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[Risk factors for pre-eclampsia in low and middle-income countries, a case study of Tanzania]

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Declaration

I hereby declare that I have composed this thesis; the work on this thesis is my own. In the acknowledgement section, I have stated the contributions made by my supervisors and other colleagues. I also declare I have not submitted this work for any other degree or professional qualification.

Signature:

Date: 27th May,2020.

Abstract

This PhD research focuses on understanding a maternal pregnancy condition known as pre-eclampsia. This condition contributes to around 14 % of the global burden of maternal mortality and fivefold of perinatal mortality in developing countries. Genetic, environmental, nutritional and socioeconomic factors are thought to disproportionately affect the burden of pre-eclampsia in low and middle-income countries (LMIC).

The first chapter gives an overview; it introduces pre-eclampsia and outlines its contribution to the burden of maternal and infant mortality and morbidity. It summarises all chapters in this thesis.

The second chapter gives the background of the literature review. This chapter describes pre-eclampsia in the context of hypertensive disorders of pregnancy. It then explains some of the risk factors of pre-eclampsia, the natural history of the disease and the health system response: current modalities in screening, the evolution of the definition pre-eclampsia, diagnosis, prevention and management.

The third chapter describes the scoping study, which aims at summarising explored risk factors in Africa, to identify potential gaps and the feasibility of conducting a systematic review. Its results showed that there were twenty studies done in Africa that had explored relatively few risk factors. These studies had methodological limitations of size and rigour, hence produced conflicting and inconclusive associations between pre-eclampsia outcome and most explored risk factors, including malaria infection. They also showed a gap in the literature regarding models built by risk factors that attempted to classify pre-eclampsia outcome in African populations.

The fourth chapter describes the systematic review that explores the relationship between malaria infection and gestational hypertension (GH) with proteinuria (pre-eclampsia) or without. It considers two pathways that malaria possibly exert its effect on causing gestational hypertension. One pathway being through a dysfunctional placenta and the other pathway is by

endothelial inflammation of blood vessels from malaria toxins. The result of the meta-analysis was a pooled odds ratio (OR) of 2.6, 95 % confidence interval (CI) 1.5 to 4.5. The odds of pre-eclampsia among pregnant women with malaria infection were 2.6 times than pregnant women without malaria infection.

The fifth chapter describes the analysis of secondary data from Tanzania. This data analysis has four objectives. The sixth chapter describes the results of the four objectives, while chapter Seven presents the discussions of the four objectives. This data analysis used data from two sources: a hospital maternity register from northern Tanzania and a clinical trial in Dar es Salaam, Tanzania. The data analysis had four objectives; objective one determined the incidence pre-eclampsia to be 1.9 %, 95 % CI 1.3 % to 2.2 % in Dar es Salaam city, Tanzania. Objective two describes the sociodemographic characteristics of women with pre-eclampsia compared with those without pre-eclampsia in northern Tanzania population. Pre-eclampsia was more frequent among women with age above 35 years, single and tertiary level education.

Objective three aimed to identify biomedical risk factors for pre-eclampsia among women in northern Tanzania. Then, I used these identified risk factors to build prediction models for pre-eclampsia. I later assessed the ability of these models to classify women with and without pre-eclampsia. Maternal age, weight before pregnancy, contraceptive intrauterine device (IUD) use, a diagnosis of malaria, diagnosis of infections, history of hypertension and HIV treatment were statistically significant predictors in some of my models. My final models in predicting pre-eclampsia in all deliveries, term and preterm delivery subgroups produced an area under the curve of 69.4 %, 71.2 % and 66.9 % respectively. The points of maximum sensitivity and specificity produced sensitivity values of 65 %, 65 % and 59 % respectively, while also producing specificity values of 63 %, 65 % and 66 % respectively. The risk factors and the prediction models were developed on a hospital-based register where the incidence of pre-eclampsia was 3.5%. Since hospital

estimates tend to overestimate the incidence compared to population survey, the results of my prediction models will differ in women populations with a high risk of pre-eclampsia.

The fourth objective describes the pregnancy outcome of women with pre-eclampsia compared to women without pre-eclampsia. The results showed there were more stillbirths among women with pre-eclampsia. The odds of stillbirth were 4.8 (95 % CI 3.7 – 6.3) times among women with pre-eclampsia than women without pre-eclampsia in all deliveries. Upon stratifying by term and preterm deliveries the odds were 2.6 (95 % CI 1.6 – 4.3) and 2.9 (95 % CI 2.0 – 4.1) respectively. The surviving offspring have worse developmental indicators compared to their counterparts in terms of low a Apgar score at 1-minute, low birth weight, small head circumference and short birth length. The odds of a low Apgar score (0 – 3) baby was 4.3 times (95 % CI 3.4 - 5.4) among pre-eclampsia women in all deliveries compared to normal Apgar score (4 – 10) babies. The association was maintained in the subgroups of term and preterm delivery (OR = 2.3, 95 % CI 1.5 – 3.5 and 2.8, 95 % CI 2.0 - 3.9 respectively).

Chapter Eight covers the conclusion and recommendations of this thesis, which are: (i) Few studies that explored risk factors in Africa, more studies are needed to resolve conflicting and inconclusive findings from these studies. (ii) Malaria infection is associated with pre-eclampsia and gestational hypertension in malaria endemic regions. Malaria control should be intensified among pregnant women and further studies should explore the causal mechanisms. (iii) The incidence of pre-eclampsia should be tracked to observe changes in its trends in the evolving urban populations of LMIC. (iv) Affordable and feasible prediction models for pre-eclampsia should be developed and assessed for performance to enable identification and provision of prevention services on women with a high risk of developing pre-eclampsia. (v) Women with pre-eclampsia should receive appropriate treatment to mitigate the negative impact on their pregnancy outcome.

Lay Summary

This PhD research aims to contribute to our understanding of a maternal condition called pre-eclampsia in low and middle-income countries (LMIC). This condition is defined by the international society for the study of hypertension in pregnancy (ISSHP) as a rise in blood pressure during pregnancy that may occur at 20 weeks onwards accompanied by either proteinuria or other organ damage. It is reported as one of the top causes of maternal and infant death. National, regional and global targets have been set in an attempt to reduce maternal and infant death. Despite the decline, some causes of maternal death have persisted in some regions of the world. I believe researching in this area would uncover the problems and pave the way for designing preventive strategies.

The World Health Organisation (WHO) reports that two-third of maternal death occur in Sub-Saharan Africa. Sub-Saharan Africa has certainly not made sufficient progress in reducing maternal deaths compared to other regions. WHO reported that from 2000 to 2017 maternal mortality dropped by 40% in Sub-Saharan Africa, while in Southern Asia it dropped by 60 %. What is holding back this region and what are the causes of these deaths in the region? Most maternal deaths arise from preventable causes such as; bleeding during and after delivery, hypertensive disorders of pregnancy, infections, obstructed labour and unsafe abortions. Unlike the above causes, deaths from hypertensive disorders of pregnancy seem more difficult to address especially in LMIC. A successful reduction of maternal deaths in LMIC has to include addressing hypertensive disorders of pregnancies, which account 14 % of global maternal deaths according to WHO data. If progress is to be made on the reduction of global maternal mortality ratios, it is important to improve our understanding of the occurrence of hypertensive disorders of pregnancy focusing on pre-eclampsia in low-income settings. This PhD research aims to contribute knowledge in this field.

Chapter One and Two sets out a general literature review on what is known about the occurrence, causes, outcomes and management of hypertensive

disorders of pregnancy, with a focus on pre-eclampsia. It outlines the complexity of this subject and then highlights the implications of this complexity for the prevention and management of this condition in low-income countries such as Tanzania, which is the case study of this PhD.

Chapter Three covers a scoping review on risk factors of pre-eclampsia in Africa; the purpose of the scoping review was to summarise existing evidence of risk factors for pre-eclampsia in Africa, identify gaps in the literature and identify a suitable systematic review topic and assess the feasibility of undertaking it. The results showed risk factors for pre-eclampsia have not been adequately explored in the African population, marked by the relatively few studies reported from my search. My literature search suggests local risk factors such as malaria infection during pregnancy may drive pre-eclampsia condition in this region. I observed a gap in lack of literature that shows how well risk factors can classify women with pre-eclampsia in African populations. Following these identified gaps, my data analysis explored malaria as a local risk factor for pre-eclampsia alongside other risk factors that are prevalent globally such as chronic hypertension. I have also assessed how prediction models build from the identified risk factors can classify pre-eclampsia outcome in northern Tanzania population.

Chapter Four is a systematic review and meta-analysis on the relationship between malaria infection and gestational hypertension with or without proteinuria. My findings from the meta-analysis showed that malaria infection was associated with gestational hypertension [with proteinuria (pre-eclampsia) and without]. Thus, I was able to suggest the potential contribution of malaria infection in the overall burden of hypertensive disorders of pregnancy in Africa.

Chapter Five covers the analysis of secondary data on risk factors for pre-eclampsia in Tanzania. I first estimated the incidence of pre-eclampsia in the urban settings of Dar es Salaam city. My age-adjusted incidence estimate (1.9 %, 95 % CI 1.3 % - 2.2 %) was similar to global estimates of pre-eclampsia. Second, I described the sociodemographic characteristics of

women with pre-eclampsia. Pre-eclampsia appeared to be more frequent among women with older age and tertiary level education in northern Tanzania. Third, I used modelling to identify risk factors of pre-eclampsia in a population of singleton women from northern Tanzania. I examined the performances of these models in classifying women with or without pre-eclampsia using area under the curve, sensitivity and specificity values. My findings showed there were differences in risk factors that predicted pre-eclampsia outcome in the term and preterm deliveries subgroups. Fourth, I described the pregnancy outcomes of women with pre-eclampsia. My findings showed that infant deaths were high in women with pre-eclampsia. Surviving offspring of women with pre-eclampsia had poor indicators of physical and mental health. Therefore, efforts to avert pre-eclampsia may have a potential change with lifelong impact in improving the survival and wellbeing of newborn infants.

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contribution is not in vain and the medical profession owes you and your daughters better maternal services.

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List of Abbreviations and Acronyms.

ADMA Assymetric dimethyl arginine.

ANC Antenatal clinic.

Apgar Appearance, Pulse, Grimace, Activity and Respiration.

ARV Anti-Retro Viral drugs.

AUC Area Under the Curve.

BMI Body Mass Index.

BP Blood Pressure.

Ca^{+} Calcium Ion.

CASP Critical Appraisal Skills Programme.

CD4 Cluster of Differentiation 4.

CI Confidence Interval.

CS Caesarean section.

CVD Cardiovascular Disease.

D. f Degrees of freedom.

DDAH Dimethyl arginine dimethyl amino hydrolase.

DIC Disseminated Intravascular Coagulopathy.

DPT-H Diphtheria, Pertussis, Tetanus, Haemophilus influenza vaccine.

Dx Diagnosis of

GDP Gross Domestic Product.

GEE Generalised Estimating Equations.

GH Gestational Hypertension.

HAART Highly Active Anti-Retroviral Therapy.

HELLP Haemolysis, Elevated Liver enzyme, low Platelets.

HIV Human Immunodeficiency Virus

Hx History of

ICD International Classification of Diseases.

IHME Institute of Health Metrics and Evaluation.

IUD Intrauterine Device.

IUFGR Intra Uterine Fetal Growth Restriction.

IVF Invitro-Fertilization.

KCMC Kilimanjaro Christian Medical Centre.

LMIC Low and Middle-Income countries.

MDGs Millennium Development Goals.

Mg^{+} Magnesium Ion.

MMR Maternal Mortality Ratio.

MUAC Mid-Upper Arm Circumference.

MUHAS Muhimbili University of Health and Allied Sciences.

NACP National AIDS Control Program.

NBS National Bureau of Statistics.

NCD Non-Communicable Disease.

NICE National Institute for Health and Care Excellence.

OR Odds Ratio.

PhD Doctor of Philosophy.

PMI Placental Malaria Infection.

PRISMA Preferred Reporting Items in Systematic Reviews and Meta-Analysis.

RBC Red Blood Cell.

RCT Randomised Clinical Trial.

ROC Receiver Operator Curve.

RR Relative Risk.

SD Standard Deviation.

SDG Sustainable Development Goal.

SPSS Statistical Package for Social Science.

TB Tuberculosis.

TDHS Tanzania Demographic Health Survey.

TNF Tumour Necrosis Factor.

UTI Urinary Tract Infection.

VEGF Vascular Endothelial Growth Factor.

VEGFR-1 Vascular Endothelial Growth Factor.Receptor-1.

WHO World Health Organisation.

Chapter 1 Thesis Overview.

This PhD research focuses on understanding a maternal pregnancy condition known as pre-eclampsia. This condition contributes to around 14 % of the global burden of maternal mortality and increases the risk of perinatal mortality fivefold in low and middle-income countries (LMIC) (López Jaramillo *et al.* 2009, Say *et al.* 2014). The World Back categorises countries with gross national income per capita (United States dollars-USD) of ≤ 1,025 as low income, 1,026 – 3,995 as lower middle income, 3,996 – 12,375 as upper middle income and ≥ 12,376 as high-income countries. There is a considerable body of research knowledge suggesting the association between pre-eclampsia and a range of genetic, environmental, nutritional and socioeconomic factors (López-Jaramillo *et al.* 1997, Williams and Morgan 2012, Elongi Moyene *et al.* 2016). These factors are thought to disproportionately affect the burden of pre-eclampsia in LMIC as observed in the difference in the burden of pre-eclampsia and eclampsia across world regions (Firoz *et al.* 2011), (Abalos *et al.* 2013).

In this thesis, I start by introducing my research on risk factors for pre-eclampsia in LMIC focusing on pre-eclampsia in the context of the wider problem of hypertensive disorders of pregnancies. The term hypertensive disorders of pregnancy (HDP) refers to a group of distinct diseases that includes; white coat hypertension, pre-eclampsia gestational hypertension, and chronic hypertension (Tranquilli *et al.* 2014). There are two important challenges in conducting research into pre-eclampsia, particularly in low resource settings. The first is that, as I explain later, the aetiology and

pathogenesis of pre-eclampsia and the other hypertensive disorders of pregnancy are not fully understood. It is unclear whether early onset and late onset pre-eclampsia share the same risk factors in LMIC populations. The second challenge is that the different hypertensive disorders of pregnancy are not always accurately diagnosed and distinguished from each other, particularly in resource-poor settings (Abalos *et al.* 2013). The risk of misclassification is therefore significant.

This thesis focuses on understanding the risk factors for pre-eclampsia, which is a subject of growing public health concern. It deserves increased attention due to ongoing lifestyle changes across populations in Sub-Saharan Africa, because of globalisation and urbanisation which affect environmental, nutritional and socioeconomic factors. I have described the epidemiology of the condition and the evolving health system response. This sets the scene for the potential contribution of my research in advancing knowledge of the condition in LMIC.

Pre-eclampsia is one of the leading global causes of maternal and infant mortality. National, regional and global targets have been set in an attempt to reduce maternal and infant death, but despite declines in global maternal mortality ratio by half (from 1990 to 2015, as seen below), some causes of maternal death have persisted in some regions of the world. Maternal deaths due to hypertensive disorders (including pre-eclampsia) have persisted around 15 % from 1994 to 2014 (as seen below). More research on

understanding the risk factors for pre-eclampsia in LMIC is an essential first step in designing effective preventive strategies.

Global maternal mortality ratios have fallen sharply over the last quarter-century. The global maternal mortality ratio (MMR) fell from 385 deaths per 100,000 live births (80 % Confidence Interval (CI), 359 to 427 per 100,000 live births) in 1990, to 216 (80 % CI, 207 – 249) deaths per 100,000 live births in 2015. However, there is marked variation between countries, the MMR still being high in many low-income countries. In 2015 the MMR ranged from 12 deaths per 100 000 live births (95 % confidence interval (CI), 11 to14 per 100,000 live births) for high-income regions to 546 (95 % CI, 511 – 652) for Sub-Saharan Africa (Alkema *et al.* 2016). Sub-Saharan Africa has certainly not made sufficient progress in reducing maternal deaths. What is holding back this region and what are the causes of these maternal deaths in the region? Most maternal deaths arise from preventable causes such as haemorrhage during and after delivery, hypertensive disorders of pregnancy (including pre-eclampsia), infections, obstructed labour and unsafe abortions (Say *et al.* 2014). In low-income countries between 1991 and 2014, maternal deaths attributable to sepsis and infections fell steeply in absolute values (20 % to 10.7 %). There was a moderate decline in maternal deaths from haemorrhage (30 % to 27.1 %) while maternal death from hypertensive disorders (15 % to 14 %) remained fairly constant (Abouzahr *et al.* 1994, Say *et al.* 2014). The decline in deaths due to infection can be accounted for by antibiotic use, which has reduced maternal deaths due to infections and abortions (Maine and Rosenfield 1999). Obstructed labour has been

addressed through improvements in obstetric care by the deployment of skilled health care workers and the establishment of referral mechanisms (WHO 2004b). Provision of blood services and uterotonic drugs has reduced deaths due to haemorrhage (Kuzume *et al.* 2017). Unlike the above causes, deaths from hypertensive disorders of pregnancy seem more difficult to address. This is partly because of complexity: this group of conditions encompasses several sub-categories that can be challenging to distinguish from one another, particularly in low-income settings, thus presenting difficulties in specifying the diagnosis and in choosing the correct management path. The relative intractability of this problem may also be impacted by broader changes to population health affecting LMIC. Growing urbanization and adoption of western diet and lifestyle in these settings is thought to drive an increase in cardiovascular disease (CVD). In 2016, CVD was the third highest cause of death in Sub-Saharan Africa, up from 5th in 1990 (IHME 2016). Similar to this, a successful reduction of maternal mortality in LMIC has to include addressing hypertensive disorders of pregnancies, which account for 14 % of global maternal deaths (Say *et al.* 2014). If progress is to be maintained on the reduction of global maternal mortality ratio, it is important to improve our understanding of the epidemiology of hypertensive disorders of pregnancy in general, and pre-eclampsia in particular, in low-income settings. This PhD research project aims to contribute knowledge in this field.

Chapter Two sets out a general literature review on what is known about the epidemiology, aetiology, outcomes and management of hypertensive

disorders of pregnancy, with a focus on pre-eclampsia. It outlines the complexity of this subject and then highlights the implications for the prevention and management of this condition in low-income countries such as Tanzania, which is the case study of this PhD research.

Chapter Three is a scoping review on risk factors for pre-eclampsia in Africa. The purpose of the scoping review was to summarise existing evidence of risk factors for pre-eclampsia in Africa, identify gaps in the literature and identify a systematic review topic and assess the feasibility of undertaking it (Arksey and Malley 2005). The results showed risk factors for pre-eclampsia have not been adequately explored in the African population, marked by the relatively small number of studies identified. This scoping review suggests that locally relevant risk factors such as malaria infection during pregnancy may drive pre-eclampsia in Sub-Saharan Africa.

In addition to the scoping review of literature, I observed a gap in the general literature of pre-eclampsia in African populations. There are no studies on the performance of prediction models using biomedical risk factors to classify women with pre-eclampsia in African populations. We do not know how well we can predict and classify pre-eclampsia outcome among African women. Following these identified gaps, my analysis of secondary data explored malaria as a regional specific risk factor for pre-eclampsia alongside other risk factors that are prevalent globally such as chronic hypertension. I have also assessed how models built from the identified risk factors can classify pre-eclampsia outcome in northern Tanzania population.

Chapter Four is a systematic review and meta-analysis of the association between malaria infection and gestational hypertension with or without proteinuria. My findings from the meta-analysis showed that malaria infection was associated with gestational hypertension (with proteinuria/pre-eclampsia and without). The strength of this association was a pooled odds ratio (OR) of 2.6, 95 % CI 1.5 to 4.5). Thus, I was able to suggest the potential contribution of malaria infection in the overall burden of hypertensive disorders of pregnancy in Africa.

Chapter Five is an analysis of secondary data on risk factors for pre-eclampsia using two data sets from Tanzania. Chapter Six is the result of the data analysis in Chapter Five, while chapter Seven is the discussion of the results in Chapter Six. The data analysis covers four objectives. I first estimated the incidence of pre-eclampsia in the urban settings of Dar es Salaam city using a data set from a clinical trial. This data set is described in detail in Chapter Five, section 5.5.3. The age-adjusted incidence estimate of pre-eclampsia was 1.9 % (95 % CI 1.3 % - 2.2 %) which was similar to global estimates of pre-eclampsia 2 % to 8 % (Abalos *et al.* 2013). I used a second data set (a hospital registry data set, which is described in Chapter Five, section 5.5.2) to describe the sociodemographic characteristics of women with pre-eclampsia. Pre-eclampsia appeared to be more frequent among women with older age and tertiary level education in northern Tanzania. A multivariable logistic regression model of all sociodemographic characteristics showed that women with age 36 - 40 and ≥ 41 years were statistically significantly associated with having pre-eclampsia (OR = 1.8 [95

% CI, 1.2 – 2.8] and 2.6 [95 % CI, 1.4 – 4.6] respectively). Independent of age, women with tertiary level education were associated with having pre-eclampsia (OR = 1.34, 95 % CI 1.06 - 1.69). Thirdly, I used logistic regression modelling to identify risk factors for pre-eclampsia in a population of singleton women from northern Tanzania. I examined the performance of these logistic regression models in classifying women with or without pre-eclampsia using area under the curve, sensitivity and specificity values. My analysis further included two sub-groups: women who delivered their babies at term and women who had preterm deliveries. My findings show there were differences in the set of risk factors that predicted pre-eclampsia outcome in the subgroups. Pre-eclampsia in term deliveries was predicted by; maternal age, the number of pregnancies, weight before pregnancy, history of hypertension, HIV treatment, contraceptive IUD use, diagnosis of infections and diagnosis of malaria. While pre-eclampsia in preterm delivery was predicted by; maternal age, history of hypertension, diagnosis of infections and weight before pregnancy. Fourthly, I described the pregnancy outcomes of women with pre-eclampsia. My findings showed that the rate of stillbirths was higher in pre-eclampsia pregnancies, compared with normal pregnancies (OR = 4.874, 95 % CI 3.75 – 6.331). Surviving offspring of pre-eclampsia pregnancies had poor indicators of physical and mental health.

- Offspring survival

- The odds of stillbirth were 4.8 (95 % CI 3.7 – 6.3) times among women with pre-eclampsia than women without pre-eclampsia in all deliveries.

- Mental and physical wellbeing of newborn

  - Pre-eclampsia was associated with a low Apgar score (OR = 4.3, 95 % CI 3.4 - 5.4) in all deliveries.

- Physical wellbeing of newborn

  - The odds of low birth weight baby were 6.4 (95 % CI 5.3 – 7.6) times among women with pre-eclampsia than women without pre-eclampsia in all deliveries.

  - In all deliveries, newborns of pre-eclampsia women were shorter in birth length than their counterparts, mean difference of 1.92cm, 95 % CI 1.54 - 2.29, $p < 0.001$. This difference was observed in both term (0.40cm, 95 % CI 0.18 - 0.63, $p < 0.001$) and preterm subgroups (3.72cm, 95 % CI 2.83 - 4.62, $p < 0.001$.

Literature in this area suggests these features indicate fetal programming in pre-eclampsia newborns thus placing the surviving newborns into health risks in their adult life (Rogvi *et al.* 2012, Palinski 2014, Ashtin *et al.* 2019). Therefore, efforts to avert pre-eclampsia may potentially improve the survival and wellbeing of newborn infants. Chapter Eight presents the conclusion and recommendation from the data analysis. This chapter also shows my plans/ way forward after completion of this research work.

Chapter 2 Background Literature Review.

This general review of the literature on pre-eclampsia is divided into the following sections:

- Hypertensive disorders of pregnancy: an overview
- Pre-eclampsia: an overview
- Prevalence, temporal trends and geographical distribution of pre-eclampsia
- The natural history of pre-eclampsia
- Risk factors for pre-eclampsia
- Global, national and health system responses to pre-eclampsia

2.1 Hypertensive disorders of pregnancy: an overview

Pre-eclampsia is a subcategory within a broad collection of conditions known as hypertensive disorders of pregnancy. High blood pressure (BP) during pregnancy encompasses several different conditions: chronic hypertension; transient gestational hypertension; gestational hypertension; pre-eclampsia; white coat hypertension and masked hypertension. Pre-eclampsia may progress to Eclampsia and HELLP (**H**emolysis, which is the breaking down

of red blood cells; **E**levated **L**iver enzymes; **L**ow **P**latelet count) and pre-eclampsia may superimpose on chronic hypertension (Brown *et al.* 2018).

Chronic hypertension is a preexisting condition of elevated systolic/diastolic blood pressure (≥ 140/90 mmHg) prior to pregnancy, which does not resolve within 12 weeks after delivery. This condition results from factors that are often unrelated to pregnancy (e.g. obesity, genetic factors) and is a lifelong condition. It is detected by high blood pressure before 20 weeks of gestation and its persistence 12 weeks after delivery. The prevalence of chronic hypertension is estimated to be 5 % of all pregnancies (Mammaro *et al.* 2009). Although unrelated to pregnancy, pregnancy tends to affect the control of chronic hypertension; hence, women with this condition require extra consideration on their management when they become pregnant. It is reported that women with severe chronic hypertension, SBP ≥ 160mmHg or DBP ≥ 110mmHg, within the first 12 weeks of pregnancy are at 50 % increased risk of developing pre-eclampsia superimposed on chronic hypertension.

Gestational hypertension is a new onset of elevation of blood pressure, without proteinuria, that occurs during pregnancy from 20 weeks onwards and persists throughout pregnancy only to subside after delivery. This condition occurs in women who were not previously hypertensive (Brown *et al.* 2018).

Transient gestational hypertension is a new onset of elevated blood pressure arises at any gestation and resolves without treatment during pregnancy

hence the name transient. It too is not characterised by proteinuria (Brown *et al.* 2018). White-coat hypertension means elevated blood pressure (≥140/ 90 mmHg) taken at health facility but normal blood pressure when measured at home or work (< 135/85 mmHg); it is not an entirely benign condition it indicates an increased risk for pre-eclampsia (Brown *et al.* 2018).

Masked hypertension is another form of hypertension, more challenging to diagnose. It is characterised by blood pressure that is normal at a health facility visit but elevated at other times, most typically diagnosed by 24 hours ambulatory blood pressure monitoring (ABPM) or automated home blood pressure monitoring (HBPM) (Brown *et al.* 2018).

The current advice is regardless of the hypertensive disorder of pregnancy, blood pressures consistently ≥ 140/90 mmHg in health facility visit or ≥ 135/85 mmHg at home should be treated. The treatment should aim for a target diastolic blood pressure of 85 mmHg in the health facility (and systolic blood pressure of 110–140 mmHg) to lower the likelihood of developing severe maternal hypertension and other complications (Brown *et al.* 2018).

The sub-categories of high blood pressure during pregnancy can easily be confused with each other and pose some challenges in their diagnosis. Each category has a unique management path; thus, misdiagnosis can result in mismanagement of the condition. The potential misclassifications of these sub-categories make it difficult to achieve exact confirmation of the cause of maternal death from hypertensive conditions (Duley 1992). This impairs the validity of aggregated data from lower level health facilities (which often do

not have the technology and expertise to make a precise diagnosis) to national level health facilities in many LMIC, thus making it difficult to establish the burden of each subcategory of hypertensive disorders of pregnancy (Firoz *et al.* 2011, Bilano *et al.* 2014).

2.2 About pre-eclampsia: an overview

Pre-eclampsia is a hypertensive condition that arises after the 20th week of gestation. It can occur in a non-hypertensive woman or it may be superimposed on preexisting chronic hypertension. In the former scenario, it is characterised by persistent high systolic (≥ 140 mmHg) or diastolic (≥ 90 mmHg) blood pressure and organ damage/proteinuria. Proteinuria levels of ≥ 0.3 g/24 hours or a dipstick result of ≥ 1 +, equivalent to 30 mg/dl in a single urine sample or spot urine protein/ creatinine ratio ≥ 30 mg protein/mmol creatinine, of new onset after 20 weeks of gestation (Tranquilli *et al.* 2014). For preexisting chronic hypertension, the blood pressure is exacerbated, and a new onset of proteinuria is observed after the 20th week of gestation. To diagnose pre-eclampsia, one requires the gestational age at the onset of high blood pressure and proteinuria. Clinical skills and tools such as sphygmomanometer (for blood pressure) and urine dipstick are necessary to obtain this diagnosis. Routine screening for blood pressure and proteinuria is necessary prior to the 20th week to pick up the new onset of these key signs. Over time, the definition of pre-eclampsia has evolved, to match with our increasing recognition of its systemic nature and its heterogeneous clinical

presentation. For example, a significant proportion of women develop severe pre-eclampsia without proteinuria being detectable while having other signs of organ damage (Barton *et al.* 2001). As a result, the Canadian hypertensive disorders of pregnancy working group reflected this change in its report. This change has led to a revised definition of pre-eclampsia to include the presence of severe features with or without proteinuria (Magee *et al.* 2014). The recommendations from different countries lead to the 2014 revised recommendations by the International society for the study of hypertension which also viewed some subcategories of hypertensive disorders of pregnancy as a spectrum rather than discrete entities (Tranquilli *et al.* 2014). However, the adoption of these new definitions depends on the ability of different national health systems to operationalise the definition in their settings. Most LMIC have continued to use the rather simple definition stated above.

The aetiology of pre-eclampsia is still unknown. There are several theories attempting to explain its causation. This lack of clear understanding of its aetiology has deterred efforts to diagnose, prevent and treat the condition effectively. Some theories have classified pre-eclampsia based on its onset during gestation, early onset (≤ 34 weeks) and late onset (≥ 34 weeks). The onset classification is useful in guiding management since early onset is associated with worse outcomes (Phipps *et al.* 2016). An alternative classification is based on the severity of raised blood pressure: mild (BP < 160/110mmHg), severe (BP ≥ 160/110mmHg). Severe pre-eclampsia is also accompanied by worse outcomes (Phipps *et al.* 2016).

It is hypothesised that not all pre-eclampsia cases have the same causal origin. Early onset pre-eclampsia is thought to differ from late onset pre-eclampsia in its risk factors. Therefore, to be able to effectively predict and screen pre-eclampsia of different onsets, we would require different prediction models for late and early pre-eclampsia onset types (Scazzocchio and Figueras 2011). Early onset pre-eclampsia (occurring in ≤ 34 weeks of gestation) is most closely associated with inadequate placentation and may well be associated with alterations in angiogenic balance. Late pre-eclampsia (occurring > 34 weeks of gestation) is most commonly associated with normal placental development and is believed to be predicted by factors associated with long term cardiovascular risk, such as obesity, diabetes and chronic hypertension (Kleinrouweler *et al.* 2012). This suggests that there may be inappropriate use of risk factors in developing pre-eclampsia prediction models if they do not consider the variations between early and late pre-eclampsia. In addition, risk factors are thought to vary across ethnicity. Davies-Tuck *et al.* (2016) showed that obesity resulted in different pregnancy complication in Australian women of European descent compared to those of South-East Asia descent. A particular risk factor may exert different outcomes in different ethnicities. Hence, it is necessary to determine what risk factors are linked to the early and late onset of pre-eclampsia in diverse ethnic groups of Africa. A clear understanding of risk factors involved in Africa will enable appropriate assignment of risk factors in the screening of early and late onset pre-eclampsia. We plan to use data from northern

Tanzania to explore these risk factors for this subpopulation of African women. The results may be generalizable to other subpopulations of LMIC.

Pre-eclampsia may progress to eclampsia, which is characterised by the onset of generalised seizures in women with pre-eclampsia, on condition that the tonic-clonic seizures are not due to other causes (e.g. epilepsy). Similar to pre-eclampsia, the pathogenesis of eclampsia is not fully known. Eclampsia data show it disproportionately complicates the pregnancies in LMIC. Five to eight percent of women with pre-eclampsia end up presenting with full blown eclampsia in LMIC (WHO 2011). In the developed countries of North America and Europe, the incidence of eclampsia is estimated to be 5-7 cases per 10,000 deliveries, while that of developing counties is much higher and varies widely from 1 case per 100 pregnancies to 1 case per 1700 pregnancies (WHO 2004a, Shah 2009). These incidence differences may be due to the efficiency of health service delivery or genetic susceptibility in converting to eclampsia among these populations. It also demonstrates that within developing countries there is significant variation in the distribution of eclampsia. Eclampsia is associated with worse pregnancy outcomes and higher mortality compared to pre-eclampsia. Eclampsia is an obstetric emergency and requires immediate intervention to save lives of the mother and, before birth, her unborn child.

HELLP syndrome occurs in 10 % – 20 % of women with severe pre-eclampsia and is associated with substantial, widespread endothelial damage. HELLP is a life and death situation. Often blood leaks from the

blood vessels and accumulates into spaces such as the brain ventricles or the liver. It may also complicate to disseminated intravascular coagulopathy (DIC), which is often fatal. The management of eclampsia and HELLP is complex and requires skilled health care workers (HCW) who are scarce in rural settings of developing countries. Therefore, eclampsia and HELLP syndrome remain as important predictors of further organ dysfunctions and mortality (WHO 2011).

Gestational hypertension, pre-eclampsia, eclampsia and HELLP are viewed as a continuous spectrum of severity in organ dysfunction and involvement rather than distinct conditions. This view has resulted in the redefinition of pre-eclampsia to encompass its systemic organ involvement. As described above, before 2013 the presence of proteinuria was essential to a diagnosis of pre-eclampsia. In the revised definition of pre-eclampsia, the borders that separate pre-eclampsia from other conditions on the spectrum of hypertensive diseases of pregnancy have shifted. However, this shift comes with cost and operational challenges. New tools and expertise are required to make an accurate diagnosis of pre-eclampsia. This poses very real challenges to resource-poor settings in LMIC and has limited the extent to which the new broad definition of pre-eclampsia in the absence of proteinuria has been adopted in some countries (Brown *et al.* 2018).

2.3 Prevalence, temporal trends and geographical distribution of pre-eclampsia

Pre-eclampsia is known to occur across all populations in the world. However, there are regional variations from the reported data. The highest prevalence among pregnant women estimates is in Africa (AFRO) region 5.6 % (3.6 % – 11.3 %) while the lowest prevalence estimates were in Eastern Mediterranean (EMRO) region 1.0 % (0.1 % – 2.6 %) (Abalos *et al.* 2013). This apparent variation in its distribution may be a result of variation in susceptibility or the distribution of risk factors. Furthermore, it could be a result of variations in diagnostic capabilities and reporting systems across regions. This section describes the prevalence of pre-eclampsia among pregnant women, evidence of temporal trends and geographical distribution across populations.

Pre-eclampsia is estimated to occur in 2 % to 8 % of all pregnancies worldwide (WHO 2011); however, there are noticeable regional variations. A systematic review estimated the regional point prevalence of pre-eclampsia among pregnant women (95 % CI) in WHO regions of Africa (AFRO), America (AMRO) and south-east Asia (SEARO) to be 5.6 % of deliveries (95 % CI 3.6 % - 11.3 %), 3.0 % (95 % CI 1.5 % - 5.2 %) and 5.1 % (95 % CI 1.9 % - 10.9 %) respectively (Abalos *et al.* 2013). Most of the estimates in this review were hospital-based which often overestimates the incidence of pre-eclampsia. Another limitation of the regional estimate is the paucity of datasets involved and their high heterogeneity. The number of datasets and

heterogeneity (I^2) for WHO regions were: 11 (I^2 = 85 %) in Africa (AFRO), 5 (I^2 = 97 %) in America (AMRO) and 5 (I^2 = 95 %) South-east Asia (SEARO) (Abalos *et al.* 2013). This limits the comparison of the observed regional estimates.

The trend and distribution of gestational hypertension, pre-eclampsia and eclampsia have been studied in high-income countries. Findings from a study conducted in the USA from 1987 to 2004 showed an increase in the incidence of gestational hypertension by 183 % (annual incidence rates per 1,000 deliveries of 10.5 (8.2 - 12.9) to 29.7 (25.4 – 33.9)) and pre-eclampsia by 28 % (annual incidence rates per 1,000 deliveries of 25.1 (21.5 – 28.6) to 32.1 (27.6 – 36.6)) during the 18-year span. The study suggested the following plausible contributors to this large increase: population level increases in known risk factors for pre-eclampsia such as pre-pregnancy overweight and obesity, diabetes, multiple births, and maternal age (Wallis *et al.* 2008). However, a study by Roberts *et al.* (2011) that explored the trend of gestational hypertension and pre-eclampsia in Australia, North Europe, North America from 1997 to 2007, showed surprisingly that contrary to expectation, there was a decrease in these conditions in all countries except in the USA. The observed increased prevalence in risk factors such as diabetes, nulliparity and maternal age was expected to result in an increase in these conditions over time (Roberts *et al.* 2011). The authors attributed their findings to the increased utilization of intervention services such as early elective delivery, use of low dose aspirin, calcium and vitamin supplementation. They also pointed out the limitation of changes in

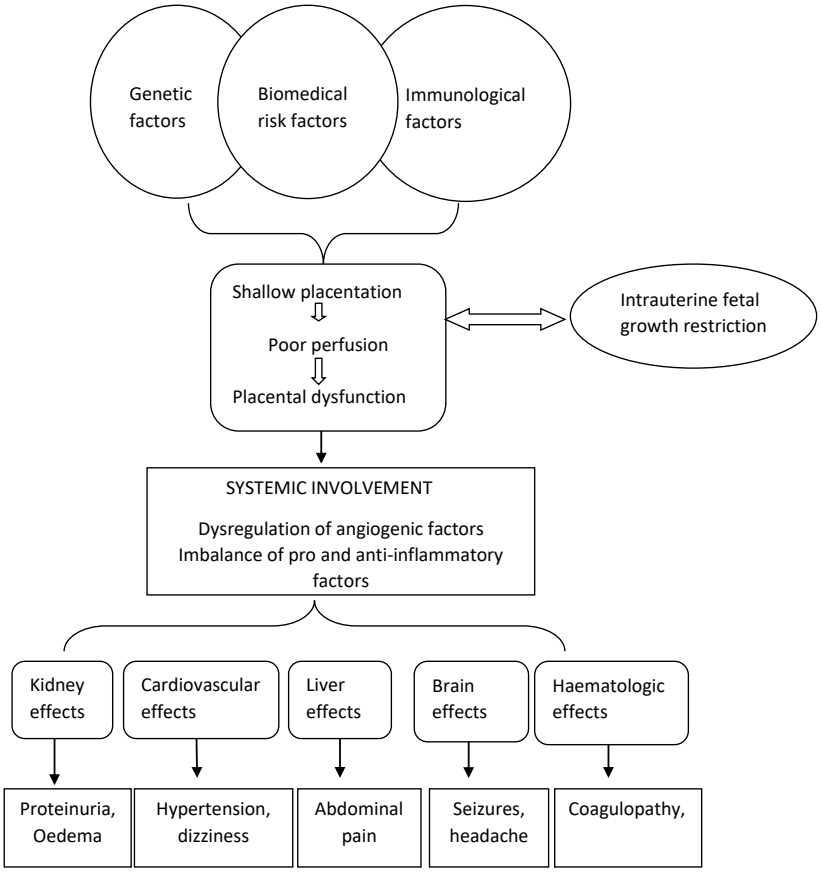
diagnostic criteria from ICD9 to ICD10 that may decrease the proportion of pre-eclampsia cases over the years. These findings may also be influenced by these countries experiencing a decreased fertility rate, furthermore, women with pre-eclampsia have even lower subsequent pregnancies. These factors may confound the nature of observed results.

In LMIC, the trend of pre-eclampsia seems to be on the rise. A hospital-based study in Ethiopia showed the proportion of women with pre-eclampsia was 2.2 % of pregnancies (95 % CI 1.9 % - 2.5 % in 2009, increasing by 154 % to 5.58 % of all pregnancies (95 % CI 5.3 % - 5.8 %) in 2013 (Wagnew *et al.* 2016). However, it is uncertain whether these results are a true increase in cases in the population or are the result of increased awareness and utilization of maternity services.

2.4 Natural history of pre-eclampsia

Pre-eclampsia often results in catastrophic outcomes, if the natural history of the disease is left without intervention. It accounts for about 14 % of maternal mortality and fivefold of perinatal mortality across the globe (Khan *et al.* 2006, López Jaramillo *et al.* 2009). It increases the risk of CVD for the surviving mother (Bellamy *et al.* 2007). Surviving infants are also prone to increased health risks in their adult lives (Palinski 2014).

Image 1: Pathogenesis of pre-eclampsia.



Genetic factors, maternal factors and immunological factors interact to cause placental dysfunction (Phipps *et al.* 2019).

2.4.1 Maternal and Infant Mortality

Pre-eclampsia can progress to a more severe form, such as eclampsia or HELLP, which have far worse outcomes for maternal and fetal mortality. Pre-eclampsia leads to intrauterine fetal growth restriction of the fetus and is also thought to cause stillbirths, premature delivery and low weight babies (Backes *et al.* 2011). As a result, pre-eclampsia accounts for a fivefold increase in perinatal mortality in LMIC compared to non-pre-eclampsia

pregnancies (López Jaramillo *et al.* 2009). Pre-eclampsia risk is threefold in a woman's first pregnancy compared to multiparous women (Hartikainen *et al.* 1998). A meta-analysis by Bartsch *et al.* (2016) showed first pregnancy has the highest population attributable risk (PAR) for pre-eclampsia among all other risk factors in their study (32.3 %, 95 % CI 27.4 % to 37 %). PAR of a given risk factor is the proportion of the incidence of the disease in the population that is due to the particular exposure. Although the risk of developing pre-eclampsia is markedly different between nulliparous and parous women, once they develop pre-eclampsia their pregnancy outcomes do not seem to differ. In a middle-income country of Jordan, Badria and Amarin (2005) showed that there was no statistically significant difference in proportions of stillbirth, neonatal deaths, assisted deliveries or low birth weight between nulliparous women with pre-eclampsia and parous women with pre-eclampsia.

Hypertensive disorders of pregnancy (including pre-eclampsia) are estimated to account for maternal deaths by 9.1 %, 95 % CI 3.9 % – 21.9 % in Africa and 9.1 %, 95 % CI 2.0 % - 34.3 % Asia, while accounting for 25.7 %, 95 % CI 7.9 % - 52.4 % of maternal deaths in Latin America and the Caribbean (Khan *et al.* 2006). These estimates show a wide confidence interval due to uncertainty from the small studies that were used to arrive at the estimates. Lack of data due to weak health information systems especially in rural parts of Africa and Asia can explain the uncertainty in their estimates. If true, the true incidence might be beyond the upper limit of the 95 % CI of the estimate based on the available data.

2.4.2 Fetal programming effect

Apart from causing infant mortality, there is growing evidence of possible intergenerational effects from maternal pre-eclampsia status, leading to an increased risk of CVD in the offspring of pre-eclampsia women in adult life. Fetal programming is thought to occur during the development of the embryo and fetus. At this stage, important physiological parameters can be reset by environmental factors. The local fetal cellular environment can alter gene expression during the developmental construction of tissues and organs, and these alterations can result in lasting consequences for the function of those tissues and organs during childhood and adulthood (Palinski 2014). This fetal programming effect is thought to be mediated through fetal growth restriction in the first trimester and abnormal offspring blood pressure and birth weight (Palinski 2014). Women born preterm or with inappropriate weight for gestational age were found to be at increased risk of gestational diabetes and pre-eclampsia in their adult life (Rogvi *et al.* 2012).

A few studies have suggested that maternal smoking, hypercholesterolaemia and obesity could cause fetal programming (Cederqvist *et al.* 1984, Noakes *et al.* 2003). They are thought to exert their programming effect partly by increased oxidative stress. Common mechanisms may also include immune programming. Smoking during pregnancy is associated with increased levels of immunoglobulins M and A in cord blood which is thought to affect fetal immune responses to allergens (Cederqvist *et al.* 1984, Noakes *et al.* 2003). This evidence links the association between early fetal programming to later-life risk of disease. As part of this thesis, I will describe the characteristics of

newborns of women with pre-eclampsia. These offspring may possess features suggestive of having experienced aversive utero environment, which may programme them to have increased health risks in later life.

2.4.3 Late effects of Maternal CVD and Cancer

Evidence from systematic reviews suggests that women who suffer from pre-eclampsia/eclampsia are at an increased risk of developing CVD in later life (Bellamy *et al.* 2007, Wu *et al.* 2017, Thilaganathan and Kalafat 2019). CVDs are a range of diseases that affect the cardiovascular system i.e. the heart, the blood vessels and blood dynamics. After a mean follow up of 14.5 years, the review found that mortality was higher in the group who had pre-eclampsia/eclampsia, relative to those who did not, the odds ratio was 1.49 (95 % CI 1.05 to 2.14) (Bellamy *et al.* 2007). However, the potential causal association of pre-eclampsia to CVD is debated. Other studies suggest that the two are not causally linked; rather they share common risk factors (Magnussen *et al.* 2007, Romundstad *et al.* 2010). Increasingly studies have suggested that pre-eclampsia is an independent risk factor for maternal cardiovascular disease (Mongraw-Chaffin *et al.* 2010). This evidence has been adopted by the Guideline of the American Health Association/American Stroke Association (Goldstein *et al.* 2011).

There is also conflicting evidence on the potential association between pre-eclampsia and cancer. In Israel, a 12 years cohort study by Calderon Margalit et al found that women with pre-eclampsia were at increased risk of going on to develop cancer (hazard ratio 1.23, 95 % CI 1.05 to 1.45)

(Calderon-Margalit *et al.* 2009). This is biologically plausible, given that pre-eclampsia is mediated by proinflammatory cytokines such as (TNFα) which is also among the markers of breast cancer (Reyes-Lopez *et al.* 2012, Weel *et al.* 2016). However, in contrast to this evidence, two meta-analyses showed pre-eclampsia was not significantly associated with the development of cancer: any cancer (relative risk 0.96, 95 % CI 0.73 - 1.27) (Bellamy *et al.* 2007) and breast cancer (RR = 0.93, 95% CI 0.82–1.06, p = 0.27)(Sun *et al.* 2018). Gestational hypertension also showed no significant association (RR = 0.95, 95% CI: 0.81–1.12, p = 0.54) (Sun *et al.* 2018). Therefore, there is insufficient epidemiological evidence linking pre-eclampsia to cancer.

2.5 Risk factors for pre-eclampsia

This section describes the commonly explored risk factors for pre-eclampsia. The study of risk factors has been influenced by the diversity in geographical location and ethnicity of the researched populations. There is regional inequality in the published research exploring pre-eclampsia risk factors, where the LMIC have been under-researched. Some risk factors have been consistent across different populations while others have shown inconclusive results. Below is a summary of risk factors commonly described in the literature and some plausible hypothesised mechanisms of action.

Obesity is among the modifiable risk factors associated with the development of pre-eclampsia. There is a growing epidemic of obesity in high-income countries and this is rapidly extending to LMIC (Misra and Khurana 2008). In the USA from 1970 to 2004, the percentage of women who are obese (BMI >

30) or overweight (BMI > 25) had increased almost by 60 % (Wang *et al.* 2008). A study based on the pregnant population in Pittsburgh showed a threefold increase in the risk of pre-eclampsia associated with obesity (Bodnar *et al.* 2005). A narrative review of the literature of articles from 1994 to 2011 involving 28 articles also showed an association of obesity and pre-eclampsia (Salihu *et al.* 2012). A 2019 published meta-analysis of 16 papers involving 5,946 women showed pre-eclampsia was associated with BMI (Kg/m^2). Healthy women had mean BMI of 25.13 (95 % CI: 23.52 - 2.74), women with mild pre-eclampsia their mean BMI was 27.42 (95 % CI: 24.4 - 30.34) while those with severe pre-eclampsia had a mean BMI of 26.33 (95 % CI: 24.52 - 28.13) (Motedayen *et al.* 2019). However, none of the included papers in the above meta-analysis was from the African population. It is still of interest to find the association of obesity and pre-eclampsia among African populations.

A possible mechanism linking obesity with hypertension is through asymmetric dimethyl arginine (ADMA). ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), an enzyme that produces nitric oxide, a potent vasodilator. High circulating plasma ADMA concentrations are also found in obese subjects (Eid *et al.* 2004, Krzyzanowska *et al.* 2004, Marliss *et al.* 2006, McLaughlin *et al.* 2006). While the exact mechanism for the increase in plasma ADMA in people with obesity is unknown, it may be mediated in part by a change in dimethyl arginine dimethyl amino hydrolase (DDAH), which is the key enzyme in the main pathway for degrading ADMA in the body.

Diabetes mellitus is a chronic disease that has long been associated with the development of pre-eclampsia. A meta-analysis of cohort studies by Bartsch *et al.* (2016) showed pre-gestational diabetes was associated with pre-eclampsia with a relative risk of 3.7, 95 % CI 3.1 to 4.3. While this association has long been established, there is also growing evidence suggesting pre-eclampsia is a risk factor of diabetes mellitus in later life. A meta-analysis by Wu *et al.* (2016) showed pre-eclampsia is associated with two-fold increase in the risk of diabetes after adjusting for BMI and gestational diabetes in later life. However, there is concern that the preclinical diabetic state could have existed prior to pre-eclampsia. (Weissgerber and Mudd 2015). It is, therefore, necessary to further research the relationship of diabetes mellitus and pre-eclampsia in different African populations to uncover their temporal relationship in these populations.

The prevalence of diabetes is increasing globally, initially in high-income countries, but also in LMIC (WHO 2017a). Insulin is now known to play a role in the regulation of the angiogenic processes through regulating endothelial cell migration proliferation (Escudero *et al.* 2017). Insulin exerts its signaling through endothelial cells and pericytes, therefore insulin may affect the formation of placental blood vessels and thus contribute to the development of pre-eclampsia.

Hyperemesis gravidarum is a clinical condition that occurs in the first trimester of pregnancy. It is characterised by severe nausea and vomiting; weight loss and dehydration may occur. It is more severe than morning

sickness commonly experienced during pregnancy. It is thought to be caused by an increase in human chorionic gonadotrophin hormone level during pregnancy. Studies have shown it is associated with a twofold increase in the occurrence of late pre-eclampsia among pregnant women who develop it (Bolin 2012). This clinical condition can be exploited in predicting high-risk pregnancies in LMIC. Currently, there are no systematic reviews that have examined the association of hyperemesis gravidarum and pre-eclampsia.

Severe anaemia (< 8.5gm/dl) is associated with an increased risk of pre-eclampsia. On the other hand, elevated levels of haemoglobin more than 14.5 gm/dl and ferritin have also been associated with poor fetal outcomes and pre-eclampsia (Gonzales *et al.* 2012). A study in Pakistan showed haemoglobin, haematocrit, serum iron, serum ferritin and transferrin saturation are increased in pregnant women at 28 – 34 weeks gestation, who later develop pre-eclampsia (Zafar and Iqbal 2008). Another study only showed serum iron concentration, ferritin, and percent saturation of transferrin to be higher among pre-eclampsia cases than controls at mean gestation age 33 weeks (Rayman *et al.* 2002). Free iron radicals are postulated as a casual factor in oxidative stress that leads to pre-eclampsia. Both studies recommend that the iron status of pregnant women should be assessed before giving iron supplements, as these may cause more harm to high-risk pre-eclampsia women. This shows that not all forms of anaemia are associated with pre-eclampsia; rather the haemolytic anaemias that release free iron radicals may be the culprit. We also know that the placenta tends to overgrow in size to maximise oxygen and nutritional supply to the fetus, in

anaemic and malnourished women. This compensatory mechanism may confound any potential association between nutritional related anaemias with pre-eclampsia. There are no systematic reviews associating anaemia with pre-eclampsia, however, a WHO multicountry study in LMIC that showed severe anaemia was associated with pre-eclampsia after adjusting for antenatal clinic visits, urinary tract infections, gestational diabetes, chronic hypertension, age, BMI and marital status (AOR: 2.98; 95 % CI 2.47 – 3.61) (Bilano *et al.* 2014). The clinical perspective regards anaemia as a symptom of an underlying disease. In tropical settings, it is meaningful to try to associate pre-eclampsia with the possible underlying disease such as malaria rather than the mere symptom of anaemia.

The prevalence of placental malaria is estimated to be 16 – 63 % among primigravidae and much less among multigravidae 12 – 33 % (Darmstadt *et al.* 2011). While malaria infection is estimated to affect 11.1 million pregnancies in Sub-Saharan Africa (WHO 2019b). A case-control study in Sudan established an association between placental malaria and pre-eclampsia (Adam *et al.* 2011). Another study in The Gambia showed a 5.4-fold increase in maternal death due to eclampsia during the malarial rainy seasons. This study also concluded that placental malaria infection is associated with non proteinuric hypertension in women living in hypo-endemic malaria areas, however in this study proteinuria values ≤ 2 were regarded as non proteinuric, this may have categorised mild pre-eclampsia as non proteinuric hypertension (Ndao *et al.* 2009). Hypo endemic areas have low and periodic/seasonal transmission of malaria infection. Malaria

infection during pregnancy is known as a major cause of maternal anaemia and low birth weight babies. Malaria has the potential of being an important risk factor in Africa since it is very prevalent and commonly affects women during the vulnerable period of pregnancy. The mechanism by which malaria may cause pre-eclampsia is through the damage of the placenta during placental malaria infection (Adam *et al.* 2011). An alternative pathway mechanism is through the haemolysis of red blood cells and the release of free radicals that damage blood vessels endothelia (Rayman *et al.* 2002, Zafar and Iqbal 2008). There are no meta-analyses studies showing the association of malaria infection with pre-eclampsia. I plan to explore further this relationship in the next chapters to understand malaria's potential association with pre-eclampsia in Africa.

Cigarette smoking may have a paradoxical effect on pre-eclampsia. Studies have shown that smoking reduces the occurrence of pre-eclampsia by 50 % in a dose dependent manner (Lucinda 2007). A meta-analysis involving 28 studies with 833,714 women showed there is a 32 % risk reduction of pre-eclampsia 95 % CI 31 % - 33 % among smokers (Conde-Agudelo *et al.* 1999). This finding was also echoed by another meta-analysis by Wei *et al.* (2015) which showed a 0.67-fold lower risk of pre-eclampsia in women who smoke regularly. Women who smoke in early pregnancy and quit do not have a reduced risk, whereas those who start smoking in late pregnancy and those who smoke throughout pregnancy are protected (Wikström *et al.* 2010). Exposure to nicotine, carbon monoxide, stimulation of nitric oxide production, lowering of antiangiogenic factors, or a decreased immune response have

been advanced as possible explanations for this observation (Conde-Agudelo *et al.* 1999, Bainbridge *et al.* 2005, Beste *et al.* 2005). Interestingly, women who smoke and develop pre-eclampsia seem to have a poorer outcome than women with pre-eclampsia who do not smoke (Salafia and Shiverick 1999). This observation suggests either a synergy between smoking and pre-eclampsia or that smoking may be masking the symptoms of pre-eclampsia in these women (Magnussen *et al.* 2007).

Pre-eclampsia has also been shown to have a seasonal variation in its occurrence. Studies done in the USA suggest that the increased prevalence of pre-eclampsia among pregnant African Americans women living in the south could be due to vitamin D deficiency, which fluctuates seasonally (Wallis *et al.* 2008). The potential mechanism for the role played by Vitamin D in causing pre-eclampsia is still not well understood; however, a proposed mechanism is that Vitamin D3 acts as an inhibitor for placental cytochrome P450scc preventing the production of lipid peroxides and excess progesterone, both of which may contribute to the etiopathogenesis of pre-eclampsia (Zabul *et al.* 2015). A prospective cohort study showed black women are at an increased risk of pre-eclampsia compared with white women (Knuist *et al.* 1998). This could be due to inherent genetic differences or due to vitamin D absorption through the skin. It is known that skin pigment causes a difference in Vitamin D concentration level between white and black people (Atkinson *et al.* 2014). There is no meta-analysis evidence to show the pooled estimate of the association between vitamin D and pre-eclampsia. The existing narrative review by Christesen *et al.* (2012) suggested vitamin D

is associated with pre-eclampsia in randomised clinical trial (RCT), cohort and case-control design studies. However, this association was not shown in some studies thus inviting suggestions that vitamin D works with other factors such as calcium in bringing out the effect (Christesen *et al.* 2012).

Pre-eclampsia risk is threefold in a woman's first pregnancy compared to parous women (Hartikainen *et al.* 1998). A meta-analysis by Bartsch *et al.* (2016) showed first pregnancy has the highest population attributable risk for pre-eclampsia among all other risk factors in their study (32.3 %, 95 % CI 27.4 % to 37 %). Pregnancy is viewed as a physiological stressor on the cardiovascular system. Women who fail to cope with these changes end up developing pre-eclampsia, those who pass this stress test on their first pregnancy are deemed fit and suffer less from pre-eclampsia in their subsequent pregnancies. However, having a previous pre-eclampsia pregnancy is associated with 7 – 15 % higher risk of pre-eclampsia in subsequent pregnancies (Hernandez-Diaz *et al.* 2009, McDonald *et al.* 2009), while women who did not have pre-eclampsia in the previous pregnancies have only a 1 % chance of developing it in their subsequent pregnancies. It is hypothesised that the risk in first pregnancy may occur more among women who have limited exposure to their partner's sperm. In support of this argument, women who had a short duration of cohabitation, those who used barrier contraception and those who used donor sperm have been seen to be most affected (Hutcheon *et al.* 2011).

Human immunodeficiency virus (HIV) infection is known to compromise the immune response. HIV affects the inflammatory process in the entire body, including blood vessels and the placenta. The introduction of highly active antiretroviral therapy (HAART) for treating HIV has posed a question about the role these drugs play in relation to developing pre-eclampsia among people with HIV. Small studies have presented conflicting evidence, some suggesting that HIV is a risk factor while others show HIV has a protective role against developing pre-eclampsia. A systematic review and meta-analysis looking at HIV status and use of HAART concluded that there is insufficient evidence to show the direction of the effects of HIV and HAART on pre-eclampsia (Adams *et al.* 2016). It is important to understand any potential associations since most of the HIV global burden is in Sub-Saharan Africa.

Micronutrient deficiency has been associated with increased risk of development of pre-eclampsia. A randomised double-blind controlled clinical trial conducted among Ecuadorian pregnant teenagers, where the population average daily calcium intake is 51 % of the daily requirement showed calcium (2 g/day) supplementation reduced the risk of pre-eclampsia by 3.2 % (12.35% in the treatment group versus 15.5% in the placebo group). The study concluded that calcium supplementation during pregnancy in a calcium deficient population reduces the risk of pre-eclampsia (López-Jaramillo *et al.* 1997). It is therefore important that future research explore the role of micronutrients in the development of pre-eclampsia in LMIC where malnutrition still prevails.

Advanced maternal age is a risk factor for pre-eclampsia according to several studies across different populations (Bilano *et al.* 2014, Tessema *et al.* 2015, O'Gorman *et al.* 2016). The association between maternal age and pre-eclampsia has been suggested to follow a U shape (Kumari 2016). Thus, women with lower age (< 20 years) are at higher risk and at the other end of the age spectrum, women aged above 35 years are also at a higher risk. However, this U shaped relationship is widely to be confounded by primiparity (Kumari 2016). Pre-eclampsia is more common among women in their first pregnancy (primiparous) and hence it corresponds to women with younger age. Upon removal of this confounding nature of primiparity in the > 20 years age group, pre-eclampsia appears to be at a higher rate in the > 35 years age group. This is in line with the recommendation by the National Institute of Health and Care Excellence (NICE) guideline that states that women in their first pregnancy and women above 35 years are at higher risk of pre-eclampsia (NICE 2019). There is scarce evidence from meta-analysis studies showing the association between maternal age and pre-eclampsia. However, a WHO multicountry survey by Bilano *et al.* (2014) showed the odds ratio for pre-eclampsia among women aged ≥ 35 years compared to those aged 20 - 35 years was 1.7, 95 % CI 1.6 - 1.9.

Prior history of hypertension is a known risk factor for pre-eclampsia. Concurrently, previous pre-eclampsia leads to a higher risk of developing pre-eclampsia in the subsequent pregnancies (M J. Mahande *et al.* 2013). A meta-analysis by Bartsch *et al.* (2016) showed prior pre-eclampsia and chronic hypertension were the first and second factors with a highest pooled

relative risk for pre-eclampsia, (8.4, 95 % CI 7.1 to 9.9) and (5.1,95 % CI 4.0 to 6.5) respectively. This association has been claimed to result from shared pre-pregnancy risk factors. Pre-pregnancy cardiovascular risk factors such as triglycerides and cholesterol levels have shown an association with pre-eclampsia (Magnussen *et al.* 2007). Several observational studies have echoed a similar association between prior history of hypertension and pre-eclampsia (Anorlu *et al.* 2005, Kiondo *et al.* 2012, Bilano *et al.* 2014). This strong relationship has even produced a specific subcategory in the hypertensive disorders of pregnancy known as pre-eclampsia superimposed on chronic hypertension (Tranquilli *et al.* 2014). Women with severe chronic hypertension within 12 weeks of gestation have been seen to be at a 50 % higher risk of developing superimposed pre-eclampsia (Mammaro *et al.* 2009).

2.6 Global, national and health system responses to pre-eclampsia

Over the years, our scientific understanding of the aetiology and pathogenesis of pre-eclampsia has advanced. There has also been a global political demand for improvement in maternal health care delivery. The United Nations through its Millennium Development Goal (MDG) number five that promoted maternal health has vividly described the call for maternal health improvement. The current Sustainable Development Goals (SDGs) have continued to highlight this as a priority through goal number three on

good health and wellbeing, and goal number five on gender equality (United Nations 2015).

2.6.1 Diagnosis, Prevention and Management of pre-eclampsia

As described above, low-income countries such as Tanzania are still using the previous definition of pre-eclampsia stated by ISSHP (2001). Thus, characterizing pre-eclampsia by persistent high systolic (≥ 140 mmHg) or diastolic (≥ 90 mmHg) blood pressure and proteinuria only. Proteinuria levels of ≥ 0.3 g/24 hours or a dipstick result of ≥ 1 +, which is equivalent to 30 mg/dl in a single urine sample or spot urine protein/ creatinine ratio ≥ 30 mg protein/mmol creatinine, of new onset at 20 weeks onwards of gestation (Brown *et al.* 2001). Although this ISSSP report had considered situations where high blood pressure could occur with other haematological features in the absence of proteinuria, this did not form an essential part of the definition. In recognition of the syndromic nature of pre-eclampsia, the diagnosis of pre-eclampsia by the ISSHP 2014 report revised its definition of pre-eclampsia to include all the above features in the ISSHP 2001 report, plus any of the additional features. The additional features are: haematological, renal and hepatic changes, the presence of utero placental dysfunction (fetal growth restriction) and a more explicit list of neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata) (Tranquilli

et al. 2014). This broad definition of pre-eclampsia is unlikely, to be adopted and operationalised successfully in resource-limited countries, as they require the support of laboratory and sonographic tests, which in most LMIC settings are absent or inaccessible.

The lack of appropriate technologies and other resources is a barrier for LMIC to detect and manage pre-eclampsia appropriately. This situation widens the inequality in service delivery between women who access to these modern services in urban areas from those residing in the rural. It also presents policy challenges calling for further investment into health budgets in rural areas to bridge the gap of inequality in health care.

2.6.2 Screening of pre-eclampsia

Improvement in the screening of high-risk women for pre-eclampsia has enabled the timely provision of preventive services in high-income countries. The United Kingdom NICE guideline on hypertension in pregnancy has outlined criteria for high risk women, defined as having any of the following conditions: hypertensive disease during a previous pregnancy, type 1 or type 2 diabetes, chronic hypertension, chronic kidney disease and autoimmune diseases such as systemic lupus erythematosis or antiphospholipid syndrome (NICE 2019). A woman is categorised as at moderate risk of hypertension in pregnancy if she has more than one of the following factors: first pregnancy; multiple pregnancies; pregnancy interval of more than 10 years; body mass index (BMI) of 35 kg/M^2 or more at first visit; family history of pre-eclampsia; age ≥ 40 years. However, the categorization into high and

moderate risk using the above risk factors may not be applicable universally across all populations. Regionally specific identification and classification of risk factors should be done to arrive at better guidelines for managing pre-eclampsia in LMIC.

In LMIC, screening for pre-eclampsia and other hypertensive disorders of pregnancy is done during routine antenatal clinic (ANC) visits. Pregnant women, among other things, are monitored for their blood pressure and protein levels in urine. The screening process is compromised by delay and lack of booking to ANC by some women. Shortage of skilled staff, tools and supplies to measure blood pressure and detect proteinuria contribute to low rates of pre-eclampsia detection.

Prevention of pre-eclampsia in pregnant women at high risk of pre-eclampsia is mainly through the administration of low dose aspirin (Firoz *et al.* 2011). The efficacy of aspirin in the reduction of preterm pre-eclampsia varies across maternal characteristics groups (Poon *et al.* 2017). Early identification of high-risk pregnancies through screening so that administration of low dose aspirin can be started promptly has been shown to result in better outcomes. A meta-analysis showed that aspirin started at 16 weeks or earlier in pregnancy was associated with a significant and greater reduction in pre-eclampsia (relative risk = 0.47, 95 % CI 0.34 - 0.65, prevalence of 9.3 % in the treated compared with 21.3 % in the control group) in women with moderate or high-risk pregnancies than aspirin started after 16 weeks of gestation (Bujold *et al.* 2010).

The mechanism of aspirin in preventing pre-eclampsia is still debated. Over time, our understanding of the mechanism of action of aspirin has evolved to enable us to explain its effects. The first pathway is that low dose aspirin (75mg-100mg per day) is able to irreversibly inhibit enzyme cyclooxygenase-1, thus inhibiting platelets to produce thromboxane A2, resulting in the antithrombotic effect of aspirin i.e. prevention of blood clotting by the aggregation of platelets (Cadavid 2017). The second pathway is that a moderate dose of aspirin (650 mg – 4 g per day) inhibits both enzymes cyclooxygenase-1 and 2, resulting in inhibition of prostaglandins production, which is a pro-inflammatory compound. In doing so, aspirin prevents inflammation in the body. A third mechanism is through generation of pro-resolving lipid mediators from arachidonic acid, called aspirin-triggered lipoxins. This compound gives aspirin further ability to resolve inflammation. Other mechanisms have been put forward, suggesting that aspirin causes anti-inflammatory effects via the induction of nitric oxide. Despite these mechanisms, epidemiological studies have shown inconsistency in the effectiveness of aspirin in preventing pre-eclampsia. Rossi and Mullin (2011) conducted two meta-analyses examining the effect of aspirin in groups of women with low risk and high-risk of pre-eclampsia. They found no statistically significant effect. Concurrently, apart from the side effects of gastric bleeding due to aspirin, there is growing concern about the effects of aspirin resistance, aspirin treatment failure and aspirin low response in study populations, aspirin resistant effect is thought to confound the effects of aspirin in the prevention of pre-eclampsia (Navaratnam *et al.* 2016). This

area requires further research, taking into account the confounding effect of aspirin resistance.

Prevention modalities may change as our understanding of pre-eclampsia develops. The hypothesis that pre-eclampsia arises because of imbalances of the factors responsible for the formation of new blood vessels has gained acceptance (Ahmed 1997). It has been shown that loss of vascular endothelial growth factor (VEGF) activity as a result of the increase in the levels of endogenous soluble(s) VEGFR-1(also known as soluble fm like tyrosine kinase-1 (sFlt-1)) antagonises the beneficial effects of VEGF (Ahmed 1997). The imbalance in anti-angiogenic factors is most strongly associated with the clinical signs of pre-eclampsia. Anti-angiogenic factors, sVEGFR-1 and soluble endoglin (sEng), also known as CD105, are increased before the clinical onset of pre-eclampsia (Levine *et al.* 2006). Thus current researchers are proposing the use of pravastatin, which is known to reduce the levels of sVEGFR-1 and thus promoting VEGF (Ahmed and Ramma 2015). This newly proposed drug for prevention is being studied further in clinical trials to evaluate its safety during pregnancy. Pravastatin is preferred to other statins as it is thought to be associated with fewer congenital anomalies (Esteve-Valverde *et al.* 2018). Statin drugs are contraindicated during pregnancy for their association with congenital anomalies. Statins are thought to decrease cholesterol synthesis. It is through this mechanism that statins may affect fetal growth to cause congenital anomalies (BMA *et al.* 1966).

Once pre-eclampsia is evident, treatment is through the administration of antihypertensive drugs. If eclampsia is already evident or develops regardless of the administration of antihypertensive drugs, the recommended management is provision of magnesium sulphate followed by delivery of the baby, the definitive treatment. There is a dilemma in deciding to induce delivery on the grounds of maternal wellbeing, especially when delaying delivery would increase the survival of the foetus. Guidelines have been developed to assist physicians in making such management decisions, with the intention of maximizing the chances of survival for both the mother and the foetus whenever possible. However, in resource-limited settings, it is difficult to adhere to such guidelines, where premature care is often absent or not sufficiently comprehensive to enable the survival of premature babies. In such settings, the entire process of treatment is subject to multiple barriers caused by a weak health system. Limited access to antenatal care, shortage of skilled health workers, inappropriate skill mix and lack of pharmaceutical support are some of the barriers to access and provision of quality prevention and treatment services for the mother and the premature baby in many LMIC (Firoz *et al.* 2011).

The mechanism of action of magnesium sulphate is not clearly understood and hence its use needs to be weighed against potential risks. The risks involved include the development of toxicity, which is more common in women with renal failure. Toxicity leads to respiratory arrest, which can be reversed with intravenous calcium gluconate. A patellar reflex test is used to assess the consciousness level, which tends to be impaired by the toxicity.

Absence of the reflex warrant administration of calcium gluconate. In declined consciousness, intubation should be performed to maintain airway patency (Anthony *et al.* 2016). Therefore, the effective control of eclampsia requires skilled personnel and equipped facilities, which are often lacking in LMIC settings.

The complexity in diagnosis and treating the sub-categories of hypertensive disorders of pregnancy is a major challenge. The management of hypertension during pregnancy is different from the management of hypertension in women who are not pregnant. This is due to the extra consideration of the foetus' wellbeing. It is not desirable to rapidly reduce maternal blood pressure because this may affect the placental blood perfusion, which may pose grave consequences for the foetus. Use of angiotensin converting enzyme inhibitors is also limited during pregnancy since this is associated with poor fetal outcome. Beta-blocker drugs are also relatively contraindicated, being regarded as an independent risk factor for the development of intrauterine growth restriction. This situation ends up narrowing the options of drugs that can be used to treat hypertension during pregnancy.

Labetalol is the recommended drug of choice in treating hypertension during pregnancy in the UK (NICE 2019). It has dual effects, acting as an alpha (α1) and beta (β1/β2) adrenergic receptor blocker. In the short term, it decreases blood pressure by decreasing systemic vascular resistance with little effects on stroke volume, heart rate and cardiac output (MacCarthy and Bloomfield

1983). In the long term, it reduces the heart rate while maintaining cardiac output by increasing stroke volume (Louis *et al.* 1984). In most LMIC settings methyldopa is the drug of choice (Anthony *et al.* 2016). It is a central acting drug and is documented to be safe during pregnancy; however, it should be avoided in women at risk of depression. Calcium channel blockers can also be used to manage hypertension in pregnancy; however, when given with magnesium sulphate in eclampsia they rarely can interact to cause prolonged neuromuscular blockage (Magee *et al.* 1996, Berdai *et al.* 2016). However, the recommended doses in common practice show nifedipine and magnesium sulphate when prescribed together do not increase the risk of magnesium sulphate side effects (Magee *et al.* 2005). The contraindication of these drugs to subgroups of women with specific challenges narrow their scope of use. It also complicates the skills required by HCWs to safely use them in the indicated subgroup of women. In addition to this mix of barriers, some LMIC do not have the pre-eclampsia related drugs in their National essential drugs list (Lalani *et al.* 2013). Lack of availability of these drugs in low levels health facilities in LMIC contributes to ineffective management of the condition, thus leading to poor outcomes. Clinicians in many LMIC are routinely faced with all these difficult choices of using what is available at that moment to address the challenges of HDP.

2.7 Summary

Pre-eclampsia's contribution to the burden of maternal and infant mortality places it as a condition of public health importance in LMIC. Most of the

research aiming to understand and tackle this problem has been done in high-income countries. Thus, the recommendations drawn from these studies may not be applicable or implementable in LMIC settings.

LMICs are faced with several challenges in their response towards tackling pre-eclampsia. It is challenging to diagnose pre-eclampsia, since it resembles other hypertensive disorders of pregnancy. It thus requires skilled staff and equipment to detect increased blood pressure and proteinuria. The lack of skilled staff and equipment hampers effective and timely diagnosis. The process of screening, prevention and treatment of pre-eclampsia is faced with similar challenges. Shortage of drug supply in health facilities is a common barrier in service delivery. It is difficult to make progress because most LMICs lack accurate epidemiological data to inform researchers and decision makers about the scale of the problem. The demand side i.e. the pregnant woman is also faced with a lack of awareness of pre-eclampsia and access limitations to antenatal and delivery services.

The LMIC of Africa experience high burdens of potential risk factors such as malaria infection and HIV that may be unique drivers in the region. Pregnancy increases women’s vulnerability to malaria and can complicate into placental malaria, which has even worse consequences. So far, there is inconclusive published evidence linking malaria with pre-eclampsia. Africa also bears most of the global HIV burden, which is important because of the potential of HIV to influence pre-eclampsia, although so far, there is conflicting evidence on the association between HIV and pre-eclampsia. In

this context, the Africa region requires attention to exploring the unique drivers for pre-eclampsia.

In order to start to address these gaps in knowledge and response systems, we first need a better understanding of the risk factors for pre-eclampsia operating in the local settings of African populations. This thesis focuses on exploring risk factors in Tanzania, which has high maternal mortality, but very limited data on pre-eclampsia. The country is also experiencing population growth with rapid urbanization and transformation of lifestyle and diet. These factors are thought to increase the incidence of pre-eclampsia. Successful identification of specific risk factors will inform prevention strategies contributing to the reduction of maternal and infant mortality and attainment of the SDG number three.

Chapter 3 Scoping study on risk factors for pre-eclampsia in Africa.

3.1 Background

Pre-eclampsia is a complex and poorly understood condition, which contributes to a significant burden of maternal and infant mortality, particularly in LMIC (Say *et al.* 2014). There is a considerable body of research suggesting associations between pre-eclampsia and a range of genetic, environmental, nutritional and socioeconomic risk factors (López-Jaramillo *et al.* 1997, Williams and Morgan 2012, Moyene *et al.* 2016). Although these factors are likely to vary between populations, most research has been conducted in high-income country populations. Little research has been done on this topic in African populations, and most systematic reviews exploring the risk factors for pre-eclampsia do not include primary research conducted in African populations. This may be due to the absence of primary studies or to the poor quality of studies that do exist. Because genetic, environmental and socioeconomic factors in African populations differ from those in high-income countries in Europe and north America, it is important to explore risk factors specific to the population in Africa. The first step in doing this is to assess the scope of available evidence from published quantitative papers that have investigated risk factors of pre-eclampsia in African populations. This chapter uses a systematic scoping review methodology to conduct such a scoping study (Arksey and Malley 2005).

Specifically, the purpose of this chapter is to conduct a structured review of the literature to identify and summarise current research into pre-eclampsia risk factors, conducted in African populations. Research questions addressed are:

- What research has been conducted in African populations on the risk factors for pre-eclampsia?
- Are there any significant gaps in the research literature?
- Based on this analysis, can a suitable topic be identified which could be further explored through a systematic review and the meta-analysis of existing data sets?

The advantage of using scoping review methodology over a narrative literature review is that a scoping review uses a rigorous, structured and systematic approach to searching and documenting retrieved literature. As such, it can provide a transparent, comprehensive, and unbiased account of relevant studies (Arksey and Malley 2005).

It is important to explore the risk factors for pre-eclampsia in LMIC, especially when Africa is undergoing rapid urbanisation and a transformation of lifestyles. Despite these changes, infectious disease burden still overwhelms the region. The dual burden of communicable and non-communicable diseases cripples the response of the health system. Research efforts in Africa are more inclined to conditions that have high direct mortality, like HIV and Ebola while leaving behind the slow killers like hypertensive diseases.

Hypertensive diseases are known to emerge and worsen during pregnancy. In most cases, hypertension will be a lifelong chronic condition that impairs quality of life. It is also a burden to the health system, which must support the individual for life. In LMIC, the chronic nature of hypertension has huge costs to the already heavily burdened health system (von Dadelszen and Magee 2016). Hypertensive disease is predicted to increase in the coming years in Africa. In some settings, it has overtaken haemorrhage as the leading cause of maternal mortality (LEE *et al.* 2012); hence, it is vital to understand the risk factors to plan prevention strategies to reduce hypertensive conditions.

It is not technically feasible to explore the risk factors for hypertensive disorders of pregnancy as one outcome because the subcategories within hypertensive disorders have different origins and hence different risk factors. Although there is an overlap of some risk factors there are distinct risk factors associated with each subcategory. Therefore, as seen in other literature; we must explore risk factors for each specific subcategory of HDP. My research focuses on exploring the risk factors for pre-eclampsia, a subcategory of hypertensive conditions during pregnancy. Women are a vulnerable group in terms of their health status, especially in LMIC, and the backbone of the society in providing health care. Therefore, a women's health status has a direct influence on the heath of her family. Hypertensive disorders of pregnancy, especially pre-eclampsia, have been associated with poor outcomes and mortality to both mother and child (Lopez-Jaramillo *et al.* 2005, Say *et al.* 2014), and also associated with later life cardiovascular morbidity

of the surviving mother and the newborn (Bellamy *et al.* 2007, Palinski 2014, Wu *et al.* 2017, Thilaganathan and Kalafat 2019).

I therefore aimed to explore the risk factors of pre-eclampsia in African populations currently documented in the literature. I have explored observational studies that have shown the statistical significance and the strength of the association between pre-eclampsia and the different risk factors. I have shown populations where the relationship was observed in an attempt to map out similarities and differences across different populations in African countries.

3.2 Methods

Arksey and Malley (2005) propose the use of scoping review studies as a methodology for summarising, disseminating and identifying gaps in a body of research literature. A scoping review is a more structured thus minimises bias compared to a narrative literature review. The process of conducting a scoping review is a highly iterative and flexible one, which is useful in the early stages of conducting a new research study.

I searched for relevant literature in the following databases; EMBASE, WHO Global Index Medicus and Web of Science core collection. Full details of the search strategy for each data base are given in the appendix (see annex 5). I limited my search to English language and studies on humans. I did not limit the search by year of publication. My study populations were all pregnant women and my outcome characteristic was pre-eclampsia.

Criterion	Included	Excluded
Population	• Pregnant women in Africa	• Non-African countries
Exposure	• Aetiology/ risk factors such as smoking, contraceptives agents, anaemia, diabetes mellitus, hypertension, cholesterol, placental malaria, HIV	
Outcome	• Pre-eclampsia	
Study Type	• All primary quantitative studies (cross-sectional, case-control, cohort, randomised controlled trial studies)	• Qualitative studies, case series and ecological studies, Genetic studies, animal studies, studies on subpopulations of women with rare conditions such as renal or liver transplant (since their findings could not be generalisable to the population of all pregnant women)
Language	• English	
Year of Publication	• All	

Papers identified from the search strategies were exported to an EndnoteX7 software library, where duplicates were removed automatically by matching papers by tittle, year of publication and author name. Then manually removal was done where titles, year of publication and author name varied though the

papers were still duplicate. The titles and abstracts of papers were then screened using the following inclusion and exclusion criteria.

Inclusion criteria

1. Epidemiological quantitative studies and randomised control trials exploring risk factors for pre-eclampsia
2. Studies published in the English language
3. Studies published in peer-reviewed academic journals

Exclusion criteria

1. Animal and laboratory (in vitro) studies
2. Studies targeting non-African populations
3. Studies that explored risk factors for pre-eclampsia in a subpopulation of women such as among women with rare conditions such as renal, liver transplant. This was because the associations observed in these subgroups could not be generalised to the general population.
4. Studies that only examined risk factors for other hypertensive disorders of pregnancy, and not specifically pre-eclampsia

Full article reviews were then conducted to assess eligibility according to my inclusion and exclusion criteria.

Unlike systematic review methodology, this scoping methodology is designed to be iterative: as the process unfolds and the researcher learns more about the topic, it is legitimate to revise the inclusion and exclusion criteria and to change the focus of the review. My initial search identified all relevant studies globally, it did not exclude studies out of Africa. However, later I focused my review search to studies conducted in African populations, by selecting studies from Africa from the initial worldwide search strategy that retrieved all studies across the globe.

The following data from eligible papers was extracted: author, year of the study, country of the study, study design, sample size, key results of the study. Following the methodology of Arksey and Malley (2005), a formal process of assessing the quality of included studies was not carried out. The purpose of a scoping review is to summarise the volume, focus, study designs and general conclusions of the literature on a particular topic, and to identify any gaps in the research, rather than to answer a specific and narrowly focused research question (Arksey and Malley 2005). In this way, scoping review methodology differs from systematic review methodology although, as in this case, a scoping review may be conducted as a precursor to conducting a systematic review. Their value in this respect is that they can identify gaps in the existing literature in a highly systematic way.

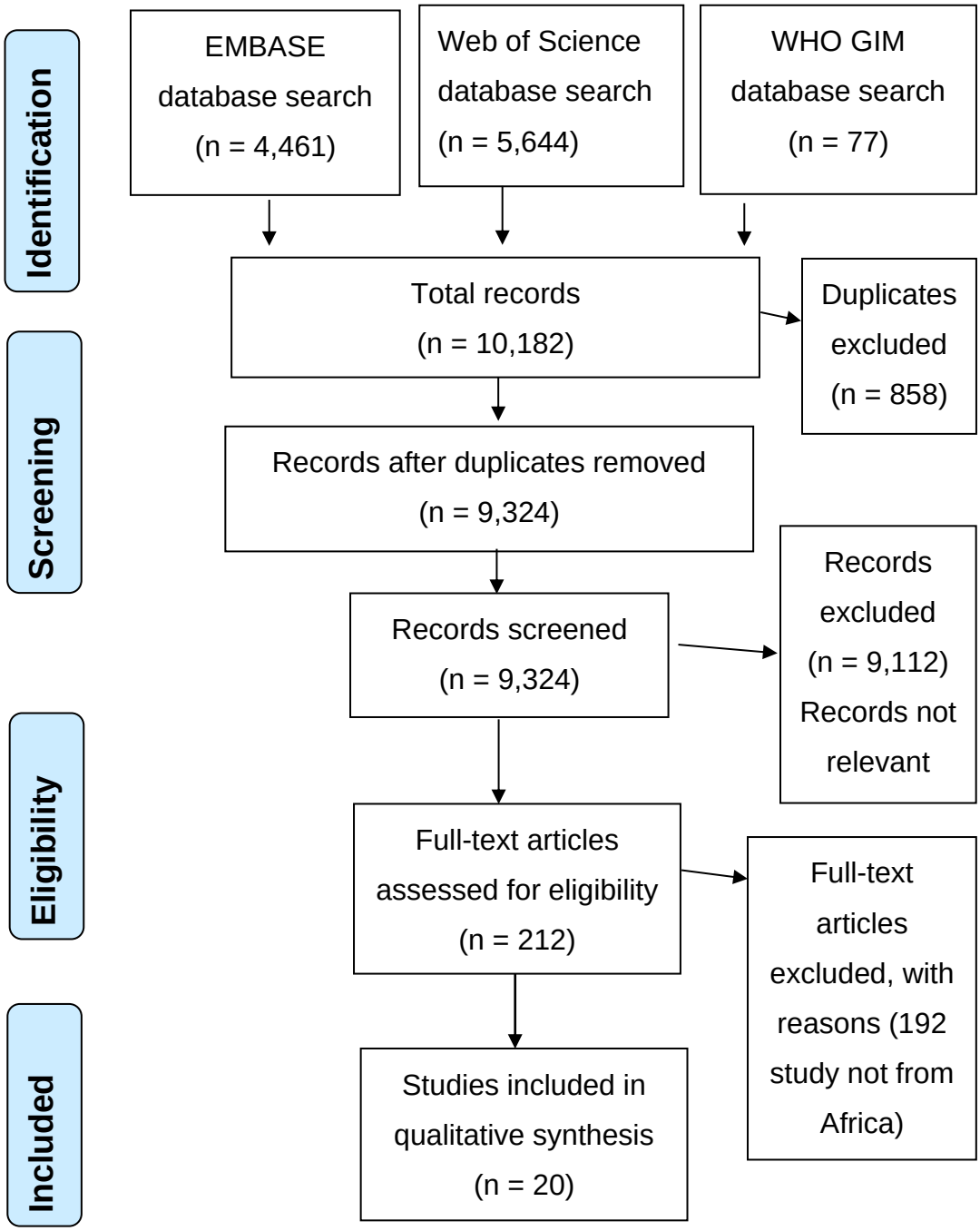
3.3 Results

This section covers the results of the literature search and the characteristics of the identified studies in terms of their study populations, study designs, sample sizes, risk factors investigated, their respective associations and statistical significance. It is split into two sections, section one presents the results of the literature search according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guide. Section two describes and summarises the identified literature on pre-eclampsia in Africa.

3.3.1 Results of the literature search

The search yielded 10,182 papers, of which 858 were duplicates, 9,112 were rejected as ineligible after a title and abstract screen and 192 after full-text screening. Twenty papers were included in the final analysis. The results of the literature search are summarised in figure 1.

Figure 1. PRISMA flow diagram of selected papers in scoping review.



3.3.2 Results of literature analysis

This section summarises the current published literature exploring the risk factors for pre-eclampsia in African populations. I identified 20 primary observational studies on risk factors for pre-eclampsia conducted in Africa, carried out in 11 countries out of the 54 countries that constitute the African region. Most of the studies (16 studies) were case-control by design. There were three cohort studies and one cross-sectional study. All twenty studies were small hospital-based studies covering a limited geographical area served by the health facility. There are no countrywide or multi-country studies identified.

The results are presented in three parts. Table one lists the studies included in the review, showing the country where the study was conducted and the study design. Table two summarises the risk factors explored by the twenty studies and their statistical significance. Finally, Table 3 shows the strength of association of the risk factors, confounding factors addressed and limitation of each of the twenty studies. The synthesis of the results is presented in the discussion.

Table 1: Summary of studies done in Africa.

Study code	Country	Study design	Author and Year	Title
S1	Sudan	Case-control	(Adam *et al.* 2011)	Malaria and pre-eclampsia in an area with unstable malaria transmission in Central Sudan
S2	Sudan	Case-control	(Elmugabil *et al.* 2016)	Serum calcium, magnesium, zinc and copper levels in Sudanese Women with pre-eclampsia
S3	Sudan	Case-control	(Adam *et al.* 2013)	Placenta praevia and pre-eclampsia: analyses of 1645 cases at Medani Maternity Hospital, Sudan
N1	Nigeria	Case-control	(Ajah *et al.* 2016)	The feto-maternal outcome of pre-eclampsia with severe features and Eclampsia in Abakaliki, Southeast Nigeria
N2	Nigeria	Case-control	(Anorlu *et al.* 2005)	Risk factors for pre-eclampsia in Lagos, Nigeria
E1	Ethiopia	Case-control	(Endeshaw *et al.* 2016a)	A family history of hypertension increases the risk of pre-eclampsia in pregnant women: a case-control study
E2	Ethiopia	Cross-sectional	(Tessema *et al.* 2015)	Pre-eclampsia and associated factors among pregnant women attending antenatal care in Dessie referral hospital, Northeast Ethiopia: a hospital-based study
E3	Ethiopia	Case-control	(Endeshaw *et al.* 2016b)	Obesity in young age is a risk factor for pre-eclampsia: a facility-based case-control study, northwest Ethiopia
G1	Ghana	Case-control	(Ephraim *et al.* 2014)	Serum calcium and magnesium levels in women presenting with pre-eclampsia and gestational hypertension: a case-control study in the Cape Coast metropolis, Ghana
T1	Northern Tanzania	Prospective cohort.	(M J. Mahande *et al.* 2013)	Recurrence of pre-eclampsia in northern Tanzania: A registry-based cohort study
ZA1	Zambia	Cohort	(George, 2015,)	Association between HIV highly active antiretroviral therapy and pre-eclampsia at

Study code	Country	Study design	Author and Year	Title
				the university teaching hospital Lusaka Zambia
Z1	Zimbabwe	Case-control	(Mahomed *et al.* 1998)	Risk factors for pre-eclampsia among Zimbabwean women; maternal arm circumference and other anthropometric measures of obesity
D1	DR Congo/ Zaire	Case-control	(Elongi Moyene *et al.* 2016)	Pre-eclampsia and toxic metals; a case-control study in Kinshasa, DR Congo
SN1	Senegal	Case-control	(Ndao *et al.* 2009)	Placental malarial infection as a risk factor for hypertension disorders during pregnancy in Africa: A case-control study in the urban area of Senegal, West Africa
SN2	Senegal	Case-control	(Sartelet, 1996)	Malaria associated pre-eclampsia in Senegal
SA1	South Africa	Cohort	(Frank *et al.* 2004)	Does human immunodeficiency virus infection protect against pre-eclampsia-eclampsia?
SA2	South Africa	Case-control	(Nieuwoudt *et al.* 2014)	Pregnancy outcomes in super-obese women – an even bigger problem? A prospective cohort study
SA3	South Africa	Case-control	(Richards *et al.* 2014)	A comparison of maternal calcium and magnesium levels in preeclamptic and normotensive pregnancies: an observational case-control study
SA4	South Africa	Case-control	(Mammen, 2005)	Glucose tolerance in rural women with pre-eclampsia
U1	Uganda	Case-control	(Kiondo, 2012)	Risk factors for pre-eclampsia in Mulago Hospital, Kampala, Uganda

Table 2 shows the risk factors by study and country. It also indicates whether a statistically significant association was found between the risk factor and pre-eclampsia. Forty-five different risk factors have been explored in relation to pre-eclampsia in African populations. Most of the studies show an inconclusive association between risk factors and pre-eclampsia in the different countries where they were explored i.e. a risk factor showed association in one study but failed to show association in another study. The small number of risk factors that were investigated in more than one country show conflicting results. The inconclusive and conflicting results may be due to inherent differences in study populations or it may be due to insufficient power in detecting a statistically significant difference.

Table 2. Pre-eclampsia risk factors examined by country.

Study code	Total	S1	S2	S3	N1	N2	E1	E2	E3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Hx of Hypertension	**3✓**					✓					✓										✓
Hx of pre-eclampsia	**3✓**					✓					✓	✓									
Hx of diabetes	**2✓1x**						✓				✓									x	
Hx of Malaria	**1✓2x**	✓								x					x						
Placental Malaria	**2✓1x**	✓													x	✓					
HIV	**2x**											x					x				

Study code	Total	S1	S2	S3	N1	N2	E1	E2	E3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
UTI	**1✓**						✓														
Periodontal diseases	**1✓**						✓														
Anaemia	**3✓1x**		✓	✓			✓		x												
Folate use	**3✓**						✓		✓												
Contraceptive use	**1x**									x											
Serum Calcium	**3✓**		✓							✓									✓		

Study code	Total	S 1	S 2	S 3	N 1	N2	E 1	E 2	E 3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Serum Magnesium	**3✓**		✓							✓									✓		
Serum Zinc	**1x**		X																		
Serum Copper	**1x**		X																		
Urine Lead level	**1✓**													✓							
Placenta Praevia	**1✓**			✓																	
Family Hx of Hypertension	**4✓**	✓					✓	✓							✓						

Study code	Total	S1	S2	S3	N1	N2	E1	E2	E3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Family Hx of Diabetes	1✓							✓													
BMI	4✓3x	x	x			✓				x	✓		✓					✓			
MUAC	2✓								✓				✓								
Plasma Vitamin C	1✓																				✓
Fruit intake	2✓								✓										✓		
Vegetable intake	1x								x												
Meat intake	1x								x												

Study code	Total	S 1	S 2	S 3	N 1	N2	E 1	E 2	E 3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Coffee intake	**1✓**								✓												
Exercise/work during pregnancy	**3x**					x	x		x												
Blood Group	1x	x																			
Antenatal care	**4✓2x**	✓		✓	✓		x				x				✓						
Education level	**5✓5x**	x		✓	✓	x	x	x	✓		✓				x						✓
Hx of Abortion	**1x**									x											

Study code	Total	S1	S2	S3	N1	N2	E1	E2	E3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Hx of past poor obstetric outcome	**1✓**														✓						
Primiparity	**4✓5x**	✓	x	✓	✓		x	x		x		✓			x						
Partner change	**2✓2x**					x	✓			x					✓						
Age	**5✓6x**	x	x	✓	x	x	✓	✓	✓	x	✓				x						
Twins/triplets pregnancies	**3✓**					✓	✓								✓						
Smoking	**1✓**																	✓			

Study code	Total	S1	S2	S3	N1	N2	E1	E2	E3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Alcohol	**1✓1x**						✓		x												
Bed nets use	**2x**	x													x						
Urban/rural residence	**3✓1x**				✓		✓		✓						x						
Occupation	**1✓3x**				✓	x	x		x												
Stressful environment	**1✓**					✓															
Poly/Monogamy marriage	**1x**				x																

Study code	Total	S1	S2	S3	N1	N2	E1	E2	E3	G1	T1	ZA1	Z1	D1	SN1	SN2	SA1	SA2	SA3	SA4	U1
Country		Sudan			Nigeria		Ethiopia			Ghana	Tanzania	Zambia	Zimbabwe	DR Congo/Zaire	Senegal		South Africa				Uganda
Marital status	**1✓3x**						x	✓	x						x						
Income	**1✓2x**						x		x						✓						

Key: ✓ = Statistically significant association found. X = Risk factor investigated but statistically significant association not found

Table 3 Results of studies showing the association of risk factors to pre-eclampsia in the African region.

These studies have shown the strength of the association of various risk factors with pre-eclampsia. Appropriate study design and analysis methods were used to measure association while controlling for some confounders. There are limitations from potential biases, measurement errors, misclassifications, chance and power due to small sample size to enable accurate estimate in multivariable risk factors analysis.

Study code	Sample size	Results	Comments
S1	143 cases/143 control	In multivariable analysis family history of hypertension (OR = 5.7, 95 % CI = 2.9 - 11.5; P = 0.001) and placental malaria (OR = 2.3, 95 % CI = 1.0 - 5.2; P = 0.04) were significantly associated with pre-eclampsia. Univariable analysis; primigravidae, lack of ANC, history of malaria was associated with pre-eclampsia	They excluded diabetes and twins. No associations between age, parity, educational level, lack of antenatal care, blood groups and body mass index and pre-eclampsia
S2	24913 deliveries. Case 50 control 50.	In binary logistic regression, lower calcium (OR = 0.73, 95 % CI = 0.56 — 0.95, P = 0.021) and higher magnesium (OR = 5.72, 95 % CI = 1.23 — 26.5, P = 0.026) levels were associated with pre-eclampsia	The possible interactions between trace elements and their carrying vehicle were not considered. Women with diabetes mellitus, other endocrine disorder, and kidney disease were excluded from both cases and controls. Temporal relations not possible to establish.

Study code	Sample size	Results	Comments
S3	1645cases/1645 controls.	Women with age > 35years (OR = 1.4, 95 % CI: 1.1 – 1.8), primiparity (OR = 3.3, 95 % CI: 2.7 – 4.0), parity > 5 (OR = 3.1, 95 % CI: 2.4 – 4.0), and anaemia (OR = 3.3, 95 % CI: 2.8 – 3.9). The risk of pre-eclampsia was inversely increased with education level and prenatal care attendance. The prevalence of placenta praevia was 0 (0 %) and 55 (3.3 %), P < 0.001 in preeclamptic and control women, respectively. Placenta praevia was a significant protective factor of pre-eclampsia (OR = 0.3, 95 % CI: 0.1 –0.7).	They excluded diabetes and twins. Did not study, history of hypertension, nor obesity. No category of severe anaemia (<7g/dl). Placenta praevia had zero cases.
N1	No prior sample calculated .207 cases/207 control	13750 births recorded. The prevalence of pre-eclampsia with severe features and eclampsia were 136 (0.99 %) and 104 (0.76 %) respectively. Pre-eclampsia with severe features and eclampsia was more common among adolescents, rural dwellers, poorly educated, unemployed, not booked to a clinic and nulliparous women. It was more associated with preterm delivery, caesarean section, low birth weight babies, maternal and perinatal mortality	The study focused on severe pre-eclampsia and eclampsia. Not pre-eclampsia per se. The association found were from a univariable analysis. No adjustment was done for the odds ratio, a regression model was not applied.
N2	Cases 128 control 240	Out of the 1803 women who delivered during the period 137 (7.6 %) had pre-eclampsia/ eclampsia. Of these, 128 (93.4 %) were analysed. Primigravidae were 91 (71.1 %). Age ≤ 19 years was not considered a risk factor. The risk factors that were associated with increased risk of pre-eclampsia were: nulliparity (OR = 4.77; 95 % CI 2.90 – 7.78), stressful work during pregnancy (OR = 2.10; 95 % CI 1.20 – 3.71), stressful home	No meaningful limitation.

Study code	Sample size	Results	Comments
		environment (OR = 1.97; 95 % CI 1.27 – 3.69). Furthermore, previous pre-eclampsia (OR = 11.68; CI 3.81 – 37.61), history of chronic hypertension (OR = 2.21; 95 % CI 1.17 – 6.20), a body weight greater than 80 kg (OR = 2.01; 95 % CI 1.05 – 3.87) and multiple pregnancy (OR = 2.71; 95 % CI 1.27 – 6.13).were also associated with pre-eclampsia.	
E1	151 cases/302 control	Pre-eclampsia was associated with: Advanced maternal age (Adjusted odds ratio (AOR) = 4.79; 95 % CI 1.031 - 22.18), family history of hypertension (AOR = 11.16; 95 % CI 5.41 - 41.43), history of diabetes mellitus (AOR = 6.17; 95 % CI 2.11 - 20.33), UTI in the current pregnancy (AOR = 6.58; 95 % CI 2.93 - 14.73). In addition, failure to comply with iron and folic acid supplement during pregnancy (AOR = 8.32; 95 % CI 3.35 - 20.62), lack of exercise (AOR = 3.33; 95 % CI 1.35 - 8.17), multiple pregnancy (AOR = 4.05; 95% CI 1.57 - 12.27), anaemia (AOR = 4.19; 95 % CI 1.27 - 13.92), and periodontal disease or gingivitis (AOR = 3.51; 95 % CI 1.14 - 10.83) were associated with pre-eclampsia.	No meaningful limitation.
E2	Cross-sectional 490 recruited	Incidence (41/490) 8.4 %. Family history of chronic hypertension (AOR = 7.19, 95 % CI 3.24 - 15.2) Past history of hypertension (AOR = 4.3, 95 % CI 1.33 - 13.9) Family history of diabetes (AOR = 2.4, 95 %CI 1.09 - 5.6), age > 35 (AOR = 4.5, 95 % CI 1.56 - 12.8) being unmarried (AOR = 3.03, 95 % CI 1.12 - 8.2)	BMI was not assessed. Recruited antenatal attendees, hence prone to survival bias.

Study code	Sample size	Results	Comments
E3	151 cases/302 control	The odds of pre-eclampsia were higher among obese (mid-upper arm circumference (MUAC) ≥ 25 cm) women than their leaner counterparts (AOR = 3.33, 95 % CI: 1.87 - 5.79).	The study used MUAC to determine obesity, not BMI. Survivor selection bias, temporal relation not certain in case-control, MUAC may not reflect weight status at conception.
G1	380 sampled (160 GH, 100 PE and 120 Normal)	Mean concentration of Mg and Ca were both significantly lower in GH and PE than the control group (p<0.001). Age > 40 was significantly associated with GH and not PE in a multivariable analysis that included (abortion, malaria, parity, paternity, gravidity, BMI, contraception, age) not Ca or Mg concentration.	Ca and Mg level may vary with gestation age hence they should have controlled it. Controls were matched for age, consecutive sampling limits generalisation. Women with; chronic hypertension, on antihypertensive drugs, diabetes, renal and autoimmune diseases were excluded.
T1	19,811singleton deliveries, 3,909 recurrence deliveries	The absolute recurrence risk of pre-eclampsia was (42/171) 24.6 %, non-recurrence risk (103/4332) 2.4 %, which amounted to a 9.2 fold relative risk (95 % CI: 6.4 - 13.2). Other 1st pregnancy characteristics lead to pre-eclampsia recurrence: chronic hypertension (RR = 8.9; 95 % CI: 5.7 - 13.8), gestational hypertension (RR = 9.8; 95 % CI: 4.9 - 19.1) and diabetes mellitus (RR = 8.4; 95 % CI: 2.7 - 26.3).	Excluded twins, excluded distant referrals. Had follow up completion rate of 58 %
ZA1	824 Cohort	177 developed pre-eclampsia, 122 were HIV positive, 104 were on HAART. Neither HIV status (OR = 0.44; 95 %CI 0.1 - 3.2) nor HAART use (OR = 0.99; 95 % CI 0.1 - 8.9). Pre-eclampsia was associated with	No meaningful limitation.

Study code	Sample size	Results	Comments
		primiparity (OR = 1.7; 95 % CI1.1 - 2.7), previous history of pre-eclampsia (OR = 3.65; 95 % CI 2.1 - 6.4)	
Z1	144cases/ 144 control	Women with the highest quantile of mid-upper arm circumference (MUAC) (28-39) were at 4.4 times more likely to have their pregnancy complicated with pre-eclampsia than women in lower quantile (21-23) cm. Significant P values for trends in BMI, height, weight and MUAC with pre-eclampsia. We would expect height to be inversely related	Not able to control gestational diabetes, also no pre-pregnancy BMI, hence used MUAC.
D1	88 Pre-eclampsia (34 rainy and 54 dry season) and 88 controls (33 rainy and 55 dry seasons)	Artisanal activities had a significant association with pre-eclampsia (OR = 2.34, 95 % CI 1.13 – 4.85, p = 0.02) and it was more in pre-eclampsia than in control. The daily urinary excretions of 14 metals were significantly higher in women with pre-eclampsia than in control women, e.g. for lead: 61 µg/day (25th–75th percentile 8–345) in women with pre-eclampsia vs 9 µg/day (25th–75th percentile 3–21) in controls (p<0·001). A significant interaction was found between season and pre-eclampsia for several elements, with higher urinary excretions in pre-eclampsia women than controls during the dry season, but not during the rainy season.	Primigravidae were excluded. Pre-eclampsia cases were admitted pts while controls were outpatients. Exposure to lead certainly varies between groups. Case-control limits temporal relationship establishment. (No data of urinary metal excretion before third trimester). Did not use serum levels of metals, instead used urinary excreted levels. No environmental data on exposure concentration in the general population. Did not control for dietary intake exposure (heavy metal).

Study code	Sample size	Results	Comments
SN1	223 cases/240 control	In multivariable analysis, Placental malaria infection (PMI) appeared to be an independent risk factor for gestational hypertension (adjusted odds ratio 2.7, 95 % confidence interval: 1.0, 7.6). PMI associated with non proteinuric hypertension and not with pre-eclampsia or eclampsia	No significant association observed for pre-eclampsia and eclampsia. Inadequate sample size calculated due to underestimation of prevalence (used 10 % but actual was 5 %). However, Univariable analysis neither showed significance association
SN2	32 cases and 220 controls	Placentas were infected in 53 % (17 of 32) of cases and 27 % (60 of 220) of controls (OR = 3.0, 95 % CI 1.3 - 6.9). After adjustment for the effects of age, the number of previous pregnancies, twin deliveries, maternity centre, and date of delivery in a logistic regression model analysis, the estimated odds ratio was 3.3 (95 % CI 1.1 - 9.5). Pre-eclampsia was significantly associated with malaria infection during pregnancy ($p < 0.03$) and 69.7 % of cases of pre-eclampsia with infected placenta might be attributable to malaria infection.	No meaningful limitation.
SA1	Cohort 2600 total (1896 HIV- by 704 HIV+)	The rates of pre-eclampsia-eclampsia were 5.2 % (98/1,896) in HIV negative and 5.7 % (40/704) in HIV positive women (P = 0.61). HIV infection not associated with pre-eclampsia rate.	Mantel Haenszel stratified analysis was performed to identify confounding explanatory variables and examined the influence of age less than 30 years, primiparity, weight less than 60 kg, haemoglobin level less than 10 mg/dL, clinic delivery, and delivery at less than 37 weeks, on the development of proteinuric hypertension. Only gestational age at delivery proved to be confounding.

Study code	Sample size	Results	Comments
			Did not control for CD4 level which could confound the association
SA2	Case-control 66morbid obese/46 super obese	The incidence of pre-eclampsia was significantly higher in the super obese group than in the morbidly obese group (23.9 % v. 9.1 %; p = 0.03). In the super obese group, 5 (10.8 %) had early onset pre-eclampsia compared with 2 (3.0 %) in the morbidly obese group; similar findings were observed for late onset pre-eclampsia (6 (13.0 %) and 4 (6.0 %), respectively).	No comparison with normal weighted pregnant women
SA3	96 cases/96 control	There was a significant difference between pre-eclampsia and control in their concentration of serum Ca and Mg although the means ± Standard Error in each was still in the normal range value for Ca and Mg serum levels. No difference was seen in the hair concentration of Ca and Mg, which indicates a long-term concentration of the elements. This finding refutes the current belief that the mechanism by which calcium supplementation reduces the risk of developing pre-eclampsia is by correcting a pre-existing nutritional deficiency	The normal level of Ca in hair is not known (No reference values). Using this test of Ca levels in hair we don't know how far back Ca was stable if the hair is tested now.
SA4	117 cases and 94 controls.	Oral glucose tolerance test was similar to both groups. Post load incremental glucose area under the curve in the pre-eclampsia group (4.16 ± 0.21) was similar to that in the normotensive group (3.95 ± 0.21, p = 0.495).	The study groups were similar in age, parity and gestational age. The pre-eclampsia group had a significantly higher body weight and body mass index (BMI).

Study code	Sample size	Results	Comments
U1	207 cases and 352 controls.	The risk factors were low plasma vitamin C (OR = 3.19, 95 % CI: 1.54 – 6.61), low education level (OR = 1.67, 95 % CI: 1.12 – 2.48), chronic hypertension (OR = 2.29, 95 % CI 1.12 – 4.66), family history of hypertension (OR = 2.25, 95 % CI: 1.53 – 3.31) and primiparity (OR = 2.76, 95 % CI: 1.84 – 4.15) and para > 5 (3.71, 95 % CI: 1.84 – 7.45). Alcohol use, Hx of Diabetes, age, Socioeconomic status, distance to hospital, smoking, HIV were not associated with pre-eclampsia	No meaningful limitation.

The above risk factors in table 3 can be grouped into seven major factors a) to f) summarised below.

Sociodemographic factors

Advanced maternal age was found to have a statistically significant association with pre-eclampsia in four case-control and one cohort studies (Adam *et al.* 2013, M J. Mahande *et al.* 2013, Tessema *et al.* 2015, Endeshaw *et al.* 2016a, Endeshaw *et al.* 2016b).

Primiparity was found to have a statistically significant association with pre-eclampsia in four case-control study (Adam *et al.* 2011, Adam *et al.* 2013, George *et al.* 2015, Ajah *et al.* 2016).

High parity and being unmarried were found to have a statistically significant association with pre-eclampsia in a case-control study in Ethiopia (Tessema et al. 2015).

Familial and medical factors

Family history of hypertension was found to have a statistically significant association with pre-eclampsia in two case-controls and one cohort study. (Adam *et al.* 2011, Tessema *et al.* 2015, Endeshaw *et al.* 2016a).

History of diabetes mellitus was statistically significant on a cohort study of recurrent pre-eclampsia in Tanzania and a case-control study in Ethiopia (M J. Mahande *et al.* 2013, Endeshaw *et al.* 2016a).

Lack of exercise and stressful environment were found to have a statistically significant association with pre-eclampsia in a case-control study in Nigeria (Anorlu et al. 2005).

Infections

Placental malaria was found to have a statistically significantly association with pre-eclampsia in two case-control studies in Senegal and Sudan (Sartelet *et al.* 1996, Adam *et al.* 2011) However, malaria was not found to be statistically significant associated with pre-eclampsia in a case-control in Ghana (Ephraim et al. 2014).

Urinary tract infection (UTI) in pregnancy was found to have a statistically significant association with pre-eclampsia in a case-control study in Ethiopia. (Endeshaw et al. 2016a).

Periodontal disease is inflammation of gums, it was found to have a statistically significant association with pre-eclampsia in a case-control study in Ethiopia (Endeshaw et al. 2016a).

Micronutrient factors

Low serum calcium and magnesium levels were found to have a statistically significant association with pre-eclampsia in three case-control studies (Ephraim et al. 2014, Richards et al. 2014, Elmugabil et al. 2016).

Environmental factors

Heavy metals pollution such as Lead was found to have a statistically significant association with pre-eclampsia in a case-control study D.R Congo/Zaire (Elongi Moyene et al. 2016).

Nutritional factors

Anaemia was found to have a statistically significant association with pre-eclampsia in three case-control studies (Adam et al. 2013, Elmugabil et al. 2016, Endeshaw et al. 2016a)

Obesity was found to have a statistically significant association with pre-eclampsia in three case-control studies (Zimbabwe, South Africa and Nigeria) and one cohort study in Tanzania (Mahomed *et al.* 1998, Anorlu *et al.* 2005, M J. Mahande *et al.* 2013, Nieuwoudt *et al.* 2014).

Evidence from cohort studies revealed the following findings. Two cohort studies had explored HIV and found no association between HIV and pre-eclampsia (Frank et al. 2004, George et al. 2015). Another cohort study explored the recurrence of pre-eclampsia where previous pregnancy experience of pre-eclampsia, chronic hypertension, gestational hypertension and diabetes mellitus were associated with increased risk of pre-eclampsia in subsequent pregnancies (M J. Mahande *et al.* 2013).

One study examined pre-eclampsia with severe features and eclampsia in Nigeria, where it showed severe pre-eclampsia and eclampsia were more

common among adolescents, rural dwellers, poorly educated, unemployed, unbooked antenatal visits and nulliparous women (Ajah et al. 2016).

3.4 Discussion

My scoping review has revealed a relatively small number of published papers exploring risk factors for pre-eclampsia in the African region. The list of risk factors explored was extensive, but the measures of association were not consistent in their statistical significance across studies from different countries. Lack of power in the small studies may have resulted in the inconclusive results observed in this review. The inconclusive evidence and small study sizes presented by some studies have led me to identify potential areas for carrying out a systematic review study to aggregate results. Most of the identified risk factors with inconclusive results have important clinical relevance since they affect a large proportion of the population. These results also underscore the need for doing more research in this area to build the epidemiological evidence base from robust study designs.

The African region has a diverse ethnic population (over 115 ethnic groups) that is underrepresented based on my findings (Shoup 2011). My scoping review identified 20 papers from only eleven countries out of the 54 counties in the African continent. This suggests that the continent is underrepresented by the findings of these few represented countries. Africa has the highest global rates of maternal and infant mortality, yet few studies have been done to understand risk factors for pre-eclampsia, which is one of the major

contributors to maternal mortality (WHO 2016). This underscores the importance of conducting further research on this topic in the African region.

The susceptibility to developing pre-eclampsia is thought to vary across genetically diverse populations (Williams and Morgan 2012). Hence, the findings of these few studies may not be generalizable to the diverse subpopulations in the continent. Most of the papers used a case-control design. This design has several advantages, including being relatively cheap and quick to conduct and being well suited to exploring multiple risk factors simultaneously; however, it is unable to establish a temporal relationship between the exposure/risk factor and the outcome disease. A temporal relationship is the sequential timing of the occurrence of the risk factors prior to the disease; it is important evidence in establishing causality. Sixteen out of the twenty papers were case-control by design, thus limiting the affirmation of the temporal relationship. This choice of design may also be a result of weakness in health information systems to enable cohort design studies from data collected routinely in antenatal services.

Cohort studies produce a higher quality of evidence due to the ascertainment of the temporal relationship between the risk factor/exposure and the outcome. I identified two cohort studies that explored HIV as a risk factor and found no association with pre-eclampsia. The other cohort study explored the recurrence rate of pre-eclampsia. In this study, recurrence of pre-eclampsia in subsequent pregnancies was associated with a history of pre-eclampsia,

chronic hypertension, gestational hypertension and diabetes in the previous pregnancy.

The results from table 2 show the different risk factors that have been examined in different countries. The table shows risk factors that were explored and the ones that were statistically significant in their association with pre-eclampsia. There is inconsistency in the significance of some key risk factors for pre-eclampsia such as obesity and malaria. Not all countries where obesity or malaria was examined showed consistency in whether there was a statistically significant association with pre-eclampsia. This may be due to variation in ethnic susceptibility towards these risk factors across the different populations. Another possible explanation could be methodological shortfalls of the studies such as small sample size. This observation warrants large studies to be done to confirm or refute these findings.

Severe anaemia has been associated with pre-eclampsia (Adam *et al.* 2011, Elmugabil *et al.* 2016, Endeshaw *et al.* 2016b). However, we do understand that anaemia is often caused by underlying conditions such as malaria and other haemolytic infections. Therefore, malaria may be the underlying condition that is causally linked to pre-eclampsia. To explore this further, in the next chapter I will present the results of a systematic review study investigating the direct association between malaria and pre-eclampsia.

Malaria infection is a potentially important risk factor in the region. Malaria is very common during pregnancy and thus affects a large population of

pregnant women in African countries. If malaria is found to be associated with pre-eclampsia, it may turn out to be a significant contributing factor to the burden of pre-eclampsia in Africa, due to the high prevalence of malaria in the region (Desai *et al.* 2007). In this scoping review, I have identified the possible association between malaria and pre-eclampsia to be a potential area for conducting a systematic review and a meta-analysis to aggregate evidence from different small studies to establish their association.

Unlike malaria infection, family history of hypertension, a diagnosis of diabetes mellitus and low serum levels of calcium and magnesium have consistently been shown to be associated with the development of pre-eclampsia in different studies. Antenatal care has been shown to have a protective role in developing pre-eclampsia, although not consistently in all the studies. Where there is consistency in the results across studies, the findings can be used to inform maternal health strategies. However, larger studies may still be required to confirm these observations.

The strength of this study is that it provides transparency, reproducibility and an unbiased review of the literature, as a result of adopting the scoping literature review methodology, which also gives flexibility in focusing or widening the search scope based on the results obtained. Initially, I used a wide search strategy that retrieved all papers that examined risk factors for pre-eclampsia across the globe and later selected papers from the African region. This approach enabled me to target the population of interest in my study without bias from the retrieved papers.

A potential limitation is that my search strategy only covered EMBASE, Web of Science, WHO-GIM databases, the inclusion of more databases like MEDLINE may have yielded more articles.

A limitation is in the few numbers of identified papers in Africa that restrict the generalization to the whole region. This may partly be a result of restricting to English language articles. However, this may not have made a substantial difference, as only approximately 2 % of retrieved papers were not in English. Another important limitation is that only peer-reviewed, published papers were included. A more comprehensive approach would involve a grey literature search and hand searching the reference lists of key papers. The reason this was not undertaken for this scoping review is that the purpose was to carry out a rapid review of the literature in order to inform a more in-depth and comprehensive systematic review.

There are limitations in the comparison of evidence presented by these papers due to differences in selection criteria of the study populations. Some studies excluded groups such as twin pregnancies and women with diabetes mellitus or chronic hypertension. The variations of study populations across studies due to different recruitment criteria limit the comparison for the observed association of some risk factors across study populations.

Another limitation is that some studies did not adjust the association for possible confounding factors. A lack of recorded data for important potential confounding variables, possibly because of weak health information systems,

could have led to failures in making necessary adjustments for the examined associations.

There were limitations in the range of risk factor variables that were examined in the cohort studies. Two cohort studies in South Africa and Zambia had both explored HIV, which showed non statistically significant association with pre-eclampsia. Of all risk factors from African based studies, HIV is the only risk factor that has been explored using a cohort design that is able to establish a temporal association between exposure and outcome. Most of my retrieved articles were case-control and thus faced this methodological pitfall.

Most of the papers were not able to categorise the examined risk factors based on early and late onset pre-eclampsia. This shortfall may have contributed to misclassification of the risk factors and hence underestimation of the association. There is also a potential for these studies to have misclassified pre-eclampsia outcome with other HDP. Table 3 shows the sample size, the measure of association and the comments of the study's results. Most of the case-control studies were small studies, with insufficient power in the multivariable analysis, as reflected in the wide confidence intervals of some multivariable analysis results. Larger and focused studies for early and late pre-eclampsia could generate results with improved validity.

The scoping review study on risk factors for pre-eclampsia in Africa identified several gaps and highlighted the feasibility for a systematic review. The first

gap observed was that the association of malaria infection and pre-eclampsia was not conclusive from the retrieved studies. Based on the observed gaps from the scoping review, I decided to address this gap by carrying out a systematic review to examine the association of malaria and pre-eclampsia (see Chapter Four). The second gap observed was that most studies did not differentiate between early and late onset pre-eclampsia in designing their analysis to identify risk factors for pre-eclampsia in Africa. In addressing this gap, I planned to conduct a secondary analysis study in one of the LMIC settings, taking into account early and late onset pre-eclampsia in identifying risk factors. Another observed gap is that none of the studies had attempted to build prediction models using identified risk factors to predict the outcome of pre-eclampsia. I, therefore, decided to explore biomedical risk factors in relation to early and late onset pre-eclampsia. A woman's biomedical parameters can conveniently be measured in hospital settings. In addition, these parameters are often recorded in patient notes. Thus, in future, these parameters may be used to develop prediction models that can easily be applied in hospital settings, unlike models that incorporate complex, expensive and hard to measure biomarkers as predictors. These more complex prediction models are not feasible in resource-poor settings in LMICs.

3.5 Summary

A history of hypertension, previous pre-eclampsia pregnancies and low levels of serum calcium and magnesium have consistently been shown to be associated with the development of pre-eclampsia across studies. Interventions could target these risks to improve screening detection and focused prevention services.

Larger studies examining many variables are needed in Africa to address the gaps of scarcity of evidence in this area. So far, there are few countries where the risk factors for pre-eclampsia have been studied, mostly using a case-control design. Further studies using cohort design would improve our understanding of the risk factors and their temporal relationship with pre-eclampsia.

Malaria infection is a potential risk factor for the development of pre-eclampsia. However, evidence from small studies has not been conclusive. In the next chapter, I explore this relationship further by conducting a systematic review and meta-analysis to aggregate the available evidence from observational studies.

It is relevant to analyse risk factors under the consideration of early and late onset pre-eclampsia to avoid potential misclassification. Most previous studies were unable to do so; my secondary data analysis in Tanzania aims to address this issue (see Chapter Five).

Chapter 4 Systematic review and meta-analysis on the association of malaria infection and gestational hypertension.

4.1 Background

Hypertensive disorders of pregnancy account for 14 % of global maternal mortality (Say *et al.* 2014). Pre-eclampsia and gestational hypertension (GH) are closely related subcategories of hypertensive disorders of pregnancy. Pre-eclampsia is characterised by a rise in blood pressure and protein loss in urine (proteinuria), with an onset at 20 weeks of gestation and onwards. GH is also characterised by a rise in blood pressure, with an onset of 20 weeks of gestation and onwards, but without proteinuria. The main difference between pre-eclampsia and GH is thus that pre-eclampsia involves multi-organ damage, characterised by the proteinuria feature. As explained in Chapter Two of this thesis, however, the fact that a significant proportion of women develop severe pre-eclampsia without proteinuria being detectable (Barton *et al.* 2001). This finding prompted the ISSHP to revise its definition of pre-eclampsia to include the presence of severe features with or without proteinuria (Tranquilli *et al.* 2014).

The aetiology of these conditions is not clearly understood; so far, there are several theories for their occurrence and different risk factors and mechanisms of action are being explored, as described earlier in this thesis. The scoping review in the previous chapter summarises the observational

epidemiological research evidence on pre-eclampsia risk factors in African populations. This identified inconclusive evidence on the association between malaria infection and pre-eclampsia. Some of these studies also showed that malaria infection was associated with GH. This suggests that malaria infection is a shared risk factor for both pre-eclampsia and GH. This understanding tallies with other studies that have shown that pre-eclampsia and GH share cardiovascular risk factors (Egeland *et al.* 2016). Following these observations, I set out to conduct a systematic review and a meta-analysis with the aim of aggregating available evidence on the relationship between malaria infection and the combined outcome of pre-eclampsia and GH.

Malaria is a protozoan blood infection that primarily affects and destroys red blood cells thus causing a release of endotoxin that triggers an inflammatory reaction (Verdecchia *et al.* 2016). Malaria is a common disease in tropical countries that accounted for 405,000 deaths globally (WHO 2019b). Over 11 million pregnant women are estimated to be exposed to malaria infection in 2018 while about 24 million children were also infected with malaria (WHO 2019b). In 2017, it was estimated to cause 34,000 (95 % CI 20,000 - 53,000) maternal death among 15 - 49 years females in Sub-Sahara Africa (IHME 2017). Malaria alone is estimated to cause 2.1 million deaths (95 % CI 1.6 – 3.2) of 1 - 49 month children globally (Liu *et al.* 2016). Its potential contribution as a risk factor to pre-eclampsia and GH deserves attention, in order to reduce pregnancy related mortality and morbidity.

What mechanisms have been proposed linking malaria infection with pre-eclampsia and GH? To date, a dysfunctional placenta is the basis of most theories for the cause of pre-eclampsia (Ahmed and Ramma 2015). Malaria infection may develop into placental malaria during pregnancy. Placental malaria changes the environment in the intervillous space of placenta. This occurs as a result of malaria infected erythrocytes binding to syncytiotrophoblast, a specialized epithelial layer that covers the interior of the villous of the placenta. Malaria infected erythrocytes bind on ST receptors known as chondroitin sulphate A (CSA) and hyaluronic acid. This binding leads to the sequestration of infected erythrocytes in the intervillous space of the placenta. Sequestration leads to secretion of chemokines resulting in inflammatory cells recruitment and cytokine production which is associated with poor pregnancy outcomes (Kidima 2015). Several studies suggest that placental malaria infection is associated with pre-eclampsia and GH, the hypothesised mechanism being that of a dysfunctional placenta affected by malaria parasites (Ndao *et al.* 2009, Adam *et al.* 2011).

This is not, however, the only proposed mechanism linking malaria to GH and pre-eclampsia. A newly proposed mechanism hypothesises that malaria infection may cause hypertension via inflammatory reaction on the endothelial lining in blood vessels. It is hypothesised that inflammatory processes on the endothelium due to malaria infection may play a role in causing hypertension (Verdecchia *et al.* 2016). Studies in Asia that examined maternal serum iron and ferritin have suggested oxidative stress caused by

free iron radicals may play a role in causing pre-eclampsia (Rayman *et al.* 2002, Zafar and Iqbal 2008). Thus, the inflammatory and oxidative stress due to malaria toxin and red blood cell (RBC) destruction may lead to pre-eclampsia. We also know that malaria infection on its own may induce proteinuria (Ehrich and Horstmann 1985). Therefore, an alternative pathway mechanism may exist where malaria infection during pregnancy may be causing both hypertension and proteinuria, which are the main features that define pre-eclampsia, without placental pathway involvement. It is also possible that malaria infection will only induce hypertension without proteinuria, to resemble gestational hypertension.

In this regard, it is worth examining the association of malaria and hypertension during pregnancy (with or without proteinuria) to identify its total effect on the burden of hypertensive disorders of pregnancy. From this point onwards in this chapter, I have regarded gestational hypertension to imply the combination of both, pre-eclampsia and gestational hypertension proper i.e. high blood pressure with an onset from 20 weeks of gestation without proteinuria. Therefore, my systematic review and meta-analysis regards my outcome of gestational hypertension to imply both conditions hypertension without proteinuria or with proteinuria (pre-eclampsia).

Earlier studies suggest an association between malaria infection in the rainy season and increased maternal mortality due to pre-eclampsia (Etard *et al.* 2003). Women are prone to develop malaria throughout pregnancy, but the peak period seems to occur before the onset of pre-eclampsia (Brabin 1983,

Moore *et al.* 2017). The peak incidence of malaria during pregnancy has been shown to occur at 13 -16 weeks of gestation, which is prior to the onset of the GH which commences at 20 weeks of gestation onwards (Brabin 1983). In Thailand, the peak of initial malaria infection occurred at 5 – 6 gestation weeks while the peak for recurrent malaria infection occurred at 14 weeks for falciparum malaria and 20 for vivax malaria (Moore *et al.* 2017). This sequential timing of the malaria risk factor and the occurrence of the disease suggests a temporal relationship between the risk factor and the outcome.

Malaria infection could be an important contributing factor to GH in tropical countries where malaria is prevalent. About 25 million pregnant women from Sub Saharan Africa are at risk of malaria infection every year, while approximately 25 % have been found with evidence of placental malaria infection at delivery (Desai *et al.* 2007). Understanding the association of gestational hypertension with malaria infection can inform prevention strategies in malaria endemic regions. Even a weak association between malaria and gestational hypertension will likely have a high population attributable risk (PAR), due to the high prevalence of malaria in these settings. PAR is the product of the magnitude of the risk (relative risk of the exposure) times the incidence of the exposure in the population. In other words, it is the incidence of the disease in the population that would be eliminated if the exposure was eliminated. I, therefore, set out to conduct a systematic review and meta-analysis with the aim to aggregate the available

evidence on this association. Evidence on the association between malaria infection and GH is likely to attract further studies to investigate causality.

4.2 Methods

REVIEW QUESTION

What is the association of malaria infection and gestational hypertension in malaria endemic area?

DATABASE SEARCHES

The following databases were searched: MEDLINE, WHO Global Index Medicus, Google Scholar, EMBASE. I extended my search to include grey literature and hand searching of referenced studies. The search date was 9th week of 2018.

KEYWORDS

Malaria, Placental malaria, Hypertension-pregnancy-induced (pre-eclampsia, eclampsia, HELLP)

The above keywords were used to search in the medical subject heading (MESH) and as keywords in the respective databases. Exposure keywords (malaria or placental malaria) were combined using the Boolean word AND with outcome keywords (gestational hypertension, pre-eclampsia). Keywords were modified to fit the respective databases.

I restricted my search to full articles, English language, and human studies. I did not apply limits on the year of study.

TYPE OF STUDIES

I explored observational studies for the association between malaria infection and gestational hypertension. I included case-control, cross-sectional, clinical trials and cohort studies, while excluding case report, case-series and ecological studies.

POPULATION

All pregnant women, who live in malaria endemic countries of Africa.

EXPOSURE

Women diagnosed with malaria during pregnancy, or post pregnancy diagnosis of placental malaria formed the exposed group. Malaria Infection is diagnosed clinically by signs and symptoms accompanied by a blood test (rapid blood test for malaria or blood slide for microscopy). Malaria infection during pregnancy can invade the placenta to cause placental malaria, which can be confirmed after delivery by examining the placenta tissue. In my study, I considered either malaria infection during pregnancy or placental malaria confirmed after delivery as my exposure of interest. The sensitivity and specificity of light microscopy in detecting malaria varies; in maternal blood the sensitivity is 41 % and specificity is 100 %, in placental blood the sensitivity is 35 % and specificity is 100 %, while in placenta tissue the

sensitivity is 33 % and specificity is 95 % (Campos *et al.* 2011). This shortfall in diagnostic tools present potential misclassification.

COMPARATOR

Women free of malaria diagnosis during pregnancy or free from post-pregnancy diagnosis of placental malaria were regarded as the unexposed group. These women were drawn from the same communities as my exposed group.

OUTCOME

The outcome of interest was a diagnosis of gestational hypertension (pre-eclampsia and GH proper). Women with this outcome were examined for the exposure of malaria infection or placental malaria infection.

INCLUSION AND EXCLUSION CRITERIA

- All observational studies that examined the association of malaria and gestational hypertension were eligible for inclusion.
    - Ecological, case series and case report studies were excluded.
    - Studies that did not ascertain the diagnosis of malaria by a laboratory test were excluded.

SELECTION PROCESS

The retrieved papers from the searched databases were exported to Endnote X7 software. Duplicates were removed first by the software and then followed by manual search. Titles and abstracts of identified studies were screened for relevance. Those not considered relevant were excluded.

Additional papers were selected from grey literature sources such as relevant institutions' repositories (e.g. universities). Reference lists of the selected papers were examined to identify additional papers.

A full-text review of the selected articles identified the eligible articles based on my inclusion and exclusion criteria. The selection process followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guideline. (PRISMA flow diagram as shown in figure 2 below)

QUALITY ASSESSMENT

Critical appraisal skills programme (CASP) checklist tools were used to guide the quality assessment of the observational studies. In case-control and cross-sectional studies, this tool was used (https://casp-uk.net/wp-content/uploads/2018/01/CASP-Case-Control-Study-Checklist-2018.pdf). While in cohort studies, this tool was used (https://casp-uk.net/wp-content/uploads/2018/01/CASP-Cohort-Study-Checklist_2018.pdf). Each

paper was assessed for its weakness and strength based on the checklist questions. The tool assessed eleven aspects of the paper:

1. Did the paper have a focused question?
2. Did the paper use the appropriate method to answer the question?
3. How was the overall recruitment process?
4. How was the recruitment of cases done?
5. How were the controls recruited?
6. Was the exposure accurately measured?
7. Were relevant confounding factors accounted for in the design and analysis?
8. What are the results of the study?
9. How precise are the results?
10. Do you believe the results (internal validity)?
11. Can the results be applied to the local population (generalizability)?
12. Do the results of this study fit with other available evidence?

The selected studies satisfied all the above quality assessment questions. Studies that did not address confounding factors in analysis and design were excluded. The CASP tools assess the quality of a paper in a holistic manner,

thus it does not use the scoring of points in the above twelve questions to justify a paper's eligibility in terms of its quality. The detailed analysis of each paper is presented on annex 7, table 55 at the end of this thesis.

POTENTIAL BIAS

Malaria infection can be subclinical; hence it can be undiagnosed leading to misclassification of the exposure status in the unexposed group. Misclassification of this sort will shift the association towards the null. My attempt to reduce this bias was by ascertaining the diagnosis of malaria when it was supported by a laboratory test: peripheral blood smear for malaria parasite, rapid diagnostic test for malaria or placenta histology for malaria. I set criteria to include studies that had ascertained the lack of malaria exposure by a laboratory test.

There is also a potential misclassification of the outcome of gestational hypertension (pre-eclampsia and GH proper), which may bias my results. To minimise this bias, I restricted my selection to only include studies that used the outcome of pre-eclampsia or GH proper, where both share the characteristic of hypertension with the onset of 20 weeks and onwards. However, there is always a challenge of ascertaining the gestation age of 20 weeks and detecting the new onset of hypertension, especially in low resource settings. This challenge presents the potential misclassification bias in the primary studies that we cannot eliminate.

DATA EXTRACTION

I extracted key information from the articles; author, year, study design, country of study, sample size and univariable odds ratios and confidence interval (unadjusted). Where it was available, multivariable (adjusted) odds ratio and confidence interval were extracted together with the list of variables used in the adjustment model.

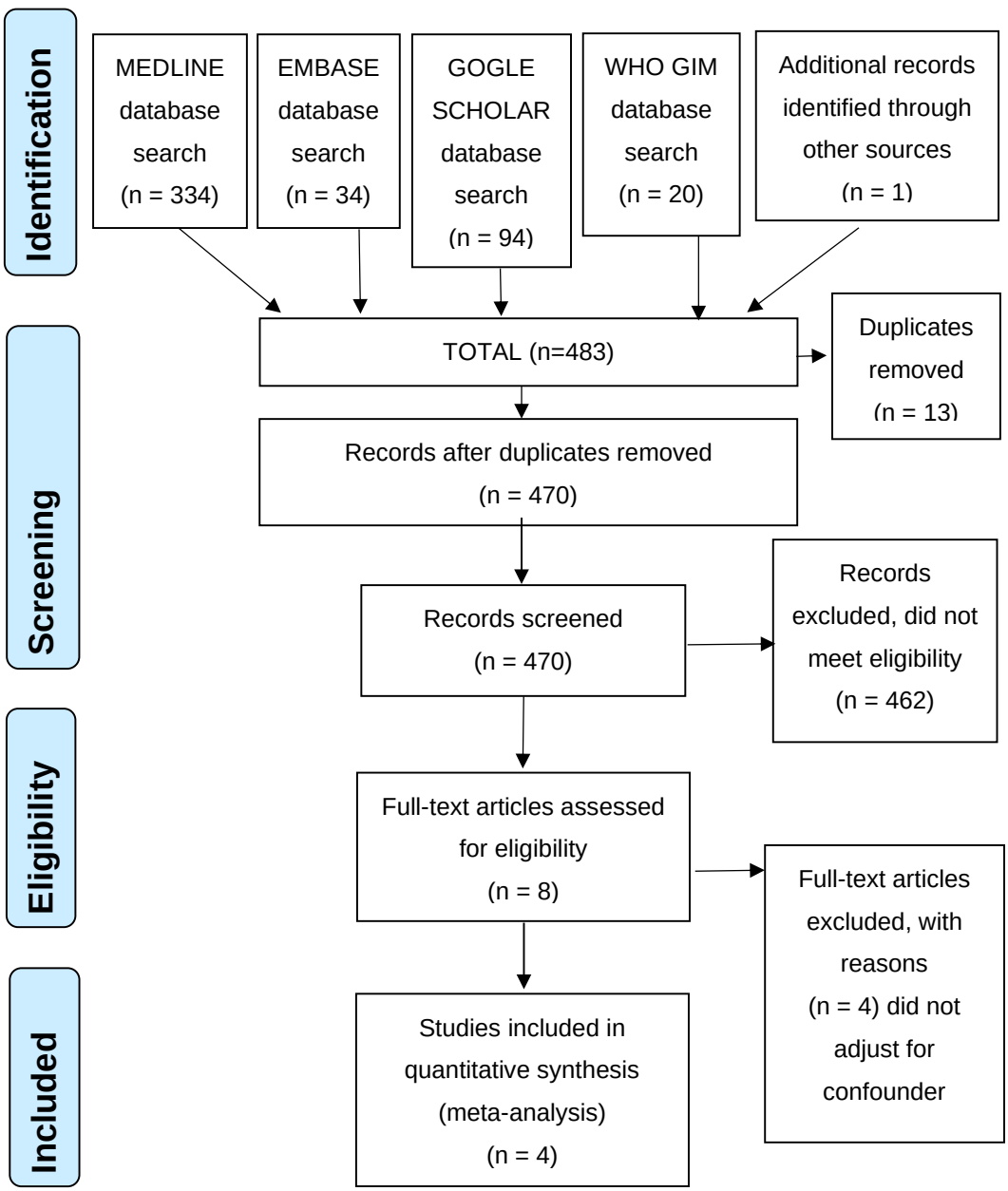
DATA ANALYSIS

A narrative synthesis was done to summarise the evidence from the gathered papers. Odds ratios or relative risks from the selected studies were directly extracted from the available data. I then assessed the heterogeneity of the included papers to determine the feasibility of a meta-analysis. I had pre-set to accommodate moderate heterogeneity (I^2 = 30 %) due to the expected population variability of the exposure outcome association. I had anticipated the association between malaria and gestational hypertension to vary widely across study populations; hence, I used the random effects model (DerSimonian and Laird 1986) to estimate the pooled effect in the meta-analysis. RevMan 5 software was used to aggregate the odds ratios from selected studies. Inverse variance weighting was used to compute the pooled odds ratio and its confidence interval. The inverse variance method estimates the amount of variation across studies by comparing each study's result with an inverse-variance fixed-effect meta-analysis result. I used a forest plot to display my study results. I also used a funnel plot to assess the publication bias of the selected papers.

4.3 Results

The results of my literature search, the screening of articles based on the inclusion and exclusion criteria, followed by a quality assessment, was in accordance to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidance. The figure below outlines findings in the identification, screening and eligibility stapes.

Figure 2. PRISMA flow diagram of selected papers in the Systematic review.



My search identified 483 papers, 462 were excluded based on title and abstract, 13 were duplicates. Eight papers were assessed for eligibility only four were suitable for the meta-analysis. These four papers were case-control studies and are approximately 1 % of all identified papers from my search strategy. There were two cohort studies and two case-controls that were excluded due to lack of addressing confounding factors in the design and analysis.

4.3.1 Narrative synthesis

My preliminary narrative synthesis paves the way for the meta-analysis of the selected studies. It describes the context of the methods and results of the meta-analysed papers. In exploring the association between malaria infection and gestational hypertension, my systematic review has identified four good quality studies that satisfied the CASP checklist (See annex 7 table 55). Studies that have adequately measured the association of malaria and gestational hypertension. All four studies showed that malaria infection increases the odds of gestational hypertension. However, some of the findings were not statistically significant, while some had a wide confidence interval of their estimates. Three studies were conducted in West Africa (Senegal and Ghana) and one in the horn of Africa (Sudan). All these countries have a tropical climate, which favors the endemicity of malaria infection. These few studies inadequately represent the African population,

which is very diverse. However, the similarity in the measure of association across these studies (homogeneity) suggests the effect of malaria on gestational hypertension is narrow in range and possibly is a fixed value across all populations.

These four studies were hospital-based, thus are prone to some selection bias on their study participants. The cases and controls were selected from the same hospital in an attempt to ensure controls are as similar as cases except having the disease. However, the exposure odds in the control group could not be ascertained to be equal to that of a general population, thus setting a possibility of bias in estimating the association.

The selection criteria of the study participants applied a restriction to some population groups that were thought to have the potential to confound the association, such as women with twin pregnancies and those with diabetes. Table 4 shows the groups that were excluded in the selection of the individual studies. Other studies attempted to match the controls with the cases using age, parity and prematurity factors known to confound the association. However, they did not do a matched analysis rather they adjusted for these factors in the analysis using a logistic regression model. Overall, all four studies had adjusted for maternal age, the number of previous pregnancies and history of hypertension. Two studies (Adam *et al.* 2011, Ephraim *et al.* 2014) had adjusted for body mass index (BMI) and diabetes. This provides sufficient confidence of adjustment of key confounders in my pooled estimates.

The ascertainment of exposure status was based on confirmation of malaria diagnosis through histological examination of the placenta remains after delivery. This ensured a minimum possibility for misclassification of disease outcome in exposed and unexposed groups, which could dilute the strength of the association. If a woman had acquired placental malaria in the course of her pregnancy, then her placenta would reveal histological changes to detect past malaria infection on the placenta. The diagnosis of placental malaria is not a routine test as it involves the collection of a sample from the placenta at the time of delivery; this may explain why few studies have embarked on exploring this relationship in these LMIC, where laboratory services are often limited. However, these few studies used appropriate laboratory tests to diagnose placental malaria. Giemsa staining test of placenta blood and placenta histological section were the methods used.

All the four studies have relatively small sample sizes ranging from 252 to 463 participants (see table 4). Most of the studies lacked detail on how they calculated their sample size. The relatively small sample sizes generate wide confidence intervals for the final estimates thus contributing to some studies giving non-statistically significant estimates, as seen on the forest plot.

These selected studies were able to adjust for the necessary confounding variables. Table 4 lists the variables that were adjusted for in the relationship. The results of these studies are more credible because the researchers were able to minimise the confounding effect by applying restriction and matching techniques in the selection and the unmatched logistic regression analysis.

All the studies adhered to the definitions of the outcome, gestational hypertension proper and pre-eclampsia. All studies used the same definition of raised blood pressure, where the cutoff was a rise of blood pressure ≥140mmHg (systolic) and ≥90mmHg (diastolic). However, there was some variation in the cut off point for proteinuria, ≥ 2+, >2 and ≥ "1+" in Ndao *et al.* (2009), Adam *et al.* (2011), Ephraim *et al.* (2014) studies respectively. This classification is key to segregate between the pre-eclampsia cases from gestational hypertension proper cases. A pregnant woman with raised blood pressure and proteinuria value = 2 would be regarded by one study as having pre-eclampsia while the other study would regard her as having gestational hypertension. This difference in cut off points on the severity of proteinuria across studies was likely to introduce misclassification in their outcome variable i.e. women with pre-eclampsia or GH.

The potential misclassification in the outcome of gestational hypertension (pre-eclampsia and GH proper) in the primary studies may still have an influence on our finding. We know that it is a challenge to ascertain the characteristic of a new onset of raised blood pressure above 140mmHg (systolic) and 90mmHg (diastolic) starting from 20 weeks of gestation, especially in poorly resourced settings. In these settings, there is high illiteracy thus some women may not recall their last menstrual date correctly.

4.3.2 Meta-analysis

Table 4 Overview of the included studies

The table shows the countries, sample size, study design, characteristics of participants and the results of the selected papers for the systematic review.

Country	Author and Year	Sample size	Participants characteristics	Results comparison
Sudan	(Adam *et al.* 2011)	143 Cases/143 control	Recruited case and controls from hospital, Excluded Twins and Diabetes.	The multivariable analysis adjusted for age, primigravidae, Hx of malaria, Family Hx of HT, BMI, Blood Group, Placental malaria, Education level, Lack of ANC
Ghana	(Ephraim *et al.* 2014)	120 Cases/160 control	Recruited case and control from hospital, excluded chronic HT, on Antihypertensive drugs, eclampsia, diabetes autoimmune, renal diseases.	Adjustment of confounder in the multivariable analysis: Age, gravidity, parity, BMI, contraceptive use, Abortion, New paternity and Malaria for GH
Senegal	(Ndao *et al.* 2009)	223 Cases/240 control	Recruited cases and control from hospital, matched them by age, parity and prematurity	Multivariable analysis, adjusted for placental malaria, residence, parity, past pregnancies, ANC visit, family Hx of HT, the period of delivery seasonality, illiteracy, marital status.
Senegal	(Sartelet, 1996)	32 Cases/220 control	Recruited cases and control from hospital, were similar on mean age, no of previous pregnancies	Adjusted for age, no of previous pregnancies, twin delivery, maternity centre and date of delivery.

Figure 3. Forest plot of the meta-analysed studies

This forest plot shows the odds ratio of the individual studies and their pooled effect in the association between malaria infection and gestational hypertension. The individual studies are weighed by the inverse variance method. The pooled odds ratio is 2.67 with 95 % CI [1.58 - 4.53]. This suggests women exposed to malaria during pregnancy had 2.67 times (95 % CI 1.58 to 4.53) the odds of gestational hypertension compared to women not exposed to malaria during pregnancy. This association was statistically significant.

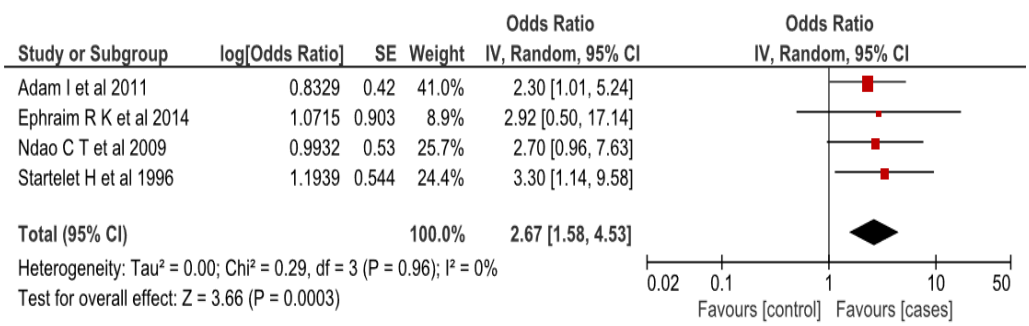
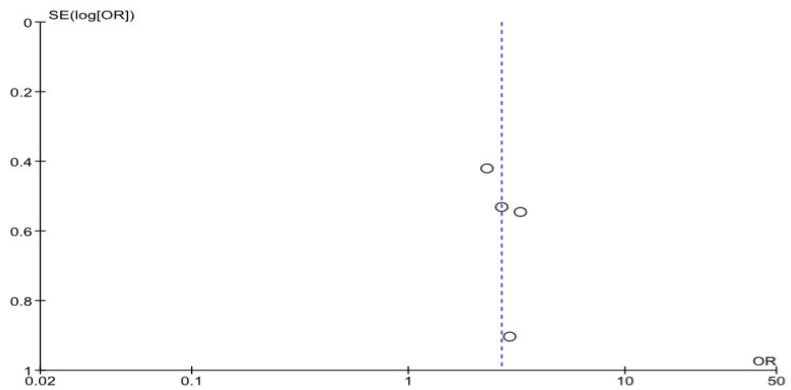


Figure 4. Funnel plot of the meta-analysed studies

This funnel plot shows that studies with large and small variance had similar estimates of the effect of malaria infection on gestational hypertension.



4.4 Discussions

The association between malaria infection and gestational hypertension has long been explored by different small studies in Africa (Sartelet *et al.* 1996, Etard *et al.* 2003). My meta-analysis has been able to show an overall statistically significant association between malaria infection and gestational hypertension, a pooled odds ratio of 2.67 (95 % CI 1.58 - 4.53). This association was drawn from 1,281 women (518 malaria cases and 763 controls). My outcome of interest was gestational hypertension with proteinuria (pre-eclampsia) or without; both being referred as gestational hypertension in my chapter. Malaria infection is a common infection in Africa, especially during pregnancy. My result suggests that it is associated with gestational hypertension. This association places malaria infection as a potentially important risk factor for gestational hypertension in malaria endemic regions.

One of the strengths of this review unlike other studies is that it explores the association of malaria infection to a broader spectrum outcome of two related

subcategories of hypertensive disorders of pregnancy: pre-eclampsia and gestational hypertension. Since malaria infection can be prevented and cured, this association sheds light on the potential benefit to maternal and infant health if this single risk factor of malaria is identified and addressed.

The search process produced four good quality papers. I used the critical appraisal skills program (CASP) checklist tool to assess the quality of the papers. (See table 55 at annex 7) These papers had a focused research question to explore the relationship between malaria infection and gestational hypertension. These studies were able to adjust for possible confounders through restriction, matching and analysis. All four studies had adjusted for parity, which confounds the association between malaria and GH. Thus, the aggregate result of my meta-analysis was able to give a credible result on the nature of the overall association.

A potential limitation may be due to residual confounding even after adjusting most of the common factors shown in the literature. Another limitation is the few studies that met my selection criteria thus necessitating further studies in the area.

The measure of heterogeneity I^2 was 0 %; this was different from my earlier assumptions. The value of I^2 shows the variability between study groups that is not explained by chance alone. A higher value of I^2 shows the between study groups difference is significant and hence the study groups are inherently different from each other. My analysis assumed the relationship

between malaria and gestational hypertension to vary widely across societies and thus I adopted the random effects model, which often produces a higher measure of heterogeneity. My findings appear to suggest that there is a constant effect of malaria on gestational hypertension across African populations because the between study groups difference is explained by chance, hence not inherently different. Three of my selected studies were from West Africa and one from Sudan. However, my analysis included only a few studies, so this limitation may have produced the observed low I^2 value.

The 95 % confidence interval of my pooled odds ratio estimate was 1.58 to 4.53, from the random effects method (DerSimonian and Laird 1986). If I were to use the fixed effects method then this 95 % confidence interval would have been narrower, closer to my point estimate of 2.67. This provides assurance that changing the method would not have resulted in a wider 95 % confidence interval.

Although my analysis involved a small number of studies, the funnel plot seems to suggest that studies with high variability in their standard errors produced similar effect sizes to studies with less variability. There is no asymmetry on the funnel plot, a feature that indicates publication bias; all studies are centred close to the common effect. This observation may be due to chance or absence of sufficient published papers from diverse populations. Therefore, it does not provide sufficient evidence to rule out the absence of publication bias.

The evidence from these studies is supported by other ecological studies that suggested an association between an increase in pre-eclampsia death during the rainy malaria seasons (Etard *et al.* 2003, Anya 2004). Dorman *et al.* (2002) also showed that malaria infection was affecting the uterine blood flow, this being a step closer in showing the effect of malaria infection on the placenta. Placenta dysfunction has been one of the theories linking malaria to pre-eclampsia.

This meta-analysis has aggregated evidence for the small studies that examined the association of malaria and gestational hypertension and, thus produced a more credible result of the existing association. If malaria is causal to GH, then my findings have potential policy implications on disease prevention, as it links infectious disease (malaria) prevention to potential benefit in preventing gestational hypertension, a non-communicable disease (NCDs). Therefore, reducing malaria during pregnancy may have a broader impact beyond that of reducing the direct effects of malaria on the mother and the unborn baby. Molecular studies exploring this causal relationship could improve our understanding and identify intervention points on the theorised causal pathways.

4.5 Summary

Malaria infection is associated with gestational hypertension in tropical Africa. This association appears to be similar in different African contexts, having fixed effects, across the studied populations. If malaria is causal to gestational hypertension, enhancing malaria control efforts on pregnant women could have immediate indirect benefits during pregnancy on averting hypertension and in long-term cardiovascular diseases. Molecular studies are needed to further explore the causal mechanism.

Chapter 5 Analysis of risk factors for pre-eclampsia in Tanzania.

This chapter builds on the findings from the two previous chapters. Chapter Three, the scoping review, summarised the evidence of explored risk factors in the African continent. Out of the many suggested risk factors from the scoping review, I selected malaria infection and further explored it by carrying out a systematic review and meta-analysis. The results are presented in Chapter Four. Chapter Five analysed data from Tanzania with the aim of estimating the incidence of pre-eclampsia in urban settings. This was performed using trial dataset with 3,767 women from the commercial capital city of Dar es Salaam, Tanzania. The remaining analysis in this chapter was performed using a dataset with 16,432 women from northern Tanzania. With this dataset, I described the sociodemographic characteristics of women with pre-eclampsia. This was to get an understanding of pre-eclampsia's distribution along sociodemographic groups. Then I embarked on identifying risk factors that are associated with early and late onset pre-eclampsia. In my analysis, I had a large sample size, and sufficient confounding variables, unlike most other studies, which lacked methodological rigour. I focused on exploring maternal biomedical characteristics as risk factors from pre-eclampsia. These maternal biomedical characteristics are collected routinely in the health information system. Furthermore, I used the statistically significant maternal risk factors to develop logistic regression prediction

models. These models were developed for all deliveries and for term and preterm deliveries subgroup. This was to account for the aetiological difference between early and late onset pre-eclampsia. The developed prediction models were used to classify the likelihood of a woman to develop pre-eclampsia outcome. The area under the curve (AUC) and maximum sensitivity and specificity values were computed for each model. Overall, all the three models performed satisfactorily in terms of the AUC, sensitivity and specificity. Moreover, there is room for further improvement in their performance, if biomarkers are integrated as predictors in these models. There is a growing interest to understand the long-term effects on newborns from pre-eclampsia pregnancies. Thus, the final piece of this analysis describes the pregnancy outcome of women with pre-eclampsia pregnancies. The results show newborns of pre-eclampsia pregnancies have far worse indicators of physical and mental wellbeing than their counterparts.

5.1 About Tanzania

My study has analysed secondary data from Tanzania, which has a population of 44,928,923 (NBS-Tanzania 2012b) spread out in a geographic area of 947,303 square kilometres (NBS-Tanzania 2012a). Tanzania is among the LMIC, its gross domestic product (GDP) per capita is 960.15 USD and ranks as the 158 poorest out of 187 countries. (IMF 2016).

The Tanzanian population is growing quickly, with a high fertility rate of 5.5 births per woman (NBS-Tanzania 2015). It is also a rapidly urbanising

country. Dar es Salaam is the main commercial city of the country with a population of 4,364,541 (NBS-Tanzania 2012b). These population changes have not been met with a corresponding growth in the health system. Tanzania has a low physician population density of 0.399 per 10,000 people. It trails behind its neighbouring countries of Kenya (1.988 per 10,000 people) and Uganda (0.908 per 10,000 people) (WHO 2014). The situation is very different in the developed counties; the United Kingdom has a physician population density of 28.058 per 10,000 people (WHO 2014).

Being a tropical country, Tanzania is prone to most common tropical diseases such as malaria and helminthiasis, which lead to anaemia and other adverse consequences, especially during pregnancy.

The maternal mortality ratio (MMR) is still high, estimated to be around 556 maternal deaths per 100,000 live births (UNICEF 2017). MMR is the number of maternal related deaths during a given period per 100,000 live births during the same period. MMRs in Sub-Sahara Africa range from 119 maternal deaths per 100,000 live births in South Africa to 1,150 maternal deaths per 100,000 live births in South Sudan (World Bank 2017). A few studies have estimated the burden of pre-eclampsia in Tanzania, which is thought to contribute to the high maternal mortality. The incidence of pre-eclampsia in northern Tanzania was estimated to be 3.5 % among women in their first pregnancies through a hospital-based study (M J. Mahande *et al.* 2013). The incidence of pre-eclampsia in some African counties was reported to range from 1.1 % in the Ivory coast to 9.3 % in South Africa (Abalos *et al.*

2013). Furthermore, this study was also hospital-based, utilizing a hospital register. Thus, both studies may underestimate the burden since a substantial proportion of women in rural settings still deliver at home and hence their information is never captured in the health system. Another study done in Dar es Salaam (an urban setting) examined the incidence of eclampsia amongst the hospitalised and non-hospitalised population. The incidence was 200/10,000 and 67/10,000 respectively (Urassa *et al.* 2006). These methodological limitations in the above studies show the uncertainty in our estimation of the burden of pre-eclampsia /eclampsia, which is a condition of public health concern.

Some potential risk factors for pre-eclampsia in Tanzania have also been documented by several reports. The prevalence of HIV is around 4.7 % (95 % CI 4.3 to 5.0) (NBS-Tanzania 2017b) while that of malaria, which is measured among children aged 6 to 59 month is 7.3 % (NBS-Tanzania 2017a).

Cardiovascular diseases, which share similar risk factors to pre-eclampsia, have been explored in rural and urban settings in Tanzania. Walker *et al.* (2010) showed the yearly stroke incidence rates were 108.6 per 100,000 (95 % CI 89.0 – 130.9) in Hai (rural Tanzania) and 315.9 per 100,000 (281.6 – 352.3) in Dar es Salaam (urban Tanzania). The rural rate was similar to that in northern Manhattan, USA study which showed the incidence of stroke to be 93 per 100,000 in white people and 223 per 100,000 in black people, but the urban rate of stroke in Tanzania was higher than the northern Manhattan

study (Walker *et al.* 2010). He suggested that this could be a result of a difference in the prevalence of risk factors. He, therefore, emphasised the importance of screening for hypertension, which is a precursor of stroke. Improving our understanding of risk factors for pre-eclampsia in African populations has the potential of providing appropriate care to the high-risk women and mitigate catastrophic outcomes of pregnancies complicated with pre-eclampsia.

Access and utilisation of reproductive health services is still a challenge in Tanzania as in many other LMIC. A study done in rural Tanzania estimated the proportion of home deliveries to be 58 % (Mrisho *et al.* 2007). The low proportion of hospital deliveries contributes to high maternal and infant mortality due to a lack of emergency care at home. On the other hand, child immunisation coverage is high in Tanzania, ranging from about 100 % BCG vaccine coverage for TB to 97 % coverage for DPT-H influenza and Hepatitis B vaccine (USAID 2017). This could indicate that there is public willingness to utilise health services if made accessible by outreach services that bring the services close to the doorsteps of these women.

5.2 Problem statement

There is limited understanding of the range and interactions of risk factors for pre-eclampsia in African countries. My scoping review study shows that few studies have been done in Africa and those that have been conducted often exhibit methodological limitations. The literature suggests there is

considerable variation in risk factors, some being more predominant in certain geographical regions while others may be universal. Genetic risk factors are also thought to play a role in the aetiology of pre-eclampsia (Williams and Morgan 2012). Black women have been shown to be at an increased risk of developing pre-eclampsia compared to white women (Knuist *et al.* 1998). Certain risk factors have been associated with different pregnancy outcomes in different ethnic groups. Obesity has been associated with gestational hypertension/pre-eclampsia in Australian born women while being associated with only shoulder dystocia in South Asian born women (Davies-Tuck *et al.* 2016). Therefore, it appears that particular risk factors may produce different outcomes in different populations and certain risk factors may only apply to a specific population.

The scarcity of literature that has explored the risk factors of pre-eclampsia in African populations warrants further research to examine the relationship between risk factors and pre-eclampsia outcome. This study will explore globally known risk factors such as chronic hypertension and body weight alongside potentially locally important risk factors, such as malaria infection. Several small studies have suggested that placental malaria is associated with pre-eclampsia (Ndao *et al.* 2009, Adam *et al.* 2011). However, the results of these studies were not conclusive. Unlike previous small studies that lacked methodological rigour, my analysis has utilised data sets that are large, enough to explore the association of risk factors routinely captured in the Tanzanian health information system. From the reviewed literature, there

is a lack of evidence on the utility of these risk factors in predicting pre-eclampsia in African populations. Hence, I am also interested in assessing the performance of a prediction model that utilises these risk factors in classifying pre-eclampsia outcome.

My scoping review shows that most studies that explored risk factors for pre-eclampsia did not differentiate early and late onset pre-eclampsia. My study will focus on identifying appropriate risk factors for early and late onset pre-eclampsia in Tanzania, with the potential to produce separate results for the two distinct disease subgroups.

At the outset of this analysis, I anticipated several potential challenges: with a rare outcome such as pre-eclampsia, including challenges in identifying risk factors and modelling the outcome and challenges due to missing data and potential misclassification of the outcome when relying on routine hospital data. On the other hand, the use of routine data for this research problem provides results that are generalizable to these settings.

There is some evidence to suggest an increasing trend in the incidence of pre-eclampsia among pregnant women in both high, low and middle-income countries (Wallis *et al.* 2008, Vata *et al.* 2015), although the picture is mixed: a large international comparative study by Roberts *et al.* (2011) suggests a decrease in some European counties but not Massachusetts, USA. Unfortunately, we lack sufficient evidence to establish the trend of pre-eclampsia in LMIC. The apparent observed rise from the Vata et al study

could be a result of increased utilization of health services or improvements in diagnostics and recording in the medical services. It is possibly due to a corresponding increase in the trends of associated risk factors such as obesity, diabetes and chronic hypertension (Wang *et al.* 2008, WHO 2017a). With this uncertainty in mind, my study sets to determine the incidence of pre-eclampsia in Dar es Salaam, an urban setting of Tanzania. I envisage that rapid urbanisation and lifestyle transformation may predispose this population to a higher incidence of pre-eclampsia. There is a lack of historical data to establish the trend of pre-eclampsia from the past years, since pre-eclampsia diagnosis is not routinely reported in the health information system. However, my study's findings could set a reference for comparing with future incidence studies to establish the future trend of the disease in Tanzania. In this objective, I set to estimate the incidence in an urban setting of Dar es Salaam, Tanzania.

In addition to estimating the incidence of pre-eclampsia, I will also describe the sociodemographic characteristics of women with pre-eclampsia. This information will provide an understanding of pre-eclampsia distribution in the population subgroups. Although the distribution of pre-eclampsia along sociodemographic characteristics has been described in several studies in Africa (Endeshaw *et al.* 2016a, Endeshaw *et al.* 2016b), each country has its own socioeconomic dynamics, thus distribution results from one country may not apply to another country. A comprehensive epidemiological understanding of the incidence, distribution, risk factors and disease

outcomes will facilitate the design of appropriate prevention interventions targeted at this population.

Several studies have revealed the potentially devastating effects on infants born from pre-eclampsia pregnancies. Fetal programming in utero is thought to mediate their later life health impairment. A cohort study in the Netherlands showed that first trimester growth restriction was associated with increased risk of developing CVD in school age children (Jaddoe *et al.* 2014). This fetal programming effect is thought to be mediated through fetal growth restriction in the first trimester, birth weight and altered offspring blood pressure (Palinski 2014). Women born preterm or with inappropriate weight for gestational age were found to be at increased risk of gestational diabetes and pre-eclampsia in their adult life (Rogvi *et al.* 2012). Little is known of pregnancy outcomes from women with pre-eclampsia in LMIC. In addition to causing perinatal mortality, we want to know about the condition of the surviving offspring from pre-eclampsia. My study aims to describe characteristics of surviving offspring of pre-eclampsia using indicators of mental and physical wellbeing at birth. These indicators contribute to the wellbeing trajectory of these offspring in later life.

5.3 Rationale

Studies have shown that risk factors interact differently across ethnic groups (African American, Hispanics and Caucasian) due to environmental and genetic factors (Knuist *et al.* 1998, Makgoba *et al.* 2012, Davies-Tuck *et al.*

2016). Therefore, identifying risk factors for early and late onset pre-eclampsia in Tanzania, may inform the design of effective intervention strategies. This research will contribute new knowledge on the status of evolving risk factors such as obesity, smoking, anaemia and malaria during pregnancy in Tanzania.

Most developing countries' health systems rely solely on maternal risk factors to identify women with a high risk of pre-eclampsia. I am interested to know how well these risk factors can classify the outcome. I have therefore developed and assessed the performance of pre-eclampsia prediction models in using maternal risk factors. This modelling is unique because it utilises local risk factor (malaria infection), in an attempt to achieve improved prediction suitable and focused to the Tanzanian population.

A comparative description of the pregnancy outcomes between women with and without pre-eclampsia demonstrated the contribution of pre-eclampsia to the burden of poor pregnancy outcomes in Tanzania. This study also shines a light on birth indicators of offspring of women with pre-eclampsia, these indicators contribute to wellbeing in adult life. This comparative description of pregnancy outcomes has also made a comparison of the subgroups of preterm and term deliveries. This was done to account for the difference in pregnancy outcome as a result of difference in the duration of the pregnancy before delivery. Therefore, pre-eclampsia women with preterm deliveries were compared with non-pre-eclampsia women who also had preterm deliveries.

This study will also estimate the incidence of pre-eclampsia among women in Dar es Salaam, Tanzania. This finding will enable us to establish the baseline of the magnitude of the condition, which will serve in future comparison when evaluating the trend of the condition in the country. I envisage that the evolving lifestyle-related risk and the changing prevalence of other risk factors in this setting necessitate periodic estimation of the burden of the pre-eclampsia. High cost attached to reforming health information systems is likely to continue to prevent integration of pre-eclampsia and other hypertensive disorders in the routinely monitored conditions in the health system. Therefore, LMIC like Tanzania will have to rely on periodic research to inform the burden of the condition. The incidence of pre-eclampsia in Dar es Salaam city is also important in raising awareness and for mobilizing resources to address this problem. Unlike previous incidence studies, which were based in tertiary hospital, my study utilised data from lower level public health facilities across Dar es Salaam, where access to maternal health services is free of charge, hence making my incidence estimate more closely equivalent to that of a population-based study.

The importance of this study:

- *It highlights the burden and the sociodemographic distribution of pre-eclampsia in LMIC. which is relevant in raising awareness and targeting prevention efforts.*
- *It has identified relevant risk factors in LMIC settings which are useful in risk stratification and prediction for population health management in LMIC*
- *It has shown the adverse health outcomes of newborns of pre-eclampsia deliveries in LMIC settings, calling on the need for intervention to mitigate pre-eclampsia in these settings*

5.4 Objectives

The key objective of this study is to determine the incidence, risk factors and pregnancy outcomes for pre-eclampsia among women in Tanzania.

Specific objectives are:

1. To determine the incidence of pre-eclampsia among women in Dar es Salaam, Tanzania from 2007 to 2009.

2. To describe the sociodemographic characteristics of women with pre-eclampsia in northern Tanzania from 2006 to 2010.

3. To identify risk factors for pre-eclampsia, term and preterm deliveries subgroups, among women in northern Tanzania from 2006 to 2010.

4. To describe the pregnancy outcomes characteristics of women with pre-eclampsia in northern Tanzania from 2006 to 2010.

5.5 Methods

5.5.1 Study design

I have used cross-sectional study design to address my four objectives. This study utilises two Tanzanian data sets.

1. The first dataset is sourced from the Kilimanjaro Christian Medical Center (KCMC) maternal register; KCMC is a tertiary level hospital, serving the Northern region of Tanzania. Its maternal register collects all the vital information on women's deliveries. I used this data set to identify the risk factors for pre-eclampsia in term and preterm deliveries and to describe the sociodemographic and pregnancy outcome characteristics of women with pre-eclampsia pregnancies. [Objective 2,3 and 4]

2. The second dataset is sourced from a clinical trial of nutritional supplements to newborns (Child 2, NCT.00421668), conducted in Dar es Salaam, Tanzania. This Child 2 trial recruited a cohort of pregnant women at antenatal clinics and collected background and pregnancy outcome data of the recruited women. I used this data set to estimate the incidence of pre-eclampsia in Dar es Salaam, Tanzania. [Objective 1]

5.5.2 KCMC register data set

The KCMC is among four tertiary level hospitals in Tanzania and caters for the northern regions of Tanzania. My dataset from this centre covers from 2006 to 2010 and has records of 16,342 women. The register is computerised and uses access software that applies field restrictions i.e. you must enter data in all the specified entry fields on the screen. Some entry fields provide selection options, these mechanisms enhance completeness and valid data entry. The dataset includes information about the women during the antenatal period and at delivery. The hospital has skilled staff and equipment to facilitate an accurate diagnosis. Despite these assurances, I cannot rule out misclassification due to sensitivity and specificity limitations of diagnostic tools.

5.5.2.1 Study population and sampling for objective two, three and four

KCMC is a tertiary level hospital that caters for the northern regions of Tanzania (Kilimanjaro, Arusha and Tanga). The population from these regions is around 5,379,602 (NBS Tanzania, 2012). Most maternal cases attending this hospital were referred from lower level health facilities from these regions. Antenatal and delivery services are offered free of charge in Tanzania. My sample data was drawn from the register based in this hospital, where all singleton deliveries from 2006 to 2010 were extracted. It represents the characteristics of women with pre-eclampsia in the northern region. However, this hospital-based dataset is not ideal in estimating the incidence

of pre-eclampsia in a population. To achieve this, I have sought the dataset from the clinical trial in Dar es Salaam solely for this purpose.

5.5.2.2 Study variables

Independent variables

Table 5 Maternal antenatal variables

This table lists all the maternal variables that my analysis utilised to assess whether they are associated with the occurrence of pre-eclampsia. These variables were selected out the KCMC maternal register dataset. The complete list of all the variables in the KCMC dataset is listed in annex 1.

Variables stating history of a condition or use of item means the woman had experienced that disease or used that item prior to her current pregnancy. It does not show the duration or how may time in the past she had used the item or experienced the condition. However, some chronic diseases once acquired, they last for life such as a history of kidney disease.

Variables stating diagnosis of a condition mean the woman had experienced that condition in her current pregnancy.

Variables			
Mothers age	History of Kidney disease	History of contraceptive IUD use	Chewing tobacco
Number of pregnancies	History of Sickle cells	History of contraceptive implant use	Smoking cigarette
History of Diabetes	Drinking Alcohol	History of contraceptive male condoms use	Result of HIV-test
History of Hypertension	History of Heart disease	History of contraceptive p-pills use	Diagnosis of Malaria
HIV treatment	Mothers height	Mothers weight before pregnancy	Diagnosis of Gest diabetes
History of Liver disease	Diagnosis of Infections	Diagnosis of Anaemia	Diagnosis of Diabetes
	Diagnosis of Heart disease	History of contraceptive injections use	

Table 6 Pregnancy outcome variables

The table below lists the variables that my study explored in describing the pregnancy outcomes of pre-eclampsia women in comparison to non pre-eclampsia women. These variables are selected from the list of variables from the KCMC register dataset. The full list of variables for this dataset (see annex 1). These pregnancy outcome characteristics are from women who had singleton deliveries at KCMC maternity register from 2006 to 2010.

Pregnancy outcome characteristic

Child delivery status	Mode of delivery	Apgar score 1 min categories	Birth weight (Bwt) categories
Live born	Spontaneous	1 (0-3score)	1 (Very low Bwt <1500g)
Transferred to paediatric	Vacuum	2 (4-6score)	2 (Low Bwt 1500-<2500g)
Stillborn	CS elective	3 (7-10score)	3 (Normal Bwt 2500-4000g)
Neonatal death	CS others Assisted breech		4 (High Bwt >4000g)
Sex of the child	Mean Fetal head circumference	Fetal length	Mean gestation age at delivery

Dependent variables

The dependent variable was diagnosis of pre-eclampsia/eclampsia in the current pregnancy. This dependent variable was analysed in its subgroups of preterm and term deliveries as a proxy to early onset pre-eclampsia (≤ 34weeks of gestation) and late onset pre-eclampsia (>34 weeks of gestation).

5.5.2.3 Sample size estimate

Dataset from the KCMC register has 16,342 records of women's deliveries. This surpasses my estimated sample size calculated hereunder. I used the formula for computing sample size for the difference in proportion of unequal groups (Kelsey 1986). I used obesity as my risk factor in calculating the sample size. The prevalence of obesity among women in Dar es Salaam, Tanzania was estimated to be 24.7 % (Shayo and Mugusi 2011). This gives the ratio (r) of exposed/ unexposed of 0.333. A prior study in Northern Tanzania by M J. Mahande *et al.* (2013) showed that the proportion of pre-

eclampsia among the exposed (obese) was 6.5 % and in the unexposed (normal weight) was 2.5 %. Using these findings and a set alpha level (α = 0.05) and power at 80 % (β = 0.2), The Kelsey method gives the required sample size is 886 i.e. [222 exposed (obese) group, 664 unexposed (normal weight) group]. I did not get data from prior studies in Tanzania to enable me to estimate the different sample size needed to satisfy the set alpha level and power for each of the variable (risk factors) I intend to explore. However, my estimated sample size of 886 women for the risk factor of obesity serves to show that my available sample size of 16,342 women is likely to meet the required sample size for all the other risk factors I plan to explore.

5.5.3 Child 2 clinical trial data set

Muhimbili University of Health and Allied Sciences (MUHAS) in collaboration with Harvard School of Public Health conducted a clinical trial on the effect of zinc and multivitamin supplementation to infants of HIV Negative mothers in Dar es Salaam, Tanzania (Registration no NCT.00421668). The trial recruited a cohort of pregnant women from antenatal clinics, randomised their infants from 2007 to 2009, and followed them up for 18 months until 2011. The trial was designed to study infants, but it had to recruit mothers first and provide standard antenatal care during their pregnancy period. In this process, data were collected about their pregnancy state and progress.

Recruitment plan

Mothers of potentially eligible infants were recruited into the study in one of two ways:

1. Pregnant women ≤ 34 weeks gestation presenting at one of the three prenatal clinics (Sinza, Magomeni and Amtulabai health centres) in Dar es Salaam were informed about the study and consented prenatally or

2. Women were recruited from the labour ward of Muhimbili National Hospital within 12 hours of delivering a healthy singleton baby.

A mother was considered enrolled after signing the consent form for the study. An infant was not enrolled until he/she was randomised.

Eligibility criteria for mothers

A woman eligible for enrollment must:

- be pregnant with a singleton fetus
- be ≥ 18 years old
- be ≤ 34 weeks gestation (as measured by last menstrual period)
- be HIV negative
- be willing to remain in Dar es Salaam for 2 years after the child's birth
- Must not have had a previous child enrolled in Child 2

Measurements and tools

Pre-eclampsia diagnosis

The study used the standard definition of pre-eclampsia. Pre-eclampsia was defined as persistent high systolic (≥ 140 mmHg) or diastolic (≥ 90 mmHg) blood pressure and proteinuria (≥0.3 g/24 hours or a dipstick result of ≥ 1+, equivalent to 30 mg/dl in a single urine sample or spot urine protein/ creatinine ratio ≥ 30 mg protein/mmol creatinine) of new onset after 20 weeks of gestation (Kleinrouweler *et al.* 2012).

Blood pressure was measured using a sphygmomanometer and a stethoscope. The systolic value was recorded on the first knocking (Korotkoff) sounds and the diastolic value was noted when the knocking sound disappears. The correct size of blood pressure cuff was used during blood pressure measurement.

Protein in urine was measured by dipstick and results recorded as Nil, Trace (5-20mg/dl), + (30mg/dl), + + (100mg/dl), + + + (300mg/dl) and + + + + (> 300mg/dl) (Kallen and Watson 2015).

Data collection

Double entry of data was conducted to minimise entry errors. Two data clerks monitored by a supervisor collected, coded and entered the trial data. All the information was stored on secure computers in the data centre. Data

collection forms used in the study that are of interest to my secondary analysis are:

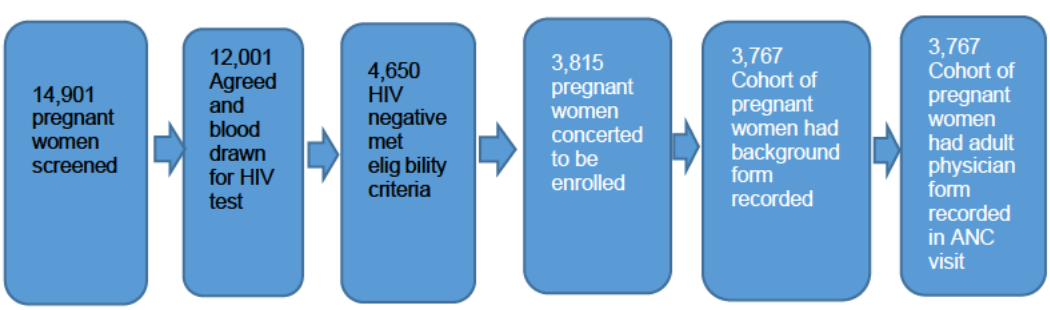
1. Background form (filled in once at enrollment into the study during ANC, it captures the background information of the participant)

2. Adult physician form (filled in during ANC follow-up visits every 4 weeks or if a pregnant mother is sick, it captures the physician notes when attending the woman)

5.5.3.1 Study population and sampling for objective one

My target population is pregnant women in Dar es Salaam. My sampled population is pregnant women who met the criteria (≥ 18years, HIV negative) and got recruited in the clinical trial child 2 study. A total of 3,815 pregnant women consented and were enrolled in the study, out of 4,650 who were eligible. (See the below recruitment flow diagram of the primary trial study). There was nonresponse rate of [1 - (3767/4650)] *100 = 19 %.

Figure 5. Recruitment flow diagram



The nonresponse rate includes women who were eligible to join the study but did not consent and women who consented to join the study but did not join for whatever reason.

5.5.3.2 Sample size estimates

In estimating the incidence of pre-eclampsia using the trial dataset, I used the formula estimating the proportion of a dichotomous outcome in a single population. My estimated incidence of pre-eclampsia among northern Tanzanian women attending ANC was 3.5 % (M. J. Mahande *et al.* 2013). I set the margin of type I error of my estimate at 1 % (α = 0.01), and a 99 % confidence interval. The calculated sample size using the Kish Leslie formula is 2,248 (Leslie 1965). My dataset from the clinical trial has records of 3,767 pregnant women, which surpasses the required sample size for a 99 % CI.

5.5.4 Data analysis

Statistical Package for Social Science (SPSS) software version 25 was used for analysis. I used the Kish Leslie formula to calculate the sample size for pre-eclampsia incidence estimation in Dar es Salaam (Leslie 1965). To calculate the required sample size for estimating risk factors for pre-eclampsia, I used the Kelsey (1986) formula for sample size estimation of unequal group of exposure/risk factor, while setting the power at 80 % and alpha level (α = 0.05).

Objective 1: (Using the child 2 trial dataset). The incidence of pre-eclampsia was determined by counting the frequency of new pre-eclampsia cases in the

cohort population in Dar es Salaam. The crude incidence was adjusted by age to give an adjusted incidence, and then a 95 % CI was estimated.

Objectives 2 and 4: (Using KCMC register dataset). Sociodemographic characteristics were compared between women with and without pre-eclampsia. Similarly, pregnancy outcome variables (stillbirth, Apgar score and prematurity.) were compared between women with and without pre-eclampsia. Significance testing was done using Chi square (χ^2) or Fisher's exact test. Continuous variables were compared by mean difference between pre-eclampsia and non-pre-eclampsia groups. T-test was done for statistical significance testing.

Objective 3: (Using KCMC register dataset). In determining risk factors for the development of pre-eclampsia in all deliveries, term and preterm subgroups, I split the dataset with 16,342 cases into 80 % for training and 20 % for testing my models. Assessment of missing data was done to explore the pattern of missing-ness to inform selection of appropriate assumptions for my analysis. There was no missing value in the outcome variable of pre-eclampsia, so the imputations were only done on some of the predictor variables. I imputed missing data in selected continuous variable using linear regression model. I first did a complete case analysis to develop my first set of prediction models using logistic regression. Then I imputed the missing dataset with single imputation method and developed a second set of logistic regression models. I compared the first (without imputed data) and second set (with imputed values) of logistic regression models. The second set

models (with imputed values) were not better than the first set models (without imputation i.e. complete case analysis). The models from complete case analysis were then internally validated on the 20 % testing dataset spared for this role. The performances of these models during validation were compared to their performance on the training datasets. The area under the curve (AUC) and its 95 % confidence intervals were used to assess the model classification performance. I used the following steps in developing my models: I conducted a univariable analysis for each biomedical risk factor with a logistic regression model. I added predictor variables with statistically significant (p values < 0.05) to a multivariable logistic regression model. I specified these predictors using “Enter method” in SPSS, when adding them to the multivariable logistic regression. I explored clinically plausible interaction terms in an attempt to fit the best multivariable model. I developed three different multivariable prediction models to predict pre-eclampsia outcome among all deliveries and in preterm and term deliveries subgroups. The best fitted model for each of these three outcome models is reported in the results section.

I tested the assumptions of logistic regression modelling. The assumption of independent observations was tested by using a generalised estimating equation model (GEE) and the result compared with that from a logistic regression model. This consideration resulted from the fact that a woman may have delivered multiple times during the period of 2006 to 2010. This possible correlation of pre-eclampsia outcome within a cluster of each

individual woman was addressed by using GEE model and then compared with the results of logistic regression models. The logistic regression modelling was done with the assumption that each pregnancy was an independent outcome, even if it was from the same woman, since she would be having different risk profile in each pregnancy.

5.5.5 Ethical considerations

Ethical approval was obtained from the owners of the two datasets:

1. The clinical trial, a trial of zinc and multivitamins to the infant of HIV unexposed pregnant women in Dar es Salaam, Tanzania (Registration no NCT.00421668).

2. The KCMC maternal register.

The owners of these datasets had already received ethical approval from the relevant ethics bodies prior to carrying out the primary studies. My request for approval is for secondary analysis of these datasets.

Neither data set contains patient names or other identifiers to ensure confidentiality of participants' identity. Level one, self-audit form was filled in and signed under by the principal research supervisor. This is in accordance with the University's ethical approval procedures. A letter from the university's ethical review committee was issued, affirming that the study was a level one. Annex 8 shows the approval letters from owners of the datasets

and the approval letter from the University of Edinburgh (Usher Institute Research and Ethics Committee).

5.5.6 Study limitations

Incidence estimation of pre-eclampsia

Because the child 2 clinical trial was designed to focus on infants, rather than on pregnancy outcomes of their mothers, there are limitations based on the enrolment criteria of excluding HIV positive pregnant women. However, systematic review evidence shows no conclusive evidence of HIV being associated with pre-eclampsia (Adams *et al.* 2016). Therefore, the incidence of pre-eclampsia among HIV negative women is unlikely to be different from those who are HIV positive. Thus, it is reasonable to assume that my estimate is representative of the whole population of pregnant women in Dar es Salaam.

Chance

I used an alpha level of 5 % in my analysis in my four objectives thus allowing a 5 % chance of type I error.

Power

My study had sufficient sample size to detect a clinically meaningful difference in proportion between unequal groups with a power of 80 % and an alpha level of 0.05. (See sample size estimates sections above)

Selection bias

The clinical trial in Dar es Salaam recruited women based on the eligibility criteria (see annex 3). The recruitment was also voluntary (nonprobability sampling) hence may not represent the target population. Recruitment was carried out among pregnant women attending public antenatal clinics. These women are likely to differ in their risk of pre-eclampsia compared to more affluent pregnant women who are attending private antenatal clinics. This aspect may bias us to have a lower estimate of the incidence of pre-eclampsia in Dar es Salaam.

Non-response bias

Non-response bias occurs when participants chosen for the study are unwilling or unable to participate and this may result in the respondents to differ from non-respondents in relation to the characteristic under investigation. There were 4,650 eligible women, out of which 3,767 were enrolled in the child 2 clinical trial, giving a nonresponse rate of 19 %. It is assumed that those who enrolled did not differ in their characteristics from all

those who were eligible, thus making the dataset representative of the target population; however, this is an untested assumption, which may not be true.

Misclassification bias

My study's validity depends on the quality of the datasets in terms of how the variables were measured, recorded, and coded in the clinical trial and the KCMC hospital maternal register. In this process, there is a possibility for misclassification of the variables. This may impair identifying associations in the analysis. In order to minimise this challenge, I inspected all variable values in my datasets to detect obvious errors. I am not able to do a diagnosis audit to validate the assigned diagnosis because my data was from past years; it would be difficult to retrieve the case files of the patients for validation. Carrying out a diagnosis quality audit at the present time would not reflect the situation in past years when the data were generated. A diagnosis quality audit checks the diagnosis status of patients in a hospital and compares the results to what has been documented in the patient's diagnosis. It offers a measure of agreement between the documented diagnosis and the true diagnosis.

Confounding factors

Confounding variables such as maternal age, parity and gestational age at delivery were recorded and were used in the multivariable analysis to adjust

the strength of associations. However, other confounding variables such as change of paternity and previous abortions were not available or accurately captured to enable adjusting for their effects. Therefore, my results may still have a limitation of residual confounding.

5.5.7 Summary

This chapter introduces the context of Tanzania, one of the low-income countries in Africa. Data from this country is used for the secondary analysis of risk factors for pre-eclampsia. This chapter also outlines the problem statement highlighting the gaps from literature. The rationale and objectives of my study are presented in this chapter. Then a detailed plan of methods for analysing the data are presented for the respective objectives. The ethical approval process is discussed with respect to the dataset acquisition. The end of this chapter presents the potential study limitations and mitigations.

Chapter 6 Results of pre-eclampsia risk factor analysis in Tanzania.

Chapter Six provides the results from the planned analysis presented in the previous chapter. The results are organised according to the four objectives of the data analysis.

- Objective one has estimated the incidence of pre-eclampsia in Dar es Salaam, to be 1.9 %, 95 % CI 1.3 % to 2.2 %.
- Objective two has shown sociodemographic distribution of pre-eclampsia in northern Tanzania to be more prevalent among women aged above 35 years, single and women with tertiary education.
- Objective three has shown there is a difference in risk factors that explain term and preterm pre-eclampsia. The identified risk factors for all types of pre-eclampsia we're; maternal age, mother's weight before pregnancy, history of hypertension, HIV treatment, contraceptive IUD use, a diagnosis of malaria and a diagnosis of infections. Prediction models developed using these risk factors, were able to satisfactorily classify women with and those without pre-eclampsia outcome.
- Objective four has shown pre-eclampsia is associated with a high proportion of stillbirths. Furthermore, the surviving newborns from pre-eclampsia pregnancies have far worse indicators of physical and mental wellbeing.

6.1 Results for objective one

My study aimed to estimate the incidence of pre-eclampsia using data from a cohort of women who participated in a clinical trial conducted in Dar es Salaam, Tanzania from 2008 to 2009. This cohort from the trial had 3,767 participants residing in Dar es Salaam. Dar es Salaam is the commercial capital of Tanzania where rapid urbanization and lifestyle changes have manifested over the years.

I calculated the crude incidence of pre-eclampsia by age categories of pregnant mothers from the cohort dataset. (See the table below)

Table 7: Proportion of pre-eclampsia across different age categories generated from the cohort data

Proportion of pre-eclampsia in age categories from cohort data

Age categories	<20	20-34	35-49	Total
Non-pre-eclampsia	125	2433	322	2880
	96.9 %	98.3 %	97.9 %	98.2 %
Pre-eclampsia (n)	4	43	7	54
%	3.1 %	1.7 %	2.1 %	1.8 %
(95 % CI)	(0.1 – 6.0)	(1.1 – 2.2)	(0.5 – 3.6)	(1.3 – 2.2)
Total	129	2476	329	2934
	4.2 %	84.2 %	11.6 %	100.0 %

The above table shows the proportion of pre-eclampsia to be highest in the < 20years age category, followed by the older age category 35-49. The overall crude incidence of pre-eclampsia is 1.8 %.

Table 8: The standardised incidence of pre-eclampsia using the Tanzania demographic health survey (TDHS) 2005

Standardised pre-eclampsia incidence using THDS data 2005

Age categories	<20	20-34	35-49	Total
Distribution of women in Tanzania	906	4013	853	5772
Percentage	15.7 %	69.5 %	14.8 %	
Pre-eclampsia Incidence derived from above table (95 % CI)	3.1 % (0.1 – 6.0)	1.7 % (1.1 – 2.2)	2.1 % (0.5 – 3.6)	1.8 % (1.3 – 2.2)
Expected number of pre-eclampsia (95 % CI)	28 (1 – 54)	68 (47 – 88)	17 (4 – 31)	113 (76 – 131)

The proportions of pre-eclampsia for different age categories calculated from cohort data (table 7) are applied to the distribution of women in the Tanzanian population from a demographic health survey (TDHS) to adjust the crude incidence of pre-eclampsia. The 5772 women from the TDHS were selected though sampling to ensure their representativeness of women age in urban Tanzania.

The adjusted pre-eclampsia incidence point estimate is 1.9 percent [113/5772 = 1.9 %]. While the lower limit of 95 % CI is [76/5772 = 1.3 %] and the upper limit is [131/5772 = 2.2 %]. The standard error formula for a single proportion is square root of [p (1-p) / n]. Where p = 1.8 % and n = 2,934. The standard error is 0.245 % and the 95 % CI is [1.3 % to 2.2 %].

Adjusting the crude incidence of pre-eclampsia by maternal age produced the incidence of pre-eclampsia of 1.9 % [95 % CI (1.3 % - 2.2 %)]. This is similar to the world estimates of the incidence of pre-eclampsia, which is 2 % to 8 % of all pregnancies (WHO 2011).

6.2 Results for objective two

Objective two describe the sociodemographic characteristics of women with pre-eclampsia in northern Tanzania. The table below shows the comparison of the sociodemographic characteristic among women with and without pre-eclampsia. It shows this comparison in all deliveries and in the subgroups of women with the term and preterm deliveries.

Table 9: Sociodemographic characteristics of women with pre-eclampsia, among all deliveries, preterm and term deliveries subgroups

Sociodemographic characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases
			Mothers Age categories			
≤ 20	1890	55, 2.8 %	1643	41, 2.4 %	247	14, 5.4 %
21 - 25	4208	125, 2.9 %	3754	70, 1.8 %	454	55, 10.8 %
26 - 30	4494	134, 2.9 %	4075	96, 2.3 %	419	38, 8.3 %
31 - 35	3229	159, 4.7 %	2864	107, 3.6 %	365	52, 12.5 %
36 - 40	1325	91, 6.4 %	1169	55, 4.5 %	156	36, 18.8 %
≥ 41	249	23, 8.5 %	212	17, 7.4 %	37	6, 14.0 %
Pearson χ^2, D. freedom, P value		77.3, 5, <0.001		56.7, 5, <0.001		25.3, 5, <0.001
Trend χ^2, D. freedom, P value		57.4, 1, <0.001		39.0, 1, <0.001		16.3, 1, <0.001
			Education level categories			
No school	201	3, 1.5 %	184	2, 1.1 %	17	1, 5.6 %
Primary education	8887	298, 3.2 %	7763	193, 2.4 %	1124	105, 8.5 %
Secondary education	757	20, 2.6 %	678	17, 2.4 %	79	3, 3.7 %
Tertiary education	5532	265, 4.6 %	5080	174, 3.3 %	452	91, 16.8 %
Pearson χ^2, D. freedom, P value		23.4, 3, <0.001		11.5, 3, 0.009		31.6, 3, <0.001
Trend χ^2, D. freedom, P value		19.1, 1, <0.001		10.5, 1, 0.001		25, 1, 0.001

Sociodemographic characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
	Marital status categories					
Married	13578	503, 3.6 %	12146	332, 2.7 %	1432	171, 10.7 %
Single	1746	79, 4.3 %	1508	51, 3.3 %	238	28, 10.5 %
Widowed	7	0, 0 %	7	0, 0 %	-	-
Remarried	2	0, 0 %	2	0, 0 %	-	-
Divorced	11	0, 0 %	10	0, 0 %	1	0, 0 %
Polygamous	5	0, 0 %	4	0, 0 %	1	0, 0 %
Fisher's Exact test value (P value)		3.8 (0.54)		4.1 (0.55)		1.48 (1.00)
	Gravidae categories					
1	5639	200, 3.4 %	5070	138, 2.6 %	569	62, 9.8 %
2	3957	114, 2.8 %	3591	70, 1.9 %	366	44, 10.7 %
3	2515	99, 3.8 %	2224	75, 3.3 %	291	24, 7.6 %
4	1351	59, 4.2 %	1170	38, 3.1 %	181	21, 10.4 %
5+	1049	67, 6.0 %	870	38, 3.1 %	179	29, 13.9 %
Pearson χ^2, D. freedom, P value		28.3, 4, <0.001		19.9, 4, 0.001		5.7, 4, 0.22
Trend χ^2, D. freedom, P value		15.5, 1, <0.001		8.4, 1, 0.004		1.1, 1, 0.292
	Abortion/ Miscarriages					
(B) History of normal pregnancies	13146	466, 3.4 %	11752	324, 2.7 %	1394	142, 9.2 %
(A) History of failed pregnancies	1365	73, 5.1 %	1173	35, 2.9 %	192	38, 16.5 %
(A/B) Odds ratio (95% CI)		1.5(1.1-1.9)		1.0(0.7-1.5)		1.9(1.3-2.8)

Sociodemographic characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
Pearson χ^2, D. freedom, P value		10.2, 1, 0.001		0.19, 1, 0.661		11.5, 1, 0.001
	Mothers occupation					
Housewife	3188	108, 3.3 %	2797	72, 2.5 %	391	36, 8.4 %
Farmer	2969	104, 3.4 %	2545	68, 2.6 %	424	36, 7.8 %
Service	840	32, 3.7 %	745	20, 2.6 %	95	12, 11.2 %
Business	3521	132, 3.6 %	3161	89, 2.7 %	360	43, 10.7 %
Professional	3002	156, 4.9 %	2781	104, 3.6 %	221	52, 19.0 %
Student	369	16, 4.2 %	340	9, 2.6 %	29	7, 19.4 %
Others	1477	37, 2.4 %	1322	23, 1.7 %	155	14, 8.3 %
Pearson χ^2, D. freedom, P value		23.2, 6, 0.001		14.3, 6, 0.026		30.2, 6, <0.001
	Fathers occupation					
Farmer	1908	63, 3.2 %	1610	39, 2.4 %	298	24, 7.5 %
Business	4326	158, 3.5 %	3888	101, 2.5 %	438	57, 11.5 %
Skilled worker	2486	94, 3.6 %	2192	68, 3.0 %	294	26, 8.1 %
Unskilled worker	60	2, 3.2 %	53	1, 1.9 %	7	1, 12.5 %
Service	2217	73, 3.2 %	1948	51, 2.6 %	269	22, 7.6 %
Official	12	0, 0 %	12	0, 0 %	-	-
Professional	3700	177, 4.6 %	3401	113, 3.2 %	299	64, 17.6 %
Student	293	9, 3.0 %	267	7, 2.6 %	26	2, 7.1 %
Unemployed	6	0, 0 %	5	0, 0 %	1	0, 0 %
Others	363	9, 2.4 %	320	5, 1.5 %	43	4, 8.5 %
Pearson χ^2, D. freedom, P value		14.6, 9, 0.102		7.8, 9, 0.551		28.1, 8, <0.001

The proportion of pre-eclampsia increases linearly across the maternal age ordinal categories. This is observed among all deliveries and in both subgroups of term and preterm deliveries.

There is a linear increase in the proportion of pre-eclampsia across mother's education ordinal categories. This trend is observed in all pre-eclampsia deliveries and in the preterm and term delivery subgroups.

The proportion of women with pre-eclampsia does not differ significantly across the marital status categories. This applied to all deliveries and in the term and preterm subgroups.

There is an increase in the proportion of pre-eclampsia across gravidae categories. This trend was observed on all pre-eclampsia and term deliveries. However, this trend was not observed in the preterm delivery subgroup.

Pre-eclampsia is associated with abortion/miscarriages in all deliveries and in preterm deliveries. Odds ratio (abortions/miscarriages vs normal pregnancy) 1.5 [95 % CI (1.1 - 1.9)] and 1.9 [95 % CI (1.3 - 2.8)] respectively. This association was not observed in the term delivery subgroup, odds ratio 1.00 [95 % CI (0.7 -1 .5)].

The proportion of pre-eclampsia differs across the nominal categories of mother's occupation. The proportion of pre-eclampsia was highest among

professional mothers. This was observed in all deliveries, and in term and preterm deliveries subgroups.

The proportion of pre-eclampsia was not different across the nominal categories of father's occupation.

The overall social demographic comparison of women with and without pre-eclampsia shows women with older age, single, higher education level, history of abortion/miscarriage, professional occupation and with a higher number of pregnancies were more likely to have pre-eclampsia. On the other hand, father's occupation categories were not associated with having pre-eclampsia.

I further analysed these sociodemographic characteristics to explore if they explained the occurrence of pre-eclampsia in all deliveries, after adjustment. The table below shows the result of a multivariable logistic regression model for sociodemographic characteristics only. I did not find any evidence of multicollinearity in the model.

Table 10: A multivariable logistic regression model for sociodemographic characteristics explaining pre-eclampsia outcome among all deliveries

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
Mothers education level (All other education levels-reference)							
Tertiary education	0.29	0.11	6.1	.013	1.34	1.06	1.69
Mothers occupation (All other occupations, reference)							
Professional	0.14	0.13	1.21	0.27	1.15	0.89	1.50
Marital status (All other categories, reference)							
Single	0.33	0.13	6.06	0.01	1.39	1.07	1.82
Fathers Occupation (Farmer, reference)			2.91	0.96			
Business	0.13	0.161	0.72	0.39	1.14	0.83	1.57
Skilled worker	0.17	0.17	1.00	0.31	1.19	0.84	1.68
Unskilled worker	-0.55	1.02	0.29	0.58	0.57	0.07	4.24
Service	<0.01	0.18	<0.01	0.97	1.00	0.70	1.43
Official	-17.84	11531	<0.01	0.99	<0.001	<0.001	.
Professional	0.15	0.17	0.75	0.38	1.16	0.82	1.63
Student	-0.04	0.36	0.01	0.90	0.95	0.47	1.94
Unemployed	-18.09	14116	<0.01	0.99	<0.001	<0.001	.
Others	-0.08	0.33	0.05	0.81	0.92	0.47	1.77
Mothers Age categories (≤20 Reference)			31.55	<0.01			
21-25	-0.10	0.16	0.35	0.55	0.90	0.65	1.25

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
26-30	-0.11	0.17	0.37	0.54	0.89	0.63	1.27
31-35	0.32	0.18	2.87	0.09	1.37	0.95	1.99
36-40	0.60	0.21	7.71	<0.01	1.82	1.19	2.79
≥41	0.95	0.29	10.55	<0.01	2.59	1.45	4.60
Gravidae categories (1, reference)			7.57	0.10			
2	-0.24	0.12	3.54	0.06	0.78	0.60	1.01
3	-0.09	0.15	0.37	0.54	0.91	0.67	1.22
4	-0.03	0.18	0.04	0.84	0.96	0.67	1.38
5+	0.18	0.20	0.87	0.35	1.20	0.81	1.79
History of Abortion/Miscarriage (Normal pregnancies, reference)	0.26	0.14	3.50	0.06	1.30	0.98	1.72
Constant	-3.66	0.18	382.1	<0.01	0.02		

The above model for pre-eclampsia outcome among all deliveries suggests that being single, maternal age and tertiary education are the only jointly statistically significant sociodemographic factors that are associated with the outcome, after adjustment. A possible explanation is that women with tertiary education have a lifestyle that predisposes them to be more at risk than their counterparts. Single women may not have gained enough exposure to their partner's sperm.

6.3 Results for objective three

Objective three aimed to identify risk factors for pre-eclampsia, term and preterm deliveries subgroups, among women in northern Tanzania. This result section contains key findings in the development and validation of the prediction model using maternal biomedical risk factors. My original dataset contained 16,342 cases form KCMC hospital. I then randomly split the dataset into two parts, 80 % (13,113 cases) for the training set and 20 % (3,229 cases) for internal validation. I did not have access to another dataset with similar predictor variables coming from an African setting, which I could use for external validation.

My analysis was focused primarily on modelling for the outcome of pre-eclampsia for all deliveries, then I went on to carry out two sub-analyses by modelling for the outcome of pre-eclampsia among term deliveries followed by pre-eclampsia among preterm deliveries.

The first set of the abovementioned models was developed using complete case analysis of the training dataset under the assumption of missing completely at random. Then the above models were applied on the same training dataset after single imputation since most of them were dichotomous in nature (yes or no for a disease). There was no improvement on the model's performance in classifying pre-eclampsia outcome when applied on the imputed training dataset. The beta coefficients of the predictors and their standard errors did not reasonably differ between the complete case and

imputed analysis outputs. The complete case analysis models developed initially were then internally validated using the 20 % data that were spared for validation purposes.

I used the Statistical Package for Social Science (SPSS) version 25 to carry out my analysis. Binary logistic regression modelling was used in my analysis. Univariable analysis was carried out for each of the biomedical factors. The results of the univariable association are presented in Tables 13, 17 and 21 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries respectively).

The statistically significant variables were then added to a multivariable model using the Enter method in SPSS. The multivariable model was revised to exclude manually non statistically significant variables in the model. The final prediction models are presented in tables 15, 19 and 23 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries respectively). The performance of the developed models was determined by the area under the receiver operator curve (ROC), presented in figures 9, 10 and 11 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries respectively).

My dataset had missing values. SPSS software omits all cases with missing variables in the analysis; therefore, my complete case analysis had a reduced sample size. In order to address this gap, I applied single imputation for missing values in my training dataset and developed similar models to the

above developed models (seen in tables 15, 19 and 23). I then compared their classification performance and beta coefficient values of the predictors. The results of the models developed on imputed training datasets are presented in tables 25, 26 and 27 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries respectively). However, there was no improvement in the classification performance from the models developed from the imputed training dataset (see figure 12, 13 and 14). The beta coefficients of my predictors did not reasonably differ from that of complete case model output. The standard errors were slightly smaller in the imputed models outputs due to an increase in sample size after imputing all missing values.

I carried out validation of the developed models. These models performed satisfactorily on the 20 % validation dataset. There was an increase in the standard error of the beta coefficients in all the models because the 20 % validation dataset is of a smaller sample size. This increase in the standard error of the beta coefficient rendered some predictor variables to have a wide confidence interval and thus become non statistically significant. However, there was little change in the values of most beta coefficients point estimates in the models developed. The results of the validated models are presented in tables 28, 29 and 30 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries respectively). The 95 % CI of the area under the curve (AUC) of the model's performance on the validation dataset was similar to that in the complete case analysis. The

results of the AUC are presented in figure 15, 16 and 17 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries respectively)

I also performed diagnostics checks for the logistic regression models; I tested the impact of potential correlation of my outcome within the cluster of each individual women by modelling the pre-eclampsia outcome using generalised estimating equation (GEE). The results of GEE univariable analysis (see table 14, 18 and 22) and later the multivariable results of GEE are presented side by side with the results of logistic regression, see table 16, 20 and 24 (all pre-eclampsia, pre-eclampsia among term deliveries and pre-eclampsia among preterm deliveries.

6.3.1 Exploration of data

I explored my data to understand the nature of the relationship between the predictor variables and the outcome. I am citing one example, where I explored the nature of the relationship between maternal age and the risk of pre-eclampsia expressed in percentage. A study by Kumari (2016) showed maternal age had a J- shape relationship with pre-eclampsia. However, this study did not factor the difference brought about by the number of pregnancies (nulliparity and parity), which is an important confounder of this relationship.

Figure 6: The relationship between women age and proportion of pre-eclampsia for all women.

I grouped women to their age and calculated the proportion of women with pre-eclampsia in that age. This resulted in a U-shaped relationship. The outliers' values were due to a few women in that age group. Example 1 out of 3 gives 33 %.

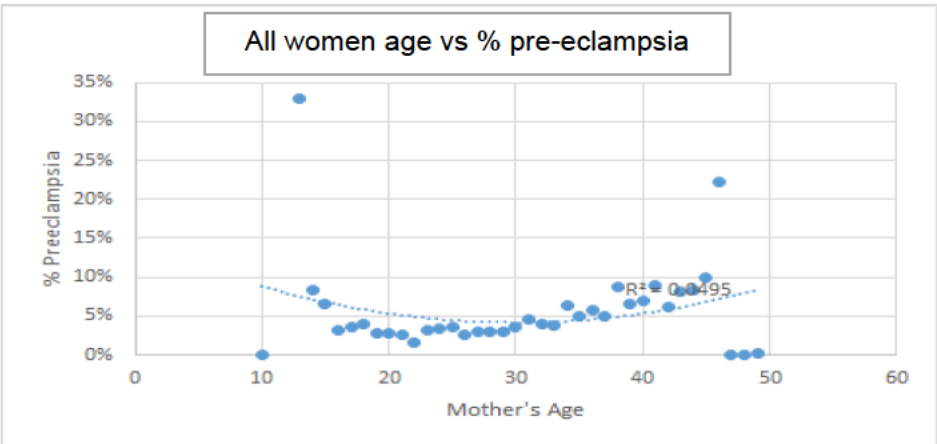


Figure 7: The relationship between maternal age and proportion of pre-eclampsia among parous women.

I selected only parous women then grouped them according to their age and calculated the proportion of women with pre-eclampsia in that age. This resulted in a linear shaped relationship.

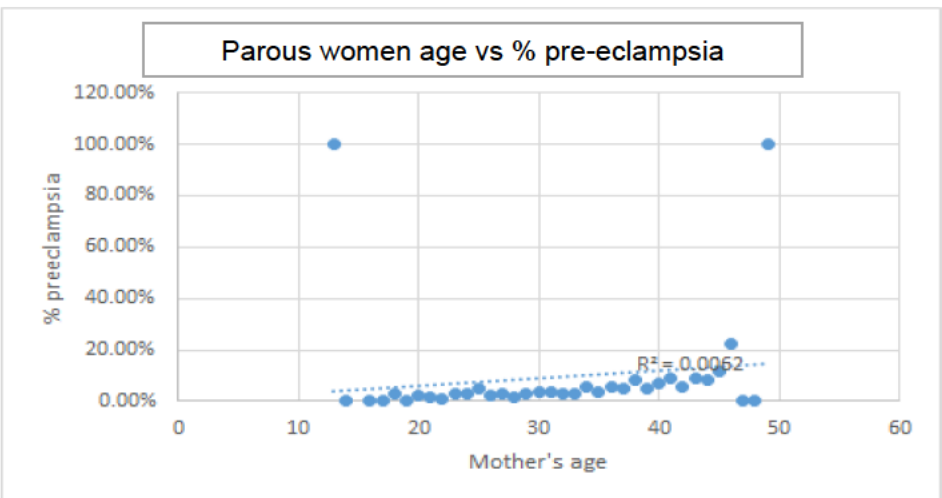
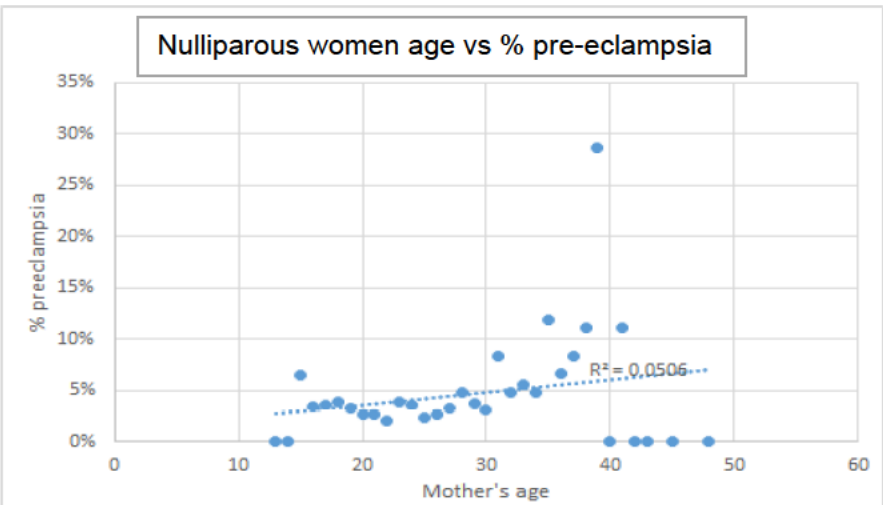


Figure 8. The relationship between maternal age and proportion of pre-eclampsia among nulliparous women.

I selected only nulliparous women then grouped them according to their age and calculated the proportion of women with pre-eclampsia in that age. This also resulted in a linear shaped relationship.



My above analysis to address the confounding effect of a number of pregnancies (nulliparity) on the relationship between maternal age and pre-eclampsia has shown a linear relationship. On this basis, I have fitted a linear relationship in my modelling of pre-eclampsia outcome while using maternal age, the number of pregnancies and other predictors.

6.3.2 Missing data

This section shows the extent of missing values in my original dataset and the measures used to reduce missingness in some of the predictor variables that had an unacceptably high proportion of missingness. I build linear regression models using other variables in the dataset to populate the

missing values in numerical data. In one variable (mother's height), I imputed the mean value of mother's height.

*- denotes the variables that were modified to reduce missingness. There was no systematic pattern to the missingness of these values

Table 11 Percentage of missing values in my sociodemographic and predictor variables.

Predictor variables		
Variable list	Valid	Missing
Maternal age	16338	4
Number of pregnancies	15391	951
Gestational age in weeks LMP*	14783	1559 (9.5 %)
Body weight at admission	16276	66
Highest educational level of mother	16318	24
Mothers occupation	16314	28
Marital status	16328	14
Fathers age	16229	113
Fathers education	16295	47
Fathers occupation	16306	36
Hx Diabetes	16342	0
Hx Hypertension	16342	0
Hx Heart disease	16342	0
Hx Epilepsy	16342	0
Hx Malaria	16342	0
Hx Anaemia	16342	0
Hx Liver disease	16342	0
Hx Kidney disease	16342	0
Hx Sickle cells	16342	0
Mothers weight before pregnancy**	13321	3021(18.5 %)
Mothers height***	13604	2738 (16.7 %)
p-pills	16342	0
injections	16342	0
implant	16342	0
Number of ANC visits	16095	247

Chewing tobacco	16320	22
Chewing tobacco during pregnancy?	16316	26
Do you Smoke	16312	30
Smoking during this pregnancy	16329	13
Do you drink alcohol beverage	16260	82
Alcohol during this pregnancy	16321	21
Result of HIV-test****	14822	1520(9.3 %)
HIV treatment	16308	34
Diagnosis of Gest diabetes	16342	0
Diagnosis of diabetes	16342	0
Diagnosis of Hypertension	16342	0
Diagnosis of Pre-eclampsia	16342	0
Diagnosis of eclampsia	16342	0
Diagnosis of epilepsia	16342	0
Diagnosis of leeding	16342	0
Diagnosis of anaemia	16342	0
Diagnosis of hyperemsis	16342	0
Diagnosis of malaria	16342	0
Diagnosis of heart disease	16342	0
Diagnosis of infections	16342	0

* Missingness reduced to 2 %, using a model with R^2 = 26.7 %.

** Missingness reduced to 7.7 %, using a model with R^2 = 57.7 %.

*** Missingness reduced to 0 %, imputed mean height = 160.4cm to 2758 missing values out of 13,584 cleaned values.

**** No imputation done, variable not used at all instead we used the below variable, HIV treatment to avoid multicollinearity effect between HIV test and HIV treatment variables in modelling.

Table 12 Percentage of missing values in my pregnancy outcome variables

Pregnancy outcome variables		
Variable list	Valid	Missing
Mothers health after delivery	16309	33
sequence for twins	16342	0
Childs status	16316	26
Sex of the child (neonates)	16284	58
birth weight	16308	34
birth length*	16104	238
head circumference**	16090	252
Mode of delivery	16323	19
Apgar 1min	16300	42

*Missingness reduced from 1.55 % to 1.4 % after imputing with a model R^2 = 45.6 % suing birthweight and head circumference. There was no significant reduction (only 0.15 %) in missingness using these variables as they also had missing values.

**I did reduce missingness in this variable since it would result in a non-significant reduction as above.

6.3.3 Complete case modelling

Table 13: Logistic regression model; univariable association of biomedical factors to all pre-eclampsia outcome.

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
Maternal age	0.05	0.007	55.4	<0.01	1.05	1.03	1.06
Number of pregnancies	0.09	0.027	11.7	0.001	1.09	1.04	1.15
History of Diabetes	-0.46	1.013	0.2	0.646	0.62	0.08	4.57
History of Hypertension	2.34	0.223	110.7	<0.01	10.42	6.73	16.12
History of Heart disease	-17.95	5926.1	0.0	0.998	<0.01	<0.01	.
History of Liver disease	-0.46	1.013	0.2	0.647	0.62	0.08	4.57
History of Kidney disease	0.90	0.429	4.4	0.035	2.47	1.06	5.73
History of Sickle cells	1.86	1.119	2.7	0.096	6.45	0.72	57.85
History of contraceptive p-pills use	0.09	0.096	0.8	0.344	1.09	0.90	1.32
History of contraceptive injections use	0.04	0.091	0.2	0.651	1.04	0.87	1.24
History of contraceptive IUD use	0.88	0.180	24.2	<0.01	2.43	1.70	3.46
History of contraceptive implant use	-0.02	0.266	0.01	0.913	0.97	0.57	1.63
History of contraceptive male condoms use	-0.47	0.266	3.1	0.077	0.62	0.37	1.05

Drinking Alcohol	-0.13	0.098	2.0	0.155	0.87	0.71	1.05
Chewing tobacco	1.86	1.119	2.7	0.096	6.44	0.71	57.76
Smoking cigarette	1.45	1.081	1.8	0.178	4.29	0.51	35.72
Result of HIV-test	-0.71	0.256	7.7	0.005	0.49	0.29	0.81
HIV treatment	-0.70	0.264	7.1	0.008	0.49	0.29	0.82
Diagnosis of Gest diabetes	-17.95	17974	0.0	0.999	<0.01	<0.01	.
Diagnosis of Diabetes	0.44	0.729	0.3	0.539	1.56	0.37	6.53
Diagnosis of Anaemia	-0.36	0.361	0.9	0.319	0.69	0.34	1.41
Diagnosis of Hyperemesis Gravidarum	-0.008	0.363	0.0	0.982	0.99	0.48	2.01
Diagnosis of malaria	-0.47	0.136	12.3	<0.01	0.62	0.47	0.80
Diagnosis of heart disease	-17.95	13397	0.0	0.999	<0.01	<0.01	.
Diagnosis of infections	-0.84	0.125	46.1	<0.01	0.42	0.33	0.54
Mothers weight before pregnancy	0.03	0.003	137.4	<0.01	1.03	1.02	1.03
Mothers height	0.02	0.007	9.2	0.002	1.02	1.00	1.03

The below table offers model comparison between the generalised estimating equation and the above logistic regression model.

Table 14: Generalised estimating equation model; univariable association of biomedical factors to all pre-eclampsia outcome.

The generalised estimating equation did not converge on univariable analysis of some variables due to few data hence they are left blank on this table.

Variables	B	S.E.	Wald	Sig	Odds Ratio (OR)	95 % C. I. for Odds ratio	
						Lower	Upper
Maternal age	0.05	0.01	29.53	<0.01	1.05	1.03	1.07
Number of pregnancies	0.08	0.03	5.49	0.01	1.09	1.01	1.17
History of Diabetes	-	-	-	-	-	-	-
History of Hypertension	2.15	0.32	43	<0.01	8.59	4.53	16.28
History of Heart disease	-	-	-	-	-	-	-
History of Liver disease	-0.26	1.01	0.06	0.79	0.76	0.10	5.62
History of Kidney disease	0.36	0.72	0.25	0.61	1.443	0.349	5.959
Diagnosis of Infection	-0.78	0.15	27.26	<0.01	0.45	0.33	0.61
History of Sickle cells	2.16	1.15	3.51	0.06	8.73	0.90	84.08
History of contraceptive p-pills use	0.03	0.10	0.09	0.76	1.03	0.83	1.28
History of contraceptive injections use	0.13	0.10	1.78	0.18	1.14	0.93	1.39
History of contraceptive implant use	-0.036	0.29	0.01	0.90	0.96	0.53	1.72

Variables	B	S.E.	Wald	Sig	Odds Ratio (OR)	95 % C. I. for Odds ratio	
						Lower	Upper
History of contraceptive IUD use	0.88	0.18	24.22	<0.01	2.43	1.70	3.46
History of using male condom	-0.47	0.265	3.13	0.07	0.62	0.37	1.05
Chewing tobacco	-	-	-	-	-	-	-
Smoking cigarette	-	-	-	-	-	-	-
Drinking Alcohol	-0.16	0.13	1.54	0.21	0.84	0.64	1.10
Result of HIV-test	1.03	0.41	6.16	0.01	2.80	1.24	6.31
HIV treatment	-0.079	0.38	4.23	0.04	0.45	0.21	0.96
Diagnosis of Gest diabetes	-	-	-	-	-	-	-
Diagnosis of Diabetes	-	-	-	-	-	-	-
Diagnosis of Anaemia	-0.371	0.416	0.79	0.37	0.69	0.305	1.56
Diagnosis of Hyperemesis Gravidalum	-0.03	0.51	<0.01	0.94	0.96	0.35	2.62
Diagnosis of Malaria	-0.414	0.18	5.09	0.02	0.66	0.46	0.94
Diagnosis of Heart disease	-	-	-	-	-	-	-
Mothers weight before pregnancy	0.03	<0.01	86.75	<0.01	1.03	1.03	1.04
Mothers height	0.02	0.01	4.97	0.02	1.02	1.00	1.04

Table 15: Logistic regression model; multivariable association of maternal age, history of hypertension, HIV treatment, diagnosis of malaria, diagnosis of infections, contraceptive use of IUD, mothers weight before pregnancy and all pre-eclampsia outcome.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-5.87	0.29	394.3	1	<0.01	0.00		
History of Hypertension (Yes)	1.77	0.28	38.3	1	<0.01	5.89	3.36	10.33
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.70	0.16	19.5	1	<0.01	0.49	0.36	0.67
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.99	0.13	58.3	1	<0.01	0.37	0.28	0.47
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.45	0.19	5.4	1	0.01	1.57	1.07	2.30
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.75	0.29	6.3	1	0.01	0.47	0.26	0.84
(No)	-	-	-	-	-	1	-	-
Maternal age	0.03	0.00	15.4	1	<0.01	1.03	1.01	1.05
Mother's weight before pregnancy	0.03	0.00	71.5	1	<0.01	1.03	1.02	1.03

Model goodness of fit (Hosmer and Lemeshow test) χ^2=8.168, d.f 8, P value 0.417

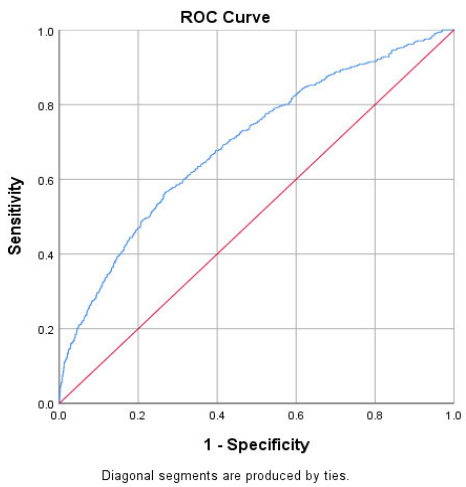
Contingency Table for Hosmer and Lemeshow Test					
	Non pre-eclampsia		Pre-eclampsia		
Decile (10%)	Observed	Expected	Observed	Expected	Total
1	1201	1206	17	11	1218
2	1201	1205	21	16	1222
3	1196	1189	15	21	1211
4	1183	1183	28	27	1211
5	1179	1178	32	32	1211
6	1173	1170	36	38	1209
7	1173	1166	39	45	1212
8	1158	1158	55	54	1213
9	1138	1145	78	70	1216
10	1066	1063	125	127	1191

The below table offers model comparison between the generalised estimating equation and the above logistic regression model.

Table 16: Generalised estimating equation model; multivariable association of maternal age, history of hypertension, HIV treatment, diagnosis of malaria, diagnosis of infections, contraceptive use of IUD, number of pregnancies, mothers weight before pregnancy and all pre-eclampsia outcome.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Intercept)	-5.87	0.31	338.8	1	<0.01	0.00	0.00	0.00
History of Hypertension (Yes)	1.77	0.28	38.6	1	<0.01	5.89	3.37	10.31
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.70	0.15	20.2	1	<0.01	0.49	0.36	0.67
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.99	0.13	58.1	1	<0.01	0.37	0.28	0.47
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.45	0.19	5.3	1	0.02	1.57	1.07	2.32
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.75	0.30	6.2	1	0.01	0.47	0.26	0.84
(No)	-	-	-	-	-	1	-	-
Maternal age	0.03	0.00	14.8	1	<0.01	1.03	1.01	1.05
Mother's weight before pregnancy	0.03	0.00	68.3	1	<0.01	1.03	1.02	1.03

Figure 9: Logistic regression model performance on a receiver operator curve. The above model predicts overall pre-eclampsia outcome.



*Area under the curve 0.694

The predicted probability cut off point that maximises sensitivity and specificity is 0.035. Youden's Index yields a sensitivity of 65 % and specificity of 63 %.

Model classification at Youden's index

		Predicted		
		Non pre-eclampsia	Pre-eclampsia	
Observed	Non pre-eclampsia	7351	4317	Specificity 63%
	Pre-eclampsia	156	290	Sensitivity 65%
Overall classification percentage				63%

Positive likelihood ratio = 1.75; Negative likelihood ratio = 0.55.

Table 17: Logistic regression model: univariable association of biomedical factors and pre-eclampsia outcome among term deliveries.

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
Maternal age	0.05	0.009	33.6	<0.01	1.05	1.03	1.07
Number of pregnancies	0.08	0.039	5.2	0.023	1.09	1.01	1.17
History of Diabetes	-17.64	7595	0.0	0.998	<0.01	<0.01	.
History of Hypertension	2.15	0.326	43.4	<0.01	8.59	4.53	16.28
History of Heart disease	-17.64	7218	0.0	0.998	<0.01	<0.01	.
History of Liver disease	0.03	1.016	0.0	0.971	1.03	0.14	7.60
History of Kidney disease	0.36	0.724	0.2	0.612	1.44	0.34	5.95
History of Sickle cells	-17.64	23205	0.0	0.999	<0.01	<0.01	.
History of contraceptive p-pills use	0.10	0.133	0.6	0.437	1.10	0.85	1.43
History of contraceptive injections use	0.02	0.127	0.03	0.850	1.02	0.79	1.31
History of contraceptive IUD use	1.06	0.205	26.7	<0.01	2.89	1.93	4.32
History of contraceptive implant use	0.32	0.313	1.05	0.303	1.38	0.74	2.54
History of contraceptive male condoms use	-0.99	0.415	5.73	0.017	0.37	0.16	0.83

Drinking Alcohol	-0.16	0.136	1.54	0.215	0.84	0.64	1.10
Chewing tobacco	-17.64	23205	<0.01	0.999	<0.01	<0.01	.
Smoking cigarette	-17.64	17974	<0.01	0.999	<0.01	<0.01	.
Result of HIV-test	-1.03	0.415	6.16	0.013	0.35	0.15	0.80
HIV treatment	-0.79	0.385	4.23	0.040	0.45	0.21	0.96
Diagnosis of Gest diabetes	-17.64	17974	<0.01	0.999	<0.01	<0.01	.
Diagnosis of Diabetes	-17.64	9220	<0.01	0.998	<0.01	<0.01	.
Diagnosis of Anaemia	-0.61	0.585	1.12	0.289	0.53	0.17	1.69
Diagnosis of Hyperemesis Gravidalum	-0.03	0.510	<0.01	0.946	0.96	0.35	2.62
Diagnosis of Malaria	-0.41	0.184	5.09	0.024	0.66	0.46	0.94
Diagnosis of Heart disease	-17.64	20096	<0.01	0.999	<0.01	<0.01	.
Diagnosis of Infections	-0.78	0.151	27.26	<0.01 0	0.45	0.33	0.61
Mothers weight before pregnancy	0.03	0.004	91.59	<0.01	1.03	1.03	1.04
Mothers height	0.02	0.009	6.24	0.012	1.02	1.00	1.04

The below table offers model comparison between the generalised estimating equation and the above logistic regression model.

Table 18: Generalised estimating equation model; univariable association of biomedical factors and pre-eclampsia outcome among term deliveries.

The generalised estimating equation did not converge on univariable analysis of some variables due to few data hence they are left blank on this table.

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
Maternal age	0.055	0.010	29.5	<0.01	1.05	1.03	1.07
Number of pregnancies	0.089	0.037	5.4	0.01	1.09	1.015	1.177
History of Diabetes	-	-	-	-	-	-	-
History of Hypertension	2.15	0.326	43.4	<0.01	8.5	4.5	16.2
History of Heart disease	-	-	-	-	-	-	-
History of Liver disease	0.037	1.01	<0.01	0.97	1.03	0.14	7.06
History of Kidney disease	0.36	0.724	0.25	0.61	1.4	0.3	5.9
History of Sickle cells	-	-	-	-	-	-	-
History of contraceptive p-pills use	0.103	0.133	0.60	0.43	1.10	0.85	1.43
History of contraceptive injections use	0.024	0.127	0.03	0.85	1.02	0.79	1.31
History of contraceptive IUD use	1.062	0.205	26.77	<0.01	2.89	1.93	4.32
History of contraceptive implant use	0.322	0.312	1.05	0.30	1.38	0.74	2.54
History of contraceptive	-0.99	0.414	5.73	0.01	0.37	0.16	0.83

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
male condom use							
Chewing tobacco	-	-	-	-	-	-	-
Smoking cigarette	-	-	-	-	-	-	-
Drinking Alcohol	-0.16	0.135	1.54	0.21	0.845	0.648	1.102
Result of HIV-test	1.030	0.414	6.16	0.01	2.8	1.2	6.3
HIV treatment	-0.79	0.384	4.23	0.04	0.4	0.2	0.9
Diagnosis of Gest diabetes	-	-	-	-	-	-	-
Diagnosis of Diabetes	-	-	-	-	-	-	-
Diagnosis of Anaemia	-0.61	0.584	1.12	0.28	0.53	0.17	1.69
Diagnosis of Hyperemesis Gravidarum	-0.03	0.510	<0.01	0.94	0.96	0.35	2.62
Diagnosis of Malaria	-0.41	0.183	5.09	0.02	0.6	0.4	0.9
Diagnosis of Infections	-0.78	0.151	27.26	<0.01	0.4	0.3	0.6
Diagnosis of Heart disease	-	-	-	-	-	-	-
Mothers weight before pregnancy	0.038	0.004	86.7	<0.01	1.038	1.030	1.047
Mothers height	0.023	0.010	4.9	0.02	1.023	1.003	1.045

Table 19:Logistic regression model; multivariable association of maternal age, history of hypertension, HIV treatment, diagnosis of infections, contraceptive IUD use, diagnosis of malaria, number of pregnancies, mothers weight before pregnancy and pre-eclampsia outcome among term deliveries.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-6.70	0.39	291.1	1	<0.01	0.001		
History of Hypertension (Yes)	1.5	0.38	16.7	1	<0.01	4.74	2.24	9.99
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.73	0.20	13.2	1	<0.01	0.48	0.32	0.71
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.98	0.16	37.1	1	<0.01	0.37	0.27	0.51
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.71	0.22	9.8	1	0.002	2.05	1.31	3.20
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.98	0.42	5.5	1	0.019	0.37	0.16	0.85
(No)	-	-	-	-	-	1	-	-
Maternal age	0.05	0.01	14.1	1	<0.01	1.05	1.02	1.07
Number of pregnancies	-0.14	0.05	6.7	1	0.009	0.86	0.77	0.96
Mother's weight before pregnancy	0.03	0.00	62.8	1	<0.01	1.03	1.02	1.04

Model goodness of fit (Hosmer and Lemeshow test) χ^2=1.858, d.f 8, P value 0.985

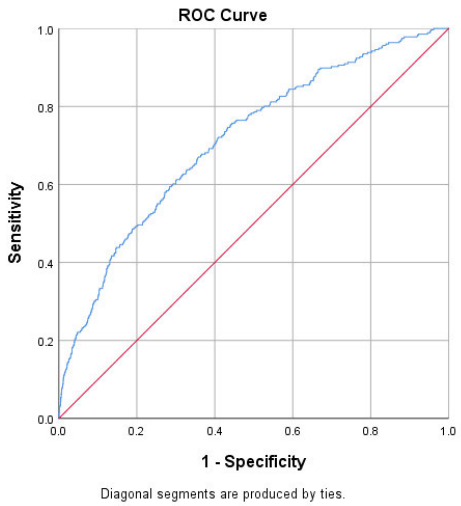
Contingency Table for Hosmer and Lemeshow Test					
	Non pre-eclampsia		Pre-eclampsia		
Decile (10%)	Observed	Expected	Observed	Expected	Total
1	994	994	6	5	1000
2	988	989	11	9	999
3	989	987	11	12	1000
4	984	983	15	15	999
5	980	979	18	18	998
6	975	976	24	22	999
7	975	974	26	26	1001
8	968	965	31	33	999
9	948	954	51	44	999
10	910	906	83	86	993

The below table offers model comparison between the generalised estimating equation and the above logistic regression model.

Table 20: Generalised estimating equation model; multivariable association of maternal age, History of hypertension, HIV treatment, diagnosis of infections, contraceptive IUD use, diagnosis of malaria, number of pregnancies, mother's weight before pregnancy and pre-eclampsia outcome among term deliveries.

							95 % Confidence Interval (OR)	
Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	Lower	Upper
(Intercept)	-6.70	0.42	253.6	1	<0.01	0.00	0.00	0.00
History of Hypertension (Yes)	1.55	0.38	16.3	1	<0.01	4.74	2.22	10.07
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.73	0.19	13.5	1	<0.01	0.48	0.32	0.71
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.98	0.16	36.8	1	<0.01	0.37	0.27	0.51
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.71	0.23	9.5	1	<0.01	2.05	1.30	3.23
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.98	0.42	5.4	1	0.01	0.37	0.16	0.85
(No)	-	-	-	-	-	1	-	-
Maternal age	0.05	0.01	12.9	1	<0.01	1.05	1.02	1.08
Number of pregnancies	-0.14	0.06	5.8	1	0.01	0.86	0.76	0.97
Mother's weight before pregnancy	0.03	0.00	64.8	1	<0.01	1.03	1.02	1.04

Figure 10: Logistic regression model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome among term deliveries.



*Area under the curve 0.712

The predicted probability cut off point that maximises sensitivity and specificity is 0.026. Youden's index yields a sensitivity of 65 % and specificity of 65 %.

Model classification at Youden's index

		Predicted		
		Non pre-eclampsia	Pre-eclampsia	
Observed	Non pre-eclampsia	6312	3398	Specificity 65%
	Pre-eclampsia	179	97	Sensitivity 65%
Overall classification percentage				64%

Positive likelihood ratio = 1.85; Negative likelihood ratio = 0.53.

Table 21: Logistic regression model; univariable association of biomedical factors and pre-eclampsia outcome among preterm deliveries

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
Maternal age	0.05	0.014	15.47	<0.01	1.05	1.02	1.08
Number of pregnancies	0.04	0.054	0.75	0.38	1.04	0.94	1.16
History of Diabetes	0.39	1.084	0.13	0.71	1.48	0.17	12.38
History of Hypertension	2.23	0.508	19.30	<0.01	9.30	3.44	25.15
History of Heart disease	-19.02	20096	<0.01	0.99	<0.01	<0.01	.
History of Liver disease	-	-	-	-	-	-	-
History of Kidney disease	1.08	1.158	0.88	0.34	2.96	0.30	28.72
History of Sickle cells	23.39	40192	<0.01	1.00	14422364222	<0.01	.
History of contraceptive p-pills use	0.06	0.206	0.09	0.76	1.06	0.71	1.59
History of contraceptive injections use	0.33	0.177	3.48	0.06	1.39	0.98	1.96
History of contraceptive IUD use	0.45	0.419	1.18	0.27	1.57	0.69	3.58
History of contraceptive implant use	-1.47	1.017	2.11	0.14	0.22	0.03	1.67
History of contraceptive male condoms use	0.43	0.371	1.34	0.24	1.53	0.74	3.18
Drinking Alcohol	0.01	0.203	<0.01	0.95	1.01	0.67	1.50

Chewing tobacco	-	-	-	-	-	-	-
Smoking cigarette	-	-	-	-	-	-	-
Result of HIV-test	-0.47	0.433	1.20	0.27	0.62	0.26	1.45
HIV treatment	-0.62	0.469	1.75	0.18	0.53	0.21	1.34
Diagnosis of Gest diabetes	-19.02	23205	<0.01	0.99	<0.01	<0.01	.
Diagnosis of Diabetes	0.10	1.064	0.01	0.92	1.10	0.13	8.93
Diagnosis of Anaemia	0.19	0.621	0.09	0.75	1.21	0.35	4.10
Diagnosis of Hyperemesis Gravidarum	-0.76	1.030	0.55	0.45	0.46	0.06	3.48
Diagnosis of Malaria	-0.27	0.276	0.95	0.32	0.76	0.44	1.31
Diagnosis of Heart disease	-19.02	40192	<0.01	1.00	<0.01	<0.01	.
Diagnosis of Infections	-0.91	0.230	15.92	<0.01	0.40	0.25	0.62
Mothers weight before pregnancy	0.03	0.006	32.41	<0.01	1.03	1.02	1.04
Mothers height	0.02	0.015	3.26	0.07	1.02	0.99	1.05

The below table offers model comparison between the generalised estimating equation and the above logistic regression model.

Table 22: Generalised estimating equation model; univariable association of biomedical factors and pre-eclampsia outcome among preterm deliveries.

The generalised estimating equation did not converge on univariable analysis of some variables due to few data hence they are left blank on this table.

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
Maternal age	0.053	0.013	16.6	<0.01	1.05	1.02	1.08
Number of pregnancies	0.047	0.054	0.76	0.38	1.04	0.94	1.16
History of Diabetes	0.393	1.083	0.13	0.71	1.48	0.17	12.3
History of Hypertension	2.230	0.507	19.30	<0.01	9.3	3.4	25.1
History of Heart disease	-	-	-	-	-	-	-
History of Liver disease	-	-	-	-	-	-	-
History of Kidney disease	1.088	1.158	0.88	0.34	2.96	0.03	28.7
History of Sickle cells	-	-	-	-	-	-	-
History of contraceptive p-pills use	0.062	0.205	0.09	0.76	1.06	0.71	1.59
History of contraceptive injections use	0.330	0.176	3.48	0.06	1.39	0.98	1.96
History of contraceptive IUD use	0.457	0.418	1.18	0.27	1.57	0.69	3.58
History of contraceptive implant use	-1.47	1.016	2.11	0.14	0.22	0.03	1.67

History of contraceptive male condoms use	0.43	0.371	1.34	0.24	1.53	0.74	3.18
Drinking Alcohol	0.012	.203	<0.01	0.95	1.012	0.67	1.507
Chewing tobacco	-	-	-	-	-	-	-
Smoking cigarette	-	-	-	-	-	-	-
Result of HIV-test	0.475	0.433	1.20	0.27	1.60	0.68	3.75
HIV treatment	-0.62	0.469	1.75	0.18	0.53	0.21	1.34
Diagnosis of Gest diabetes	-	-	-	-	-	-	-
Diagnosis of Diabetes	0.104	1.064	0.01	0.92	1.10	0.13	8.93
Diagnosis of Anaemia	0.194	0.621	0.09	0.75	1.21	0.35	4.10
Diagnosis of Hyperemesis Gravidarum	-0.76	1.029	0.55	0.45	0.46	0.06	3.48
Diagnosis of Malaria	-0.27	0.276	0.95	0.32	0.764	0.44	1.31
Diagnosis of Heart disease	-	-	-	-	-	-	-
Diagnosis of Infections	-0.91	0.229	15.92	<0.01	0.40	0.25	0.62
Mothers weight before pregnancy	0.034	0.006	28.2	<0.01	1.03	1.02	1.04
Mother's height	0.027	0.020	1.75	0.18	1.02	0.98	1.06

Table 23: Logistic regression model; multivariable association of maternal age, history of hypertension, diagnosis of infection, mother's weight before pregnancy and pre-eclampsia outcome among preterm deliveries.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-4.81	0.52	84.6	1	<0.001	0.008		
History of hypertension (Yes)	1.84	0.53	11.8	1	0.001	6.30	2.21	17.98
(No)						1		
Diagnosis of Infections (Yes)	-0.90	0.23	14.6	1	<0.001	0.40	0.25	0.64
(No)						1		
Maternal age	0.03	0.01	5.3	1	0.021	1.03	1.00	1.06
Mother's weight before pregnancy	0.02	0.00	20.6	1	<0.001	1.02	1.01	1.04

Model goodness of fit (Hosmer and Lemeshow test) χ^2=5.675, d.f 8, P value 0.684

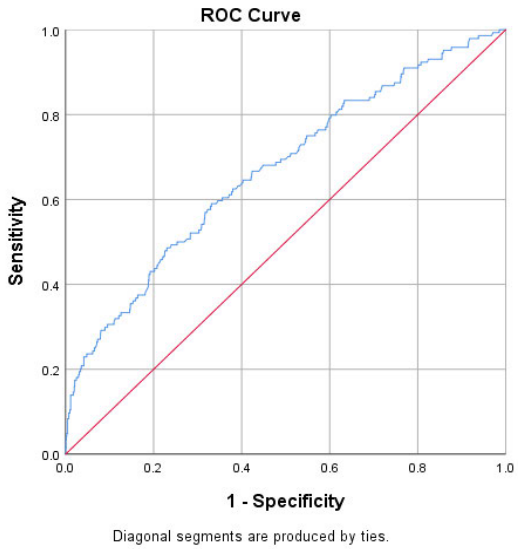
Contingency Table for Hosmer and Lemeshow Test					
	Non pre-eclampsia		Pre-eclampsia		
Decile (10%)	Observed	Expected	Observed	Expected	Total
1	129	130	6	4	135
2	128	128	7	6	135
3	123	125	11	8	134
4	125	125	10	9	135
5	126	125	11	11	137
6	125	122	11	13	136
7	119	119	15	14	134
8	116	117	19	17	135
9	121	113	14	21	135
10	96	98	40	37	136

The below table offers model comparison between the generalised estimating equation and the above logistic regression model.

Table 24: Generalised estimating equation model; multivariable association of maternal age, history of hypertension, diagnosis of infections, mother's weight before pregnancy and pre-eclampsia outcome among preterm deliveries.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Intercept)	-4.81	0.52	83.8	1	<0.001	0.00	0.00	0.02
History of hypertension (Yes)	1.84	0.51	12.7	1	<0.001	6.30	2.29	17.30
(No)						1		
Diagnosis of Infections (Yes)	-0.90	0.23	14.3	1	<0.001	0.40	0.25	0.64
(No)						1		
Maternal age	0.03	0.01	5.9	1	0.015	1.03	1.00	1.06
Mother's weight before pregnancy	0.02	0.00	19.0	1	<0.001	1.02	1.01	1.04

Figure 11: Logistic regression model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome among preterm deliveries.



*Area under the curve 0.669

The predicted probability cut off point that maximises sensitivity and specificity is 0.107. Youden's index yields a sensitivity of 59 % and specificity of 66 %.

Model classification at Younden's index

		Predicted		
		Non pre-eclampsia	Pre-eclampsia	
Observed	Non pre-eclampsia	713	495	Specificity 66%
	Pre-eclampsia	59	85	Sensitivity 59%
Overall classification percentage				59%

Positive likelihood ratio = 1.73; Negative likelihood ratio = 0.62.

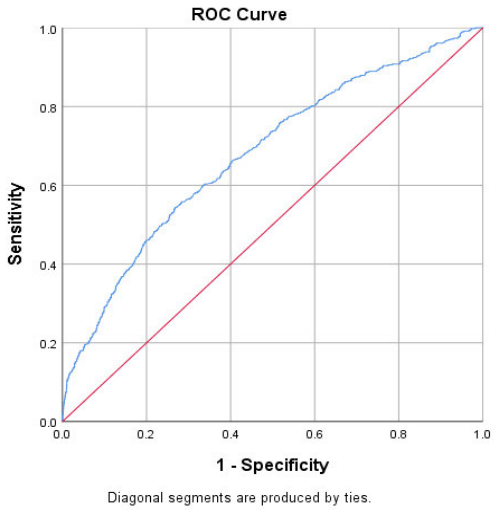
6.3.4 Imputed cases modelling

Table 25 Multivariable association of maternal age, history of hypertension, HIV treatment, diagnosis of malaria, mother's weight before pregnancy and pre-eclampsia outcome. This model was developed using imputed data.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-7.68	0.37	421.0	1	<0.01	<0.01		
History of Hypertension (Yes)	1.73	0.27	39.6	1	<0.01	5.68	3.30	9.76
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.63	0.15	17.4	1	<0.01	0.53	0.39	0.71
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	0.98	0.12	61.5	1	<0.01	2.66	2.08	3.41
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.32	0.20	2.4	1	0.11	1.37	0.92	2.05
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.72	0.28	6.3	1	0.01	0.48	0.27	0.85
(No)	-	-	-	-	-	1	-	-
Maternal age	0.02	0.00	11.8	1	0.001	1.02	1.01	1.04
Mother's weight before pregnancy	0.03	0.00	77.9	1	<0.01	1.03	1.02	1.03

Model goodness of fit (Hosmer and Lemeshow test) χ^2=13.659, d.f 8, P value 0.9

Figure 12 Model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome using imputed data.



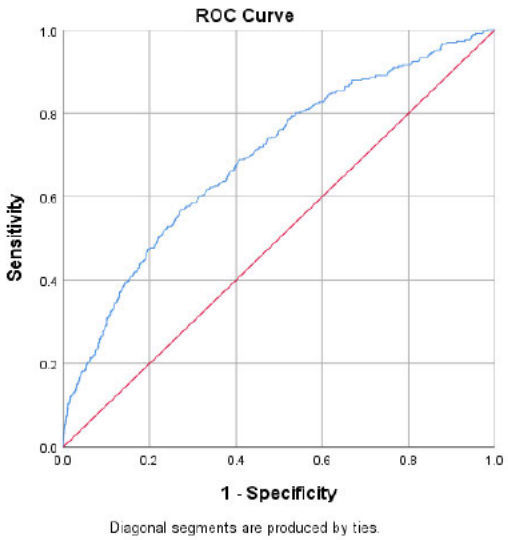
*Area under the curve 0.680, 95 % CI 0.655 - 0.705.

Table 26 Multivariable association of maternal age, number of pregnancies, history of hypertension, HIV treatment, diagnosis of malaria, mother's weight before pregnancy and pre-eclampsia outcome among term deliveries. This model was developed using imputed data.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-6.29	0.35	317	1	<0.01	<0.01		
History of Hypertension (Yes)	1.81	0.32	30	1	<0.01	6.10	3.22	11.57
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.62	0.18	12	1	<0.01	0.53	0.37	0.76
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.99	0.15	43	1	<0.01	0.36	0.27	0.49
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.50	0.23	4	1	0.03	1.65	1.04	2.62
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.96	0.38	6	1	0.01	0.38	0.17	0.81
(No)	-	-	-	-	-	1	-	-
Maternal age	0.03	0.01	9	1	<0.01	1.03	1.01	1.06
Number of pregnancies	-0.11	0.05	5	1	0.02	0.89	0.80	0.98
Mother's weight before pregnancy	0.03	0.00	69	1	<0.01	1.03	1.02	1.04

Model goodness of fit (Hosmer and Lemeshow test) χ^2=7.502, d.f 8, P value 0.484.

Figure 13 Model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome among term deliveries using imputed data.



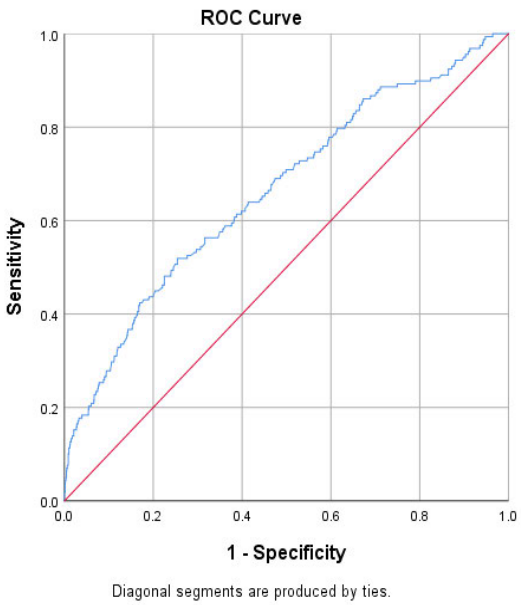
*Area under the curve 0.693, 95 % CI 0.664 - 0.723.

Table 27 Multivariable association of maternal age, history of hypertension, mother's weight before pregnancy and pre-eclampsia outcome among preterm deliveries. This model was developed using imputed data.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-4.73	0.50	86	1	<0.01	<0.01		
History of hypertension (Yes)	1.46	0.57	6	1	0.01	4.31	1.39	13.33
(No)						1		
Diagnosis of Infections (Yes)	-0.95	0.22	17	1	<0.01	0.38	0.24	0.60
(No)						1		
Maternal age	0.03	0.01	5	1	0.01	1.03	1.006	1.063
Mother's weight before pregnancy	0.02	0.00	19	1	<0.01	1.02	1.01	1.04

Model goodness of fit (Hosmer and Lemeshow test) χ^2=4.018, d.f 8, P value 0.855.

Figure 14 Model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome among preterm deliveries using imputed data.



*Area under the curve 0.664, 95 % CI 0.617 - 0.711.

6.3.5 Internal validation of models

This section validates the three models (All deliveries, term and preterm deliveries) developed from the training data set (80 %). The below results show the performance of the models on the testing data set (20%).

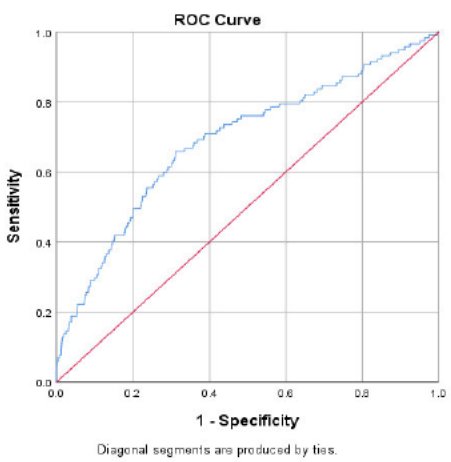
Table 28 Validation of model predicting all pre-eclampsia outcomes.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-5.37	0.57	88.3	1	<0.01	<0.01		
History of Hypertension (Yes)	2.46	0.45	29.1	1	<0.01	11.73	4.79	28.67
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-0.92	0.34	7.4	1	0.006	0.39	0.20	0.77
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.76	0.24	9.9	1	0.002	0.46	0.29	0.75
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	0.15	0.47	0.1	1	0.745	1.16	0.46	2.95
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.74	0.60	1.5	1	0.217	0.47	0.14	1.54
(No)	-	-	-	-	-	1	-	-
Maternal age	0.02	0.01	2.5	1	0.109	1.02	0.99	1.06
Mother's weight before pregnancy	0.02	0.00	14.7	1	<0.01	1.02	1.01	1.03

Model goodness of fit (Hosmer and Lemeshow test) χ^2=10.31, d.f 8, P value 0.244.

Contingency Table for Hosmer and Lemeshow Test					
	Non pre-eclampsia		Pre-eclampsia		
Decile (10%)	Observed	Expected	Observed	Expected	Total
1	289	291	6	3	295
2	287	290	8	4	295
3	290	288	5	6	295
4	289	286	5	7	294
5	294	288	4	9	298
6	289	285	7	10	296
7	283	283	12	11	295
8	278	280	17	14	295
9	277	278	19	17	296
10	253	254	34	32	287

Figure 15 Logistic Regression model performance on a receiver operator curve. The above model predicts overall pre-eclampsia outcome.



Youden's index yields maximum sensitivity of 65 % and specificity of 67 % is attained at a cut of point of 0.039 predicted probability.

Model Summary

Area Under the Curve	0.689
95 % Confidence Interval	0.636 - 0.743

Model classification at Youden's index

		Predicted		
		Non pre-eclampsia	Pre-eclampsia	
Observed	Non pre-eclampsia	1896	933	Specificity 67%
	Pre-eclampsia	41	76	Sensitivity 65%
Overall classification percentage				67%

Positive likelihood ratio = 1.97; Negative likelihood ratio = 0.52.

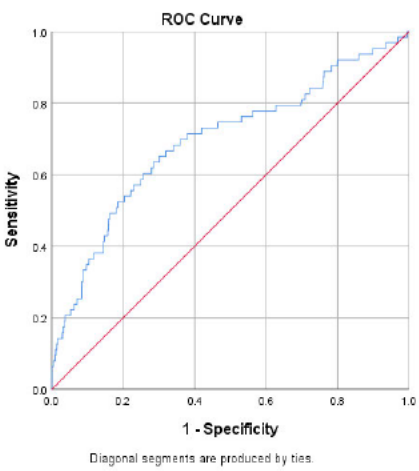
Table 29 Validation of model predicting pre-eclampsia among term deliveries.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-6.30	0.80	61.0	1	<0.01	<0.01		
History of Hypertension (Yes)	2.47	0.57	18.6	1	<0.01	11.85	3.86	36.39
(No)	-	-	-	-	-	1	-	-
Diagnosis of Malaria (Yes)	-1.05	0.47	4.9	1	0.02	0.34	0.13	0.88
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-0.64	0.31	4.1	1	0.04	0.52	0.28	0.97
(No)	-	-	-	-	-	1	-	-
Contraceptive use IUD (Yes)	-0.13	0.67	0.0	1	0.83	0.87	0.23	3.25
(No)	-	-	-	-	-	1	-	-
HIV treatment (Yes)	-0.60	0.73	0.6	1	0.41	0.54	0.12	2.31
(No)	-	-	-	-	-	1	-	-
Maternal age	0.04	0.02	2.8	1	0.09	1.04	0.99	1.10
Number of pregnancies	-0.08	0.11	0.5	1	0.46	0.91	0.73	1.15
Mother's weight before pregnancy	0.02	0.00	10.7	1	<0.01	1.02	1.01	1.04

Model goodness of fit (Hosmer and Lemeshow test) $\chi^2 = 12.90$, d.f 8, P value 0.115.

Contingency Table for Hosmer and Lemeshow Test					
	Non pre-eclampsia		Pre-eclampsia		
Decile (10%)	Observed	Expected	Observed	Expected	Total
1	239	240	3	1	242
2	240	240	3	2	243
3	237	240	7	3	244
4	241	238	1	3	242
5	240	237	2	4	242
6	240	236	2	5	242
7	237	235	5	6	242
8	235	234	7	7	242
9	230	232	12	9	242
10	222	224	21	18	243

Figure 16: Logistic regression model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome among term deliveries.



Youden's index yields maximum specificity of 66 % and specificity of 66 % is attained at a cut of point of 0.025 predicted probability.

Model Summary

Area Under the Curve	0.695
95 % Confidence Interval	0.620 - 0.769

Model classification at Youden's index

		Predicted		
		Non pre-eclampsia	Pre-eclampsia	
Observed	Non pre-eclampsia	1558	803	Specificity 66%
	Pre-eclampsia	21	42	Sensitivity 66%
Overall classification percentage				66%

Positive likelihood ratio = 1.94; Negative likelihood ratio = 0.51.

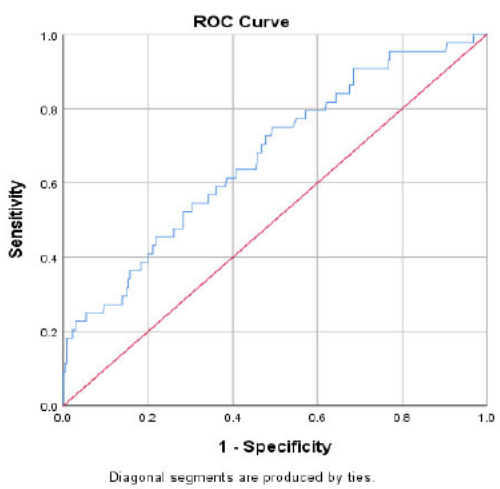
Table 30 Validation of model predicting pre-eclampsia among preterm deliveries.

Parameter	B	S. E	Wald Chi-Square	df	Sig.	Odds Ratio (OR)	95 % Confidence Interval (OR) Lower	Upper
(Constant)	-3.83	1.06	12.9	1	<0.01	0.02		
History of Hypertension (Yes)	3.72	1.28	8.4	1	<0.01	41.51	3.34	515.54
(No)	-	-	-	-	-	1	-	-
Diagnosis of Infections (Yes)	-1.30	0.49	6.8	1	<0.01	0.27	0.10	0.72
(No)	-	-	-	-	-	1	-	-
Maternal age	0.01	0.02	0.3	1	0.58	1.01	0.96	1.07
Mother's weight before pregnancy	0.02	0.01	3.5	1	0.06	1.02	0.99	1.05

Model goodness of fit (Hosmer and Lemeshow test) $\chi^2 = 4.03$, d.f 8, P value = 0.854.

Contingency Table for Hosmer and Lemeshow Test					
	Non pre-eclampsia		Pre-eclampsia		
Decile (10%)	Observed	Expected	Observed	Expected	Total
1	31	31	2	1	33
2	32	30	0	1	32
3	28	29	4	2	32
4	29	28	3	3	32
5	28	28	4	3	32
6	27	27	5	4	32
7	27	27	5	4	32
8	27	26	5	5	32
9	27	25	5	6	32
10	24	23	11	11	35

Figure 17: Logistic regression model performance on a receiver operator curve. The above model predicts pre-eclampsia outcome among preterm deliveries



Youden's index yields maximum sensitivity of 61 % and specificity of 62 % is attained at a cut of point of 0.13 predicted probability.

Model Summary

Area Under the Curve	0.672
95 % Confidence Interval	0.585 - 0.758

Model Classification at Youden's index

		Predicted		
		Non pre-eclampsia	Pre-eclampsia	
Observed	Non pre-eclampsia	174	106	Specificity 62%
	Pre-eclampsia	17	27	Sensitivity 61%
Overall classification percentage				62%

Positive likelihood ratio = 1.60; Negative likelihood ratio = 0.62.

6.3.6 Diagnostics of the models

Independent observation assumption

In this logistic regression analysis, I have regarded the KCMC register data as a cross-sectional study design assuming that all the recorded pregnancies on my dataset (16,342) at KCMC hospital register from 2006 to 2010 were independent of each other. They were all singleton deliveries that were recorded on the register. This assumption was derived from the idea that each pregnancy separated in time, even if from the same mother, would be linked to different values of predictor variables. Even if the woman was the same, her age would differ between subsequent deliveries, so it is likely that her weight, hypertension status, infection status and other predictor variables might also differ.

However, I made an effort to reanalyse the KCMC dataset using generalised estimating equation (GEE). This modelling technique can model datasets with cluster-correlated measurements, thus removing the possible correlation that results from the same women coming in for two or more subsequent deliveries in KCMC hospital. I used the hospital ID number to identify women who may have been delivered multiple times and recorded in the maternity register.

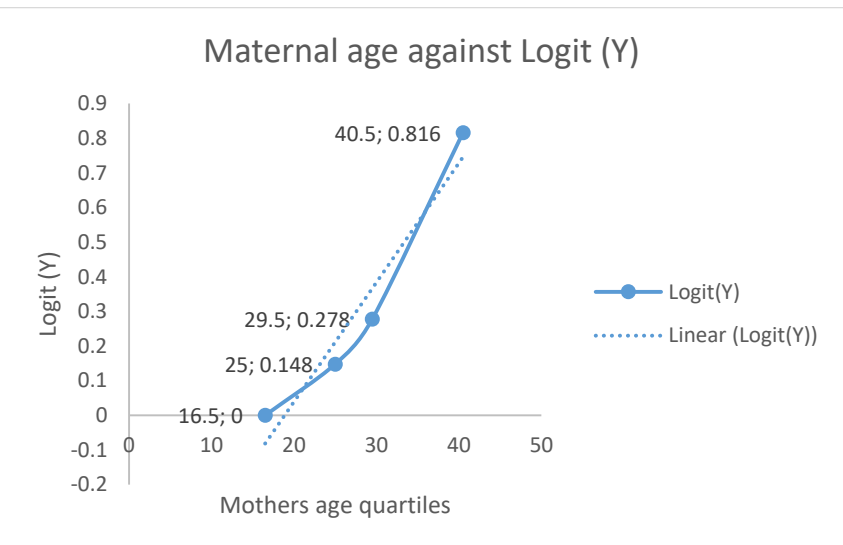
The result from the generalised estimating equation modelling is presented side by side with the result of the logistic regression model for complete case analysis, all deliveries and in term and preterm subgroups. There was no

meaningful difference between the results of the two models i.e. logistic regression and generalised estimating equation.

Linearity assumption

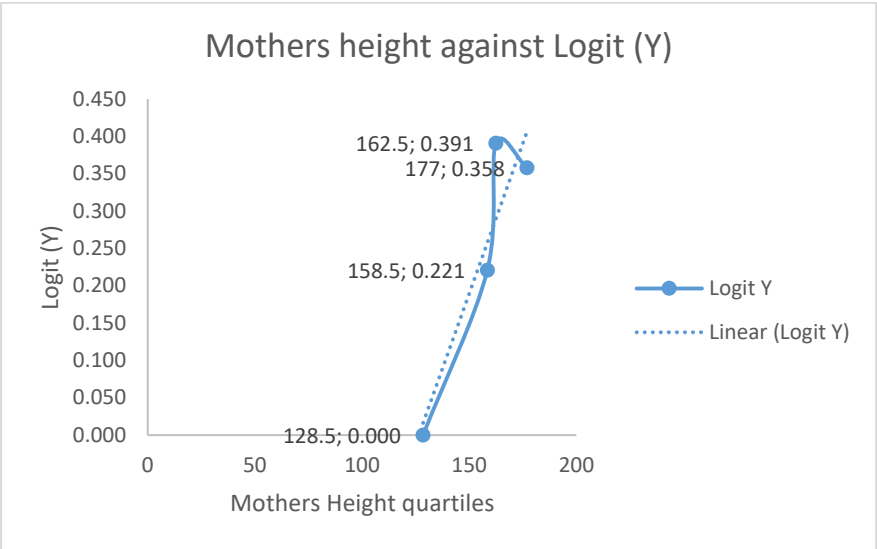
My analysis includes conducting diagnostics for my models. Figures 18, 19 and 20 show the relationship between the continuous predictor variables and their Logit outcome in a univariable regression model. This analysis tests the assumption of my model that the predictor variable has a linear relationship with the logit of the outcome. My continuous predictor variables were maternal age, mother's height and mother's weight before pregnancy, while my outcome variable is a diagnosis of pre-eclampsia.

Figure 18 Maternal age against the Logit (Y)



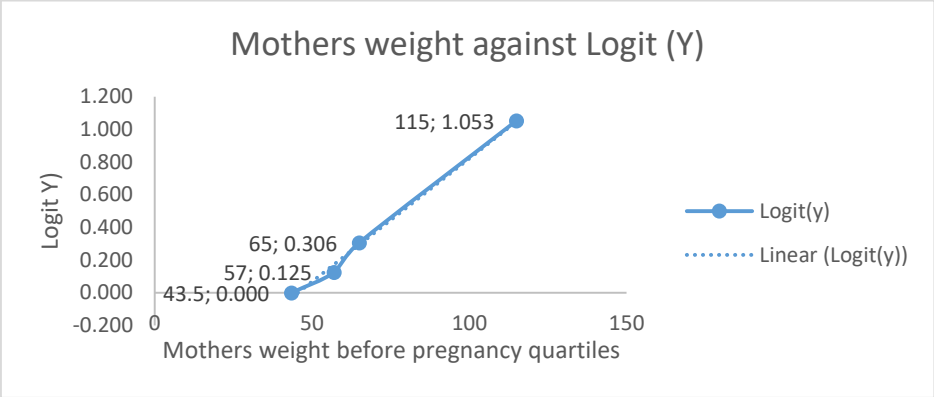
This figure suggests there is a linear relationship between maternal age and the logit of pre-eclampsia

Figure 19 Mother's height against the Logit (Y)



This figure suggests that there is an approximately linear relationship between the mother's height and the logit of pre-eclampsia. However, the linear relationship seems not to hold on extreme levels of mother's height depicted by the upper "S-shaped" bending, a non-monotonic relationship.

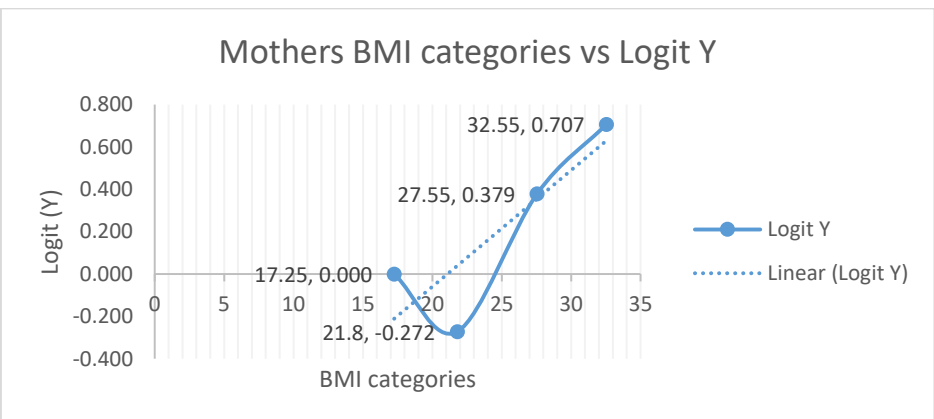
Figure 20 Mother's weight against the Logit (Y)



This figure suggests that a mother's weight before pregnancy has a linear relationship with the logit of pre-eclampsia.

I attempted to resolve the nonlinearity observed in figure 19 by creating a composite variable of body mass index (BMI) which aggregates mother's weight and height variables. The diagnostic of this new BMI variable is shown below. There is a non-monotonic relationship in the low values of BMI, suggesting BMI lower than 25 corresponds with negative logit of Y i.e. decreased log odds of pre-eclampsia. While BMI higher than 25 increases the log odds of pre-eclampsia.

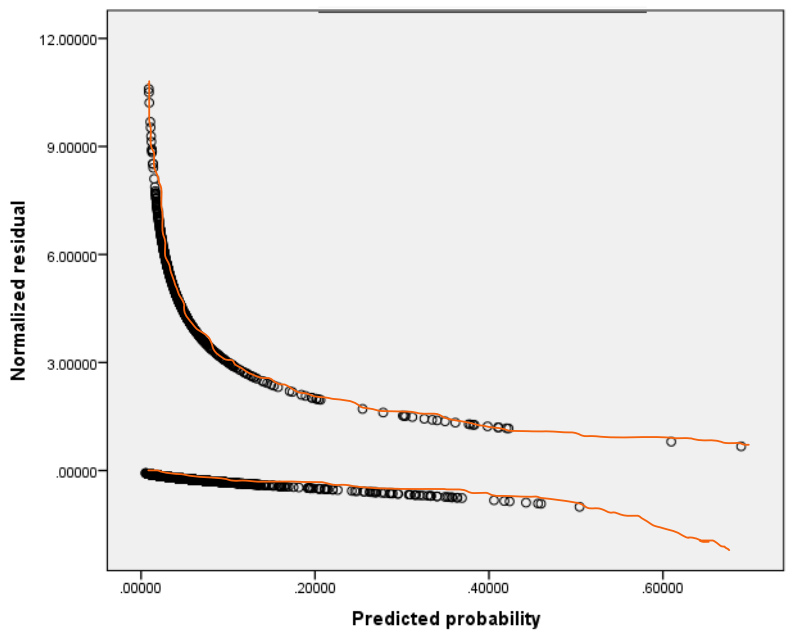
Figure 21 Mother's BMI categories against the Logit (Y)



Since BMI did not show to have a better linear relationship with pre-eclampsia compared to mother's weight and mother's height separately. I did not fit BMI in my prediction model rather I continued to use the weight and height variables independently.

Residual analysis

Figure 22 Residual analysis of the model predicting all pre-eclampsia outcomes, complete case analysis



*Expected normal residual trend in orange line ___

This figure suggests the residuals from the model do follow the expected normal trend. A normalised residual is a ratio: the difference between the observed count and the expected/predicted count and the standard deviation of the expected count. Homoscedasticity is not an assumption of logistic regression.

Multicollinearity

This table below shows two variables to be highly correlated: results of HIV test and HIV treatment. All my models included only one of these variables to avoid multicollinearity. Tolerance is 1 minus the squared multiple correlation of the variable with all other independent variables in the regression equation.

Table 31 Assessment of multicollinearity of the predictor variables. The tolerance and variance inflation values (VIF) are presented.

Variable	Tolerance	VIF
Mother's weight before pregnancy	0.772	1.295
Mother's height	0.863	1.158
Maternal age	0.593	1.687
number of pregnancies	0.552	1.813
History of Hypertension	0.966	1.035
History of Diabetes	0.587	1.705
History of Heart disease	0.991	1.009
History of Malaria	0.969	1.032
History of Anaemia	0.975	1.025
History of Gyn. disease	0.974	1.027
History of liver disease	0.995	1.005
History of kidney disease	0.995	1.005
History of sickle cells	0.998	1.002
History of epilepsy	0.996	1.004
Use of contra. p-pills	0.889	1.125
Use of contra. injections	0.822	1.216
Use of contra. implant	0.988	1.012
Chewing tobacco	0.874	1.144
Smoking cigarette	0.874	1.145
Drinking alcohol	0.954	1.048
Result of HIV-test	0.143	7.006
HIV treatment	0.142	7.028
Diagnosis of gest diabetes	0.974	1.027

Variable	Tolerance	VIF
Diagnosis of diabetes	0.586	1.706
Diagnosis of hypertension	0.977	1.024
Diagnosis of anaemia	0.989	1.012
Diagnosis of hyperemesis	0.992	1.008
Diagnosis of malaria	0.968	1.033
Diagnosis of heart disease	0.993	1.007
Diagnosis of infections	0.965	1.037

Goodness of fit of the models

Goodness of fit of the models was assessed by the Hosmer and Lemeshow test. The results of each model are presented below the respective model tables. None of the fitted models failed the test i.e. p value < 0.05.

Improving the model performance based on diagnostic findings

I attempted to improve my prediction model for pre-eclampsia among all deliveries (first model) by substituting mother's weight with my composite variable BMI. However, the performance of this new BMI model (see table 32 and 33 below) was not significantly better compared to my first model in table 19 and figure 9 above.

Table 32 Multivariable association of BMI, maternal age, contraceptive IUD use, history of hypertension, HIV treatment, diagnosis of malaria, diagnosis of infections and pre-eclampsia outcome in all deliveries.

Variables	B	S.E.	Wald	Sig.	Odds Ratio (OR)	95 % C. I. for Odds Ratio	
						Lower	Upper
BMI	0.07	0.01	67.8	<0.01	1.07	1.05	1.09
Maternal age	0.03	0.01	19.9	<0.01	1.03	1.02	1.05
Contraceptive IUD use	0.49	0.19	6.6	0.01	1.64	1.12	2.40
History of Hypertension	1.76	0.28	38.3	<0.01	5.85	3.34	10.24
HIV treatment	-0.75	0.29	6.4	0.01	0.46	0.26	0.84
Diagnosis of Malaria	-0.68	0.16	18.5	<0.01	0.50	0.36	0.68
Diagnosis of infection	-0.98	0.13	57.0	<0.01	0.37	0.29	0.48
Constant	-5.8	0.30	385.0	<0.01	0.003		

Table 33 BMI Model performance summary

Model Summary

Area Under the Curve	0.691
95 % Confidence Interval	0.665 - 0.717

6.3.7 Comparison of the final models; (first model) all deliveries, (second model) term deliveries and (third model) preterm deliveries

Table 34 Comparison of the final models for predicting pre-eclampsia in all deliveries and in term and preterm deliveries subgroups.

Variables	Pre-eclampsia-All	Pre-eclampsia-Term deliveries	Pre-eclampsia-Preterm deliveries
	1st Model OR	2nd Model OR	3rd Model OR
Maternal age (1year unit)	1.035	1.05	1.036
Number of pregnancies (1 pregnancy unit)	-	0.86	-
History of hypertension (Yes) (No)	5.896	4.74	6.308
HIV treatment(Yes) (No)	0.470	0.37	-
Diagnosis of Malaria (Yes) (No)	0.493	0.48	-
Diagnosis of infections (Yes) (No)	0.370	0.37	0.405
Contraceptive IUD use (Yes) (No)	1.577	2.05	-
Mothers weight before pregnancy (1kg unit)	1.030	1.03	1.029
Area Under the Curve	**0.694**	**0.712**	**0.669**

*Variable not in the model (-)

Area Under the Curve classification scores are interpreted as follows:

0.5 – < 0.6 = Failure; 0.6 – < 0.7 = Weak classifier; 0.7 - <0.8 = Moderate classifier; 0.8 - < 0.9 = Good classifier and 0.9 – 1 = Excellent classifier.

Table 35 Internal validation of the models on the testing dataset.

Performance of models in the validation dataset

Pre-eclampsia	1st Model	2nd Model	3rd Model
Area Under the Curve	0.689	0.695	0.672
95 % Confidence Interval	0.636 - 0.743	0.620 -0.769	0.585 - 0.758

Key findings

The strength of the association shown for each predictor should be interpreted as the change in odds of pre-eclampsia outcome for a unit change in the predictor variable, conditional on the other variables in the models. The findings obtained from the logistic regression models are to be interpreted as the average population values of the odds ratio for each predictor variable. This means any woman picked at random from the study population will have the stated odds of developing pre-eclampsia. Therefore, according to results in table 34 above:

1. A five-year increase in mothers age increased the odds of pre-eclampsia on average by 18 % [95 % CI (9 - 29) %], 27 % [95 % CI (10 - 40) %], and 19 % [95 % CI (2 - 38) %] among all deliveries, term deliveries and preterm deliveries subgroups respectively. The five years increase in odds ratio was obtained from the one-year increase in odds ratio raised to the power of five. i.e. $(1.035)^5 = 1.187$,

equivalent to a 18 % increase in odds for a five-year increase in maternal age.

2. A positive history of hypertension increased the odds of pre-eclampsia by 489 % [95 % CI (236 - 933) %], 374 % [95 % CI (124 - 899) %] and 530 % [95 % CI (121-1698) %] among all deliveries, term deliveries and preterm deliveries subgroups respectively.

3. A ten-kilogram increase in mother's weight increased the odds of pre-eclampsia by 34 % [95 % CI (25 - 43) %], 34 % [95 % CI (21 - 48) %] and 33 % [95 % CI (18 - 50) %] among all deliveries, term deliveries and preterm deliveries subgroups respectively.

4. A mother on HIV treatment was at a decreased odd of pre-eclampsia by 53 % [95 % CI (16 - 74) %] and 63 % [95 % CI (15 - 84) %] among all deliveries and term deliveries respectively. However, HIV treatment was not a significant predictor among preterm deliveries.

5. A positive diagnosis of malaria decreased the odds of pre-eclampsia by 51 % [95 % CI (33 - 64) %] and 52 % [95 % CI (29 - 68) %] among all deliveries and term deliveries respectively. However, malaria diagnosis was not a significant predictor among preterm deliveries.

6. The number of pregnancies was only a significant predictor on the term deliveries model, where an additional pregnancy to a mother reduced the odds of pre-eclampsia by 14 % [95 % CI (4 - 23) %].

7. The three models produced an area under the curve of 69.4 %, 71.2 % and 66.9 % in all deliveries, term and preterm deliveries respectively (see table 34 above).

8. The performances of all the three models were internally validated using the 20 % testing dataset and their area under the curve performance results, seen in table 35, was similar to the AUC results in the training dataset (table 34). The point estimates of the AUC of the training dataset were contained within the 95 % confidence interval of the AUC results in the validated dataset.

9. The results from the GEE models on all deliveries, term and preterm subgroups was similar to that shown from above logistic regression models. Thus, suggesting the potential correlation was not altering our conclusion on the strength of association of our predictors and pre-eclampsia outcome.

10. The positive likelihood ratio values of my models were weak likelihood in separating true positives over false positives. Approximately in every two true positives, there was one false positive. The same was observed of the negative likelihood ratio values, they were also weak in separating false negatives over true negatives. Approximately, for every one false negative, there were two true negatives.

6.4 Results for objective four

Objective four describe the pregnancy outcome characteristics of women with pre-eclampsia in northern Tanzania. The table below shows the comparison of the pregnancy outcome characteristic among women with and without pre-eclampsia. It shows this comparison in all deliveries and in the subgroups of women with term and preterm deliveries.

Table 36 Pregnancy outcome characteristics of women with pre-eclampsia, among all deliveries, preterm and term deliveries subgroups.

Pregnancy outcome characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases
			Child delivery status			
Live born	12928,84.1%	349, 59.5%	11932,87.1%	299, 77.5%	996,59.5%	50, 24.9%
Transferred to paediatric ward	1960,12.7%	161, 27.4%	1493,10.9%	69, 17.9%	467,27.9%	92, 45.8%
Stillborn	442,2.9%	74, 12.6%	246,1.8%	18, 4.7%	196,11.7%	56, 27.9%
Neonatal death	48,0.3%	3, 0.5%	33,0.2%	0, 0%	15,0.9%	3, 1.5%
Pearson χ^2, D. freedom, P value		299.3, 3, <0.001		37.9, 3, <0.001		93.2, 3, <0.001
			Sex of the child			
Male	7988	304, 3.7%	7081	214, 2.9%	907	90, 9.0%
Female	7360	280, 3.7%	6593	170, 2.5%	767	110, 12.5%

Pregnancy outcome characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases
Odds ratio (95% CI)		1.0 (0.8-1.1)		0.8 (0.6-1.0)		1.4 (1.0-1.9)
Pearson χ^2, D. freedom, P value		0.0, 1, 0.99		2.3, 1, 0.127		6.0, 1, 0.014
Mode of delivery						
Spontaneous	10418,67.7%	352, 60.2%	9402,68.6%	248, 64.4%	1016,60.7%	104, 52%
Vacuum	108,0.7%	5, 0.9%	100,0.7%	4, 1%	8,0.5%	1, 0.5%
CS elective	443,2.9%	21, 3.6%	402,2.9%	13, 3.4%	41,2.4%	8, 4%
CS others	4370,28.4%	199, 34%	3774,27.5%	118, 30.6%	596,35.6%	81, 40.5%
Assisted breech	41,0.3%	8, 1.4%	27,0.2%	2, 0.5%	14,0.8%	6, 3%
Fisher's Exact test value (P value)		26.0 (<0.01)		5.7 (0.187)		11.8 (0.013)
Apgar score 1 min categories						
1 (0-3score)	653,4.3%	94, 16%	393,2.9%	25, 6.5%	260,15.6%	69, 34.5%
2 (4-6score)	654,4.3%	45, 7.7%	496,3.6%	20, 5.2%	1589.5%	25, 12.5%
3 (7-10score)	14056,91.5%	447, 76.3%	12803,93.5%	341, 88.3%	1253,75%	106, 53%
Pearson χ^2, D. freedom, P value		196.9, 2, <0.001		20.0, 2, <0.001		50.0, 2, <0.001

Pregnancy outcome characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases
	Birth weight (Bwt) categories					
1 (Very low Bwt <1500g)	208,1.4%	81, 13.8%	15,0.1%	6, 1.6%	193,11.6%	75, 37.5%
2 (Low Bwt 1500-<2500g)	1224,8%	147, 25.1%	748,5.5%	60, 15.5%	476,28.5%	87, 43.5%
3 (Normal Bwt 2500-4000g)	1352988%	337, 57.5%	12539,91.5%	300, 77.7%	990,59.3%	37, 18.5%
4 (High Bwt >4000g)	4132.7%	21, 3.6%	402,2.9%	20, 5.2%	11,0.7%	1, 0.5%
Pearson χ^2, D. freedom, P value		739.4, 3, <0.001		133.3, 3, <0.001		151.3, 3, <0.001
	Mean Fetal head circumference, (Mean Standard error)					
Non-pre-eclampsia	34.3cm (0.01)		34.5cm (0.01)		33.1cm (0.07)	
Pre-eclampsia	33.3cm (0.11)		34.4cm (0.07)		31.1cm (0.28)	
T test, degrees of freedom, P value	8.3, 591.3, <0.001		1.3, 397.8, 0.179		6.9, 200.0, <0.001	
Mean diff(95%CI), p value	0.993 (0.758-1.227), P <0.001		0.099 (-0.046-0.245), P = 0.153		2.020 (1.444-2.597), P < 0.001	
	Number ratio (Non-pre-eclampsia: Pre-eclampsia)					
	15512: 578		13594: 380		1599: 178	
	Fetal length, (Mean standard error)					
Non-pre-eclampsia	48.3cm (0.01)		48.5cm (0.01)		46.3cm (0.10)	

Pregnancy outcome characteristic	All pre-eclampsia women (No, %)		Pre-eclampsia women in Term deliveries (No, %)		Pre-eclampsia women in Preterm deliveries (No, %)	
	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases
Pre-eclampsia	46.3cm (0.18)		48.1cm (0.11)		42.6cm (0.44)	
T test, D. freedom, P value	10.1, 589.3, <0.001		3.5, 391.6, <0.001		8.1, 196.7, <0.001	
Mean diff (95%CI), p value	1.920 (1.548-2.292), P < 0.001		0.406 (0.182-0.631), P < 0.001		3.729 (2.831-4.628), P < 0.001	
	Number ratio (Non-pre-eclampsia: Pre-eclampsia)					
	15524: 580		13603: 380		1602: 179	
	Mean gestation age at delivery					
Mean in weeks	38.9		39.6		33.9	
Standard error of mean	0.02		0.01		0.06	
Number	16020		14106		1880	
	Gestational age at delivery					
Preterm	1679	201, 11.9%				
Term	13720	386, 2.7%				
Odds ratio (95% CI)		0.23 (0.19-0.28)				
Pearson χ^2, D. freedom, P value		296.7, 1, <0.001				

The strength of association between pre-eclampsia and some key pregnancy outcomes has been explored by computing the odds ratio. This allows comparison with other research findings from LMIC.

Table 37 Relationship between stillbirth outcome and pre-eclampsia.

	Stillbirth No (%)	Livebirth No (%)	Total No
Pre-eclampsia	74 (12.8%)	513 (87.4%)	587
Non pre-eclampsia	442 (2.9%)	14,936 (97.1%)	15378
Total	516	15449	15965

Pearson Chi-square test 171, d.f. 1, p value < 0.001.

Odds ratio 4.8, 95 % CI 3.7 – 6.3.

The odds of having stillbirth delivery are 4.8 times among women with pre-eclampsia compared to those without. This was observed among all deliveries combined.

Table 38 Relationship between stillbirth outcome and pre-eclampsia term.

	Stillbirth No (%)	Livebirth No (%)	Total No
Pre-eclampsia	18 (4.7%)	368 (95.3%)	386
Non pre-eclampsia	246 (1.8%)	13,458 (98.2%)	13,704
Total	264	13,826	14,090

Pearson Chi-square test 16.7, d.f. 1, p value < 0.001.

Odds ratio 2.6, 95 % CI 1.6 – 4.3.

The odds of having stillbirth delivery among term deliveries was reduced compared to above table. The odds here were 2.6 times among women with pre-eclampsia compared to those without.

Table 39 Relationship between stillbirth outcome and pre-eclampsia preterm.

	Stillbirth No (%)	Livebirth No (%)	Total No
Pre-eclampsia	56 (27.9%)	145 (72.1%)	201
Non pre-eclampsia	196 (11.7%)	1,478 (88.3%)	1,674
Total	252	1,623	1,875

Pearson Chi-square test 40.2, d.f. 1, p value < 0.001.

Odds ratio 2.9, 95 % CI 2.1 – 4.1.

The odds of having stillbirth delivery among preterm deliveries was also reduced compared to all deliveries, see above table. The odds here were 2.9 times among women with pre-eclampsia compared to those without.

There was no statistically significant association between women with pre-eclampsia and neonatal death outcome. However, this was neonatal death was for the period when the neonate was still admitted in the maternity ward. Thereafter, they were either transferred to paediatric ward or discharged to home.

Table 40 Relationship between neonatal death outcome and pre-eclampsia among live birth

	Neonatal death No (%)	Neonatal alive No (%)	Total No
Pre-eclampsia	3 (0.6%)	510 (99.4%)	513
Non pre-eclampsia	48 (0.3%)	14,888 (99.7%)	14,936
Total	51	15,398	15,449

Fisher exact test p value = 0.24.

There was no statistically significant association between women with preeclampsia and neonatal death outcome in all deliveries. Odds ratio 1.8, 95% CI 0.5 - 5.8.

Table 41 Relationship between neonatal death outcome and pre-eclampsia among live birth term.

	Neonatal death No (%)	Neonatal alive No (%)	Total No
Pre-eclampsia	0 (0%)	368 (100%)	368
Non pre-eclampsia	33 (0.2%)	13,425 (99.8%)	13,458
Total	33	13,458	13,826

Fisher exact test p value = 1.0.

There was no statistically significant association between women with preeclampsia and neonatal death outcome in term deliveries. Odds ratio 0.9, 95 % CI 0.9 – 0.9.

Table 42 Relationship between neonatal death outcome and pre-eclampsia among live birth preterm.

	Neonatal death No (%)	Neonatal alive No (%)	Total No
Pre-eclampsia	3 (2.1%)	142 (97.9%)	145
Non pre-eclampsia	15 (1.0%)	1,463 (99.0%)	1,478
Total	18	1,605	1,623

Fisher exact test p value = 0.2.

There was no statistically significant association between women with preeclampsia and neonatal death outcome in preterm deliveries. Odds ratio 2.0, 95 % CI 0.5 - 7.1.

Table 43 to 45 explore the association between women with pre-eclampsia and the mode of delivery. Pre-eclampsia was associated with assisted delivery in preterm deliveries and not in term deliveries.

Table 43 Relationship between mode of delivery and pre-eclampsia.

	Assisted Delivery No (%)	Normal Delivery No (%)	Total No
Pre-eclampsia	233 (39.8%)	352 (60.2%)	585
Non pre-eclampsia	4,962 (32.3%)	10,418 (67.7%)	15,380
Total	5,195	10,770	15,965

Pearson Chi-square test 14.6, d.f. 1, p value <0.001.

The odds of experiencing assisted delivery was 1.3 times among women with pre-eclampsia in all deliveries (odds ratio 1.3, 95 % CI 1.1 - 1.6).

Table 44 Relationship between mode of delivery and pre-eclampsia term.

	Assisted Delivery No (%)	Normal Delivery No (%)	Total No
Pre-eclampsia	137 (35.6%)	248 (64.4%)	385
Non pre-eclampsia	4,303 (31.4%)	9,402 (68.6%)	13,705
Total	4,440	9,650	14,090

Pearson Chi-square test 3.042, d.f. 1, p value = 0.081.

There was no statistically significant association between women with pre-eclampsia and experiencing assisted delivery in term deliveries subgroup. Odds ratio 1.2, 95 % CI 0.9 – 1.4.

Table 45 Relationship between mode of delivery and pre-eclampsia preterm.

	Assisted Delivery No (%)	Normal Delivery No (%)	Total No
Pre-eclampsia	96 (48%)	104 (52%)	200
Non pre-eclampsia	659 (39.3%)	1,016 (60.7%)	1,675
Total	755	1,120	1,875

Pearson Chi-square test 5.56, d.f. 1, p value = 0.018.

The odds of experiencing assisted delivery was 1.3 times among women with pre-eclampsia in preterm deliveries (odds ratio 1.42, 95 % CI 1.06 – 1.9).

Table 46 to 48 show there was a strong association between women with pre-eclampsia and low birth weight outcome in all deliveries, term and preterm deliveries subgroups.

Table 46 Relationship between low birth weight (< 2500gm) and pre-eclampsia.

	Low birthweight No (%)	Normal birthweight No (%)	Total No
Pre-eclampsia	228 (40.4%)	337 (59.6%)	565
Non pre-eclampsia	1,432 (9.6%)	13,529 (90.4%)	14,961
Total	1,660	13,866	15,526

Pearson Chi-square test 540.2, d.f. 1, p value < 0.001.

The odds of low birth weight were 6.4 times among women with pre-eclampsia in all deliveries (odds ratio 6.4, 95 % CI 5.3 – 7.6).

Table 47 Relationship between low birth weight (< 2500gm) and pre-eclampsia term.

	Low birthweight No (%)	Normal birthweight No (%)	Total No
Pre-eclampsia	66 (18%)	300 (82%)	366
Non pre-eclampsia	763 (5.7%)	12,539 (94.3%)	13,302
Total	829	12,839	13,668

Pearson Chi-square test 94.5, d.f. 1, p value < 0.001.

The odds of low birth weight were reduced in term deliveries compared to all deliveries above. Odds ratio 3.6, 95% CI 2.7 – 4.7.

Table 48 Relationship between low birth weight (< 2500gm) and pre-eclampsia preterm.

	Low birthweight No (%)	Normal birthweight No (%)	Total No
Pre-eclampsia	162 (81.4%)	37 (18.6%)	199
Non pre-eclampsia	669 (40.3%)	990 (59.7%)	1,659
Total	831	1,027	1,858

Pearson Chi-square test 121.3, d.f. 1, p value < 0.001.

Like in term deliveries, the odds of low birth weight were reduced in preterm deliveries subgroup compared to all deliveries. Odds ratio 6.4, 95 % CI 4.4 – 9.3.

Table 49 to 51 explore the association between women with pre-eclampsia and high birth weight outcome. The association was observed in all deliveries and term deliveries, not in preterm deliveries.

Table 49 Relationship between high birth weight (> 4000gm) and pre-eclampsia.

	High birthweight No (%)	Normal birthweight No (%)	Total No
Pre-eclampsia	21 (5.9%)	337 (94.1%)	358
Non pre-eclampsia	413 (3%)	13,529 (97%)	13,942
Total	434	13,866	14,300

Pearson Chi-square test 10, d.f. 1, p value = 0.002.

Odds ratio 2.0, 95 % CI 1.3 – 3.2.

Table 50 Relationship between high birth weight (> 4000gm) and pre-eclampsia term.

	High birthweight No (%)	Normal birthweight No (%)	Total No
Pre-eclampsia	20 (6.3%)	300 (93.8%)	320
Non pre-eclampsia	402 (3.1%)	12,539 (96.9%)	12,941
Total	422	12,839	13,261

Pearson Chi-square test 10, d.f. 1, p value = 0.002.

Odds ratio 2.0, 95 % CI 1.3 – 3.3.

Table 51 Relationship between high birth weight (> 4000gm) and pre-eclampsia preterm.

	High birthweight No (%)	Normal birthweight No (%)	Total No
Pre-eclampsia	1 (2.6%)	37 (97.4%)	38
Non pre-eclampsia	11 (1.1%)	990 (98.9%)	1,001
Total	12	1,027	1,039

Fisher exact test, p value = 0.3.

There was no statistically significant association between pre-eclampsia and high birth weight in preterm deliveries subgroup (odds ratio 2.4, 95 % CI 0.3 - 19.2).

Table 52 to 54 show there is a strong association between women with pre-eclampsia and giving birth to newborns with low Apgar score (0 – 3) outcome.

Table 52 Relationship between Apgar score and pre-eclampsia.

	Low Apgar (0 – 3) No (%)	High Apgar (4 – 10) No (%)	Total No
Pre-eclampsia	94 (16%)	492 (84%)	586
Non pre-eclampsia	653 (4.3%)	14,710 (95.7%)	15,363
Total	747	15,202	15,949

Pearson Chi-square test 175, d.f. 1, p value < 0.001.

The odds of having a newborn with low Apgar score was 4.3 times among women with pre-eclampsia than women without pre-eclampsia. Odds ratio 4.3, 95 % CI 3.4 – 5.4.

Table 53 Relationship between Apgar score and pre-eclampsia term.

	Low Apgar (0 – 3) No (%)	High Apgar (4 – 10) No (%)	Total No
Pre-eclampsia	25 (6.5%)	361 (93.5%)	386
Non pre-eclampsia	393 (2.9%)	13,299 (97.1%)	13,692
Total	418	13,660	14,078

Pearson Chi-square test 16.9, d.f. 1, p value < 0.001.

The odds of having newborn with low Apgar score were reduced in term deliveries compared to all deliveries. The strength of the association was halved, Odds ratio 2.3, 95 % CI 1.5 – 3.5.

Table 54 Relationship between Apgar score and pre-eclampsia preterm.

	Low Apgar (0 – 3) No (%)	High Apgar (4 – 10) No (%)	Total No
Pre-eclampsia	69 (34.5%)	131 (65.5%)	200
Non pre-eclampsia	260 (15.6%)	1,411 (84.4%)	1,671
Total	329	1,542	1,871

Pearson Chi-square test 44.2, d.f. 1, p value < 0.001. OR = 2.8, 95 % CI 2.0 – 3.9.

Key findings

The proportion of children with poor health indicators was higher among women with pre-eclampsia compared to women without pre-eclampsia. This outcome worsened among women with pre-eclampsia and preterm deliveries.

1. The relative risk of stillbirth was higher among women with pre-eclampsia compared to women without pre-eclampsia was 4.4, 2.5 and 2.3 in all deliveries, term and preterm deliveries subgroups respectively. The association of pre-eclampsia and stillbirth appear to be partly mediated through prematurity. Controlling for prematurity dropped the strength of association. The odds ratio was 4.8, 95 % CI 3.7 – 6.3 (All deliveries). It dropped to 2.6, 95 % CI 1.6 – 4.3 and 2.9, 95 % CI 2.0 – 4.1 in term and preterm deliveries.

2. Similarly, the relative risk of neonatal deaths was higher among women with pre-eclampsia compared to women without pre-eclampsia. It was 1.6 in all deliveries and preterm deliveries. Examination of the strength of association between pre-eclampsia and these poor pregnancy outcomes showed stillbirth were strongly associated with pre-eclampsia pregnancies (odds ratio = 4.8, 95 % CI

3.7 – 6.3, p value < 0.001) while there was no association between neonatal death and pre-eclampsia pregnancy.

3. There was no difference in the sex proportions of children born from all women with pre-eclampsia and term women with pre-eclampsia. However, there were more girls born from women with pre-eclampsia in the preterm delivery subgroup (OR = 1.4 95 % CI 1.0 – 1.9, P value 0.014).

4. The proportion of assisted deliveries (vacuum, CS elective, CS others and assisted breech) was higher in women with pre-eclampsia among all deliveries (OR = 1.38, 95 % CI 1.17 - 1.64, p value < 0.001). However, this association was only statistically significant in preterm deliveries subgroup (OR = 1.42, 95 % CI 1.06 – 1.9, p value = 0.018) and not in term deliveries subgroup (OR = 1.2, 95 % CI 0.97 – 1.49, p value = 0.081).

5. The relative risk of children with poor Apgar (0 – 3) scores in the first minute of life was higher among women with pre-eclampsia compared to women without pre-eclampsia. It was 3.8, 2.3 and 2.2 in all deliveries, term and preterm deliveries subgroups. Apgar score of 4 - 10 was considered normal and the reference group in calculating the odds ratio. There is a strong association between pre-eclampsia and low Apgar score (OR = 4.3 95 % CI 3.4 – 5.4). When stratified into term and preterm subgroups, the odds ratio was 2.343, 95 % CI 1.5 –

3.5 and 2.8, 95 % CI 2.0 – 3.9 respectively. This suggests part of the effect of pre-eclampsia on Apgar score is partially mediated through prematurity.

6. The relative risk of children with “very low” birthweight was higher among women with pre-eclampsia compared to women without pre-eclampsia. It was 10, 16 and 3.2 in all deliveries, term and preterm deliveries subgroups respectively. Similarly, the relative risk of children with “low” birthweight was higher among women with pre-eclampsia compared to women without pre-eclampsia by 3, 2.8 and 1.5 times in all deliveries, term and preterm deliveries respectively. Upon combining the very low birth (< 1500gm) and low birth (1500 - < 2500gm) categories into one category of low birth weight (< 2500gm), the new low birth weight category was strongly associated with pre-eclampsia (OR = 6.4, 95 % CI 5.3 – 7.6). When this effect was stratified in the term and preterm subgroups, the strength of the association was odds ratio of 3.6, 95 % CI 2.7 – 4.7 and 6.4, 95 % CI 4.4 – 9.3 respectively. An interesting note from my result is that pre-eclampsia was apparently associated with high birth weight (> 4000gm). The odds ratio was 2.04, 95 % CI 1.3 – 3.2, p value = 0.002 and 2.07, 95 % CI 1.3 – 3.3, p value = 0.002 among all deliveries and term deliveries respectively. This association was lost in the preterm delivery subgroup OR = 2.4, 95 % CI 0.3 – 19.2, p value = 0.362.

7. Mean head circumference of children born from women with pre-eclampsia was smaller than in children of women without pre-eclampsia among all deliveries (33.3 cm ± 0.11 SD vs 34.3cm ± 0.01 SD, p value < 0.001).The mean difference was 0.9 (0.75 - 1.22), P < 0.001 in all deliveries. The mean difference was 2.02 (1.44 - 2.59), P < 0.001 in preterm deliveries. However, this difference was not statistically significant in term deliveries 0.09 (-0.04 - 0.24), P = 0.15.

8. The mean fetal length of children born of women with pre-eclampsia was shorter than in children born from women without pre-eclampsia in all deliveries (46.3 cm ± 0.18 SD vs 48.3 cm ± 0.01SD, p value < 0.001). This was the case for children born of women with pre-eclampsia with both preterm and term delivery subgroups (42.6 cm ± 0.28 SD vs 46.3 cm ± 0.1 SD, p value < 0.001) and (48.1 cm ± 0.11 SD vs 48.5 cm ± 0.01SD, p value < 0.001) respectively. The mean difference was 1.9 (1.5 - 2.2), P < 0.001in all deliveries. The mean difference was also observed in term deliveries 0.4 (0.1- 0.6), P < 0.001 and was more pronounced in preterm deliveries 3.7 (2.8 - 4.6), P < 0.001.

9. The proportion of women with pre-eclampsia was higher among preterm than term deliveries, 11.9 % and 2.7 % respectively. This difference in proportion was statistically significant (Pearson chi-square test = 296.7, d.f = 1, p value < 0.001).

The mean gestation age at delivery was 33.9 weeks for the preterm subgroup and 39.6 weeks for the term subgroup. The overall gestation age at delivery was 38.9 weeks.

6.4.1 Summary

This chapter starts by showing the results of the estimation of the incidence of pre-eclampsia in Dar es Salaam, Tanzania. The data used for this estimation was drawn from Child 2 clinical trial that was conducted in Dar es Salaam. In northern Tanzania, a hospital registry data was used to sample singleton deliveries for secondary analysis. The analysis shows that maternal age ≥ 35 years, single and education level are the sociodemographic factors that are jointly associated with pre-eclampsia outcome. In objective three, which identifies risk factors for pre-eclampsia; maternal age, history of hypertension, history of contraceptive IUD use, HIV treatment, diagnosis of malaria, diagnosis of infections and mother weight before pregnancy are the statistically significant predictors in all deliveries. I observed a difference in the set of predictors in term and preterm deliveries subgroups, which supports the hypothesis of difference in aetiology between early and late onset pre-eclampsia. The above predictors were obtained from logistic regression modelling; similarly, a generalised estimation equation modelling was used to compare the results in an attempt to explore potential correlations in the dataset. AUC was used to assess and compare the classification ability of the developed logistic regression models. My models showed moderate classification performance of AUC scores around 0.7 (see

AUC reference scores at section 6.3.7 above). This modelling aspect of the analysis also included performing diagnostic tests for key model assumptions. Lastly, this chapter addresses the fourth objective which shows pregnancy outcomes of women with pre-eclampsia are worse that non pre-eclampsia women. This comparison is done on perinatal survival indicators of stillbirth and early neonatal death. A comparison is also made on physical wellbeing indicators such as birth weight, head circumference and birth length. Apgar score comparison is also made which also suggests that surviving newborns from pre-eclampsia pregnancies have poorer mental wellbeing.

Chapter 7 Discussion on pre-eclampsia risk factor analysis in Tanzania.

Chapter Seven discusses the results shown in the previous chapter. This chapter is also organised according to the four objectives of the analysis.

- Objective one estimated the incidence of pre-eclampsia in Dar es Salaam to be 1.9 %, 95 % CI 1.3 % to 2.2 % after adjusting for age distribution of the urban population from the Tanzania Health Demographic Survey 2005 data. This finding was on the lower side of global estimates of pre-eclampsia, 2 % to 8 %. The estimates were based on the classical definition of pre-eclampsia, raised blood pressure with proteinuria. Adoption of modern definitions of pre-eclampsia may uncover a higher estimate of the incidence of pre-eclampsia.
- Objective two showed the sociodemographic distribution of pre-eclampsia to be more prevalent among women aged above 35 years, single and those with tertiary education. Tertiary education may be a proxy of unknown risk factor, thus requiring further studies to explore this observation.
- Objective three has revealed several maternal characteristics to be associated with pre-eclampsia. These factors jointly can partly explain the variability in pre-eclampsia occurrence. There is a need to improve pre-eclampsia prediction. I propose to achieve this by using

biomarkers, by introducing new diagnostic methods to improve detection of conditions such as gestational diabetes.

- Objective four shows pre-eclampsia is associated with stillbirth, neonatal deaths and poor indicators of physical and mental wellbeing among surviving newborns of pre-eclampsia pregnancies. Follow-up studies may be done to these newborns to observe the health implication of pre-eclampsia in their adult life.

7.1 Discussion objective 1

To determine the incidence of pre-eclampsia among women in Dar es Salaam, Tanzania.

My study estimates the age adjusted incidence of pre-eclampsia in Dar es Salaam, from 2007 to 2009, was around 1.9 % (95 % CI 1.3 % - 2.2 %). This finding is within the lower range of global estimates. The eligibility criteria of my study participants posed a limitation that may have biased my estimates, as explained in more details below. The strength of my study is that I used cohort data with a sufficient number of participants, and I adjusted for age, a common confounder. Despite the limitations, my result showed pre-eclampsia is a problem in urban settings of Dar es Salaam by affecting more than two percent of all women attending antenatal services.

A systematic review involving around 39 million women from 40 countries, from 2002 through to 2010, showed a global point estimate of 4 % for the incidence of pre-eclampsia (95 % CI of 2 % to 8 %) (Abalos *et al.* 2013). My

estimates in Dar es Salaam of 1.9 % (95 % CI 1.3 % - 2.2 %) fall in the lower range of this global estimate. However, this global review study pointed out the challenges of estimating the incidence due to limitations in data from health systems of LMIC. A study in Mozambique estimated the incidence of pre-eclampsia to be 2.3% (Magee *et al.* 2018). This finding is comparable to my finding in Dar es Salaam 1.9 % (95 % CI 1.3 % - 2.2 %).

My estimated incidence in Dar es Salaam was drawn from women participating in a clinical trial with the following eligibility criteria; being HIV negative, > 18 years, residents of Dar es Salaam for next two years. The recruited trial participants were from three public antenatal clinics in Dar es Salaam. This may not be the accurate reflection of the true incidence of pre-eclampsia in the total population in Dar es Salaam because the women enrolled on the trial may differ in important respects from the general population. For example, women who attend public antenatal clinics are likely to be of lower socioeconomic status than those attending private antenatal clinics, which may impact on their risk of pre-eclampsia. Women with higher socioeconomic status are thought to live a sedentary lifestyle, which may predispose them to have a higher incidence of pre-eclampsia (Hoirisch-Clapauch and Benchimol-Barbosa 2011). The limitation of not being able to include women from private antenatal clinics in Dar es Salaam may have underestimated the incidence of pre-eclampsia. I was not able to adjust for socioeconomic status because the information was not captured in my dataset. Contrary to this hypothesis, two studies in Ethiopia showed no

statistically significant difference in univariable analysis between income and pre-eclampsia (Endeshaw *et al.* 2016a, Endeshaw *et al.* 2016b). Another study in Senegal actually showed women with remunerative activity had a lower proportion of pre-eclampsia (Ndao *et al.* 2009). It is uncertain whether this potential bias resulting from not including women attending private antenatal would affect my estimate. This uncertainty about the direction of the association of socioeconomic status and pre-eclampsia in African populations may be explained by some risk factors such as; stress, hypertension and diabetes that are linked with both, high and low socioeconomic status.

The incidence of pre-eclampsia in Tanzania has been estimated by a few studies. One hospital-based study done in a northern Tanzania referral hospital estimated the incidence to be as high as 3.5 % among women delivering in hospital (M J. Mahande *et al.* 2013). The limitation of this estimate is that women who were referred to deliver in this facility are likely to be more at risk for maternal complications including pre-eclampsia, thus overestimating the incidence. However, Mrisho *et al.* (2007) showed hospital delivery is still low, at 42 %, in Tanzania. Therefore, a sizable proportion of women in this population may have developed maternal complications, including pre-eclampsia, without being recorded on hospital data systems.

The estimation of pre-eclampsia in Dar es Salaam was drawn from HIV negative trial participants. This may have biased my estimate. However, this potential bias is likely to be minimal because the prevalence of HIV positive

people aged 15 - 49 years in Dar es Salaam is only 4.7 %, 95 % CI 3.7 – 5.6 (NBS-Tanzania 2017b). Furthermore, there is no conclusive evidence to suggest whether HIV positive women are at an increased or decreased risk of developing pre-eclampsia compared to HIV negative women. A systematic review by Adams *et al.* (2016) involving 13 studies, seven studies being from Africa, and 21,200 women showed an inconclusive association of HIV and pre-eclampsia. In this regard, it seems unlikely that the omission of HIV positive women would have significantly biased my estimate of the incidence of pre-eclampsia in Dar es Salaam.

The strength of my estimated result is that I used data from a cohort of pregnant women who were recruited in a multicentre clinical trial. This provided quality data with a sufficient number of participants for estimating the incidence. This data set had a lot of missing values on maternal variables which made it unfit for modelling the risk factors. Unlike other studies that have estimated the incidence of pre-eclampsia using tertiary hospital data, which were likely to contain most women with obstetric complication, thus biasing their representativeness of the population estimates. The participants from my study were recruited from antenatal clinic of lower level health facilities, thus making my estimates closer to a population-based study. A total of 3,767 pregnant women were involved in my study. I also reweighted for maternal age, which could influence the incidence estimation in the population. Despite the uncertainty resulting from the inclusion of study participants, the incidence around two percent shows that pre-eclampsia is a

problem in Dar es Salaam-Tanzania, as in other urban settings of LMIC. The result provides the baseline reference for the years around 2007 to 2009. Having established this as a baseline, future studies will be able to compare the future incidence of pre-eclampsia in urban Tanzania.

Further research could be done in urban settings of LMIC to estimate the incidence of pre-eclampsia using a broad systemic definition of pre-eclampsia adopted by the International Society of the Study of Hypertension in Pregnancy (ISSHP) (Tranquilli *et al.* 2014). This will enable accurate estimation of the burden of the condition. However, an important limitation to the adoption of this definition in LMIC is its reliance on the use of modern technological equipment such as ultrasound and laboratory tests that are scarcely available at a population level in LMIC. The discrepancy between incidence estimates drawn using the two definitions of pre-eclampsia could inform the health system about the number of missed cases of pre-eclampsia as a result of using the simple definition.

7.2 Discussion objective 2

To describe the sociodemographic characteristics of women with pre-eclampsia in northern Tanzania.

My study has shown that there are sociodemographic differences between women with pre-eclampsia and those without pre-eclampsia in the northern Tanzania population. Further analysis showed that women with pre-eclampsia in the subgroups of term and preterm delivery did not differ from one another in their sociodemographic characteristics. The reason for conducting this subgroup analysis was to explore the idea that early and late pre-eclampsia may arise from different mechanisms and hence may differ in their risk factors and present with different sociodemographic distributions (Kleinrouweler *et al.* 2012). In the northern Tanzanian population single marital status, mothers aged above 35 years and mothers with tertiary education level were significantly associated with pre-eclampsia occurrence. My findings add to the pool of knowledge from studies examining sociodemographic characteristics of women with pre-eclampsia in Africa. Some of my results agree, while other conflicts, with results from other parts of Africa.

My initial results suggest that women with older age, history of abortion/miscarriage, tertiary education level and professional occupation are more likely to have pre-eclampsia. This observation is plausible in the Tanzanian setting, where women who pursue education to tertiary level are

likely to bear their first pregnancy at an older age than their less educated counterparts are. They also are likely to acquire professional occupations, which are associated with a sedentary lifestyle.

Pre-eclampsia was associated with women with a history of abortion/miscarriage (OR = 1.5, 95 % CI 1.1 - 1.9) in a univariable analysis. In a multivariable analysis of all sociodemographic factors, history of abortion/miscarriage was non-significantly associated with pre-eclampsia (OR = 1.30, 95 % CI 0.98 - 1.72, p value 0.06). However, a cohort study involving 20,846 women in Norway showed two or more (spontaneous or induced) abortions reduces the risk of pre-eclampsia, OR = 0.73 (95 % CI 0.55 – 0.97) after adjusting for maternal age, smoking in pregnancy, infertility treatment, pre-pregnancy BMI and education (Trogstad *et al.* 2008). Subgroup analysis showed two or more induced abortion reduces the odds of pre-eclampsia while two or more spontaneous abortion increased the risk of pre-eclampsia (OR = 0.26, 95 % CI 0.04 - 1.91 vs OR = 1.03, 95 % CI 0.62 - 1.64 respectively). Although this subgroup analysis was not statistically significant, it shows opposite directions of association for the two types of abortions with pre-eclampsia. In the context of my study population in northern Tanzania, legal induced abortion services are limited. We are inclined to suggest that the self-reported history of abortion/miscarriage that is linked to increased odds of pre-eclampsia is predominantly due to spontaneous abortions/miscarriages.

Father's occupation category showed a statistically significant difference in the proportion of women with pre-eclampsia (Pearson chi-square test, p value = 0.102 respectively). This may imply that the spouse socioeconomic status does not change women's risk of pre-eclampsia in this population.

Pre-eclampsia seemed to occur more with maternal age increase (Chi-square trend p value < 0.001) and the number of children she has borne (Chi-square trend p value < 0.001).

Upon combining, all the above sociodemographic variables in a multivariable logistic regression model, single marital status women, women with older age (≥ 36 years) and those with tertiary education were the only statistically significant sociodemographic variables that explained pre-eclampsia. Women aged 36 - 40 and ≥ 41 years were more associated with having pre-eclampsia than younger women ≤ 20 years (OR = 1.8 [95 % CI, 1.2 – 2.8] and 2.6 [95 % CI, 1.4 – 4.6] respectively). Independent of age, women with tertiary level education were associated with having pre-eclampsia (OR = 1.34, 95 % CI 1.0 - 1.6).

Women who are single in marital status were associated with the development of pre-eclampsia. Their odds of developing pre-eclampsia were 1.3 times those in all other marital categories combined (OR = 1.3, 95 % CI 1.07 – 1.82). This finding was also observed in Ethiopia by Tessema *et al.* (2015). However, studies from Ethiopia (Endeshaw *et al.* 2016a, Endeshaw *et al.* 2016b) and Senegal by Ndao *et al.* (2009) did not find a statistically

significant association. A plausible explanation could be that women with single marital status may not have gained enough exposure to their partner's sperms (Hutcheon *et al.* 2011).

Maternal age was also associated with pre-eclampsia in different studies in Africa (Tessema *et al.* 2015, Endeshaw *et al.* 2016a, Endeshaw *et al.* 2016b). One of these studies done in Sudan showed an OR = 1.4 (95 % CI, 1.1 - 1.8) for women > 35 years after adjusting for parity, education level, prenatal care, anaemia and placenta praevia (Adam *et al.* 2013). This observation concurs with Framingham study that showed an increase in age is a risk factor for a wide range of cardiovascular diseases (Kannel and McGee 1979). My study showed that women with tertiary education were at increased odds of having pre-eclampsia independent of their age (OR = 1.34, 95% CI 1.0 - 1.6). Contrary to my results, most studies in Africa showed that a low education level was associated with pre-eclampsia (Kiondo *et al.* 2014, Ajah *et al.* 2016). In Ethiopia Endeshaw *et al.* (2016b) showed that women who could not read or write had an increased odds of pre-eclampsia in a univariable analysis (OR = 2.18, 95 % CI 1.19, 4.62). However, this association was no longer statistically significant in multivariable analysis. My finding suggests that tertiary education in women of this population may be a proxy to some unknown risks. It also implies that unknown risk factors associated with having tertiary education outweigh the unknown risk factors associated with lower levels of education. This seems to be an interesting

area for further exploration in understanding the evolving dynamics of pre-eclampsia distribution in sociodemographic strata in African populations.

Furthermore, my study compared the sociodemographic characteristics of women with pre-eclampsia in the subgroups of term and preterm deliveries. The findings of this subgroup analysis showed that women with pre-eclampsia differed from those without pre-eclampsia in a similar manner across the term and preterm deliveries subgroups. A study in Ethiopia showed similar results that women with early and late onset pre-eclampsia did not differ on sociodemographic characteristics (Endeshaw *et al.* 2016b).

My study adds to our understanding of the sociodemographic relations with pre-eclampsia in northern Tanzania. Mothers aged above 35 years and those with tertiary education can be targeted by prevention strategies for pre-eclampsia. Women can be informed of this evidence to enable them to make an informed decision on when they wish to have babies. Women with tertiary education may also be encouraged to avoid underlying risk factors such as sedentary lifestyle or stress to reduce their risk of pre-eclampsia. This study adds to the scarce body of knowledge on the understanding of early and late pre-eclampsia in an African population. My findings support an earlier study in Ethiopia that the sociodemographic relationship with pre-eclampsia is similar for early and late onset pre-eclampsia.

7.3 Discussion objective 3

To identify risk factors for pre-eclampsia, term and preterm deliveries subgroups, among women in northern Tanzania.

Theories on the aetiology of pre-eclampsia have suggested several mechanisms for its occurrence. Biological and medical risk factors have been explored in various populations. My study explored some of the maternal biomedical risk factors in the northern Tanzanian population. My findings show that maternal age, weight before pregnancy, history of hypertension, HIV treatment, contraceptive IUD use, diagnosis of infection and diagnosis of malaria significantly predicted the occurrence of pre-eclampsia in all deliveries. The subgroup analysis on term and preterm deliveries showed some differences in risk factors. Pre-eclampsia in term deliveries was predicted by maternal age, the number of pregnancies, weight before pregnancy, history of hypertension, HIV treatment, contraceptive IUD use, diagnosis of infection and diagnosis of malaria. Pre-eclampsia in preterm deliveries was predicted by maternal age, history of hypertension, diagnosis of infection and weight before pregnancy. To the best of my knowledge, this study is the first to develop and assess the performance of prediction models for pre-eclampsia, term and preterm deliveries subgroups, in an African population, relying only on maternal biomedical factors.

The comparison of performance of the prediction models was assessed by the area under the curve (AUC) which is a relatively simple approach to

quantify the performance of a model (Hanley and McNeil 1982). AUC is obtained after plotting the sensitivity values against 1- specificity values of a test or model. The value of AUC summarises the overall probability of the model in correctly assigning high probability to cases and low probability to non-cases and thus, correctly classifying them. The fist model predicting pre-eclampsia among all deliveries produced an area under the curve of 69.4 %. The second model predicting pre-eclampsia among term delivery was 71.2 %, while the third model predicting pre-eclampsia among preterm deliveries was 66.9 %. The performance of my models was satisfactory when compared to findings from a similar study which had an area under the curve of 73 % (Myatt *et al.* 2012). This study was done in the USA and used biomedical factors plus biomarkers to predict pre-eclampsia outcome among low risk women. Biomarkers are known to be more accurate in measuring clinical parameters than biomedical factors gathered from participants' history or self-reported. The study also used quality data from a clinical trial, where misclassification was expected to be minimal. Despite these assuring steps, their findings were not far superior to those of my models that only relied on biomedical factors. This suggests that the unexplained variability in predicting pre-eclampsia is likely due to genetic factors or unmeasured or yet unknown biomedical factors that were not examined in either their study or mine. Another study done in the United Kingdom showed an area under the curve of 80 % for early onset pre-eclampsia (< 37 weeks) and 74.5 % for late onset pre-eclampsia (≥ 37 weeks) when using maternal characteristics alone.

Later, they combined maternal characteristics and several biomarkers specific for gestation age of 11 - 13 weeks. This improved the area under the curve of the model to 90 % for early onset and 79.6 % for late onset pre-eclampsia (O'Gorman *et al.* 2016). These results also pointed out that biomarkers levels were more pronounced among early onset pre-eclampsia cases than late onset cases, hence improved the prediction of early onset much greater than late onset pre-eclampsia. Contrary to their finding, my study showed maternal biomedical factors were slightly better in predicting late onset (AUC 71.2 %) than early onset (AUC 66.9 %) pre-eclampsia in my study population. However, this difference could be due to differences in sample sizes of term and preterm deliveries subgroups.

The purpose of my prediction models is to classify women with and those without pre-eclampsia. I selected the Youden's index, a point on the receiver operator curve for each model that would simultaneously maximise my prediction sensitivity and specificity values. In my first model that predicts pre-eclampsia among all deliveries, a cut-off point of 0.035 on my predicted probability resulted in a sensitivity of 65 % and a specificity of 63 %. My second model that predicts pre-eclampsia among term deliveries subgroup, a cut-off point of 0.026 on my predicted probability resulted in a sensitivity of 65 % and specificity of 65 %. My third model that predicts pre-eclampsia among preterm deliveries subgroup, a cut-off point of 0.107 on my predicted probability resulted in a sensitivity of 59 % and a specificity of 66 %. I am not aware of similar studies that explored model performance on pre-eclampsia

prediction in African countries, to provide a comparison of results. My study contributes to the scarce literature on predicting pre-eclampsia using maternal biomedical factors in African populations.

The above models were each subjected to a validation process using the internal validation dataset with 20 % (3,229) women. The performance of the first model (All deliveries) was similar to that observed above from the training dataset; the maximum sensitivity and specificity at a cut point of 0.039 were 65 % and 67 % respectively. The performance of the second model (term deliveries) on the validation dataset produce similar results to that of the training dataset. At a cut of point of 0.025, the sensitivity was 66 % and the specificity was 66 %. The third model for preterm deliveries also produced similar results to that observed in the training dataset: at a cut of point of 0.13, the sensitivity was 61 % and specificity was 62 %. In the short time span of my research, I was not able to access a dataset from another African population to facilitate external validation of my models, a process that would have shown the extent to which my models were generalisable in other populations.

The strength of my study is that we have used routinely captured data with its limitations in missing values and measurement errors to develop my models, unlike models developed using high quality trial data. Thus, my models portray a generalisable result of using these predictors elsewhere on Tanzanian health facilities to predict pre-eclampsia outcome. Furthermore, I have used simple linear relationships for my continuous predictors, such as

maternal age and number of pregnancies, in modelling the log odds of pre-eclampsia outcome (as shown in my diagnostic section of these assumptions). Interaction of variables such as maternal age by the number of pregnancies, malaria and HIV treatment did not produce significant interactions. I acknowledge that a more complex relationship could be fit between my predictors and log odds of pre-eclampsia but this effort would likely improve slightly the overall performance at a cost of a more mathematically complex relationship.

My models had moderate sensitivity and specificity values. This limitation is because it was developed in a low risk population, where the incidence of pre-eclampsia in my data was around 3.5 %. Therefore, these models are suited for predicting the outcome in low risk populations. Another limitation of my analysis is that some variables were self-reported, and this led me to rely on my clinical intuition to interpret the result of these variables based on the context. I interpreted the observed protective association of the variables diagnosis of malaria and diagnosis of infections on pre-eclampsia as having a history of ‘treated malaria and treated infections’ to be protective of pre-eclampsia. Furthermore, I was unsuccessful at factoring in some known predictors such as diabetes mellitus, gestational diabetes and change of paternity, due to limitations in the hospital information system, from which my data was drawn. Therefore, my study results are likely to have a limitation of residual confounding since not all factors were accounted for in the modelling.

7.3.1 Predictor variables in my models

The odds ratio output from my logistic regression and GEE models represents the population average change in odds of having pre-eclampsia per the respective predictor. This means, if all women in the population had a unit change in any given predictor then the odds of pre-eclampsia outcome would change by that amount for the whole population on average. This population average effect can also be expressed as the average change in odds of any women picked at random from that population. The magnitude of change in odds of pre-eclampsia as a result of a unit change of a predictor is to be regarded as a change that occurs when other predictors in that model are kept constant. There is an agreement on the role of age as a predictor across different studies. In my study's first model (all deliveries) a five-year increase in age of any woman picked at random from this population was associated with an increase in the odds of pre-eclampsia on average by 16 % (equivalent to OR = 1.03, 95 % CI 1.017 – 1.053, per year). In the second model (term deliveries subgroup) and third model (preterm deliveries subgroup) the five years increase in age increased the odds of pre-eclampsia by 28 % (equivalent to OR = 1.05, 95 % CI 1.02 – 1.07, per year), and 16 % (equivalent to OR = 1.03, 95 % CI 1.005 – 1.068, per year) respectively. Similar studies conducted in African populations showed that maternal age was associated with an increase in the odds of pre-eclampsia (Tessema *et al.* 2015, Endeshaw *et al.* 2016a, Endeshaw *et al.* 2016b). However, these studies analysed age as a categorical variable, making it difficult to compare

their results directly with mine in terms of the incremental change one year makes to the odds of pre-eclampsia. The understanding that age increases pre-eclampsia tallies with the Framingham cohort study, where the risk of cardiovascular disease was doubled among the older group (65 - 94years) compared to the young group (35 - 64 years) with the same systolic blood pressure (Vokonas *et al.* 1988). We cannot stop people from growing old, but in the case of pre-eclampsia reduction, we can recommend women to consider the risks of pregnancy at a relatively older age.

My study found that having a positive history of hypertension was associated with an increase in the odds of pre-eclampsia. My first, second and third models showed the odds are increased by 489 % (OR = 5.89, 95 % CI 3.36 – 10.33), 374 % (OR = 4.74, 95 % CI 2.24 – 9.99) and 530 % (OR = 6.30, 95 % CI 2.21 – 17.98) respectively. This finding tally with established clinical knowledge, where having chronic hypertension does increase one's risk of developing pre-eclampsia (O'Gorman *et al.* 2016, NICE 2019).

A ten-kilogram increase in mother's body weight before pregnancy was associated with an increased odd of pre-eclampsia of 34 % (equivalent to OR = 1.03, 95 % CI 1.023 – 1.037, per kilogram) in my first model (all deliveries). The odds of pre-eclampsia were increased by 34 % (equivalent to OR = 1.03, 95 % CI 1.02 – 1.04, per kilogram) in my second model (term deliveries) and 33 % (equivalent to OR = 1.029, 95 % CI 1.01 – 1.04, per kilogram) in my third model (preterm deliveries). My findings are similar to studies done in Africa that have shown heavy body weight increases the risk of pre-

eclampsia; however, these studies used MUAC or body mass index obtained at delivery or during pregnancy. Different from them, my study has used pre-pregnancy body weight as a predictor of pre-eclampsia. Pre-pregnancy body weight provides the advantage of identifying the risk group early on in pregnancy and even before pregnancy, thus providing room to modify this risk factor. Other studies that have used pre-pregnancy BMI in Asian populations and have echoed similar findings to mine (Savitri *et al.* 2016, Shao *et al.* 2017). A cohort study in Indonesia showed that pre-pregnancy BMI is associated with an increase odd of pre-eclampsia (OR = 1.09, 95 % CI 1.04 – 1.14). They further showed pre-pregnancy BMI determined the baseline SBP (0.25 mmHg/Kg/M^2, 95 % CI 0.17 – 0.34) and DBP (0.18mmHg/Kg/M^2, 95 % CI 0.13 – 0.24) but not their change during the pregnancy period. Another cohort study in China showed that pre-pregnancy BMI and excessive gestational weight gain (GWG) were two independent risk factors for pre-eclampsia. Women with pre-pregnancy obesity alone vs normal BMI were at increased risk for pre-eclampsia (OR = 1.81, 95 % CI 1.37 – 2.39). Women with normal pre-pregnancy BMI and excessive GWG vs normal BMI and normal GWG were at increased risk of pre-eclampsia too (OR = 2.28, 95 % CI 1.7 – 3.05). Women with both obese pre-pregnancy BMI and excessive GWG vs normal BMI and normal GWG were at a much higher increased risk of pre-eclampsia (OR = 3.78, 95 % CI 2.65 – 5.41), thus speculating potential interaction between obese pre-pregnancy BMI and excessive GWG. We were unable to address this potential confounding

factor in my study because my data did not capture weight gain during pregnancy. However, based on the above findings, we can ascertain that pre-pregnancy weight is a risk factor irrespective of the pregnancy weight gain factor.

Women with a positive diagnosis of malaria during pregnancy had a decreased odd of pre-eclampsia by 51 % (OR = 0.49, 95 % CI 0.36 – 0.67) among all deliveries and 52 % (OR = 0.48, 95 % CI 0.32 – 0.71) among term deliveries. Malaria diagnosis was not a significant predictor of pre-eclampsia among preterm deliveries. Malaria infection is prevalent in tropical countries thus placing women at risk of malaria related complications. It is estimated that 38.9 million pregnancies occur in Sub-Sahara Africa, out of which 11.1 million pregnancies are infected with malaria annually (WHO 2019b). Therefore, malaria's potential contribution towards pre-eclampsia is of considerable proportion in these populations. Malaria had been associated with pre-eclampsia in some observational studies in Africa but there was no conclusive evidence from the individual small size studies (Sartelet *et al.* 1996, Ndao *et al.* 2009, Adam *et al.* 2011, Ephraim *et al.* 2014). My finding drawn from a sample of 16,342 pregnancies, adds up to the body of evidence linking malaria to pre-eclampsia among term deliveries. However, the direction of the association contradicts that of previous studies. My study suggests that malaria infection decreased the odds of pre-eclampsia, where the other studies (and my systematic review) have suggested that it does increase the odds of pre-eclampsia (Sartelet *et al.* 1996, Ndao *et al.* 2009,

Adam *et al.* 2011, Ephraim *et al.* 2014). A plausible explanation is that the reported malaria cases in my dataset were also treated for malaria and hence they had a decreased odd of pre-eclampsia compared to the control. Some of the control might have subclinical malaria and thus have a higher risk of placental infection that was undetected, which may have led to pre-eclampsia than the cases who got treated for malaria and cleared the placental infection. The policy of giving two doses of Sulfadoxine Pyrimethamine (SP) for presumptive treatment for malaria infection to all pregnant women was adopted in Tanzania since year the 2000, this presumptive treatment factor could affect the observed relationship. An evaluation study done in Tanzania in 2015 showed 63 % of pregnant women were unwilling to take SP. Clinic results showed 54% of pregnant women (30 – 40 weeks) took a single dose, 34 % took two doses. It was also found that SP was not administered under direct observed therapy in 86 % of women (Ayubu and Kidima 2017). In my analysis, I lacked the data of women who might have received the presumptive treatment for malaria infection. Therefore, SP treatment may have confounded the observed direction of the association.

My analysis was unable to show an association between malaria infection and pre-eclampsia among preterm deliveries (< 37weeks). Brabin (1983) had suggested that the peak incidence of malaria during pregnancy occurred at 13 - 16 weeks of gestation, which is prior to early onset pre-eclampsia (arising from 20 – 34 gestation weeks). One plausible explanation is that

perhaps there is a wide window period from the time the placenta is infected with the malaria parasite to when pre-eclampsia manifests, therefore obscuring the link between malaria infection and early onset pre-eclampsia. An alternative explanation is that my subgroup analysis did not have sufficient power to detect an existing weak association. My preterm deliveries subgroups had 153 malaria cases and 1357 controls and yielded OR = 0.76, 95 % CI 0.44 – 1.31, p value = 0.32 in a univariable analysis. I recommend further research studies to address these limitations by generating credible epidemiological evidence on the relationship between malaria and early onset pre-eclampsia.

Women who had a diagnosis of infection during their pregnancy period were associated with decreased odds of pre-eclampsia by 63 % (OR = 0.37, 95 % CI 0.28 – 0.47) in all deliveries. This finding was also echoed in the subgroup analysis, in term deliveries the odds were decreased by 63 % (OR = 0.37, 95 % CI 0.27 – 0.51) and in preterm deliveries by 60 % (OR = 0.4, 95 % CI 0.25 - 0.64). My dataset did not unpack the diagnosis of infection to indicate the specific infections included. This became a challenge in comparing and interpreting my results in relation to the existing literature. A meta-analysis on maternal infections and pre-eclampsia by Conde-Agudelo *et al.* (2008) showed that urinary tract infection and periodontal disease were associated with pre-eclampsia. The review also cited trial studies from Germany (Fischer *et al.* 1970) and Croatia (Drazancić *et al.* 1989), which showed that treatment with antibiotics for urinary tract infection was associated with a reduced odds

of pre-eclampsia (OR = 0.22, 95 % CI 0.17 - 0.30 and OR = 0.36, 95 % CI 0.20 - 0.64). With this evidence in mind, it is possible that the observed reduction in odds of pre-eclampsia from my analysis may imply that women who were diagnosed as having infection were also treated and hence they were at a reduced odds of pre-eclampsia compared to their counterparts (control group) who may have had a subclinical infection that went undiagnosed. Another possible explanation is that infections may lower maternal immunity thus lower the possibility of maternal fetal immune incompatibility, which is thought to cause pre-eclampsia (Kenny and Kell 2018). In my review of African studies on pre-eclampsia risk factors, only one study in Ethiopia showed that urinary infection and periodontal disease were associated with pre-eclampsia (Endeshaw *et al.* 2016a). Therefore, contrasting this finding with my study's finding it is not conclusive whether maternal infection increases or decreases the odds of pre-eclampsia in African populations. Further studies could explore this relationship by unpacking the specific infections and accounting for the immunological aspect of the relationship.

My analysis revealed that use of an intrauterine contraceptive device (IUD) was associated with an increased odd of pre-eclampsia in all deliveries and in term deliveries (OR = 1.57, 95 %CI 1.07 – 2.30 and OR = 2.05, 95 %CI 1.31 – 3.20 respectively). There is scarce evidence on the relationship between IUD and pre-eclampsia globally. A case-control study conducted in the United Kingdom by Parker *et al.* (2016) showed IUD decreased the odds

of pre-eclampsia (OR = 0.76, 95 %CI 0.58 – 0.98). The contrast observed with my study finding may be explained by the difference in types of IUD used in the UK and northern Tanzania. In the UK, copper containing IUDs and hormone releasing IUDs are both available while in northern Tanzania around the study period of 2006 to 2010, only copper containing IUDs were used. It is, therefore, necessary to conduct further studies to examine this potential association of pre-eclampsia and IUDs of both types. IUDs are growing in popularity as they offer a long-term reversible contraceptive option to most women in both developing and developed countries.

Women receiving HIV treatment were at a decreased odd of developing pre-eclampsia by 53 % (OR = 0.47, 95 % CI 0.26 - 0.84) among all deliveries and by 63 % (OR = 0.37, 95 % CI 0.16 – 0.85) among term deliveries. HIV treatment was not a significant predictor of pre-eclampsia among preterm deliveries. There have been conflicting results from studies whether HIV increases or decreases the likelihood of developing pre-eclampsia (Adams *et al.* 2016). My findings show that women on HIV treatment are at decreased odds of pre-eclampsia. The Tanzania HIV treatment guideline indicates, that women who test HIV positive are to be initiated on antiretroviral drugs (ARVs) to prevent mother to child transmission (NACP 2005). I cannot disentangle the source of the observed effect of a decrease in odds of pre-eclampsia, whether it results from having a positive HIV status, the HIV treatment or their interaction i.e. being positive and then receiving ARVs. I was also limited in my analysis while attempting to disentangle these components because of

the high correlation between women being HIV positive and simultaneously being on treatment, which brought a multicollinearity effect.

Some studies have attempted to disentangle HIV status and HIV treatment in relation to pre-eclampsia. In South Africa, Frank *et al.* (2004) found no association between untreated HIV positive status and the risk of pre-eclampsia., In a cohort of 2,600 women, the rate of pre-eclampsia was 5.2 % in HIV negative and 5.7 % in HIV positive women (p = 0.61). In Zambia, George *et al.* (2015) attempted to establish whether there was an association of HIV status, and/or HIV treatment with pre-eclampsia. In a cohort of 824 women, neither HIV positive status (OR = 0.44, 95 % CI 0.1 - 3.2) nor HIV treatment (HAART) use was statistically significantly associated with the risk of pre-eclampsia (OR = 0.99, 95 % CI 0.1 - 8.9). However, this study had a small sample size, resulting in a wide confidence interval, and hence may have obscured a statistically significant association. Contrast to these findings my study shows; HIV treatment is statistically significantly associated with reduced odds of pre-eclampsia by 53 % (OR = 0.47, 95 % CI 0.26 - 0.84) among all deliveries and by 63 % (OR = 0.37, 95 % CI 0.16 – 0.85) among term deliveries.

Women with a higher number of pregnancies were associated with decreased odds of pre-eclampsia. One additional pregnancy was associated with 14 % (OR = 0.86, 95 % CI 0.77 – 0.96) decrease in the odds of the women developing pre-eclampsia. This finding is in line with the understanding that pre-eclampsia is predominantly a condition affecting the

first pregnancy (nulliparous women) (Hartikainen *et al.* 1998). The relationship between the number of pregnancies and pre-eclampsia is confounded by maternal age. Women on their first pregnancies tend to be younger while also women on their fourth or fifth pregnancy tend to be older. In addressing this potential confounding effect of maternal age on number of pregnancies, I included both variables in my modelling. My results showed an increase in the number of pregnancies is associated with decreased odds of pre-eclampsia after adjusting for maternal age. This relationship was statistically significant only among term deliveries (OR = 0.86, 95 % CI 0.77 – 0.96). Several studies in African populations, that explored the relationship between the number of pregnancies and pre-eclampsia, have produced similar result showing the risk of pre-eclampsia is higher on first pregnancies (Adam *et al.* 2013, George *et al.* 2015, Ajah *et al.* 2016, Elmugabil *et al.* 2016).

7.3.2 Statistically non-significant predictors

A series of variables have been shown to be statistically significant predictors of pre-eclampsia in literature. However, some of them have not been statistically significant in my models. In this section, I am discussing the possible explanation that rendered them non-statistically significant in my analysis.

Diabetes mellitus and gestational diabetes are risk factors that have been shown to explain the occurrence of pre-eclampsia in numerous populations

(Tessema *et al.* 2015, Endeshaw *et al.* 2016a). My univariable analysis showed neither of these variables; a history of diabetes, a diagnosis of diabetes or a diagnosis of gestational diabetes was statistically associated with pre-eclampsia in all deliveries, term and preterm deliveries subgroups (See table 13, 17 and 21). This could be a result of very few cases with a positive history or diagnosis of diabetes. My dataset showed very few women had reported a history of diabetes (42 cases vs 16,298 control), a diagnosis of gestational diabetes (5 cases vs 16,333 control) or a diagnosis of diabetes (35 cases vs 16,307 control). The observed very low prevalence of self-reported cases of history of diabetes and the number of diagnosed cases of diabetes and gestational diabetes can be explained by a weak health system to diagnose diabetes in these settings. A cross-sectional household study done in the same setting (Kilimanjaro region, northern Tanzania) by Stanifer *et al.* (2016) showed the prevalence of diabetes was 5.7 %; 95 % CI 3.3 - 9.4 and that of glucose impairment was 21.7 %; 95 % CI 15.2 – 29.8. This population-based study had measured haemoglobin A1c in 481 adult participants from 346 urban and rural households. The study also reported a low awareness of diabetes (35.6 %) and low treatment uptake among diabetics (33.3 %) (Stanifer *et al.* 2016).

Unlike the method used in this research study, the routine method used to screen pregnant women of diabetes in Tanzania is the urine dipstick test for sugar. The sensitivity of the urine test is low, and this may have led to misdiagnosis of diabetes cases. A systematic review involving five studies

concluded that urine dipstick test is insufficient to diagnose diabetes mellitus (Wei and Teece 2006). Currently, the Tanzanian government is in the process of adopting a new guideline that recommends routinely screening pregnant women using fasting blood glucose. In future, after this guideline has been implemented countrywide, it will warrant the revision of my prediction models for pre-eclampsia to incorporate the diagnosis of diabetes as a predictor in these models. Currently, it will be of no practical value to incorporate a predictor variable that is not measured sensitively enough to explain my outcome of pre-eclampsia in these settings.

Smoking cigarettes has been documented in the literature as having a protective effect towards the development of pre-eclampsia. A systematic review involving six studies by Lucinda (2007) showed that smoking is a protective factor in pre-eclampsia. My analysis was not able to show an association of either smoking cigarettes or chewing tobacco with the development of pre-eclampsia. This observation may be attributed to the very low number of self-reported cases that professed to be smoking cigarettes (7 cases/smokers vs 16,302 control/non-smokers) or chewing tobacco (5 cases vs 16,312 control) in my dataset. I was not able to find literature that has explored the prevalence of smoking among women of reproductive age in these settings, but I speculate that it has been under-reported in my dataset. This potential reporting bias could be caused partially by the hospital setup where the information was extracted: participants may not have felt at ease to disclose the information to their health care providers.

Other factors could have influenced my results of identified risk factors in this population.

1. Change of paternity in subsequent pregnancies.

This factor has been documented to influence the risk of a woman developing pre-eclampsia since the genetic makeup of the fetus is new to the mother's immune system (Hutcheon *et al.* 2011). The feto-maternal immune incompatibility, because of changing a father, may lead to placenta dysfunction and hence pre-eclampsia. My dataset did not capture this variable, so I was unable to factor it in my analysis.

2. Aspirin consumption during pregnancy.

I did not have medical records that could enable me to identify women who may have consumed aspirin for other conditions. aspirin has been used to prevent pre-eclampsia in women at high risk of developing the condition (Bujold *et al.* 2010, Poon *et al.* 2017). However, it will be challenging for any study done in LMIC to account for this factor due to weaknesses in health systems. In most settings, it is possible to access aspirin without a prescription and even where it is prescribed, there is no central heath information system to enable tracking of the patient's intake of such drugs in dispensaries and health centres across the region.

3. In vitro fertilization and interpregnancy interval

The literature from developed countries has suggested factors such as In vitro fertilization (IVF) and inter-pregnancy interval to play a role as risk factors in their populations. I do not think these factors would have made a difference if explored in LMIC settings because IVF is a very rare intervention. Inter-pregnancy interval has been shown to matter after a ten-year period of spacing pregnancies, where the risk of pre-eclampsia equals that of a nulliparous women (Skjaerven *et al.* 2002). Most LMIC are experiencing very high fertility rates. In Tanzania, the average fertility rate is five children per women. Given the fact that the reproductive age is from 15 to 49 years, this high fertility rate can hardly be attained with interpregnancy spacing of more than ten years to make it worth factoring it into my analysis.

My conclusion is that prediction models relying on maternal biomedical characteristics alone can be useful to predict the occurrence of pre-eclampsia in LMIC, although with moderate sensitivity and specificity. The addition of biomarkers as shown in studies elsewhere is likely to improve the prediction performance. The predictors that have been statistically significant and adopted in my model to predict pre-eclampsia in all deliveries are; maternal age, weight before pregnancy, history of hypertension, HIV treatment, contraceptive IUD use, diagnosis of infection and diagnosis of malaria. My subgroup analysis showed there is a difference in the set of predictors that predict pre-eclampsia in term deliveries and preterm deliveries, thus, supporting a difference in aetiology of the two subgroups. My

findings differed from other studies since I was unable to include diabetes mellitus or gestational diabetes as predictor variables due to limitations in the health system in Tanzanian settings. Other predictors such as IVF that are often used in developed countries were not relevant in the context of LMIC. Currently, there are diagnostic challenges facing these potential predictors, diabetes mellitus and gestational diabetes. In future, I may revise these models to include gestational diabetes and diabetes mellitus to improve model prediction. Prediction models that rely on existing routine clinical data are likely to be affordable in LMIC and offer an opportunity of improving targeted care delivery to women at high risk of pre-eclampsia.

7.4 Discussion objective 4

To describe the pregnancy outcomes characteristics of women with pre-eclampsia in northern Tanzania.

Our understanding of the effects of pre-eclampsia on the unborn child has increased over the years. Pre-eclampsia has been reported to contribute fivefold to perinatal mortality in developing countries (López Jaramillo *et al.* 2009). Furthermore, there is also a report of increased risk of stroke in adult life among the offspring of pre-eclampsia pregnancies, thus suggesting the possible long term effects of pre-eclampsia on the surviving offspring (Kajantie *et al.* 2009). The WHO is tracking the attainment of the SDG on child health through global health observatory data. The infant mortality data

showed 4.1 million deaths (75 % of under-five deaths) occurred in the first year of life. The infant mortality rate in Africa (51 death per 1000 live births) was six times that of Europe (8 deaths per 1000 live births), showing marked regional variation. The WHO also reported that 47 % of all under-five deaths were neonatal deaths, in the first 28 days of life (WHO 2017b). I have described the characteristics of pregnancy outcomes of women with pre-eclampsia in comparison with women without pre-eclampsia using data from northern Tanzania. Furthermore, I have carried out a subgroup analysis for term and preterm deliveries subgroups to account for the difference in gestational age at delivery of the newborn. The gestational age at delivery is known to confound the survival and ill-being of the newborn (Osrin *et al.* 2001). My overall finding has shown that pregnancy outcomes of women with pre-eclampsia are far worse than women without pre-eclampsia. This finding has remained valid even after accounting for the gestational age at delivery in my term and preterm subgroup analysis. The literature suggests that there is no difference in the pregnancy outcome of primiparous and multiparous women with pre-eclampsia (Badria and Amarin 2005). I have not made a distinction in characterizing the pregnancy outcomes of primiparous and multiparous women with pre-eclampsia. My findings are a combination of primiparous and multiparous pre-eclampsia women.

My initial exploration of stillbirth outcome among women with pre-eclampsia in northern Tanzania showed that the rate of stillbirth was (74/587) 12.6 %, 95 % CI 10 % - 15.2 % of pregnancies. This was similar to a rather small

study in the Lake zone of Tanzania that examined only eclampsia outcomes of 78 women, and showed stillbirth was 12.2 % (10/78) of all eclampsia outcome (Ndaboine *et al.* 2012). I then examined the strength of the association. The odds of stillbirth were 4.8 (95 % CI 3.7 – 6.3) times among women with pre-eclampsia than women without pre-eclampsia in all deliveries. Upon stratifying by term and preterm deliveries the odds were 2.6 (95 % CI 1.6 – 4.3) and 2.9 (95 % CI 2.0 – 4.1) respectively. The drop in the strength of association appears to suggest that part of the effect of pre-eclampsia on stillbirth is mediated by prematurity which is an integral outcome of pre-eclampsia. The overall association between pre-eclampsia and stillbirth finding is consistent with other research findings. A WHO multi-country survey involving around 313,030 women by Abalos *et al.* (2014) showed pre-eclampsia (OR = 3.1, 95 % CI 2.7 - 3.5) and eclampsia (OR = 3.9, 95 % CI 3.1 - 4.8) were both associated with fetal death (stillbirths). The observed strong association between pre-eclampsia and stillbirth outcomes may suggest the necessity to tackle pre-eclampsia as a probable root cause of stillbirth outcomes.

I also examined neonatal death within 24-hours outcome among women with pre-eclampsia. Neonatal death was 0.5 % (3/587) of all deliveries among women with pre-eclampsia in KCMC hospital, northern Tanzania. Upon examination of the strength of the association, my finding showed pre-eclampsia was not statistically significantly associated with neonatal death in all deliveries (p value =0.2), neither in term (p value =1) or preterm (p value

=0.2) deliveries subgroups. My finding was different from that drawn from the WHO survey where pre-eclampsia (OR = 2.7, 95 % CI 2.2 -3.2) and eclampsia (OR = 6.5, 95 % CI 4.9 – 8.8) were both associated with early neonatal death (Abalos *et al.* 2014). I may speculate that this lack of difference in early neonatal deaths within 24 hours between women with and those without pre-eclampsia is a result of quality neonatal care services or due to bias since children born with complications were transferred to the pediatric ward at my study Centre, KCMC hospital. The neonatal deaths that could have occurred in the pediatric ward could not be established from my data set. However, neonatal deaths may be worse in lower level health facilities. A small study in rural western Tanzania showed the proportion of neonatal death among women with severe pre-eclampsia and eclampsia was as high as 12.8 % (9/70) (Mooij *et al.* 2015).

In addition to causing neonatal death and stillbirth, pre-eclampsia is associated with low Apgar scores of the surviving offspring. Low Apgar score at 1 minute is an indicator of the mental wellbeing of the newborn immediately after birth. Poor metal wellbeing at birth may lead to lifelong mental impairment in the surviving infant. My findings show pre-eclampsia is associated with a low Apgar score (OR = 4.3, 95 % CI 3.4 - 5.4) in all deliveries. The association was maintained in the subgroup of term and preterm deliveries (OR = 2.3, 95 % CI 1.5 – 3.5 and 2.8, 95 % CI 2.0 - 3.9 respectively). Prematurity being an integral part of pre-eclampsia outcome appears to mediate part of the pre-eclampsia effect in causing a low Apgar

score. A study in Ghana showed hypertensive disorders of pregnancy were associated with low Apgar score at birth, With an odds ratio of 1.8, 95 % CI 1.1 – 3.1 after adjusting for maternal age, parity, number of antenatal visits, gestational age at delivery and mode of delivery (Adu-Bonsaffoh *et al.* 2017). This study did not present the association of pre-eclampsia with low Agpar score, a subcategory of hypertensive disorders of pregnancy, but showed the proportion of low Agpar score was higher among pre-eclampsia subcategory (45.7 %) compared to other subcategories combined (26.8 %). Therefore, the strength of the association between pre-eclampsia and low Apgar score is likely to be higher than the one presented for the entire group of hypertensive disorders of pregnancies. A larger multinational survey by Abalos *et al.* (2014) also echoed the proportion of low Apgar score was higher in newborns of pre-eclampsia (20.6 %) and eclampsia (25.5 %) compared to newborns of non-hypertensive women (5.3 %).

The negative effect of pre-eclampsia on surviving offspring is also observed in the low birthweight (< 2500gm) indicator. Low birth weight, which often results from either preterm delivery or intrauterine fetal growth restriction or both, is thought to influence the survival of the newborn in the early days in life (Osrin et al. 2001). Low birth weight depicts fetal nutrition and is also a developmental indicator that has long term health effect on the sufferers in adult life (Barker et al. 1993, Lucas 1994). My result showed pre-eclampsia was strongly associated with low birth weight in all deliveries, term and preterm deliveries subgroups, with odds ratios of 6.4, 95 % CI 5.3 – 7.6: 3.6,

95 % CI 2.7 – 4.7 and 6.4, 95 % CI 4.4 – 9.3 respectively. I compared the strength of the association between term and preterm deliveries and observed that the strength is nearly double in the preterm subgroup compared to the term subgroup. What could explain this marked effect on low birth weight of pre-eclampsia newborns compared to non-pre-eclampsia newborns when both are born preterm? A plausible explanation for observing a much stronger association between low birth weight and pre-eclampsia in the preterm subgroup than in the term deliveries subgroup is due to some confounding factor. In my cases, the co-existence of IUFGR in the preterm deliveries with pre-eclampsia may be the confounding factor. A study by Sharma *et al.* (2017) showed IUFGR was associated with pregnancies with pre-eclampsia and preterm delivery compared with pregnancies with pre-eclampsia alone. In the second paragraph below, I have embarked on exploring features of IUFGR among newborns of pre-eclampsia in term and preterm subgroups in order to substantiate this confounding relationship.

The other side of the birth weight spectrum suggests that pre-eclampsia is associated with macrosomia (high birth weight > 4000gm) as well. The strength of the association was 2.0, 95 % CI 1.3 – 3.2 in all deliveries and 2.0, 95 % CI 1.3 – 3.3 in term deliveries. In preterm the association was not statistically significant due to small sample size for the group, this is evidenced by the wide confidence interval (OR = 2.4, 95 % CI 0.3 – 19.2, p value = 0.36). This observation could suggest a number of plausible explanations, one of them being the confounding effect of IUFGR. If we

assume that pre-eclampsia is associated with macrosomia then we may expect in the preterm subgroup, where IUFGR related to pre-eclampsia strongly exists, will counter and nullify the effects that would result in macrosomia (high birth weight). Therefore, we are only able to observe the macrosomia effect of pre-eclampsia in the term subgroup where the IUFGR influence is reduced. A second explanation is that the relationship between pre-eclampsia and macrosomia is confounded by another factor. Both conditions may share the same risk factor such as gestational diabetes. There is scarce literature on this relationship between pre-eclampsia and macrosomia in LMIC. However, a study in Ghana by Adu-Bonsaffoh *et al.* (2017) demonstrated that macrosomia exists across the different subcategories of hypertensive disorders of pregnancy. Another study in the USA by Langford *et al.* (2011) suggested that macrosomia and pre-eclampsia had a shared risk factor: overweight women who gained weight above 25 lb were more at risk of developing pre-eclampsia (RR = 1.7, 95 % CI 1.5 - 1.9) and macrosomia (RR = 2.1, 95 % CI 1.9 - 2.3) compared to those who gained recommended weight (15 - 25 lb). There is a need for more research to further explore this relationship between pre-eclampsia and macrosomia in LMIC populations.

I examined features of intrauterine fetal growth restriction, head circumference and birth length, by comparing newborns of pre-eclampsia and non-pre-eclampsia deliveries in term and preterm subgroups. The average gestational age of preterm deliveries was 33.9 weeks, while that of term

deliveries was 39.6 weeks. The average head circumference for preterm newborns of pre-eclampsia women was smaller compared to those of their counterparts' i.e. preterm newborns of non-pre-eclampsia women. The mean difference of head circumferences was 0.993 (0.758 - 1.227), $p < 0.001$ in all deliveries. The mean difference was 2.020 (1.444 - 2.597), $p < 0.001$ in preterm deliveries. However, this difference was not statistically significant in term deliveries 0.099 (-0.046 - 0.245), $p = 0.153$.

These findings only show the comparison of average head circumference between newborns of women with and without pre-eclampsia. They do not offer a comparison with the standard values of the fetal head circumference with respect to gestational age that is used to quantify intrauterine fetal growth restriction. A limitation to my analysis is that I was unable to compare the deviation of head circumference of newborns of pre-eclampsia women with the standard fetal head circumferences for the respective gestational age that range from 26 – 40 weeks. This analysis would enable us to show the deviation of pre-eclampsia newborns from the standard head circumferences across the range of gestational age. In future, I plan to focus no this analysis to be able to observe the presence and the extent of intrauterine fetal growth restriction experienced by newborns of pre-eclampsia women in northern Tanzania.

Similarly, I explored birth length, another feature of intrauterine fetal growth restriction, in relation to pre-eclampsia. My results showed that newborns of pre-eclampsia women were on average shorter in length than newborns of

non-pre-eclampsia women (46.3 cm ± 0.18 SD vs 48.3 cm ± 0.01SD, p value < 0.001). This finding was also observed in both preterm and term subgroups. (42.6 cm ± 0.28 SD vs 46.3 cm ± 0.1 SD, p value < 0.001) and (48.1 cm ± 0.11 SD vs 48.5 cm ± 0.01SD, p value < 0.001) respectively. The mean differences for birth length in all deliveries was 1.920 (1.548 - 2.292), p < 0.001. The mean difference was also observed in term deliveries 0.406 (0.182 - 0.631), p < 0.001 and was more pronounced in preterm deliveries 3.729 (2.831 - 4.628), P < 0.001.

Unlike head circumference presented above, birth length has shown a statistically significant difference in all deliveries, term and preterm deliveries subgroup. Both indicators are measured using the same tool i.e. a measuring tape around the head in the case of head circumference and crown to heel in the case of birth length. A reason for the discrepancy observed by these two indicators of IUFGR may be measurement errors. This may mask an existing difference in head circumferences but be insufficient to mask the difference in birth lengths because the existing difference is inherently smaller in head circumferences than in birth length. Therefore, I suggest that birth length is a more sensitive indicator to detect IUFGR.

The alliance for maternal and newborn health improvement study has shown that use of transcerebellar diameter measurement in late pregnancy can improve the estimation of gestation age with appropriate weight for gestation and those with small for gestation age in LMIC (B J Wylie et al 2020). This

valuable finding, if applied has the potential to accurately identifying IUFGR after ascertaining appropriate gestation age of a newborn.

Pre-eclampsia appears to present a constraint in the health delivery system. This is such an important aspect especially in the LMIC, where resources for health care are scarce. I have observed that pre-eclampsia is associated with assisted deliveries in all deliveries (OR = 1.38, 95 % CI 1.1 – 1.6). This finding also implies that assisted delivery services are required preferentially to support pre-eclampsia women to deliver safely to mitigate the negative impacts of pre-eclampsia on the mother and the newborn. In the event such services are not available then the consequence in terms of stillbirth and neonatal deaths will be more severe for mothers with pre-eclampsia. The magnitude of the association between pre-eclampsia and these negative consequences (stillbirth, neonatal death, Apgar score etc.) is highly influenced by the quality of intervention services. Even the less obvious interventions seem to make a difference in the outcome. There is evidence from a study in India showing the maternal and fetal outcome of women with severe pre-eclampsia undergoing emergency cesarean section differed depending on the anaesthesia technique used in the surgery (Suman *et al.* 2014). This underlines the contribution of interventions in influencing the extent and types of outcomes from pre-eclampsia pregnancies. My results are from a tertiary hospital in northern Tanzania where most of the comprehensive emergency obstetric services are available and accessible. The situation is likely to be much worse in rural health settings, where such

intervention services are absent or not timely accessed. A study in Nigeria showed both maternal and perinatal death due to eclampsia were worse when patients presented after 12 hours from the onset of convulsion (Yakasai and Gaya 2011). In light of this thinking, I advocate for investment in addressing pre-eclampsia in LMIC, where the magnitude of its consequences is likely to be more devastating than the presented picture of my results. My results also highlight the effects on the surviving offspring in terms of their developmental indicators such as Apgar score and birth weight. This further emphasises investing in pre-eclampsia to avert these developmental challenges to surviving newborns, thus giving them a lifelong benefit.

7.5 Summary

The analysis of secondary data from Tanzania aimed firstly, to estimate the incidence of pre-eclampsia in Dar es Salaam city, Tanzania. I estimated the incidence in the year 2009 to have been 1.9 %, 95 % CI 1.3 % to 2.2 %. This estimate serves as a baseline for future monitoring of the trend of the condition in this rapidly evolving urban setting of Tanzania. Secondly, I described the sociodemographic characteristic of women with pre-eclampsia in northern Tanzania. The observed results showed that pre-eclampsia was independently associated with pregnant women above 35 years, single and those with tertiary level education. The increased risk among women with tertiary education may be due to either lifestyle change or increased health-

seeking behaviour. Qualitative studies could explore this social group to understand this dynamic relationship. Thirdly, I analysed the risk factors associated with pre-eclampsia in northern Tanzania, which revealed that different sets of risk factors explain term and preterm pre-eclampsia outcomes in this population. This tally with findings elsewhere, which also suggested different aetiology for early and late pre-eclampsia onset. In my analysis, pre-eclampsia in term deliveries was explained by maternal age, number of pregnancies, history of hypertension, a diagnosis of malaria, diagnosis of infection, contraceptive IUD use, HIV treatment and pre-pregnancy body weight. In preterm deliveries, pre-eclampsia was explained by maternal age, pre-pregnancy body weight, diagnosis of infection and history of hypertension. The prediction of pre-eclampsia outcome using models including these risk factors yielded a Youden's index with a sensitivity of 65 % and specificity of 63 % in classifying pre-eclampsia outcome in all deliveries. This raises the concern for the need to integrate modern markers and tools to improve the prediction of pre-eclampsia. Most LMIC rely on a similar list of risk factors to screen for pre-eclampsia. Integrating modern markers and tools in predicting pre-eclampsia may improve identification of cases and allow timely provision of prevention services. Fourthly, I described the characteristics of pre-eclampsia pregnancy outcomes. This revealed that women with pre-eclampsia experience higher percentage of stillbirth and neonatal death. Their surviving offspring also had worse Apgar scores and

birth weight, which are important indicators setting them into a poor developmental trajectory in later life.

This work contributes to the scarce literature on risk factors for pre-eclampsia in LMIC. It also captures the aspect of difference in risk factors in early and late pre-eclampsia onset in the region. In an attempt to explore the local drivers, I examined malaria infection as a unique local risk factor for this region, alongside other risk factors. The revelation of an existing relationship between malaria infection and pre-eclampsia may encourage further research to explore this important factor in this region. The observed results of poor pre-eclampsia pregnancy outcome and moderate sensitivity of screening tools in the region calls for further investment in the health system to mitigate the impact of the disease, so as to attain the SDGs for improved maternal and child health.

Chapter 8 Conclusion on pre-eclampsia risk factor analysis in Tanzania.

8.1 Conclusion

This section concludes the discussions of this thesis in line with its main areas of research: the scoping review study on risk factors of pre-eclampsia in Africa, the systematic review and meta-analysis of the association of malaria infection and gestational hypertension and lastly, the analysis of risk factors for pre-eclampsia from Tanzania.

The scoping review showed that risk factors for pre-eclampsia have been under-researched in African populations, despite the fact that such populations are experiencing high levels of maternal and infant mortality. The risk factors associated with pre-eclampsia showed inconsistent results across populations. Malaria infection, a locally important potential risk factor, showed inconclusive results in its association with pre-eclampsia and gestational hypertension, thus compelling me to undertake a meta-analysis of the primary studies. I observed methodological limitations in the studies since most studies were case-control with small sample size. Very few cohort studies were able to examine the temporal relationship between the risk factor and outcome. There was scarce evidence that took into account early and late onset of pre-eclampsia in analysing risk factors. Furthermore, there were no assessments of model performance in classifying pre-eclampsia

outcome in these populations. Following identification of these gaps, I set out to address them in the systematic review and in my risk factor analysis study using data from Tanzania.

The systematic review and meta-analysis of primary studies that examined the association of malaria and pre-eclampsia plus gestational hypertension in African populations revealed a strong association after adjusting for key confounding variables. The odds of having GH were more than double among women with malaria infection, compared to women without malaria infection. In this, meta-analysis pre-eclampsia and gestational hypertension proper were combined into one outcome of gestational hypertension. This approach enabled me to identify the contribution of malaria as a risk factor in the overall burden of hypertensive disorders of pregnancy, as it was linked to both the pre-eclampsia and gestational hypertension sub-categories. Malaria infection was observed to exert constant effects across the studied populations. The ongoing interventions that are controlling malaria infection on pregnant women could have an indirect benefit of reducing gestational hypertension and, in the long-term, cardiovascular diseases to women in the region. However, since my study only shows an association, there is a need for conducting molecular studies to further explore the causal mechanism.

The analysis of secondary data from Tanzania showed the incidence of pre-eclampsia in Dar es Salaam Tanzania in 2009 was 1.9 %, 95 % CI 1.3 % to 2.2 %, which is similar to the WHO global estimates of between 2 % and 8 %. This estimate serves as a reference for future estimation of the incidence in

the evolving urban setting of Tanzania. The sociodemographic characteristics that were independently associated with pre-eclampsia in northern Tanzania were women aged 35 years and over, single and with tertiary level education. Further qualitative studies could examine the dynamics of this relationship between tertiary education level and pre-eclampsia to inform the public on prevention strategies. This analysis showed that different sets of risk factors explain term and preterm pre-eclampsia outcomes in the northern Tanzanian population. This tally with findings elsewhere, which also suggest different aetiologies for early and late pre-eclampsia onset. Pre-eclampsia in term deliveries was explained by; maternal age, the number of pregnancies, history of hypertension, a positive diagnosis of malaria infection, HIV treatment, contraceptive IUD use, diagnosis of infection and pre-pregnancy body weight. Pre-eclampsia in preterm deliveries was explained by maternal age, pre-pregnancy body weight, diagnosis of infection and history of hypertension. The prediction of pre-eclampsia outcome in all deliveries, term and preterm deliveries subgroups using models with these risk factors showed an AUC of 69.4 %, 72.1 % and 66.9 % respectively. The three models yielded Younden's index with sensitivity values of 65 %, 65 % and 59 % respectively and specificity values of 63 %, 65 % and 66 % respectively in classifying pre-eclampsia outcomes in the three groups. The AUC scores show the models were moderate (≥ 70 % - < 80 %) and weak classifiers (≥ 60 % - <70 %) of the outcome. This finding prompts the need to integrate modern markers to improve the prediction of pre-eclampsia in LMIC, which

continue to rely solely on maternal biomedical risk factors for screening. My analysis also revealed that women with pre-eclampsia experience a higher percentage of stillbirth and neonatal death. Their surviving offspring also had worse Apgar scores and birth weight, which are important indicators setting them to a poor developmental trajectory in later life.

This work contributes to the scarce literature on risk factors for pre-eclampsia in LMIC. It also illustrates the difference in risk factors in early and late pre-eclampsia onset in the region. The observed results of poor pregnancy outcome in pre-eclampsia deliveries and moderate sensitivity of prediction models calls for further investment in the health system to mitigate the impact of the disease, so as to attain SDGs for improved maternal and child health.

8.2 Recommendations

As a result of this study, I have identified five key recommendations, as articulated below.

1. The diverse populations in the African region will benefit from research with methodological rigour that aims to explore the risk factors of pre-eclampsia in order to resolve the conflicting and the inconclusive results observed in the literature for some important risk factors.

2. There is a need to conduct molecular studies to understand potential causal links between malaria infection and both pre-eclampsia and gestational hypertension.

3. It is necessary to conduct studies periodically to estimate the incidence of pre-eclampsia, especially in urban settings where rapid lifestyle changes may result in an increasing trend in the condition. Alternatively, establishing accurate diagnostic procedures for pre-eclampsia and efficient data recoding in the health information systems in most LMIC may provide a reliable means of monitoring the condition using routine data.

4. It is important for LMIC to develop prediction models for pre-eclampsia that have higher sensitivity and specificity compared to my models that only relied on maternal biomedical characteristics because I was attempting to offer a cheap and feasible way to predict the outcome.

High sensitivity and specificity models may be achieved through incorporating biomarkers, suited to the target population. However, the end models ought to be affordable and feasible to implement in the region.

5. The mortality and morbidity proportions of infants born from pre-eclampsia women are unacceptably high from the Tanzania data. Indicators of wellbeing among surviving offspring of pre-eclampsia were poor, hinting at a poor health trajectory in their adult life. We do not know the long-term effects because of fetal programming on the surviving offspring. This calls for further studies to explore the impact in later life among offspring in LMIC. Establishment of routine data systems that can link maternal records and adulthood morbidity and mortality records will enable follow up studies that explore fetal programming effects in LMIC.

8.3 Way forward

I plan to collaborate with other researchers in Tanzania and incorporate pre-eclampsia related questions in the ongoing surveillance studies to enable an up-to-date estimation of the incidence of pre-eclampsia and related risk factors in Tanzania.

I plan to revise the developed prediction models by incorporating more risk factors, such as gestational diabetes after a new maternal health guideline is implemented in Tanzania. The guideline adopts the use of blood sugar test

rather than a urine sugar test in detecting gestational diabetes. This will enable better prediction of pre-eclampsia.

I plan to disseminate my results through publications; I have earmarked the following areas of my thesis for publication.

a. The systematic review and meta-analysis on the association of malaria infection and gestational hypertension. This paper has been submitted to the Journal of Global Health. Malaria association reviewers have reviewed it. Later on, I will embark on addressing the reviewer's comments.

b. The risk factors that explain pre-eclampsia occurrence in northern Tanzania.

c. Description of pregnancy outcomes of women with pre-eclampsia in northern Tanzania.

I plan to advocate for the process of developing a national guideline for preventing and managing hypertensive disorders of pregnancy in Tanzania. This will harmonise care delivery and promote necessary preventive approaches to reduce maternal and infant mortality and morbidity.

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ANNEX.

Annex 1: list of research variables in the KCMC dataset:

Variable code	Position	Variable label	Variable type
ID	1	Mother ID number	Scale
CID	2	Childs unique ID number (for matching)	Scale
DOB	3	Birth date child	Scale
HOSNUM	4	Hospital number mother	Scale
motherage	5	Mothers age	Scale
grav	6	number of pregnancies	Nominal
para	7	number of previous children	Nominal
gaweek	8	gest age in weeks LMP (26145-26155 added from prevCs orig)	Scale
gaday	9	Gestation age in days LMP	Scale
BODWGTAD	10	body weight at admission	Scale
MTHEDU	11	Highest educational level of mother	Nominal
MTHOCC	12	Mothers occupation	Nominal
MARIST	13	Marital status	Nominal
FTHAGE	14	Fathers age	Scale
FTHEDU	15	Fathers education	Nominal
FTHOCC	16	Fathers occupation	Nominal
MHBP01	17	Diabetes	Nominal
MHBP02	18	Hypertension	Nominal
MHBP03	19	Heart disease	Nominal
MHBP04	20	Epilepsy	Nominal
MHBP05	21	Malaria	Nominal
MHBP06	22	Anemia	Nominal
MHBP07	23	Gyn. disease	Nominal
MHBP08	24	Liver disease	Nominal
MHBP09	25	Kidney disease	Nominal
MHBP12	26	Sickle cells	Nominal
BLDTRA	27	<none>	Nominal
BODWGT	28	Mothers weight before pregnancy	Scale

Variable code	Position	Variable label	Variable type
BODHGT	29	Mothers height	Scale
FAMPLN	30	Used family planning?	Nominal
FPLPIL	31	p-pills	Nominal
FPLINJ	32	injections	Nominal
FPLIUD	33	IUD	Nominal
FPLFCO	34	female condom?	Nominal
FPLCON	35	condoms	Nominal
FPLIMP	36	implant	Nominal
FPLLAC	37	lactation	Nominal
FPLWIT	38	withdrawal	Nominal
FPLNAT	39	natural	Nominal
FPLABS	40	abstained	Nominal
FPLTRA	41	traditional	Nominal
FPLOTH	42	other family planning	Nominal
FPLTRY	43	Months tried to get pregnant	Scale
ANCARE	44	Antenatal care this pregnancy?	Nominal
ANCNUM	45	Number of ANC visits	Scale
LASTMP	46	LMP date	Scale
ULTSND	47	Ultrasound performed?	Nominal
ESTDOD	48	US est date of delivery	Scale

Variable code	Position	Variable label	Variable type
SMOKCT	49	Chewing tobacco (9 recorded as missing)	Nominal
SMOKCB	50	Chewing tobacco during pregnancy?	Nominal
SMOKIN	51	Do you Smoke	Nominal
SMOPRE	52	Smoking during this pregnancy	Nominal
SMOKNO	53	Cigarettes per day	Nominal
ALCOHO	54	Do you drink Alcohol bevarage	Nominal
ALCPRE	55	Alcohol during this pregnancy	Nominal
ALCOCC	56	Freq of drinking	Nominal
ALCOCP	57	Freq of drinking during pregnancy	Nominal
HIVREC	58	HIV status recorded	Nominal
HIVRES	59	Result of HIV-test	Nominal
tblBirths_HIVTRE	60	HIV treatment	Nominal
MHDP01	61	Gest diabetes	Nominal
MHDP02	62	Diabetes	Nominal

Variable code	Position	Variable label	Variable type
MHDP03	63	Hypertension	Nominal
MHDP04	64	Pre-eclampsia	Nominal
MHDP05	65	Eclampsia	Nominal
MHDP06	66	Epilepsia	Nominal
MHDP07	67	Bleeding	Nominal
MHDP08	68	Anemia	Nominal
MHDP09	69	Hyperemesis	Nominal
MHDP10	70	Malaria	Nominal
MHDP15	71	Heart disease	Nominal
MHDP18	72	Infections	Nominal
MTHHEA	73	Mothers health after	Nominal
ESTGAG	74	estimated gestational week	Scale
SEQUCE	75	sequence for twins	Nominal
CSTATUS	76	child's status	Nominal
STILC1	77	Stillborn time of	Nominal
STILC2	78	Stillborn admission	Nominal
STILC3	79	Stillborn condition	Nominal

Variable code	Position	Variable label	Variable type
STILPM	80	Stillbirth, Post mortem?	Nominal
NEONDE	81	Time of neonatal death	Nominal
SEX	82	Sex of the child (neonates)	Nominal
WEIGHT	83	birth weight	Scale
LENGTH	84	birth length	Scale
HEADCI	85	head circumference	Scale
DEMODE	86	Mode of delivery	Nominal
APGR01	87	Apgar 1min	Nominal
APGR05	88	Apgar 5min	Nominal
APGR10	89	Apgar 10min	Nominal
MALFOR	90	Birth defects	Nominal
INJURI	91	Injuries	Nominal
DESEAS	92	Diseases	Nominal
BIRDAT	93	Birth date child	Scale
DEADAT	94	Date of death	Nominal

Annex 2: standard antenatal care

According to the guidelines set by WHO and adopted by the Tanzanian Ministry of Health, all women and children seen in the public or private Clinics need to have some basic care standards met. Study Clinics need to fall within these requirements of health care provision whether using the local Clinic system or via research activities. The provisions set forth here are those recommended by the WHO Model for Antenatal Care in Randomised Clinic Trials:

- All women will get MCH care free of charge
- All pregnant women will attend ANC monthly in accordance with Ministry of Health guidelines.
- Women received Tetanus Toxoid vaccinations according to the following schedule:

  Dose #1: First antenatal visit

  Dose #2: after 4 weeks

  Dose #3: after 6 months

  Dose #4: after 1 year

  Dose #5: after 1 year
- Women are given malaria prophylaxis: Fansidar tablets at 20 weeks and 30 weeks gestational age. N.B. Not to be administered during first trimester.
- Women receive iron (60mg elemental iron) and folic acid (at least 0.25 mg) to be taken daily as a FeFol combination tablet.
- Women will be checked for syphilis and blood group typing during the first antenatal visit.
- Urine will be tested at first ANC visit with a multiple dipstick for bacteriuria and proteinuria. Repeat proteinuria (Albumin stick) test at second visit if client is nulliparous or has a history of hypertension, pre-eclampsia or eclampsia. Continue to check for bacteriuria until infection is gone.
- Haemoglobin concentration will be determined every 4 weeks of antenatal.

Annex 3: Enrolment to child 2 study

A mother was considered enrolled when she signs the consent form for the study. An infant was not enrolled until he/she was randomised.

The trial was a 2X2 factorial, double-blind, randomised controlled trial, 2400 infants who were 6 weeks of age and born to HIV-negative mothers were randomly assigned to receive daily oral supplementation of **Multivitamins** (vitamin B complex and vitamins C and E), **zinc, zinc + Multivitamins** or **placebo** for 18 months. Study nurses assessed morbidity at monthly visits and by physicians every 3 month and/or when the child was acutely ill.

Eligibility

Eligibility was determined by the medical history information obtained, by physical exam, and by laboratory results. Subjects who fail to meet the eligibility criteria were informed by study staff and thanked. Enrolled subjects whose eligibility was verified by the screening tests were informed by study staff and scheduled to return for an enrollment visit.

Inclusion Criteria for Mothers

Eligibility of women was evaluated using the Confirmation of Maternal Eligibility form. This form was completed at the Enrollment Visit (V1).

A woman eligible for enrollment must:

- be pregnant with singleton foetus
- be ≥ 18 years old
- be ≤ 34 weeks gestation (as measured by last menstrual period)
    - The original gestational age criteria were 28-34 weeks gestational age. I started enrolling women less than or 34 weeks on 21 January 2008.
- be HIV negative
- be willing to remain in Dar es Salaam for 2 years after the child's birth
- Must not have had a previous child enrolled in Child2 study

Inclusion Criteria for Children

Eligibility of children will be evaluated using the confirmation of eligibility form. This form will be completed at the Visit 11 appointment.

Inclusion criteria for children:

- Singleton, live-born infants
- Born to HIV negative women
- Mothers intend to stay in Dar es Salaam for at least 2 years after delivery
- 5 to 7 weeks of age

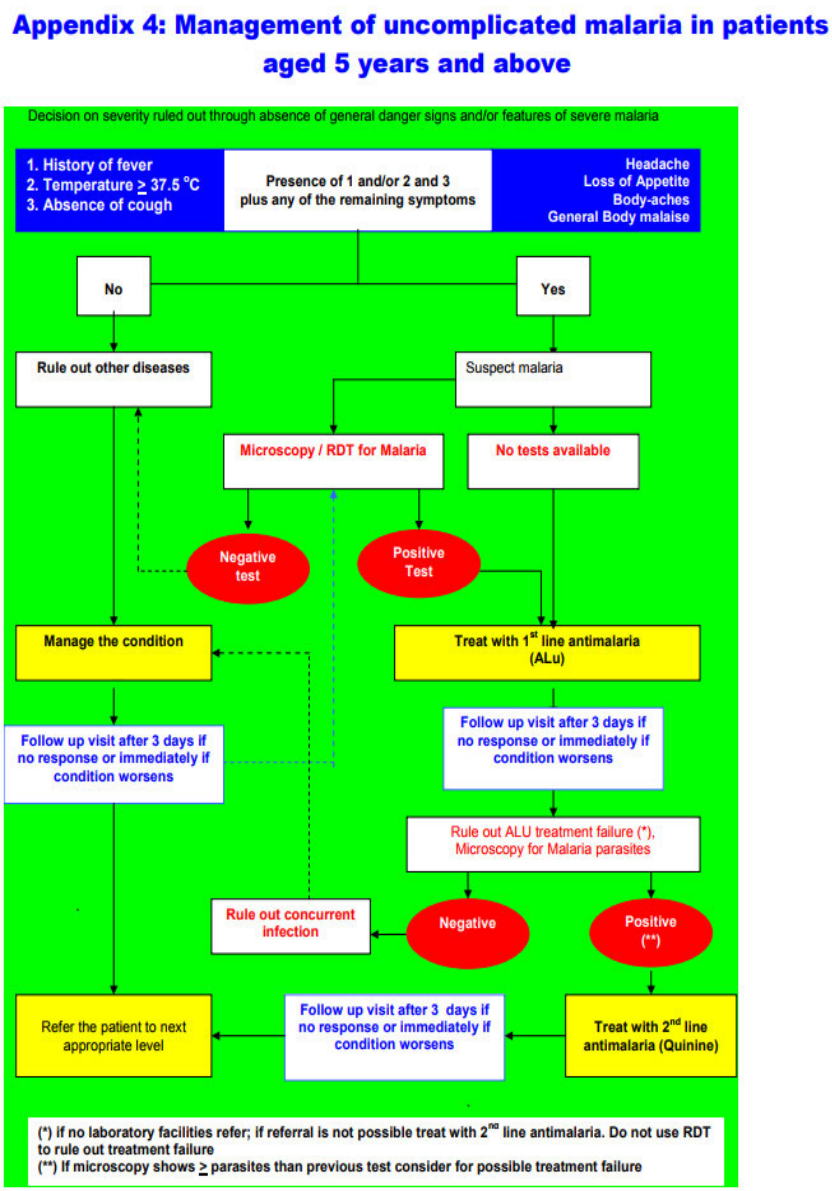
Exclusion criteria for children:

- serious congenital anomalies (e.g., cyanotic congenital heart disease, spinal bifida, or other nervous system malformations)
- serious medical conditions (e.g., Down syndrome, other genetic condition that would interfere with compliance with study procedures)
- inability to comply with study regimen (e.g., inability to take oral supplement)
- inability to comply with study procedures (e.g., mother unable to attend monthly follow-up Clinic visits)

Annex 4:

Figure 23. Malaria diagnosis algorithm.

Rapid Diagnosis test for malaria was performed during consultation with the doctor to diagnose malaria



Annex 5: search strategy for scoping study risk factors for pre-eclampsia.

A. EMBASE;

1. exp risk factor/
2. exp etiology/
3. exp contraceptive agent/
4. exp smoking/
5. exp anemia/
6. exp diabetes mellitus/
7. exp hypertension/
8. exp cholesterol/
9. exp placental malaria/
10. exp Human immunodeficiency virus/
11. 1 or 2
12. 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
13. 11 or 12
14. exp pre-eclampsia/
15. exp eclampsia/
16. 14 or 15
17. 13 and 16
18. limit 17 to english language
19. limit 18 to human
20. limit 19 to full text

B; WHO GLOBAL HEALTH LIBRARY.

(etiology OR risk factor) AND (Pre-eclampsia OR pre-eclampsia) AND (instance:"ghl") AND (db:("LILACS" OR "WPRIM" OR "IMEMR" OR "IMSEAR" OR "AIM") AND la:("en"))

C; WEB OF SCIENCE.

Set	Web of Science Core Collection Search History - " final risk f preE"
#8	(#5 NOT #6) *AND* **LANGUAGE:** (English) Refined by: **Open Access:** (YES) *DocType=All document types; Language=All languages;*
#7	(#5 NOT #6) *AND* **LANGUAGE:** (English) *DocType=All document types; Language=All languages;*
#6	TS=animal$ *DocType=All document types; Language=All languages;*
#5	(#4 AND #1) *AND* **LANGUAGE:** (English) *DocType=All document types; Language=All languages;*
#4	(#3 OR #2) *AND* **LANGUAGE:** (English) *DocType=All document types; Language=All languages;*
#3	TS=(smoking OR diabetes OR hypertension OR anemia OR malaria OR HIV OR contraceptive) *DocType=All document types; Language=All languages;*
#2	TS=("risk factor*" OR etiology OR cause*) *DocType=All document types; Language=All languages;*
#1	(TI=(pre-eclampsia OR pre-eclampsia)) *AND* **LANGUAGE:** (English) *DocType=All document types; Language=All languages;*

Annex 6: applied search strategies in systematic review.

MEDLINE

Database: Ovid MEDLINE(R) <1946 to February Week 2 2018>
Search Strategy:
---

1 hypertension, pregnancy-induced/ or eclampsia/ or hellp syndrome/ or pre-eclampsia/ or pregnancy complications, cardiovascular/ or pregnancy complications, hematologic/ or pregnancy complications, infectious/ (87919)
2 malaria.mp. or MALARIA/ (76629)
3 pregnancy induced hypertension.mp. or Hypertension, Pregnancy-Induced/ (5188)
4 1 or 3 (88851)
5 2 and 4 (1086)
6 limit 5 to (english language and full text and humans) (334)

EMBASE

Database: Embase <1980 to 2018 Week 08>
Search Strategy:
---

1 malaria/ or malaria.mp. (101474)
2 pregnancy induced hypertension.mp. or maternal hypertension/ (16362)
3 1 and 2 (95)
4 limit 3 to (full text and human and english language) (34)

WHO GLOBAL INDEX MEDICUS

Search date:03/03/2018 (20)
(tw:(pregnancy induced hypertension)) OR (tw:(maternal hypertension)) OR (tw:(pre-eclampsia)) AND (tw:(malaria)) OR (tw:(placental malaria)) AND (instance:"ghl") AND (db:("LILACS" OR "WPRIM" OR "IMEMR" OR "IMSEAR" OR "WHOLIS" OR "AIM" OR "BBO") AND type of study:("case-control" OR "cohort" OR "systematic reviews" OR "clinical trials") AND limit:("humans") AND la:("en"))

GOOGLE SCHOLAR

Search date:03/03/2018 (94)
("Pre-eclampsia" OR "pregnancy induced hypertension") AND ("malaria" OR "Placental malaria")

Annex 7:

Table 55. Quality assessment checklist of the selected papers with CASP tool

Author, Year	*Focused question*	*Appropriate method to answer the question*	*Recruitment of cases*	*Recruitment of controls*	*Recruitment Overall*	*Exposure accurately measured*	*Confounding factors accounted for*	*Confounding factors taken into account in the design/analysis*	*What are the results?*	*How precise are the results?*	*Do you believe the results?*	*Can the results be applied to the local population?*	*Do the results of this study fit with other available evidence?*
Adam I et al 2011	Yes	Yes	Hospital-based recruitment	Control selected from same hospital, some bias compared to exposure in community.	No sample size or power calculation. Case and control not comparable in their primigravidae status, family hx of HT [more % in cases], control lacked ANC care more than cases.	Exposure accurately measured, Giemsa stain of placental blood and placenta histology	Excluded Twins and Diabetes, known predictors.	Multivariable analysis adjusted for age, primigravidae, Hx of malaria, Family Hx of HT, BMI, Blood Gp, Placental malaria, Education level, Lack of ANC	Multivariable analysis Family Hx of HT OR=5.7 95%CI [2.9-11.5]. Placental malaria OR=2.3 95 %CI [1.0-5.2]	Results are precise, P=0.04 for placental malaria, moderate strength of the association OR 95 %CI 1.0-5.2. Just above 1.	Yes	Internal validity Yes.	Generalizability, yes results are comparable with previous studies in Africa.

Author, Year	*Focused question*	*Appropriate method to answer the question*	*Recruitment of cases*	*Recruitment of controls*	*Recruitment Overall*	*Exposure accurately measured*	*Confounding factors accounted for*	*Confounding factors taken into account in the design/analysis*	*What are the results?*	*How precise are the results?*	*Do you believe the results?*	*Can the results be applied to the local population?*	*Do the results of this study fit with other available evidence?*
Ephraim R et al 2014	Yes	Yes, case-control	Multicentre hospital-based recruitment. Hypertensive with or without proteinuria post 20wks	Age matched controls normal tensive and without proteinuria post 20wks.	No sample size calculated prior the study	Malaria dx not explained	Adjustment of confounder in the multivariable analysis, Age, gravidity, parity, BMI, contraceptive use, Abortion, New paternity and Malaria for GH not PE	excluded chronic HT, on Antihypertensive drugs, eclampsia, diabetes autoimmune, renal diseases.	malaria not associated with GH, OR = 2.92 95 %CI [0.5 to 17.15]	Non-significant and wide CI, possibly insufficient power.		Internal validity cannot be ascertained, no number of malaria exposed case and control	

Author, Year	*Focused question*	*Appropriate method to answer the question*	*Recruitment of cases*	*Recruitment of controls*	*Recruitment Overall*	*Exposure accurately measured*	*Confounding factors accounted for*	*Confounding factors taken into account in the design/analysis*	*What are the results?*	*How precise are the results?*	*Do you believe the results?*	*Can the results be applied to the local population?*	*Do the results of this study fit with other available evidence?*
Sartel et H et al 1996	Association of pre-eclampsia and placental malaria?	Yes, case-control design	Hospital-based two centres. No exclusion of participants. Did not use the current definition of pre-eclampsia, BP and proteinuria.	controls similar to cases, when compared on mean age and no of previous pregnancy.	No sample size precalculated,	placental malaria accurately measured. Outcome measured in old standards, diastolic BP only. No proteinuria.	Adjusted for age, no of previous pregnancies, twin delivery, maternity centre and date of delivery.	Adjusting for date of delivery/seasonality is unique and valuable in malaria hypo endemic area	Multivariable odds ratio 3.3 95 %CI [1.1 to 9.5.]	Significant results with moderate CI, possibly due to low power after adjusting multiple variables. The univariable CI was narrow. 3.0 95 %CI [1.3 - 6.9].	Yes, case-control design	Internal validity Yes, Generalizability Yes.	Yes, a pioneer study.

Author, Year	*Focused question*	*Appropriate method to answer the question*	*Recruitment of cases*	*Recruitment of controls*	*Recruitment Overall*	*Exposure accurately measured*	*Confounding factors accounted for*	*Confounding factors taken into account in the design/analysis*	*What are the results?*	*How precise are the results?*	*Do you believe the results?*	*Can the results be applied to the local population?*	*Do the results of this study fit with other available evidence?*
Ndao C T et al 2009	Relationship between placental malaria and hypertension during pregnancy.	case-control design	Hospital-based study	Control matched to cases, age parity and prematurity	case and control were matched for Age, parity and date of delivery(prematurity). But did not do a matched analysis, rather unconditioned logistic reg. 69% drop in recruiting eligible cases/control due to data missing, but suggested to be missing completely at random.	Placental malaria accurately measured. Also the outcome well categorised pre-eclampsia, eclampsia, gestational hypertension.	Compared case and control on many aspects, they were comparable: Insecticide treated bed nets use, quinine prophylaxis use by urine test, residence, age, religion, period of delivery-seasonality.	Variables included in the analysis, placenta malaria, residence, parity, past pregnancies, ANC visit, family Hx of HT, period of delivery-seasonality, illiteracy, marital status.	Multivariable OR = 2.7 95 %CI [1.0 - 7.6]	narrow CI, hence results can be trusted.	Yes	Internal validity Yes, there may be variabilities across different endemicity regions.	Generalizable results, and in agreement with other studies.

Annex 8: Approval letters to use primary datasets and University ethical clearance

KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE
(Constituent College of Tumaini University Makumira)
All correspondences should be
Addressed to the **Provost**
P. O. Box 2240, **MOSHI**, Tanzania.
Telephone 255-759-929965.
Fax: 255-55-2751351.
Email:jmmahande@gmail.com
Website: http://www.kcmuco.ac.tz.

Date: 17 April 2018

To: Henry Mruma
P.O Box 65301
Dar es Salaam

Dear Sir
Re: Use of Kilimanjaro Christian medical birth registry for your PhD Thesis

Reference is made to your letter dated on 27th March 2018 requesting to use the Kilimanjaro Medical Birth Registry Data. I am happy to inform you that, these data are open to both local and international researchers interested in maternal and New-born health. However, in order to sustain running the registry, we do charge user fee depending on the level. For your case as PhD students (local) you will need to pay 2000 US $. In addition, we prefer researcher (s) from our institution to be part of the authors for all the publications which use the medical registry. To avoid being a ghost author, we normally like to be involved in all processes from manuscript preparation to publication. Based on our experience in using the medical registry data, we always ready to provide technical experts during the design and analysis of the data.

Sincerely Yours,

Michael J. Mahande (PhD)
Head, Department of Epidemiology and Biostatistics- KCMUCo

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Ref. No: MUHAS/PCH/KPM/PF

17th February,2018.

TO WHOM IT MAY CONCERN

RE: PERMISSION TO USE DATA FROM OUR STUDY NCT.00421668 FOR PHD THESIS BY DR. HENRY MRUMA.

I, the undersigned hereby give permission to Dr. Henry Mruma a PhD student at the University of Edinburgh(s0968240) and faculty member at the Community Health Department, Muhimbili University of Health and Allied Sciences (MUHAS) to use data from our dataset repository from our Randomized Clinical Trials in Tanzania for his thesis that examines "Risk factors for pre-eclampsia in Tanzania". He plans to use the dataset as part of his secondary analysis study.

However, we shall retain the control of all publications resulting from this secondary analysis as we deem necessary.

Please feel free to contact me for any details or clarification.
Yours Sincerely

Prof. Karim Manji
PI-Child1/Child2

cc. Director of Research and Publications, MUHAS .

THE UNIVERSITY *of* EDINBURGH

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30 August 2017

HENRY ABRAHAM MRUMA

Dear Henry

Re: RISK FACTORS FOR PRE-ECLAMPSIA IN TANZANIA

This is to confirm that the Level 1 Ethics Self-Audit undertaken by you with respect to the above study (as submitted on **30/08/2017**) demonstrates that the proposed research poses no reasonably foreseeable ethical risks. Within our research governance process, this means that the research proposed (as outlined on the Level 1 form) does not require formal ethical review by the Usher Research Ethics Group (UREG) – i.e. it can be considered to be 'exempt'.

You may forward this letter, together with the Level 1 form completed, to any collaborating data owner who requires reassurance as to ethical oversight of the research proposed.

Yours sincerely

Diane White
Usher Research Ethics Group Administrator