3):130–7. Available from: http://dx.doi.org/10.1053/j.semperi.2009.02.010
2. Vousden N, Lawley E, Seed PT, Gidiri MF, Goudar S, Sandall J, et al. Incidence of eclampsia and related complications across 10 low-and middlere source geographical regions: Secondary analysis of a cluster randomised controlled trial. PLoS Med. 2019;16(3):1–15.
3. Gathiram P, Moodley J. Pre-eclampsia: Its pathogenesis and pathophysiolgy. Cardiovasc J Afr. 2016;27(2):71–8.
4. Zeeman GG. Neurologic Complications of Pre-eclampsia. Semin Perinatol [Internet]. 2009;33(3):166–72. Available from: http://dx.doi.org/10.1053/j.semperi.2009.02.003
5. Hypertension G. Clinical Management Guidelines for Obstetrician – Gynecologists Gestational Hypertension and. 2019;133(1):1–25.
6. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi J. and management. 2011;467–74.
7. Ghulmiyyah L, Sibai B. Maternal Mortality From Preeclampsia/Eclampsia. YSPER [Internet].2012;36(1):56–9.Availablefromhttp://dx.doi.org/10.1053/j.semperi.2011.09.011
8. Brown MA, Magee LA, Kenny LC, Karumanchi SA, Mccarthy FP, Saito S, et al. The hypertensive disorders of pregnancy : ISSHP classi fi cation , diagnosis & management recommendations for international practice. 2018;(xxxx).
9. Ndwiga C, Osoti A, Pooja S, Odwe G, Ogutu O, Charlotte W. Retrospective cohort study : clinical presentation and outcomes of pre- eclampsia and eclampsia at kenyatta national hospital , nairobi , kenya ending eclampsia study report. 2018;(january):1–28.
10. Health N. Pre-eclampsia , eclampsia and adverse maternal and perinatal outcomes : a

secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. 2014;14–24.

11. T SS. MATERNAL AND PERINATAL OUTCOME IN PATIENTS WITH ECLAMPSIA AT KENYATTA NATIONAL HOSPITAL.
12. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of Preeclampsia. Annu Rev Pathol Mech Dis. 2010;5(1):173–92.
13. Tomimatsu T, Mimura K, Matsuzaki S, Endo M, Kumasawa K, Kimura T. Preeclampsia: Maternal systemic vascular disorder caused by generalized endothelial dysfunction due to placental antiangiogenic factors. Int J Mol Sci. 2019;20(17):1–18.
14. Maynard SE, Karumanchi SA. Angiogenic Factors and Preeclampsia. Semin Nephrol [Internet]. 2011;31(1):33–46. Available from: http://dx.doi.org/10.1016/j.semnephrol.2010.10.004
15. Cipolla MJ, Kraig RP. Seizures in women with preeclampsia: Mechanisms and management. Fetal Matern Med Rev. 2011;22(2):91–108.
16. Hammer ES, Cipolla MJ. Cerebrovascular Dysfunction in Preeclamptic Pregnancies. Curr Hypertens Rep. 2015;17(8):1–13.
17. Brussé I, Duvekot J, Jongerling J, Steegers E, De Koning I. Impaired maternal cognitive functioning after pregnancies complicated by severe pre-eclampsia: A pilot case-control study. Acta Obstet Gynecol Scand. 2008;87(4):408–12.
18. Schwartz RB, Feske SK, Polak JF, DeGirolami U, Iaia A, Beckner KM, et al. Preeclampsia-eclampsia: Clinical and neuroradiographic correlates and insights into the pathogenesis of hypertensive encephalopathy. Radiology. 2000;217(2):371–6.
19. Richards A, Graham D, Bullock R. Clinicopathological study ofneurological

complications due to hypertensive disorders of pregnancy. 1988;(table 1):416–21.

20. Hecht JL, Ordi J, Carrilho C, Ismail MR, Zsengeller ZK, Karumanchi SA, et al. The pathology of eclampsia: An autopsy series. Hypertens Pregnancy. 2017;36(3):259–68.

21. Vigil-De Gracia P. Maternal deaths due to eclampsia and HELLP syndrome. Int J Gynecol Obstet [Internet]. 2009;104(2):90–4. Available from: http://dx.doi.org/10.1016/j.ijgo.2008.09.014

22. Martin JN, Thigpen BD, Moore RC, Rose CH, Cushman J, May W. Stroke and severe preeclampsia and eclampsia: A paradigm shift focusing on systolic blood pressure. Obstet Gynecol. 2005;105(2):246–54.

23. Cunningham FG, Twickler D. Cerebral edema complicating eclampsia. Am J Obstet Gynecol [Internet]. 2000;182(1 I):94–100. Available from: http://dx.doi.org/10.1016/S0002-9378(00)70496-0

24. Florkowski CM, Chew-Harris JSC. Methods of estimating GFR - Different equations including CKD-EPI. Clin Biochem Rev. 2011;32(2):75–9.

25. Vousden N, Lawley E, Seed PT, Gidiri MF, Goudar S, Sandall J, et al. Incidence of eclampsia and related complications across 10 low-and middlere source geographical regions: Secondary analysis of a cluster randomised controlled trial. PLoS Med. 2019;16(3):1–15.

26. Fischer M, Schmutzhard E. Posterior reversible encephalopathy syndrome. J Neurol. 2017;264(8):1608–16.

27. Haram K, Svendsen E, Abildgaard U. BMC Pregnancy and Childbirth The HELLP syndrome : Clinical issues and management . A Review. 2009;15:1–15.

28. Rimaitis K, Grauslyte L, Zavackiene A, Baliuliene V. Diagnosis of HELLP Syndrome : A

10-Year Survey in a Perinatology Centre. 2019;(Table 1):1–9.

29. Parizel PM, Makkat S, Jorens PG, Ozsarlak O, Cras P, Van Goethem JW, et al. Brainstem hemorrhage in descending transtentorial herniation (Duret hemorrhage). Intensive Care Med. 2002;28(1):85–8.

30. Zeidman LA, Videnovic A, Bernstein LP, Pellar CA. Lethal pontine hemorrhage in postpartum syndrome of hemolysis, elevated liver enzyme levels, and low platelet count. Arch Neurol. 2005;62(7):1150–3.

31. Lin L Te, Tsui KH, Cheng JT, Cheng JS, Huang WC, Liou WS, et al. Increased Risk of Intracranial Hemorrhage in Patients with Pregnancy-Induced Hypertension. Med (United States). 2016;95(20):1–7.

32. Kutlesic M, Mostic-Ilic T, Ilic D, Kutlesic R. A case of eclampsia complicated by cerebral haemorrhage and iatrogenic hypopharyngeal oedema and haemathoma. Vojnosanit Pregl. 2017;74(9):884–90.

33. Dubey SR. Intracranial haemorrhage in pregnancy. Int J Nurs Educ Res. 2020;8(4):559–63.

34. Oudghiri N, Behat M, Elchhab N, Doumiri M, Tazi AS aou. Spontaneous subdural hematoma associated with preeclampsia: a case report and litterature review. Pan Afr Med J. 2014;19:213.

35. Rahaman P, Bigio MR Del. Histology of Brain Trauma and Hypoxia-Ischemia. 2018;(May):539–54.

36. Gg Z, Jl F, Dm T, Fg C, Gynecol AJO. Cerebral infarction in eclampsia. 2004;89–102.

37. Iadecola C, Davisson RL. Hypertension and Cerebrovascular Dysfunction. Cell Metab. 2008;7(6):476–84.

Appendix I: Autopsy procedures

Autopsy, standard techniques, gross pathology, histopathology sections

Autopsy Number: _____

Study Number: _____

Date of Autopsy: _____

Time of Autopsy: _____

Clinical Diagnosis: _____

Autopsy Diagnosis: _____

Principal investigator: _____

Informed consent

This will be obtained from close relatives by the principle investigator. Include permission to retain organs.

Identification

1. Matching of the identification details on tags appended on the deceased with available documentation.

2. Identification by the next of kin of the deceased in life.

External examination

Bi-coronal incision of the scalp, excision of the calvarium with evisceration of the brain

Central Nervous System: Eviscerated brain is weighed, the circle of Willis, examined, and then fixed in 10% buffered formalin for 2 weeks prior to dissection.

Description of brain features

The **brain** weight _____ g. The leptomeninges are *thin and transparent with no vascular congestion, subarachnoid hemorrhage, or exudate/other.* The circle of Willis and other basal

vasculature is *intact and normally formed/other*. The vessels are *intact, patent, and thin walled/other*. The cranial nerves are *intact and normally distributed/other*. The dorsal convexities of the brain are *symmetrical with a well-developed gyral pattern/other*. The brainstem and cerebellum *show the usual/other external configuration*. There is *no localized external softening or contusion of the brain/other*. There is no displacement of the *cingulate gyrus, medial temporal lobe, or cerebellar tonsils/other*.

Multiple coronal sections of the cerebrum show *an intact cortical ribbon of appropriate thickness/other.* The internal architecture shows *the usual pattern/other without focal lesions or hemorrhage/other.* The ventricular system is of appropriate *configuration and size/other.* The transverse sections of the brainstem show *a well pigmented substantia nigra and locus caeruleus/other.* The pons shows *well-defined pyramids and inferior olivary nuclei/other.* Sections of the cerebellum *show prominent inferior olivary nuclei/other,* the hemispheres show *the usual foliar pattern and appearance of the dentate nuclei/other.*

Specimens for histopathology

1. Parasagittal frontal lobe from anterior horn of lateral ventricle to midline apex (sampling corpus callosum, lateral ventricular wall, cingulated gyrus, indusium griseum, Parasagital neocortex and Centrum semiovale)
2. Temporal lobe including hippocampus at level of the lateral geniculate body (samples hippocampus, transitional allocortex, temporal neocortex, lateral geniculate body, temporal horn wall, choroid plexus and tail of the caudate nucleus.
3. Midline mammilary bodies through the insular cortex (samples hypothalamus, anterior thalamus, third ventricular wall, internal capsule, optic tract, globus pallidum, putamen, claustrum, insular cortex an both external and extreme capsules)

4. Midbrain (samples crux cerebri, substantia nigra, aqueduct of Sylvius, red nucleus and decussation of the brachium conjuctivum)
5. Pons at the level of fifth nerve exit (samples pontine tegmentum, floor of fourth ventricle, trapezoid body [ascending sensory pathways] pyramidal tract and cerebellar afferent nuclei and tracts)
6. Medulla Oblongata (samples pyramidal tracts, inferior olivary nuclei, medial lemniscus, various cranial nerve nuclei, medial longitudinal fasciculus, choroid plexus, floor of the fourth ventricle and inferior cerebellar peduncle)
7. Cerebellum (samples vermis and neo cerebellar cortex, white matter and dentate nucleus)
8. Any additional sections (specify)_____.

Appendix II: Staining SOPs

Harris Haematoxylin and Eosin Staining

Principle

The involves application of hemalum, a complex formed from aluminum ions and oxidized hematoxylin. This colours nuclei of cells blue. Counterstaining is with an aqueous or alcoholic solution of eosin Y, which colors eosinophilic other structures in various shades of red, pink, and orange.

Procedure

- Deparaffinize the sections by dipping them in xylene 3 times for 3 minutes each.
- The sections are then rehydrated in decreasing concentration of alcohol (100%, 95%, 80%, 70%) and water. Blot excess water from slide holder before going into hematoxylin.
- Hematoxylin staining procedure:

1. Stain in Harris Haematoxylin for 5 minutes

2. Rinse with running tap water for 1 minute
3. Dip in 1 % acid alcohol (1%HCL in 70% alcohol) for 3-5 minutes
4. Wash with running tap water for 1 minute
5. Counter stain in eosin Y for 5 minutes
6. Rinse with running tap water for 1 minute
7. Dehydrate with increasing alcohol concentrations (70%, 80%, 100%)
8. Clear with 3 changes of xylene.
9. Mount in DPX
10. Observe under a microscope

Results

Cytoplasm: pink

Nuclei: blue

Fibrin: deep pink

RBCS: orange red

Quality control

Haematoxylin will be filtered daily and changed after 3 weeks

Eosin is changed after 6 days

Alcohols and xylene changed if they are cloudy

MASSON'S TRICHROME STAINING PROTOCOL

Principle: This method is used for the detection of collagen fibers in tissues on formalin-fixed, paraffin-embedded sections, and may be used for frozen sections as well. The collagen fibers will be stained blue and the nuclei will be stained black and the background is stained red.

Procedure

Fixation: 10% formalin or Bouin's solution

Section: paraffin sections at 5 um.

1. Deparaffinize and rehydrate through 100% alcohol, 95% alcohol 70% alcohol.
2. Wash in distilled water.
3. For Formalin fixed tissue, re-fix in Bouin's solution for 1 hour at 56 C to improve staining quality.
4. Rinse running tap water for 5-10 minutes to remove the yellow color.
5. Stain in Weigert's iron hematoxylin working solution for 10 minutes.
6. Rinse in running warm tap water for 10 minutes.
7. Wash in distilled water.
8. Stain in Biebrich scarlet-acid fuchsin solution for 10-15 minutes. Solution can be saved for future use.
9. Wash in distilled water.
10. Differentiate in phosphomolybdic-phosphotungstic acid solution for 10-15 minutes or until collagen is not red.
11. Transfer sections directly (without rinse) to aniline blue solution and stain for 5-10 minutes. Rinse briefly in distilled water and differentiate in 1% acetic acid solution for 2-5 minutes.
12. Wash in distilled water.

13. Dehydrate very quickly through 95% ethyl alcohol, absolute ethyl alcohol (this step will wipe off Biebrich scarlet-acid fuchsin staining) and clear in xylene.

14. Mount with resinous mounting medium.

Results:

Collagen: Blue

Nuclei: Black

Muscle, cytoplasm, keratin: Red

Appendix III: Consent explanation and form

Study title: Autopsy brain findings in preeclampsia and eclampsia at Kenyatta National Hospital

Principle investigator: Dr. Waithera Mbau

Introduction

My name is Dr. Waithera Mbau, a postgraduate student studying Human pathology at the University of Nairobi. I will conducting a study on persons who have died from preeclampsia and eclampsia and examining the brain findings with the aim of identifying the neurological complications which will inform on prevention and management of the same.

Purpose of the study

The purpose of this study is to examine the brain changes occurring in women who have died from hypertension in pregnancy.

Type of research intervention

This study involves examination of the brain tissue. The brain of the person who has died will be removed and suspended in formalin for two weeks. It will then be sliced into small sections, and tissue samples taken to the histology laboratory for processing.

Risks

There are no risks expected in this study. The autopsy method used ensures there is very minimal disfiguration of the body and after the autopsy, the body will be treated with formalin to prevent decomposition and thereafter sutured while maintaining aesthetics.

Voluntary participation

Your participation in this study is voluntary and you have a right to accept or decline. You can withdraw from the study at any time even after consenting. Should you decline to participate in the study, this will not affect the services rendered to you or the deceased in this facility.

Right of the investigator to withdraw you from the study

The investigator has a right to withdraw you from the study without your approval.

This will occur in situations where the investigator will not find adequate clinical information such as laboratory results in the medical records.

Cost and compensation

There will no cost incurred or compensation for participating in the study.

Use of study material for further studies.

Based on the findings of the study, the brain tissues samples collected may be used for further studies. You have a right to agree or decline to this. Should you agree, the brain tissue samples will be archived for future use.

Contact persons

If you have questions, complaints, or concerns about this study, you can contact the principal investigator from University of Nairobi, School of Medicine, Department of Human Pathology, Postgraduate program: Dr. Waithera Mbau +254725159885 Email: waitherambau@gmail.com or my Supervisor Dr. Edwin Walong +254738509623 or you can contact the KNH-UoN ERC at uonknh_erc@uonbi.ac.ke.

Thank you for your participation in this research and your help is greatly appreciated.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions and the answers given to my satisfaction. I voluntarily agree to take part in this study.

Name of participant …….....

Signature/Mark……. …… Date.…….…….

Signature of investigator ……. Date…….…….

Consent to future used of the tissue sample for future studies.

By signing this, I voluntarily agree to the use of the tissue sample obtained during the autopsy for future studies.

Name of participant …….....

Signature/Mark……. …… Date.…….…….

Signature of investigator ……. Date…….…….

Kichwa cha somo: Matokeo ya ubongo wa Autopsy katika preeclampsia na eclampsia huko KNH

Upelelezi wa kanuni: Dk Waithera Mbau

Utangulizi

Jina langu ni Dk Waithera Mbau, mwanafunzi wa uzamili anayesomea ugonjwa wa Binadamu katika Chuo Kikuu cha Nairobi. Nitafanya uchunguzi juu ya watu ambao wamekufa kutokana na ugonjwa wa preeclampsia na eclampsia na kuchunguza matokeo ya ubongo kwa lengo la kubaini matatizo ya neva ambayo yataarifu juu ya kuzuia na usimamizi wa huo.

Kusudi la utafiti

Kusudi la utafiti huu ni kuchunguza mabadiliko ya ubongo yanayotokea kwa wanawake ambao wamekufa kutokana na preeclampsia na eclampsia.

Aina ya uingiliaji wa utafiti

Utafiti huu unajumuisha uchunguzi wa tishu za ubongo. Ubongo wa mtu aliyeamua utafutwa na kutengenezwa kwa formalin kwa wiki mbili. Kisha itagawanywa, na sampuli za tishu zinachukuliwa kwa maabara ya histology kwa usindikaji.

Hatari

Hakuna hatari zinazotarajiwa katika utafiti huu. Njia ya uchunguzi wa mwili inayotumiwa inahakikisha kuna mabadiliko kidogo sana ya mwili na baada ya uchunguzi, mwili utatibiwa na formalin ili kuzuia kuoza na baadaye kushonwa wakati wa kudumisha uzuri.

Ushiriki wa hiari

Ushiriki wako katika utafiti huu ni wa hiari na una haki ya kukubali au kukataa. Ukikataa kushiriki katika utafiti, hii haitaathiri huduma zinazotolewa kwako au kwa marehemu.

Haki ya mpelelezi kukuondoa kwenye utafiti

Mpelelezi ana haki ya kukuondoa kwenye masomo bila idhini yako. Hii itatokea katika hali ambazo mchunguzi hatapata habari za kliniki za kutosha kama vile matokeo ya maabara kwenye rekodi za matibabu.

Gharama na fidia

Hakuna gharama inayopatikana au fidia ya kushiriki katika utafiti.

Matumizi ya nyenzo za kujifunzia kwa masomo zaidi.

Kulingana na matokeo ya utafiti, sampuli za tishu zilizokusanywa zinaweza kutumiwa kwa masomo zaidi. Una haki ya kukubali au kukataa hii. Ukikubali, sampuli za tishu zitahifadhiwa kwa matumizi ya baadaye

Mawasiliano

Ikiwa una maswali, malalamiko, au wasiwasi juu ya utafiti huu, unaweza kuwasiliana na mpelelezi mkuu kutoka Chuo Kikuu cha Nairobi, Shule ya Tiba, Idara ya Patholojia ya Binadamu, mpango wa Uzamili: Dk Waithera Mbau +254725159885 Barua pepe: waitherambau@gmail.com au Msimamizi wangu Dk. Edwin Walong +254738509623 au unaweza kuwasiliana na KNH-UoN ERC kwa uonknh_erc@uonbi.ac.ke.

Asante kwa ushiriki wako katika utafiti huu na msaada wako unathaminiwa sana.

IDHINI

Kwa kusaini fomu hii ya idhini, ninathibitisha kuwa nimesoma habari katika fomu hii ya idhini na nimepata nafasi ya kuuliza maswali na majibu yaliyopewa kuridhika kwangu. Ninakubali kushiriki kwa hiari kushiriki katika utafiti huu.

Jina la mshiriki …… .....

Saini / Alama ……. …… Tarehe.…….…….

Saini ya mchunguzi ……. Tarehe…….…….

Idhini ya matumizi ya baadaye ya sampuli ya tishu kwa masomo ya baadaye.

Kwa kusaini hii, ninakubali kwa hiari utumiaji wa sampuli ya tishu iliyopatikana wakati wa uchunguzi wa mwili kwa masomo ya baadaye.

Jina la mshiriki …… .....

Saini / Alama ……. …… Tarehe.…….…….

Saini ya mchunguzi ……. Tarehe…….…….