DETERMINANTS OF PRETERM BIRTH, SMALL FOR GESTATIONAL AGE AND FOETAL GROWTH IN A MARGINALIZED POPULATIONS ON THAI-MYANMAR BORDER: A 30 YEAR POPULATION COHORT STUDY

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Abstract

Preterm birth (PTB) and small for gestational age (SGA) have serious implications for neonatal health, particularly in low- and middle-income countries (LMICs) where healthcare access is restricted, and socioeconomic disparities are prevalent. This thesis aimed to investigate the factors influencing PTB, SGA, and foetal growth among marginalized pregnant women along the Thai-Myanmar border.

The research objectives were to identify demographic, infectious, medical, and obstetric factors associated with poor birth outcomes (Objective 1); explore the determinants of PTB and SGA in a 30-year cohort of refugee and migrant pregnant women (Objective 2); identify the demographic, infectious (malaria infection), and non-infectious factors (preeclampsia) associated with poor foetal growth (Objective 3); and explore interventions to optimize foetal growth in low-resource settings (Objective 4).

Retrospective data analysis utilized secondary data from the Shoklo Malaria Research Unit (SMRU), including antenatal care and delivery records, and prospective pregnancy cohort studies. The findings aligned with existing literature, highlighting a high prevalence of teenage pregnancies and a dual burden of malnutrition (overweight and underweight) linked to adverse pregnancy outcomes. Risk factors for PTB included teenage pregnancy, low Body Mass Index (BMI), preeclampsia, a history of previous PTB, and non-malaria bacterial infection. Risk factors for SGA included teenage primigravida, advanced maternal age, low BMI, smoking, malaria infection, and preeclampsia. Increased maternal height and BMI were protective factors for both PTB and SGA.

Additionally, maternal overweight and carrying a male fetus were associated with higher mean abdominal circumference (AC) z-scores, while the height of taller women and overweight mothers influenced head circumference (HC), biparietal diameter (BPD), and femur length (FL) z-scores. Preeclampsia had a significant adverse impact on AC and FL z-scores. Malaria infection did not significantly affect HC and BPD, but vivax-infected pregnancies showed lower mean AC and FL z-scores temporarily after infection, all of which were treated.

In conclusion, these findings emphasize the importance of comprehensive healthcare approaches to reduce the burden of PTB and SGA in LMICs. At SMRU, malaria control effort has successfully decreased the adverse impact from malaria, whereas non-infectious causes are becoming more prevalent. Addressing identified risk factors, along with comprehensive healthcare strategies, can contribute to improving birth outcomes in these settings. As these findings emerged from this thesis three strategies have been implemented in the population: i) avoiding repeat teenage pregnancy after childbirth, ii) aspirin and calcium for prevention of pre-eclampsia and iii) most recently screening all women for gestational diabetes using a two-step approach. Government led service provision would be ideal however the civil war following the February 2021 *coup d'etat* threatens gains obtained in the prior decade. There is real danger malaria will re-emerge due to conflict. Malaria redirects limited resources to fight a single scourge and will inevitably increase maternal and child death.

Statement of contribution

The analyses in this thesis used two different data sources. Firstly, Chapters 3 and 4 was based on the routinely collected data from ANC clinics at SMRU to describe the baseline characteristics of marginalized pregnant women, as well as the trends of important risk factors and adverse pregnancy outcomes over a 30-year period. The data cleaning process for the SMRU birth cohort dataset, spanning from 1986 to 2020, was conducted by the Maternal and Child Health (MCH) team, led by Professor Rose McGready and other SMRU researchers. I conducted the analyses for the description of the SMRU birth cohort and the trends of important maternal risk factors, PTB, and SGA, with the kind assistance of Dr. Makoto Saito.

Secondly, Chapter 5 utilized data from prospective pregnancy cohorts, namely the Impact of Malaria Infection in Pregnancy on Foetal and Newborn Growth (UPS), INTERBIO 21st (Foetal Study), and Molecular Signature in Pregnancy (MSP). I would like to extend my gratitude to Dr. Marcus Rijken, Dr. Tobias Brummaier, and the INTERBIO-21st Study Team for kindly granting the permission to utilize the clean ultrasound data of foetal biometric measurements. The statistical analysis plan was drafted after discussions with Professor Julie Simpson and Dr. Makoto Saito, and I managed the data analysis with the support of Dr. Makoto Saito.

Furthermore, I conducted a systematic literature review on placental histopathology in preterm birth with confirmed maternal infection, which has been published in a peer-reviewed journal. It was modified from the manuscript and added as Appendix A. I would like to acknowledge my supervisors, Professor Rose McGready and Professor Francois Nosten, for their help with the screening of full-text articles as

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Published article

1. Min AM, Saito M, Simpson JA, Kennedy SH, Nosten FH, McGready R. Placental histopathology in preterm birth with confirmed maternal infection: A systematic literature review. PLoS One. 2021 Aug 12;16(8): e0255902. doi: 10.1371/journal.pone.0255902. PMID: 34383833; PMCID: PMC8360573.

Unrelated articles published (not first author) during DPhil

1. Lee SJ, Hashmi AH, Min AM, Gilder ME, Tun NW, Wah LL, Wah M, Win E, Ner M, Charunwatthana P, Nosten FH, Carrara VI, McGready R. Short maternal stature and gestational weight gain among refugee and migrant women birthing appropriate for gestational age term newborns: a retrospective cohort on the Myanmar-Thailand border, 2004-2016. BMJ Glob Health. 2021 Feb;6(2): e004325. doi: 10.1136/bmjgh-2020-004325. PMID: 33597278; PMCID: PMC7893649.
2. Saito M, Briand V, Min AM, McGready R. Deleterious effects of malaria in pregnancy on the developing fetus: a review on prevention and treatment with antimalarial drugs. Lancet Child Adolesc Health. 2020 Oct;4(10):761-774. doi: 10.1016/S2352-4642(20)30099-7. PMID: 32946830.

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who collected the data while simultaneously providing quality healthcare to the vulnerable population. Without their selfless and endless efforts, I wouldn't have been able to analyse a 30-year cohort data. I must also thank all the pregnant women participating in the SMRU birth cohort. I really hope that this DPhil will return, in kind, helpful suggestions for improving birth outcomes for this marginalized population – we have a direction for what we need to prioritise.

Last but not the least, I would like to immensely thankful my loved ones. My parents invested in me to pursue a future career. My lovely wife, Nhoi, has been taking on more responsibilities for our two little sons and patiently supporting me throughout my DPhil. Without her, I would not have been able to make it through successfully.

Abbreviations

AC	Abdominal circumference
ALSO	Advanced Life Support in Obstetrics
ANC	Antenatal care
AOR	Adjusted odd ratio
BHF	Borderland Health Foundation
BMI	Body mass index
BPD	Biparietal diameter
CI	Confidence interval
DBM	Double burden of malnutrition
EGA	Estimated gestational age
FL	Femur length
GDM	Gestational Diabetes Mellitus
HC	Head circumference
HDP	Hypertensive disorders of pregnancy
HICs	High income countries
IQR	Interquartile range
KDHW	Karen Department of Health and Welfare
LMICs	Low-and middle-income countries
LMP	Last day of first menstrual period
MMR	Maternal mortality ratio
NMR	Neonatal mortality ratio
OR	Odd ratio

PE	Preeclampsia
PIH	Pregnancy induced hypertension
PTB	Preterm birth
SDG	Sustainable Development Goal
SGA	Small for gestational age
SMRU	Shoklo Malaria Research Unit
UNHCR	United Nations High Commissioner for Refugees
WHO	World Health Organization

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Chapter 1

Introduction

1.1 Chapter overview

This chapter summarizes the outline of the thesis and provides a review of relevant literature of adverse pregnancy outcomes overall, and specifically in relation to preterm birth (PTB), small for gestational age (SGA) and foetal growth. Literature on non-communicable and infectious diseases during pregnancy and their impact on PTB, SGA and foetal growth are also summarized. Finally, the study aim, objectives and rationale are presented.

1.2 Thesis structure

This thesis contains six chapters, as follows:

Chapter 1: Introduction

The introduction provides a summary of the literature relevant to the three main adverse outcomes under analysis in this thesis: PTB, SGA and foetal growth. As adverse pregnancy outcomes are inter-related, other outcomes are briefly presented. PTB, SGA and foetal growth, and their relationship to infectious and non-communicable disease are summarized. Reference to the difficulties of identifying PTB, SGA and foetal growth in resource-limited settings are provided.

Chapter 2: Population and Methods

This chapter sets the scene for the following chapters and presents how data has been collected. Sources of data and variable definition used in the Shoklo Malaria Research Unit (SMRU) birth cohort from 1986 to 2020 and the three prospectively

pregnancy cohorts for foetal growth are described. The pregnant women population of refugee and migrant women, study site and staff on the Thai-Myanmar border are also presented.

Chapter 3: Cohort profile: maternal and newborn characteristics including PTB and SGA in marginalized pregnant women along Thai-Myanmar border between 1986-2020

Characteristics within a population can impact pregnancy outcomes for example maternal factors such as age and the proportion who are teenage will be associated with the proportion of newborn who are premature. This chapter provides a description of standardized maternal and newborn characteristics in the SMRU cohort from 1986 to 2020. The newborn characteristics will include PTB and SGA, as well as other indicators such as abortion, stillbirth, neonatal death. Globally, maternal death reflects population health and health services so these will also be presented. Hence, this chapter will provide a complete picture of the maternal and newborn health situation and provide important background for Chapters 4 and 5.

Chapter 4: Determinants of PTB and SGA of a population of refugee and migrants on the Thailand-Myanmar border from 1986 to 2020.

This chapter is at the core of the thesis. It describes in detail the most important determinants of PTB and SGA providing a breakdown of contributing factors over this 30-year time frame. When considering possible interventions to mitigate the impact of these factors, the population attributable risks are presented for PTB and SGA.

Chapter 5: Determinants of foetal growth in marginalized population: pool data analysis of three pregnancy cohort studies

This chapter focuses on the determinants of foetal growth using INTERGROWTH-21st standard by primarily using Z score of foetal biometrics measurement (Abdominal circumference (AC), head circumference (HC), Biparietal diameter (BPD) and femur length (FL). Then, the association between AC Z score and malaria infection in pregnancy, which is assumed as time dependent variable, will be explored. Furthermore, the relationship between AC z-score and pre-eclampsia (PE) will be examined.

Chapter 6: Discussion and conclusion

In this chapter, I explain the relevance of the results from previous chapters and their implications for the marginalized population in this area as well as in other resource limited setting, limitations of the data and future direction for research to reduce PTB, SGA and foetal growth restriction.

1.3 Introduction to PTB, SGA and foetal growth

Intrauterine foetal growth during pregnancy is a critical determinant of neonatal outcomes, with suboptimal gestation or birthweight or both, and being associated with poor neonatal outcomes. Many factors have been implicated in contributing to growth restriction in utero, including infection during pregnancy, non-communicable diseases (NCDs) such as hypertensive disorders of pregnancy (HDP)[2] and gestational diabetes[3]. Of these, infection during pregnancy has been identified as a significant contributor to foetal growth restriction (FGR) and poor neonatal outcomes, including PTB and SGA[4, 5].

PTB and SGA are the leading causes of perinatal mortality and long-term health consequences, including impaired motor and neurocognitive development[6]. When small newborns are better classified, it is smallness due to prematurity that exerts the greatest threat to survival. Evidence from resource limited setting on the burden and impact of being born too early (PTB) or too small (SGA) is scarce, primarily due to the difficulty of accurately dating pregnancies[7]. However, studies from various countries have reported that the burden of PTB and SGA is higher in resource limited setting compared to high-income countries (HICs). Moreover, the proportion of PTB and SGA attributable to maternal infections during pregnancy, such as malaria, is higher in resource limited setting[4, 5]. In addition, non-communicable diseases such as HDP[8] and gestational diabetes[9] have been reported to be increasing in prevalence in resource limited setting, further contributing to the burden of IUGR, PTB, and SGA.

1.4 Adverse pregnancy outcomes

1.4.1 Preterm birth (PTB)

Preterm birth (PTB), either spontaneous or medically indicated, is commonly defined as the delivery of a liveborn infant before 37 completed weeks of gestational age and it can be further classified based on gestational age into extremely preterm (<28th week), very preterm (28th- <32nd week), and late preterm (32nd -37th week)[10]. PTB is a major public health concern worldwide and the leading cause of neonatal mortality and under-5 child mortality worldwide[6]. The multifactorial nature of spontaneous PTB has recently been characterized phenotypically into five categories: maternal conditions, foetal conditions, placental pathologic conditions, signs of initiation of labour, and pathways of delivery (either spontaneous or medically

indicated PTB)[11]. A better understanding of these factors can help identify women at higher risk of PTB and develop effective interventions to prevent it.

However, reliable gestational age assessment in resource limited setting is a significant obstacle to effective prevention and management of PTB. Poor data recording, accurate last menstrual period recognition, and low access to ultrasound dating scans contribute to this challenge, particularly in rural areas[7]. Therefore, efforts to improve gestational age assessment methods and data recording in these settings are essential.

The estimated global PTB rate was reported as 11.1% (14.9 million) in 2010[12], and it decreased to 10.6% (14.84 million) of all live PTBs in 2014[13], which suggests some limited progress in the reduction of PTB burden. However, there are significant variations between regions and countries, with the majority of PTBs occurring in Sub-Saharan Africa and South Asia, where data recording and birth registration are poor and reliable gestational age assessment methods are often not available. The burden of PTB is high, with approximately 1 million babies dying every year from complications related to PTB[14]. A systematic review of 107 countries found that the lowest recorded PTB rate was in Belarus (4.3%, 3.4-5.4) and the highest was in Cyprus (18.7%, 15.4-22.3), while Thailand (12.7%, 10.1-15.6) and Myanmar (10.4%, 8.7-11.9) had similar rates[13]. This variation in PTB rates may be due to differences in access to healthcare, socioeconomic status, maternal age, parity, and reported variables particularly medical indications.

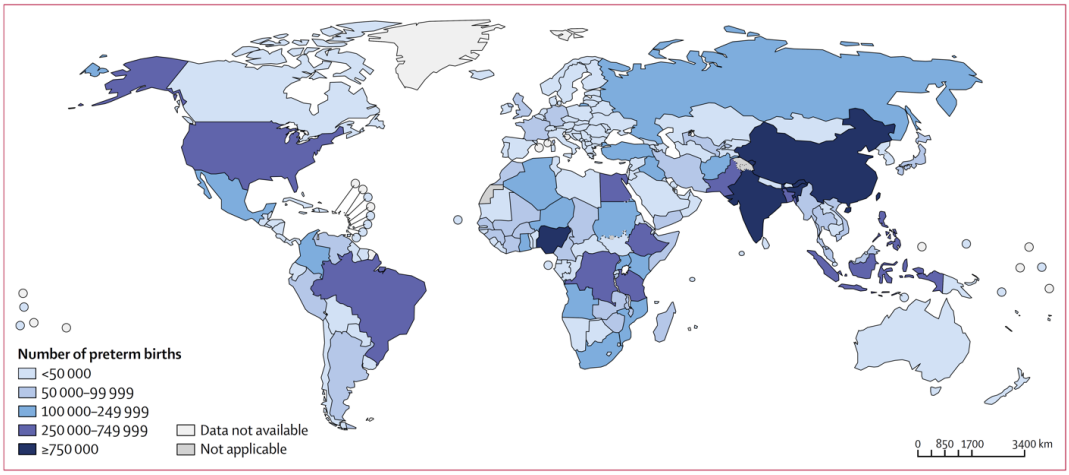


Figure 1-1: Global estimate of PTB rates in 2014. This Figure has been reproduced from Chawanpaiboon et al. (2019)[12].

The most recent systematic review conducted to date (Feb 2023) examined 44 potential risk factors for spontaneous PTB in 81 low and middle income countries and determined that the significant associations included: maternal undernutrition which had the highest population attributable fraction (PAF) (17.5%), followed by maternal infections (16.6%), environmental exposures (16%) and pregnancy history (8.7%)[4]. Maternal infections included HIV, Malaria, Syphilis, Chlamydia, asymptomatic bacteriuria, periodontal infection, bacterial vaginosis and group B streptococcus colonization. Environmental exposures included ambient air pollution, indoor air pollution, intimate partner violence and tobacco smoking. Pregnancy history included a combination of short birth interval, young maternal age (<18y) and primiparity, and older maternal age. The article concluded that there was a significant gap in data from low- and middle-income countries.

1.4.2 Low birth weight (LBW)

Low birth weight (LBW) is a public health indicator used for decades to assess maternal nutrition and physical health, antenatal care and impact of infection or diseases during pregnancy[15]. It is defined as a birth weight less than 2,500 g, regardless of gestational age[16]. While it has been used for international comparisons, it is not suitable for clinical management of patients as it does not provide sufficient direction for targeting interventions[17]. The incidence of LBW varies between 6% and 18% across countries, with the highest rate found in South Central Asia[15]. However, the global estimate of LBW may be underestimated and biased, particularly in developing countries where most deliveries occur outside health facilities and birth weight is frequently not measured in a timely way[18].

1.4.3 Small for gestational age (SGA)

Small for gestational age (SGA) is a term used to describe infants who are born below the 10th percentile of birthweight for gestational age and sex of a reference population [19]. SGA babies can be either term or preterm, and represents the end-point of intrauterine growth restriction[20].

There are various underlying causes of SGA, and some infants may be constitutionally small but still healthy, while others may be ill due to infections, placental-mediated growth restriction, structural or chromosomal abnormalities, maternal malnutrition, and exposure to toxic substances[21]. In addition, maternal medical conditions such as hypertension and diabetes can also contribute to SGA. The incidence of SGA varies among different populations and is influenced by several factors, including maternal age, parity, socioeconomic status, and ethnicity.

According to estimates, approximately 32.4 million SGA babies, accounting for 27 percent of all live births, were born in LMICs in 2010[22]. However, the Child Health Epidemiology Reference Group (CHERG) reported that 23.3 (17.6 to 31.9) million infants were born SGA in LMICs in 2012 using the INTERGROWTH-21s standard and the highest burden of SGA was found in South Asia, where the prevalence was about 34%[23].

SGA is a significant contributor to neonatal mortality, particularly in LMICs. In 2012, an estimated 606 500 (21.9%) (495 000 to 773 000) neonatal deaths were attributable to SGA, and the population attributable fraction of SGA to neonatal deaths was 26%[23]. Accurate estimation of gestational age is essential in identifying SGA infants. The method used to estimate gestational age can significantly affect the prevalence of SGA. The INTERGROWTH-21s standard is currently recommended for estimating gestational age in LMICs[24].

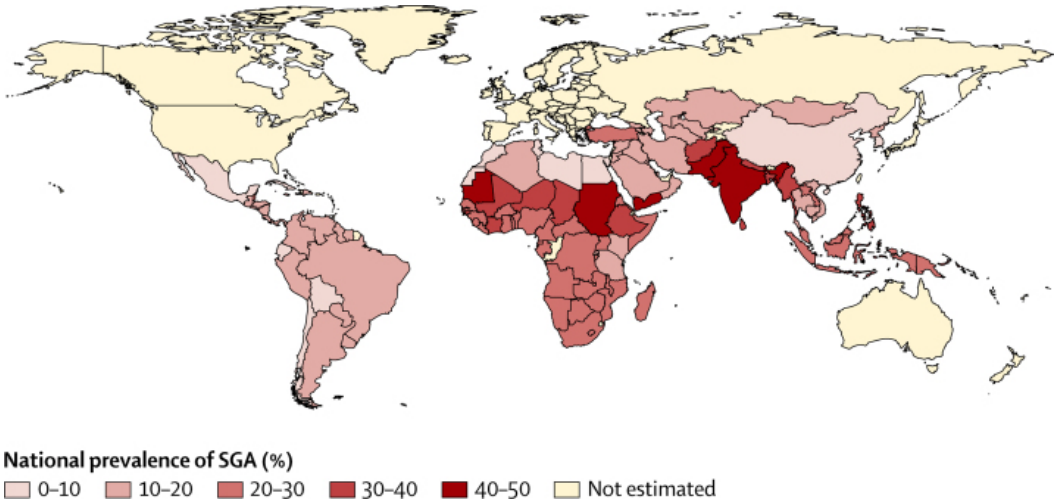


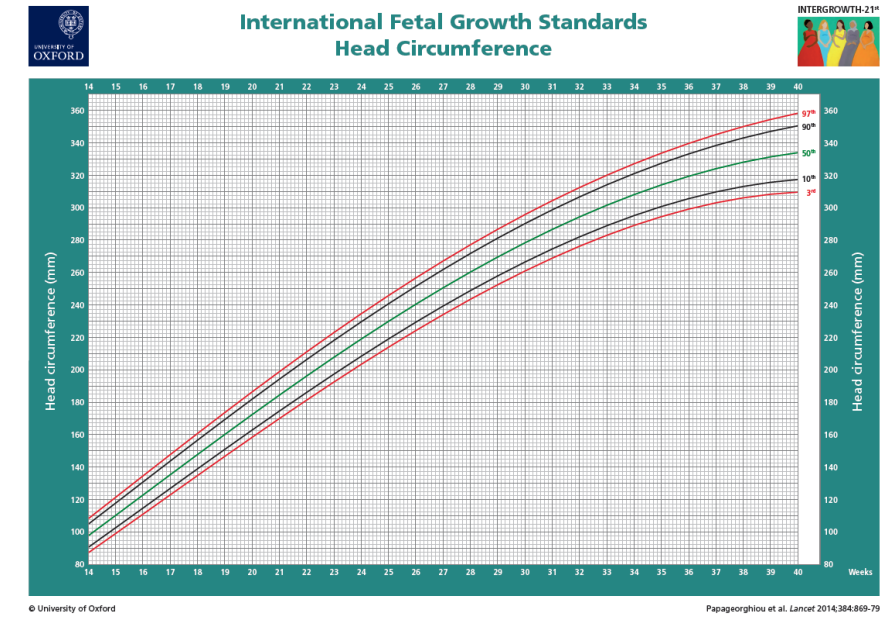
Figure 1-2: Estimated prevalence of SGA births in 138 low-income and middle-income countries[24].

1.4.4 Foetal growth

Foetal growth restriction (FGR), also known as intrauterine growth restriction (IUGR), is associated with perinatal mortality and long-term health outcomes[26]. The identification of a growth restricted foetus during the antenatal period, can permit an active management approach, which balances the risks of stillbirth and prematurity.

Traditionally, symphysio-fundal height measurement has been used as a universal screening tool to monitor foetal growth during pregnancy and foetal growth restriction should be considered if there is a discrepancy between estimated gestational age and fundal height measurement greater than 3 centimeters[27]. Maternal obesity, multiple pregnancies, uterine abnormalities, foetal position, distended bladder and inter-observer variations can affect the accuracy of correct measurement hence limiting applicability of this method[27].

A single ultrasound scan can provide an estimate of gestation of a foetus but two scans can provide a profile of growth and two weeks is the accepted minimum time between scans to observe foetal growth. Biometric measurements of a foetus by ultrasound by well-trained sonographers is a better screening tool for impaired foetal growth. Centile charts are used to plot foetal growth by gestational age for specific biometric parameters such as head and abdominal circumference[28] (**Figure 1-3**).



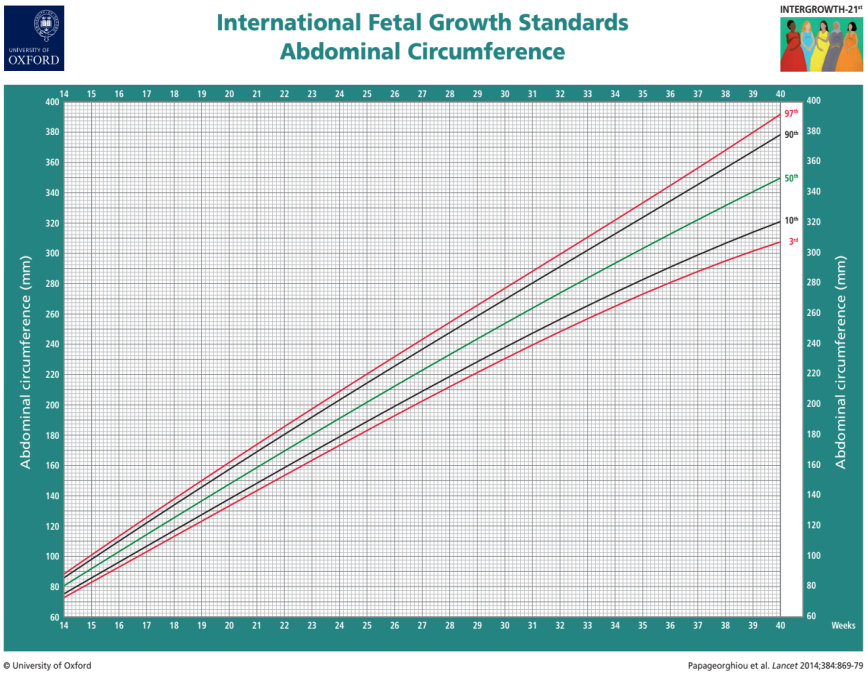


Figure 1-3: INTERGROWTH International foetal growth standards for head circumference and abdominal circumference[28]. (The images are retrieved from https://intergrowth21.tghn.org/foetal-growth/)

While a single scan can determine if the foetal head and abdominal circumference is less than the 10th centile, only a series of scans can determine if the foetus is falling off its projected growth curve. Once the diagnosis of foetal growth restriction has been made, foetal wellbeing tests and umbilical artery doppler velocimetry should be performed to know the optimal timing of delivery and reduce the perinatal mortality[26] by as much as 29%[19].

Foetal anomaly scan may have some role in screening and management of growth restriction as small foetuses may be associated with structural and genetic abnormalities[21]. Many studies have shown that there were no effective preventive strategies for foetal growth restriction including nutritional and dietary supplements, bedrest and use of aspirin to prevent placental insufficiency[30]. Timing and mode of delivery of small foetuses depends on the gestational age, clinical findings, ultrasound examination results and maternal comorbidities[31]. Settings with well-developed neonatal intensive care units could reduce the risk of neonatal death by caring for preterm infants. Settings without neonatal intensive care units will likely invoke later induction, risking intrauterine death, rather than having an infant that dies from PTB complications as a result of induction of labour.

1.4.5 Mortality

Mortality remains a definitive adverse pregnancy outcome for mother and newborn and high mortality rates are indicative of problems in maternal and child health service provision or may be related to the presence of specific diseases within communities, or both.

1.4.5.1 Maternal mortality

Maternal mortality refers to the death of a woman within 42 days of the termination of pregnancy, regardless of the duration of pregnancy. Maternal mortality is measured as a maternal mortality ratio (per 100,000 livebirths). In 2017, LMICs and HICs had vastly different rates of maternal mortality, with LMICs experiencing an estimated 462 maternal deaths per 100,000 livebirths annually compared to just 11 maternal deaths per 100,000 livebirths annually in HICs[32]. Over 90% of maternal deaths occur in LMICs, especially in sub-Saharan Africa and southern Asia[33]. Adolescent pregnancy is a significant risk factor for maternal death, particularly for mothers under the age of 15[34].

Maternal death can be categorized as direct or indirect. The leading causes of direct maternal deaths are severe bleeding per vagina before and during pregnancy, bacterial sepsis, hypertensive disorders, obstetric complications during labour, and unsafe abortion. Approximately 27% of maternal deaths are due to indirect causes, including pre-existing medical conditions, HIV infection, malaria, diabetes, and other indirect causes[35, 36].

Preventing maternal deaths is crucial and can be achieved through proper antenatal care, safe delivery, and availability of family planning methods. While there has been a significant reduction in maternal mortality worldwide, it has not met the target number of sustainable development goal (SDG3), which is “reducing the goal MMR to less than 70 per 100,000 livebirths, with no country having a maternal mortality rate of more than twice the global average”[37].

1.4.5.2 Neonatal mortality

Neonatal death is a critical public health concern that requires urgent attention to reduce the global burden of child mortality. According to the World Health Organization, neonatal death is defined as "the death of a live-born infant within the first 28 completed days of life"[38]. This definition can be further classified into early neonatal death, which occurs during the first seven days of life, and late neonatal death, which occurs after seven to 28 days of age[38].

Early and late neonatal deaths tend to have different causes, which can help inform appropriate interventions and prioritization of programs to address the underlying issues. Prematurity and jaundice are common causes of early neonatal death, while birth asphyxia and sepsis are more common causes of late neonatal death. It is essential to adhere to these definitions to accurately track neonatal mortality and develop effective strategies to prevent these deaths[39].

Globally, neonatal mortality has decreased significantly over the past few decades, with the rate declining from 36.6 deaths per 1,000 live births in 1990 to 17 deaths per 1,000 live births in 2017[38]. However, neonatal mortality remains high in resource-limited settings, particularly in Sub-Saharan Africa and Southern Asia, where rates are 25 and 27 deaths per 1,000 live births, respectively[38]. To address this issue, the Sustainable Development Goal 3.2 aims to reduce the global neonatal mortality rate to less than 12 deaths per 1,000 live births by 2030[37].

1.4.6 Stillbirth

According to international standards, stillbirth is defined as the delivery of a baby with no signs of life at or after 28 weeks of gestation, which allows for

international comparisons[40]. However, in some developed countries, the definition of stillbirth includes foetal loss before the 20th week of gestation, but does not count terminations of pregnancy for medical reasons[41]. Over the past four decades, there has been a decreasing trend in the global stillbirth rate, which was 35 per 1,000 live births in 1980 and decreased to 15 per 1,000 live births in 2015[42]. Most stillbirths occur in developing countries, and it is estimated that half of all stillbirths occur during the intrapartum period due to inadequate antenatal care, a lack of skilled birth attendants, insufficient emergency obstetrics care, and inadequate facilities for caesarean deliveries[43]. However, there are limitations in the accuracy of these stillbirth data due to variable definitions of stillbirth among countries, underestimations resulting from home deliveries in remote areas of LMICs, and inconsistencies between medical definitions and reporting criteria for stillbirth[43].

1.4.7 Pregnancy loss

Spontaneous pregnancy loss is a term used to describe the non-viable intra-uterine pregnancy that occurs at or before 22 weeks of gestation or with a birthweight of less than 500g. This phenomenon is commonly referred to as early pregnancy loss, and clinical practice often uses the terms miscarriage or abortion interchangeably. However, there is no consistent cut-off gestational age for pregnancy loss, as it varies from less than 20, 22, or 24 weeks in high-income countries to less than 28 weeks in developing countries[44].

The survival of babies born before 28 weeks of gestation presents a significant challenge in developing countries due to the lack of facilities for intensive newborn

care and medical expertise. Consequently, the true global prevalence of pregnancy loss is unknown since the cut-off gestational age varies.

1.5 Global disease patterns and health risks

The global disease pattern and mortality are changing radically resulting in a reduction of infectious disease mortality, and increases in non-communicable diseases including cancers, chronic lung diseases, diabetes and cardiovascular diseases in high- and middle-income countries[45]. However, infections such as respiratory tract infections, HIV/AIDS, malaria and tuberculosis, are still the major cause of death in low-income countries (LICs).

1.6 Infectious diseases in pregnancy

Pregnancy is a normal and healthy state[46]. However, infections including bacterial, viral, parasite and fungal are more likely to occur during pregnancy and can cause adverse pregnancy outcomes. Immunological changes during pregnancy could lead to immunosuppressed condition in order to avoid rejection of the foetus and body defence immune system is inadequate to respond to the infection. Furthermore, there are other factors (pregnancy hormones, impact of stress and microbiome) that influence immune response to the infection. Maternal infection could reach to intrauterine environment causing intrauterine infection by vertical transmission (hematogenous spread), ascending infection from genital tract and transmission from the abdominal cavity through the fallopian tube, which is less common. Maternal infections and subsequent placental inflammation can lead to poor pregnancy outcomes such as PTB, LBW and SGA.

As part of understanding the evidence on infection in pregnancy, I completed a systematic review (Appendix 1) to explore the association between placental histopathological abnormality and PTB in the presence of confirmed infection[47]. Databases including PubMed/Medline, Scopus, Web of Science and Embase were searched using the keywords related to PTB, placental histopathology and infection. Of 1529 articles, the titles and abstracts were screened, and the full texts of eligible articles were reviewed to extract and summarise data. Only 23 studies (13 bacterial, 6 viral and 4 parasitic) were included despite use of 7 different gestational age windows for PTB, and 20 different histopathological classification systems, precluding data pooling. Histopathological chorioamnionitis, and funisitis (when examined) were commonly observed in PTB complicated by confirmed bacterial or viral, but not parasitic, infection. The presence of malaria parasites but not pigment in placenta was reported to increase the risk of PTB, but this finding was inconclusive. One in three studies were conducted in LMICs. An array of definitions of PTB subgroups, histological classification systems, histopathologic abnormalities and diagnostic methods to identify infections were reported in this systematic review. Commitment to using standardised terminology and classification of histopathological abnormalities associated with infections is needed to identify causality and potential treatment of PTB. Studies on PTB needs to occur in high burden countries and control for clinical characteristics (maternal, foetal, labour, and placental) that may have an impact on placental histopathological abnormalities.

1.6.1 Parasitic infection

Malaria infection is the most common parasitic infection of mankind. Around 247 millions of malaria cases in 2021 had been reported worldwide and the highest burden is found in the African region[48]. The global decreasing trend of malaria cases and malaria related deaths observed in every region of the world until 2019 has worryingly given way to rising trends.

Malaria infection in pregnancy contributes a substantial health risk on maternal, foetal and newborn babies. The prevalence of malaria infection in pregnancy is highest in Africa with an estimated 11 millions of pregnancy infected[49]. Plasmodium and plasmodium vivax are the two most common malaria infection of the four species of malaria parasites, detected by blood smear (gold standard). Severe malaria and maternal death are primarily caused by plasmodium falciparum and the risk is highest particularly in low/unstable transmission and during pandemic. Approximately, 10-20% of pregnancies were affected by falciparum malaria and 303,000 maternal deaths occurred in 2015 worldwide[50]. The majority of these maternal deaths occurred in malaria endemic areas and in areas where antenatal care is suboptimal[49]. Placental malaria is associated with maternal anaemia and adverse pregnancy outcomes such as PTB, stillbirth, LBW and SGA[51].

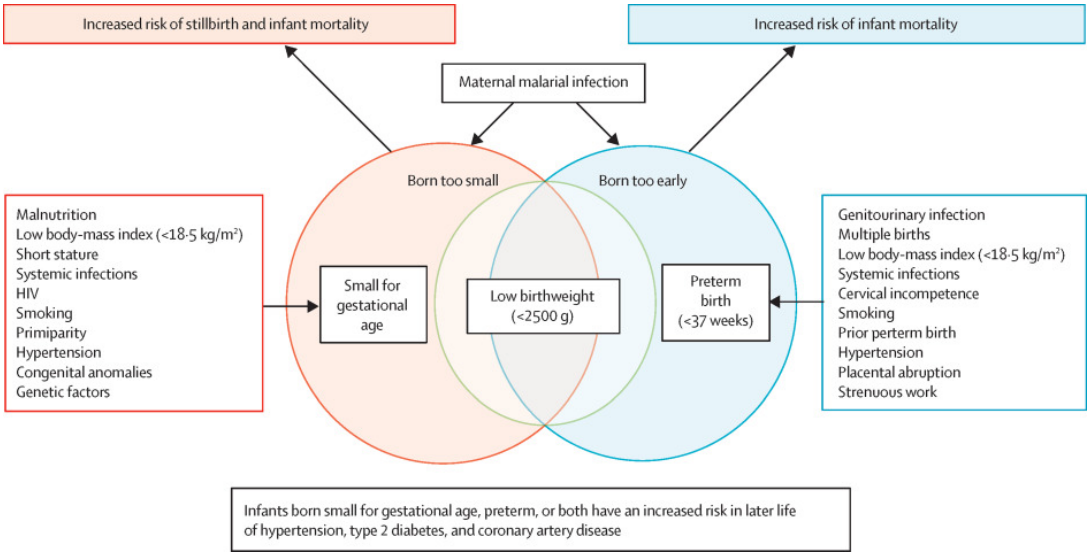


Figure 1-4: The adverse pregnancy outcomes: PTB, SGA and low birthweight in relate to malaria infection in pregnancy[51].

1.6.2 Bacterial infections

Bacterial infections, particularly urinary tract infections (UTIs), are a significant concern during pregnancy, as they can lead to sepsis and potentially fatal outcomes for both mother and foetus. According to recent research, bacterial infections are a leading cause of maternal, foetal, and neonatal mortality, particularly when diagnosis and treatment are delayed or insufficient[53].

Sepsis is defined as the presence of systemic manifestations of infection, while sepsis with septic-induced organ dysfunction or tissue hypoperfusion that does not respond to adequate fluid therapy is called septic shock. UTIs are a common bacterial infection associated with sepsis during pregnancy[54]. Women are more susceptible to UTIs due to their shorter urethra (3-4 cm in length) and its proximity to the vagina and anus. Moreover, anatomical and physiological changes during pregnancy make pregnant women more prone to UTIs[55].

UTIs during pregnancy can be categorized as asymptomatic bacteriuria, where there is the presence of asymptomatic infection in the urinary bladder, and symptomatic UTI, which can lead to cystitis, pyelonephritis, and significant morbidity for both mother and developing fetus[56]. The most common organisms that cause UTIs during pregnancy are Escherichia coli, Klebsiella pneumonia, Proteus, Acinetobacter, Saprophyticus Staphylococcus, Streptococcus Group B and Pseudomonas aeruginosa[57]. Studies indicate that up to 20% of pregnant women may suffer from symptomatic UTI during the antenatal period and UTI during pregnancy is significantly associated with PTB, foetal growth restriction, and Caesarean delivery[54,55].

If not treated appropriately, bacterial sepsis can follow UTI, potentially leading to maternal death. Moreover, antimicrobial-resistant strains of bacteria are a growing concern that may further complicate treatment[53]. In some settings, up to 20-30% of pregnancies complicated by symptomatic UTIs, highlighting the importance of prompt and adequate treatment[58]

Chorioamnionitis, also known as intra-uterine infection, is a condition where the foetal membranes and the amniotic fluid are infected by microorganisms. This condition can lead to PTB and other complications for both the mother and the newborn. It is primarily caused by bacterial infections, which are polymicrobial in nature. Diagnostic methods for chorioamnionitis are varied including amniocentesis, blood tests, and ultrasound imaging. However, these methods have limitations and the diagnosis of chorioamnionitis can be challenging [47].

Assuming 25-40% of PTB occurs due to ascending intrauterine bacterial infections, several bacterial species have been identified as causing chorioamnionitis,

including Mycoplasma species, Urea plasma species, bacterial vaginosis, and Group B streptococcus (GBS)[59]. GBS is the primary cause of neonatal sepsis and death and antibiotic prophylaxis for GBS is indicated in high-risk cases. These cases include women who have had a previous baby with GBS disease, women who have GBS in their urine during the current pregnancy, women who go into labour before 37 weeks, and women who have a fever during labor[60].

1.6.3 Viral infections

The adverse pregnancy outcomes, including pregnancy loss/spontaneous abortion, PTB, LBW/SGA, stillbirth, congenital anomaly, maternal and perinatal mortality, can also be caused by viral infections in pregnancy. Transplacental transmission of infection from mother to child is also the consequence of viral infections in pregnancy, and this can occur with HIV, hepatitis B, and ToRCH infections (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex)[61].

In recent years, the global outbreak of COVID-19 has had a profound impact on global health. The pandemic began at the end of 2019 and is caused by respiratory droplets containing SARS-CoV2 variants. According to a report in December 2022, the pandemic has affected 657 million people and caused 6.6 million deaths[62]. Multiple mutations to the genetic code have occurred and five variants of SARS-CoV2 have been identified, namely the alpha, beta, gamma, delta, and omicron dominated the early waves of the pandemic. The delta variant is associated with more severity and death compared to other variants[63]. Pregnant women are at a higher risk of suffering severe illness, particularly in late pregnancy. This includes ICU admission, mechanical ventilation, and maternal death, compared to non-pregnant women[64]. In addition,

COVID-19 infection is associated with an increased risk of PTB and stillbirth, but not with pregnancy loss before 20 weeks' gestation[64]. However, COVID-19 vaccination during pregnancy can be administered any time as a preventive measure, preferably in the second and third trimester, with no evidence of adverse effects on pregnancy and the foetus. It can also be safely given to lactating mothers, and no adverse effects have been reported so far[64]. COVID-19 had differential effects on HICs and LMICs that are still being elucidated although the increased risk of pre-eclampsia was described across these settings[65].

One of the most significant viral infections is HIV infection affecting 36.9 millions of people globally[66]. WHO estimated that 1.3 millions of HIV positive women are pregnant annually and more than 90% of cases are from sub-Sahara Africa region[67]. The prevalence of HIV positive pregnant women in HICs is low (0.1-1/1000) but it is as high as 29% in LICs[66]. However, the global trend of HIV infection in pregnancy is decreasing due to global action of increased awareness in general population, prevention, universal screening and provision of life-long anti-retroviral therapy (ART)[66]. The prevalence of HIV infection in Thailand is around 1.1% and Thailand is the first country in southeast Asia region to reduce mother to child transmission of HIV infection as low as less than 2%[68]. Untreated maternal HIV infection increased the risk of mother to child transmission, PTB, LBW, SGA, stillbirth, maternal and perinatal mortality [69]. Treating HIV infection with ART is crucial to reduce the maternal and perinatal mortality, mother to child transmission and adverse pregnancy outcomes[69].

Hepatitis B virus infection (HBV) is also the global concern to tackle; estimating 296 millions of people with chronic HBV (Hepatitis B surface antigen

positive) worldwide and around 820,000 deaths annually as of 2019 due to the consequences of developing cirrhosis, hepatic failure and hepatocellular carcinima[70]. Again, the global prevalence of HBV is varied depending on the region and the highest is seen in Asia and sub-Sahara Africa region (>8%)[71]. Reducing the transmission of HBV by 90% of new infection and mortality by 65 % at 2030 is the global aim by WHO[70]. The transmission of HBV occurs contact with infected blood or body fluids, sharing sharp instruments including needles and transplacental transmission of mother to child, which is the most common mode of transmission in high endemic areas. Prevention of mother to child transmission is one of the strategies to reduce the transmission of HBV. Providing hepatitis B vaccine at birth, use of antiviral agents (Tenofovir) during pregnancy and giving hepatitis B immunoglobulin to the newborn babies if the mother is highly infectious (Hepatitis B e antigen detected) are used to reduce the transmission of mother to child[70]. In some settings HBV in pregnancy was reported to increase the risk of PTB and gestational diabetes mellitus[72, 73].

1.7 Non-communicable diseases (NCDs) in pregnancy

Non-communicable diseases (NCDs) are chronic medical diseases, which have complex aetiologies and multiple risk factors, that cannot be transmitted person to person, insidious onset and longer duration of the disease process resulting in long-term disabilities and premature death. There are several types of NCDs including cardiovascular diseases, respiratory diseases, neurological disorders, musculoskeletal problems, HDP, metabolic diseases (diabetes, thyroid), haematological disorders (sickle cell, thalassaemia) and nutrition related health problems. Mental health disorders and acute injuries that could lead to long term disabilities are sometimes

defined as NCDs. Cardiovascular diseases, respiratory diseases and diabetes are three leading causes of deaths and account for 71% of all global NCD deaths. The emerging burden of NCDs is a global concern and the reproductive age group between 30-69 years (including older fathers) are increasingly affected. Common NCDs during pregnancy include HDP, pre-existing or newly diagnosed gestational diabetes mellitus, haematological disorders and nutritional deficiencies.

1.7.1 Hypertensive disorders of pregnancy (HDP)

Hypertension in pregnancy is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg in two measurements at least 4-6 hours apart. Up to 15-20% of pregnancies are complicated by hypertensive disorders. The definitions of different types of hypertensive disorders are listed in **Table 1-1**. Gestational hypertension, PE, chronic hypertension and PE superimposed by chronic hypertension depend on the presence of proteinuria (protein excretion is more than 300g per 24-hour urine collection).

Table 1-1: Classification of Hypertensive disorders of pregnancy (HDP)[3].

	Definition
Chronic hypertension	Pre-exiting or onset of hypertension before 20 weeks' gestation
Gestational hypertension or Pregnancy induced Hypertension (PIH)	New onset of hypertension after 20th weeks' gestation without proteinuria and absence of haematological or biochemical abnormalities
Pre-eclampsia (PE)	Defined as gestational hypertension after 20 weeks' gestation accompanied by one or more of the following new-onset conditions: ▪ Proteinuria (++ proteinuria on dipstick test, protein: creatinine ratio >30 mg/mmol, albumin: creatinine ratio >8 mg/mmol, or >300 mg protein/24 hr) Other maternal end-organ dysfunction, including: ▪ Neurologic complications (e.g., eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, or persistent visual scotomata) ▪ Pulmonary oedema ▪ Hematologic complications (e.g., platelet count <150,000/µl, DIC, haemolysis) ▪ Acute kidney injury (e.g., creatinine .90 µmol/litre or .1 mg/dl) ▪ Liver involvement (e.g., elevated aminotransferase levels, such as ALT or AST >40 IU/litre) with or without right-upper quadrant or epigastric abdominal pain) ▪ Uteroplacental dysfunction (e.g., placental abruption, angiogenic imbalance, foetal growth restriction, abnormal umbilical-artery Doppler waveform analysis, or intrauterine foetal death)
Eclampsia	Progression of severe PE with generalized seizure, agitation and loss of consciousness
HELLP syndrome	Haemolysis, elevated liver enzyme, low platelet count (<100,000/mm3)

Urinary protein excretion is increased during pregnancy, but it should not exceed 300g per 24 hours in normal pregnancy. Performing urinary dipstick (≥1+ or

more) two times 4-6 hour apart or urinary protein/creatinine ratio of 30 ng/mmol are alternative methods to detect proteinuria if 24-hour urine collection is not possible[74].

The pathophysiology of hypertensive disorder is complex, and chemical mediators and pregnancy related hormones are responsible for the occurrence of placental insufficiency that can lead to adverse pregnancy outcomes (intrauterine growth restriction, PTB and intrauterine foetal death). PE is diagnosed if there is a new onset of hypertension after the 20th week of gestation with proteinuria. Severe PE is the major cause for maternal morbidity and mortality, and it is associated with extreme PTB (EGA <32 week) and intrauterine growth restriction. It can damage multiple organs (liver, renal and haematological system) and resulting in an increased risk of developing HELLP syndrome: haemolysis, elevated liver enzymes, Low platelet count. Eclampsia occurs when the central nervous system is damaged and convulsion or seizure develop[75]. A large study in Chinese women (>200,000 pregnant women) reported that earlier onset of gestational hypertension/pre-eclampsia was more detrimental to both LBW and SGA[76].

1.7.2 Gestational Diabetes Mellitus (GDM)

Glucose metabolism is altered during pregnancy, and pregnancy itself is a diabetogenic state due to pregnancy related hormones (Placental lactogen hormone, growth hormone, cortisol, prolactin and progesterone) in relation to the foetal growth. Insulin resistance develops as a result of the pregnancy related hormones especially in the second and third trimester of pregnancy[77]. WHO defines gestational diabetes as "carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy and resolved after delivery"[77]. Despite the various

diagnostic and screening methods for GDM, the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study, an international multicentre cohort, recommended a 75-hour oral glucose tolerance test for diagnosing GDM. It can be performed any-time during pregnancy but proposed to best for screening between the 24th week and 28th week of gestation based on risk factors rather than universal screening[78]. The risk factors of GDM includes history of previous GDM, macrosomia, pre-pregnancy BMI ≥ 25mg/kg, gestational weight gain, family history of diabetes, history of stillbirth, pregnancy induced hypertension, maternal age > 40 year, and certain ethinicity[78]. Lee et al. estimated that the prevalence of GDM in Asia was 11.5%[79]. The consequences of GDM include the increased risks of PTB, operative delivery, foetal macrosomia as well as growth restriction, and polyhydramnios, as well as increasing the rate of PE[80-83]. Childhood obesity is also associated with GDM. Obesity is a growing health issue worldwide and it increases the chance of having diabetes. Early recognition and timely treatment are important for reducing the risks of adverse pregnancy outcomes such as macrosomia, large for gestational age, perinatal mortality, birth trauma and admission to neonatal special care unit. The prevalence of GDM in LMICs ranges between 0.4% and 17.4%[84].

1.7.3 Anaemia in pregnancy

Anaemia in pregnancy is a global health burden affecting both HICs and LMICs. In 2019, the global estimate of anaemia in pregnancy aged 15-49 was 36% and the highest rates of anaemia were observed in two regions: South Central Africa and South Asia[85]. The diagnosis of anaemia in pregnancy is defined as a Haemoglobin (Hb)

less than 11g/dl in first and third trimester, and less than 10.5 g/dl in second trimester [86].

The causes of anaemia in pregnancy are multifactorial and can be categorized into nutritional, physiological, infectious, and genetic factors. Nutritional anaemia is caused by deficiencies of vitamins, folic acid, and iron, which are essential for erythropoiesis and iron deficiency anaemia is the most common factor[87]. Physiological anaemia occurs especially during second trimester as a result of dilutional effect of increased plasma volume 40-50% and expansion of red blood cell up to 15-20%[88]. Malaria infection, which is the leading cause of anaemia in pregnancy in malaria endemic areas, and soil transmitted helminths are both common infections causing anaemia[89]. Additionally, genetic factors including malabsorption and haemoglobinopathies, such as sickle cell disease and thalassaemia, can also cause anaemia[90]. Anaemia in pregnancy is a preventable risk factor for bleeding after childbirth (also known as post-partum haemorrhage), which is one of the leading causes of maternal morbidity and mortality[91, 92]. It is also associated with adverse foetal and neonatal outcomes such as SGA, PTB and neonatal mortality[93].

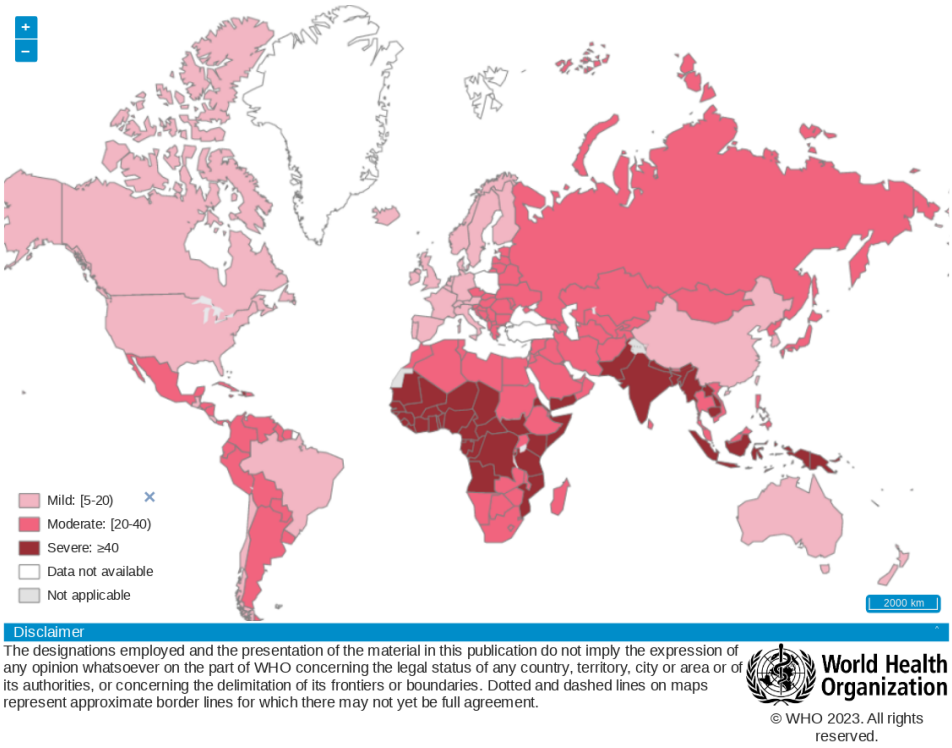


Figure 1-5: The global prevalence of anaemia in pregnancy (aged between 15-49). This Figure is retrieved from (https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-anaemia-in-pregnant-women-%28-%29).

1.7.4 Double burden of malnutrition (DBM)

Maternal nutrition is one of the important factors for foetal growth and development. The double burden of malnutrition (DBM) refers to the coexistence of high prevalence of underweight and overweight or obesity in the same population[94]. A high proportion of underweight women has been an important health concern in LMICs for decades. In the most recent decade, there has been a change in epidemiological patterns from underweight to overweight in LMICs due to urbanization, globalization, and changes in lifestyle and behavior[95]. DBM during pregnancy can lead to the

complications such as anaemia, gestational diabetes, PE, PTB and poor foetal growth, which are associated with morbidity and mortality for the mother and neonate.

Most multicentre pregnancy studies are based on WHO classification of body mass index with few utilising the Asia-Pacific recommended guidelines (**Table 1-2**). This is problematic for studies including Asian women: the 3-5% higher body fat in Asians (particularly South Asians) compared to Europeans, likely increases their risk or type 2 diabetes and cardiovascular disease.

Table 1-2: Body mass index (BMI) classification by WHO and Asia-Pacific guidelines[95].

	WHO BMI	Asia-Pacific BMI
Underweight	<18.5 kg/m^2	<18.5 kg/m^2
Normal weight	18.5-24.9 kg/m^2	18.5-22.9 kg/m^2
Overweight	25-29.9 kg/m^2	23-24.9 kg/m^2
Obesity	≥ 30 kg/m^2	≥ 25 kg/m^2

1.8 The additive effects of the infectious and non-communicable diseases (NCDs) on PTB, SGA and foetal growth

Studies from high income countries describe worse birth outcomes when pregnant women have co-existing hypertension and gestational diabetes[97, 98]. The PTB rate in women with both chronic hypertension and pre-gestational diabetes was 35.5% compared to 25.5% in women with chronic hypertension, and 19.4% in women with pre-gestational diabetes[99]. The rate of SGA was 18.2% in women with both versus

18.3% in women with chronic hypertension versus 9.7% in women with pre-gestational diabetes.

In resource limited setting, where the burden of infectious diseases is high and NCDs are expanding, there is a data gap on the interaction between these diseases and their impact on pregnancy outcomes. A recent study, investigating the association between malaria infection and HDP in approximately 23,000 singleton pregnancies presented in 1st trimester, found that the falciparum (but not vivax) malaria in first trimester was associated with PE/eclampsia in primigravidae (but not in multigravida) and PIH in multigravida (but not in primigravida)[100]. The effect of falciparum malaria appeared greater if the infection occurred early in pregnancy, but this was not conclusive. While other studies in marginalized population have described the association of malaria with PTB and small for gestation age, as well as miscarriage, stillbirth, neonatal and maternal death [51, 101-106], it is still not known if infectious disease such as malaria and NCDs such as HDP have an additive impact on PTB and SGA.

1.9 Study aims, objectives and rationale

1.9.1 Aims and objectives

This research aims to identify the changes over time of maternal characteristics and birth outcomes, modifiable risk factors, the determinants of PTB, SGA, and foetal growth among pregnant women, who are living on Thai-Myanmar border. The objectives are as follows:

Objective 1. Identify demographic, infectious, medical and obstetric factors associated with poor birth outcomes (Chapter3)

Objective 2. Explore the determinants of PTB and SGA in a 30-year cohort of refugee and migrant pregnant women (Chapter 4)

Objective 3. Identify demographic, infectious, and non-infectious factors associated with poor foetal growth (Chapter 5)

Objective 4. Explore options for interventions to optimize foetal growth in low resource settings. (Chapter 6)

1.9.2 Research rationale

The research rationale for investigating risk factors for PTB, SGA and foetal growth, specifically the preventable causes such as infection in tropical settings, is based on the significant burden and long-term consequences that it poses. The identification of risk factors is essential as it can potentially lead to treatment options or timely interventions to mitigate the negative impact on maternal and foetal health outcomes. By understanding the preventable risk factors and implementing measures to address them, it is possible to improve the health outcomes of pregnant women and their foetuses. Therefore, it is important to conduct research in this area to increase the awareness about the impact of preventable risk factors on PTB, SGA and foetal growth, and improve clinical management of pregnancies, particularly in tropical settings where the incidence of infection is higher. Ultimately, this research can contribute to the development of effective strategies to prevent impaired foetal growth and improve the health outcomes of mothers and their offspring. (**Figure 1-6**).

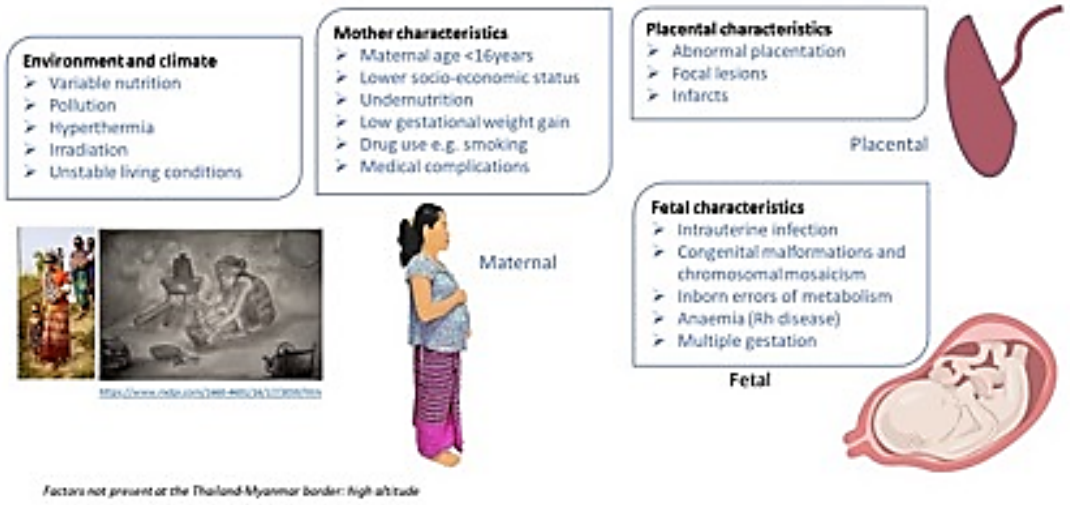


Figure 1-6: Causes of foetal growth restriction (FGR)/Intra-uterine growth restriction (IUGR)[106].

1.10 Chapter summary

The current literature highlights the substantial burden of adverse birth outcomes, including PTB and SGA, and their contributing factors such as infectious diseases and NCDs in LMICs, particularly in the regions of Africa and Asia. However, there is a significant gap in knowledge regarding the PTB, SGA, and foetal growth data from resource-limited settings, utilizing accurate gestational age assessment. The determinants of PTB, SGA and foetal growth have not been studied holistically in the Thai-Myanmar border population as yet. Therefore, this study aims to provide comprehensive information on the factors associated with PTB, SGA and foetal growth of the marginalized pregnant women. The findings of this study may have significant implications for improving maternal and child health outcomes in LMICs, particularly in the Thai-Myanmar border region.

Chapter 2

Population and Method

2.1 Introduction

This chapter provides information on the study sites, population and the methods used to collate data and explains the conduct of the three pregnancy clinical trials, which are analysed in Chapter 5.

2.2 Sources of data for this thesis

In this thesis, two different data sources were available to analyse. Firstly, in chapter 3 and 4, I used data from the entire unselected population of pregnant women attending routine antenatal care at Shoklo Malaria Research Unit (SMRU) clinics commencing in 1986 and delivered by 31-Dec-2020. Secondly, in chapter 5, I used pooled data from three registered prospective clinical trials[108, 109] embedded within the population cohort with more intense prospective investigation of foetal growth.

2.3 Study setting and population

2.3.1 Study setting

The 327 kilometres long Thai-Myanmar border is divided by the Moei river, covering nearly the entire length of the western boundary of Tak Province, Thailand and eastern boundary of Kayin state, Myanmar (Fig 2-2).

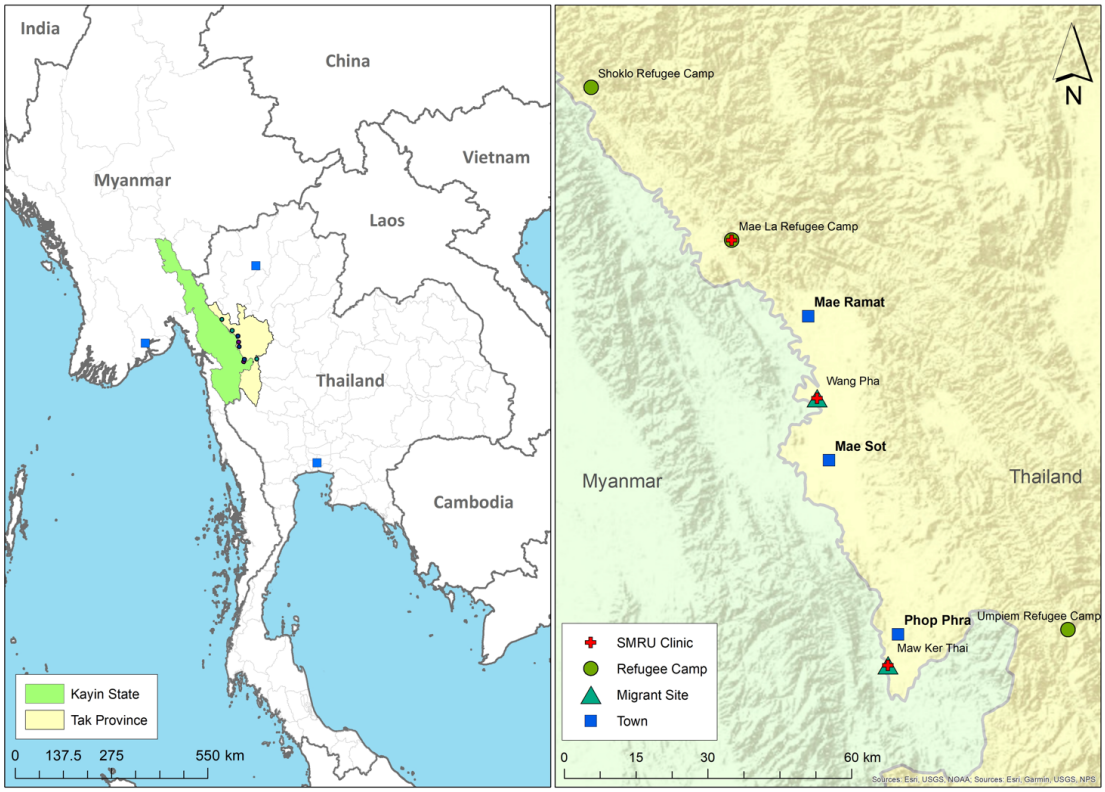


Figure 2-1:Kayin state and Tak Province at the Thailand Myanmar border.

In this area, there are citizens of Myanmar and Thailand as well as a sizeable, marginalized population of refugees and migrants, some of whom participated to these studies. Families may have members on both sides of the border and lives have adapted with decades of conflict between the Karen National Army and the Myanmar military government. As a result of prolonged civil war, hundreds of thousands of people in Kayin state have been forced to take the shelters in Thailand since 1980. Even though the nationwide cease fire agreement (NCA) was signed in 2016, health problems remain a significant challenge in the post-conflict and unstable border and rural settings that have purposively been deprived of Government support. There are significant barriers and challenges to meet the standard of sustainable development goals for Myanmar people including those who are undocumented in Thailand. On top of pre-existing

health problems, the COVID-19 pandemic and the most recent military *coup d'etat* in Myanmar on 1 Feb 2021, had a huge impact on the population, including those at the borderlands. For mother and child health, few quality healthcare options are available particularly in rural areas in the event of unexpected obstetric emergencies.

2.4 Study population

2.4.1 Refugee population

More than 90,000 refugees are currently living in 9 different refugee camps along Thai Myanmar border and Mae La refugee camp is the largest, which hosts 34,215 refugees according to United Nations High Commissioner for Refugees (UNHCR) latest report. Although a program for facilitating voluntary repatriation had been agreed between the Thailand and Myanmar governments in 2016, less than 1,000 refugees have returned to Myanmar. Refugees remain in the border camps, none of which have closed since the agreement was put in place but neither has the population number decreased. Life in refugee camps is hard after cuts to aid and as officially they are not allowed to take paid employment.

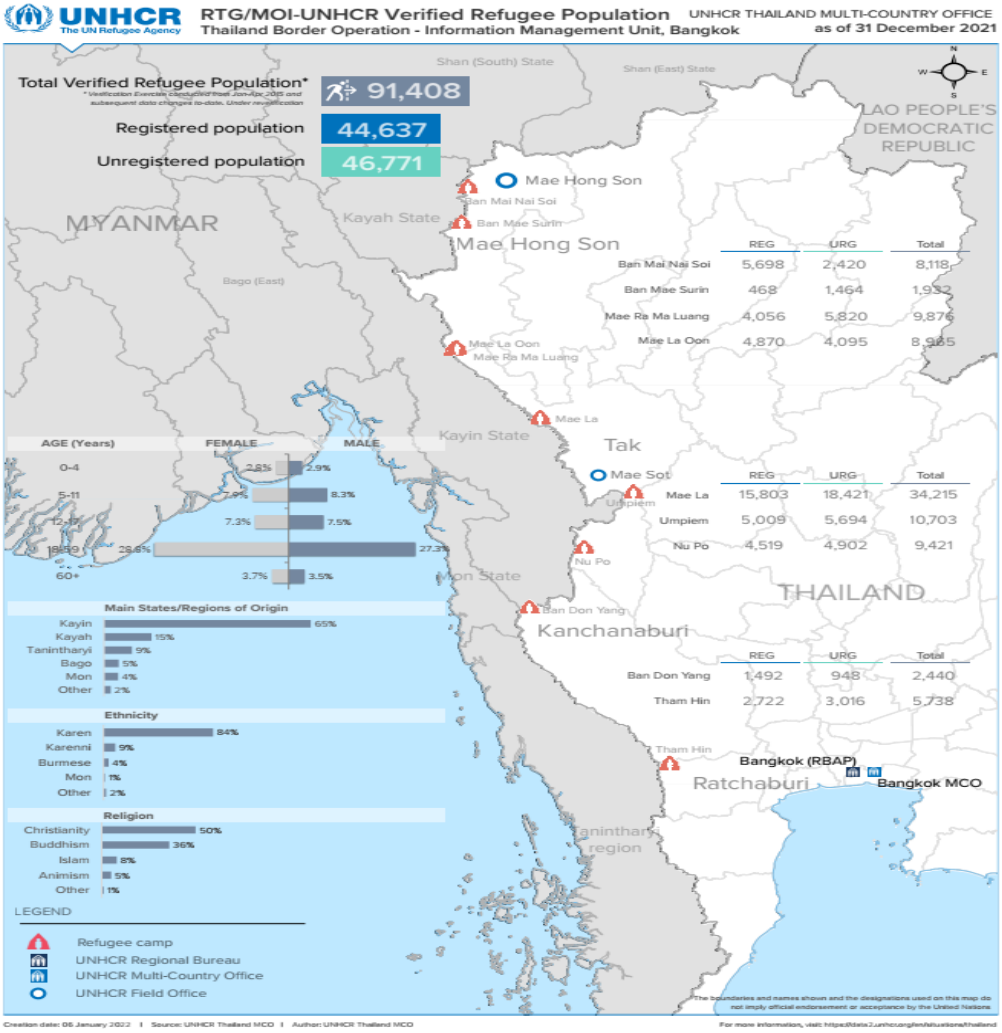


Figure 2-2:Map showing verified refugee population along Thai-Myanmar border.

2.4.2 Migrant population

The political instability and economic stagnation of Myanmar have driven millions of migrant workers to the neighbouring countries. It is estimated that there were 2.1 million Burmese migrant workers in Thailand in 2019, and Tak Province is home to an estimated 200,000 of these workers[110]. It is necessary for migrants to obtain a work permit prior to enrolling in the migrant health insurance scheme resulting in the

exclusion of undocumented migrants. Attempts to estimate the proportion of registered migrants suggest that the official counts represent a large underestimation of the population. Furthermore, 40-45% of migrants in Thailand are women and safe motherhood programs are required[110] but scarce. Most undocumented migrants are working in the agriculture sector in Tak province and depend on daily wages. Out of pocket expense was the only way to obtain medical care for undocumented migrants for many decades.

2.5 Shoklo Malaria Research Unit (SMRU)/ Borderland Health Foundation (BHF)

SMRU is primarily an academic research organization, which was established in Shoklo camp on the Thai Myanmar border since 1986. SMRU is a field station of the faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand and part of Mahidol Oxford Research Unit (MORU), which was located in Mae Sot city, Tak Province, north-western Thailand Myanmar border until 2022 and then moved to Mae Ramat district (fig. 2-3). SMRU has been consistently funded through the Wellcome Trust of UK, via the MORU with additional support from various sources such as the European Union, DFID, The Global Fund and the Bill & Melinda Gates Foundation.

The main objective of SMRU is to provide quality health care to the marginalized, vulnerable populations living on both sides of the Thai-Myanmar border by performing research activities and humanitarian services; infectious diseases such as malaria and tuberculosis and on maternal and child health (MCH). The clinical research findings have a direct benefit to the local population resulting in new or updated implementation of care and services. The research is shared to the world through publications in international scientific journals. SMRU publications can be assessed through the following website (www.shoklo-unit.com/research-activities/publications). SMRU has participated to WHO treatment guidelines with much of the current guideline based on the pioneering work on artemisinin derivatives conducted at the border.

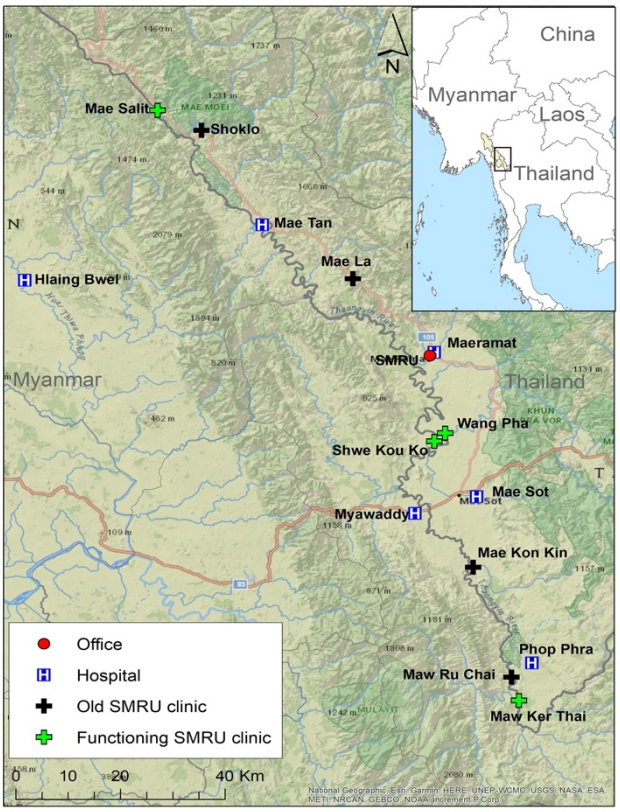


Figure 2-3:Map of SMRU office, old and new clinics and referral hospitals.

The Borderland Health Foundation (BHF) was established in December 2017 with the aim of scaling up humanitarian work, while SMRU mainly focuses on academic research. The primary objective of BHF is to promote public health among underprivileged populations by collaborating with Thai public health and other partner organizations such as Mae Tao Clinic (MTC) and Karen Department of Health and Welfare (KDHW). BHF focuses on the health projects addressing malaria, tuberculosis,

and maternal and child health (MCH). Additionally, the research findings conducted by SMRU will be translated and applied to the same population on a larger scale. More information is available on the BHF website (https://bhf-th.org).

2.5.1 SMRU clinics and Laboratory facility

The central SMRU office and laboratory facilities (microbiology, haematology, malaria microscopy and in-vitro) are in Mae Ramat district, which is 36 kilometre north of Mae Sot (Figure 2-1). SMRU clinics and field laboratories are located in rural and remote areas where most of the migrant population including pregnant women finding access to care difficult. The location of existing MCH SMRU clinics are as follows (Figure 2-3):

(1) Wang Pha clinic is located west of Mae Sot in Mae Ra Mat district and serves for the migrant population.

(2) Mawker Thai clinic is located on the South and 56 kilometres away from Mae Sot in Phop Phra district and serve for the migrant population.

(3) Mae La clinic was in the Maela refugee camp (between 1996 and 2016) and serves for the refugee population. The clinic shut down on end of 2016 and handed over to American Refugee Committee Thailand (ARC). Shoklo Refugee camp was amalgamated to Maela refugee camp in 1996 and care was provided by Médecins Sans Frontières (or *Doctors Without Borders* in English). Maela was initially a small refugee camp of approximately 4,000 people but due to cross border conflicts many small refugee camps were amalgamated to Maela starting in 1995 with Shoklo being the last to arrive in 1996, and eventually making it the largest refugee camp on the border.

(4) Mae Salit clinic is located near Klo Yaw Lay, Myanmar and SMRU collaborates with Karen Department of Health and Welfare (KDHW) and serving MCH for the population.

Multiple malaria posts (village tract health centres) in Myanmar have been set up since 2014 for the purpose of malaria elimination and the epidemiology of malaria infection at Thai-Myanmar border has significantly changed. For the duration of this thesis the malaria incidence and prevalence trends have been downwards as reported in the international literature[111].

2.5.2 SMRU staff and health trainings

Most of the clinical and laboratory care at SMRU is carried out by local healthcare workers under the supervision of expatriate doctors, who support the work of the medics (see below for the definition), nurses, midwifes (also known as skilled birth attendant), sonographers, counsellors, basic health workers and laboratory technicians.

The population has significant barriers to receiving formal education as they are undocumented in Thailand (either refugees or migrants). Becoming a certified healthcare professionals by formal medical training is challenging for local people as their schooling is not recognised by Myanmar or Thailand. However, local people who are interested to provide healthcare can become medics, nurses, midwives, sonographers or counsellors after completing an appropriate theory and practical training delivered by local NGOs or by SMRU-BHF. More information of their duties and training is as follows[112]:

Medics: A ‘medic’ takes the role of doctor by doing clinical examinations, ordering laboratory tests, making clinical diagnosis, prescribing treatment and management plan of the patients. They have normally received a minimum of 6–12 months of theory training and a further 6–12 months of on-the-job practical training[113, 114].

Nurse: A ‘nurse’ works in the same capacity as nurses in developed countries and have normally received 3–6 months of theory and 3–6 months of practical training.

Midwife: A ‘midwife’ (or skilled birth attendant) provides antenatal and delivery care services. SMRU developed its first curriculum in 1994, with the current course taking a minimum of 15 months training and a minimum requirement of 30 normal vaginal deliveries to become a midwife[115].

Sonographer: SMRU trains local staff in ultrasound gestational age assessment. They have the hardest entrance test compared to the other health staff and the quality of their work has been assessed in more than 4,000 scans[116]. Sonographers have a 16-week theory and practice course followed by 6 months of supervised internship.

Counsellor: A ‘counsellor’ provides antenatal care counselling particularly in explaining screening for pregnant women (blood tests, gender-based violence, mental health, nutrition).

Doctors: As SMRU is an international organization, the doctors working at SMRU, permanent or volunteer, are certified from different countries and the majority are from Myanmar. The doctors are clinically involved with daily patient management as well as supervising and training the clinic staff, seeking grants support and managing the health projects taking place at SMRU, and conducting clinical research.

On the Thai-Myanmar border, several community-based organizations (CBO) such as Mae Tao and non-government organizations (NGOs) such as Première Urgence - Aide Médicale Internationale (PU-AMI), International Rescue Committee (IRC), as well as SMRU have been providing health-related trainings for the local population. SMRU utilizes the established curriculums and aims to produce the competent healthcare providers after completion of trainings. American Academy of Family Physicians (AAFP) approved certificate in emergency obstetrics was established with the support of the Australasian branch of Advanced Life Support in Obstetrics (ALSO) in 2008. This emergency obstetrics training (ALSO) including theory and practical drills has been conducted approximately annually since its inception and requires updating every three years. All midwives and medics are required to take ALSO training. Evidently, the participants consistently receive high score on the practical component but achieving the high score to pass the theory examination is quite challenging for them largely due to language barriers as English is frequently a 3rd or 4th languge[112].

Figure 2-4:Guidelines used to standardize clinical practice on the Thailand-Myanmar border.

Part of maintaining systematic patient care and management has been through guidelines which SMRU has regularly updated for the border. These guidelines include

the SMRU malaria treatment guidelines, Burmese border guidelines (BBG), the SMRU Obstetric Manual, the SMRU Paediatric and Neonatal Guidelines and laboratory guidelines **(**Figure 2-4**)**. Staff are trained according to the guidelines, which are used in daily practice.

Continuous medical education and monitoring of SMRU staffs are regularly performed by the training department led by experienced medical doctors. Regarding clinical research, Good clinical practice (GCP) training including face-to-face and online training, has been provided to all SMRU staff and renewed as necessary.

2.5.3 SMRU Antenatal care (SMRU ANC)

SMRU antenatal care has offered basic, standardised care over the years with minimal variation in frequency of antenatal visit and laboratory testing for malaria and anaemia. **(**Figure 2-5**)**. The frequency of antenatal visits at SMRU was unique as every pregnant woman was previously encouraged to come the clinic to check malaria infection and anaemia, due to a profound malaria-related maternal mortality ratio of 1,000 per 100,000 live births before the introduction of weekly antenatal care visits[117]. This frequent number of ANC visits had been distinct from recommendation of World Health Organization (WHO) for control of malaria infection in pregnancy with insecticide treated bed nets and passive case management[118]. The rationale for the frequent visit policy adopted at SMRU was based on the life cycle of the *Plasmodium falciparum* parasite, which has an incubation period of 11-15 days[119], and SMRU has observed maternal deaths with women being absent for as little as one week.[120] After achieving malaria infection controlled in the non-pregnant population, the frequency of antenatal visits was decreased; firstly once a week to once every two weeks (2008), and

then to the current policy, which is aligned with WHO recommendation of a minimum eight ANC visits (2017). Currently, malaria infection screening is stopped if first three ANC visits indicate no malaria infection and only performed if pregnant women are symptomatic. Pregnant women detected with malaria infection at the first three visits are screened at every ANC visit until delivery due to the risk of recurrence[121].

HIV antibody screening provides a good opportunity to prevent the transmission of virus from mother to child and it was introduced at SMRU in 1998. HIV screening was initially done under an "opt-in" approach (Doctor or healthcare provider offered counselling and testing and it was only performed after getting informed consent). It was later changed to an "opt-out" screening approach where screenings are routine, but women still have the right to refuse because pregnant women in the community were not aware of the risks of HIV infection[122]. Syphilis (TPHA) and Hepatitis B (Hepatitis B surface antigen) testing was initiated in August 2012 and is also based on "opt-out" approach.

Blood pressure (BP) monitoring is an essential part of antenatal care in order to detect and prevent the complications of hypertensive disorders during pregnancy. Making a definite diagnosis of hypertensive disorders is achieved by taking two blood pressure readings 30-60 minutes apart after laying down for 5-10 minutes and checking urine for proteinuria. Screening for hypertensive disorders started since the beginning of ANC at SMRU.

At every antenatal visit, pregnant women are checked for maternal complications and foetal wellbeing including foetal growth. Maternal medical and obstetrics complications arising during pregnancy are managed by the teams of

midwives, medics, nurses and doctors and referred to either Thai or Myanmar hospital if required.

2.5.4 Antenatal ultrasound

Ultrasound dating scan is used to detect viability, number of foetuses, pregnancy location and get an estimated gestational age (EGA) if first ANC is between 9^{+0} and 13^{+6} weeks of gestation. A second ultrasound scan is scheduled between 18^{+0} and 23^{+6} weeks of gestation and confirmation of gestational age, foetal viability, placental localization and routine cervical length measurement were done by trans-abdominal scan. The ultrasound assessment of maternal and foetal health is in consistent with WHO recommendations[123]. Dubowitz gestational age assessment was done after delivery to estimate gestational age of new-born babies when ultrasound dating scan could not have been done before 24^{+0} weeks of gestation.

Antenatal ultrasound scanning was first introduced at SMRU in late 2001 using Dynamic imaging (2001), Fukuda Denshi UF 4100 (2002), Toshiba Power vision 700 (2006), Samsung SonoAce R3 (2012). Voluson GE 3D and Philip 3D. Sonographers are required to capture 5 ultrasound images of each foetal biometric: head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL) twice a year to maintain their skills and minimize the inter-observer variation[124]. Furthermore, SMRU obstetrics ultrasound manual and protocol are based on recommendations of British Medical Ultrasound Society[125], INTERGROWTH 21st and INTERBIO 21st ultrasound manual[126].

2.5.5 Delivery

SMRU aims to provide safe delivery for pregnant women. Therefore, normal deliveries are managed by trained midwives and obstetrics doctors, using a modified WHO partograph and high-risk pregnancies in labour are managed according to SMRU obstetric manual, with referral if required.

2.5.6 Referral system on the Thai-Myanmar border

There are approximately 2,000 deliveries annually, of which 6-7% are caesarean sections at the nearest hospital, mostly in Thailand but in the last decade also in Myanmar. Thai territory hospitals were the only referral option for emergency obstetrics patients between1986 and 2016 because border crossings in the daytime took time and it was impossible to cross the border at night. However, with improved collaboration of Thai and Myanmar health authorities before the coup, Myawaddy hospital became another option for high-risk pregnancy referral during the daytime. Each SMRU clinic has a 24-hour standby car to take the emergency obstetrics cases to the nearest hospital for proper management purposively to avoid to delayed referral. Myanmar migrant women in Thailand are required to pay hospital costs in full even in government hospital, while in Myanmar there are co-payments required alongside the public health care system; and these costs are normally incurred to humanitarian organizations working along the border and have been a significant burden for them.

There was a novel strategy launched in 2017 by DreamLopments, called The Migrant Fund (M-FUND https://www.dreamlopments.com/) program to support migrant heath care for those can’t afford to pay hospital costs. After making affordable monthly payment to M-fund, migrant people can get quality healthcare from either Thai

or Myanmar hospital without additional payment. SMRU also provides referral coordinators at the hospitals on both side of border to overcome the language barrier and help the patients with administrative issues.

Staff are supervised by doctors, who are available in person for consultations during office hours, and by phone (1986-1994 by radio advice) beyond office hours. If needed, Doctors will spend the night in the clinics to manage the high-risk patients. Sites for antenatal care were higher in number than sites for safe delivery with trained skilled birth attendants (**Table 2-1**).

2.5.7 Post-natal care

The complications of new-born babies such as neonatal jaundice, neonatal sepsis, transient tachypnoea of new-born (TTN) and feeding difficulties are managed following SMRU neonatal guidelines in special care baby unit (SCBU). SMRU established SCBU in Maela refugee camp since 2008, in Wang Pha clinic since 2009 and in Maw Ker Thai since 2010. The quality of health care by staff (medic, nurse, midwife) has been assessed regularly[122, 127, 128]. SMRU extended services to include provision of contraceptives including short- and long-term contraceptive methods. These are available at the clinics after proper counseling[129, 130].

Table 2-1: Sites of refugee camp and migrant antenatal care and birth rooms

	Country	Timeframe	Maximum population	Antenatal care	Labour rooms
Shoklo camp	Thailand	1986 – 1995 (camp closure, amalgamated to Maela)	9,000	Weekly	24 hours per day, 365 days per year
Bonoklo camp	Thailand	1986 – 1995 (camp closure, amalgamated to Maela)	5,000	Weekly	24 hours per day, 365 days per year
Mae Salit Gray Tah camp	Thailand	1986 – 1995 (camp closure, amalgamated to Maela)	3,000	Weekly	Home birth, obstetric complications referred to Shoklo clinic
Htee Wah Kee (Klee Maw Klu) camp	Myanmar	1994-1995	2,500	Weekly	Home birth, obstetric complications referred to Shoklo clinic
Maela camp	Thailand	1995-2016	40,000	5 days/week	From 1995 - 2016
Mae Salit	Myanmar	2017-current	2,000	5 days/week	Jan 2017-current
Maw Ker Thai (MKT)	Thailand	1998-current	unknown	5 days/week	Dec 2007-current
Wang Pha (WPA)	Thailand	1999-current	unknown	5 days/week	April 2010-current

2.5.8 Data management of the ANC routinely collected clinical data.

SMRU switched from a paper-based records system, which was originally developed by the non-profit organization called Technologies Sans Frontières, to computer-based patient record system in 2008. Real time data entry is performed by clinic staff and it can prevent the data loss, which could occur with paper records, due to floods or fire. ANC paper records have been entered into electronic database and remained in SMRU archives. If data queries or clarifications are required, they can be verified from the paper-based records **(Figure. 2-6).**

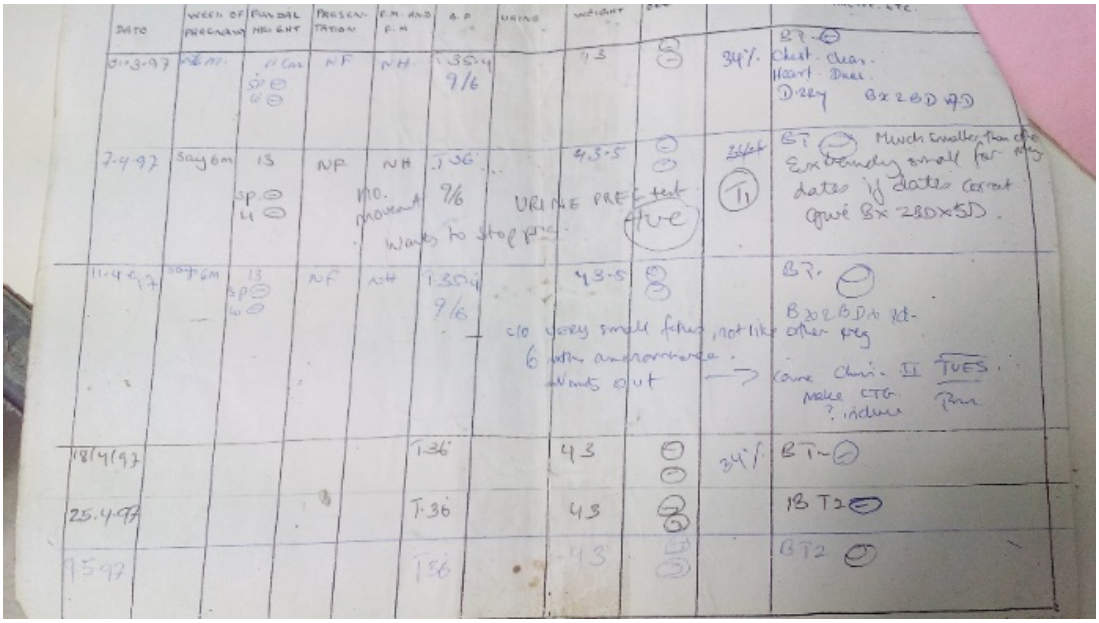


Figure 2-5:Example of antenatal records from 1986.

Figure 2-6: SMRU team working together for data verification of paper-based records.

2.6 Description of non-study cohort data in SMRU.

Archived data of pregnant women including demographic, maternal characteristics, obstetrics and medical factors, and birth outcomes from 1986 to Dec 2020 were included. Variables available for data analysis were mentioned in **Table 2-2**. The anonymized data was used for analysis.

Table 2-2: Definitions and categories of variables.

Variables	Definition	Type	Categories or units of measurement
Demographic factors			
Maternal age	Age of the mother in years, at first ANC visit.	Continuous	Age in years; also categorized <20 (teenagers), 20-34 and ≥35 years or in combination with gravidity
Ethnicity	Self-nominated ethnicity	Categorical	Karen, Burmese, Burmese Muslim, Other
Living Status	The location of the clinic attended: either in a refugee camp, as a visitor to the refugee camp, or a migrant community.	Categorical	Refugee, Migrant
Literacy	Self-reported literacy (regardless of language/s), with best reading language reported. *Note* this measure of literacy is highly specific (95.9%) but has a low sensitivity (56.2%) for identifying low health literacy in this population	Categorical	Karen, Burmese, English or Thai)
Lifestyle factors			
Smoker	Currently smoking status at the first antenatal consultation, and routinely collected from 1997.	Categorical	Yes; No; self-reported

Weight	Weight measured at first and subsequent ANC visit by mechanical Salter scales with 0.5 kg precision.	Continuous	kg
Height	Height measured at first ANC using a mechanical stadiometer with 5 mm precision until 2010 when these were replaced with electronic stadiometers with 1 mm precision.	Continuous	cm
Body mass index	First ANC weight (kg) / height^2 (meters)	Continuous	kg/m^2
BMI category	Categories in the literature vary with recommended categories for Asians set at lower BMI than other populations.	Categorical	Underweight (<18.5), Normal (18.5-23.5), Overweight (>23.5-27.5), Obesity (>27.5)
Obstetric factors			
Parity	Number of pregnancies resulting in live or still birth (≥28weeks gestation) and excluding miscarriage	Categorical	Nulliparous, Multiparous
Gravidity	Number of pregnancies a woman has had, including the current pregnancy.	Categorical	Primigravida, Multigravida
Miscarriage	Pregnancy loss prior to 28 weeks' gestation	Categorical	Previous miscarriage (in multigravida)
Medical factors			

Malaria first ANC	Presence of asexual parasites in the peripheral blood (per 500 leukocytes or 1000 erythrocytes) detected by microscopy. For each malaria episode, additional variables were collected: date; antimalarial treatment; plasmodium species; symptoms (fever or history of fever in the past 48 hours).	Categorical	Yes; No
Malaria in pregnancy	As above, measured weekly up to 1998, then changed to biweekly	Categorical	Yes; No
Non-malaria infection	Suspected bacterial infection is defined as the presence of febrile illness and receiving the antibiotics.	Categorical	No fever and antibiotics, suspected bacterial infection and suspected virus infection
Anaemia first ANC	Anaemia determined by haematocrit: ratio of the volume of red blood cells to the total blood volume; with defined as <30%; measured fortnightly.	Categorical	Yes; No
Anaemia in pregnancy	As for Anaemia first ANC	Categorical	Yes; No
Hypertension	Hypertension was two confirmed episodes of high blood pressure > 6 h apart with systolic BP ≥ 140 or diastolic	Categorical	Yes; No

Gestational hypertension	Gestational hypertension was new hypertension that occurred after 20 weeks' gestation with no/trace of proteinuria.	Categorical	Yes; No
Chronic hypertension	Chronic hypertension was hypertension by medical history or documented before 20 weeks' gestation.	Categorical	Yes; No
Pre-eclampsia	Pre-eclampsia was new onset hypertension that occurred after 20 weeks' gestation with proteinuria (≥1+ on a dipstick), without alternative explanation. For women with a history of chronic hypertension, the onset of new proteinuria was after 20 weeks' gestation.	Categorical	Yes; No
Eclampsia	Eclampsia was observed generalized convulsion (i.e., "fitting") in a pregnant woman without other clear explanation, with a record of proteinuria or hypertension.	Categorical	Yes; No
Hypertensive disorder of pregnancy	Hypertensive disorder of pregnancy was a combined outcome of gestational hypertension, pre-eclampsia, or eclampsia	Categorical	Eclampsia and/ or pre-eclampsia, pregnancy induced hypertension, chronic hypertension; no known or

			measured hypertension
HIV, Hepatitis B, Syphilis	Confirmed laboratory evidence of infection	Categorical	Yes; No
Delivery factors			
Place of birth	Place of childbirth	Categorical	Home, SMRU (or on the way to SMRU), Hospital (Thai or Myanmar)
Pregnancy outcome known	Final outcome of pregnancy known (miscarriage, delivered, maternal death undelivered) or unknown (lost to follow-up)	Categorical	Yes; No
Miscarriage (if outcome known)	Binary variable for embryo or foetal death before 28 weeks' gestation.	Categorical	Yes; No
Singleton birth	Only one neonate at childbirth	Categorical	Yes; No
Stillbirth	Binary variable for foetal death after 28 weeks' gestation (stillbirth), either in utero (antepartum stillbirth) or during labour (intrapartum stillbirth).	Categorical	Yes; No
Birthweight measured in 72 hours	Clinic born infants weighed in 30 minutes but infants born at home present late (included are those within 72hours)	Categorical	Yes; No
Birth weight	Birthweight of the new-born (grams), using electronic SECA medical scales (precision 10 grams, calibrated fortnightly).	Continuous	Gram

Gestational age at birth	The best estimate of gestational age based on the following algorithm 1) Ultrasound <24 weeks 2) Dubowitz examination 3) Fundal height formula 4) LMP. NB: The gestational age at the time of any event during pregnancy (e.g., the first antenatal consultation, or a malaria episode) is back-calculated using the estimated gestational age at birth, the date of birth and the date of the event.	Continuous	length of pregnancy at birth in days
Method of gestational age assessment	Method of estimating gestational age	Categorical	Ultrasound, Dubowitz Assessment, Fundal height or last menstrual period.
Preterm birth	Birth before 37 weeks' gestational age	Categorical	Yes; No; also early preterm birth, late preterm birth, term and post-term
Small for gestational age (SGA)	Birthweight-for-gestational-age <10th centile, determined from international standards (INTERGROWTH-21st) and local standards.	Categorical	<10th centile Intergrowth-21 international standards
Neonatal death (NND)	Death of a liveborn infant in the first 28 days of life	Categorical	Yes; No

Normal new-born	Without congenital malformations based on surface examination and heart auscultation: coded using the ICD-10	Categorical	Yes; No

2.7 Description of study cohort data in SMRU.

Three prospective, observational pregnancy cohort studies[109, 131, 132] with similar eligibility criteria provided the basis of the foetal growth data (**Table 2-3**). Inclusion criteria that were similar in each prospective study cohort were: more than 18 years of age, singleton pregnancy, gestational age assessment by first trimester dating scan, willingness to participate in the study, can attend every 4-6 weeks follow up for foetal growth assessment plus foetal wellbeing. The INTERBIO-21st study was an international multi-centre observational cohort study, and SMRU participated as one of the study sites. The other two studies were initiated by SMRU. The information collected in each study is listed in **Table 2-4**, which is similar to those collected routinely, but ultrasound scan was more frequently and more strictly quality controlled.

Table 2-3:Profile of three observational pregnancy cohorts

	Observational cohorts		
	Impact of malaria infection in pregnancy on foetal and new-born growth (UPS)	**INTERBIO 21st (foetal study)**	**Molecular signature in Pregnancy (MSP)**
Objective	Impact of malaria infection on foetal growth	To define the phenotype of PTB and SGA	To predict PTB using the molecular signature
Field researcher	Marcus Rijken	Aung Myat Min	Tobias Brummaier
Date of enrolment	February 2009 to May 2011	January 2012 to December 2013	September 2016 to July 2018
Population	Migrant (100%)	Migrant (63%) and refugee (37%)	Migrant (100%)
Protocol sample size	400	500	400
Sample size Enrolled	396	578	412
Premature discontinuation	Lost to follow-up (N=28)	Lost to follow-up (N=38)	Lost to follow-up (N=21)
	NA	Miscarried (N=3)	Miscarried (N=5)
Delivered	368	537	386
Inclusion	GA 9+0 to 13+6 weeks	GA 9+0 to 13+6 weeks	GA 8+0 to <14+0 weeks
	≥18 years, Singleton, Viable foetus	≥18 years, Singleton, Viable foetus, BMI< 35	18-49 years, Singleton, Viable foetus
Foetal growth monitoring	Every 5 weeks	Every 5 weeks	Every 6 weeks

Ultrasound Machines used for scanning	General Electric Voluson i (GE Healthcare, Austria) with a RAB2-5-RS, 2–5 MHz real-time 4D probe	General Electric Voluson i (GE Healthcare, Austria) with a RAB2-5-RS, 2–5 MHz real-time 4D probe Philips HD-9, Philips Ultrasound, USA with curvilinear abdominal transducers C5-2, C6-3, V7-3	General Electric Voluson i (GE Healthcare, Austria) with a RAB2-5-RS, 2–5 MHz real-time 4D probe
Infants follow up	1 year	2 years	2 years

Table 2-4: Data collected in the longitudinal pregnancy cohorts.

		UPS	IB 21st (foetal study)	MSP
Maternal characteristics	Gravidity and parity, age, status (refugee and migrant), smoking, literacy	√	√	√
Obstetric risk factors	History of previous (pregnancy loss (abortion, stillbirth), caesarean section, preterm labour, abnormal placentation, still birth)	√	√	√
Nutrition	At enrolment - BMI, maternal height, and weight	√	√	√
Medical morbidities	Anaemia (iron deficiency, inherited red cell disorders (thalassaemia and G6PD deficiency, response to haematinics (prophylactic or treatment)), chronic hypertension, hypertensive disorders of pregnancy, gestational diabetes, asthma, epilepsy,	√	√	√
Infection	Malaria (active surveillance), HIV (active surveillance), tuberculosis (passive surveillance), sexually transmitted infections e.g., syphilis (active), urinary tract infection, respiratory tract infection, dengue fever.	√	√	√
Foetal growth scan (4-6 weekly)	Head circumference, abdomen circumference, estimated foetal weight, dopplers, AFI	√	√	√

Foetus at birth	Placenta, live born, stillborn (antepartum or intrapartum), sex, gestation, birth weight, congenital abnormality	√	√	√
Labour	Induction, augmentation, partogram, length ROM (spontaneous, artificial), PPH, perineal, length 2nd stage, place of birth (skilled birth attendant or homebirth)	√	√	√
Socio-economic status	Ethnicity, religion, education, number of households, employment, household income, ownership e.g., livestock, material goods e.g., motorbike	√	√	√
Neonatal	Alive or dead, birth weight, APGAR score	√	√	√
Infant	Alive or dead, Weight, Height, infection, anaemia	√	√	√

2.8 Data management for analyses

An ACCESS database was used to extract data for analyses in Chapters 3 and 4. All routinely collected variables were amalgamated over the years into the ACCESS file and new data from the ANC platform is appended to the ACCESS file on a regular basis. In Chapter 5, the electronic ANC database was merged with the combined dataset of the three prospective pregnancy studies. The data of three ultrasound studies were stored separately and I have standardized them and merged them together. The data of INTERGROWTH-21st foetal study collected at SMRU were electronically entered and cleaned by the INTERGROWTH-21st team at the Department of Obstetrics and

Gynaecology, University of Oxford and they provided the clean dataset for this thesis. For UPS and MSP studies, I used the raw data. The analysis dataset does not include any confidential information. The analysis dataset was coded: and will only be traceable using the ID number with a linkage file, which is stored separately.

Stata 15.1 SE for Mac (StataCorp, USA) was mainly used for data management, statistical analyses and producing the Figures. Different statistical methods were used for each research question and the detailed methodology described in each chapter.

2.9 Ethics approval

For study cohort data, ethical approval has been obtained from Oxford Tropical Research Ethics Committee (OxTREC) and Ethical committee of Faculty of Tropical Medicine, Mahidol university (FT-MEC). Ethical approval letters for each study cohort are shown in the appendix.

For retrospective analysis of non-study cohort data (ANC records), ethical approval was granted by OxTREC (reference: OXTREC-29:08) and Tak Community Advisory Board (TCAB).

Chapter 3

Maternal and newborn characteristics including preterm birth and small for gestational age in marginalized pregnant women on the Thailand-Myanmar border: SMRU birth cohort profile, 1986-2020

3.1 Chapter overview

This chapter provides a detailed cohort profile of marginalized pregnant women who received antenatal or safe delivery care at Shoklo Malaria Research Unit (SMRU) clinics, including maternal characteristics, morbidity during pregnancy, pregnancy outcomes plus newborn characteristics, and delivery factors over a 34-year period. In addition, this chapter also presents the changes in the population over the study period, such as teenage pregnancy, under- and over-weight pregnant women, smoking during pregnancy, malaria infection in pregnancy, pre-eclampsia (PE), and maternal anaemia, as well as pregnancy outcomes including preterm birth (PTB) and small for gestational age (SGA).

3.2 Background

Pregnancy is a normal, healthy state for both the mother and the foetus, during which maternal physiological changes occur[46], and foetal growth and development commence with first-trimester organogenesis, progressing throughout the pregnancy[133]. Achieving a healthy pregnancy means that a healthy mother gives birth to a normal birthweight baby with no congenital abnormality at term; between 37^{+0} and 41^{+6} weeks' gestation. Any exposures/risks during the antenatal period (smoking, undernutrition,

chemical and radiation exposures, medical and obstetrics complications) may affect maternal, foetal, neonatal and childhood outcomes[133].

Birth cohort studies are the most appropriate method to examine causal relationships between potential maternal exposure/risk factors and pregnancy outcomes. Furthermore, birth cohort studies allow examination of the long-term consequences of exposures to the newborn, up to childhood and potentially adulthood, as poor foetal growth can lead to the occurrence of metabolic diseases in adult life[134]. Good examples of birth cohorts are the Danish national Birth Cohort and the Norwegian Mother and Child Cohort study, which are the largest pregnancy cohorts containing 100,000 pregnancies each, in Europe[135, 136]. Some of the important findings from these two cohorts include: folate supplementation may prevent autism[137], mobile phone use in pregnancy is not associated with child development[138], maternal low iodine intake increased the risk of delayed development[139]. Although there are many birth cohorts from high income countries (HICs), not all findings from HICs may be directly applicable or at all feasible for low- and middle-income countries (LMICs). This is because the exposure/risk factors during pregnancy (maternal characteristics, socioeconomic status, chemical exposure, infectious and non-infectious diseases) may not be the same between HICs and LMICs[140]. To date, there are 23 birth cohorts in Asia (known as Birth Cohort Consortium of Asia) and the majority are from HICs[141] (**Table 3-1**). Birth cohort studies require a considerable amount of time and resources (including administration, trained healthcare workers, data management, and finance) to establish[134]. There are studies from HICs reporting on pregnancy outcomes of

resettled refugee and migrant populations, but studies from the original areas of these populations are scarce[142-145].

The objective of this chapter is to provide a comprehensive birth cohort profile of the marginalized population attending SMRU clinics and to investigate trends in maternal factors that are known to contribute significantly to PTB and SGA.

Table 3-1: Birth Cohort Consortium of Asia (BiCCA) [141].

Country	Birth Cohort	Enrolment period	No. of Children enrolled	No. of Mothers enrolled
China	Laizhou Wan Birth Cohort (LWBC)	2010-2013	773	773
	Nanjing Medical University Birth Cohort (NJMUBC)	2014-2016	26,000	30,000
	Shanghai Birth Cohort (SBC)	2013-2015	3,000	4,000
Japan	Hamamatsu Birth Cohort	2007-2011	1,258	1,138
	Hokkaido Cohort	2003-2013	20,818	20,929
	Sapporo Cohort	2002-2005	504	514
	The Tohoku study of child development (TSCD)	2001-2006	1,348	1,323
Korea	Children's health and environmental chemicals in Korea (CHECK)	2011-2013	352	352
	Childhood origin of Asthma and allergic diseases (COCCA)	2007-2016	2,400	2,400
	Environment and development of children study (EDC)	2008-2014	698	698

	The mothers and children's environmental health study (MOCEH)	2006-2010	1,751	1,751
	Panel study on Korean children (PSKC)	2008	2,150	2,150
Malaysia	University Sains Malaysia (USM)	2010-2011	159	188
Nepal	Nepali birth cohort study in Chitwan valley	2008 September-October	100	100
Philippines	Cebu longitudinal health and nutritional survey (CLHNS)	1983-1984	3,080	3,327
Singapore	Growing up in Singapore towards healthy outcomes (GUSTO)	2009-2010	1,190	1,247
Sri Lanka	Kalutara children's health study (KCHS)	2014-2015	450	450
Taiwan	Taiwan birth panel study (TBPS)	2004-2005	486	486
	Taiwan early-life Cohort (TEC)	2001-2005	1,589	1,589
	Taiwan maternal and infant cohort (TMICS)	2000-2014	1,616	2,577
Vietnam	BienHoa Dioxin Cohort study	2012 September-November	200	200
	Dioxin and development of children in Vietnam (DaD0CiV)	2008-2013	200	200
	DaNang Dioxin Cohort study	2008-2010	241	241

3.3 Methods

3.3.1 Study setting, design and participants

This is a retrospective analysis of de-identified data extracted from antenatal care records (paper archives) and an electronic database of the Shoklo Malaria Research Unit (SMRU). No study sample size was preselected – all women who registered to antenatal care at SMRU were included. Originally, Shoklo and later Maela refugee camps have remained the most heavily populated camps on the western border of Thailand, although in the early years there were up to 20 refugee settlements. Provision of maternal and child health services, and data collection started in 1986 in refugee and 1998 in migrant populations. More detailed information about the study setting, population, data collection and acquisition were previously given in Chapter 2.

3.3.2 Terminology and definitions

Pregnancy outcome is a broad terminology describing maternal, foetal and neonatal health status. In this chapter, it is frequently used to encompass maternal and neonatal mortality, miscarriage/pregnancy loss, PTB, SGA, stillbirth and neonatal death (NND). Maternal characteristics, obstetric and medical information and pregnancy outcomes of all pregnant women who attended SMRU clinics were prospectively collected.

The routinely collected data included maternal age, gravidity, estimated gestational age (EGA) at 1st ANC visit, weight and height at 1st ANC visit, body mass index (BMI), living status (refugee or migrant), ethnicity, total number of ANC visits, literacy (can read or not), smoking during pregnancy, malaria infection in pregnancy, maternal anaemia at 1st ANC visit, pre-eclampsia/eclampsia during pregnancy, EGA at

birth, mode of delivery, place of delivery, foetal sex, birthweight and adverse pregnancy outcomes such as PTB, SGA, stillbirth and neonatal death. Detailed information regarding the assessment and definitions of maternal characteristics and pregnancy outcomes were described in methodology chapter 2 (**Table 2-3**).

Although the definition of pre-eclampsia (PE) has been modified in recent years, the previous definition of PE has been used throughout this analysis for all study years to ensure consistency. PE is defined as the new onset of hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) and the presence of proteinuria (≥1+ on a dipstick), with or without pitting oedema after 20 weeks' gestation. For women with a history of chronic hypertension, the onset of new proteinuria is after 20 weeks' gestation. The severity of PE has not been considered.

Maternal death is defined as "any deaths related to or aggravated by pregnancy or its management (excluding accidental or incidental causes) during pregnancy and 42 days after childbirth, irrespective of duration and site of pregnancy, expressed per 100,000 livebirths for a specific time period"[146]. It is normally expressed as Maternal Mortality Ratio and calculated as: Maternal Mortality Ratio (MMR) = number of maternal deaths (recorded or estimated)/ number of livebirths multiplied by 100,000[146].

Neonatal death is defined as "deaths among live births, regardless of gestational age at birth, during the first 28 completed days of life and it is measured as number of deaths during the first 28 completed days of life per 1,000 live births in a given year"[147] and calculation was done as following: Neonatal Mortality Rate (NMR) = number of neonatal death/number of live births multiplied by 1,000[147]. Despite the use of various lower gestational age cut-offs for assessing the viability and survival of newborn babies

across different countries, this study employs a 28-week gestation cut-off enabling the calculation of neonatal mortality rates for international comparisons. Sex ratio at birth is the number of male live births divided by number of female live births (for the same geography and year) multiplied by 100[148].

3.3.3 Statistical analysis

This retrospective data analysis describes the cohort profile including maternal characteristics, morbidity during pregnancy, and pregnancy outcomes such as PTB, SGA, stillbirth, and neonatal death during the study period between 1986 and 2020. Maternal characteristics, morbidity during pregnancy and pregnancy outcomes were presented for the refugee and migrant populations. The prevalence of maternal factors and risk of pregnancy outcomes of the whole study population (refugee and migrant) such as teenage pregnancy, low maternal weight (<40kg), under-and over-weight pregnancy, smoking during pregnancy, malaria infection in pregnancy, PE, PTB and SGA were calculated for each year. To assess whether there were any linear temporal trends in these maternal factors and their effect on pregnancy outcomes over 1986-2020, we performed a variance-weighted least-squares regression (VWLS). Compared to traditional least-squares regression, which assumes that all data points have equal variance, VWLS weights the data points according to their estimated variance, allowing for a more accurate estimation of trends over time, particularly when the data exhibit heteroscedasticity or unequal variance[149]. In this analysis, the weights were the number of pregnant women attending the SMRU clinics each year as the number varied from year to year over the study period.

Additionally, calendar year was categorized into three groups, 1986-2000, 2001-2010 and 2011-2020 to describe the MMR and NMR as these periods of time represent the improved maternal and newborn care over the time (in addition to the temporal trend). A modified definition of NMR (Singleton pregnancies with babies born alive and EGA $\geq 28^{+0}$ were included) was used for this cohort. In order to take account for lost-to-follow-up before the first 28 completed days of life, the Kaplan Meier method was used to estimate NMR with the assumption that the risk of neonatal mortality was the same for followed babies and lost-to-follow-up babies.

For PTB and SGA, the analysis was restricted to singleton pregnancies with known pregnancy outcomes; the babies born between 28^{+0} and 42^{+0} weeks' gestation. Only newborns with a birthweight measurement within 72 hours of birth were included in calculation of SGA.

IBM SPSS Statistics 25 and Stata 15.1 SE for Mac were used to perform the statistical analyses.

3.3.4 Ethics approval

Ethics approval for accessing and analysing the data of the SMRU birth cohort study was obtained from Oxford Tropical Research Ethics Committee (reference: OXTREC 28–09) and Tak Community Advisory Board (reference: TCAB 202002).

3.4 Results

3.4.1 Description of SMRU birth cohort

Table 3-2 describes the SMRU birth cohort profile between 1986-2020. A total 82,030 ANC records were available for analysis, of which 45,391 (55.3%) and 36,639

(44.7%) were from refugee and migrant populations, respectively. Maternal demographics and pregnancy outcomes in both populations were similar and clinically insignificant. The majority of ethnic groups in both populations were Karen and Burmese. Median [inter-quartile range (IQR)] maternal weight and height were slightly lower in the refugee population, 48 kg [43-53] and 150 cm [147-154], than in migrants, 49 kg [45-55] and 152 cm [148-155], respectively. Smoking during pregnancy was more common in refugee (27.1%, 8,679/31,998) compared to migrant women (18.1%, 6,627/36,523). Overall, 10-15% of both populations had malaria (12.6% of refugee women, 5,709/45,391 and 14.1% of migrant women, 5,171/36,639) with a much smaller proportion with suspected febrile bacterial infections (2.7%, 359/13,259 and 2.7%, 724/27,189).

More than 60% of pregnant women from both populations had a normal vaginal delivery at SMRU clinics with skilled attendance at birth. Notably, the caesarean section rate in both these marginalized populations was low at approximately 6%. There was no meaningful difference between two populations and further analysis includes combined two population as one.

The overall MMR between 1986-2020 was 179 (95%CI 162-196) but decreasing MMR can be seen during the last decade (2011-2020) and similarly, the overall NMR was 25 (95%CI 23-27) and NMR decreased over time (**Table 3-3**)

Table 3-2: Profile of SMRU birth cohort by refugee and migrant between 1986-2020.

	Refugee (N=45,391)	**Migrant (N=36,639)**
Maternal characteristics		
*Age (years), median [IQR]	25 [21-31]	25 [21-31]
*Age category, years		
< 20	7,535 (16.6)	6,079 (16.6)
20 to 34	31,212 (68.9)	24,822 (67.8)
≥ 35	6,538 (14.5)	5,719 (15.6)
Primigravida	11,582 (25.5)	11,235 (30.7)
Inadequate number of ANC visits (< 8)	15,815 (34.8)	19,727 (53.8)
*ANC booking at 1st trimester	22,443 (49.4)	14,717 (40.2)
*Ethnicity		
Karen	16,878/20,040 (84.2)	13,204/25,028 (52.8)
Burmese	666/20,040 (3.3)	10,061/25028 (40.2)
Others	2,496/20,040 (12.5)	1,763/25028 (7.0)
*Smoker	8,679/31,998 (27.1)	6,627/36,523 (18.1)
*Literate (can read)	6,946/11,168 (62.2)	15,444/25,683 (60.1)
*Maternal weight (kg), median [IQR]	48 [43-53]	49 [45-55]
*Height (cm), median [IQR]	150 [147-154]	152 [148-155]
*Maternal weight (<40 kg)	3,227 (8.8)	4,421 (9.9)
*BMI in kg/m^2		
Underweight (< 18.5)	2,363/22,521 (10.5)	4,540/33,812 (13.4)
Normal weight (18.5-22.9)	19,344/22,521 (57.2)	12,914/33,812 (57.2)
Overweight (≥ 23)	7,244/22,521 (32.2)	9,928/33,812 (29.4)
Gestational age assessment method		
Ultrasound	22,108 (50.2)	29,683 (81.6)
Fundal height	8,478 (19.3)	1,118 (3.1)
Dubowitz	8,937 (20.3)	4,225 (11.6)
LMP	4,485 (10.2)	1,354 (3.7)

Morbidity during pregnancy		
Malaria infection in pregnancy	5,709 (12.6)	5,171 (14.1)
*Non-malaria febrile illness		
Suspected bacterial infection	359/13,259 (2.7)	724/27,189 (2.7)
Suspected viral infection	413/13,259 (3.1)	545/27,189 (2.0)
*HIV infection	11/6,057 (0.2)	114/23,716 (0.5)
*Syphilis	20/6,346 (0.3)	173/18,979 (0.9)
*Maternal anaemia	4,850/37,154 (13.1)	3,774/36,440 (10.4)
Pre-eclampsia	594 (1.3)	596 (1.6)
Pregnancy outcomes		
*EGA at birth (weeks), median [IQR]	38 [28-40]	38 [31-39]
*Birth weight (gram), mean (SD)	2,942 (486)	2,943 (472)
*Foetal sex (Male)	18,730/35,895 (52.2)	11,959/23,240 (51.5)
*Type of outcome		
Miscarriage	4,266/41,086 (10.4)	2,334/25,659 (9.1)
Singleton pregnancy	36,435/41,086 (88.7)	23,006/25,659 (89.7)
Multiple pregnancy	385/41,086 (0.9)	319/25,659 (1.2)
[a]PTB	1,802/22,676 (8.0)	3,199/31,858 (10.1)
[b]SGA	6,896/29,610 (23.3)	4,217/17,973 (23.3)
*Stillbirth	540/36,688 (1.5)	273/23,258 (1.2)
Delivery		
*Mode of delivery		
**Vaginal delivery	30,887/32,766 (94.3)	21,692/23,293 (93.1)
Instrumental delivery	586/32,766 (1.8)	368/23,293 (1.6)
Caesarean section	1,293/32,766 (3.9)	1,233/23,293 (5.3)
*Place of delivery		
SMRU clinics	22,992/34,256 (67.1)	15,711/25,433 (61.8)
Hospital (Thai or Myanmar)	2,529/34,256 (7.4)	3,131/25,433 (12.3)
Home	8,735/34,256 (25.5)	6,591/25,433 (25.9)

Categorial variables are mentioned as number (%) and continuous variables are described as mean (SD) or median [IQR].

*Missing data expressed in number (%) by refugee and migrant: Age (19 (0.1), 106 (0.2)), Ethnicity (25,351 (55.9), 11,611 (31.7)), Smoking during pregnancy (13,393 (29.5), 116 (0.3)), Literacy (34,223 (75.4), 10,596 (29.9)), BMI (22,870 (50.4), 2,287 (7.7)), Maternal height (22,821 (50.3), 2,789 (7.6)), Maternal weight (56 (0.1), 616 (1.7)), HIV infection (39,334 (86.7), 12,923 (35.3)), Syphilis (39,045 (86.0), 17,660 (48.2)), maternal anaemia (8,237 (18.2), 199 (0.5)), EGA at birth (1,383 (3.0), 259 (0.7), birthweight (10,596 (23.3), 13,660 (37.3),Foetal sex (9,496 (20.9), 13,399 (36.6)), Type of outcome (4,305 (9.5), 10,980 (29.9)), Stillbirth (8,703 (19.2), 13,354 (36.5)), Mode of delivery (12,625 (27.8), 13,346 (36.4)), Place of delivery (11,135 (24.5), 11,206 (30.6)).

[a]PTB calculation is restricted to singleton pregnancy between 28^{+0} and 42^{+0} weeks' gestation with known birth outcomes.

[b]SGA calculation is restricted to singleton pregnancy between 28^{+0} and 42^{+0} weeks' gestation with known birth outcomes and birthweight measuring within 72 hours of birth.

BMI at first ANC visit was first calculated in 2004. Literacy data was start recorded in 2008 but routine data collection was commenced in 2010. HIV & syphilis were routinely tested after 2010.

Maternal anaemia at first ANC consultation was used and haematocrit <30 g/dl was defined as anaemia.

**Face, compound and breech presentation were combined in the vaginal delivery.

Abbreviations: ANC antenatal care; BMI body mass index; cm centimetre; kg/m^2 kilogram/$metre^2$; NVD normal vaginal delivery; PTB preterm birth; SGA small for gestational age; SMRU Shoklo malaria research unit

Table 3-3:Maternal Mortality Ratio (MMR) and Neonatal Mortality Rate (NMR) over time for SMRU birth cohort.

	Ratio and rate over three decades			Overall
	1986-2000	**2001-2010**	**2011-2020**	**1986-2020**
Number of live births (n)	15,057	22,065	22,038	59,160
Number of maternal deaths (n)	32	40	34	106
Number of neonatal deaths (n)	261	282	204	747
MMR per 100,000 live births (95% CI)	212 (139-285)	181 (125-237)	154 (103-205)	179 (162-196)
*NMR per 1,000 live births (95% CI)	44 (38-51)	22 (19-25)	21 (18-24)	25 (23-27)

*Singleton pregnancy born alive after 28 weeks' gestation were included and the Kaplan Meier method was used to estimate NMR.
Abbreviation: SMRU Shoklo malaria research unit; CI – Confidence Interval

3.4.2 Maternal characteristics and pregnancy changes over time

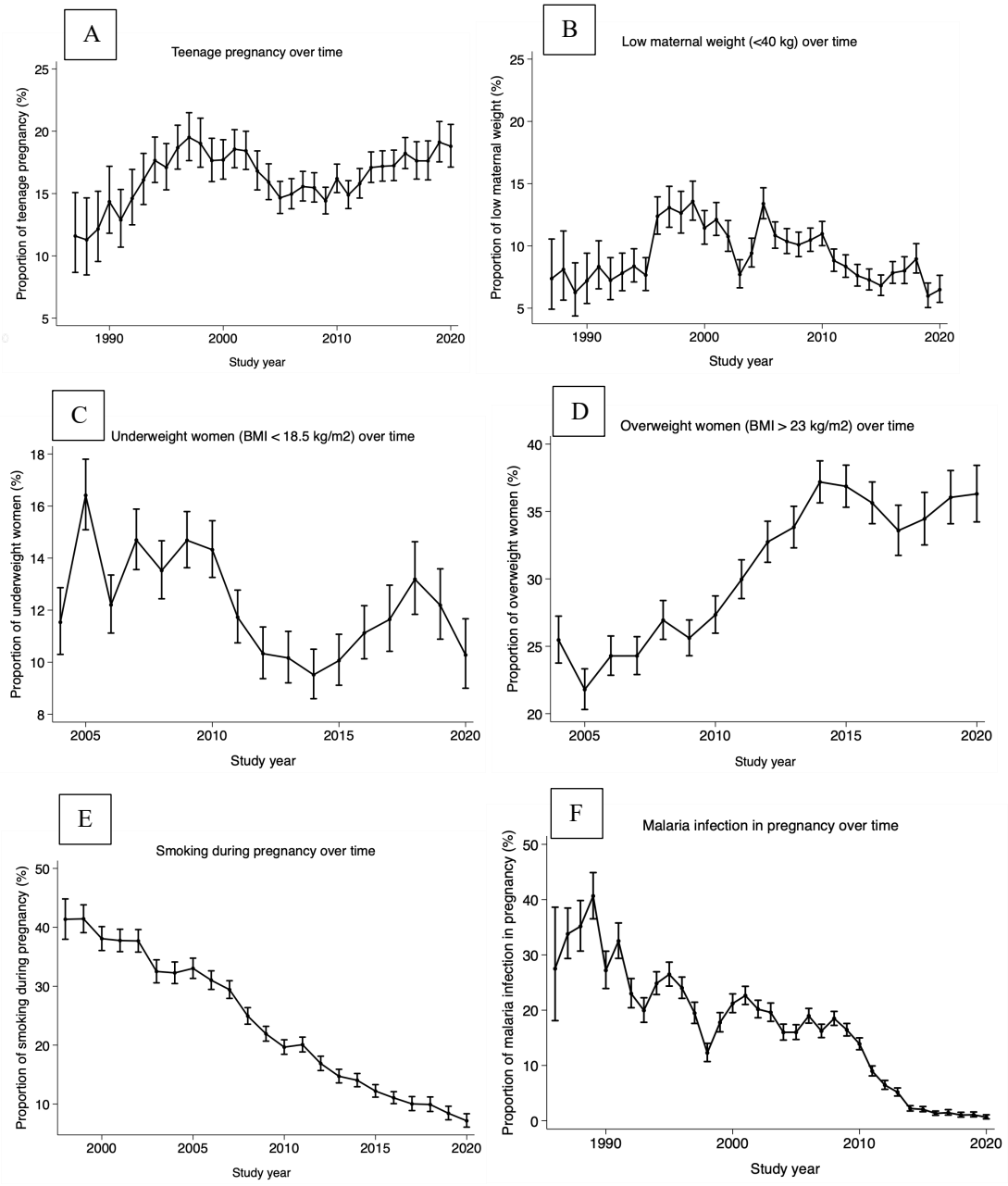
Table 3-4 provides an overview of the trends in selected maternal characteristics and pregnancy outcomes. To see the changes over time of selected maternal factors and pregnancy outcomes, migrants and refugees were combined for the linear trends analysis. The results indicate a significant decline in underweight women, malaria infection during pregnancy, smoking during pregnancy, maternal anaemia, PTB, and SGA. The study period revealed a substantial and consistent decrease in malaria infection ($p < 0.001$) and smoking ($p < 0.001$) during pregnancy at a rate of one percent per year, while the proportion of overweight pregnant women showed a significant annual increase of one percent ($p < 0.001$). The proportion of PTB and SGA also demonstrated a consistent decrease over time with average annual

reductions of 0.23% (p <0.001) and 0.34% (p <0.001), respectively. However, the results indicated a slight increase in the proportion of teenage pregnancy (0.05% per year) and preeclampsia (0.05% per year) over the study period.

Table 3-4:Linear trends in prevalence of maternal factors and risk of pregnancy outcomes over 1986-2020 for SMRU birth cohort.

Variables of interest	Estimated mean change in percent* per year (95%CI)	p value	Trend
Teenage pregnancy	0.05 (0.02, 0.08)	0.001	Increasing
Low maternal weight (<40 kg)	-0.09 (-0.12, -0.07)	<0.001	Decreasing
Underweight (BMI <18.5 kg/m^2)	-0.23 (-0.29, -0.17)	<0.001	Decreasing
Overweight (BMI >23.0 kg/m^2)	1.03 (0.94, 1.11)	<0.001	Increasing
Smoking during pregnancy	-1.68 (-1.72, -1.63)	<0.001	Decreasing
Malaria infection in pregnancy	-1.03 (-1.05, -1.01)	<0.001	Decreasing
PE	0.05 (0.04, 0.06)	<0.001	Increasing
Maternal anaemia	-0.75 (-0.79, -0.73)	<0.001	Decreasing
PTB	-0.23 (-0.25, -0.20)	<0.001	Decreasing
SGA	-0.34 (-0.38, -0.29)	<0.001	Decreasing

Abbreviations: BMI body mass index; cm centimetre; kg/m2 kilogram/$metre^2$; CI Confidence Interval; PE pre-eclampsia; PTB preterm birth; SGA small for gestational age; SMRU Shoklo malaria research unit
*Calculated using variance weighted least squares regression.


A
Teenage pregnancy over time
Proportion of teenage pregnancy (%)
Study year
B
Low maternal weight (<40 kg) over time
Proportion of low maternal weight (%)
Study year
C
Underweight women (BMI < 18.5 kg/m2) over time
Proportion of underweight women (%)
Study year
D
Overweight women (BMI > 23 kg/m2) over time
Proportion of overweight women (%)
Study year
E
Smoking during pregnancy over time
Proportion of smoking during pregnancy (%)
Study year
F
Malaria infection in pregnancy over time
Proportion of malaria infection in pregnancy (%)
Study year

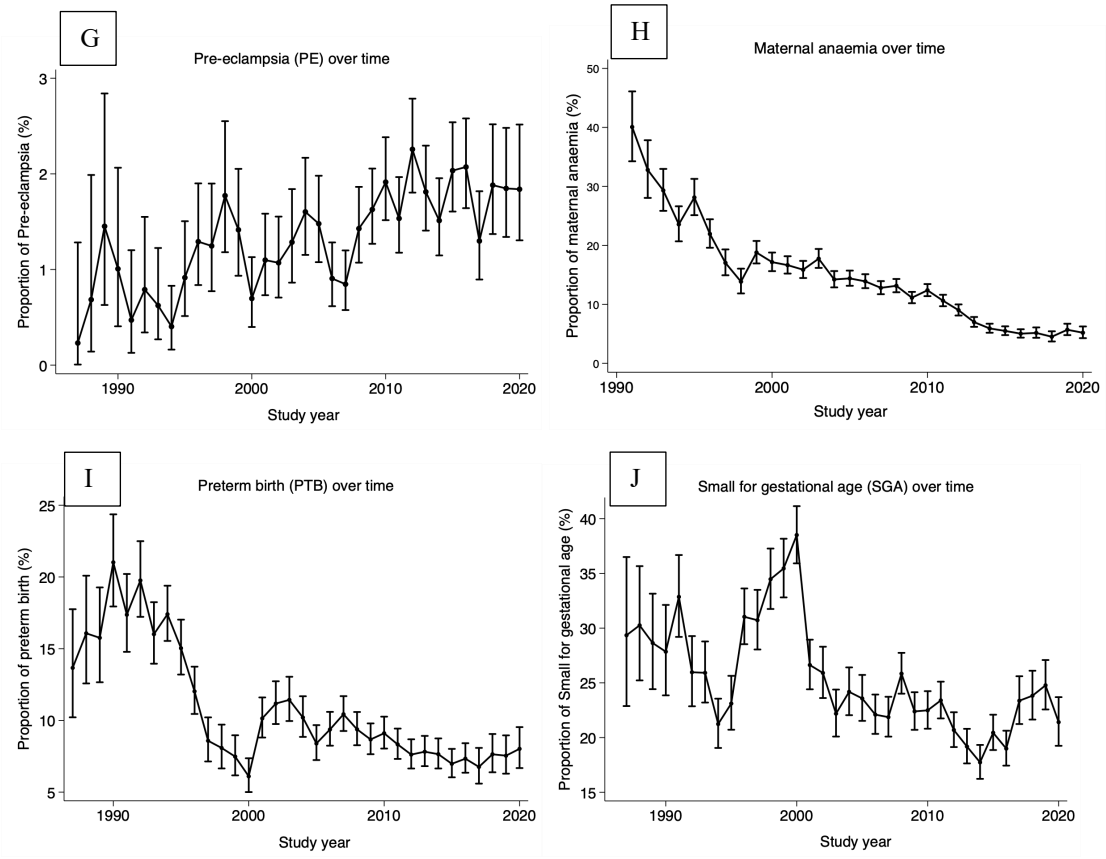


Figure 3-1:Changes in maternal characteristics and pregnancy outcomes over time in whole population (combined refugee and migrant population): A. Teenage pregnancies, B. Low maternal weight, C.Underweight (BMI<18.5 kg/m^2) D. Overweight (BMI>23.0 kg/m^2) E. Smoking during pregnancy, F. Malaria infection in pregnancy, G. Pre-eclampsia (PE), H. maternal anaemia, I. Pre-term birth (PTB), J. Small for gestation age (SGA).

3.4.3 Time periods and stratified refugee and migrant analysis

Changes in maternal factors and birth outcomes in the SMRU birth cohort were also examined in three time periods, over the last three decades (1986-2000, 2001-2010 and 2011-22) and detailed in appendix 3-1 (**Table A3-1**). The proportion of maternal factors, and pregnancy outcomes per year stratified by populations was also shown (Figure A3-2).

3.5 Discussion

This retrospective analysis using real world data from the SMRU birth cohort over 30 years is one of the largest birth cohort studies from marginalized populations residing in low-income countries and the largest in Asia. Many studies have previously reported on a specific issue along the Thailand-Myanmar border within limited time frames such as: literacy and pregnancy outcomes[150], trends and birth outcomes of teenage pregnancies[151], PTB and SGA[152, 153]; nutritional problems during pregnancy[154], early neonatal mortality and outcomes of resuscitated newborns[155], association of falciparum malaria and hypertensive disorders of pregnancy (HDP)[100] and malaria in pregnancy of marginalized pregnant women[120,103, 156]. This chapter provides an overview of SMRU birth cohort, and the trends of selected maternal factors and pregnancy outcomes with all available data over 30 years in this population.

Refugee and migrant populations are often considered as marginalized because they have both faced significant barriers to access healthcare, education and employment. In the SMRU birth cohort, the prevalence of maternal characteristics and risk of pregnancy outcomes between these two populations were similar and any small differences were clinically insignificant (**Table 3-2**).

The prevalence of teenage pregnancy rose to a peak (18%) between 1997-2002, and remained high, ranging between 15% and 18% in the last 5 years (Figure 3-1A). The association of teenage pregnancy and adverse pregnancy outcomes, has been well established internationally[151,157, 158]. There could be multiple factors leading to the high proportion of teenage pregnancy including limited access to education and healthcare, cultural and social norms, and poverty within this population.

In addition to communicable diseases, which used to be the predominant health issue in tropical areas, the double burden of nutritional problems during pregnancy[154, 159] (under- and over-weight) present a mixed picture as underweight has decreased to approximately 10% of women, while overweight still appears to be rising (Figure 3-1 C, D). The trend of increasing high maternal weight is a global phenomenon, including in Asia where the risk of cardiometabolic syndrome is higher at lower body mass index than in Caucasian populations[160]. The increasing prevalence of obesity is causing major health concerns in current pregnancies and may lead to future health problems for both the individual and future generations[161]. The hypotheses of foetal origins of adult diseases states that growth restricted foetuses may not achieve the potential growth in adulthood and are more likely to develop metabolic diseases in later life[162]. Meanwhile, underweight pregnant women are more likely to have SGA babies, overweight pregnant women may have an increased risk of having LGA babies and operative delivery[163, 164].

The incidence of smoking during pregnancy, malaria infection in pregnancy, and maternal anaemia, which were high in earlier years, have decreased significantly and appear to have reached a consistent, low level (Figure 3-1 E, F, and H). Malaria elimination efforts in the general population in this area[165] combined with frequent ANC visits, early diagnosis and treatment have contributed to reducing the prevalence of malaria and associated maternal anaemia. The decrease in smoking may be the result of a passive approach to smoking reduction which has been used at all SMRU antenatal clinics since 1998. On enrolment, women were asked about their smoking habits and encouraged to reduce or quit. Smoking cheroots rather than commercial cigarettes is common in this population because it is cheap and made up of dried betel leaf wrapper

filled with coarse ground tobacco and a blended herb filter that gives a sweet taste[166]. It yields high carbon monoxide (114 mg per cheroot) and nicotine (3.8 mg per cheroot), which is six times higher than cigarette smoke[167]. As far as we are aware, there is no other organization or campaign specifically targeting the reduction of smoking in pregnant women and smoking has not been a common social drug habit in South-East Asia. The association of reduction of birthweight with smoking (159 g (95% CI 83–235 g)) and with malaria (123 g (95% CI 34–212)) between 1986-1989 had been reported previously[167].

PTB and SGA are global health concerns due to their contribution to neonatal morbidity and mortality as well as long-term health consequences for society. An overall prevalence of PTB in this population was 9.4%, which was within the range of PTB rate in South Asia (10.4%)[13]. The proportion of PTB was as high as 20% in the earlier years followed by a significant reduction in 2000 (6.1%). Then, it increased up to 11.4 % and has remained stable in the last 5 years, ranging from 6.9% to 8.0% (Figure 3-1 I). This finding concurs with global decreasing trends of PTB. The decreasing proportion of PTB in this area may be associated with a decrease in smoking during pregnancy, malaria infection in pregnancy, maternal anaemia and improving antenatal care such as pregnancy dating by ultrasound, routine cervical length measurement abdominally to detect cervix shortening, implementation of preventive measures for PTB such as early diagnosis and treatment of bacterial infection, administration of steroids and uterotonic drugs. In the sub-group analysis, medically indicated PTB was increasing over the study period, less than 1% before 2000 to 1.8% in 2020 while there was an overall 14% reduction of spontaneous PTB: 21% in 1990 to 7% in 2020 (Figure

A3-1). Further investigation of this data is required as preterm rupture of membranes has been included in medically indicated PTB, however, this data requires a separate significant record review.

Similarly, the overall prevalence of SGA in this population between 1986-2020 was 23.3% and it was lower than South Asia (34.2%), where SGA prevalence was highest[23]. The proportion of SGA decreased from 29% in 1987 to 21% in 1994. Between 1996 and 2002, there was a surge in proportion of SGA as high as 38% in 2000 and it could possibly be related with the high proportion of malaria in migrants (follow-up started in 1998), teenage pregnancy and low maternal weight at the same period. Factors associated with PTB and SGA, and their population attributable risks will be explored in Chapter 4.

MMR declined in the last decade (154/100,000 live births in 2011-2020) but it remains far from meeting the sustainable development goal (SDG 3.1, MMR <70/100,000 live births by 2030). It is still challenging for the marginalized pregnant women to receive quality ANC care as most of them are residing at hard-to-reach areas, and the geographical distance remains a problem[168]. The general healthcare system for marginalized population in either Myanmar or Thailand is sub-optimal[169, 170].

NMR appears to be decreasing (21/1,000 live births in 2011-2020) but greater effort is required to meet the SDG 3.2 (NMR < 12/1,000 live births) with the caveat that SMRU starts to count neonatal mortality at an estimated gestation age of 28 weeks. The decline in NMR may be associated with the introduction of special care baby units (SCBU) in SMRU clinics in 2008 in Mae La refugee camp and 2010 in migrant clinics as reported by Turner et al[128]. However, NMR in this study may potentially be

underestimated due to the utilization of lower gestational age cut-off (at or beyond 28 weeks' gestation) and NMR under 28th weeks' gestation has not been taken account in this study. High neonatal mortality rates have been reported in the extremely preterm group (babies born before 28 weeks' gestation), even with appropriate management in neonatal intensive care in HICs. It remains challenging for LMICs to provide adequate management for the survival and long-term health consequences of extremely preterm babies. The sex ratio at birth (number of males per 100 females) was stable throughout the study period suggesting that abortion by foetal sex selection is uncommon in this population (Figure A3-3).

3.5.1 Strengths and limitations

The main strength is the size of this cohort, which is based on the unselected routine pregnant women attending ANC and delivery care in a marginalized setting. The data quality, accuracy and consistency of method of collection of data were good considering some structural missing data and consistent data on all variables was not available across the entire study period. For example, smoking during pregnancy, BMI and literacy were collected over different time periods. Information on gestational diabetes mellitus (GDM) was limited as screening of GDM was based on risk factors in early years[159] and risk factor-based screening was started after 2016 and universal screening only commenced in 2021. Another limitation of this study is that the information for neonatal morbidity (admission to SCBU due to neonatal sepsis) and maternal outcomes (induction of labour, post-partum haemorrhage) was available but not included in this cohort profile. Biological samples for studying human genetics were not routinely collected like in other birth cohorts. Also, there are no data on air pollution

including household or environmental, water and sanitation, socioeconomic status, interpregnancy interval and maternal mental health. A further limitation of this cohort might be the stable proportion of registered pregnancies with an unknown pregnancy outcome mostly due to home birth, a characteristic of the village midwife system in Myanmar[171]. In the migrant population, there are barriers to receive the safe delivery and newborn care such as long distance, feasibility of transportation, and risk of arrest on the way to SMRU clinics. Hence, there is a possibility of under-reporting for MMR and NMR.

3.5.2 Conclusion

This chapter provides an overview of the SMRU birth cohort. Using this birth cohort, the determinants and population attributable risks of PTB and SGA will be explored in Chapter 4. Moreover, the findings from this birth cohort will be beneficial to the local population, healthcare providers, who are working in this area including NGOs and CBOs, stakeholders and policy makers. Moving forwards, the analysis of data in the last decade provides target areas that can be used to improve birth outcomes in this population. These findings will be applicable for the border population living on the border shared by Myanmar and Thailand (more than 2000 km).

Chapter 4

Determinants and population attributable risks of preterm birth (PTB) and small for gestational age (SGA) in migrant and refugee populations along the Thai-Myanmar border (1986-2020): A retrospective analysis

4.1 Chapter overview

Preterm birth (PTB) and small for gestational age (SGA) are significant adverse pregnancy outcomes. This chapter describes: (1) the determinants of PTB and SGA in migrants and refugees along the Thai-Myanmar border and (2) the population attributable risks of PTB and SGA over a 30-year period (1986-2020).

4.2 Introduction

4.2.1 Preterm birth

PTB (livebirth before the 37th completed week or 259 days of gestation) is one of the leading causes of neonatal mortality and second leading cause of under-5 mortality worldwide[13]. Lifelong consequences of PTB such as visual, hearing and cognitive impairments can develop even if the preterm baby survives. Approximately 15 million PTBs occur every year and 11% of all liveborn babies in low and middle income countries (LMICs) in 2010[172] are preterm. Sub-Saharan Africa and South Asia have the highest proportions of PTB[13], which is classified as spontaneous or medically indicated (healthcare provider initiated). Spontaneous PTB follows a natural onset of labour with or without pre-labour rupture of membrane and accounts for approximately two-thirds of all PTBs[59]. The cause of spontaneous PTB is not completely understood.

The possible triggers include activation of the foetal hypothalamic pituitary adrenal (HPA) axis due to maternal and foetal stress; an inflammatory response due to infection or genital tract microbiome alteration; uterine overdistension and cervical incompetence[173]. Medically indicated PTB is defined as a medical or surgical intervention due to medical or obstetric complications accounting for the remaining one-third of all PTBs[59].

Multiple aetiological factors for PTB have been reported and they can generally be classified as maternal, foetal and placental factors. Maternal factors include extra-uterine infection (e.g., malaria, sexually transmitted infections) and intra-uterine infection (chorioamnionitis), medical conditions such as cardiac, thyroid and respiratory diseases, and hypertensive disorders during pregnancy (pregnancy induced hypertension, pre-eclampsia, eclampsia and HELLP syndrome). Foetal factors include foetal distress, foetal anomaly and foetal growth restriction (FGR). Antepartum haemorrhage (vaginal bleeding after 15 weeks' gestation) is regarded as the key placental contributor to PTB[174].

A better understanding of the risk factors and their contribution to PTB is required to reduce PTB related mortality. One recent study[4] assessing the population attributed fraction (PAF) for risk factors of PTB in 81 LMICs identified forty-four risk factors and the top three PAFs, which are maternal nutrition (17.5%), maternal infection (16.6%) and environmental exposures (16%) . This study also suggested that PTB related risk factors may vary in different populations and that there is an urgent need to reduce the burden of PTB[4].

4.2.2 Small for gestational age

SGA is defined by a birthweight for gestational age less than the 10th centile of an international standard or reference population, which is used as a proxy for FGR[175]. An accurate dating scan, serial antenatal ultrasound scans to monitor foetal growth and wellbeing, and umbilical artery Doppler are required to define FGR. Hence, SGA is more suitable to describe foetal growth problems in resource limited areas where ultrasound machines are available. In 2012, there were an estimated 23.3 million (19.3%) SGA babies born (as defined by the INTERGROWTH-21st Standard) and 606,500 (21%) neonatal deaths due to SGA in LMICs[23]. The highest burden was found in South Asia (34.2% of all births) and Sub-Saharan Africa (16.5%), respectively. SGA babies may have delayed physical development up to 2 years of age, learning difficulties, and behavioural and emotional problems during childhood[176]. In addition, SGA infants with poor growth are more likely to develop cardiovascular (hypertension, ischaemic heart disease and stroke) or metabolic diseases (hyperlipidaemia, diabetes mellitus) in adult life[177, 178].

Studies have shown that maternal age > 35 years, primigravida, Asian ethnicity, maternal short stature and underweight, cigarette smoking and substance abuse are associated with SGA[4, 179, 180]. Chronic medical conditions (e.g., chronic hypertension, renal disease and anti-phospholipid syndrome) and obstetric complications (e.g., bleeding in early pregnancy, preeclampsia, placental abruption) are also associated with increased risk of SGA. In addition, malaria infection in pregnancy and TORCH (Toxoplasmosis, Rubella, Cytomegalovirus and Herpes) infection result in SGA[179]. Reducing the high prevalence of SGA is a global concern and it is crucial to have a

better understanding of the associated risk factors and the population attributable risks in order to decrease the high burden of SGA[5].

4.3 Objectives

The objectives of this analysis were to quantify the determinants of PTB and SGA and to estimate the contribution of these risk factors to PTB and SGA in a marginalized population along the Thai-Myanmar border.

4.4 Method

4.4.1 Study site and population

This study is based on a retrospective, population-based, cohort of pregnant women living on the Thai-Myanmar border between 1986-2020. Detailed information on the study site and population were provided in **Chapter 2**.

4.4.2 Assessment of gestational age

Before year 2000, gestational age estimation was mostly calculated from the first day of the last menstrual period (LMP) if known or by measuring the fundal height during the pregnancy. Routine pregnancy dating scans were initiated in all clinics in 2000. Different types of ultrasound machines (Toshiba, GE Voluson I and Samsung Sono-Ace R3) have been used for pregnancy dating, foetal growth monitoring, and assessing foetal wellbeing (biophysical profiling and umbilical Dopplers). Anomaly scans were not routinely performed. The Dubowitz test within 72 hours of delivery was administered to assess the gestational age at birth in cases of late ultrasound dating scan (e.g. $>24^{+0}$ weeks' gestation). Sonographers, who were locally trained and can speak both Karen and Burmese languages, performed pregnancy dating scan, foetal biometric

measurements and foetal wellbeing tests. Their scanning skills were assessed twice a year to decrease inter-observer variations[4].

4.4.3 Terminology and definition of PTB

Babies born less than 28 weeks' gestation cannot survive without getting proper management in a neonatal intensive care unit, which is not available in this setting. For the purpose of the present analysis, 28 weeks is used as the lowest gestational age cut-off for PTB. Early PTB is defined as PTB between 28^{+0} and 33^{+6} weeks, and late preterm between $\geq 34^{+0}$ and $<37^{+0}$ weeks' gestation. Induction of labour and delivery by elective Caesarean section between $\geq 28^{+0}$ and $<37^{+0}$ weeks' gestation are classified as medically indicated PTB or healthcare provider initiated PTB[181].

4.4.4 Variables

Maternal age and gravidity are two important risk factors for adverse pregnancy outcomes and these two variables are strongly correlated. Hence, maternal age and gravidity were combined as one variable (maternal age & gravidity) and six categories were created (maternal age <20 years primigravida & multigravida, maternal age between 20 and 34 years primigravida & multigravida, maternal age (>35 years) primigravida & multigravida). Suspected bacterial infection was defined as the presence of fever treated with antibiotics. Suspected viral infection was defined as the presence of fever without antibiotic treatment. Maternal weights and heights at the first antenatal clinic (ANC) visit were available after 2004, and used to derive Asian-pacific BMI categories[96] as follows: underweight (<18.5 mg/kg^2), normal weight (18.5-22.9 mg/kg^2) and, overweight & obese (≥23.0 mg/kg^2). Maternal anaemia at the first ANC visit was defined as a haematocrit <30%. Smoking during pregnancy (since 2004),

history of PTB (since 2008) and non-malaria infection (since 2010) were systematically collected at different periods.

4.4.5 Statistical analysis

A retrospective data analysis was performed using de-identified data extracted from the antenatal care database of the Shoklo Malaria Research Unit (SMRU). These data include socio-demographic, maternal characteristics, medical and obstetric problems in the present and past pregnancy, and maternal and newborn outcomes recorded in paper form. From 2008 onwards, real-time data were recorded using a web-based application downloaded into an electronic ANC database.

Singleton pregnancies liveborn between 28^{+0} and 42^{+0} weeks' gestation with known outcomes were included in this analysis. Twin pregnancies, pregnancies with congenital abnormalities and those with unknown birth outcomes were excluded. SGA was defined by the INTERGROWTH-21st standard[24], and only birthweight measured within 72 hours of birth was included. A flow chart detailing the number of pregnancies included and excluded in the data analyses of PTB and SGA is shown in Figure 4-1 and 4-2. The proportion and number of PTB, including spontaneous or indicated, and SGA are presented.

Logistic regression was performed to estimate the unadjusted odds ratio (OR) and adjusted odds ratio (AOR) of PTB and SGA. The correlation between the independent variables was checked by Pearson's correlation coefficient. Gestational assessment methods (LMP, fundal height measurement, ultrasound, Dubowitz test), calendar year, average ambient temperature of each trimester and newborn sex were included in all regression models. A fractional polynomial function of study period

(calendar year) was used in the multivariable logistic regression models to control for the non-linear association between calendar year and the log odds of PTB and SGA[182]. In the model selection, some known risk factors such as non-malaria infection were included regardless of P-values, and other factors were selected based on the backward elimination method[182]. Two models of multivariable logistic regression were performed for each pregnancy outcome (PTB and SGA): Model A included the variables that are available throughout the study period (1986-2020) and Model B included only recent data from 2004-2020 for SGA and 2008-2020 for PTB. This is because some of the clinically important risk factors were systematically collected only after 2004 (smoking and BMI), 2008 (history of PTB) and 2008 (non-malaria infection). Average ambient temperature for each trimester was calculated by using daily temperature data from the Mae Sot weather station that was added in the multivariable models. Sub-group analyses for PTB were performed for ultrasound dated PTB, spontaneous PTB, medically indicated (healthcare provider initiated preterm birth) early and late PTB.

4.4.6 Calculating population attributable risk (PARs)

Population attributable risk is the number of cases or proportion of the whole population attributable to the risk factor/exposure.

PAR= incidence rate in total population - incidence rate in unexposed population

PARs were calculated based on the estimates of the AOR from the multivariable analyses of PTB and SGA, preferably model B for each outcome, in order to understand PARs in the last decade[183]. Multivariable models were built for each factor adjusting for confounding factors identified from the current literature. PARs of PTB and SGA

for different time periods were also calculated by dividing the data by every 5 years based on multivariable logistic regression model A (i.e., all data from 1986-2020).

4.4.7 Software used for data analysis

IBM SPSS Statistics 25 and STATA 15.1 SE were used to analyse the data. The graphs showing population attributable risks of PTB and SGA were also produced using STATA 15.1 SE.

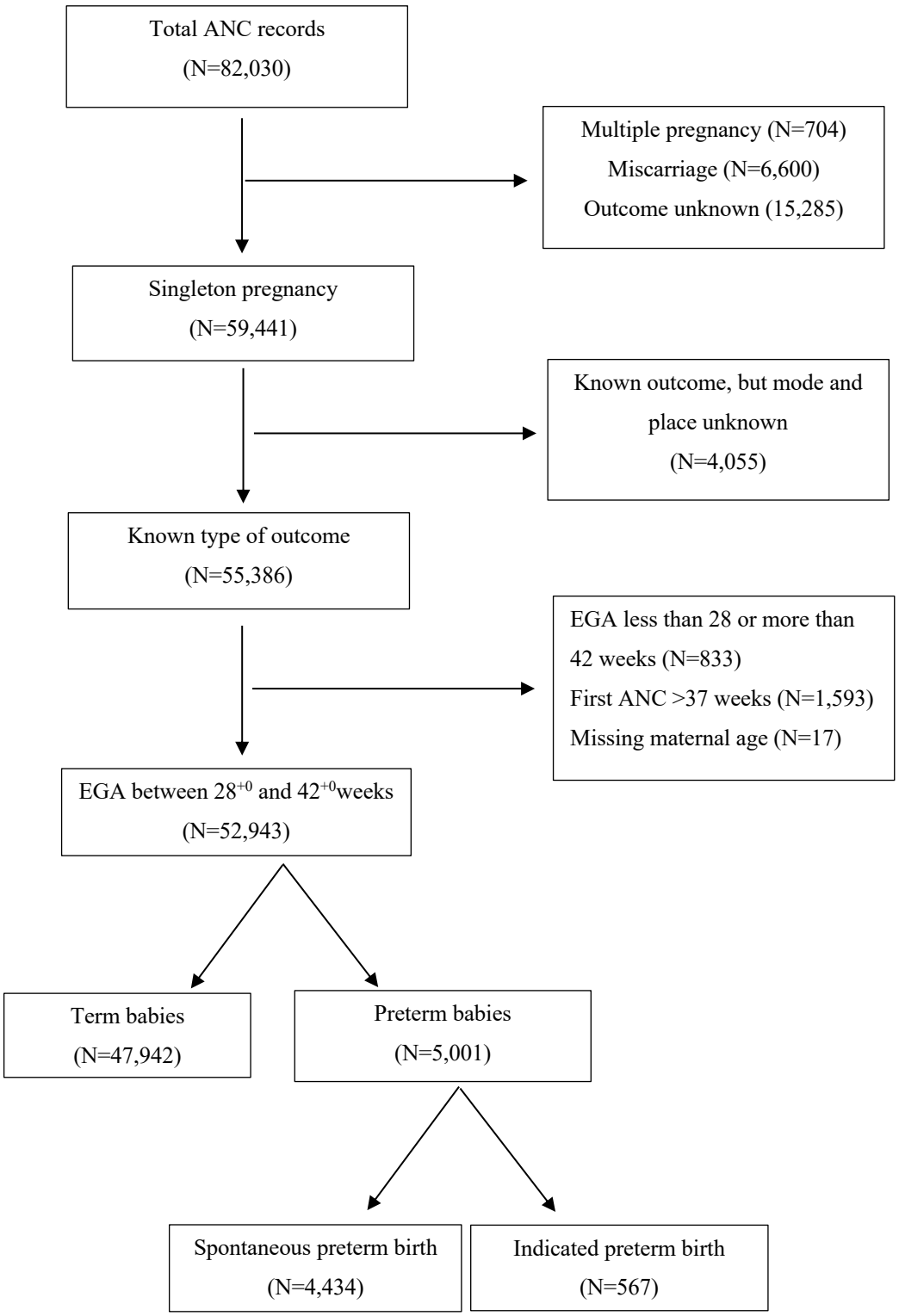


Figure 4-1:Flow chart for data-analysis (Preterm birth).

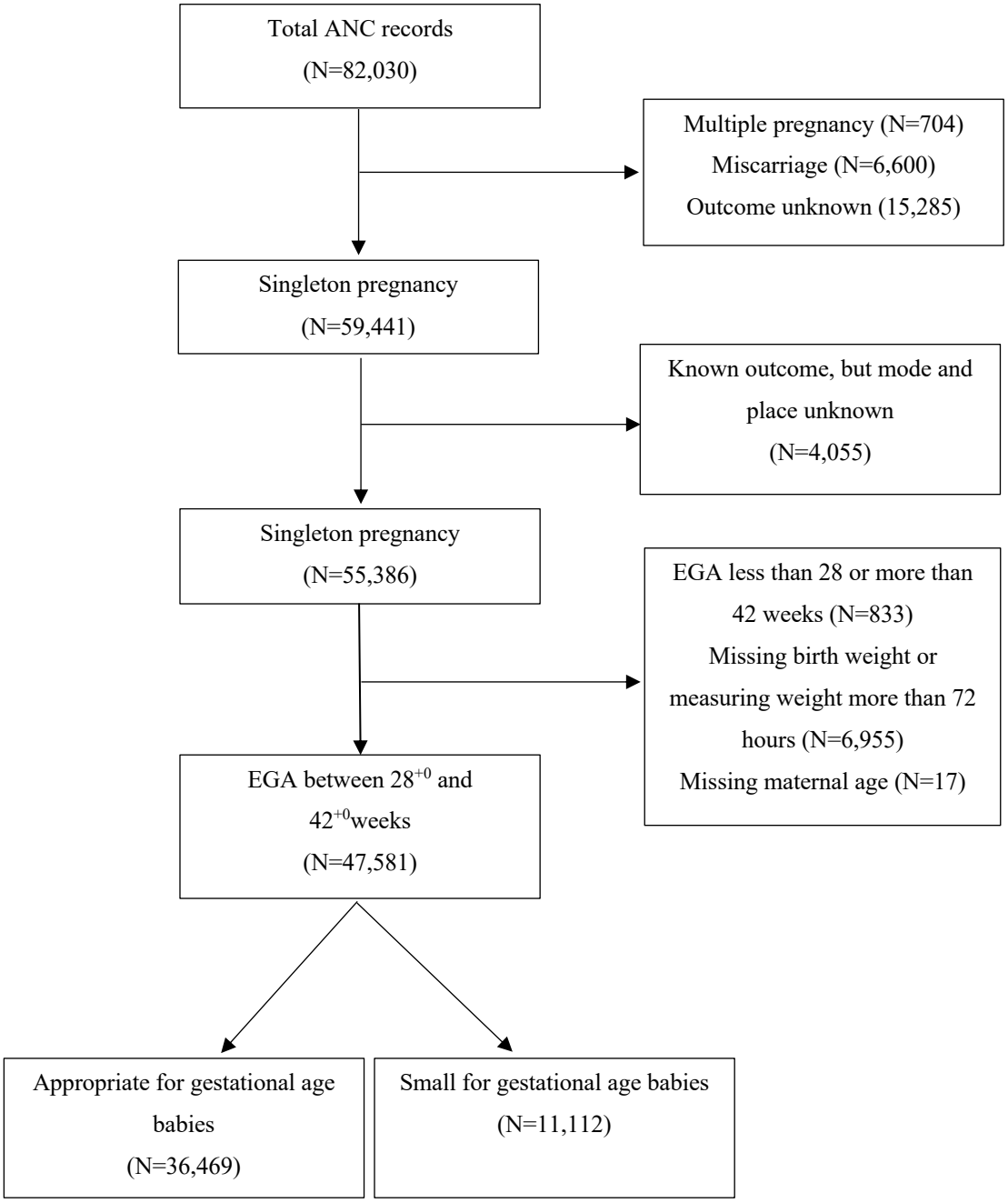


Figure 4-2:Flow chart for data analysis (Small for gestational age).

4.4.8 Ethical approval

Ethics approval for accessing and analysis of this cohort study was obtained from the Oxford Tropical Research Ethics Committee (reference: OXTREC 28–09) and Tak Community Advisory Board (reference: TCAB 202002).

4.5 Results

4.5.1 Baseline characteristics of preterm birth

Table 4-1 presents the baseline characteristics of PTB and term births. Of the total 82,030 ANC records in the SMRU database, 52,943 singleton births (90.6% (47,942) term and 9.4% (5,001) PTB) were eligible for the data analysis after excluding multiple pregnancies, miscarriages, unknown birth outcomes and any birth < 28^{+0} and >42^{+0} weeks' gestation (Figure 4-3). In the PTB group, 88.7% (4,434) and 11.3% (567) were spontaneous and indicated PTBs, respectively. The median maternal age of PTB and term births was 25 years [inter-quartile range (IQR): 21-31] and 23 years [IQR 19-30], respectively. A higher proportion of teenage pregnancies was seen in the PTB group compared to term pregnancy (27.7% vs 16.2%). Ultrasound was used to estimate the gestational age of 60.5% PTBs and 64.7% term births.

4.5.2 Determinants of preterm birth

Similar AORs were observed in both multivariable logistic regression model A (pregnancies recorded between 1986-2020) and B (pregnancies recorded between 2008-2020) (**Table 4-3**). The odds of having a PTB amongst teenage mothers (primigravida and multigravida) was two times higher compared to multigravida aged between 20-34 ($p < 0.001$). The highest odd of PTB was associated with pregnancies complicated by PE and a history of PTB ($p < 0.001$). Although malaria infection in

pregnancy was significantly associated with PTB in univariable and multivariable regression model A, it was no longer a risk factor for PTB in model B (p=0.514). Suspected bacterial infection was significantly associated with increased risk of PTB in both univariable (p <0.003) and multivariable model B (p=0.004). Sub-group analyses for assessing the associated risk factors for ultrasound dated PTB, early and late PTB, and spontaneous PTB are presented in **Appendix C**.

Table 4-1: Baseline characteristics of SMRU birth cohort for term and preterm deliveries between 1986-2020.

	Number (%) of total births	Term birth	Preterm birth
Total	**N=52,943**	**N=47,942**	**N=5,001**
Baseline			
Maternal age, median [IQR]	52,943 (100.0)	25 [21-30]	23 [19-30]
Age Category, years			
< 20	9,169 (17.3)	7,784 (16.2)	1,385 (27.7)
20-34	36,477 (68.9)	33,498 (69.9)	2,979 (59.6)
≥ 35	7,297 (13.8)	6,660 (13.9)	637 (12.7)
Primigravida	15,078 (28.5)	13,115 (27.4)	1,963 (39.2)
Attend ANC in first trimester	24,684 (46.6)	22,662 (47.3)	2,022 (40.4)
ANC consultation <8 times	13,444 (24.4)	11,153 (23.3)	2,291 (44.8)
[1]**Smoker**	9,782 (21.5)	8,843 (21.2)	939 (24.5)
[2]**Literate**	15,037 (62.1)	13,953 (62.4)	1,084 (58.7)
Living Status			
Migrant	21,879 (41.3)	20,077 (41.9)	1,802 (36.0)
Refugee	31,064 (58.7)	27,865 (58.1)	3,199 (64.0)
[3]**Ethnicity**			
Karen	21,409 (69.7)	19,529 (69.2)	1,880 (74.7)
Burmese	6,330 (20.6)	6,194 (20.9)	415 (16.7)
Other	2,963 (9.7)	2,858 (9.9)	190 (7.6)
Gestational age assessment method			
Ultrasound	34,518 (64.2)	31,493 (64.7)	3,025 (60.5)
Dubowitz	11,677 (22.0)	10,927 (22.8)	750 (14.9)
Fundal height measurement	5,163 (9.8)	4,299 (8.9)	864 (17.3)
LMP	1,588 (3.0)	1,224 (2.6)	364 (7.3)
[4]**Height in cm, Median [IQR]**	38,244 (72.0)	151.2 [148-155]	150.3 [147-154]

[5]**Weight in kg*, Median [IQR]**	52,895 (99.9)	49 [45-54.4]	47 [43-52]
[6]**BMI (kg/m^2)**			
Underweight (< 18.5)	4,323 (11.7)	3,885 (11.5)	438 (14.4)
Normal (18.5-23)	21,378 (57.7)	19,478 (57.3)	1,900 (62.3)
Overweight (≥ 23)	11,325 (30.6)	10,615 (31.2)	710 (23.3)
[7]**History of preterm birth**	1,350 (4.8)	1,127 (4.4)	223 (9.9)
[8]**Maternal anaemia at first ANC**	5,493 (11.2)	4,863 (10.9)	630 (14.7)
Malaria in pregnancy	7,121(13.5)	6,251 (13.0)	870 (17.4)
Pre-eclampsia and eclampsia	1,044 (1.98)	829 (1.6)	215 (3.9)
Mode of delivery			
Cephalic vaginal delivery	48,989 (92.5)	44,607 (93.0)	4,382 (87.6)
Breech vaginal delivery	758 (1.4)	527 (1.1)	231 (4.6)
Instrumental delivery	890 (1.7)	849 (1.8)	41 (0.8)
Caesarean section	2,249 (4.3)	1,904 (4.0)	345 (6.9)
Others**	73 (0.1)	67 (0.1)	6 (0.1)
[9]**Foetal Sex**			
Male	27,201 (51.7)	24,592 (51.5)	2,609 (53.3)
Female	25,445 (48.3)	23,159 (48.5)	2,286 (44.7)
[10]**SGA**	10,774 (23.4)	9,932 (23.6)	842 (20.7))
[11]**Neonatal death**	583 (1.9)	243 (0.9)	340 (11.5)
[12]**Place of delivery**			
SMRU	32,751 (64.4)	30,322 (66.4)	2,429 (54.0)
Home	12,801 (24.5)	11,447 (24.1)	1,354 (30.7)
Hospital	4,542 (9.1)	3,911 (8.5)	631 (14.3)

Singleton pregnancies which were delivered at estimated gestational age between ≥ 28 weeks & ≤ 42 weeks with known outcomes were included in this analysis. Data is n (%) unless stated otherwise.

Missing data expressed per term and preterm group (n(%); [1]Smoker (6,218 (12.9), 1,174 (23.5)); [2]Literate (25,573 (53.3), 3,155 (63.1); [3]Ethnicity (20,115 (40.6), 2,516 (50.3)); [4]Height (14,341 (28.9), 1947 (38.9)) ; [5]Weight (70 (0.1), 25 (0.5)) ; [6]BMI (13,964 (29.1), 1,953 (39.0)); [7]History of PTB (22,109 (46.1), 2765 (54.3)); [8]Maternal anaemia at first ANC (3,201 (6.7), 711(14.2)); [9]Foetal Sex (191 (0.4), 106 (2.1)); [10]SGA (5,936 (12.4), 925 (18.5)); [11]Neonatal death (19,936 (41.5), 2056 (41.1)); [12]Place of delivery (2,262 (4.7), 587 (11.7))

Weight measurement in first trimester was used. BMI at any time during pregnancy was first calculated in 2004.

Literacy data began to be recorded in 2008 but routine data collection was commenced after 2010.

Maternal anaemia is defined as haematocrit <30% as measured at first ANC visit.

**Face and compound presentation but delivered vaginally

Abbreviation: ANC antenatal care; BMI body mass index; cm centimetre; IQR interquartile range; kg kilogram; LMP last menstrual period, SGA small for gestational age, SMRU Shoklo Malaria Research Unit

Table 4-2:Univariable and multivariable analysis for preterm birth.

		Univariable analysis		Multivariable analysis (model A)		Multivariable analysis (model B)	
		OR (95%CI)	**p-value**	**AOR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Age and Gravidity	52,943			N=42,844		N=22,841	
<20 Primigravida	7,012	2.28 (2.11-2.46)	<0.001	2.59 (2.36-2.85)	<0.001	2.64 (2.31-3.01)	<0.001
<20 Multigravida	2,157	2.02 (1.78-2.30)	<0.001	2.06 (1.76-2.41)	<0.001	2.30 (1.83-2.89)	<0.001
20-34 Primigravida	7,882	1.52 (1.39-1.65)	<0.001	1.69 (1.53-1.87)	<0.001	1.67 (1.46-1.92)	<0.001
20-34 Multigravida	28,595	Reference		Reference		Reference	
≥ 35 Primigravida	184	1.87 (1.21-2.88)	0.004	1.77 (1.04-3.01)	0.033	1.75 (0.94-3.27)	0.077
≥ 35 Multigravida	7,113	1.18 (1.07-1.29)	0.001	1.23 (1.10-1.37)	<0.001	1.35 (1.16-1.58)	<0.001
First ANC in 1st Trimester	52,943	0.76 (0.71-0.80)	<0.001	0.77 (0.71-0.83)	<0.001	0.78 (0.70-0.87)	<0.001
Low maternal weight (<40kg)	4,955	1.40 (1.28-1.53)	<0.001	1.64 (1.47-1.83)	<0.001	NA	
Maternal height (per 10cm)	37,036	0.71 (0.66-0.76)	<0.001	NA		0.72 (0.66-0.79)	<0.001
BMI (kg/m^2)							
Underweight (<18.5)	4,323	1.16 (1.04-1.29)	0.009	NA		1.22 (1.06-1.41)	0.007
Normal (18.5-22.9)	21,379	Reference				Reference	

Overweight (≥ 23)	11,325	0.69 (0.63-0.75)	<0.001	NA		0.74 (0.66-0.84)	<0.001
Maternal anaemia at 1st ANC visit	49,032	1.41 (1.29-1.54)	<0.001	1.24 (1.11-1.39)	<0.001	1.27 (1.07-1.52)	0.007
Malaria infection in pregnancy	52,944	1.40 (1.30-1.52)	<0.001	1.29 (1.16-1.43)	<0.001	1.07 (0.88-1.30)	0.514
Non-malaria infection							
Non-febrile and no antibiotics	26,615	Reference				Reference	
Suspected bacterial infections	620	1.48 (1.14-1.91)	0.003	NA		1.52 (1.14-2.03)	0.004
Suspected viral infections	694	1.15 (0.89-1.51)	0.307	NA		1.10 (0.82-1.49)	0.501
PE	52,944	2.56 (2.19-2.99)	<0.001	3.05 (2.58-3.61)	<0.001	3.63 (2.96-4.46)	<0.001
Smoker	45,552	1.21 (1.12-1.31)	<0.001	NA		1.53 (1.32-1.76)	<0.001
History of preterm labour	28,070	2.43 (2.09-2.82)	<0.001	NA		3.28 (2.74-3.93)	<0.001
SGA	46,083	0.84 (0.78-0.91)	<0.001	0.78 (0.72-0.85)	<0.001	0.84 (0.74-0.95)	0.003
New-born sex (male)	52,647	1.07 (1.01-1.14)	0.017	1.08 (1.01-1.16)	0.028	1.09 (0.99-1.20)	0.07
Average ambient temp during 1st trimester	52,943	0.94 (0.92-0.96)	<0.001	0.94 (0.92-0.96)	<0.001	0.99 (0.96-1.02)	0.54
Average ambient temp during 2nd trimester	52,943	0.94 (0.92-0.95)	<0.001	0.96 (0.94-0.98)	0.001	1.02 (0.98-1.05)	0.351
Average ambient temp during 3rd trimester	52,943	0.97 (0.95-0.98)	<0.001	0.98 (0.96-1.01)	0.145	1.05 (1.02-1.08)	0.003

Multivariable models adjusted for living status (refugee or migrant), gestational assessment methods and calendar year (fractional form).

Abbreviation: ANC antenatal care; BMI body mass index; PE pre-eclampsia; SGA small for gestational age, temp temperature

4.5.3 Population attributable risks (PARs) of preterm birth

Six potential risk factors of PTB were identified from multivariable logistic regression model B and PARs were calculated (**Table 4-5**). The overall risk of PTB for the total population between 2008 and 2020 was highest for teenage pregnancies: 11.3 per 1,000 livebirths (95%CI 9.4, 13.0), followed by PE (3.5 per 1,000 livebirths). The PAR due to malaria infection in pregnancy was 0.8 per 1,000 births (95%CI -0.1, 1.7), which was the lowest risk. Figure 4-5 shows PAR of PTB every 5 years in order to understand the changes over time. PAR of PTB due to malaria infection in pregnancy was approximately 35 per 1,000 births between 1991 and 1995 and less than 5 per 1,000 births after 2000. However, teenage primigravida attributable PTB was persistently high throughout the study period.

Table 4-3:Population attributable risks (PARs) of PTB.

Risk factors	Study period 1986-2020 (Based on Model A)	Study period 2008-2020 (Based on Model B)
	N=42,844	N=26,221
	PAR per 1,000 births (95% CI)	PAR per 1,000 births (95% CI)
Teenage pregnancy	11.1 (9.7, 12.6)	11.3 (9.4, 13.0)
Low maternal weight (<40 kg)	3.9 (3.0, 4.8)	NA*
PE	2.7 (2.1, 3.2)	3.5 (2.7, 4.2)
Underweight (BMI<18.5)	NA	3.0 (1.8, 4.3)
Smoking	NA	2.7 (1.3, 4.2)
Maternal anaemia at first ANC	1.8 (0.8, 2.8)	1.3 (0.3, 2.3)
Malaria in pregnancy	1.7 (0.6, 2.8)	0.8 (-0.1, 1.7)

*PAR for low maternal weight in place of BMI in Model B = 2.8 (1.6, 4.0). NA-not available

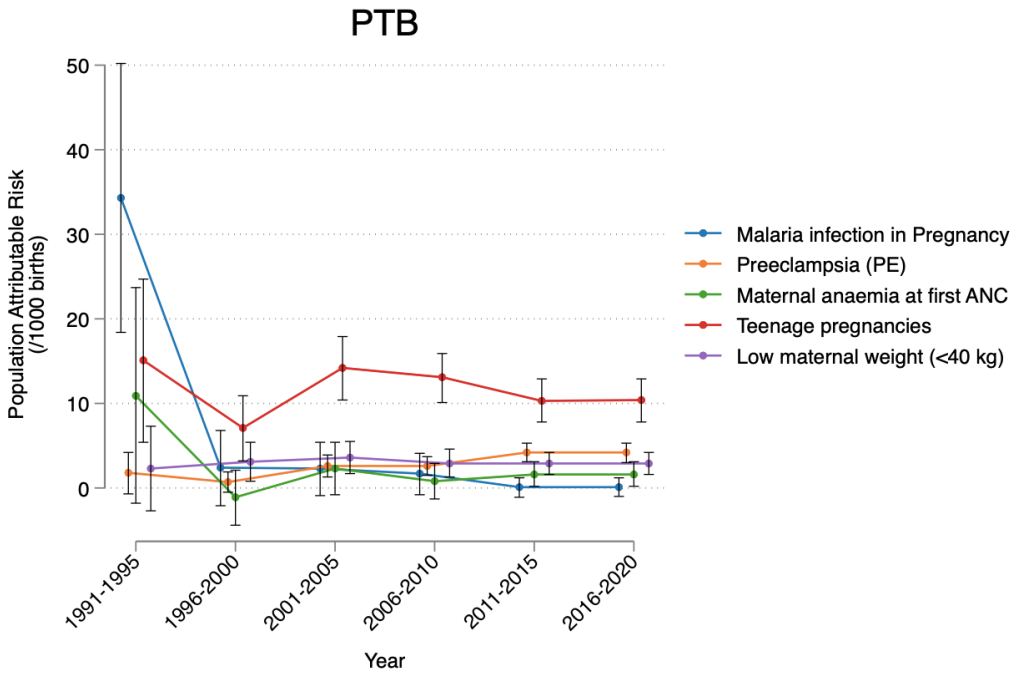


Figure 4-3:Population attributable risks of PTB between 1991-2020.

4.5.4 Baseline characteristics of SGA

Baseline characteristics by appropriate for gestational age (AGA) and SGA are presented in **Table 4-2**. After excluding multiple pregnancies, unknown birth outcomes, missing birthweights or those measured >72 hours after birth, and any births occurring <28^{+0} and >42^{+0} weeks' gestation, 47,581 singleton pregnancies were eligible for SGA data analysis: 76.6% (36,469) AGA and 23.4% (11,112) SGA (Figure 4-4). The median maternal age in both the AGA and SGA groups was 25 years [IQR: 20-30]. However, there were more teenage pregnancies in the SGA group (20.9% vs 16.4%). Higher proportions of primigravida (36.9% vs 27.1%), smoking during pregnancy (27.5% vs 17.7%), malaria infection in pregnancy (14.9% vs 10.4%), maternal anaemia (12.6% vs 9.9%), and PE (3.3% vs 1.8%) were found in SGA compared to AGA babies.

Table 4-4: Baseline characteristics of SMRU birth cohort for small for gestational age between 1986-2020.

	Number (%) of total births	Appropriate for gestational age (AGA)	Small for gestational age (SGA)
Total	N=47,581	N=36,469 (76.6)	N=11,112 (23.4)
Baseline			
Maternal age (median and IQR)	47,581 (100.0)	25 [21-30]	25 [20-30]
Age Category, years			
< 20	8,299 (17.5)	5,975 (16.4)	2,324 (20.9)
20-34	32,702 (68.7)	25,532 (70.0)	7,170 (64.5)
≥ 35	6,580 (13.8)	4,962 (13.6)	1,618 (14.6)
Primigravida	13,993 (29.4)	9,887 (27.1)	4,106 (36.9)
Attend ANC in first trimester	21,945 (46.1)	16,916 (46.4)	5,029 (44.3)
ANC consultation <8	12,652 (26.6)	9,831 (26.9)	2,821 (24.4)
[1]Smoker	8,203 (19.9)	5,600 (17.7)	2,603 (27.5)
[2]Literate	14,554 (63.3)	11,669 (64.3)	2,885 (59.7)
Living Status			
Migrant	17,972 (37.8)	13,755 (37.7)	4,217 (37.9)
Refugee	29,609 (62.2)	22,714 (62.3)	6,895 (62.1)
[3]Ethnicity			
Karen	20,262 (69.5)	15,588 (69.6)	4,674 (69.3)
Burmese	5,997 (20.6)	4,594 (20.5)	1,403 (20.8)
Other	2,888 (9.9)	2,217 (9.9)	671 (9.9)
Gestational age assessment method			
Ultrasound	29,889 (62.8)	23,464 (64.3)	6,425 (57.8)
Dubowitz	12,690 (26.7)	9,207 (24.3)	3,483 (31.4)
Fundal height measurement	3,780 (7.9)	2,778 (7.6)	1,002 (9.0)

LMP	1,222 (2.6)	1,020 (2.8)	202 (1.8)
[4]**Height in cm, Median [IQR]**	33,810 (71.1)	152 [148-155]	150 [146-153.3]
[5]**Weight* in kg, Median [IQR]**	47,499 (99.8)	50 [45-55]	46 [42.5-51]
[6]**BMI (kg/m^2)**			
Underweight (< 18.5)	3,706 (11.0)	2,534 (9.6)	1,172 (16.2)
Normal (18.5-22.9)	19,043 (56.4)	14,572 (54.9)	4,471 (61.6)
Overweight ((≥ 23)	11,025 (32.6)	9,414 (34.5)	1,611 (22.2)
[7]**Maternal anaemia at first ANC**	4,686 (10.6)	3,376 (9.9)	1,310 (12.6)
Malaria in pregnancy	5,429 (11.4)	3,775 (10.4)	1,654 (14.9)
PE	1005 (2.1)	641 (1.8)	364 (3.3)
Mode of delivery			
Cephalic vaginal delivery	43,818 (92.1)	33,551 (92.0)	10,267 (92.4)
Breech vaginal delivery	725 (1.5)	459 (1.3)	266 (2.4)
Instrumental delivery	910 (1.9)	754 (2.1)	156 (1.4)
Caesarean section	2,057 (4.3)	1,653 (4.5)	404 (3.6)
Others**	71 (0.2)	52 (0.1)	19 (0.2)
Foetal Sex			
Male	24,622 (51.8)	18,808 (51.6)	5,814 (52.3)
Female	22,959 (48.2)	17,661 (48.4)	5,298 (47.7)
Preterm birth	4,076 (8.6)	3,234 (8.9)	842 (7.6)
Stillbirth	395 (0.8)	213 (0.6)	182 (1.6)
[8]**Neonatal death**	475 (1.7)	305 (1.4)	170 (2.7)
[9]**Place of delivery**			
SMRU	33,681 (74.4)	25,965 (74.9)	7,716 (72.8)
Home	7,677 (16.9)	5,631 (16.2)	2,046 (19.3)
Hospital	3,923 (8.7)	3,088 (8.9)	835 (7.9)

Singleton pregnancies which were delivered at estimated gestational age between ≥28 weeks & ≤42 weeks with known outcomes were included in this analysis. Data is shown n (%) unless stated otherwise.

[1-9]Missing data expressed per AGA and SGA (n(%); [1]Smoker (4,836 (13.3), 1,649 (14.8)); [2]Literate (18,313 (50.2), 6,283 (56.5); [3]Ethnicity (14,070 (38.6), 4,364 (39.3)); [4]Height (9,928 (27.2), 3,854 (34.7)) ; [5]Weight (65 (0.2), 17 (0.2)); [6]BMI (9,949 (27.3), 3,858 (34.7)); [7]Anaemia in pregnancy (2,597 (7.1), 731 (6.6)); [8]Neonatal death 14,199 (38.9), 4,818 (43.4)); [9]Place of delivery (1,785 (4.9), 515 (4.6)).

Weight measurement in first trimester was used. BMI at any time during pregnancy was first calculated in 2004. Literacy data was start recorded in 2008 but routine data collection was commenced after 2010.

**Face and compound presentation but delivered vaginally

Abbreviation: ANC antenatal care; BMI body mass index; cm centimetre; IQR interquartile range; kg kilogram; LMP last menstrual period, SGA small for gestational age, SMRU Shoklo Malaria Research Unit

4.5.5 Determinants of SGA

The univariable and multivariable analyses for SGA are presented in **Table 4-4**. The direction of points of estimate are the same in both multivariable models but the magnitudes are slightly lower in model B. The logistic regression model B is assumed as the final model because it has more exposure variables such as BMI, smoking and non-malaria febrile illness than model A. In model B, five risk factors are identified for SGA: primigravida regardless of maternal age, smoking during pregnancy, low BMI, malaria infection in pregnancy and PE. Although maternal anaemia is significantly associated with SGA in model A (AOR 1.13, 95% 1.02-1.24), it was no longer a risk factor for SGA in model B. Primigravida with advanced maternal age (>35 years) was the highest odds of having SGA (AOR 2.46, 95%CI 1.65-3.67, p <0.001), followed by pregnancies with PE that were significantly associated with SGA (AOR 2.30, 95%CI 2.30, p <0.001). Women with a low BMI had 1.6 times higher odds of having an SGA baby compared to normal BMI women (AOR 1.58, 95%CI 1.44-1.74, p <0.001). Conversely, taller and obese women had a decreased risk of SGA in the models (p <0.001). ANC consultation in the first trimester had a protective effect in the univariable analysis but not in both multivariable models.

Table 4-5: Univariable and multivariable analyses for SGA.

		Univariable analysis		Multivariable analysis (Model A)		Multivariable analysis (Model B)	
		OR (95% CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
	N			N=33,540		N=26,104	
Age and Gravidity	47,581						
< 20 Primigravida	6,405	1.67 (1.57-1.78)	<0.001	1.53 (1.41-1.66)	<0.001	1.57 (1.43-1.72)	<0.001
< 20 Multigravida	1,894	1.28 (1.15-1.43)	<0.001	0.91 (0.77-1.06)	0.256	0.91 (0.76-1.10)	0.334
20-34 Primigravida	7,416	1.67 (1.58-1.78)	<0.001	1.85 (1.72-1.99)	<0.001	1.93 (1.78-2.10)	<0.001
20-34 Multigravida	25,286	Reference		Reference		Reference	
≥ 35 Primigravida	172	2.72 (2.00-3.69)	<0.001	2.73 (1.91-3.90)	<0.001	2.46 (1.65-3.67)	<0.001
≥ 35 Multigravida	6,408	1.29 (1.21-1.38)	<0.001	1.41 (1.30- 1.52)	<0.001	1.34 (1.21-1.47)	<0.001
First ANC in 1st Trimester	47,581	0.96 (0.92-0.99)	0.037	0.98 (0.92-1.04)	0.445	0.94 (0.87-1.01)	0.100
Maternal height (per 10 cm)	33,799	0.53 (0.51-0.56)	<0.001	0.56 (0.53-0.59)	<0.001	0.52 (0.49-0.56)	<0.001
Low maternal weight (<40kg)	47,499	2.44 (2.29-2.61)	<0.001	1.89 (1.73-2.06)	<0.001	NA	
BMI (kg/m2)	33,774						
Underweight (< 18.5)	3,706	1.51 (1.40-1.63)	<0.001	NA		1.58 (1.44-1.74)	<0.001
Normal (18.5-22.9)	19,049	Reference		Reference		Reference	

Overweight (≥ 23)	11,025	0.59 (0.56-0.64)	<0.001	NA		0.55 (0.51-0.59)	<0.001
***Maternal anaemia at 1st ANC**		1.30 (1.22-1.40)	<0.001	1.13 (1.02-1.24)	0.015	1.06 (0.94-1.19)	0.322
Malaria infection in pregnancy	47,581	1.51 (1.42-1.61)	<0.001	1.31 (1.19-1.44)	<0.001	1.19 (1.05-1.35)	0.005
Non-malaria febrile illness	24,214						
Non-febrile and no antibiotics	22,945	Reference		Reference		Reference	
Suspected bacterial infection	605	1.06 (0.87-1.29)	0.562	NA		0.98 (0.8-1.2)	0.846
Suspected viral infection	664	1.06 (0.88-1.27)	0.548	NA		0.93 (0.76-1.12)	0.443
PE	47,581	1.89 (1.66-2.16)	<0.001	2.03 (1.74-2.36)	<0.001	2.30 (1.94-2.73)	<0.001
Smoker	41,096	1.76 (1.67-1.86)	<0.001	NA		1.86 (1.7-2.03)	<0.001
PTB	47,581	0.84 (0.78-0.91)	<0.001	0.79 (0.71-0.88)	<0.001	0.82 (0.73-0.93)	0.002
Foetal sex (male)	47,581	1.03 (0.99-1.08)	0.166	0.98 (0.93-1.03)	0.487	1.00 (0.94-1.06)	0.972
Average ambient temp during 1st trimester	47,581	0.99 (0.98-1.01)	0.718	1.01 (0.99-1.03)	0.11	1.01 (1-1.03)	0.122
Average ambient temp during 2nd trimester	47,581	0.99 (0.98-1.00)	0.027	1.01 (0.99-1.02)	0.307	1.01 (0.99-1.03)	0.333
Average ambient temp during 3rd trimester	47,581	0.97 (0.96-0.98)	<0.001	0.99 (0.97-1.00)	0.07	0.99 (0.97-1.00)	0.132

Multivariable models adjusted for living status (refugee or migrant), gestational assessment methods and calendar year (fractional form).

*Maternal anaemia at 1st ANC visit was used and it is defined as haematocrit < 30%.

Abbreviation: ANC antenatal care; BMI body mass index; PE pre-eclampsia; PTB preterm birth; temp temperature

4.5.6 Population attributable risks of SGA

Teenage pregnancy, underweight (BMI<18.5), smoking, malaria infection in pregnancy, and PE were identified as risk factors of SGA, and PARs were calculated using AOR from model A & B (**Table 4-6)**. Between 2004 and 2020, PAR of SGA attributable to smoking was highest: 14.6 per 1,000 births (95%CI 4.3, 8.9) followed by low BMI (13.2, 95%CI 11.5,14.9). PAR of SGA due to PE was lowest: 3.5 per 1,000 births (95%CI 2.7-4.3). Figure 4-6 shows the changes of PARs of SGA every 5 years.

Table 4-6: Population attributable risks (PARs) of SGA.

Risk factors	**Study period 1986-2020 (Based on Model A)**	**Study period 2004-2020 (Based on Model B)**
	N=33,540	**N=26,104**
	PAR per 1,000 births (95% CI)	**PAR per 1,000 births (95% CI)**
Smoking	NA	14.6 (13.8, 18.2)
Underweight (BMI<18.5)	NA	13.2 (11.5, 14.9)
Low maternal weight (<40 kg)	16.9 (14.5, 18.3)	NA*
Teenage pregnancy	8.6 (6.9, 10.2)	7.1 (4.3, 8.9)
Malaria in pregnancy	6.8 (4.2, 8.3)	4.7 (3.3, 6.1)
PE	3.2 (2.5, 3.8)	3.5 (2.7, 4.3)

* PAR for low maternal weight in place of BMI in Model B = 11.2 (9.5, 12.8)

NA, not available; PE, pre-eclampsia

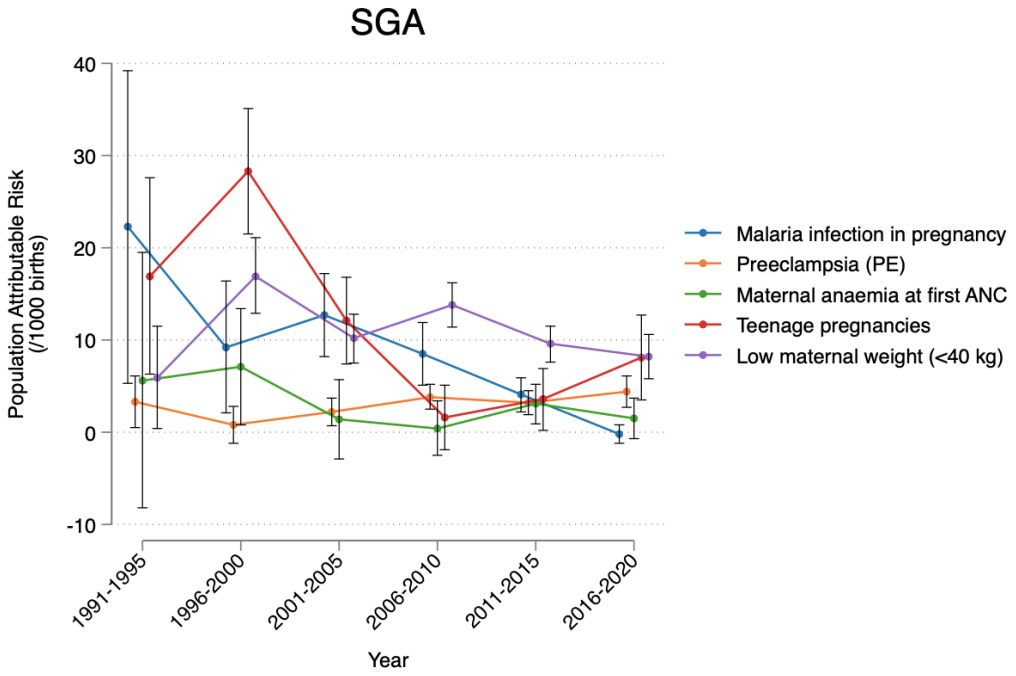


Figure 4-4: Population attributable risks of SGA between 1991-2020.

4.6 Discussion

The overall incidence of PTB of 9.4% (spontaneous to indicated ratio 7:1) was low compared to other resource limited setting[172] while the high incidence of SGA (23.4%) was similar to other regions in Asia[23]. Apart from a history of PTB and maternal anaemia, the teenage and advanced maternal age (<20 & >35 years) regardless of gravidity, smoking during pregnancy, low BMI (<18.5 kg/m^2), and PE were significantly associated with PTB and SGA. Increasing maternal stature (every 10 cm) and obesity (BMI >23.5 kg/m^2) had a protective effect on PTB and SGA (p <0.001). The findings were aligned with previous local and international studies[4, 152, 153, 184]. In the present analysis, suspected bacterial infection increased the risk of early but not late PTB (Appendix 4-1 (Table A4-2 & A4-3)).

A further objective of this chapter was to estimate the PARs of PTB and SGA. The largest sustained reduction in PAR was with malaria infection in pregnancy where PAR fell 35-fold from 1991-1995 to 2000 onwards for PTB and 22-fold for SGA in a similar timeframe to malaria infection in pregnancy. The key reason for this large reduction is the early detection and treatment with artemisinin-combination therapy in the general population since 1994, which reduced the proportion of malaria infection during pregnancy from 14.9% in 1991-95 to 1.4% in 2016-2020. There were no specific preventive measures offered to the general pregnant population over the 30 years[185]. Bryce et al. described that the top five population attributable fraction (PAF) for risk factors of PTB in South Asia region were maternal anaemia (14.9%), indoor air pollution (9.2%), bacterial vaginosis (9.1%), periodontal infection (4.5%) and low BMI (4.2%). Similarly, low BMI and maternal anaemia were attributed to PTB in this study but the PAR of PTB due to maternal anaemia was very low (1.3/1,000 livebirths). The underlying reason for this may be the reduction of malaria infection-related maternal anaemia, routine screening and treatment of worm infestations, and provision of prophylactic iron or treatment (if indicated) with iron and folate supplements throughout the pregnancy as the part of routine ANC.

It is important to note that the PARs for PTB and SGA may potentially be overestimated due to the higher incidence of PTB and SGA. Using relative risk instead of odds ratios could provide a more accurate reflection of the association, especially for outcomes with a higher frequency. However, in this analysis, the PARs was calculated by using the STATA command “regpar”, which proves particularly useful in the

presence of numerous confounders, as opposed to situations involving only a single confounder[186].

The leading population attributable fraction of SGA in South Asia region were maternal nutritional status (30.56%), including short stature, low pre-pregnancy BMI, low gestational weight gain, environment and other exposure (15.27%), such as alcohol consumption, smoking and air pollution, and pregnancy history (11.68%), which includes maternal age <18 years, primigravida and birth interval <18 months[5]. These findings concur with PARs for SGA in this analysis; smoking during pregnancy (14.6/ 1,000 livebirths) followed by low BMI (13.2/1,000 livebirths) and teenage primigravida (7.1/1,000 livebirths).

The two most persistent PARs in relation to PTB and SGA were teenage pregnancies and low BMI when each 5-year PAR was explored. The high prevalence of teenage pregnancy (16.6%) in this setting is likely due to the lack of further educational opportunities for teenagers (male and female) after 16 years of age in the refugee camp, as well as cultural barriers and misconceptions concerning contraceptives for migrant adolescents. Discrimination at pregnancy services for adolescents needs to be reduced to improve access to contraceptive services. Maternal height was associated with both PTB and SGA and requires a generational shift in nutrition to improve. Having a pregnancy with a healthy weight is a good strategy for community engagement in the future given the double burden of malnutrition identified in previous work with both underweight and overweight resulting in adverse pregnancy outcomes. As a part of our preventive measures for preterm birth, cervical length measurements are routinely conducted in our clinics. This involves transabdominal

measurements for all pregnant women, and transvaginal measurements for those at high risk for preterm birth. The cervical length is measured between 18^{+0} and 24^{+0} weeks' gestation, and serial measurements are conducted as necessary. Moreover, the pregnant women presented with threatened preterm labour are screened for UTI and vaginal infection according to SMRU obstetrics guidelines. The clinical and ultrasound information is used to guide the timely administration of tocolytics and corticosteroids, which can help prevent PTB and improve foetal outcomes.

4.6.1 Limitations of this study

The precise causes of infections could not be specified in this analysis The data for some of the risk factors investigated were not available for the entire 30-year period, such as smoking, literacy, BMI, previous history of PTB and non-malaria febrile illness. For this reason, two multivariable logistic regression models (model A & B) were performed to assess the determinants of PTB and SGA separately. Although the sample size was decreased, multivariable logistic regression model B was assumed as the final model for both pregnancy outcomes because it included more risk factors compared to model A. The drawback of using model B is that most of the malaria infected pregnancies, which occurred prior to 2004 (5,706 (52.4%) out of 10,880), were not included. Nevertheless, the point estimates of two models (A & B) were similar and indicate the same direction of the association.

Different gestational age assessment methods were used throughout study period but estimation of gestational age by ultrasound was consistently used after 2000. However, the determinants of PTB were similar in the sub-group analysis of ultrasound-dated pregnancies for PTB (Appendix 4-1, Table A4-1). Defining SGA is challenging

in low-resource settings because of home births, which prevent timely birthweight measurement. For this analysis SGA was determined based on a birthweight measurement within 72 hours of delivery. A recent systematic review mentioned that 7-8% of birth weight changes of term babies can be expected within 72 hours after birth, and therefore the estimate of SGA of 23.3% in this analysis may be an overestimate. Despite the controversy over the use of local references or international standards, the determinants of SGA were the same (Appendix 4-1, Table A4-5). There are likely to be changes occurring over the 30-year period for which data are not available including environmental/chemical exposure, climate change and socio-economic status. Calendar year and average maximum temperature per pregnancy were adjusted for in the analyses to account for temporal confounding and climate change. While socio-economic status is a known risk factor for PTB and SGA, it is assumed that there are no great disparities in these marginalized populations.

4.6.2 Additional considerations and implications for future research

Reducing the incidence of PTB and SGA is crucially important for lowering neonatal and under-5 mortality rates in order to meet Sustainable Development Goal 3 (MDG-3)[187]. Unlike other resource limited setting, accurate gestational age estimation to define PTB and SGA, and the quality of ANC data, are not a challenge for the SMRU ANCs. An urgent intervention for teenage pregnancies, low BMI, smoking and PE will be required to reduce the incidence of PTB and SGA. Dissemination of these findings would be beneficial for other healthcare providers working in resource limited setting especially on the Thai-Myanmar border, stakeholders and policymakers. Although maternal age is not a modifiable risk factor, provision of improved reproductive health

education and services in this border population, especially targeting teenagers and providing family planning services in an effective way at scale, should be carried out to reduce the prevalence of teenage pregnancies. Nutritional support for underweight pregnant women and a smoking cessation programme would be an option to tackle low BMI and smoking-related SGA. Furthermore, many studies have shown that low-dose aspirin is effective in preventing severe PE in high-risk pregnancies[188, 189]. In the SMRU ANCs, low-dose aspirin was introduced in 2020 at 20 weeks' gestation, for women at high risk of developing PE/eclampsia and willing to take it. Even though an association between maternal-foetal infection and both PTB and SGA is well established in high-income countries, the association in LMICs is still poorly understood because diagnosing infection and estimating gestational age accurately remain challenging[47]. Also, future studies should address the additional risk factors, such as climate change, air pollution, attributed PTB and SGA in resource limited setting especially in this vulnerable population. Depending on the identified risk factors, strategic interventions at scale should be considered.

4.6.3 Conclusion

This analysis is a first step, using SMRU ANC data over 30 years, to clarify the determinants and estimate PARs of each risk factor for PTB and SGA. Evidently, the results demonstrate that most of the identified risk factors in this border population are modifiable and strategies to tackle these factors at scale will be required to implement the preventive measures.

Chapter 5

Determinants of foetal growth in the marginalized population: A pooled data analysis of three pregnancy cohort studies

5.1 Introduction

Foetal growth is a critical indicator of the progress of a pregnancy, and it can be utilised to optimise outcomes for mother and baby, as well as understand the impact of exposures during pregnancy. Foetal growth monitoring, through repeated measures during pregnancy, is one of the important objectives of antenatal care (ANC) to make sure that the foetus is growing at an appropriate rate. Regulation of foetal growth is a complex process involving maternal (e.g., hormones), foetal (e.g., genetic factors) and placental factors. A wide range of hormones including insulin and placental growth hormone are responsible for foetal growth during pregnancy[190]. Apart from hormones regulating foetal growth, the placental factor is the main cause involved in impaired foetal growth because the placenta serves as a barrier for transmission of infections and mediates transferring nutrients from mother to foetus[190]. When placental insufficiency or dysfunction (failure to supply adequate nutrients and oxygen) develops, the umbilical blood flow to foetus is diminished resulting in foetal growth disruption[191]. Foetal growth is also influenced by genetic, epigenetic, environmental and maternal factors, which have both positive and negative effects on foetal growth.

Historically, low birth weight (LBW) and small for gestational age (SGA), both of which are measured at birth, were used as a proxy to describe intra-uterine growth restriction particularly in low- and middle-income countries (LMIC)[192]. LBW requires

accurate and timely weight measurement of the newborn and SGA requires accurate assessment of gestational age in addition to the birth weight. While most LMIC report LBW, less report SGA which also means there are limitations to define accurately impaired foetal growth[193]. Accurate gestational assessment and repeated ultrasound measurements are essential to assess foetal growth (such as head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL)) and the doppler indices[194].

In Chapter 4, I have shown that some (co-)morbidities were associated with a higher risk of SGA. Among them, pre-eclampsia (PE) and malaria in pregnancy are of particular importance as they are modifiable. The impact of PE on adverse pregnancy has been well-established and the effect is more significant in severe and early onset PE[195]. The deleterious effects of malaria infection on the adverse pregnancy outcomes, such as miscarriage, low birth weight, preterm birth, small for gestational age and stillbirths are also established[52, 100, 103, 156]. These adverse effects are considered higher in lower transmission areas where premunition, or effective maternal antimalarial immunity, is low[52]. Similarly, some studies reported the impact of malaria infection on the intrauterine foetal growth considering different foetal biometrics and timing of malaria in relation to the ultrasound scan. A recent report from "*Preconceptional Retard de Croissance Intrauterin et Paludisme* (RECIPAL)" cohort study from South Benin, indicated that maternal BMI, parity and newborn sex influenced foetal growth, but no significant association was found between first trimester malaria infection and adjusted z-scores of the foetal biometrics. However, third trimester malaria was more likely to have an impact on HC, BPD and AC rather than FL[196, 197].

This chapter explores the impact of maternal characteristics and morbidity during pregnancy (with a particular focus on malaria infection during pregnancy and PE) on foetal growth in marginalized populations along the Thai-Myanmar border, where transmission of malaria is low and seasonal. The primary objectives are 1) to assess the maternal factors associated with foetal growth (AC z-score), 2) to detect the effect of malaria infection in pregnancy and PE on foetal growth (AC z-score). The secondary objective is to assess the effect of malaria infection in pregnancy and PE on foetal HC, BPD and FL z-scores. The rationale for using foetal AC z-score as the primary outcome is that AC measurement is the most sensitive parameter to detect the foetal growth trajectories compared to other biometrics measurement[198, 199].

5.2 Methods

5.2.1 Study sites and population

The Population and method section (Chapter 2) details the information about study setting, overview of border population, profiles of the three pregnancy cohort studies conducted at SMRU, and data collection and management. The three pregnancy cohort studies were: 1) Impact of malaria infection in pregnancy on foetal and new-born growth (UPS study)[152], 2) INTERBIO-21st (foetal study)[131] and 3) Molecular signature in pregnancy (MSP study)[109] all had similar eligibility criteria, a process for foetal growth monitoring, prospective follow up from first trimester to birth and systematic data collection of maternal and obstetric characteristics at baseline and throughout pregnancy. The detailed information for each pregnancy cohort was previously mentioned in Chapter 2 (**Table 2-3 & 2-4**).

5.2.2 Procedure

The pregnant women attending SMRU antenatal care clinic in the first trimester between 9^{+0} and 13^{+6} weeks' gestation, with a maternal age more than 18 years, willing to participate in the study and to comply with study procedures, were invited to participate. Multiple pregnancies and known foetal congenital anomaly were excluded. All pregnant women were given detailed information (written documents and verbal explanation) of the study including study aims, procedures, schedule of follow-up visits, advantages and disadvantages of participating, in appropriate language either Karen or Burmese. Maternal information at enrolment containing maternal characteristics, medical and obstetrics history (past and current pregnancies), and socio-economic status was recorded in case record forms. At every follow-up visit, the physical and obstetric examination plus foetal growth scan were done and any adverse events were captured. Furthermore, pregnancy outcomes and biological samples (maternal blood, cord blood and placental tissue) were collected at the time of delivery.

Crown-rump length (CRL) was measured between 9^{+0} and 13^{+6} weeks' gestation and gestational age was estimated using the Robinson and Fleming formula[200]. This baseline enrolment criteria and method of the assessment of gestation in early pregnancy were fundamental requirements of these foetal growth studies. The participants were then scheduled for five-weekly scans (± one week) to assess the foetal growth after the dating scan within the possible ranges of 14-18, 19-23, 24-28, 29-33, 34-38, and 39-42 weeks' gestation. At each follow-up visit, foetal biometrics such as HC, BPD, AC and FL were measured three times and umbilical artery doppler examination was performed. In UPS study, extra scans were performed 2 weeks after

each malaria infection as it mainly focused on impact of malaria infection and foetal growth. The number of the ultrasound scan per patient for each study is shown in Figure 5-1.

The ultrasound technique for foetal biometric measurements of these cohorts were followed the INTERGROWTH-21st ultrasound manual[29]. Briefly, the HC and BPD were measured at the level of the thalami, and the callipers were correctly placed on the outer-to-outer border of the parietal bones at the widest part of the skull bone for BPD and on the outer border of the skull bone for HC measurement. The foetal head should be oval in shape, big enough to be visible (at least 30% of the ultrasound monitor), and the thalami should be symmetrically seen on either side of the midline. For AC measurement, the foetal abdomen had to be as circular as possible on cross-sectional view (using less pressure on the transducer to achieve the circular shape), and the calliper placement on the outer border of the abdomen must be correct. In addition, the umbilical vein at the anterior one-third of the abdomen and the stomach bubble must be visible, together with the foetal spine at either the 3 o'clock or 9 o'clock position. However, the kidney and bladder should not be seen. The foetal thigh should be the closest to the ultrasound probe in the horizontal plane, and the full length of the femur bone should be clearly visible. Again, the correct placement of the callipers (on the outer-to-outer border of the femoral diaphysis) and a large image (at least 30% of the monitor) are needed.

The pregnancy dating and follow-up scans were performed by locally trained sonographers using GE Voluson-i and Philips HD-9 ultrasound machines, and their quality control assessment was done regularly[124].

5.2.3 Assessment of exposures

Definitions of exposure of interests, i.e., malaria infection in pregnancy, PE, smoking and additional covariate such as maternal age, gravidity, ethnicity, body mass index, foetal sex, and pregnancy outcomes were also provided in **Chapter 2** (Methods).

Briefly, malaria infection was defined as the presence of asexual parasites in the peripheral blood (per 500 leukocytes or 1000 erythrocytes) detected by microscopy. Malaria screening was carried out at every ANC visit. For each malaria episode, additional variables were collected: date; antimalarial treatment; plasmodium species; symptoms (fever or history of fever in the previous 48 hours). If malaria parasites were detected, women were treated with supervised antimalarial treatment (chloroquine for vivax malaria, and quinine plus clindamycin for falciparum malaria in the first trimester and artemisinin-based treatment in second and third trimester).

Pre-eclampsia was defined by the new onset of hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) that occurred after 20 weeks' gestation with proteinuria (≥1+ on a urine dipstick), without alternative explanation. For women with a history of chronic hypertension, pre-eclampsia was defined as the onset of new proteinuria after 20 weeks' gestation.

5.2.4 Outcomes of interests

The primary outcome included AC z-score at different time point. Secondary outcomes include z-scores of HC, BPD and FL at different gestational age. Foetal biometric (HC, BPD, AC and FL) z-scores were calculated using INTERGROWTH-21st standard[29].

5.2.5 Eligibility

Women who miscarried, or with no repeated ultrasound measurements (<2 measurements) were excluded from this analysis (Figure 5-1). For stillbirths, ultrasound records were excluded when foetal heartbeat was not detectable at the time of ultrasound scan.

5.2.6 Statistical analysis

Baseline maternal and foetal characteristics of each pregnancy cohort were described by plotting the raw data of foetal biometrics (HC, BPD, AC and FL) with percentile reference lines using INTERGROWTH-21st charts[29]. Also, these raw data stratified by malaria species (PF, PV) and PE were plotted.

In this analysis, three-level mixed-effects linear regression was performed to assess the association between AC z-score and maternal characteristics and environmental factors. The data comprised three levels: one ultrasound measurement for each visit for each pregnant woman (level 1: record); multiple (repeated) ultrasound measurements of pregnant women over pregnancy (level 2: woman); and three pregnancy cohort studies (level 3: cohort). Random intercept was applied to these three levels.

After conducting the univariable analyses, three different multivariable models were built for the different objectives listed above. Firstly, as an exploratory analysis, any factors statistically associated with AC z-score were identified. All variables with p-value of <0.05 were included in the multivariable model. The second and third models specifically focused on assessing the impact of PE and malaria infection in pregnancy, respectively. The selection of confounders in the final multivariable models for PE and

malaria infection in pregnancy was identified by drawing directed acyclic graphs (DAG)[201]. The confounders identified *a priori* for PE and malaria infection in pregnancy were shown in Figure 5-3 & 5-4. In all three models, estimated gestational age and calendar year were always adjusted.

PE was assumed to be present for the whole pregnancy: in other words, PE was handled as non-time-dependent variable based on established pathophysiology[202].

Malaria infection was handled as time-dependent variables (i.e. for each ultrasound scan, the malaria infections occurred before the scan were accounted), differentiated by species (PF and PV), to examine the effect of malaria infection on foetal growth. In the first and second models, malaria infection at any gestational age before the ultrasound measurement was considered. In the third model, further analyses were conducted to assess whether there were any temporal changes of the impact of malaria infection. Malaria infection was grouped by the timing before the ultrasound scan: malaria occurred 1, 2, 3, 4, 5, 6, ≥7 weeks before the measurement. Falciparum (mono-infection or co-infection of falciparum and other species) and vivax (and other non-falciparum malaria) were differentiated. If any malaria occurred after an ultrasound scan, that measurement was regarded as "no malaria before the scan". Because the number of malaria episodes was reported to affect the foetal growth, a sensitivity analysis was conducted by excluding the ultrasound measurements which were exposed to malaria two or more times. In this analysis, only measurements which were exposed to one episode of malaria or never exposed were included.

Maternal age & gravidity were combined as one variable as there is a positive correlation between them. Calendar year was adjusted using fractional polynomial methods.

5.2.7 Ethics approval

UPS and INTERBIO 21st study were approved by Oxford Tropical Research Ethics Committee (OXTREC: 14-08) and Faculty of Tropical Medicine Ethics Committee (FTMEC: 2008-028). MSP study was granted by Oxford Tropical Research Ethics Committee (OXTREC: 33–15) and Faculty of Tropical Medicine Ethics Committee (FTMEC: 15–062).

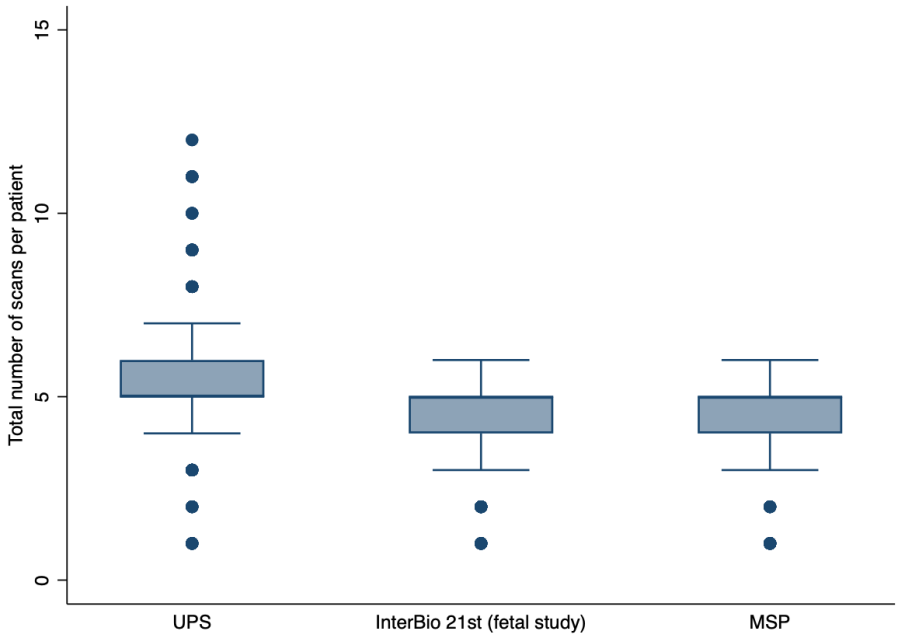


Figure 5-1: The number of ultrasound scans per patient.

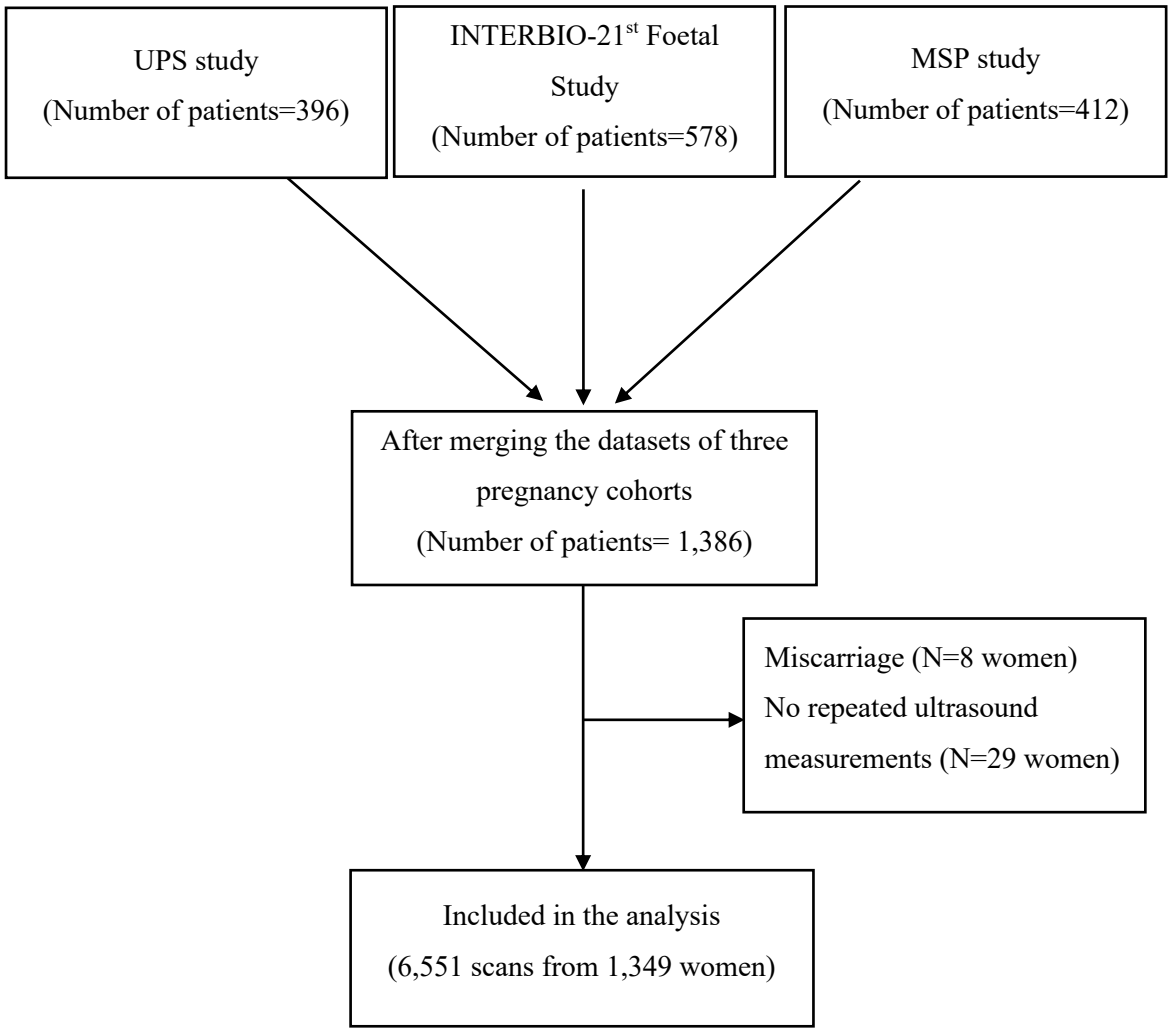


Figure 5-2: Flow chart of data analysis.

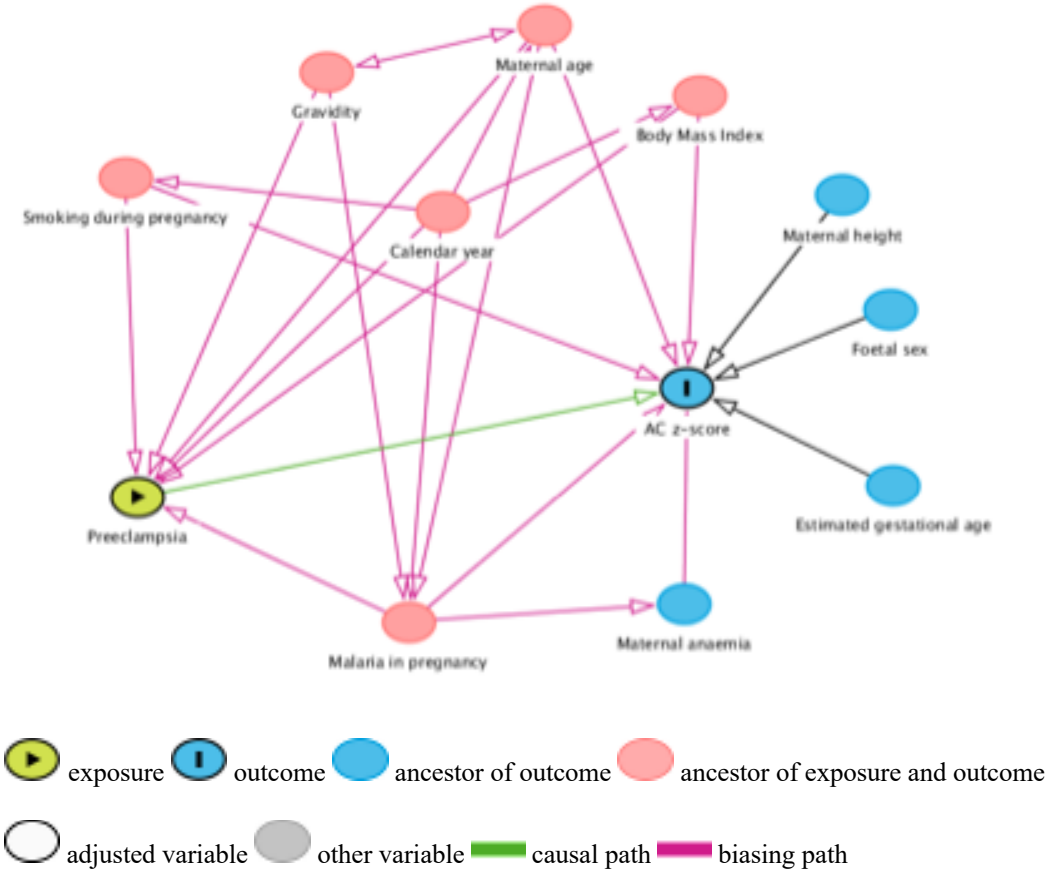


Figure 5-3: Causal pathway diagram of PE and AC z-score.

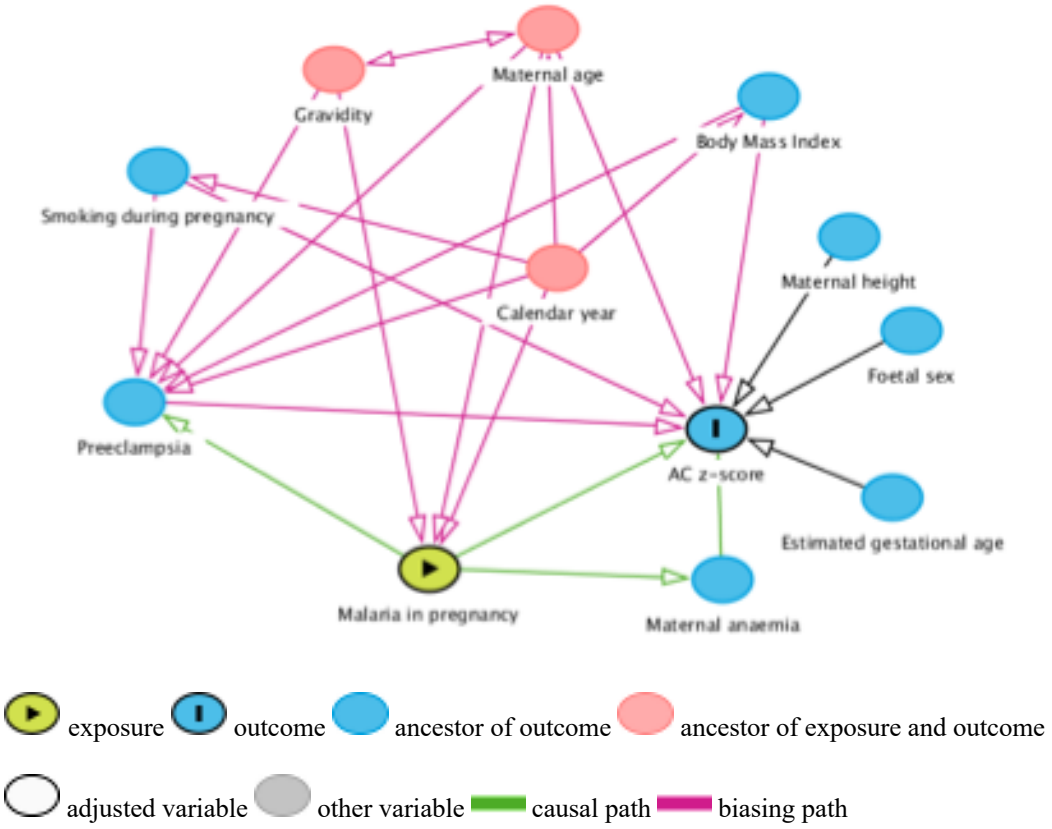


Figure 5-4: Causal pathway diagram of malaria infection in pregnancy and AC Z score.

5.3 Results

After merging the datasets of three pregnancy cohort studies, a total of 6,601 scans from 1,386 women were available. After excluding miscarriages and scans with less than two ultrasounds (Figure 5-2), a total of 1,349 patients (6,551 scans) were eligible. The baseline characteristics of pregnant women and birth outcomes were similar across the three prospective pregnancy cohorts (**Table 5-1**). The median age of the study population was 25 years [IQR 21-30], and approximately 30% of pregnant women were primigravidae. The median maternal height and body mass index were 152 cm [IQR 148-155] and 20.5 kg/m^2 [IQR 18.7-23.0], respectively.

A total of 9.5% (128/1,349) of pregnant women had at least one episode of malaria infection, including 1.9% (25/1,349) with falciparum malaria and 7.6% (103/1,349) with vivax malaria. Of the total number of malaria cases, 60.9% (78/128) of the first malaria infection were detected in the first trimester (<14 weeks' gestation), 27.3% (35/128) in the second trimester, and 11.7% (15/128) in the third trimester respectively. Additionally, 62.5% (80/128) of the malaria cases had multiple malaria episodes (>2) and 26.3% (21/80) of them had both falciparum and vivax malaria episodes (separately) during pregnancy. The proportion of pregnancies complicated by preeclampsia was 1.8% (25/1,349).

In general, the population falls below the 50th percentile of the INTERGROWTH-21st reference charts for all foetal biometric measurements (Figure 5-6). The raw data for abdominal circumference scatterplots (Figure 5-7) for malaria reveal a low number of *P. falciparum* infections compared to *P. vivax*, and a low number of PE cases. The scatterplots suggest abdominal circumference measurements are more frequently below the 50th percentile for *P. falciparum* and PE, compared to non-malaria and non-PE respectively, but not for *P. vivax*.

Table 5-1: Baseline characteristics of three pregnancy cohorts.

	UPS (N=387)	INTERBIO 21st (N=566)	MSP (N=396)	Combined three cohorts (N=1,349)
Maternal age in years, Medium [IQR]	26 [21-30]	25 [21-30]	25 [21-30]	25 [21-30]
Primigravida	122 (31.5%)	186 (32.9%)	108 (27.3%)	416 (30.8)
Maternal Height (cm), Medium [IQR]	152 [148-156]	152 [148-155]	152 [148-155]	152 [148-155]
BMI (kg/m^2), Medium [IQR]	20.2 [18.6-22.2]	20.5 [18.8-22.5]	20.5 [18.9-23.2]	20.4 [18.8-22.6]
Smoker	46 (11.9)	56 (10.0)	30 (7.6)	132 (9.8)
Literacy (can read)*	156/252 (61.9)	371 (65.6)	255 (64.4)	782/1214 (64.4)
Maternal anaemia	14 (3.6)	8 (1.4)	1 (0.3)	23 (1.7)
Malaria infection in pregnancy*				
Non-malaria	291 (75.1)	540 (95.4)	394 (99.5)	1221 (90.5)
PF	18 (4.7)	7 (1.2)	NA	25 (1.9)
PV	78 (20.2)	19 (3.4)	6 (1.5)	103 (7.6)
PE	12 (3.0)	9 (1.6)	4 (1.0)	25 (1.8)

Number of scans per patient, Medium [IQR]	5 [5-6]	5 [4-5]	5 [4-5]	5 [4-5]
Birthweight (g), mean (SD)	2,927 (543)	3,015 (494)	3,021 (526)	2,994 (518)
Foetal sex* (male)**	197/367 (53.7)	272/535 (50.8)	184/381 (48.0)	653/1,283 (50.8)

Number (%) were described for categorical variables. Medium [IQR] and mean (SD) were presented for continuous variables. The denominators (the numbers assessed) were 387 (UPS), 566(IB), 396 (MSP) and 1,349 (total) unless otherwise specified.

Abbreviation: BMI, body mass index; IQR, Interquartile range; PE, pre-eclampsia; PF, *Plasmodium falciparum*; PTB, preterm birth; PV, *Plasmodium vivax*; SGA, small for gestational age

*Malaria infection before the last ultrasound scan were taken account. 5 malaria cases were occurred after the last scan and regarded as non-malaria.

*135 of literacy data was missing in UPS study

*** foetal sex status was missing 20 in UPS, 31 in IB-21st, 15 missing in MSP study because of lost follow up. Hence, there were no outcomes.

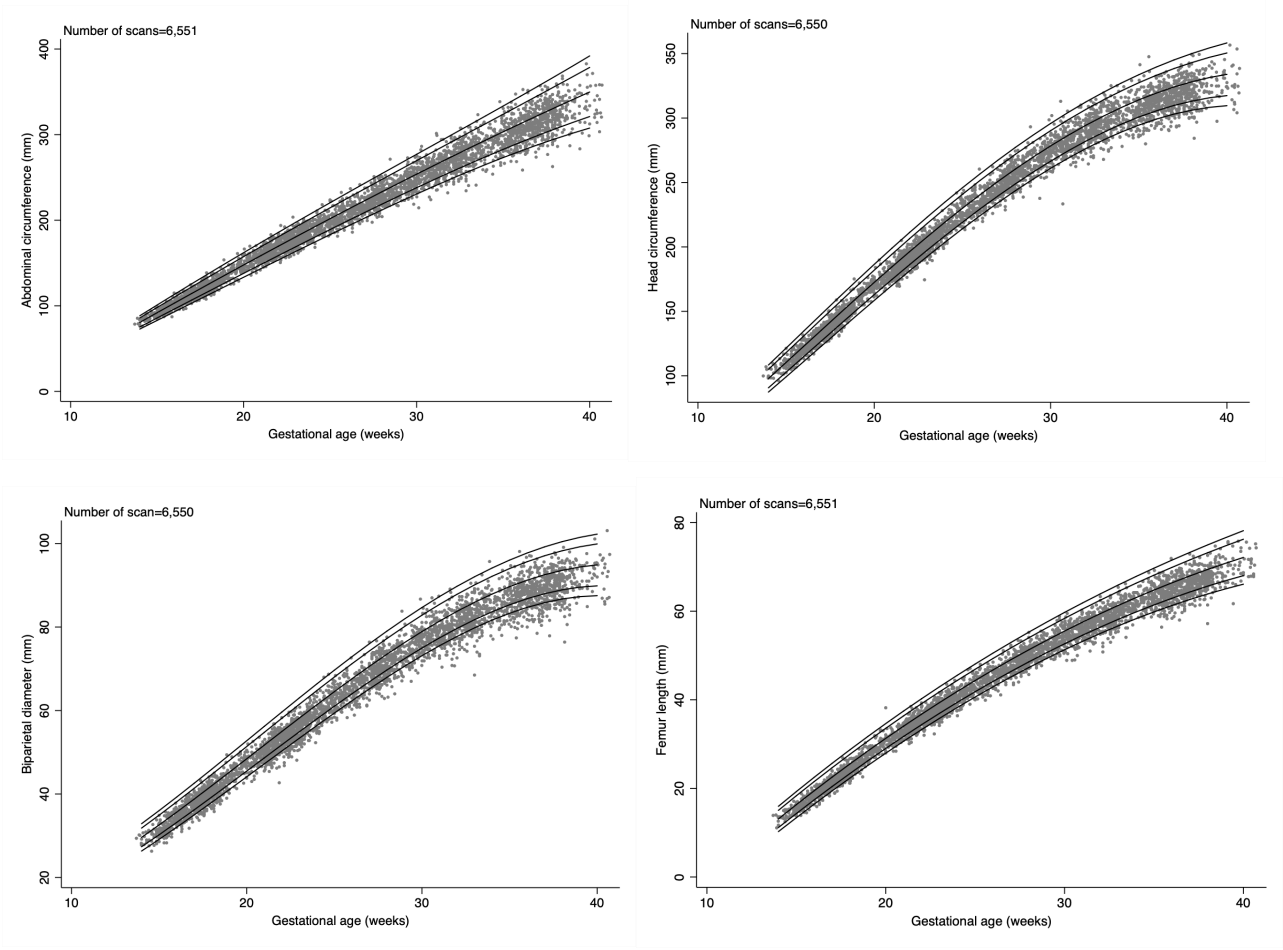


Figure 5-5: Scatterplots of foetal biometrics (AC, HC, BPD and FL) with reference lines based on INTERGROWTH 21st standard.

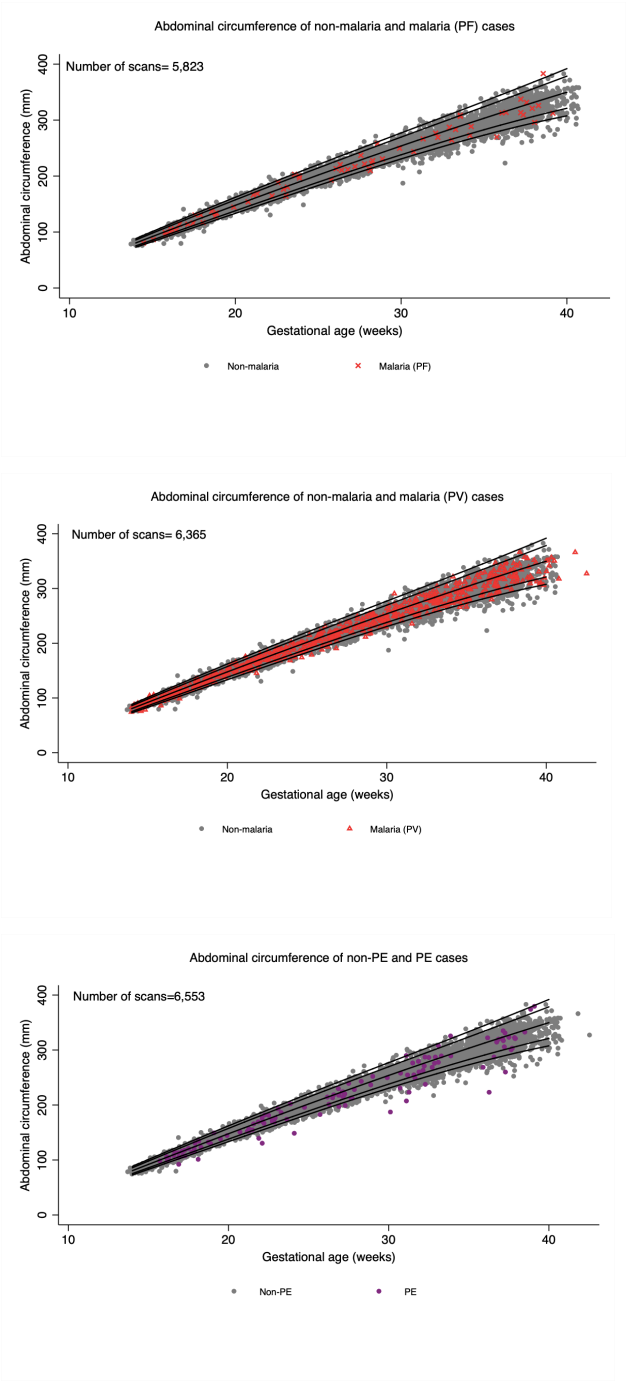


Figure 5-6: Scatterplots of abdominal circumference by the status of malaria infection in pregnancy and PE with INTERGROWTH 21st standard.

5.3.1 Association between maternal factors and foetal growth

Women who were overweight and mothers with a male foetus were factors significantly associated with increased mean predicted AC z-scores in both univariable and multivariable models ($p < 0.05$). The univariable analysis showed teenage primigravida was significantly associated with lower AC z-scores but it was no longer significant in the multivariable model (**Table 5-2**).

Higher HC z-scores were also observed in teenage pregnancy regardless of gravidity ($p < 0.05$). Taller maternal height women and mothers with a male foetus were factors significantly associated with increased HC z-scores. Similarly, significantly higher z-scores of BPD and FL were found in taller and in overweight mothers (**Table A5-2 & A5-3, Appendix D**).

Table 5-2: Univariable and multivariable three-level mixed linear regression analysis of AC z score and maternal characteristics.

			Univariable analysis		Multivariable analysis	
	Number of patients	**Number of scans**	**Mean predicted AC z-score (95% CI)**	**p-value**	**Mean predicted AC z-score (95% CI)**	**p-value**
Age and gravidity						
< 20 years, primigravida	148	701	-0.15 (-0.28, -0.02)	0.020	-0.10 (-0.23-0.03)	0.122
< 20 years, multigravida	47	225	0.06 (-0.17, 0.26)	0.576	0.13 (-0.08-0.34)	0.216
20-34 years, primigravida	265	1,275	-0.05 (-0.15, 0.06)	0.382	-0.05 (-0.15-0.05)	0.34
20-34 years, multigravida	741	3,627	Reference		Reference	
≥ 35 years, primigravida	3	15	-0.36 (-1.19, 0.47)	0.393	-0.31 (-1.10, 0.47)	0.435
≥ 35 years, multigravida	145	708	0.07 (-0.06, 0.19)	0.319	0.08 (-0.04-0.21)	0.203
BMI (kg/m^2)						
Underweight (< 18.5)	279	1,351	-0.08 (-0.18, 0.02)	0.118	-0.09 (-0.18, 0.01)	0.091
Normal (18.5-22.9)	770	3,770	Reference:		Reference	
Overweight (≥ 23)	300	1,430	0.22 (0.12, 0.32)	<0.001	0.20 (0.10, -0.30)	<0.001

Maternal height (per 10 cm)	1,349	6,551	0.04 (-0.04, 0.12)	0.288	NA	
Smoker	1,349	6,551	-0.02 (-0.15, 0.11)	0.720	NA	
Maternal anaemia	1,349	6,551	0.21 (-0.09, 0.52)	0.173	NA	
***Malaria infection**						
Non-malaria	1,216	5,671	Reference		NA	
PF	25	161	-0.06 (-0.33, 0.34)	0.694	NA	
PV	103	719	-0.07 (-0.22, 0.07)	0.325	NA	
PE	1,349	6,551	-0.28 (-0.57, 0.01)	0.059	-0.33 (-0.61, -0.06)	0.018
Foetal sex (Male)	1,266	5,947	0.40 (0.32, 0.48)	<0.001	0.39 (0.32, 0.47)	<0.001

*non-time dependent (any malaria infection during pregnancy before the last ultrasound scan, regardless of timing of malaria).

Number of patients=1,283 and number of scans=6,345 in multivariable model. The best fit model was based on backward elimination using p-value of 0.05 as the cut-off. Gestational age, and calendar year were adjusted in the multivariable model.

Abbreviation: AC abdominal circumference; BMI body mass index; NA not applicable; PE pre-eclampsia; PF *plasmodium falciparum*; PV *plasmodium vivax*

Table 5-3: Univariable and multivariable three-level mixed linear regression analysis of PE and AC z-score.

	Univariable analysis				Multivariable analysis			
	Number of patients	**Number of scans**	**Mean predicted AC z-score (95% CI)**	**p-value**	**Number of patients**	**Number of scans**	**Mean predicted AC z-score (95% CI)**	**p-value**
PE	1,349	6,551	-0.28 (-0.57, 0.01)	0.059	1,349	6,551	-0.33 (-0.62, -0.05)	0.023

*The cofounder identified by DAG such as Age and Gravida, smoking, BMI, malaria infection, and calendar year were adjusted in the multivariable model.

Abbreviation: AC, abdominal circumference; CI, confidence interval; PE, preeclampsia

5.3.2 Effect of PE on foetal growth

The pregnant women with preeclampsia had negative associations with z-scores of HC, BPD, AC, and FL in univariable analyses, which were significant ($p < 0.05$). After adjusting for the confounders according to DAG, PE was found to be significantly associated with lower AC z-scores ((-0.33 (95%CI -0.62, -0.05), p=0.023)). Additionally, PE was also associated with lower FL z-scores ((-0.33 (95%CI -0.60, -0.05), p=0.022)) but not with HC (-0.21 (95%CI -0.47, 0.05), p=0.101) and BPD z-score ((-0.11 (95%CI -0.39, 0.18, p=0.459)) in multivariable analyses (refer to **Appendix D** for details).

5.3.3 Effect of malaria infection in pregnancy on foetal growth

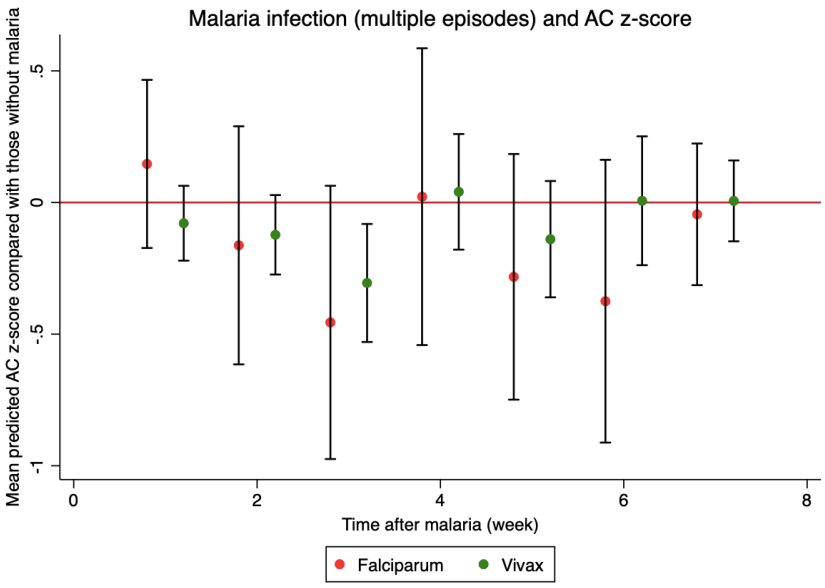
Table 5-4 presents the association between the timing of AC measurements after malaria infection with reference to measurements without malaria. Although the AC z-score decreased after malaria infection in both malaria species, the significant association was only seen in vivax malaria (N=37 scans, three weeks after infection), not falciparum malaria (N=6 scans, three weeks after infection). The mean predicted AC z-score significantly decreased two weeks (-0.16 (95% CI -0.31, -0.01), p=0.038) and three weeks after *P. vivax* (-0.33 (95% CI -0.55, -0.10), p=0.005) compared to non-infected women in univariable analysis. The association remained significant in the multivariable model, particularly for three weeks after vivax malaria (-0.31 (95%CI -0.53, -0.08), p=0.008). Nevertheless, no significant associations were found when the analysis was restricted to patients who had a single episode of malaria infection (Figure 5-7). There were no significant associations between the HC and BPD z-scores and the timing of malaria infection (**Table A5-7, A5-8 in Appendix D**). However, the model predicted a significant decrease in the mean FL z-score seven weeks after falciparum (-0.25 (95%CI -0.50, -0.01), p=0.045) and two

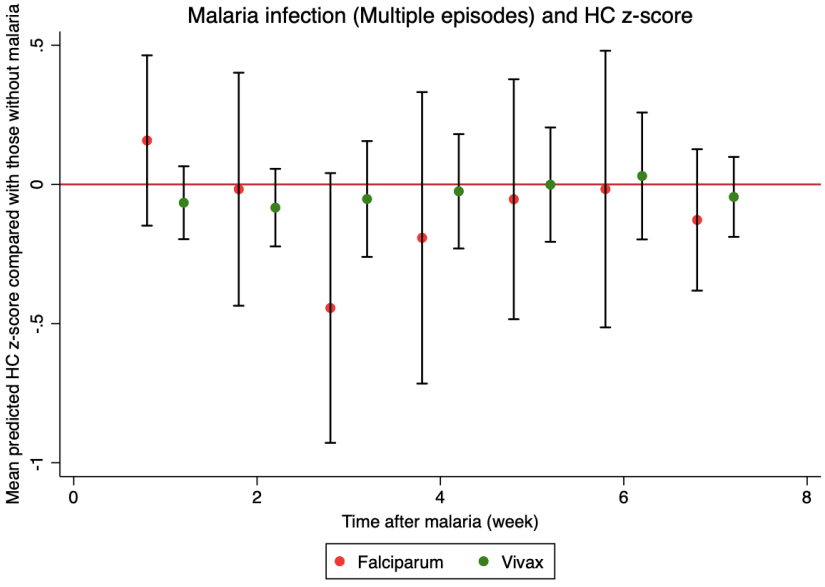
weeks after vivax malaria compared to non-malaria cases (-0.13 (95%CI -0.26, -0.01), p=0.040). Detailed information is available in **Table A5-9** (**Appendix D**).

Table 5-4: Univariable and multivariable linear regression assessing the timing of AC measurements after malaria infection with reference to measurements without malaria before.

AC z-score		**Univariable analysis**		**Multivariable analysis***	
	Scans	Mean predicted AC z-score (95% CI)	**p value**	Mean predicted AC z-score (95% CI)	**p value**
Falciparum malaria infection (PF)					
No malaria infection before	6,372	Reference		Reference	
One week after PF	18	0.19 (-0.13, 0.50)	0.247	0.15 (-0.17, 0.47)	0.368
Two weeks after PF	8	-0.10 (-0.55, 0.35)	0.649	-0.16 (-0.60, 0.30)	0.481
Three weeks after PF	6	-0.43 (-0.95, 0.09)	0.106	-0.45 (-0.97, 0.06)	0.085
Four weeks after PF	5	0.01 (-0.55, 0.57)	0.976	0.02 (-0.54, 0.59)	0.939
Five weeks after PF	8	-0.25 (-0.71, 0.22)	0.297	-0.28 (-0.75, 0.18)	0.236
Six weeks after PF	6	-0.42 (-0.96, 0.11)	0.120	-0.37 (-0.91, 0.16)	0.171
Seven weeks or more after PF	116	-0.07 (-0.33, 0.20)	0.627	-0.04 (-0.31, 0.22)	0.744
Vivax malaria infection (PV)					
No malaria infection before	5,846	Reference		Reference	
One week after PV	182	-0.11 (-0.25, 0.04)	0.144	-0.08 (-0.22, 0.06)	0.277
Two weeks after PV	146	-0.16 (-0.31, -0.01)	0.038	-0.12 (-0.27, 0.03)	0.111
Three weeks after PV	37	-0.33 (-0.55, -0.10)	0.005	-0.31 (-0.53, -0.08)	0.008*
Four weeks after PV	41	0.01 (-0.21, 0.23)	0.924	0.04 (-0.18, 0.26)	0.717
Five weeks after PV	42	-0.18 (-0.40, 0.04)	0.113	-0.14 (-0.36, 0.08)	0.217
Six weeks after PV	31	-0.01 (-0.26, 0.23)	0.909	0.01 (-0.24, 0.25)	0.958
Seven weeks or more after PV	214	-0.09 (-0.25, 0.06)	0.237	0.01 (-0.15, 0.16)	0.937

*In multivariable analysis, the cofounders identified by DAG, such as age, gravida, and calendar year were adjusted.


Malaria infection (multiple episodes) and AC z-score
Mean predicted AC z-score compared with those without malaria
-1
-.5
0
.5
0
2
4
6
8
Time after malaria (week)
Falciparum
Vivax


Malaria infection (Multiple episodes) and HC z-score
Mean predicted HC z-score compared with those without malaria
-1
-.5
0
.5
0
2
4
6
8
Time after malaria (week)
Falciparum
Vivax

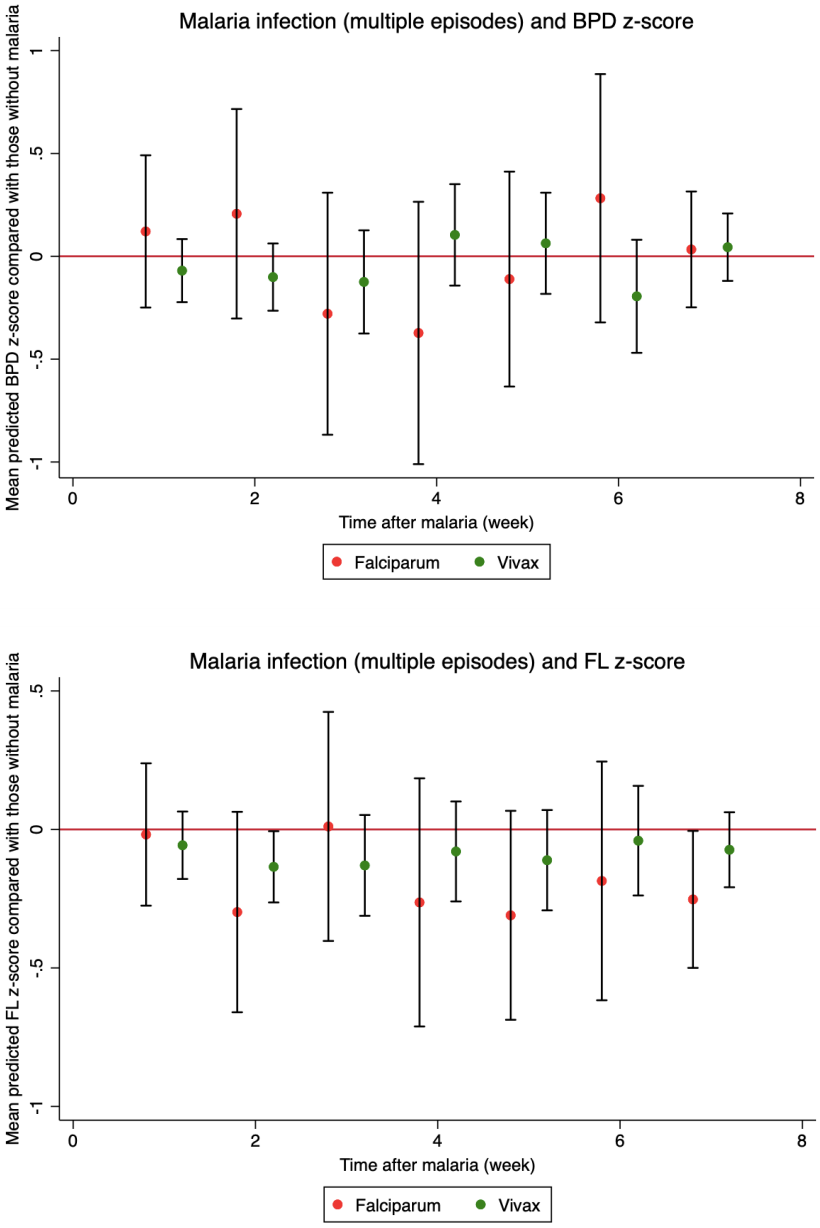


Figure 5-7: Mean predicted difference in AC, HC, BPD and FL z-scores of malaria infected (multiple episodes) pregnancies, stratified by malaria species and the interval between malaria and ultrasound measurement, with reference to those in women without malaria before.

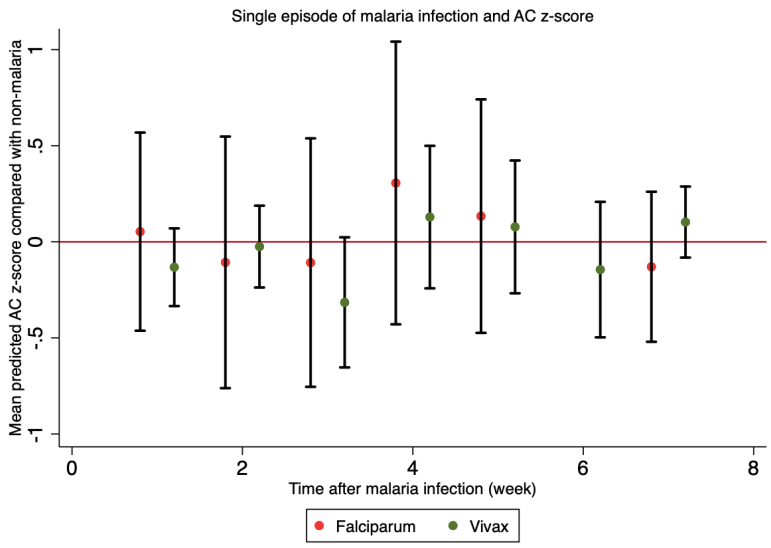


Figure 5-8: Mean predicted difference in AC z-score of measurements after single malaria infection for each malaria species compared with those without malaria.

5.4 Discussion

This chapter provides the determinants of foetal growth and the impact of malaria infection in pregnancy and PE on foetal growth. AC z-score was the primary outcome and other foetal biometric measurements (HC, BPD and FL) were secondary outcomes following accurately dated gestation and 5 weekly foetal growth scans. The estimated foetal weight (EFW) and AC are used to diagnose the foetal growth alterations and there is no consensus on which measurement is better[199]. EFW is calculated based on HC, BPD, AC and FL measurements and associated with the errors in an estimation of foetal size and growth particularly if the foetal weight is less than 1500 g or more than 4000 g[203]. On the other hand, AC is a sensitive and reliable measure of foetal growth, which is less influenced by other factors such as foetal position rather than other biometric measurements (HC, BPD, and FL). Additionally, AC measurement is relatively easy to obtain and can reflect the growth of liver and subcutaneous fat, both of which are affected by diminished oxygen and nutrient supply to the foetus[199, 203, 204]. The use of z-scores in this study also allows for

the comparison of foetal growth across different gestational age, making it a useful tool for providing valuable insights into the factors influencing the foetal growth and development.

The study found that higher mean AC z-score was significantly associated with overweight pregnant women, and male foetus. Similarly, teenage primigravid, taller and overweight women and male foetus were positively associated with mean HC and BPD z-scores. The significant association between higher HC and BPD z-scores, and teenage pregnancy in this study is unexpected. Typically, teenage pregnancies are linked to adverse perinatal outcomes. This finding prompts questions about the underlying mechanisms. Further research is needed to explore factors influencing foetal growth in teenage pregnancies. The unexpected nature of this result underscores the complexity of these dynamics and highlights the need for caution in interpreting and applying these findings in clinical practice until additional research replicates and elucidates the observed relationship. Furthermore, the taller and overweight women have a positive impact on mean FL but there was no foetal sex difference. There are few studies examining foetal growth in malarious areas. A similar result to this pooled cohort has been reported by a recent study conducted in Beninese women[197], who had *falciparum* malaria in first trimester (number of malaria infected pregnancy=45).

Furthermore, lower AC and FL z-scores was significantly associated with the pregnancies complicated by PE but not with HC & BPD z-scores. The severity and onset of PE were not considered in the analysis. A possible explanation could be a “brain sparing” effect, which is an adaptative mechanism and it occurs when the foetus is deprived of sufficient oxygen and nutritional supply, prioritizing the blood flow to the vital organs including the brain, heart, and adrenal gland. This leads to a redistribution of blood flow away from other organs, including the kidneys, liver, and gastrointestinal tract, which are less critical for immediate survival[205]. As pre-

eclampsia affects the development of spiral arterioles since early pregnancy[100, 202] , a further chronic "brain sparing" effect on FL may be possible.

The effect of malaria on mean AC z-score trended downwards at weeks two and three post malaria infection (multiple episodes) for both species. The effect was significant for vivax but not falciparum malaria at three weeks post malaria infection (Figure 5-8). This pattern of a transient decrease in mean AC z-score in both malaria species, was followed by a quick recovery at four weeks after malaria infection; suggesting the ability of the foetus to recover from the impact of infection when the mother is treated.

In the subgroup analysis including the single episode of both species, there were no significant associations between malaria infection and AC z-score although the trend observed at one, two and three weeks post malaria infection is apparent (Figure 5-8). Caution is needed to interpret this subgroup analysis results of no association: when measurements after single malaria episode was included, more than half of exposed women had malaria in the first trimester (57/101 in vivax, 18/29 in falciparum). The foetal growth observations in this study are compatible with the previous finding by Moore et. al. on the number and timing of malaria episodes with a positive linear association observed with increasing number of episodes of malaria, in both species, and an increased odds of SGA and preterm birth[103]. In that study, the impact of malaria in early pregnancy did not increase the risk of SGA whereas it was prominent in later pregnancy (i.e., in the third trimester). Other African studies using the longitudinal ultrasound data found that malaria infection in first trimester or before 20th weeks' gestation did not have the impact on foetal growth[196, 197].

Although Rijken et. al. concluded that the expected foetal BPD was significantly lower in both malaria species, which occurred in first half of pregnancy, compared to non-malaria pregnant women, this study did not find an effect of malaria on foetal head size. However, the significant

effect of falciparum and vivax malaria on mean FL z score were observed at seven weeks and two weeks after the infection respectively (Figure 5-8). Briand et. al. concluded that foetal growth velocity (EFW velocity z-score) was negatively associated with malaria infected pregnancy compared to non-malaria, irrespective of the timing of malaria infection, i.e., early or late pregnancy. The ultrasound assessment in second half of pregnancy (26, 30, and 36 weeks' gestation) and the larger sample size of malaria infected pregnancies (N=388) in their study allowed to detect the delayed effect as well as immediate effect of malaria infection on foetal growth[206].

5.4.1 Strengths and limitations

The main strength of this analysis is the use of quality ultrasound foetal biometric data. Although these studies were conducted in different timeframes, the ultrasound measurement errors were less likely because the experienced trained sonographers used the same ultrasound machines following INTERGROWTH-21st and INTERBIO-21st ultrasound manual. Furthermore, the inter-observer and intra-observer variability was minimized by performing the continuous quality control assessment, which means 10% of ultrasound images were randomly selected and reviewed by the senior sonographer and one obstetrics doctor every 6 months. Moreover, these cohorts used similar inclusion criteria reducing the risk of selection bias. The hierarchical and complex data of this study was analysed by using the multilevel mixed model, which is suitable for analysing data with multiple levels of variation, such as the repeated ultrasound measurements of three pregnancy cohorts, and both random and fixed effects were accounted. The random effects that refer to individual differences between participants or differences between the measurements can introduce variability that was not of interest, but it has been taken account in the analysis. By

including the random effects in the model, the analysis provided more accurate estimates of the fixed effects of exposures or factors that are influenced on the foetal growth.

One of the limitations is the small sample size of malaria cases that were available for assessment of foetal growth, particularly falciparum malaria (number of patients=25 and number of scans=166) and this study cannot confirm that the non-significant findings of the effect of falciparum malaria on the foetal growth is true or not. The majority of malaria, including falciparum and vivax, were detected and treated in first trimester implying that the lowering number of malaria cases over the last three decades in this area were likely to be due to the early diagnosis and treatment. The coincidence of significant efforts directed to successful malaria elimination programs, while ultrasound capacity was increasing in the study area, is a definite limitation for this type of study albeit good news for local populations. The previous studies reported that foetal growth alteration due to malaria is obvious in the 3rd trimester of the pregnancy, when the foetal growth velocity is highest[197, 206, 207]. Perhaps, this pooled cohort is compromised by the low number of malaria cases in the 3rd trimester (number of patients=15). In addition, the malaria parasitaemia, severity, clinical presentation, and the EGA or trimester of the first episode of malaria infection were not accounted in this analysis. Previously, the analysis was planned to examine the additive effects of malaria infection in pregnancy and PE on foetal growth, but this was not possible because of the low number of patients have had both malaria infection and PE during pregnancy (N=4). There is emerging evidence that early onset of PE has an underlying placental pathology, that is associated with foetal growth restriction while the late onset PE cases have different pathology and less impact on foetal growth[208]. Therefore, one of the limitations of this analysis is that the severity and onset of PE has not been considered.

A better option to further explore the findings of this study would be to conduct additional research to confirm the observed effects of malaria on foetal growth. This could involve a larger sample size, including a more diverse population and different geographical locations to increase the generalizability of the results. Additionally, future research could investigate the potential mechanisms behind the observed effects, including the immune responses elicited by malaria infection and their impact on foetal growth. Understanding the mechanisms behind the observed effects could inform the development of targeted interventions to improve the outcomes of pregnant women and their babies in malaria-endemic regions.

5.4.2 Conclusion

This study concluded that maternal BMI and foetal sex influence on AC z-score. PE effects the foetal growth characteristics with brain sparing effect. The biometric marker of foetal growth (AC) is reduced maximally three weeks post malaria infection but recovers by four weeks with early detection and treatment.

Chapter 6

Discussion and conclusion

The main objective of this thesis was to explore the determinants of preterm birth (PTB), small for gestational age (SGA) and foetal growth, as well as the changes over time of maternal factors, and birth outcomes in the marginalised pregnant women along Thai Myanmar border. Following a brief reminder of the aims and objectives of the research, the following themes are discussed in light of the strengths and limitations of each chapter presented earlier. This chapter presents the principal findings of this thesis and explore potential health interventions in relation to policy, practice and feasibility. Recommendations for future research are also provided.

6.1 Introduction

This research aimed to identify the changes over time of maternal characteristics and birth outcomes, modifiable risk factors, the determinants of PTB, SGA, and foetal growth among pregnant women, who are living on Thai-Myanmar border. The objectives were to:

Objective 1. Identify demographic, infectious, medical and obstetric factors associated with poor birth outcomes. (Chapter 3)

Objective 2. Explore the determinants of PTB and SGA in a 30-year cohort of refugee and migrant pregnant women. (Chapter 4)

Objective 3. Identify demographic, infectious, and non-infectious factors associated with poor foetal growth. (Chapter 5)

Objective 4. Explore options for interventions to optimize foetal growth in low resource settings. (Chapter 6)

6.2 Summary of the important findings

Chapter 3 reported that a high proportion of teenage pregnancies and a double burden of malnutrition (overweight and underweight) among the study population, which are associated with adverse pregnancy outcomes. Other important risk factors, such as smoking and malaria in pregnancy, showed a decreasing trend over time. Moreover, there was a reduction in both maternal and neonatal mortality rate over time, but further effort is needed to achieve the Sustainable Development Goals 3 (SDG 3)[187]. Similarly, the proportion of PTB decreased over time in accordance with global trends but remained at around 9.4%. The proportion of SGA in this population has remained consistently high (>20%) over the last 20 years.

Chapter 4 concluded that maternal age (teenage pregnancy less than 20 years regardless of gravidity), smoking during pregnancy, low body mass index (BMI), and preeclampsia (PE) were significantly associated with PTB. In addition, a history of PTB in the previous pregnancies and non-malaria bacterial infection were significantly associated with PTB, particularly early PTB (28^{th}-34^{th} weeks' gestation). Similarly, teenage primigravida and advanced maternal age (>35 years), low BMI, smoking, malaria infection in pregnancy and PE were significantly associated with an increased risk of small for gestational age (SGA). Every 10 cm increase in maternal height and a high BMI have a protective effect on both adverse pregnancy outcomes (**Table 6-1**). Furthermore, the leading population attributable risks (PARs) for PTB in this population were teenage pregnancy, PE, maternal underweight, smoking, maternal anaemia, and malaria infection. Similarly, the PARs for SGA were smoking during pregnancy, underweight women, teenage pregnancy, malaria infection, and PE (**Table 6-2**).

Chapter 5 demonstrated that maternal overweight and male foetus were associated with a higher mean abdominal circumference (AC) z-score, whereas taller women and overweight

mothers also have an influence on head circumference (HC), biparietal diameter (BPD), and femur length (FL) z-scores. Foetal sex did not have an effect on mean FL z-score. The impact of preeclampsia (PE) on mean AC and FL z-scores was significant, but not on HC nor BPD (**Table 6-3**). Neither *Plasmodium falciparum* nor *vivax* had a significant effect on HC and BPD in relation to ultrasound scans after malaria infection. In *vivax* infected pregnancies, lower mean AC and FL z-score were observed three and two weeks after the infection respectively. There were no significant associations between mean AC, HC and BPD z-scores and *falciparum* malaria. Nevertheless, lower FL z-score was found seven weeks after *falciparum* (**Table 6-4**).

Table 6-1: Summary findings for the determinants of PTB and SGA.

Determinants	PTB		SGA	
	AOR (95%CI)	p-value	AOR (95%CI)	p-value
Age and Gravidity				
< 20 Primigravida	2.64 (2.31-3.01)	<0.001	1.57 (1.43-1.72)	<0.001
< 20 Multigravida	2.30 (1.83-2.89)	<0.001	0.91 (0.76-1.10)	0.334
20-34 Primigravida	1.67 (1.46-1.92)	<0.001	1.93 (1.78-2.10)	<0.001
20-34 Multigravida	Reference		Reference	
≥ 35 Primigravida	1.75 (0.94-3.27)	0.077	2.46 (1.65-3.67)	<0.001
≥ 35 Multigravida	1.35 (1.16-1.58)	<0.001	1.34 (1.21-1.47)	<0.001
Maternal height (per 10 cm)	0.72 (0.66-0.79)	<0.001	0.52 (0.49-0.56)	<0.001
BMI (kg/m2)				
Underweight (< 18.5)	1.22 (1.06-1.41)	0.007	1.58 (1.44-1.74)	<0.001
Normal (18.5-22.9)	Reference		Reference	
Overweight (≥ 23)	0.74 (0.66-0.84)	<0.001	0.55 (0.51-0.59)	<0.001
Smoking	1.53 (1.32-1.76)	<0.001	1.86 (1.7-2.03)	<0.001
Malaria infection in pregnancy	1.07 (0.88-1.30)	0.514	1.19 (1.05-1.35)	0.005
PE	3.63 (2.96-4.46)	<0.001	2.30 (1.94-2.73)	<0.001
History of preterm labour	3.28 (2.74-3.93)	<0.001	NA	
Non-malaria bacterial infection	1.52 (1.14-2.03)	0.004	0.98 (0.8-1.2)	0.846

This is a summary table of multivariable analysis model B from Chapter 4 and the principal findings were shown.

Table 6-2: Population attributable risks (PARs) of PTB and SGA.

Risk factors	PTB	SGA
	N=26,221	N=26,104
	PAR per 1,000 births (95% CI)	PAR per 1,000 births (95% CI)
Teenage pregnancy	11.3 (9.4, 13.0)	7.1 (4.3, 8.9)
Underweight (BMI < 18.5)	3.0 (1.8, 4.3)	13.2 (11.5, 14.9)
Smoking	2.7 (1.3, 4.2)	14.6 (13.8, 18.2)
Malaria in pregnancy	0.8 (-0.1, 1.7)	4.7 (3.3, 6.1)
PE or eclampsia	3.5 (2.7, 4.2)	3.5 (2.7, 4.3)
Maternal anaemia at first ANC	1.3 (0.3, 2.3)	NA

Abbreviation: ANC antenatal care; BMI body mass index; CI confidence interval; PE preeclampsia; PTB preterm birth; SGA small for gestational age

Table 6-3: Summary findings of the association between Preeclampsia (PE) and mean z-scores of foetal biometrics (AC, HC, BPD and FL).

	Multilevel multivariable analysis							
	Mean predicted AC z-score (95% CI)	p-value	Mean predicted HC z-score (95% CI)	p-value	Mean predicted BPD z-score (95% CI)	p-value	Mean predicted FL z-score (95% CI)	p-value
PE	-0.33 (-0.62, -0.05)	0.023*	-0.21 (-0.47, 0.05)	0.120	-0.11 (-0.39, 0.18)	0.459	-0.33 (-0.59, -0.04)	0.022*

*p-value<0.05 and significant

Abbreviation: AC abdominal circumference; BPD biparietal diameter; CI confidence interval; HC head circumference; FL femur length; PE preeclampsia

Table 6-4: Summary findings of the association between the time after malaria and mean z-scores of foetal biometrics (AC, HC, BPD and FL).

	Multilevel multivariable analysis							
	Mean AC z-score (95% CI)	**p value**	**Mean HC z-score (95% CI)**	**p value**	**Mean BPD z-score (95%CI)**	**p value**	**Mean FL z-score (95%CI)**	**p value**
Falciparum malaria infection (PF)								
No malaria infection before	Reference		Reference		Reference		Reference	
One week after PF	0.15 (-0.17, 0.47)	0.368	0.16 (-0.15, 0.46)	0.312	0.12 (-0.25, 0.49)	0.521	-0.02 (-0.28, 0.24)	0.889
Two weeks after PF	-0.16 (-0.60, 0.30)	0.481	-0.02 (-0.44, 0.40)	0.936	0.21 (-0.30, 0.72)	0.426	-0.29 (-0.66, 0.06)	0.106

Three weeks after PF	-0.45 (-0.97, 0.06)	0.085	-0.44 (-0.93, 0.04)	0.073	-0.28 (-0.86, 0.31)	0.352	0.01 (-0.40, 0.42)	0.959
Four weeks after PF	0.02 (-0.54, 0.59)	0.939	-0.19 (-0.72, 0.33)	0.472	-0.37 (-1.01, 0.26)	0.252	-0.26 (-0.71, 0.18)	0.249
Five weeks after PF	-0.28 (-0.75, 0.18)	0.236	-0.05 (-0.48, 0.38)	0.809	-0.11 (-0.63, 0.41)	0.678	-0.31 (-0.69, 0.07)	0.107
Six weeks after PF	-0.37 (-0.91, 0.16)	0.171	-0.02 (-0.51, 0.48)	0.948	0.28 (-0.32, 0.88)	0.359	-0.19 (-0.62, 0.07)	0.398
Seven weeks or more after PF	-0.04 (-0.31, 0.22)	0.744	-0.13 (-0.38, 0.13)	0.325	0.03 (-0.25, 0.31)	0.816	-0.25 (-0.50, -0.01)	0.045*
Vivax malaria infection (PV)								
No malaria infection before	Reference		Reference		Reference		Reference	
One week after PV	-0.08 (-0.22, 0.06)	0.277	-0.06 (-0.19, 0.07)	0.324	-0.07 (-0.22, 0.08)	0.374	-0.06 (-0.18, 0.06)	0.358
Two weeks after PV	-0.12 (-0.27, 0.03)	0.111	-0.08 (-0.22, 0.06)	0.241	-0.10 (-0.26, 0.06)	0.226	-0.13 (-0.26, -0.01)	0.040*
Three weeks after PV	-0.31 (-0.53, -0.08)	0.008*	-0.05 (-0.26, 0.15)	0.622	-0.12 (-0.37, 0.13)	0.331	-0.13 (-0.31, 0.05)	0.162
Four weeks after PV	0.04 (-0.18, 0.26)	0.717	-0.02 (-0.20, 0.20)	0.814	0.10 (-0.14, 0.35)	0.407	-0.08 (-0.26, 0.10)	0.388
Five weeks after PV	-0.14 (-0.36, 0.08)	0.217	-0.01 (-0.19, 0.26)	0.994	0.06 (-0.18, 0.31)	0.614	-0.11 (-0.29, 0.07)	0.229
Six weeks after PV	0.01 (-0.24, 0.25)	0.958	0.03 (-0.19, 0.26)	0.759	-0.19 (-0.47, 0.08)	0.166	-0.04 (-0.24, 0.16)	0.688
Seven weeks or more after PV	0.01 (-0.15, 0.16)	0.937	-0.04 (-0.19, 0.10)	0.540	0.04 (-0.12, 0.21)	0.595	-0.07 (-0.21, 0.06)	0.288

*p-value<0.05 and significant

Abbreviation: AC abdominal circumference; BPD biparietal diameter; CI confidence interval; HC head circumference; FL femur length; PF plasmodium falciparum, PV plasmodium vivax

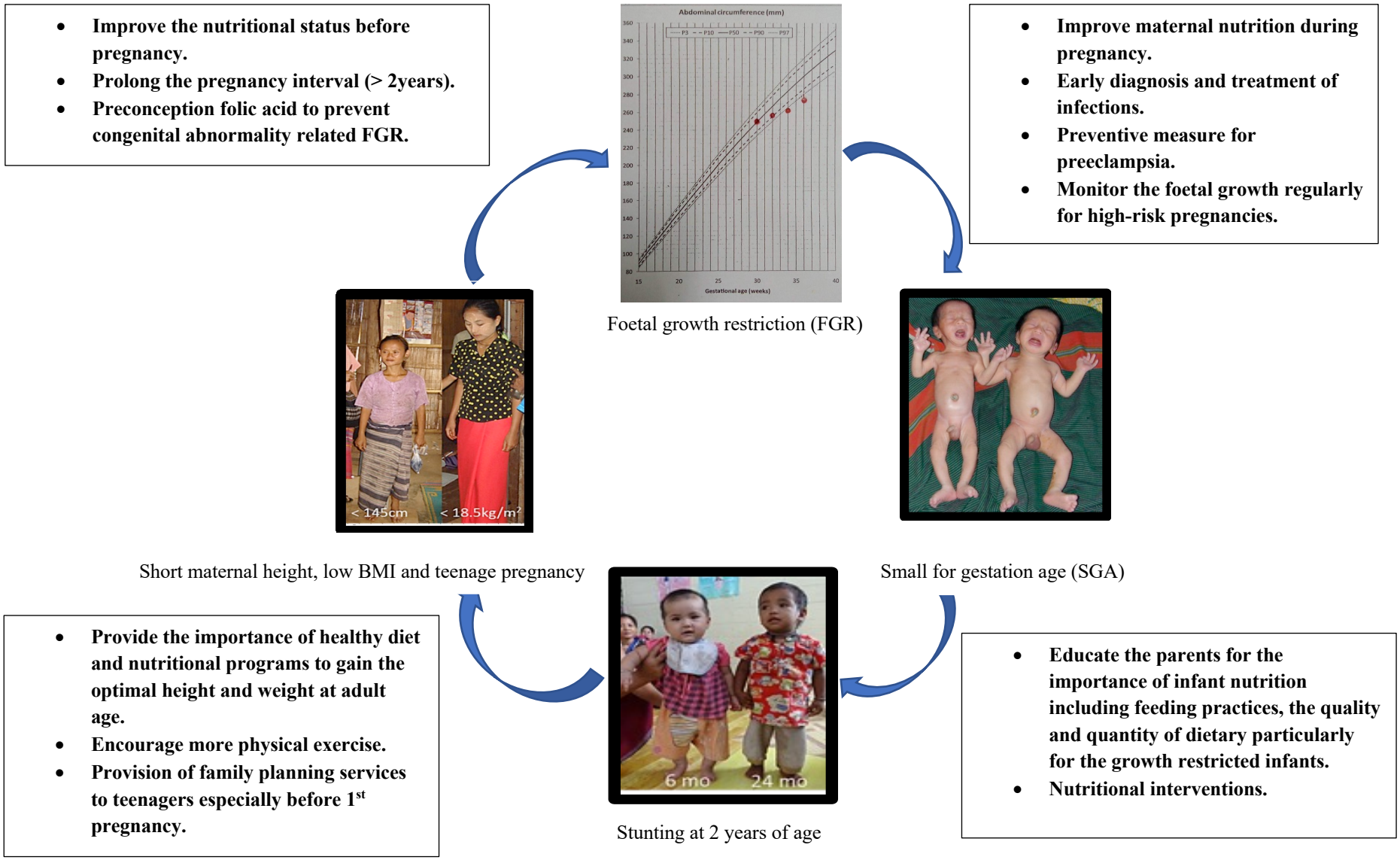


Figure 6-1: Possible interventions at different stage of life to improve the maternal and child health.

6.2 Discussion

A better understanding of the association between maternal factors or exposure during pregnancy and, PTB, SGA and foetal growth in resource limited setting is pre-requisite for future research to understand the causal relationship and develop the preventive strategies in order to reduce the burden of PTB or SGA. As a consequence of lacking ultrasound dated pregnancy data in most resource limited setting, there is an evidence gap of PTB and SGA particularly in Sub-Saharan Africa and South Asia, where the prevalence is highest. This work presents one of the first studies from Southeast Asia to describe the characteristics of marginalised pregnant women, and explore the factors associated with PTB, SGA and foetal growth. Marginalised populations have social, economic, and/or political challenges that limit their access to quality healthcare services, education, and other resources.

This study comprised two data sources: 1) routinely collected antenatal care and delivery data of Shoklo Malaria research Unit (SMRU) between the year 1986-2020 and 2) the first trimester inclusion with ultrasound dated pregnancy data of three observational prospective cohorts. Overall, this work presents one of the largest birth cohorts from Asia. A total of 82,030 antenatal records allowed a rich description of the SMRU cohort profile and assessment of the trends of the important risk factors for PTB and SGA. Regarding the data from the prospective cohorts, 1,349 pregnant women with 6,551 scans were available to assess the determinants of foetal growth and the impact of PE and malaria infection on foetal growth. The findings of this study might be applicable to populations beyond the Thai Myanmar border. A number of factors should be considered in terms of the extent to which the findings can be generalised to other resource limited setting and elsewhere as maternal characteristics, socioeconomic status and level of maternal and child health care may be differ.

SMRU was established to address health concerns of direct relevance to the local population. SMRU has conducted high quality epidemiological research and clinical trials with research results returning directly back to the community. Research staff are predominantly (> 95%) from within the local community and have contributed significantly to improve the health situation in the one of the most vulnerable populations globally over 30 years. SMRU staff and the wider community are very familiar with the research process and understand the importance of the research deriving local benefits in this area. In addition, the mutual understanding and trust between SMRU staff and the community have become are stronger over the time. As discussed in Chapter 3, SMRU has carried a larger burden of humanitarian health care in the marginalized population over time while seeking to improve the quality of care delivered. For this reason, I explored the changes of maternal factors and birth outcomes dividing into three decades; 1986-2000, 2001-2010 and 2011-2020 in addition to the temporal trends of some important exposure and outcome variables. Before year 2000, SMRU was primarily working in the refugee population and focusing on the antenatal care (ANC) where the burden of mortality from malaria was high (estimated at 1,000 per 100,000 live births in 1985). The improvement in maternal health care was clearly seen during three decades in some areas such as reduced maternal death from malaria, decreased prevalence of malaria and anaemia, decreased smoking. SMRU introduced ultrasound machines to date the pregnancy accurately and extended its service to provide the safe delivery by setting up delivery rooms in several places after year 2000. In the last decade, the special care baby units were introduced in SMRU clinics, and ANC practices were upgraded in accordance with the recommendations of WHO[209]. These included screening and treatment of common infection and medical problems during pregnancy such as sexually transmitted infections (HIV, Hepatitis B and syphilis), soil transmitted helminths, anaemia in pregnancy, gestational diabetes mellitus (GDM)

and prevention and treatment of PTB. The effect of GDM on PTB and SGA has not been accessed and it is one of the limitations of this study. Pregnant women presenting with febrile illness were investigated appropriately to understand the underlying causes and treated accordingly. In addition, monitoring and evaluation of clinical practice are regularly performed by upgrading the clinical guidelines and providing the refresher health training as well as the emergency obstetrics trainings (Advanced Life Support Obstetrics®). The staff are also encouraged to record the data in an efficient way and take good clinical practice (GCP) trainings particularly for those participating in the clinical studies. For the reasons listed above, the data quality is high and the findings of the SMRU cohort accurately reflect the situation of maternal and child health in this area.

As discussed in Chapter 4, most of the associated risk factors of PTB and SGA in this study were modifiable. Maternal age, parity and height are not modifiable risk factors. However, there are possibilities to tackle these non-modifiable risk factors, but an enormous effort is required. For instance, a comprehensive approach is needed to address the complex factors contributing to teenage pregnancy; support the young people in making healthy and informed decisions about their sexual health, provide education that keeps them in school, ensure an adequate compensation for parental work that children are not required to leave to school to support the family and ultimately, reduce the number of unintended pregnancies especially in teenage population. The maternal short stature is one of the important obstetrics risk factors and associated with difficult labour, PTB and infant SGA[210]. The maternal height in this population is relatively short; median height 152 cm [interquartile range 148-155], and it did not change much during last three decades. The individual's height is influenced by both genetic and non-genetic factors including environmental factors such as maternal nutrition, feeding practices, dietary quality and quantity,

and infection. While genetic factors can't be modified, environmental factors may be improved by promoting maternal nutrition during pre-conception, pregnancy and lactation period, and treating appropriately for the infections that may have an impact on foetal and infant growth. In addition, informing the parents about the feeding practices and selection of dietary quality and quantity during post-partum period is crucial to achieve the optimal infant growth. Information is not enough if parents cannot pay for protein foods such as milk that contribute to building skeletal bone[211].

Some known or potential risk factors were not available, including household air pollution, chemical exposure, social economic status and maternal mental health. Malaria infection in pregnancy was included in the study but malaria species *plasmodium falciparum* or *vivax* were differentiated only in the analyses on foetal growth. In addition to malaria, the suspected bacterial infection (defined as the presence of febrile illness and receiving the antibiotics with or without haemoculture result) was included in this analysis and it was significantly associated with early PTB. It represents systemic as well as localized bacterial infection such as urinary tract infection, suspected chorioamnionitis and genital tract infection but the specific contributing infection to PTB is not known in this study. However, Turner et al. previously reported that the overall GBS carriage rate, detected by both haemoculture and polymerase chain reaction (PCR), was 12% in 549 refugee pregnant women along the Thai-Myanmar border[212]. Additionally, they found no significant association between risk factors (such as fever in labour, prematurity, and prolonged rupture of membranes) and GBS. The data cleaning is in process for investigating the association of urinary tract infection and PTB. Another study, investigating the association between PTB and vaginal microbiome in this marginalized population, concluded that detection of higher levels of *Prevotella buccalis* and lower levels of *Lactobacillus crispatus* and *Finegoldia* accompanied by

low levels of cytokines such as tumour necrosis factor (IFN)- α, interferon (IFN)-γ and Interleukin-4 were predictive for PTB[213].

A high proportion of small for gestational age (SGA) babies is a concerning issue that needs to be tackled urgently. There is a strong association between SGA and stunted growth in early childhood[214]. Stunting can have the long-term effects on a child's cognitive development and physical health, including a weakened immune system, increased susceptibility to infections, and impaired school performance[215, 216]. The evidence suggests that the growth impairment in early life could lead to permanent damage resulting in sub-optimal growth in adult life and potentially impact on subsequent generations[215, 217]. Furthermore, the metabolic diseases are more likely to develop in adult life as the consequences of SGA[177, 178]. Maternal nutrition during pregnancy and early childhood nutrition are critical to mitigate these growth problems (Figure 6-1). Obviously, an enormous effort from the stakeholders, including governments, non-government organizations (NGOs), community-based organizations (CBOs), local authorities, policy makers and healthcare providers, is required to mitigate the health problems of the marginalised population in relate to political instability, economic stagnation and prolonged civil war.

6.3 Recommendation for future research or work

The findings of this thesis suggest several potential areas for future research. One is to investigate the potential interventions or programs aimed at reducing the high proportions of teenage pregnancies, double burden of malnutrition (DBM), and pre-eclampsia (PE), all of which are leading attributable risk factors for preterm birth (PTB) and small-for-gestational-age (SGA) in this study population. Implementation research is ongoing at SMRU on highly effective long-acting reversible contraceptives in teenage pregnancy (at clinic and the community level); aspirin and calcium for prevention of PE; screening all women for GDM at 24-28 weeks' gestation using

a 2-step approach; and providing the nutritional supplement (Asia Mix) for underweight pregnant women. Despite smoking and malaria infection pregnancy in this population decreased over time and continued efforts to maintain this condition is still required. Recommendations for LMICs to prevent small vulnerable newborns includes multiple micronutrient supplementation, balanced protein and energy supplementation, low-dose aspirin, progesterone provided vaginally, education for smoking cessation, malaria prevention, treatment of asymptomatic bacteriuria, and treatment of syphilis[218].

The teenage birth rate has significantly decreased from 64.5 births per 1,000 women in 2000 to 42.5 births per 1,000 women in 2021 globally[219]. However, some regions, such as Sub-Saharan Africa and Latin America and the Caribbean, still experience high rates of adolescent pregnancy, with 101 births and 53.2 births per 1,000 women, respectively[219]. Teenage pregnancy is a known risk factor for adverse pregnancy outcomes, including preterm birth, low birth weight, SGA and maternal mortality[157, 220]. It can also have negative long-term health, social, and economic consequences for both the mother and child[221, 222]. For example, teenage mothers may have limited educational and employment opportunities, leading to long-term economic disadvantage. The interventions aimed at improving access to comprehensive sexual and reproductive health services, education, and gender equality may be effective in reducing teenage pregnancy rates[157]. However, the barriers to accessing healthcare services, including contraception, persist particularly in LMICs. Socioeconomic and cultural factors, such as poverty, early marriage, and lack of education, can also contribute to high rates of teenage pregnancy[223]. Therefore, a comprehensive approach that addresses these factors is essential to reduce teenage pregnancy rates and improve the health and well-being of teenage mothers and their children.

The coexistence of undernutrition and overweight or obesity (DBM), is a growing public health concern particularly among reproductive-aged women in LMICs[95]. While underweight is associated with an increased risk of PTB and SGA, the overweight contributes significantly to large for gestational age (LGA), stillbirth and increased caesarean delivery[224]. The findings of decreasing trends in underweight and the rising prevalence of overweight among women from 55 LMICs coincides with this study findings[95]. The overall prevalence of underweight and overweight in South and Southeast Asia was 22.9 % and 21.3% with the annual reduction rate of 1.3% and 8.4% respectively[94]. The studies suggested that interventions aimed at improving maternal nutrition and addressing underlying socioeconomic factors, such as poverty and food insecurity, may be effective in reducing the burden of DBM[225, 226].

The World Health Organization (WHO) reported that the global incidence of PE varies between 2-8 % of pregnancies, and it is more common in LMICs rather than developed countries[227]. One of the devasting consequence of PE is maternal death and it is also significantly associated with foetal growth restriction (FGR), PTB and SGA. In recent years, several studies had developed new strategies to predict PE in early pregnancy by using biomarkers, which are pregnancy associated plasma protein A (PAPP-A)[228]; placental growth factor (PIGF)[229]; plasma protein 13[230]; and human chorionic gonadotrophin (hCG)[231]. One recent study discovered that a triple test, which incorporates maternal risk factors, mean arterial pressure, uterine artery pulsatile index, and PIGF, has proven more effective in predicting early PE (prior to 32 weeks' gestation) compared to screening solely based on risk factors.[232]. However, the applicability of triple test in resource limited setting is considerable due to the cost effectiveness[233]. The significant reduction of PE and PTB were associated with early administration of low dose aspirin [234-236]. Moreover, other interventions including nutritional supplement, dietary and lifestyle modification have some

protective effect on PE [237]. The calcium supplementation is also effective to prevent PE and PTB especially in pregnant women with low calcium intake[238]. Regarding the lifestyle modification, one recent study reported that the risk of hypertensive disorders of pregnancy was significantly reduced by having higher vegetables consumption in first trimester, increased physical activities and more sleep in the third trimester[239]. It is interesting to know the impact of non-harmful preventive measures on PE in resource limited setting in addition to administration of aspirin and calcium supplements in high-risk pregnancies.

One recent meta-analysis had shown that vaginal progesterone is effective to reduce the spontaneous PTB in singleton pregnancy with short cervix (<25 mm) without having any impact on the childhood development[240]. In our clinics, the routine transabdominal (all pregnant women) or transvaginal (high risks for PTB) cervical length measurement is carried out between 18^{+0} and 24^{+0} weeks' gestation as a part of the preventive measures for PTB, including a serial measurement of cervix as necessary and, the administration of tocolytics and corticosteroid. It would be important to explore the usefulness of vaginal progesterone in pregnant women with cervical shortening in this setting if further reduction of PTB is expected. The international literature reported that 35-40% of spontaneous PTB occurred due to urogenital tract infection[59]. The impact of specific bacterial infections (urinary tract infection, vaginal infection including bacterial vaginosis, Group B streptococcus (GBS) etc.) causing PTB in this population should be considered in future research.

Given the consistent high prevalence of SGA over the last 20 years, further research should focus to explore the additional risks that are not included in this study, and potential interventions to improve outcomes for these infants. The evaluation of effectiveness of current guidelines and recommendations for managing high risks pregnancies particularly for PTB and SGA would be

beneficial. The air quality in Thailand has become worse over time due to pollution, cultural burning, forest fire, biomass burning and transboundary haze from Myanmar[241]. Therefore, a better understanding of the effects of climate change and air pollution on pregnancy outcomes is required, along with studies that implement reductions in heat exposure during critical periods of pregnancy.

Finally, future research could build upon the findings presented in Chapter 5 by exploring the potential impact of other maternal factors, such as maternal nutrition or environmental exposures, on foetal biometrics. Additional investigation for foetal growth pattern after malaria infection could help to confirm the findings of this work using larger sample size of malaria infected pregnancies.

6.4 Conclusion

I truly believe that this thesis provides a new perspective towards the understandings of the prevalence and the attributable risk factors of PTB and SGA to minimize the neonatal mortality and the long-term consequences of PTB and SGA in resource limited setting. Improving maternal and child health is a critical goal, particularly in marginalized populations where access to healthcare and other resources are limited. This thesis's findings and recommendations can be informative for the development of health interventions and policies aimed at improving maternal and child health outcomes in marginalized communities along the Thai-Myanmar border. Future research in this area could build on this work by exploring additional factors that may contribute to maternal and child health outcomes, as well as evaluating the effectiveness of interventions aimed at improving these outcomes.

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Appendix A

Placental histopathology in preterm birth with confirmed maternal infection: A systematic literature review

Overview

This section presents the methods and findings of a systematic literature review exploring the association between placental histopathological abnormality and preterm birth in the presence of confirmed infection. The systematic review synthesises evidence around the studies that make an effort to genuinely triangulate these three factors, not just associating two of them (common) leaving evidence incomplete. While histopathological chorioamnionitis, and funisitis (when examined) were commonly observed in preterm birth complicated by confirmed bacterial or viral, but not parasitic, infection. For malaria the most common parasitic infections of humans, the presence of malaria parasites but not pigment in placenta was reported to increase the risk of PTB, but this finding was inconclusive. Commitment to using standardised terminology and classification of histopathological abnormalities associated with infections is needed to identify causality and potential treatment of preterm birth. Studies on preterm birth needs to occur in high burden countries and control for clinical characteristics (maternal, foetal, labour, and placental) that may have an impact on placental histopathological abnormalities. Only one in three studies were conducted in low- and middle-income countries despite the burden of preterm birth of four in five neonates in these same countries, hence representing a significant gap in the current evidence.

Aims

This systematic review of the literature aims to provide a comprehensive overview of the evidence on placental histological abnormalities in PTB cases with a confirmed infection. The review aims to identify studies reporting the triad of placental histopathology, infection and PTB, allowing an inference on the causal pathways.

This review seeks to address the following questions:

(1) What is the association between placental histopathological abnormalities and PTB in the presence of confirmed bacterial, viral or parasitic infection?

(2) Are there associations between the identified microorganism and specific placental histopathological abnormalities?

Methods

Search strategy and selection criteria

A systematic literature review following PRISMA guidelines [1] was conducted to identify studies reporting placental histopathology in PTB with a confirmed infection. Four databases: (PubMed/Medline, Scopus, Web of Science and Embase) were searched. The original search was conducted in March 2019 and continually updated until February 2021 focusing on three components: placental histopathology, PTB and infection. The search strategy and data bases are detailed in **Table A1-1**. The use of "AND" was significant to the search strategy to confirm triangulation "pregnan* AND (preterm* OR premature*) AND placenta*AND (histopatholog* OR histology* patholog*) AND (Infect* OR microorganism OR bacteria* OR virus* OR parasite*)". As "infection" encompasses a broad range of causative pathogens and studies typically had a narrow range of interest, the term "infection" was divided into three groups according to the following infectious agents: bacteria, viruses or parasites.

The rationale, objective and search strategy of this systematic review were registered in the International Prospective Registers of Systematic Reviews (PROSPERO) under the registration number CRD42019137099[2].

Table A1-1: Electronic databases searched and search strategy used for systematic literature review.

Pubmed/Medline (1946 to present) (((((Pregnan*) AND ((preterm*) OR prematur*))) AND ((placenta*) AND (((patholog*) OR histopatholg*) OR histolog*)))) AND ((((infect*) OR microorganism*) OR bacteria*) OR virus*) OR parasite*)))))
Web of science (1900-present) ALL=(pregnan*) AND ALL=(preterm* OR premature*) AND ALL=(placenta*) AND ALL=(histolog* OR histopatholog* OR histolog*) AND ALL=(infect* OR microorganism* OR bacteria* OR virus* OR parasite*)
Scopus (2004-present) (((TITLE-ABS-KEY (pregnan*)) AND (TITLE-ABS-KEY (preterm* OR premature*))) AND ((TITLE-ABS-KEY (placenta*)) AND (TITLE-ABS-KEY (h209istolog* OR h209istopathog* OR 209histolog*)))) AND (TITLE-ABS-KEY (infect* OR microorganism* OR bacteria* OR virus* OR parasite*))
Embase (1974-present) ALL=(pregnan*).mp. AND ALL= (preterm* OR premature*).mp. AND ALL=(placenta*).mp. AND ALL= (histolog* OR histopatholog* OR histolog*).mp. AND ALL= (infect* OR microorganism* OR bacteria* OR virus* Or parasite*).mp.
No temporal, geographical, country income or language restrictions were applied to the search.

Inclusion and exclusion criteria

Interventional and observational studies were included if the histopathological findings were related to PTB and any type of infection (bacterial, viral or parasitic). The inclusion criteria used for full text screening were English language, full text availability, original research describing PTB/PPROM, placental histopathology assessed, micro-organisms reported, and most

importantly that the study described the association between placental histopathology and infection in PTB/PPROM.

Screening of searched articles

The author screened the titles and abstracts of all papers identified and the full texts were obtained for all potentially relevant studies. Full text screening was conducted by the author, and full texts were independently screened by a member of the author's supervisorial team to ensure accuracy. Full texts were classified either as included or excluded. During screening, studies that did not unambiguously include the three components in triangulation, i.e. histopathological examination, infection and PTB, were excluded. Histopathological placental findings reported in isolation or as part of a series of investigations were included if the other conditions were satisfied. Systematic reviews on histopathology and PTB were checked for any possible articles that may have been missed by the primary search.

In the event of discrepancies during the selection process, a second assessment was conducted to resolve the inconsistency. The bibliographic management software, EndNote (version X9.3.3), was used to organise the articles.

Data extraction

After inclusion, the following data were extracted: year, country, economic country profile (HIC or LMIC), study design, timing of inclusion of participants (prospective at time of infection or at birth), estimated gestational age (EGA) of included PTBs, method of gestational age assessment, presence of rupture of membranes (ROM), sample size, histopathological classification and reporting system, confirmation of infection, and methodology and blinding of pathologist. Reference to the histopathological abnormality classification system was extracted when provided. High numbers of papers were excluded because they compared only two of three inclusion criteria

in their analysis: placental histopathology and PTB, or microbial infection and histopathology, or microbial infection and PTB.

Quality assessment

The methodological quality of the studies was assessed by evaluating study design, inclusion criteria and the blinding of investigators to pregnancy outcomes or causation. To address the risk of bias, the Newcastle-Ottawa quality assessment scale for case-control and cohort studies was used [3]. The author and a supervisor independently appraised the selected studies; in the event of a discrepancy, a third supervisor was consulted. Any encountered quality issues or concerns were addressed by referring to the particular section and grading the severity of the bias (moderate, serious or critical). Twenty selected clinical characteristics (maternal, fetal, delivery and infection) associated with PTB were extracted and a binary score was applied for presence or absence.

Results

Results of the literature search

A total of 3277 records were identified through database searching. After removing duplicates, 1529 articles were screened against the defined eligibility criteria (**Figure A1-1**).

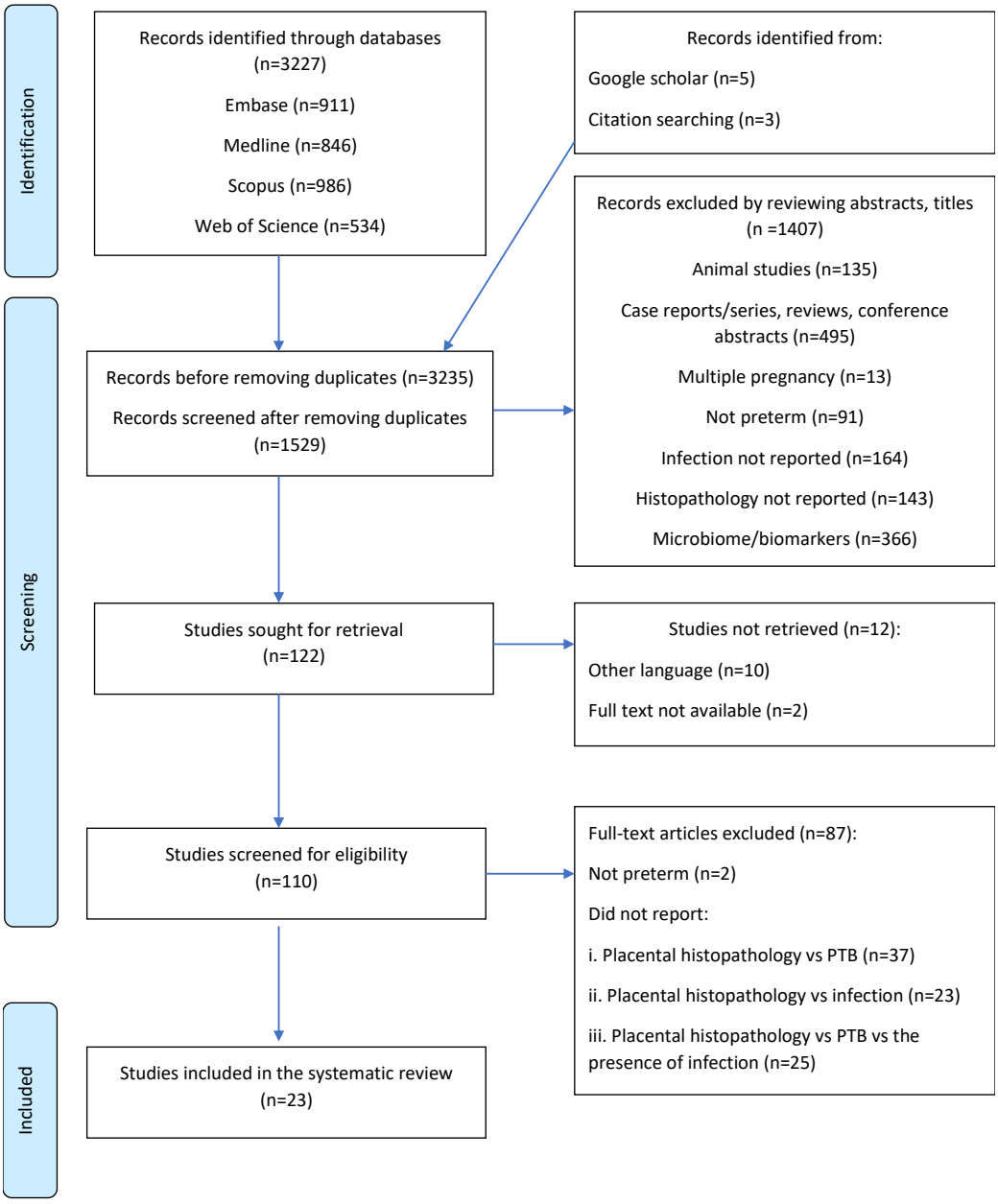

Figure A 1-1: Prisma flow chart

Of these, 1407 were excluded after screening the study title and abstract: animal studies = 135; case reports, case series or review articles = 495; articles that did not report confirmed infection = 164; articles focused on microbiome, proteomic or serum markers = 366; multiple pregnancies = 13; not preterm = 91; placental histopathology not reported = 143. This left only 23 studies for

inclusion (bacteria, viral and parasitic) [4-26]. Of included research articles that were published between 1988 and 2020, 15/23 studies (65.2%) were reported in the last 10 years. Only 8 (34.8%) were conducted in LMICs or LICs. (**Figure A 1-2**). Study characteristics, PTB definition, placental histopathology classification system, range of infective organisms, and sample size are summarised in **Table A 1-2**.

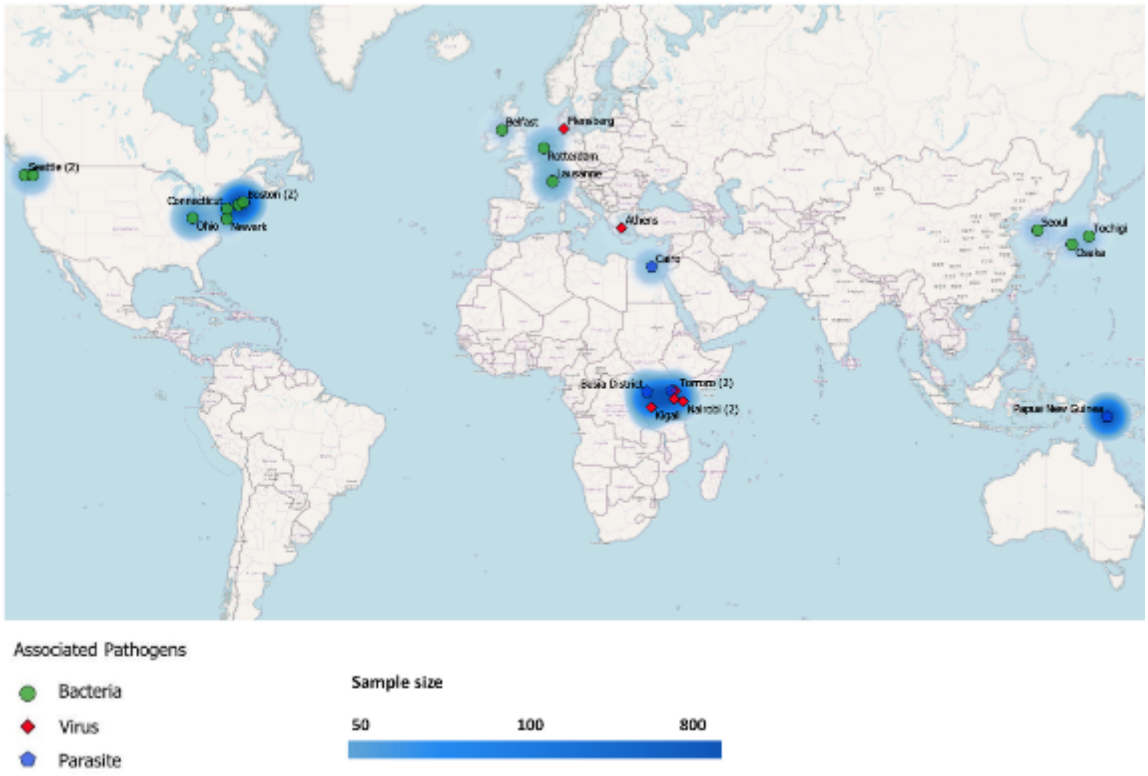

Figure A 1-2: Map of countries where studies were conducted.

Table A 1-2: Study characteristics of included articles in systematic literature review.

Author name, Year*	Type of infection	PTB definition (weeks)	Type of study	Retrospective/ Prospective	No. PTB (%)	Country	GNI** per capita	Blinding of pathologist
Cox, 2016 [6]	Bacteria	13-37	Case Control	Retrospective	57/57 (100%)	Ireland	HIC	Yes
Dammann, 2003 [7]	Bacteria	Not clearly defined	Case Control	Retrospective	464/464 (100%)	USA	HIC	Yes
Hecht, 2008 [11]	Bacteria	<28	Cohort	Retrospective	835/835 (100%)	USA	HIC	Yes
Hillier, 1988 [13]	Bacteria	<37	Case control	Retrospective	38/74 (51%)	USA	HIC	Yes
Hillier, 1991 [12]	Bacteria	<34	Case control	Retrospective	112/268 (42%)	USA	HIC	Yes
Honma, 2007 [14]	Bacteria	<32	Cohort	Retrospective	105/105 (100%)	Japan	HIC	Not mentioned
Ingrid, 2011 [15]	Bacteria	<32	Cohort	Prospective	304/304 (100%)	Netherland	HIC	Yes
Kwak, 2014 [17]	Bacteria	<37	Cohort	Prospective	179/179 (100%)	Korea	HIC	Not mentioned
Namba, 2010 [20]	Bacteria	<32	Cohort	Prospective	151/151 (100%)	Japan	HIC	Yes
Patel, 2018 [22]	Bacteria	23-33+6	Cohort	Retrospective	181/181 (100%)	USA	HIC	Not mentioned
Pettker, 2007 [23]	Bacteria	<37	Case Control	Prospective	183/183 (100%)	USA	HIC	Yes
Queiros da Mota, 2013 [24]	Bacteria	<37	Cohort	Prospective	202/376 (53.7%)	Switzerland	HIC	Not mentioned
Sweeney 2016 [25]	Bacteria	<37	Cohort	Prospective	535/535 (100%)	USA	HIC	Not mentioned
Ategeka, 2019 [5]	Virus	<37	Sub-analysis of RCT	Prospective	18/191 (9.4%)	Torroro	LMIC	Yes
Feist, 2020 [8]	Virus	<37	Cohort	Retrospective	14/50 (28%)	Germany	HIC	Not mentioned

Gichangi, 1993 [9]	Virus	<37	Case control	Prospective	117/467 (31.4%)	Kenya	LMIC	Not mentioned
Ladner, 1998 [18]	Virus	<37	Cohort	Prospective	39/275 (14%)	Rwanda	LMIC	Not mentioned
Ombimbo, 2019 [21]	Virus	<37	Cohort	Prospective	81/101 (80.2%)	Kenya	LMIC	Yes
Tsekoura, 2010 [26]	Virus	<37	Case Control	Prospective	37/58 (63.8%)	Greece	HIC	Yes
Ategeka, 2020 [4]	Parasite	<37	Sub-analysis of RCT	Prospective	37/637 (5.8%)	Uganda	LMIC	Not mentioned
Kapisi, 2017 [16]	Parasite	<37	Sub-analysis of RCT	Prospective	26/282 (9.2%)	Uganda	LMIC	Yes
Lufele, 2017 [19]	Parasite	<37	Cohort	Prospective	81/962 (8.4%)	Papua New Guinea	LMIC	Yes
Saad, 2017 [10]	Parasite	<37	Cohort	Prospective	37/240 (15.4%)	Egypt	LMIC	Yes

Abbreviations: GNI, gross national income; HIC, high-income country; HIV, Human immunodeficiency virus; LMIC low- to middle-income country; RCT, randomised controlled trial; *year of publication; **gross national income based on World Bank data. https://data.worldbank.org/indicator/NY.GNP.PCAP.CD?locations=TH.

The majority (56.5%, 13/23) of studies examined the association between bacterial infection and placental histopathology in PTB [6, 7, 11-15, 17, 20, 22-25], six studies (26%) examined viral infection [5, 8, 9, 18, 21, 26], and four studies (17%) examined studied parasites [4, 10, 16, 19]. Most studies mentioned or clarified only a few of the clinical characteristics (maternal, foetal, delivery and infection) associated with PTB: median score of 6 [range 1-12] out of a possible total of 20 (**Table A1-3**).

Quality assessment

Of the included studies, 13 were cohort studies, seven were case-control studies and three were sub-analysis of randomised controlled trials. Most included studies (65%, 15/23) were conducted prospectively and 14 mentioned the blinding of the pathologist(s) to clinical data [5-7,

[10-13, 15, 16, 19-21, 23, 26] (**Table A1-2**). The gestational age assessment by ultrasound was clearly specified in 12 articles [4, 5, 7, 12, 13, 15, 16, 18, 19, 21, 23, 26]. The risk of bias was low using the Newcastle-Ottawa quality assessment scale: median 6 (maximum score 7) for cohort studies and median 7 (maximum 8) for case-control studies (**Table A1-4**).

Table A1-3: Clinical characteristics associated with placental histopathology.

	maternal								foetal	delivery					infection							
	Smoking / Drugs	History PTB (PROM)	BMI or Stature	Cervical incompetence	Gravidity / parity	Age / teenage	Preeclampsia / eclampsia	Medical complications e.g.	Foetal monitoring / foetal distress	Antenatal corticosteroid	Caesarean section *vs* vaginal	PTB spontaneous	PTB induced	Premature PROM	Study treatment	Number of VE	ANC screening for BV	ANC screening for GBS	Treatment for PROM /	Length of ROM	Total (maximum score 20)	comment
Bacteria																						
Cox, 2016 [1]	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	
Dammann, 2003 [2]	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	1	1	7	*U. urealyticum* culture routine if bw <1501g
Hecht, 2008[3]	0	0	0	1	0	0	1	0	1	1	1	0	1	0	0	0	0	1	0	1	7	
Hillier, 1988 [4]	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	1	1	6	
Hillier, 1991 [5]	1	1	0	0	1	0	1	1	0	0	1	1	0	1	0	0	0	0	0	1	9	
Honma, 2007 [6]	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	
Ingrid, 2011 [7]	0	0	0	0	1	1	1	0	1	0	1	1	0	1	1	0	0	0	1	0	9	
Kwak, 2014 [8]	0	0	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	7	
Namba, 2010 [9]	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	4	
Patel, 2018 [10]	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	0	0	1	1	8	
Pettker, 2007 [11]	0	1	0	0	1	1	0	0	1	1	1	1	1	1	0	1^	0	0	1	1*	12	*Interval amniocentesis to delivery in hours;

																						^ no VE in PPROM
Queiros da Mota, 2013[12]	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	5	
Sweeney 2016 [13]	0	0	0	1	1	1	0	0	0	0	1	0	0	1	1	0	0	0	1	0	7	
Virus																						
Ategeka, 2019 [14]	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	All on ARV, Stage 1, RCT Cotri+Dp vs Cotri, p=0.07
Feist, 2020 [15]	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1	6	
Gichangi, 1993 [16]	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	
Ladner, 1998 [17]	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	5	Women treated for STI but not HIV
Ombimbo, 2019 [18]	0	0	0	0	0	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	6	All on ARV, no mention of Cotri
Tsekoura, 2010 [19]	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	4	
Parasite																						
Ategeka, 2020 [20]	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	5	
Kapisi, 2017 [21]	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	5	
Lufele, 2017 [23]	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	5	IPTp (SP+Az) or curative dose (SP+CQ)
Saad, 2017 [22]	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	5	

Abbreviations: Ampi, Ampicillin; ANC, antenatal care; AROM, artificial rupture of membranes; ARV, antiretroviral; Az, azithromycin; BV, bacterial vaginosis; Cotri, cotrimoxazole; CQ, chloroquine; DP, dihydroartemisinin-piperaquine; GBS, group B streptococcus; HIV, human immunodeficiency virus; IPTp, intermittent preventive treatment pregnancy; Pen, Penicillin; PROM, preterm rupture of membranes; PPROM preterm premature rupture of membranes; PT, preterm; PTB, preterm birth; RCT, randomised controlled trial; ROM, rupture of membranes; SP, sulphadoxine-pyrimethamine; STI, sexually transmitted infection; VE, vaginal examination

Table A1-4a. Risk of bias assessment using Newcastle – Ottawa Scale (NOS): Cohort studies.

Author, Year, Reference	Selection				Comparability		Outcome			Total Stars
	Representativeness	Selection of non-exposed cohort	Ascertainment of exposure	Outcome of interest not present at start of study	Most important clinical character-istics	Additional character-istics	Assessment of outcome	Was follow-up long enough for outcomes	Adequacy of follow-up of cohorts	
Ategeka, 2019 [$]	*	*	*	*		*	*	NA	NA	6
Ategeka, 2020 [$]	*	*	*	*		*	*	NA	NA	6
Feist, 2020	*	*	*			*	*	NA	NA	5
Hecht, 2008	*	*	*	*	*	*	*	NA	NA	7
Honma, 2007	*	*	*	*			*	NA	NA	5
Ingrid, 2011	*	*	*	*	*	*	*	NA	NA	7
Kapisi, 2017 [$]	*	*	*	*		*	*	NA	NA	6
Kwak, 2014	*	*	*	*	*	*	*	NA	NA	7
Ladner, 1998	*	*	*	*		*	*	NA	NA	6
Lufele, 2017	*	*	*	*		*	*	NA	NA	6
Ombinbo, 2019	*	*	*	*		*	*	NA	NA	6
Namba, 2010	*	*	*	*			*	NA	NA	5

Patel, 2018	*	*	*	*	*		*	NA	NA	6
Querios da Mota, 2013	*	*	*	*		*	*	NA	NA	6
Saad, 2017	*	*	*	*		*	*	NA	NA	6
Sweeney, 2016	*	*	*	*		*	*	NA	NA	6

$: Sub-analysis of randomised control trial of malaria prevention studies. *Star: The NOS tool uses a star system to assess the methodological quality based on three criteria; i) participants' selection (4 stars), ii) comparability of study groups (2 stars), and iii) assessment of outcome/exposure (3 stars). Therefore, the highest total score for a cohort study was 7. Follow-up long enough for outcomes and adequacy of follow-up of cohorts are not applicable in these reviewed articles. NA: Not applicable.

Table A 1-4b: Risk of bias assessment using Newcastle – Ottawa Scale (NOS): Case-Control studies.

Author, Year, Reference	Selection				Comparability		Exposure			Total Stars
	Case definition	Representativeness	Selection of control group	Definition of control group	Most important clinical factors	Additional factors	Ascertainment of exposure	Method of ascertainment	Non-response rate	
Cox, 2016	*	*	*	*			*	*	NA	6
Dammann, 2003	*	*	*	*	*	*	*	*	NA	8
Gichangi, 1993	*	*	*	*			*	*	NA	6
Hillier, 1988	*	*	*	*		*	*	*	NA	7
Hillier, 1991	*	*	*	*	*	*	*	*	NA	8
Pettker, 2007	*	*	*	*	*	*	*	*	NA	8
Tsekura, 2010	*	*	*	*			*	*	NA	6

*Star: The NOS tool uses a star system to assess the methodological quality based on three criteria; i) participants' selection (4 stars), ii) comparability of study groups (2 stars), and iii) exposure (2 stars). Therefore, the highest total score for a case-control study was 8. Follow-up long enough for outcomes and adequacy of follow-up of cohorts are not applicable in these reviewed articles. NA: Not applicable

Variations of study participants

While an estimated gestational age of less than 37 weeks was the most common PTB definition, various lower cut-offs were used: seven different gestational age windows were described in 23 studies. Moreover, eight studies recruited early and extremely preterm newborns as the study participants and the classification of PTB was not uniform [6, 7, 11, 13-15, 20, 22].

PTB subcategories (spontaneous PTB with intact membranes, PPROM and indicated or induced due to maternal or foetal complications) were frequently not mentioned (**Table A 1-3**). Only 11 studies were designed to investigate the associations of placental histological abnormalities and infections in PTB [6, 7, 9, 11-13, 15, 17, 20, 22, 23].

Histopathological methods

Placental tissue processing techniques varied and 20 different publications [27-46] were used to support histopathological classification systems, which were reported in the included studies (**Table A1-5a**). Six studies used standardised histopathological definitions: four [5, 11, 15, 25] using Redline *et al.* 2003 [41] and two using Khong *et al.*[36]. Seven studies used their own definitions of histological chorioamnionitis [12-14, 17, 18, 23, 24, 26] and three malaria studies [4, 16, 19] used four classifications [39]. Evidence of reporting of the severity of placental histopathological abnormality was limited to eight (34.8%) of studies [4, 8, 11, 15, 20, 23-25].

Of the included studies, 13 were cohort studies, seven were case-control studies and three were sub-analysis of randomised controlled trials. Most included studies (65%, 15/23) were conducted prospectively and 14 mentioned the blinding of the pathologist(s) to clinical data [5-7, 10-13, 15, 16, 19-21, 23, 26] (**Table A1-2**). The gestational age assessment by ultrasound was clearly specified in 12 articles [4, 5, 7, 12, 13, 15, 16, 18, 19, 21, 23, 26]. The risk of bias was low using the Newcastle-

Ottawa quality assessment scale: median 6 (maximum score 7) for cohort studies and median 7 (maximum 8) for case-control studies (**Table A1-4**).

Table A1-3: Clinical characteristics associated with placental histopathology.

	maternal								foetal	delivery					infection							
	Smoking / Drugs	History PTB (PROM)	BMI or Stature	Cervical incompetence	Gravidity / parity	Age / teenage	Preeclampsia / eclampsia	Medical complications e.g.	Foetal monitoring / foetal distress	Antenatal corticosteroid	Caesarean section *vs* vaginal	PTB spontaneous	PTB induced	Premature PROM	Study treatment	Number of VE	ANC screening for BV	ANC screening for GBS	Treatment for PROM /	Length of ROM	Total (maximum score 20)	comment
Bacteria																						
Cox, 2016 [1]	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	
Dammann, 2003 [2]	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	1	1	7	*U. urealyticum* culture routine if bw <1501g
Hecht, 2008[3]	0	0	0	1	0	0	1	0	1	1	1	0	1	0	0	0	0	1	0	1	7	
Hillier, 1988 [4]	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	1	1	6	
Hillier, 1991 [5]	1	1	0	0	1	0	1	1	0	0	1	1	0	1	0	0	0	0	0	1	9	
Honma, 2007 [6]	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	
Ingrid, 2011 [7]	0	0	0	0	1	1	1	0	1	0	1	1	0	1	1	0	0	0	1	0	9	
Kwak, 2014 [8]	0	0	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	7	
Namba, 2010 [9]	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	4	
Patel, 2018 [10]	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	0	0	1	1	8	
Pettker, 2007 [11]	0	1	0	0	1	1	0	0	1	1	1	1	1	1	0	1^	0	0	1	1*	12	*Interval amniocentesis to delivery in hours;

																						^ no VE in PPROM
Queiros da Mota, 2013[12]	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	5	
Sweeney 2016 [13]	0	0	0	1	1	1	0	0	0	0	1	0	0	1	1	0	0	0	1	0	7	
Virus																						
Ategeka, 2019 [14]	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	All on ARV, Stage 1, RCT Cotri+Dp vs Cotri, p=0.07
Feist, 2020 [15]	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1	6	
Gichangi, 1993 [16]	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	
Ladner, 1998 [17]	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	5	Women treated for STI but not HIV
Ombimbo, 2019 [18]	0	0	0	0	0	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	6	All on ARV, no mention of Cotri
Tsekoura, 2010 [19]	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	4	
Parasite																						
Ategeka, 2020 [20]	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	5	
Kapisi, 2017 [21]	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	5	
Lufele, 2017 [23]	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	5	IPTp (SP+Az) or curative dose (SP+CQ)
Saad, 2017 [22]	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	5	

Abbreviations: Ampi, Ampicillin; ANC, antenatal care; AROM, artificial rupture of membranes; ARV, antiretroviral; Az, azithromycin; BV, bacterial vaginosis; Cotri, cotrimoxazole; CQ, chloroquine; DP, dihydroartemisinin-piperaquine; GBS, group B streptococcus; HIV, human immunodeficiency virus; IPTp, intermittent preventive treatment pregnancy; Pen, Penicillin; PROM, preterm rupture of membranes; PPROM preterm premature rupture of membranes; PT, preterm; PTB, preterm birth; RCT, randomised controlled trial; ROM, rupture of membranes; SP, sulphadoxine-pyrimethamine; STI, sexually transmitted infection; VE, vaginal examination

Table A1-4a. Risk of bias assessment using Newcastle – Ottawa Scale (NOS): Cohort studies.

Author, Year, Reference	Selection				Comparability		Outcome			Total Stars
	Representativeness	Selection of non-exposed cohort	Ascertainment of exposure	Outcome of interest not present at start of study	Most important clinical character-istics	Additional character-istics	Assessment of outcome	Was follow-up long enough for outcomes	Adequacy of follow-up of cohorts	
Ategeka, 2019 [$]	*	*	*	*		*	*	NA	NA	6
Ategeka, 2020 [$]	*	*	*	*		*	*	NA	NA	6
Feist, 2020	*	*	*			*	*	NA	NA	5
Hecht, 2008	*	*	*	*	*	*	*	NA	NA	7
Honma, 2007	*	*	*	*			*	NA	NA	5
Ingrid, 2011	*	*	*	*	*	*	*	NA	NA	7
Kapisi, 2017 [$]	*	*	*	*		*	*	NA	NA	6
Kwak, 2014	*	*	*	*	*	*	*	NA	NA	7
Ladner, 1998	*	*	*	*		*	*	NA	NA	6
Lufele, 2017	*	*	*	*		*	*	NA	NA	6
Ombinbo, 2019	*	*	*	*		*	*	NA	NA	6
Namba, 2010	*	*	*	*			*	NA	NA	5

Patel, 2018	*	*	*	*	*		*	NA	NA	6
Querios da Mota, 2013	*	*	*	*		*	*	NA	NA	6
Saad, 2017	*	*	*	*		*	*	NA	NA	6
Sweeney, 2016	*	*	*	*		*	*	NA	NA	6

$: Sub-analysis of randomised control trial of malaria prevention studies. *Star: The NOS tool uses a star system to assess the methodological quality based on three criteria; i) participants' selection (4 stars), ii) comparability of study groups (2 stars), and iii) assessment of outcome/exposure (3 stars). Therefore, the highest total score for a cohort study was 7. Follow-up long enough for outcomes and adequacy of follow-up of cohorts are not applicable in these reviewed articles. NA: Not applicable.

Table A 1-4b: Risk of bias assessment using Newcastle – Ottawa Scale (NOS): Case-Control studies.

Author, Year, Reference	Selection				Comparability		Exposure			Total Stars
	Case definition	Representativeness	Selection of control group	Definition of control group	Most important clinical factors	Additional factors	Ascertainment of exposure	Method of ascertainment	Non-response rate	
Cox, 2016	*	*	*	*			*	*	NA	6
Dammann, 2003	*	*	*	*	*	*	*	*	NA	8
Gichangi, 1993	*	*	*	*			*	*	NA	6
Hillier, 1988	*	*	*	*		*	*	*	NA	7
Hillier, 1991	*	*	*	*	*	*	*	*	NA	8
Pettker, 2007	*	*	*	*	*	*	*	*	NA	8
Tsekura, 2010	*	*	*	*			*	*	NA	6

*Star: The NOS tool uses a star system to assess the methodological quality based on three criteria; i) participants' selection (4 stars), ii) comparability of study groups (2 stars), and iii) exposure (2 stars). Therefore, the highest total score for a case-control study was 8. Follow-up long enough for outcomes and adequacy of follow-up of cohorts are not applicable in these reviewed articles. NA: Not applicable

Variations of study participants

While an estimated gestational age of less than 37 weeks was the most common PTB definition, various lower cut-offs were used: seven different gestational age windows were described in 23 studies. Moreover, eight studies recruited early and extremely preterm newborns as the study participants and the classification of PTB was not uniform [6, 7, 11, 13-15, 20, 22].

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Histopathological methods

Placental tissue processing techniques varied and 20 different publications [27-46] were used to support histopathological classification systems, which were reported in the included studies (**Table A1-5a**). Six studies used standardised histopathological definitions: four [5, 11, 15, 25] using Redline *et al.* 2003 [41] and two using Khong *et al.*[36]. Seven studies used their own definitions of histological chorioamnionitis [12-14, 17, 18, 23, 24, 26] and three malaria studies [4, 16, 19] used four classifications [39]. Evidence of reporting of the severity of placental histopathological abnormality was limited to eight (34.8%) of studies [4, 8, 11, 15, 20, 23-25].

Table A 1-5a: Placental histopathological sample collection and classification system.

Author name, Year*	Details of sample collection	Classification system used for placental histopathology
Cox, 2016 [26]	"Tissue samples were collected from the sub-chorionic plate area on the foetal side of the placenta"	Kraus, 2004 [57] categorised the histopathological samples as the presence or absence of chorioamnionitis by PMN leucocytes. Severity of HP changes-not done
Dammann, 2003 [27]	"Formalin-fixed, paraffin-embedded, haematoxylin and eosin-stained sections of the umbilical cord, placental membranes and placental parenchyma."	Kaplan, 1991 [55] based presence of chorioamnionitis on the report by the College of American pathologists conference XIX. Severity of HP changes-not done.
Hecht, 2008 [31]	"Sections of the umbilical cord and a membrane roll and full-thickness sections from the centre and a paracentral zone of the placental disc."	Driscoll SG, 1991 [51] defined histologic chorioamnionitis based on the report by the College of American pathologist's conference XIX, while inflammation of the membrane using the scoring system similar to that proposed by *Redline, 2003 [10] Severity of HP changes-done and used for comparison.
Hillier, 1988 [33]	"The foetal membranes (chorioamnion) were grasped with forceps, and a strip 2 to 3 cm wide was cut from the point of rupture to the placental margin. A "membrane role" was made by rolling the foetal membranes onto a 1-mm wooden dowel. The membrane was fixed in 10 percent neutral buffered formalin, imbedded in paraffin, and stained with haematoxylin and eosin."	Author own: "≧ 10 PMN leucocyte per 10 non-adjacent field (x400 power)" Severity of HP changes-not done.
Hillier, 1991 [32]	"The foetal membranes were grasped with forceps, and a 2 to 3 cm strip was cut from the point of rupture to the discoid placental margin to prepare a "membrane roll" for histologic examination."	Author own: "≧ 10 PMN leucocyte per 10 non-adjacent field (x400 power)." Severity of HP changes-not done.
Honma, 2007 [34]	Not stated	Author own: "PMN leucocyte detected in chorion, amnion, subchorion and funisitis if PMN leucocytes found in umbilical vessels wall." Severity of HP changes-not done.
Ingrid, 2011 [35]	"Immediately after delivery, placentas and membranes were fixed in formalin for at least 6 hours. Sampling was performed with two membrane	*Redline, 2003 [10] was used to categorised the placental inflammation according to 3 stages of

	rolls, two cross sections of the cord and three representative blocks of the placental disk as a minimum. The tissues were embedded in paraffin until histopathological examination."	maternal inflammatory response and /or foetal inflammatory response. Severity of HP changes- done and used for comparison.
Kwak, 2014 [37]	Not stated	Author own: "presence of acute inflammatory changes of membranes of the placenta after delivery" Severity of HP changes-not done.
Namba, 2010 [40]	Not stated	Blanc, 1981 [63] Severity of HP changes- done.
Patel, 2018 [42]	Not stated	**Khong, 2016 [56] Severity of HP changes-not done.
Pettker, 2007 [43]	"An approximately 1 cm^2 portion of amnion-chorion taken at a location away from the site of membrane rupture (amnion–chorion biopsy), and an approximately 1 cm^3 partial-thickness section from the foetal side of the placenta (placenta biopsy)."	Author own (modified): "Three histologic stages of chorioamnionitis (stage I: intervillositis, stage II: chorionic inflammation, and stage III: full-thickness inflammation of both chorion and amnion) were complemented by the histologic grading system devised by Salafia *et al.* [60,65], which includes four grades of inflammation of the amnion, chorion– decidua, and umbilical cord." Severity of HP changes-done and used for comparison.
Queiros da Mota, 2013 [44]	"Each placenta was fixed in 10% buffered formalin solution for 48 h. After examination of the appearance, colour and insertion of the amniotic membranes, a 10 cm-wide membrane roll was rolled with the amnion inside, from the rupture site to the placental margin. Samples were taken at two levels of the roll, each containing the rupture site and the placental margin. Three samples were taken (at the two extremities and in the middle). The placental disk was measured and weighed after removal of the membranes and cord, and the foetal and maternal surfaces examined. Placental tissue was sampled, each 5–10 mm, including the area at the cord insertion, and at least three full-thickness villous	Kalousek, 1992 [54] Severity of HP changes-done and used for comparison.

	tissue samples. All samples were less than 1 cm thick. Microscopic examination was performed using H&E-stained sections."	
Sweeney 2016 [45]	Placentae were refrigerated under strict aseptic conditions within 24 hours of delivery. The external placental surface was decontaminated using 70% alcohol, and areas where the amnion had detached from the placenta were avoided, to minimise contamination. An incision was made into the amnion (ie, uppermost) membrane, and the interface between the amnion and chorion was identified. Chorioamnion tissue was also excised and placed into sterile cryogenic vials. All samples were stored at −80°C prior to analysis.	*Redline, 2003 [10] Severity of HP changes-done and used for comparison
Ategeka, 2019 [25]	"All placental sampling was done within 30 minutes of delivery. Placental specimens were fixed in 10% Neutral Buffered Formalin for 24 hours, and stored at room temperature in 70% ethanol prior to tissue processing. Collected tissue included: placental membranes ("membrane roll"), umbilical cord (two cross-sectional slices, one proximal and one distal to where the cord inserts into the placental disc), and chorionic plate with villous parenchyma. Specimens were dehydrated through a series of ethanol washes, cleared in xylene and embedded in paraffin wax blocks. A 3 μm thick section from each tissue block was obtained using a rotary microtome and sections mounted onto glass slides via a floatation water bath. Slides were baked in a hot air oven at 60˚C for 30 minutes, de-paraffinized in xylene, dehydrated through a series of ethanol washes, stained with Haematoxylin and Eosin (H&E), and mounted with organic mounting media."	*Redline, 2003 [10] Severity of HP changes-not done.
Feist, 2020 [28]	"At least two paraffin blocks of the umbilical cord tissue and the foetal membranes and at least four blocks from the placental parenchyma were produced for histological investigations."	**Khong, 2016 [56] and Baergen RN, 2011 [64] were used to classify the histological lesions. Severity of HP changes- done (high and low grade) and not used for comparison.
Gichangi, 1993 [29]	"Placenta obtained at delivery were fixed in 10% formalin for a period of two weeks to one month.	Placental leucocytic infiltration was reported as 0=no infiltration, 1= less than 5 PMN, 2= 2-5 PMN and 3= more than 10 PMN on high power

	Free membranes, umbilical cord and placenta were processed."	field microscopy as described by Benirschke, 1961 [49]. Severity of HP changes-not done.
Ladner, 1998 [38]	"Tissues from membrane and placenta were fixed in 10% formalin and stained with haemtoxylin and eosin. Tissues from membranes and placenta."	Author own: "≧ 10 PMN leucocyte in the amnion, chorioamniotic plate or both per several non-adjacent x400 power fields" Severity of HP changes-not done.
Ombimbo, 2019 [41]	Six biopsies of the placentas and foetal membranes were obtained immediately after delivery (2 central from either side of the cord insertion and 4 from peripheral aspects of the placenta at the 12, 3, 6, and 9 o'clock positions) and fixed in 10% neutral buffered formalin. The membranes were removed from the placentas and made into rolls; the rolls and placental biopsies were dehydrated in increasing concentrations of alcohol (70% -100%), 1 hour per solution. Then, they were cleared in trichloroethane (TCE) for 2 hours and infiltrated with paraffin for 12 hours. Finally, the specimens were embedded in fresh molten paraffin for 12 hours overnight. Blocks, which were sectioned and stained. Five micrometre serial sections were cut using a Leitz Wetzlar sledge microtome, floated in warm water, mounted on glass slides, and dried in a hot air oven at 40°C overnight. The sections were stained with Masson's trichrome, picrosirius red, or haematoxylin and eosin (H&E). H&E was used to demonstrate the general histoarchitecture. Masson's trichrome highlighted the connective tissue components. Picrosirius red stained collagen fibres.	Hromatka, 2013 [53] was used to describe the general structural organization of the amnion, chorion, and placenta was recorded, and the amount of fibrin deposition was noted; while other features were also scored and included villus degeneration, syncytiotrophoblast delamination, vascularity, red blood cell adhesion to terminal villi, intermediate mature to mature villi, syncytial knotting, villitis, and deciduitis. Terminal villi were scored using the method described in Altshuler, 1984 [47]. Severity of HP changes-not done.
Tsekoura, 2010 [46]	"Samples from the peripheral membranes stained with routine haematoxylin-eosin were blinded and evaluated by light microscopy to diagnose histological chorioamnionitis."	Author own "The presence of neutrophils in the membranes (chorion and/or amnion) was used to define chorioamnionitis." Severity of HP changes-not done.
Ategeka, 2020 [24]	"The basal plate was trimmed into a 3-mm slice and dehydrated through a series of ethanol washes, cleared in xylene, and embedded in paraffin wax blocks. A 3-µm thick section from each tissue block	Bulmer, 1993 [50] defined as uninfected (no evidence of parasites or pigment), active infection (parasites detected, no malaria pigment in fibrin), active-chronic (parasites detected and malaria

	was obtained using a rotary microtome, and sections were mounted onto glass slides via a flotation water bath. Slides were baked in a hot air oven at 60°C for 30 minutes, deparaffinized in xylene, dehydrated through a series of ethanol washes, stained with hematoxylin and eosin, and mounted with organic media. The histopathological process underwent extensive quality assurance to eliminate formalin pigment and minimise the effect of other artifacts.	pigment in fibrin), or past-chronic (parasites not detected, malaria pigment in fibrin). Muehlenbachs, 2010 [59] describes a simplified score that for grading the histologic features of placental malaria (PM). *A*, Categories of maternal inflammation in the intervillous spaces: I, minimal; II, present; and III, massive. *B*, Categorizing malarial pigment deposition in intervillous fibrin. Severity of HP changes-done and not used for comparison.
Kapisi, 2017 [36]	"Collection of specimens within 1 h of delivery, including placental tissue. Formalin-fixed paraffin-embedded placental biopsies were processed in duplicate for histological evidence of placental malaria."	First the reader is referred to Kakuru, 2016 [66] which then refers the reader to two manuscripts. The first is Rogerson, 2003 [67] with 5 categories: 1. Not infected - No malaria parasites or pigment, 2. Past infection- No parasite, only pigment, 3. Parasites present and pigment in fibrin, 4. Parasites, pigment in monocyte ± fibrin, 5. Parasite, no pigment in monocyte or fibrin. Muehlenbachs, 2010 [59] as described above. Severity of HP changes-not done.
Lufele, 2017 [39]	Placental biopsies were collected from the maternal side of the placenta. Incisions extended from the maternal to the foetal side of the placenta without reaching the foetal membrane. Biopsies were fixed and transported in 10% neutral buffered formalin. Sections were stained with Giemsa, cover slipped and returned to PNG for analyses.	Ismail, 2000 [58] Rogerson, 2003 [67] as described above. Severity of HP changes-not done.
Saad, 2017 [30]	"The placenta was collected in a clean labelled container and was preserved in 10% formaldehyde. After 24 hours of 10% formaldehyde fixation, the coded samples were processed for routine paraffin embedding. Multiple sections of 4 μm thickness were cut on a rotary microtome from the middle of each specimen and mounted on clean gelatinized slides for H&E staining." Ten fields in 4 examined slides (ie total of 40 fields) were examined for each sample. In each field; the 10 smallest terminal villi (less than 80	Tntbirojn, 2009 [62] was used to classify the inflammatory changes, infarction, intervillous thrombosis, chorionic villitis, haemorrhagic endovasculitis, placental intravascular thrombi, trophoblast degenerative knots, perivillous fibrin deposition and fibrinoid necrosis and villous oedema. Both Gordijn, 2008 [52] and Khong, 2003 [65] were referenced to support scoring systems reported in "Quality of placental pathology reports" by giving

	μm in diameter) were microscopically evaluated, and then the mean was calculated.”	1 point to each of the previous criteria. The mean score was finally calculated for each group. Severity of HP changes-not done.

Abbreviations: PMN; polymorphonuclear

*see Redline 2003 detailed in Table A 1-5b (below)

**see Khong 2016 detailed in Table A 1-5c (below)

Table A1-5b: **Redline *et al.*, 2003: nomenclature and definitions of placenta reaction patterns related to amniotic fluid infection [10].**

Diagnostic categories	Suggested diagnostic terminology	Definitions
Maternal inflammatory response		
Stage		
1—Early	Acute subchorionitis or chorionitis	PMN in subchorionic fibrin and/or membrane trophoblast
2—Intermediate	Acute chorioamnionitis	Diffuse-patchy PMN in fibrous chorion and/or amnion
3—Advanced	Necrotizing chorioamnionitis	PMN karyorrhexis, amniocyte necrosis, and/or amnion basement membrane thickening/hypereosinophilia
Grade		
1—Mild–moderate	No special terminology required	Not severe as defined below
2—Severe	Severe acute chorioamnionitis or with subchorionic micro-abscesses	Confluent PMN (≥ 10 x 20 cells in extent) between chorion and decidua; ≥ 3 isolated foci or continuous band
Other	Chronic (or subacute) chorioamnionitis	Sub-amnionic mononuclear cell infiltrate with occasional PMN (meconium and hemosiderin-laden macrophages excluded)
Foetal inflammatory response		
Stage		
1—Early	With chorionic vasculitis or umbilical phlebitis	Intramural PMN-chorionic vessels and/or umbilical vein
2—Intermediate	With umbilical vasculitis (one or two arteries and vein) or umbilical pan vasculitis (all vessels)	Intramural PMN-umbilical artery or arteries (umbilical vein)
3—Advanced	With (subacute) necrotizing funisitis or with concentric umbilical perivasculitis	PMN associated debris in concentric bands-rings-halos around one or more umbilical vessels
Grade		
1—Mild–moderate	No special terminology required	Not severe as defined below

2—Severe	With a severe foetal inflammatory response or with intense chorionic (umbilical) vasculitis	Near confluent intramural PMN-chorionic and/or umbilical vessels with attenuation/degeneration of VSMC
Other	With associated foetal vessel thrombi	Recent thrombosis associated with intramural PMN
Other specific features	Peripheral funisitis Acute villitis Acute intervillositis with intervillous abscesses Decidual plasma cells	Focal aggregates of PMN at the umbilical cord surface PMN in villous stroma (or between trophoblast and stroma) Patchy-diffuse PMN in intervillous space Unequivocal plasma cells in decidua basalis or capsularis

PMN; polymorphonuclear leucocyte, VSMC;

Table A 1-5c: Khong *et al.,* 2016: Staging and Grading of the Maternal and Foetal Inflammatory Responses in Ascending Intrauterine Infection by Amsterdam placental workshop group consensus 2016 [56]

Maternal Inflammatory Response	
Stage	**Grade**
Stage 1—acute subchorionitis or chorionitis	Grade 1—not severe as defined
Stage 2—acute chorioamnionitis: polymorphonuclear leukocytes extend into fibrous chorion and/or amnion	Grade 2—severe: confluent polymorphonuclear leukocytes or with subchorionic micro abscesses
Stage 3—necrotizing chorioamnionitis: karyorrhexis of polymorphonuclear leukocytes, amniocyte necrosis, and/or amnion basement membrane hyper eosinophilia	
Foetal Inflammatory Response	
Stage 1—chorionic vasculitis or umbilical phlebitis	Grade 1—not severe as defined
Stage 2—involvement of the umbilical vein and one or more umbilical arteries	Grade 2—severe: near-confluent intramural polymorphonuclear leukocytes with attenuation of vascular smooth muscle
Stage 3—necrotizing funisitis	

Pathogen detection methods

Culture technique was used in 11 bacterial studies [7, 11-14, 17, 20, 22-25] and molecular methods were performed in three studies to identify the infective bacterial organisms [6, 15, 25]. The majority of studies used a single sampling method (placental tissue or placental swab or vaginal swab) while three studies used two or more sampling techniques [13, 22, 23]. For studies targeting human immunodeficiency virus (HIV) infection, two studies used an enzyme linked immunosorbent assay (ELISA) as a diagnostic test [9, 18], while another two studies did not mention the diagnostic methods [5, 21]. In another two studies that targeted viral infection, polymerase chain reaction (PCR) was performed to detect adenovirus, human papilloma virus (HPV) and enterovirus [8, 26]. Two of three malaria studies confirmed infection by peripheral blood films, placental blood microscopy, and Loop-mediated isothermal application (LAMP) [4, 16] but the histopathological detection of malaria parasites and pigment in placenta was used in all three malaria studies [4, 16, 19].

Placental histopathologic findings in studies of preterm birth with bacterial infection

There were 13 studies that reported placental histopathology and PTB with bacterial infection. A wide range of bacterial species (n=54) were reported with *Ureaplasma* species (7/13), Group B *streptococcus* (5/13) and *Mycoplasma hominis* (6/13) being the most common. Four articles specifically investigated only *Ureaplasma* and *Mycoplasma* species [7, 14, 17, 20]. Another two studies examined the association between selected microorganisms (*Chlamydia trachomatis* and group B *Streptococcus* (*GBS*)) and PTB [15, 22]. Histological chorioamnionitis was the most common placental histopathological abnormality in PTB with bacterial infection [5-7, 9, 11-15, 17, 18, 22-25]. Four studies reported both chorioamnionitis and funisitis [7, 9, 11, 23].

Ingrid *et al*. used PCR to identify *C. trachomatis* and studied its association with placental histopathology in PTB. They reported that a chlamydia infection was two times as likely to be

64 associated with placental histological inflammation in preterm cases compared to absence of
65 chlamydia infection [15]. The rates of histological chorioamnionitis between *GBS* positive and
66 negative groups of PPROM were not significantly different, as described by Patel *et al*. [22].
67 Pettket *et al.* reported that in preterm cases with a positive amniotic fluid culture, acute
68 histological chorioamnionitis and funisitis were more pronounced when compared to preterm
69 amniotic fluid culture negative cases [23]. Querios da Mota *et al.* showed that the proportion of
70 histological chorioamnionitis with positive bacteriological culture in preterm placentas was 25%
71 and more than 50% of microbial culture results came from preterm placentas [24]. Sweeney *et al.*
72 also concluded that histological chorioamnionitis was more likely to be associated with
73 moderate/late PTB, complicated by an infection with *Ureaplasma* species, the most common
74 organisms identified in placental culture [25]. More detailed information on these studies is
75 provided in **Table A 1-6.**

76 **Table A 1-6: Identified bacterial infection and histopathological changes in preterm birth.**

Author, Year	Reported pathogenic microorganism	Diagnostic method	No. PTB (%)	Infection	Histopathological abnormalities	Triad of histopathology, infection and PTB
Cox, 2016 [6]	*Mycoplasma, Ureaplasma (parvum, urealyticum), GBS, Gardnerella*	RT-PCR (placental tissue)	57/57 (100%)	*U. parvum* was detected in 11 (19.3%), *G. vaginalis* in 10 (17.5%), *GBS* in 9 (15.8%), and *U. urealyticum* in 2 (3.5%) placental samples. However, *M. hominis and M. genitalium* were not detected. *U. urealyticum and GBS* were more common in second trimester, and G. *vaginalis* was more detected in third trimester samples.	Histological chorioamnionitis was found in 24/57 (42.1%) preterm placentas and 26/57 (45.6%) preterm placentas had at least one bacterial infection. *U. urealyticum* and GBS in 2nd trimester placental sample and *G. vaginalis* in 3rd trimester were not associated with histological chorioamnionitis.	Only *U. parvum* was significantly associated with histological chorioamnionitis in preterm placentas (OR 5.0; 95%CI 1.2-21.5, P=0.002).
Damma nn, 2003 [7]	*U. urealyticum M.hominis*	Culture (placental swab)	464/464 (100%)	*U. urealyticum* and *M. hominis* were detected in 139/464 (30%) and 27/464 (6%) of placentas, respectively. Both microorganisms were positive in 21/464 (4.5%) placental samples.	Foetal vasculitis was associated with *U. urealyticum* (53%, P ≤0.001) and *M. hominis* (14%, P ≤0.001) compared to those without foetal vasculitis.	In multivariate analysis (adjusted by gestational age, duration of membrane rupture), *U. urealyticum* culture positive placenta was associated with increased risk of foetal vasculitis (aOR 3.4; 95%CI 2.1-5.7) compared to *U. urealyticum* culture

						negative placenta: risk increased with a short duration of membrane rupture and with a gestational age >28 weeks.
Hecht, 2008 [11]	*Actinomyces spp, Prevotella spp, Corynebacterium spp, E.coli, Lactobacillus spp, staphylococcus spp, GBS, group D streptococcus, Alpha hemolytic streptococcus, anaerobic streptococcus, G. vaginalis, Mycoplasma spp, U. urealyticum*	Culture (placental tissue)	835/835 (100%)	41% of cultured preterm placentas were positive; *E. coli and Mycoplasma spp* were most common microorganisms.	The microorganisms associated with high grade/stage inflammation of chorionic plate and foetal vasculitis were *Actinomyces spp, Prevotella spp, Corynebacterium spp, E. coli, Lactobacillus spp, staphylococcus spp, GBS, group D streptococcus, Alpha hemolytic streptococcus, anaerobic streptococcus, G. vaginalis, Mycoplasma spp, U. urealyticum.*	In preterm birth delivered by Caesarean section, high grade/stage inflammation of the chorionic plate (OR 4.6; 95%CI 3.2-6.7), chorionic vasculitis (OR 3.9; 95%CI 2.6-5.8) and umbilical cord vasculitis (OR 3.4; 95%CI 2.2-5.4) were more associated with bacterial infection compared to placentas without inflammation.
Hillier, 1988 [13]	*U. urealyticum, M. hominis, G. vaginalis, Mobiluncus spp, Peptostreptococcus spp, Bateroides spp, streptococci, Lactobaccilli*	Culture (placental swab and vaginal swab) Gram stain	38/74 (51%)	The most common microorganisms found in preterm placentas were *U. urealyticum* 18/38 (47%) and *G. vaginalis* 10/38 (26%).	Bacterial vaginosis was also associated with histological chorioamnionitis (OR 2.6, 95%CI 1.0-6.6).	After controlling for demographic and obstetric variables, preterm birth was related to bacterial infections of placentas (OR 3.8; 95% CI 1.5-9.9) and with histological chorioamnionitis (OR 5.0; 95%CI 1.6-15.3). Any microorganisms recovered from the placentas (OR 7.2;

						95%CI 2.7-19.5) and *U. urealyticum* with or without other organisms (OR 9.8; 95%CI 3.5-19.5) were significantly associated with histological chorioamnionitis, regardless of gestational age. Preterm birth did not alter the result.
Hillier, 1991 [12]	*Genital mycoplasma (U. Urealyticum, M. hominis), Facultative bacteria (GBS, E.coli, G. vaginalis, Streptococci, Enterococcus, Lactobacillus), Anaerobic bacteria (Peptostreptococcus, Fusobacterium, Bacteroides, Actinomyces, Mobiluncus), Yeast (C. Albicans)*	Culture (placental swab)	112/268 (42%)	Microorganisms were detected 36/112 (32%) and 29/156 (19%) placentas in cases (≤ 34 weeks' gestation) and controls (>34 weeks' gestation), respectively. Two or more bacteria were identified from 17 (15%) of 112 placentas in the case group (≤ 34 weeks' gestation) and 12 (8%) of 156 placentas in the control group (>34 weeks' gestation) (P=0.05).	Placental histological chorioamnionitis was detected in 66/112 (59%) of cases and 35/156 (22%) of controls.	Histological chorioamnionitis and bacterial infection (*U. urealyticum* not included) were strongly associated in cases after controlling for cofounding variables e.g., mode of delivery, duration of membrane rupture and bacterial vaginosis (OR 7; 95%CI 3.0-16.4). *U. urealyticum, E. coli, Bacteriodes* were significantly associated with histological chorioamnionitis (P<0.05). *GBS* but not *Peptostreptococcus* was also associated with both preterm

						delivery and histological chorioamnionitis.
Honma, 2007 [14]	*U. urealyticum*	Culture (placental swab)	105/105 (100%)	*U. urealyticum* was positive in 17 placentas (25.8%) of non-chronic lung disease (N-CLD), and 15 placentas (38.5%) of chronic lung disease group. Other microorganisms detected in 12 placentas (18.2%) with or without *U. urealyticum* were *coagulase-negative Staphylococcus* (CoNS), *Enterococcus*, *Candida*, *α-Streptococcus*, *Enterobacter*, *Bacillus* and *Mycoplasma hominis*.	39/105 (37%) preterm placentas (<32 weeks' gestation) had histological chorioamnionitis. 21/39 (54%) placentas with histological chorioamnionitis had colonisation of *U. urealyticum* (OR 6.73, 95% CI:1.89-23.91).	In multivariate analysis, histologic chorioamnionitis was associated with premature rupture of membrane (OR 10.19; 95%CI: 3.10 – 33.56), placental colonization of U. urealyticum (OR 6.73, 95%CI: 1.89 – 23.91), neonatal colonization of other microorganisms (OR 7.33, 95%CI: 1.22 – 44.13).
Ingrid, 2011 [15]	*Chlamydia trachomatis*	PCR (placental tissue)	304/304 (100%)	*C. trachomatis* was harboured in 76/304 (25%) preterm placentas (≤32 weeks' gestation).	Histological evidence of placental inflammation was found in 123/304 (40%) preterm placentas: 64/123 (52%) had inflammation of maternal and foetal placental tissue, 50/123 (41%) had only maternal tissue inflammation and 4/123 (3%) had	*C. trachomatis* infection was more associated with placental histological inflammation: 41/76 (54%) placentas with *C. trachomatis* versus 82/228 (36%) preterm placentas without *C. trachomatis* infection (OR 2.1; 95%CI 1.2-3.5).

					inflammation of foetal placental tissue. Other abnormal placental findings such as peripheral funisitis, acute villitis, acute intervillositis with intervillous abscesses were detected in 5/123 (4%) placentas.	
Kwak, 2014 [17]	*U. urealyticum, M. hominis*	Culture (vaginal swab)	179/179 (100%)	112/179 (62%) of vaginal fluid cultures were positive for genital mycoplasma (99 cases were only positive for *U. urealyticum* and 13 were positive for both *U. urealyticum* and *M. hominis*). But no samples were positive for *M. hominis*.	50/179 (28%) of preterm placentas had histological chorioamnionitis. However, 36/112 (32%) of *U. urealyticum* positive cases had histological chorioamnionitis, which was statistically significant (P<0.05).	Patients with culture positive for both *U. urealyticum* and *M. hominis* 13/112 (12%) had a significantly higher proportion of preterm birth (100% (13/13) vs 73% (73/99), P<0.001) and histological chorioamnionitis (62% (8/13) vs 29% (29/99), P=0.019) than patients only positive for *U. urealyticum*.
Namba, 2010 [20]	*Ureaplasma spp*	Culture (placental swab) PCR (placental tissue)	151/151 (100%)	63/151 (42%) of preterm placentas (<32 weeks' gestation) harboured *Ureaplasma spp* compared to 10/41 (24%) term placentas (P<0.05).	Histological chorioamnionitis was more common in preterm placentas of *Ureaplasma spp* positive (52/63, 83%) compared to placentas without infection (29/63, 46%) , P<0.001. Histological chorioamnionitis with funisitis	In multivariate analysis, the association between *Ureaplasma spp* positivity in preterm placentas and histological chorioamnionitis was significant (OR 8.6; 95%CI 3.72-19.89).

					was also significantly associated with *Ureaplasma* positive preterm placentas than negative group (46% vs 7%, P<0.001).	
Patel, 2018 [22]	*GBS*	Culture (vaginal and rectal swab)	181/181 (100%)	*GBS* was positive in 55/181 (29.4%) of preterm cases.	The rate of histological chorioamnionitis was not significantly different between *GBS* negative and positive groups (81/126 (64.2%) vs 38/55 (69%), P=0.62). Histologic chorioamnionitis was associated with earlier gestational age (29.2 versus 31.3 weeks, P<.0001) at birth. Earlier gestational age (<30 weeks) was associated with histological chorioamnionitis (P<0.001).	This single-centered, retrospective cohort study demonstrated that genital GBS colonization does not appear to be associated with an increased rate of Histologic chorioamnionitis in patients with PPROM <34 weeks of gestation.
Pettker, 2007 [23]	*U. urealyticum, Bacteriodes, Gram(+) anaerobes, E.coli, Group B Streptococcus, Peptostreptococcus spp, Streptococcus Pneumoniae*	Culture (amniocentesis microbial culture, placental tissue biopsy, placental swab)	183/183 (100%)	Of 29/56 amniotic culture positive preterm samples, the most common micro-organism was *U. urealyticum* (12/29,31.5%).	Amniotic fluid culture negative with preterm birth was more associated with histological chorioamnionitis and funisitis than term pregnancy control group (P<0.001).	"Positive amniotic fluid cultures with preterm birth had higher severities (median grades) of funisitis and acute histologic chorioamnionitis compared with those with negative amniotic cultures and

						term pregnancy controls group (P<0.05)".
Queiros da Mota, 2013 [24]	*Bacteria species including gram positive and negative*	Culture (placental swab)	202/376 (53.7%)	73/376 (19.4%) placentas were bacterial culture positive; 38/73 (52%) were preterm placentas. 193 microorganisms were detected in 152 positive cultures and *gram positive cocci* was the most common.	101/376 (26.9%) placentas had histological chorioamnionitis; 43/101 (42%) of term and 53/101 (52%) of preterm placentas. The rate of positive cultures was higher in placentas with histological chorioamnionitis compared to those without (27% vs 16%, P=0.01).	Preterm deliveries (extremely, plus very, plus moderate, plus late preterm) in which the proportion of histologic chorioamnionitis with positive and negative cultures was 25% (14/56) and 75% (42/56) respectively.
Sweeney 2016 [25]	*U. parvum, U. urealyticum, GBS, Bacteroides, E. coli, G. vaginalis, Bifidobacterium spp, Propionibacterium spp*	Culture (placental swab) 16s rRNA PCR (placental tissue)	535/535 (100%)	Microorganisms were identified in 57/535 (10.6%) placentas. A total of 61 microorganisms were isolated from these placentas and *U. parvum* (36/61, 59%) was the most prevalent.	Placentas infected by microorganisms were more likely to have histological chorioamnionitis than noninfected placentas (31/47, 54.4% vs 90/427, 18.8%; P <0.001), irrespective of gestational age and ethnicity (P=0.528).	Histological chorioamnionitis was significantly associated with moderate/late preterm birth in the presence of *Ureaplasma spp* (P<0.001).

Abbreviations*:* AC, amniocentesis; *C. albican, candida albican; C. trachomatis, Chlamydia trachomatis; E.coli, Escherichia coli; GBS, group B streptococcus; G. vaginalis, Gardnerella vaginalis; M. hominis, Mycoplasma hominis; M. genitalium, Mycoplasma genitalium; spp, species; U. urealyticum, Ureaplasma urealyticum; U. parvum, Ureaplasma parvum*

Placental histopathology in studies of preterm birth with viral infections.

The main placental abnormality in four of six viral infection studies was histologically confirmed chorioamnionitis [5, 9, 18, 26]. Ategeka *et al*. reported that the risk of PTB was significantly increased with histological evidence of severe maternal acute chorioamnionitis in HIV infected pregnancies but not associated with foetal acute chorioamnionitis [5]. Obimbo *et al.* reported that fibrinoid deposition with villous degeneration, syncytiotrophoblast delamination, increased red blood cell adhesion to terminal villi and increased number of capillaries were significantly associated with preterm placentas in HIV infection and/or antiretroviral therapy [21]. Gichangi *et al*. reported that the likelihood of having moderate to severe histological chorioamnionitis and moderate to severe funisitis was significantly increased in preterm placentas from HIV seropositive pregnant women compared to HIV negative women, and concluded that HIV infection was a risk factor for histological chorioamnionitis in preterm deliveries [9]. Feist *et al*. did not find an association between HPV and enteroviruses in the development of placental histological abnormalities (i.e., villitis of unknown origin and chronic deciduitis) in both preterm and term pregnancies [8]. Tsekoura *et al.* reported a significant association between histological signs of chorioamnionitis and an adenovirus infection in preterm placentas compared to adenovirus negative samples [26]. Other viruses commonly causing placental lesions and adverse birth outcomes include cytomegalovirus, herpes simplex virus, and parvovirus B19. However, no study investigating these viruses met the inclusion criteria of this review. More information on placental histological findings and viral infection can be seen in **Table A 1-7**

100 **Table A 1-7: Identified viral infection and histopathological changes in preterm birth.**

Author, Year	Reported microorganism	Diagnostic method	No. PTB (%)	Infection	Histopathological abnormalities	Triad of histopathology, infection and PTB
Ategeka, 2019 [5]	*HIV*	Not mentioned	18/193 (9.4%)	All women had *HIV* and treated with combination of ART for >90 days (67.4%), and were WHO *HIV* disease stage 1 (asymptomatic) (94.8%).	102/193 (52.8%) of placentas had either maternal and/or foetal histological chorioamnionitis. 44.5% (22.5% mild, 11.0% moderate, 11.0% severe) and 28.0% (17.6% mild, 9.3% moderate, 1.0% severe) of placentas had maternal and foetal histological chorioamnionitis, respectively.	Risk of preterm birth was significantly associated with severe maternal histological chorioamnionitis compared to none-mild in HIV infected women (28.6% vs 6.0%; aOR 6.04; 95%CI 1.87-19.5, P=0.003). Risk of preterm birth was not associated with foetal histological chorioamnionitis.
Feist, 2020 [8]	*HPV, enterovirus*	HPV-PCR and RT-PCR for enterovirus	14/50 (28%)	HPV and enterovirus were not detected in any specimen with abnormal placental histopathology findings (VUE and CD).	20 cases with VUE and 30 cases with chronic deciduitis with plasma cells were included. CD (but not VUE) was associated with PTB (4/30,15%) and PPROM (7/30, 26%).	A causal role for enterovirus and HPV in the development of VUE and CD was unlikely.
Gichangi, 1993 [9]	*HIV*	Enzyme immunoassay (EIA), confirmed by Western Blot	117/467 (31.4%)	Maternal HIV-1 positive rate was 3.1% in 216 liveborn term and 8.6% in 117 preterm births. Maternal HIV-1 was independently associated with preterm	Preterm birth was strongly associated with histological chorioamnionitis (OR 2.3; 95%CI 1.4-3.8, P<0.001), funisitis (OR 6.7; 95%CI 3.2-14.2, P<0.001) and villitis (OR 7.8; 95%CI 12.0-35.5, P<0.001).	In HIV positive mothers, preterm placentas were associated with moderate to severe chorioamnionitis (OR 3.2; 95%CI 1.1-9.5, P<0.05) and moderate to severe funisitis (OR 6.1;

				birth OR 2.1 95%CI 1.1-4.0).		95%CI 1.2-42.7, P<0.05) compared to HIV negative preterm placentas.
Ladner, 1998 [18]	*HIV*	Enzyme link immunosorbent assay (ELISA)	39/275 (14%)	275 HIV negative and 286 HIV positive placenta were examined. The rate of STDs (24-28 weeks gestation, and all treated) was not statistically different by HIV serostatus.	Histological chorioamnionitis was not associated with serostatus: 27 (9.8%) HIV positive and 28 (9.8%) HIV negative women. No statistical association, independent of HIV serostatus, was found between histological chorioamnionitis and STDs.	In HIV positive women but not HIV negative women, the risk of preterm birth and premature rupture of membranes was higher in histological chorioamnionitis than in controls (RR 3.0; 95%CI 1.5-6.3, P=0.003) and (RR 2.9; 95%CI 1.4-6.1, P=0.01).
Ombimbo, 2019 [21]	*HIV*	Not mentioned	81/101 (80.2%)	38/81 (47%) preterm placentas were HIV positive cases and 43/81 (53%) were HIV negative.	Placental histopathological features including immature villi, syncytial knotting, villitis and deciduitis were not significantly different between HIV positive and negative preterm placentas.	The following placental histopathological changes between HIV positive and HIV negative preterm placentas were significantly different; fibrinoid deposition with villous degeneration (59% vs 27%, P=0.026), syncytiotrophoplast delamination (46% vs 9%, P=0.006), increased red cell adhesion to terminal villi (50% vs 9%, P=0.003) and increased number of capillaries (32% vs 0%, P<0.05).
Tsekoura, 2010 [26]	*Adenovirus*	PCR (placenta tissue)	37/58 (63.8%)	Detection of adenovirus was higher in preterm (29/71, 40.8%) than term placentas	Histological chorioamnionitis was more common in preterm than term placentas (49% vs. 19%; P=0.025). In	In adenovirus PCR-positive placentas, histological chorioamnionitis was more frequent in preterm than term

				(25/122, 20.5%), (OR 2.7; 95%CI 1.4-5.1, P=0.002).	preterm placentas, histological chorioamnionitis was more common in adenovirus PCR-positive than adenovirus negative samples (75% vs. 36%; P=0.026).	placentas (75% vs. 36%; P=0.003). However, in adenovirus PCR-negative placentas, abnormal histological findings did not differ significantly between preterm and term (36% vs. 20%; P=0.488).

Abbreviations: ART, anti-retroviral therapy; CD, chronic deciduitis*; HIV, human immunodeficiency virus; HPV, human papilloma virus;* OR, Odds ratio; PCR, polymerase chain reaction; VUE, villitis of unknown origin

Placental histopathology in studies of preterm birth with parasitic infection

The placental histopathological abnormality with *P. falciparum* malaria infection in pregnancy was the presence of malarial parasites and/or pigment, while no histological correlate of chorioamnionitis with PTB was described in three malaria studies [4, 16, 19]. Ategeka *et al.* [4] concluded that the risk of PTB was not increased with histological evidence of placental malaria pigment in malaria infected pregnant women from Uganda compared to those not-infected. However, placental malaria pigment is significantly associated with small for gestational age and low birth weight, and it was recommended to measure adverse birth outcomes in malaria intervention studies, especially in malaria high-transmission areas[4]. Kapisi *et al.* mentioned that the risk of PTB was significantly higher if malaria parasites were detected in the placenta in comparison with non-malaria infected cases, and placental malaria pigment was not associated with preterm deliveries [16]. Lufele *et al*. also reported an association between histopathological confirmed placental malaria infection and adverse birth outcomes. A chronic infection, defined as the presence of malaria parasites and pigment deposition in monocytes and/or fibrin, was significantly associated with increased risk of PTB, while acute placental infection, defined as the presence of malaria parasites but not pigment, increased the risk of PTB at a non-significant level [19]. Saad *et al*. investigated an association between a *Toxoplasma gondii* infection and PTB by using the mean histopathological score based on abnormalities such as fibrin deposition, intervillous haemorrhage, villous degeneration and fibrosis, haemorrhage and nearby inflammatory cellular infiltrate and infarction [10]. They reported that the highest pathological score was observed in anti-toxoplasma IgM positive cases compared to anti-toxoplasma IgM/IgG negative cases [10]. No other studies on parasites such as soil transmitted helminths, *Schistosomiasis*, *Trichomonas vaginalis*,

125 *Giardia*, *Cryptosporidium* were identified for this review. The detailed information on included
126 studies is shown in **Table A 1-8**.

127 **Table A 1-8: Identified parasitic infection and histopathological changes in preterm birth.**

Author, Year	Reported pathogenic microorganism	Diagnostic method	No. of PTB (%)	Infection	Histopathological abnormalities	Triad of histopathology, infection and PTB
Ategeka, 2020 [4]	*P. falciparum*	Placental blood smear, placental blood for LAMP, placental histopathology	37/637 (5.8%)	51.8% had microscopic parasitaemia, and 82.3% had microscopic or sub microscopic parasitaemia.	Women randomized to IPTp with SP had a significantly higher prevalence of malaria at delivery compared with women randomised to IPTp with DP: evidence of parasites or malaria pigment by histopathology (61.7% vs 28.2%, P< 0.001).	By binary classification, any malaria parasite or pigment detected by placental histopathology did not increase the risk of preterm birth (aRR 1.09; 95%CI 0.54-2.2, P=0.82) but was associated with an increased risk for SGA (aRR 2.11; 95%CI 1.25-3.54, P=0.005). By using the Bulmer classification system, acute-chronic infection (parasite and pigment detected) was not associated with PTB (aRR 1.64; 95%CI 0.47-5.76, P=0.44), SGA (aRR 0.88; 95%CI 0.21-3.64, P=0.86) or LBW (aRR 2.01; 95%CI 0.59-6.88, P=0.27) compared to malaria uninfected samples. However, past-chronic infection (only malaria pigment detected) was associated with increased risk for SGA (aRR 2.14; 95%CI 1.27-3.60, P=0.004) but not PTB (aRR 1.06; 95%CI 0.51-2.18, P=0.88).

Kapisi, 2017 [16]	*P. falciparum*	Microscopy of placental blood smear, LAMP detection of parasite DNA in placental blood, placental histopathology	26/282 (9.2%)	Of 282 women, 52 (18.4%) had no episodes of symptomatic malaria or asymptomatic parasitaemia during the pregnancy, 157 (55.7%) had low malaria burden (0–1 episodes of symptomatic malaria and < 50% of samples LAMP+), and 73 (25.9%) had high malaria burden during pregnancy (≥ 2 episodes of symptomatic malaria or ≥ 50% of samples LAMP+).	Compared to women with no malaria exposure during pregnancy, the risk of placental malaria by histopathology was higher among low and high burden groups (aRR 3.27; 95%CI 1.32–8.12 and aRR 7.07; 95%CI 2.84–17.6). Detection of placental parasites by any method was significantly associated with PTB (aRR 5.64; 95%CI 1.46–21.8), irrespective of the level of malaria burden during pregnancy.	After adjustment (gravidity, IPTp), PTB was significantly associated with any malaria burden during pregnancy plus placental parasites detected by microscopy of placental blood smear, LAMP detection of dried placental blood spot and placental histopathology (aRR 5.88; 95%CI 1.02-34.0, P=0.048). Risk of PTB was not significantly associated with any malaria burden during pregnancy when pigment only was detected by placental histopathology (aRR 1.74; 95%CI 0.34-8.96, P=0.51).
Lufele, 2017 [19]	*P. falciparum*	Peripheral blood smear (thick and thin films), placental histopathology	81/962[a] (8.4%)	11.9% (172/1448) experienced symptomatic malaria during their pregnancy.	Of 1451 placentas examined, 18.5% (269/1451) showed evidence of current or past PM. There were 7.5% active infections [3.7% (54/1451) acute, and 3.8% (55/1451) chronic], and 11.0% (160/1451) past infections.	After adjustment for cofounding variables, women with chronic placental malaria infection significantly had an increased risk of PTB compared to malaria uninfected women (OR 3.92; 95%CI 1.64-9.38, P=0.002). Although acute placental malaria infection was more likely to have PTB, the finding was not significant (OR 2.33; 95%CI 0.86-6.35, P=0.097).

Saad, 2017 [10]	*Toxoplasma Gondii*	Anti-*toxoplasma gondii* IgM and IgG by Indirect haemagglutination assay (sensitivity threshold 8 IU/ml), placental histopathology	37/240 (15.4%)	60 in each group defined as Group A: anti-*Toxoplasma* IgM and IgG negative; Group B: positive anti-*Toxoplasma* IgM or IgM seroconversion in mother and neonate; Group C: rising anti-*Toxoplasma* IgG during pregnancy; Group D: fixed low (non-rising) anti-*Toxoplasma* IgG during pregnancy.	The common placental histopathological findings in the IgM-positive group (group B) were fibrin deposition, intervillous haemorrhage, villous degeneration and fibrosis, haemorrhage and nearby inflammatory cellular infiltrate and infarction. Group C exhibited higher percentages of inflammatory changes then group D.	The detected abnormal placental findings were calculated and reported as the pathological mean score. This score was significantly higher (P< .001) in IgM-positive cases who ended with miscarriage or PTB (9.28 ± 0.83 and 6.80 ± 0.68, respectively) compared with IgG rising cases with the same pregnancy outcomes (4.88 ± 0.35 and 4.05 ± 0.65, respectively).

Abbreviations: ACA, (acute) chorioamnionitis; aRR, adjusted risk ratio; BMT, basal membrane thickening; *CMV, cytomegalovirus*; *GBS, group B streptococcus*; *HPV, human papilloma virus*, IAI, intra-amniotic inflammation; LAMP, Loop-mediated isothermal amplification; MIAC, microbial invasion of the amniotic cavity; OR, odds ratio; PLMN, polymorphonuclear leukocyte; PPROM, preterm premature rupture of membranes; PRBC, parasitised red blood cell; PTB, preterm birth; SGA, small for gestational age

[a] Total sample size was 1451 participants but only 962 patients had ultrasound scans to determine preterm birth

Discussion

Discussion of the main findings, strengths and limitations

This is the first systematic literature review to summarise placental histological abnormalities in PTB cases with a confirmed infection as far as I am aware. The strength of this review is that it includes studies reporting the triad of placental histopathology, infection and PTB, allowing an inference on the causal pathways. However, the heterogeneity across studies meant it was not possible to combine the results in a meta-analysis as the included studies had different study designs, employed various diagnostic techniques to confirm causative pathogens with different test sensitivities, and used various systems for placental histopathological classification. Additionally, several PTB subcategories (spontaneous PTB with intact membranes, PPROM and indicated or induced due to maternal or foetal complications) were investigated in most of the reviewed studies, again preventing data pooling. Only 12 articles specified in the Methods section that gestational age was determined by ultrasound. Notably, only one in three studies were conducted in an LMIC, where neonatal mortality from PTB is highest [47]. Another limitation is that this systematic review fails to explore the association between specific causative microorganisms and specific placental histopathological changes due to study diversity.

Histopathological examination of the placenta and infection

Histological examination of the placenta is a very useful tool for assessing the multifactorial aetiology of PTB [48] as well as other adverse pregnancy outcomes (e.g., stillbirth and SGA) [49]. While placental tissue sample collection and the classification system of placental histological abnormalities varied, histological chorioamnionitis was common and strongly associated with PTB in the presence of both bacterial and viral infection in this systematic review. In malaria, histopathology can confirm infection, although it is not usually described as

chorioamnionitis. The placental malaria confirmed by histopathological examination (presence of malaria infected red blood cells or malaria pigment in the intervillous space) is a better diagnostic tool for malaria infection rather than peripheral blood film due to subpatent infections and it is frequently used to assess the adverse pregnancy outcomes in malaria studies [30, 38, 39, 42, 50]. Lufele *et al*. found that chronic placental malaria infection increased the risk of PTB but the risk of PTB was not increased in patients with acute placental malaria infection [19]. Similarly, Kapisi *et al.* found that the risk of PTB was significantly higher if malaria parasites, but not pigment, were detected in the placenta. Although the association between PTB and placental malaria remains inconclusive, it was significantly associated with other adverse birth outcomes such as SGA and miscarriage [4, 16, 19]. The only other study in the parasitic subgroup was of toxoplasmosis in which placental histopathological findings were more similar to acute and chronic inflammatory changes reported for bacteria, although the authors created their own detailed scoring system [10].

A methodological limitation in the process of diagnosing histological chorioamnionitis is that selection of a piece of placental tissue may lead to sampling of a segment with missing focal lesions in the placenta. Additionally, an assumption poorly supported by strong evidence is that not enough time may have passed for the placental histopathological abnormality to develop, especially in the early stages of an infection and it should be considered in future studies [24]. Pettker *et al*. concluded that histopathological examination of the placenta and microbiological tests fail to diagnose intra-amniotic inflammation and that caution is required in vaginal deliveries due to bacterial contamination or colonisation[23]. For an accurate interpretation of histopathological findings, clinical and procedural details should be reported. This includes exposure to antibiotics or antivirals, length of membrane rupture, number of vaginal examinations, and ascending

bacterial infection. These important clinical factors were not adequately described in most of the included studies (**Table A 1-3**) and remain an area for improvement in future PTB studies.

Diagnostic tools for causative pathogens

Causality between histopathological changes in the placental tissue, PTB and infection can only be established if the causative pathogen is identified. The development of more sensitive, non-selective diagnostic tools over the last few years, provides researchers with additional options to unravel the link between the triad of PTB, infection and placental histopathological abnormalities [51]. Diagnostic tools reported in the reviewed studies included bacteriological culture, nucleic amplification techniques, immunohistochemical staining of placental tissue, with only one study using 16S rRNA sequencing[25]. Improved methods will increase the range of detected pathogens and lead to an improved understanding of gestational tissue infection.

However, the presence of contaminants complicates research into invasive organisms. Contamination of the placenta and membranes can occur due to PPROM or during passage through the vagina, or skin during Caesarean section, and sequencing methods do not distinguish between viable and non-viable bacteria [52, 53]. Some authors in this review indicated that the role of infection in PTB may be underestimated due to limitations in the diagnostic methods [7, 23, 25], the difficulty of distinguishing between colonisation and contamination, [11] and decreased bacterial abundance due to antibiotic treatment prior to tissue sampling [32, 34, 35].

Evidence gaps

No manuscript in this review reached the ideal "gold standard" to link the pathogen and placental histopathology of "amniotic fluid culture/polymerase chain reaction (PCR) and placental pathology" [48]. Only one study included amniotic fluid culture [23] and only four used a PCR test to establish the presence of microorganisms [6, 15, 20, 26].

Implications of the findings

Current consensus is that PTB is a syndrome, not a single clinical entity, that involves inherited predisposition, and maternal, foetal factors and placental factors, as well as signs of initiation of parturition and mode of delivery [54,55]. Suggestions to improve future research on this subject include:

i) adherence to internationally recognised classification of PTB subgroups;

ii) adherence to standard methods of placental sampling and histopathology classification (including severity);

iii) a careful approach to study design as well as sensitive and specific infection detection methods, and

iv) an effort to associate (triangulate) placental histopathology, PTB and infection.

Summary

This chapter presents a review of currently published literature on the association between preterm birth, placental histopathological abnormality, and infection. Despite four in five neonatal deaths of preterm in low- and middle-income countries and a known role of infection in preterm birth most titles and abstracts were screened out because they did not include all three aspects in design and methods. Of the 23 studies included of 1529 screened manuscripts only one in three were undertaken in low- and middle-income countries. Histopathological chorioamnionitis and funisitis (when examined) were commonly observed in preterm birth complicated by confirmed bacterial or viral but not parasitic infection. There was variation in the definitions of preterm birth, methods of detection of infection, and modifications of recommended guidelines for reporting histopathology, preventing pooling of data. To determine causality study design needs to improve and to take place in high burden low- and middle-income settings.

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Appendix B

SMRU birth cohort profile

Table A3-1: SMRU birth cohort profile including maternal characteristics, morbidity during pregnancy, pregnancy outcomes and delivery factors in last three decades.

	1986-2000	2001-2010	2011-2020
	N=18,120	N=31,971	N=31,939
Maternal characteristics			
*Age, years			
< 20	3,046 (16.9)	5,087 (15.9)	5,481 (17.2)
20 to 34	12,695 (70.5)	21,821 (68.3)	21,518 (67.4)
≥ 35	2,275 (12.6)	5,058 (15.8)	4,924 (15.4)
Primigravida	4,381 (24.2)	8,124 (25.4)	10,312 (32.3)
Number of ANC visits (<8)	5,992 (33.1)	11,202 (35.0)	18,348 (57.5)
*ANC booking at 1st trimester	6,685 (38.8)	17,676 (56.6)	12,799 (40.1)
*Ethnicity			
Karen	1,860 (86.6)	11,379 (73.1)	16,843 (61.6)
Burmese	228 (10.6)	2,577 (16.5)	7,922 (30.0)
Others	61 (2.8)	1,624 (10.4)	2,574 (9.4)
Living status			
Refugee	17,239 (95.1)	19,134 (59.9)	9,018 (28.2)
Migrant	881 (4.9)	12,837 (40.1)	22,921 (71.8)
*Smoker	1,903 (39.8)	9,202 (28.9)	4,201 (13.2)
*Literate (can read)	NA	2,596 (52.7)	19,794 (62.0)
Maternal weight (cm), median [IQR]	48 [43-52]	48 [43-53]	50 [45-56]
*Maternal weight <40 kg	1,819 (10.3)	3,381 (10.7)	2,448 (7.7)
Height (cm), median [IQR]	152 [148-155]	151 [148-155]	151.6 [148-155]
*BMI in kg/m^2			

Underweight (< 18.5)	NA	3,423 (14.0)	3,467 (10.9)
Normal weight (18.5-22.9)	NA	14,874 (60.8)	17,365 (54.6)
Overweight (≥ 23)	NA	6,179 (25.3)	10,989 (34.5)
Morbidity during pregnancy			
Malaria infection in pregnancy	4,178 (23.1)	5,605 (17.5)	1,097 (3.4)
*HIV	NA	NA	106 (0.4)
*Syphilis	NA	NA	185 (0.8)
*Maternal anaemia	2,166 (21.0)	4,360 (13.8)	2,098 (6.6)
PE or eclampsia	178 (0.9)	431 (1.4)	581 (1.8)
Pregnancy outcomes			
EGA at birth (weeks), median [IQR]	39.1 [37.1-40.2]	38.4 [33.6-39.6]	38.6 [34.0-39.6]
Birth weight (gram), mean (SD)	2,922 (536)	2,983 (595)	2,999 (556)
*Foetal sex (Male)	7,697 (42.5)	11,495 (35.9)	11,497 (36.0)
*Type of outcomes			
Singleton pregnancy	15,286 (91.0)	22,112 (87.2)	22,043 (89.6)
Multiple pregnancy	162 (1.0)	287 (1.1)	255 (1.0)
Miscarriage	1,342 (8.0)	2,961 (11.7)	2,297 (9.4)
[a]PTB	1,374 (12.6)	2,048 (9.5)	1,579 (7.2)
[b]SGA	2,773 (29.5)	4,159 (23.1)	4,147 (20.6)
*Stillbirth	268 (1.8)	293 (1.3)	252 (1.1)
Delivery factors			
*Mode of delivery			
**Vaginal delivery	10,938 (96.0)	21,168 (94.6)	20,473 (91.8)
Instrumental delivery	129 (1.1)	420 (1.9)	405 (1.8)
Caesarean section	327 (2.9)	787 (3.5)	1,412 (6.4)
*Place of delivery			
SMRU clinics	4,923 (49.1)	14,448 (57.6)	19,332 (78.7)
Hospital (Thai or Myanmar)	887 (8.8)	2,471 (9.8)	2,302 (9.4)
Home	4,225 (42.1)	8,176 (32.6)	2,925 (11.9)

Categorial variables are mentioned as number (%).

*Missing data expressed per consecutive time periods: Age (104, 5, 16); ANC visit at 1st trimester (904, 738, 0); Ethnicity (15,971, 16,391, 4,600); Smoking during pregnancy (13,341, 150, 18); Literacy

(18,120, 27,041, 18); Maternal weight (1,819, 3,381, 2,448); BMI (18,120, 7,495, 118); HIV infection (NA, NA, 3,704); Syphilis (NA, NA, 7,491); Maternal anaemia (7,804, 476, 156); Foetal sex (3,596, 9,654, 9,645); Type of outcome (1,330, 6,611, 7,344); Stillbirth (268, 293, 252); Mode of delivery (6,726, 9,596, 9,649); Place of delivery (8,085, 6,876, 7,380).

[a]PTB calculation is restricted to singleton pregnancy between 28 and 42 weeks' gestation with known birth outcomes.

[b]SGA calculation is restricted to singleton pregnancy between 28 and 42 weeks' gestation with known birth outcomes and birthweight measuring within 72 hours of birth.

BMI at first ANC visit was first calculated in 2004. Literacy data was start recorded in 2008 but routine data collection was commenced in 2010. HIV & syphilis were routinely tested after 2010.

Maternal anaemia at first ANC consultation was used.

**Face, compound and breech presentation were combined in the vaginal delivery.

Abbreviation: ANC antenatal care; BMI body mass index; cm centimetre; kg/m2 Kilogram/metre^2; NVD normal vaginal delivery; SGA small for gestational age; SMRU Shoklo malaria research unit

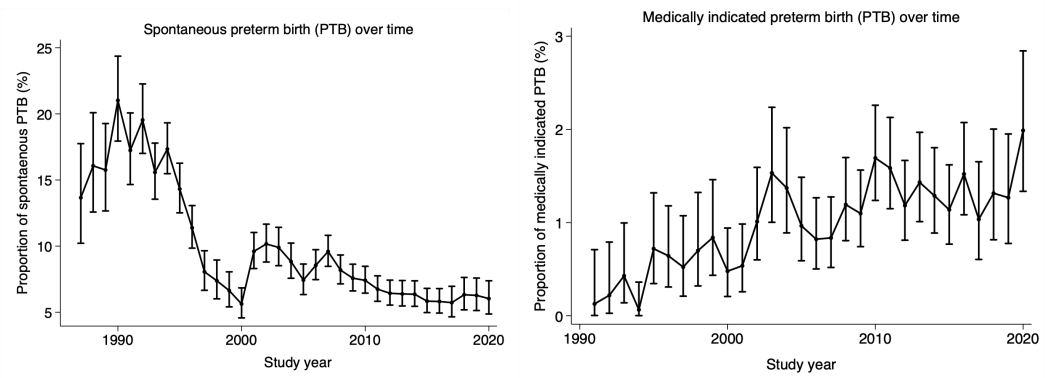


Figure A3-1: Trends of sponatenous PTB and medically indicated PTB in whole population (combined refugee and migrant)

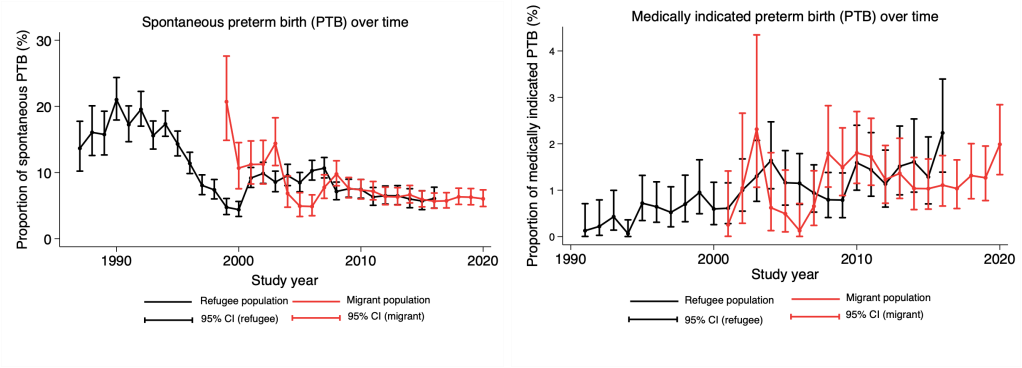
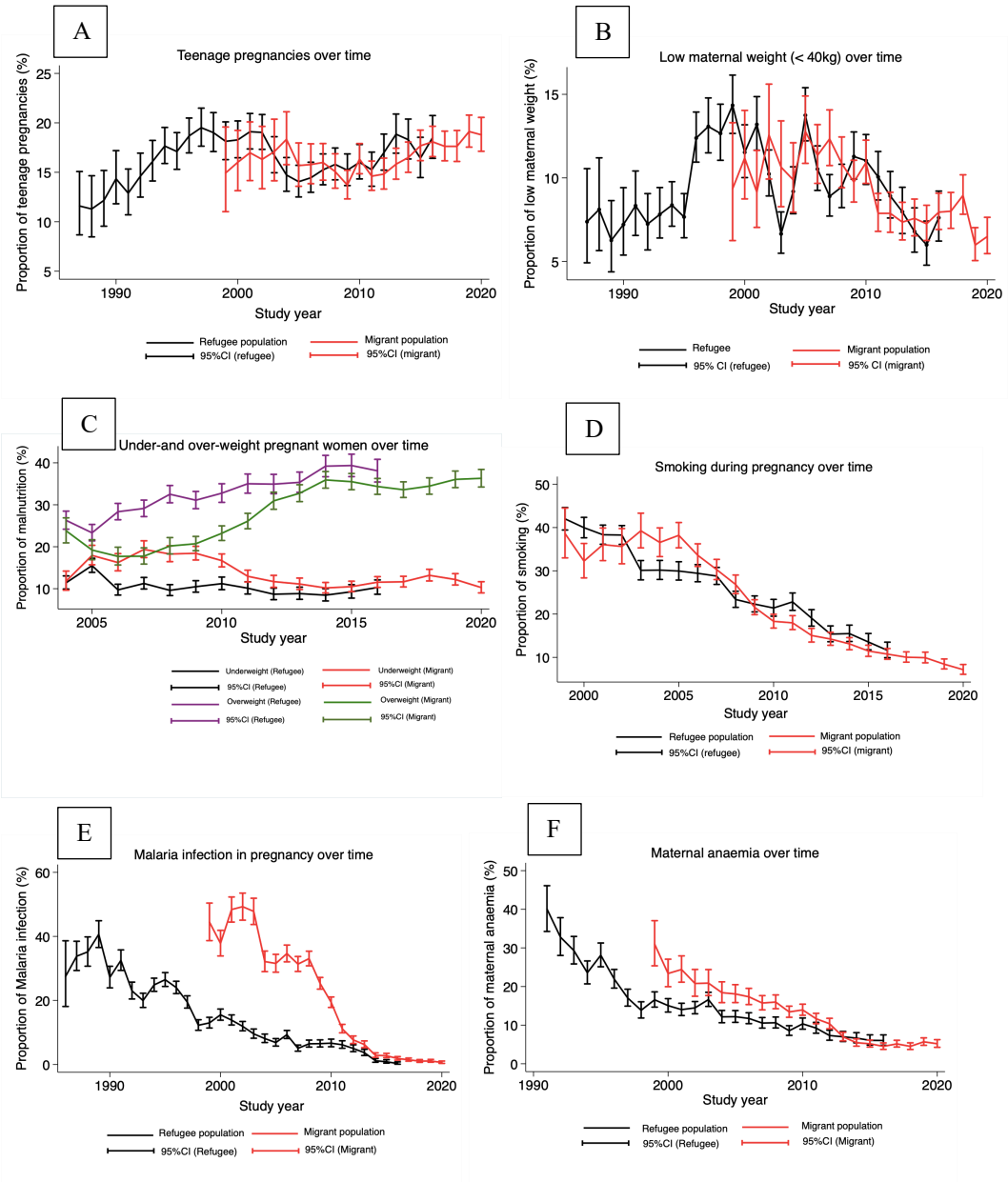


Figure A3-2: Trends of sponatenous PTB and medically indicated PTB stratified by refugee and migrant population



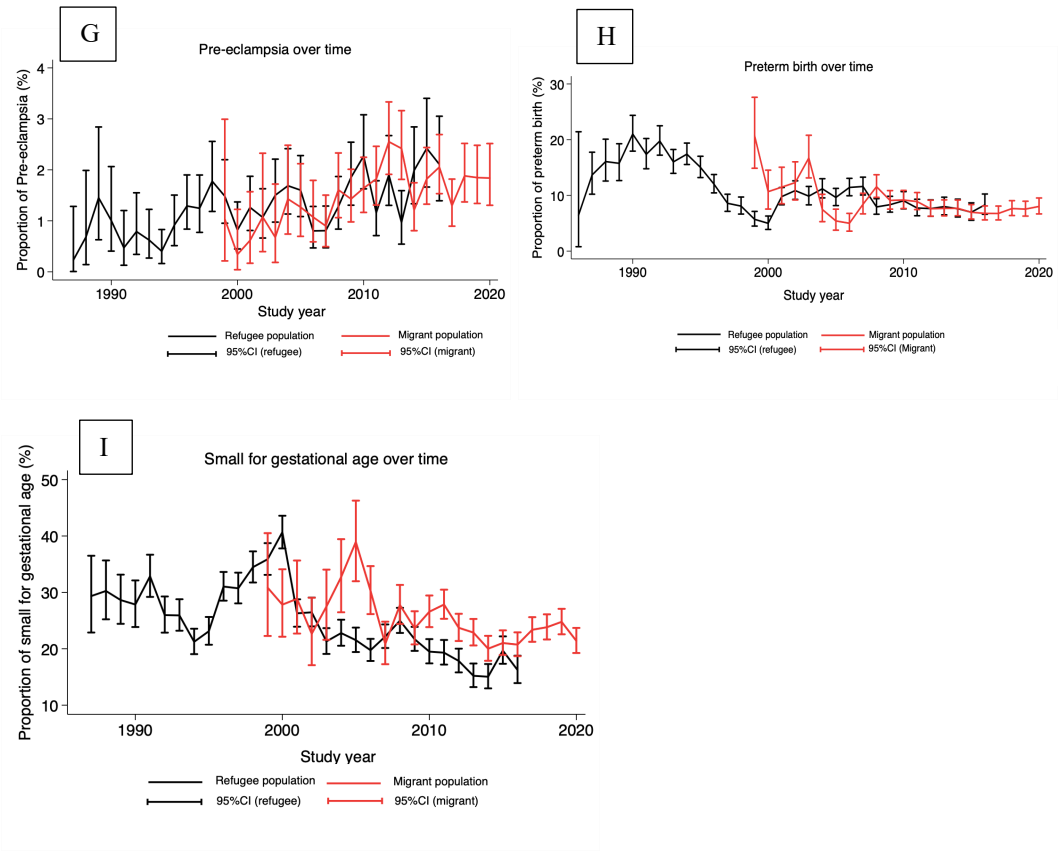


Figure A3-3: Changes of maternal characteristics and prgnancy outcomes over time stratified by refugee and migrant population: A. teenage pregnancies, B. low maternal weight (<40kg) C. under-and over-weight pregnant women, D. smoking, E. malaria infection in pregnancy, F. maternal anaemia, G. PE or eclampsia, H. preterm birth, I. SGA

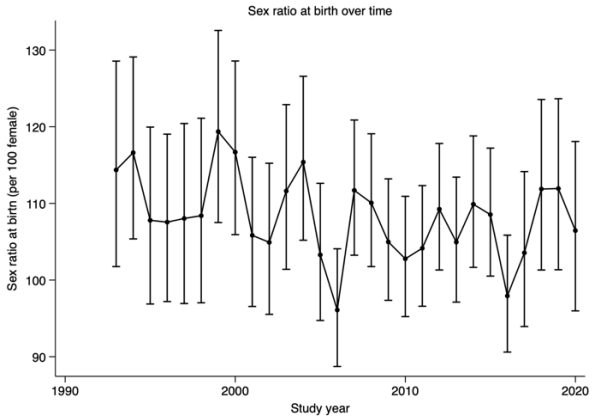


Figure A3-4: Sex ratio at birth (per 100 female) between 1986-2020

Appendix C

Subgroup analysis of preterm birth (PTB) and small for gestational age (SGA)

Table A 4-1: Univariable and multivariable analysis of PTB* (only included US dated pregnancy)

		Univariable analysis		Multivariable analysis (Model B)	
		OR (95%CI)	**p-value**	**AOR (95%CI)**	**p-value**
	N			**N=18,163**	
Age and Gravidity					
< 20 Primigravida	4,610	2.73 (2.47-3.01)	<0.001	2.93 (2.50-3.43)	<0.001
< 20 Multigravida	1,338	2.32 (1.96-2.74)	<0.001	2.44 (1.89-3.14)	<0.001
20-34 Primigravida	5,229	1.60 (1.44-1.79)	<0.001	1.72 (1.45-2.03)	<0.001
20-34 Multigravida	18,421	Reference		Reference	
≥ 35 Primigravida	135	1.95 (1.16-3.03)	0.012	2.04 (0.96-4.34)	0.063
≥ 35 Multigravida	4,785	1.29 (1.14-1.45)	<0.001	1.28 (1.07-1.53)	0.008
First ANC in 1st trimester	34,519	0.78 (0.72-0.83)	<0.001	0.77 (0.69-0.88)	<0.001
Maternal height per 10 cm	31,451	0.72 (0.67-0.78)	<0.001	0.77 (0.69-0.86)	<0.001
BMI (kg/m2)					
Underweight (<18.5)	4,188	1.11 (0.99-1.25)	0.062	1.25 (1.06-1.47)	0.007
Normal (18.5-22.9)	18,757	Reference		Reference	
Overweight (≥ 23)	8,500	0.76 (0.69-0.83)	<0.001	0.77 (0.67-0.89)	<0.001
Maternal anaemia at 1st ANC	34,316	1.36 (1.20-1.53)	<0.001	1.37 (1.14-1.71)	0.005
Malaria infection in pregnancy	34,519	1.07 (0.96-1.20)	0.236	0.96 (0.77-1.20)	0.744
Non-malaria infection					
Non-febrile and no antibiotics	21,566	Reference		Reference	
Suspected bacterial infection	515	1.21 (0.90-1.61)	0.203	1.33 (0.96-1.84)	0.082

Suspected viral infection	633	0.92 (0.69-1.24)	0.588	0.97 (0.70-1.34)	0.858
PE	34,519	3.09 (2.58-3.70)	<0.001	4.01 (3.17-5.09)	<0.001
Smoker	34,422	1.16 (1.06-1.27)	0.002	1.47 (1.24-1.74)	<0.001
History of preterm labour	22,869	2.40 (2.04-2.83)	<0.001	3.37 (2.73-4.12)	<0.001
SGA	29,704	0.82 (0.74-0.91)	<0.001	0.71 (0.61-0.82)	<0.001
Foetal sex (male)	34,462	1.13 (1.05-1.22)	0.002	1.14 (1.02-1.28)	0.025
Average ambient temp during 1st trimester	34,519	0.99 (0.97-1.01)	0.457	1.00 (0.97-1.04)	0.93
Average ambient temp during 2nd trimester	34,519	0.99 (0.98-1.02)	0.703	1.02 (0.98-1.07)	0.253
Average ambient temp during 3rd trimester	34,519	0.99 (0.98-1.02)	0.699	1.04 (0.99-1.08)	0.054

In multivariable models, living status (refugee or migrant), gestational assessment methods and calendar year (fractional form)

*There is no model A as it is included only ultrasound dated pregnancies

Abbreviation: ANC antenatal care; AOR adjusted odd ratio; BMI body mass index; OR odd ratio; PE pre-eclampsia; PTB preterm birth; SGA small for gestational age.

Table A 4-2: Univariable and multivariable analysis of early PTB

Early preterm birth		Univariable analysis		Multivariable analysis (Model A)		Multivariable analysis (Model B)	
		OR (95%CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
	N			N=42,844		N=22,841	
Age & gravidity	52,943						
< 20 Primigravida	7,012	2.41 (2.09-2.80)	<0.001	2.73 (2.29-3.25)	<0.001	2.74 (2.15-3.50)	<0.001
< 20 Multigravida	2,157	2.49 (1.99-3.12)	<0.001	2.61 (1.99-3.43)	<0.001	2.21 (1.50-3.29)	<0.001
20-34 Primigravida	7,882	1.52 (1.29-1.78)	<0.001	1.58 (1.29-1.92)	<0.001	1.62 (1.25-2.11)	0.001
20-34 Multigravida	28,595			Reference		Reference	
≥ 35 Primigravida	184	2.18 (1.02-1.46)	0.045	1.34 (0.42-4.25)	0.621	1.91 (0.58-6.30)	0.285
≥ 35 Multigravida	7,113	1.22 (1.02-1.46)	0.26	1.31 (1.06-1.61)	0.014	1.42 (1.08-1.87)	0.011

ANC visit at 1st ANC	52,943	0.71 (0.63-0.79)	<0.001	0.70 (0.61-0.81)	<0.001	0.70 (0.57-0.85)	<0.001
Low maternal weight (< 40 kg)	4,955	1.58 (1.34-1.86)	<0.001	2.02 (1.66-2.45)	<0.001	-	
Height per 10 cm	37,036	0.68 (0.60-0.77)	<0.001	NA		0.71 (0.60-0.84)	<0.001
BMI (kg/m^2)							<0.001
Underweight (< 18.5)	4,323	1.12 (0.92-1.37)	0.257	NA		1.15 (0.88-1.50)	0.294
Normal weight (18.5-22.9)	21,379	Reference		NA		Reference	
Overweight (≥ 23)	11,325	0.56 (0.47-0.67)	<0.001	NA		0.53 (0.42-0.67)	<0.001
Maternal anaemia at 1st ANC	49,032	1.59 (1.35-1.87)	<0.001	1.41 (1.16-1.73)	0.001	1.44 (1.06-1.94)	0.018
Malaria in pregnancy	52,944	1.61 (1.40-1.85)	<0.001	1.37 (1.13-1.66)	0.001	1.17 (0.84-1.63)	0.343
Non-malaria infection							
Non-febrile and no antibiotics	26,615	Reference		NA		Reference	
Suspected bacterial infection	620	2.07 (1.39-3.07)	<0.001	NA		2.19 (1.43-3.34)	<0.001

Suspected viral infection	694	1.20 (0.75-1.93)	0.448	NA		1.08 (0.65-1.81)	0.763
PE	52,944	2.59 (1.96-3.43)	<0.001	3.16 (2.34-4.27)	<0.001	3.49 (2.28-5.33)	<0.001
Smoker	45,552	1.17 (1.01-1.35)	0.04	NA		1.38 (1.07-1.79)	0.014
History of preterm birth	28,070	2.90 (2.26-3.73)	<0.001	NA		3.62 (2.71-4.85)	<0.001
SGA	46,083	0.56 (0.47-0.66)	<0.001	0.53 (0.45-0.64)	<0.001	0.53 (0.41-0.66)	<0.001
Foetal sex (male)	52,647	1.25 (1.12-1.40)	<0.001	1.30 (1.13-1.48)	<0.001	1.34 (1.12-1.59)	0.001
Average ambient temp during 1st trimester	52,943	0.94 (0.91-0.97)	<0.001	0.96 (0.92-0.99)	0.031	0.97 (0.91-1.02)	0.226
Average ambient temp during 2nd trimester	52,943	0.96 (0.93-0.99)	0.013	0.98 (0.93-1.02)	0.325	0.98 (0.92-1.05)	0.607
Average ambient temp during 3rd trimester	52,943	0.96 (0.93-0.99)	0.025	0.99 (0.95-1.03)	0.619	0.99 (0.94-1.05)	0.919

In multivariable models, living status (refugee or migrant), gestational assessment methods and calendar year (fractional form) are adjusted.

Abbreviation: ANC antenatal care; AOR adjusted odd ratio; BMI body mass index; OR odd ratio; PE pre-eclampsia; PTB preterm birth; SGA small for gestational age.

Table A 4-3: Univariable and multivariable analysis of late PTB

Late preterm birth		**Univariable analysis**		**Multivariable analysis (Model A)**		**Multivariable analysis (Model B)**	
		OR (95%CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
	N			N=42,844		N=22,841	
Age & gravidity	52,943						
<20 Primigravida	7,012	2.23 (2.04-2.44)	<0.001	2.54 (2.28-2.82)	<0.001	3.13 (2.67-3.68)	<0.001
<20 Multigravida	2,157	1.86 (1.60-2.17)	<0.001	1.87 (1.56-2.26)	<0.001	2.33 (1.78-3.06)	<0.001
20-34 Primigravida	7,882	1.52 (1.38-1.67)	<0.001	1.74 (1.55-1.95)	<0.001	2.03 (1.72-2.39)	<0.001
20-34 Multigravida	28,595			Reference		Reference	
≥35 Primigravida	184	1.77 (1.07-2.92)	0.026	1.94 (1.09-3.46)	0.025	2.18 (1.08-4.42)	0.031
≥35 Multigravida	7,113	1.16 (1.04-1.29)	0.007	1.20 (1.06-1.37)	0.005	1.33 (1.10-1.60)	0.002
ANC visit at 1st ANC	52,943	0.77 (0.72-0.83)	<0.001	0.79 (0.72-0.86)	<0.001	0.82 (0.73-0.94)	0.005

Low maternal weight (<40 kg)	4,955	1.34 (1.21-1.49)	<0.001	1.51 (1.34-1.72)	<0.001	-	
Height per 10 cm	37,036	0.72 (0.66-0.78)	<0.001	NA		0.74 (0.66-0.82)	<0.001
BMI (kg/m^2)							<0.001
Underweight (<18.5)	4,323	1.17 (1.03-1.33)	0.016	NA		1.25 (0.88-1.50)	0.009
Normal weight (≥18.5-22.9)	21,379	Reference		NA		Reference	
Overweight (≥23)	11,325	0.73 (0.66-0.4)	<0.001	NA		0.85 (0.74-0.98)	0.024
Maternal anaemia at 1st ANC	49,032	1.35 (1.22-1.50)	<0.001	1.18 (1.04-1.34)	<0.001	1.22 (1.06-1.94)	0.07
Malaria in pregnancy	52,944	1.34 (1.22-1.46)	<0.001	1.25 (1.11-1.41)	<0.001	0.93 (0.74-1.18)	0.555
Non-malaria infection							
Non-febrile and no antibiotics	26,615	Reference		NA		Reference	
Suspected bacterial infection	620	1.13 (0.82-1.57)	0.456	NA		1.08 (0.75-1.56)	0.679

Suspected viral infection	694	1.04 (0.75-1.43)	0.814	NA		1.01 (0.71-1.42)	0.976
PE	52,944	2.54 (2.14-3.04)	<0.001	2.94 (2.43-3.56)	<0.001	3.59 (2.84-4.55)	<0.001
Smoker	45,552	1.22 (1.12-1.34)	<0.001	NA		1.56 (1.32-1.84)	<0.001
History of preterm birth	28,070	2.25 (1.88-2.69)	<0.001	NA		3.15 (2.56-3.87)	<0.001
SGA	46,083	0.94 (0.87-1.03)	0.223	0.87 (0.79-1.01)	0.124	0.97 (0.85-1.11)	0.658
Foetal sex (male)	52,647	1.02 (0.95-1.09)	0.55	1.30 (1.13-1.48)	<0.001	1.02 (0.91-1.14)	0.711
Average ambient temp during 1st trimester	52,943	0.94 (0.92-0.96)	<0.001	0.97 (0.95-0.99)	0.023	0.97 (0.91-1.02)	0.226
Average ambient temp during 2nd trimester	52,943	0.93 (0.91-0.95)	<0.001	0.98 (0.96-1.01)	0.226	0.98 (0.92-1.05)	0.607
Average ambient temp during 3rd trimester	52,943	0.97 (0.95-0.98)	<0.001	1.02 (0.99-1.04)	0.227	0.99 (0.94-1.05)	0.919

In multivariable models, living status (refugee or migrant), gestational assessment methods and calendar year (fractional form) are adjusted.

Abbreviation: ANC antenatal care; AOR adjusted odd ratio; BMI body mass index; OR odd ratio; PE pre-eclampsia; PTB preterm birth; SGA small for gestational age.

Table A 4-4: Univariable and multivariable analysis of spontaneous PTB.

Spontaneous preterm birth		Univariable analysis		Multivariable analysis (Model A)		Multivariable analysis (Model B)	
		OR (95%CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
	N			N=42,844		N=22,841	
Age & gravidity	52,943						
< 20 Primigravida	7,012	2.40 (2.22-2.61)	<0.001	2.68 (2.39-2.99)	<0.001	3.53 (2.97-4.19)	<0.001
< 20 Multigravida	2,157	2.16 (1.89-2.47)	<0.001	1.97 (1.63-2.39)	<0.001	2.49 (1.87-3.33)	<0.001
20-34 Primigravida	7,882	1.53 (1.40-1.66)	<0.001	1.80 (1.60-2.03)	<0.001	2.21 (1.85-2.65)	<0.001
20-34 Multigravida	28,595			Reference		Reference	
≥ 35 Primigravida	184	1.45 (0.88-2.40)	0.144	1.49 (0.73-3.06)	0.274	1.25 (0.45-3.48)	0.662
≥ 35 Multigravida	7,113	1.02 (0.92-1.14)	0.658	1.10 (0.95-1.27)	0.189	1.20 (0.98-1.48)	0.082
ANC visit at 1st ANC	52,943	0.73 (0.68-0.77)	<0.001	0.76 (0.69-0.84)	<0.001	0.81 (0.71-1.48)	0.004

Low maternal weight (<40 kg)	4,955	1.45 (1.32-1.59)	<0.001	1.58 (1.39-1.80)	<0.001	-	
Height per 10 cm	37,036	0.69 (0.65-0.75)	<0.001	NA		0.72 (0.64-0.80)	<0.001
BMI (kg/m2)							<0.001
Underweight (< 18.5)	4,323	1.22 (1.09-1.37)	0.001	NA		1.38 (1.15-1.66)	<0.001
Normal weight (≥18.5-22.9)	21,379	Reference		NA		Reference	
Overweight (≥ 23)	11,325	0.60 (0.54-0.66)	<0.001	NA		0.80 (0.69-0.93)	0.004
Maternal anaemia at 1st ANC	49,032	1.45 (1.32-1.59)	<0.001	1.18 (1.03-1.35)	0.016	1.24 (0.99-1.55)	0.065
Malaria in pregnancy	52,943	1.52 (1.40-1.65)	<0.001	1.30 (1.15-1.48)	<0.001	1.01 (0.79-1.30)	0.885
Non-malaria infection							
Non-febrile and no antibiotics	26,615	Reference		NA		Reference	
Suspected bacterial infection	620	1.41 (1.07-1.87)	0.015	NA		1.07 (0.72-1.59)	0.754
Suspected viral infection	694	1.08 (0.81-1.45)	0.6	NA		1.06 (0.73-1.52)	0.772

PE	52,943	1.11 (0.89-1.37)	0.355	1.34 (1.02-1.75)	0.033	1.69 (1.22-2.33)	0.002
Smoker	45,552	1.24 (1.14-1.35)	<0.001	NA		1.72 (1.45-2.06)	<0.001
History of preterm birth	28,070	2.41 (2.05-2.84)	<0.001	NA		3.33 (2.66-4.16)	<0.001
SGA	46,083	0.76 (0.70-0.83)	<0.001	0.82 (0.74-0.91)	<0.001	0.89 (0.77-1.04)	0.135
Foetal sex (male)	52,647	1.07 (1.01-1.14)	0.028	1.00 (0.92-1.09)	<0.001	1.01 (0.89-1.15)	0.836
Average ambient temp during 1st trimester	52,943	0.93 (0.91-0.94)	<0.001	0.96 (0.93-0.99)	0.033	0.99 (0.95-1.03)	0.699
Average ambient temp during 2nd trimester	52,943	0.92 (0.91-0.94)	<0.001	0.97 (0.95-1.01)	0.111	1.04 (0.99-1.08)	0.099
Average ambient temp during 3rd trimester	52,943	0.96 (0.94-0.97)	<0.001	1.01 (0.98-1.04)	0.473	1.07 (1.03-1.12)	0.001

In multivariable models, living status (refugee or migrant), gestational assessment methods and calendar year (fractional form) are adjusted.

Abbreviation: ANC antenatal care; AOR adjusted odd ratio; BMI body mass index; OR odd ratio; PE pre-eclampsia; PTB preterm birth; SGA small for gestational age.

Table A 4-5: Univariable and multivariable analysis of SGA using local customized charts.

SGA		Univariable analysis		Multivariable analysis (Model A)		Multivariable analysis (Model B)	
		OR (95%CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
	N			N=43,674		N=23,857	
Age & gravidity							
< 20 Primigravida	6,427	1.69 (1.56-1.84)	<0.001	1.66 (1.52-1.82)	<0.001	1.55 (1.35-1.78)	<0.001
< 20 Multigravida	1,900	1.16 (0.99-1.36)	0.063	1.01 (0.85-1.19)	0.937	0.85 (0.64-1.14)	0.278
20-34 Primigravida	7,430	1.71 (1.58-1.85)	<0.001	1.88 (1.73-2.04)	<0.001	2.16 (1.92-2.43)	<0.001
20-34 Multigravida	25,344			Reference		Reference	
≥ 35 Primigravida	172	2.96 (2.05-4.28)	<0.001	3.31 (2.25-4.87)	<0.001	2.25 (1.27-3.99)	0.005
≥ 35 Multigravida	6,421	1.48 (1.36-1.61)	<0.001	1.52 (1.39-1.67)	<0.001	1.20 (1.32-1.73)	<0.001
ANC visit at 1st ANC	46,990	0.89 (0.84-0.94)	<0.001	0.81 (0.76-0.87)	<0.001	0.81 (0.73-0.90)	<0.001

Low maternal weight (< 40 kg)	46,917	2.76 (2.55-2.99)	<0.001	2.99 (2.74-3.25)	<0.001	-	
Height per 10 cm	33,393	0.48 (0.45-0.52)	<0.001	NA		0.50 (0.46-0.54)	<0.001
BMI (kg/m^2)							<0.001
Underweight (< 18.5)	3,706	1.63 (1.48-1.81)	<0.001	NA		1.75 (1.54-1.99)	<0.001
Normal weight (18.5-22.9)	19,050	Reference		NA		Reference	
Overweight (≥ 23)	11,031	0.52 (0.47-0.57)	<0.001	NA		0.52 (0.46-0.58)	<0.001
Maternal anaemia at 1st ANC	43,723	1.47 (1.34-1.60)	<0.001	1.19 (1.08-1.31)	<0.001	1.17 (0.99-1.37)	0.058
Malaria in pregnancy	47,005	1.71 (1.58-1.85)	<0.001	1.46 (1.34-1.60)	<0.001	1.38 (1.17-1.63)	<0.001
Non-malaria infection	23,942						
Non-febrile and no antibiotics	22,696	Reference		NA		Reference	
Suspected bacterial infection	592	1.05 (0.80-1.39)	0.717	NA		0.96 (0.72-1.28)	0.791
Suspected viral infection	656	1.35 (1.06-1.72)	0.013	NA		1.15 (0.90-1.50)	0.256

PE	47,005	2.47 (2.12-2.88)	<0.001	2.52 (2.15-2.95)	<0.001	3.26 (2.66-4.00)	<0.001
Smoker	40,598	2.01 (1.88-2.16)	<0.001	NA		1.99 (1.77-2.25)	<0.001
PTB	47,005	1.27 (1.15-1.41)	<0.001	1.11 (0.74-0.91)	<0.001	1.24 (1.05-1.47)	0.011
Foetal sex (male)	25,747	1.01 (0.95-1.07)	0.782	1.01 (0.95-1.07)	0.756	1.02 (0.94-1.12)	0.596
Average ambient temp during 1st trimester	47,005	0.96 (0.95-0.98)	<0.001	1.01 (0.99-1.03)	0.594	1.02 (0.99-1.05)	0.231
Average ambient temp during 2nd trimester	47,005	0.96 (0.94-0.98)	<0.001	0.97 (0.95-1.01)	0.111	1.02 (0.98-1.05)	0.31
Average ambient temp during 3rd trimester	47,005	0.94 (0.93-0.96)	<0.001	1.01 (0.98-1.04)	0.473	0.97 (0.94-1.00)	0.058

In multivariable models, living status (refugee or migrant), gestational assessment methods and calendar year (fractional form) are adjusted.

Abbreviation: ANC antenatal care; AOR adjusted odd ratio; BMI body mass index; OR odd ratio; PE pre-eclampsia; PTB preterm birth; SGA small for gestational age.

Appendix D

Secondary outcomes of Chapter 5

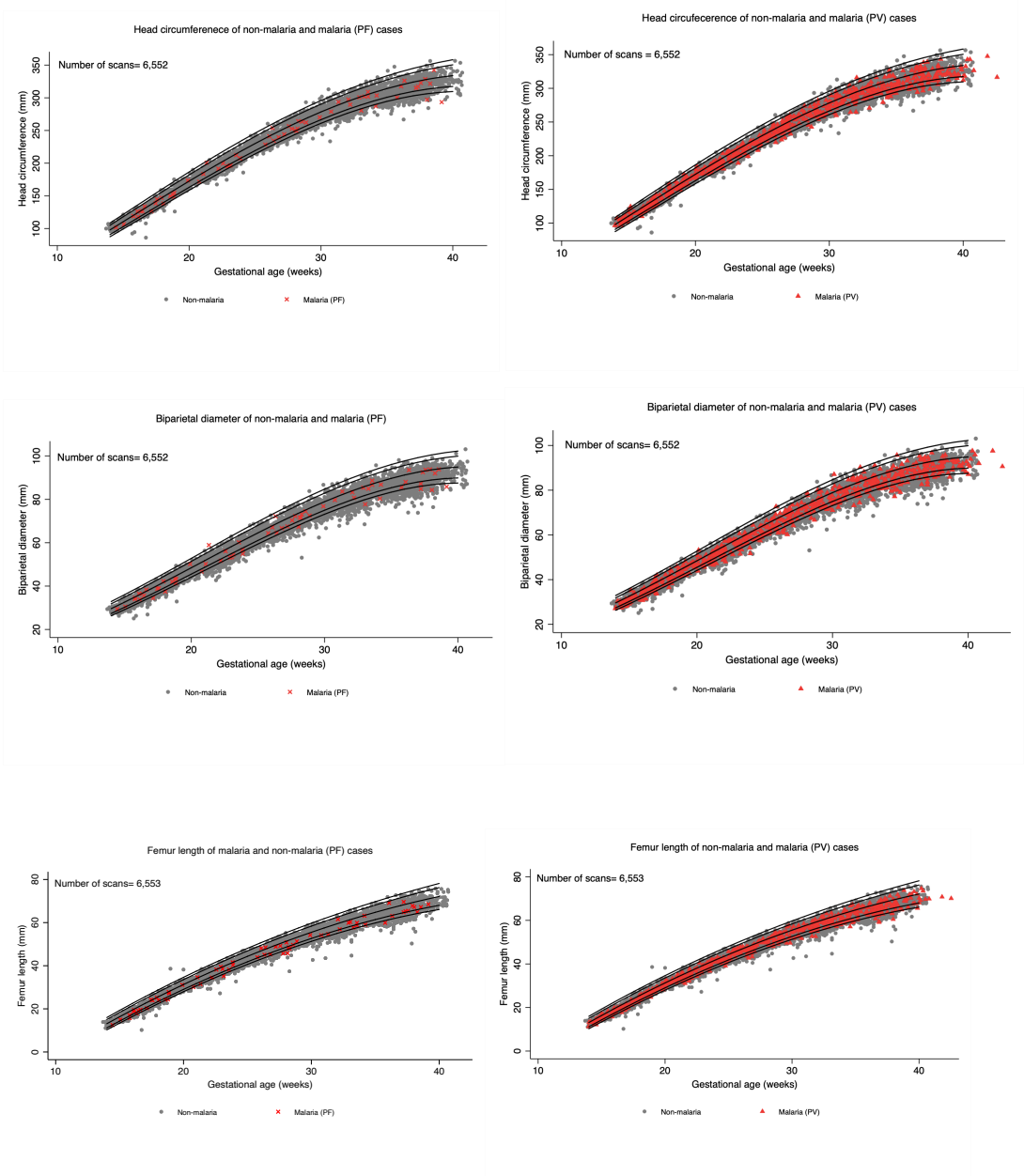
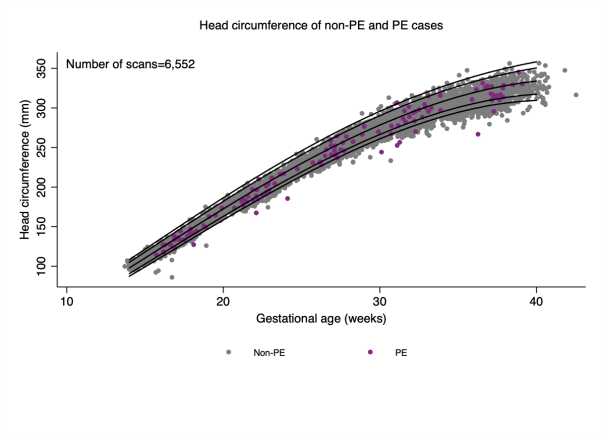
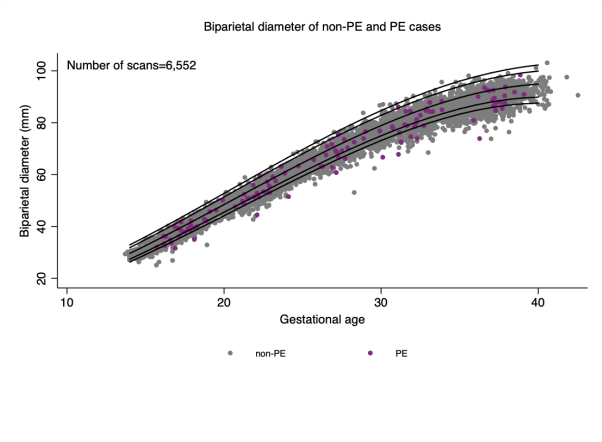


Figure A 5-1: Serial HC, BPD and FL measurements by malaria infection status using INTERGROWTH 21st standard (97%, 90%, 50%, 10% and 3%-tile lines are shown).





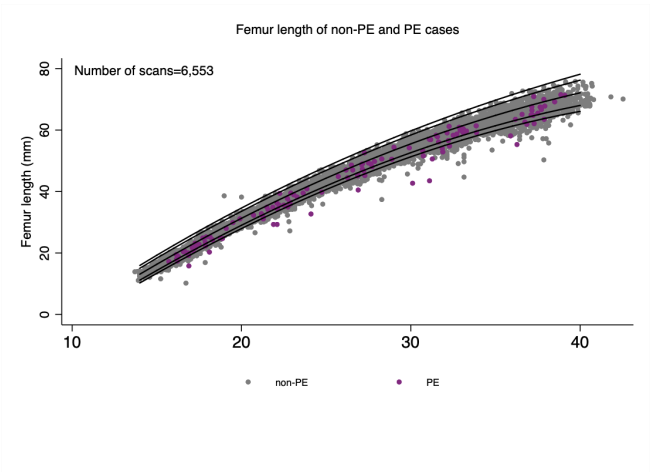


Figure A 5-2: Serial HC, BPD and FL measurements of non-PE and PE cases using INTERGROWTH 21st standard (97%, 90%, 50%, 10% and 3%-tile lines are shown).

Table A 5-1: Univariable and multivariable analysis of maternal characteristics and HC z-score.

			Univariable analysis		Multivariable analysis	
	Number of patients	**Number of scans**	**Predicted mean HC z-score (95% CI)**	**p-value**	**Predicted mean HC z-score (95% CI)**	**p-value**
PE	1,349	6,550	-0.29 (-0.58, -0.01)	0.042	NA	
Age and Gravida						
< 20 years, primigravida	148	701	0.15 (0.37, 0.27)	0.009	0.15 (0.04, 0.26)	0.009
< 20 years, multigravida	47	225	0.24 (0.05, 0.43)	0.012	0.25 (0.07-0.44)	0.007
20-34 years, primigravida	265	1,275	0.12 (0.03, 0.21)	0.010	0.09 (-0.01-0.18)	0.051
20-34 years, multigravida	741	3,627	Reference		Reference	
≥ 35 years, primigravida	3	15	-0.15 (-0.90, 0.59)	0.683	-0.21 (-0.91, 0.48)	0.548
≥ 35 years, multigravida	145	708	0.02 (-0.10, 0.14)	0.733	0.04 (-0.07, 0.15)	0.515
BMI (kg/m^2)						
Underweight (< 18.5)	279	1,351	0.02 (-0.07, 0.12)	0.613	NA	
Normal (18.5-22.9)	770	3,770	Reference:		Reference	

Overweight and obese (≥ 23)	300	1,430	0.06 (-0.09, 0.09)	0.158	NA	
Maternal height (per 10 cm)	1,349	6,551	0.08 (0.02, 0.15)	0.016	0.07 (0.01-0.14)	0.024
Smoker	1,349	6,551	-0.05 (-0.18, 0.07)	0.389	NA	
Maternal anaemia	1,349	6,551	-0.10 (-0.36, 0.17)	0.469	NA	
Malaria infection*						
Non-malaria	1,216	5,671	Reference		Reference	
PF	26	161	-0.10 (-0.35, 0.14)	0.41	NA	
PV	107	719	0.01 (-0.11, 0.14)	0.821	NA	
Male foetal sex	1,283	6,345	0.39 (0.33, 0.46)	<0.001	0.40 (0.33, 0.47)	<0.001

*non-time dependent (any malaria infection during pregnancy regardless of timing of malaria).

Number of patients=1,283 and number of scans=6,345 in multivariable model. The best fit model was based on backward elimination using p-value of 0.05 as the cut-off. Gestational age, and calendar year were adjusted in the multivariable model.

Abbreviation: BMI body mass index; HC head circumference; NA not applicable; PE pre-eclampsia; PF *plasmodium falciparum*; PV *plasmodium vivax*

Table A 5-2: Univariable and multivariable analysis of maternal characteristics and BPD z-score.

			Univariable analysis		Multivariable analysis	
	Number of patients	**Number of scans**	**Predicted mean BPD z-score (95% CI)**	**p-value**	**Predicted mean BPD z-score (95% CI)**	**p-value**
PE	1,349	6,550	-0.11 (-0.40, 0.17)	0.442	NA	
Age and Gravida						
< 20 years, primigravida	148	701	0.15 (0.24, 0.27)	0.019	0.16 (0.03, 0.28)	0.012
< 20 years, multigravida	47	225	0.10 (-0.11, 0.31)	0.354	0.15 (-0.05, 0.36)	0.149
20-34 years, primigravida	265	1,275	0.06 (-0.04, 0.16)	0.238	0.04 (-0.06, 0.13)	0.462
20-34 years, multigravida	741	3,627	Reference		Reference	
≥ 35 years, primigravida	3	15	-0.40 (-1.21, 0.41)	0.333	-0.43 (-1.20, 0.33)	0.271
≥ 35 years, multigravida	145	708	-0.03 (-0.15, 0.10)	0.697	-0.01 (-0.13, 0.11)	0.873
BMI (kg/m^2)						
Underweight (< 18.5)	279	1,351	0.04 (-0.06, 0.14)	0.426	NA	

Normal (18.5-22.9)	770	3,770	Reference:		NA	
Overweight and obese (≥ 23)	300	1,430	0.03 (-0.07, 0.12)	0.559	NA	
Maternal height (per 10 cm)	1,349	6,550	0.18 (0.11, 0.25)	<0.001	0.17 (0.10-0.24)	<0.001
Smoker	1,349	6,550	-0.06 (-0.19, 0.07)	0.345	-0.02 (-0.15, 0.11)	0.799
Maternal anaemia	1,349	6,550	0.18 (-0.11, 0.47)	0.221	0.19 (-0.11, 0.49)	0.21
Malaria infection*						
Non-malaria	1,216	5,671	Reference		NA	
PF	26	161	-0.07 (-0.34, 0.20)	0.61	NA	
PV	107	719	-0.09 (-0.23, 0.06)	0.229	NA	
Male foetal sex	1,266	5,947	0.42 (0.35, 0.50)	<0.001	0.43 (0.35, 0.50)	<0.001

*non-time dependent (any malaria infection during pregnancy regardless of timing of malaria)

Number of patients=1,283 and number of scans=6,345 in multivariable model. The best fit model was based on backward elimination using p-value of 0.05 as the cut-off. Gestational age, and calendar year were adjusted in the multivariable model.

Abbreviation: BPD Biparietal diameter; BMI body mass index; NA not applicable; PE pre-eclampsia; PF plasmodium falciparum; PV plasmodium vivax

Table A 5-3: Univariable and multivariable analysis of maternal characteristics and FL z-score.

			Univariable analysis		Multivariable analysis	
	Number of patients	**Number of scans**	**Predicted mean FL z-score (95% CI)**	**p-value**	**Predicted mean FL z-score (95% CI)**	**p-value**
PE	1,349	6,550	-0.29 (-0.58, -0.01)	0.042	-0.32 (-0.60, -0.04)	0.024
Age and Gravida						
<20 years, primigravida	148	701	-0.13 (-0.25, -0.01)	0.046	-0.08 (-0.21, 0.04)	0.199
<20 years, multigravida	47	225	-0.03 (-0.24, 0.17)	0.759	0.01 (-0.21-0.21)	0.997
20-34 years, primigravida	265	1,275	-0.01 (-0.10, 0.10)	0.946	0.01 (-0.09-0.11)	0.865
20-34 years, multigravida	741	3,627	Reference		Reference	
≥ 35 years, primigravida	3	15	1.02 (0.22, 1.83)	0.013	1.11 (0.33, 1.89)	0.006
≥ 35 years, multigravida	145	708	-0.02 (-0.15, 0.11)	0.749	-0.03 (-0.15-0.10)	0.698
BMI (kg/m^2)						
Underweight (<18.5)	279	1,351	0.02 (-0.07, 0.12)	0.613	-0.01 (-0.11, 0.09)	0.842
Normal (18.5-22.9)	770	3,770	Reference:			

Overweight and obese (≥23)	300	1,430	0.26 (0.17, 0.36)	<0.001	0.25 (0.15, 0.34)	<0.001
Maternal height (per 10 cm)	1,349	6,550	0.18 (0.11, 0.25)	<0.001	0.17 (0.10-0.24)	<0.001
Smoker	1,349	6,550	-0.06 (-0.19, 0.07)	0.345	NA	
Maternal anaemia	1,349	6,550	0.18 (-0.11, 0.47)	0.221	NA	
Malaria infection*						
Non-malaria	1,216	5,671	Reference		NA	
PF	26	161	-0.26 (-0.53, 0.01)	0.06	NA	
PV	107	719	-0.05 (-0.19, 0.09)	0.49	NA	
Male foetal sex	1,266	5,947	0.02 (-0.06, 0.09)	0.668	NA	

*non-time dependent (any malaria infection during pregnancy regardless of timing of malaria)

Number of patients=1,283 and number of scans=6,345 in multivariable model. The best fit model was based on backward elimination using p-value of 0.05 as the cut-off. Gestational age, and calendar year were adjusted in the multivariable model.

Abbreviation: BMI body mass index; FL femur length; NA not applicable; PE pre-eclampsia; PF plasmodium falciparum; PV plasmodium vivax

Table A 5-4: Univariable and multivariable three-level mixed linear regression analysis of HC z score and PE.

	Univariable analysis				Multivariable analysis			
	Number of patients	**Number of scans**	**Mean predicted HC z-score (95% CI)**	**p-value**	**Number of patients**	**Number of scans**	**Mean predicted HC z-score (95% CI)**	**p-value**
PE	1,349	6,551	-0.19 (-0.45, 0.07)	0.155	1,349	6,551	-0.21 (-0.47, 0.05)	0.120

*The cofounder identified by DAG such as age and gravida, smoking, BMI, malaria infection, and calendar year were adjusted in the multivariable model.

Table A 5-5: Univariable and multivariable three-level mixed linear regression analysis of BPD z score and PE.

	Univariable analysis				Multivariable analysis			
	Number of patients	**Number of scans**	**Mean predicted BPD z-score (95% CI)**	**p-value**	**Number of patients**	**Number of scans**	**Mean predicted BPD z-score (95% CI)**	**p-value**
PE	1,349	6,551	-0.11 (-0.40, 0.17)	0.442	1,349	6,551	-0.11 (-0.39, 0.18)	0.459

*The cofounder identified by DAG such as age and gravida, smoking, BMI, malaria infection, and calendar year were adjusted in the multivariable model.

Table A 5-6: Univariable and multivariable three-level mixed linear regression analysis of FL z score and PE.

	Univariable analysis				Multivariable analysis			
	Number of patients	**Number of scans**	**Mean predicted FL z-score (95% CI)**	**p-value**	**Number of patients**	**Number of scans**	**Mean predicted FL z-score (95% CI)**	**p-value**
PE	1,349	6,550	-0.29 (-0.58, 0.01)	0.042	1,349	6,550	-0.33 (-0.59, -0.04)	0.022*

*The cofounder identified by DAG such as age and gravida, smoking, BMI, malaria infection, and calendar year were adjusted in the multivariable model.

Table A 5-7: Univariable and multivariable analysis of HC z score and malaria infection stratified by species.

		Univariable analysis		Multivariable analysis*	
	Scans	**Coefficient (95% CI)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Falciparum malaria infection (PF)					
No malaria infection before	6,372	Reference		Reference	
One week after PF	17	0.18 (-0.11, 0.47)	0.228	0.16 (-0.15, 0.46)	0.312
Two weeks after PF	8	0.04 (-0.36, 0.43)	0.847	-0.02 (-0.44, 0.40)	0.936
Three weeks after PF	6	-0.44 (-0.93, 0.04)	0.074	-0.44 (-0.93, 0.04)	0.073
Four weeks after PF	5	-0.24 (-0.72, 0.24)	0.324	-0.19 (-0.72, 0.33)	0.472
Five weeks after PF	8	-0.06 (-0.49, 0.37)	0.797	-0.05 (-0.48, 0.38)	0.809
Six weeks after PF	6	-0.08 (-0.54, 0.38)	0.729	-0.02 (-0.51, 0.48)	0.948
Seven weeks or more after PF	116	-0.16 (-0.41, 0.08)	0.193	-0.13 (-0.38, 0.13)	0.325
Vivax malaria infection (PV)					
No malaria infection before	5,846	Reference		Reference	
One week after PV	182	-0.07 (-0.20, 0.06)	0.284	-0.06 (-0.19, 0.07)	0.324

Two weeks after PV	146	-0.08 (-0.22, -0.05)	0.234	-0.08 (-0.22, 0.06)	0.241
Three weeks after PV	37	-0.05 (-0.26, 0.16)	0.637	-0.05 (-0.26, 0.15)	0.622
Four weeks after PV	40	-0.01 (-0.20, 0.20)	0.981	-0.02 (-0.20, 0.20)	0.814
Five weeks after PV	42	0.01 (-0.19, 0.21)	0.905	-0.01 (-0.19, 0.26)	0.994
Six weeks after PV	31	0.06 (-0.17, 0.28)	0.628	0.03 (-0.19, 0.26)	0.759
Seven weeks or more after PV	214	-0.04 (-0.18, 0.11)	0.617	-0.04 (-0.19, 0.10)	0.54

*In multivariable analysis, the cofounders identified by DAG, such as age, gravida, and calendar year were adjusted.

Abbreviation: BMI body mass index; CI confidence interval; HC head circumference; PE pre-eclampsia; PF plasmodium falciparum; PV plasmodium vivax

Table A 5-8: Univariable and multivariable analysis of BPD z score and malaria infection stratified by species.

		Univariable analysis		Multivariable analysis	
	Scans	**Coefficient (95% CI)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Falciparum malaria infection (PF)					
No malaria infection before	6,373	Reference		Reference	
One week after PF	17	0.14 (-0.21, 0.49)	0.448	0.12 (-0.25, 0.49)	0.521
Two weeks after PF	8	0.27 (-0.20, 0.75)	0.261	0.21 (-0.30, 0.72)	0.426
Three weeks after PF	6	-0.27 (-0.86, 0.32)	0.366	-0.28 (-0.86, 0.31)	0.352
Four weeks after PF	5	-0.27 (-0.85, 0.32)	0.369	-0.37 (-1.01, 0.26)	0.252
Five weeks after PF	8	-0.11 (-0.63, 0.42)	0.692	-0.11 (-0.63, 0.41)	0.678
Six weeks after PF	6	0.10 (-0.45, 0.65)	0.729	0.28 (-0.32, 0.88)	0.359
Seven weeks or more after PF	116	0.01 (-0.27, 0.28)	0.973	0.03 (-0.25, 0.31)	0.816
Vivax malaria infection (PV)					
No malaria infection before	5,847	Reference		Reference	

One week after PV	182	-0.06 (-0.21, 0.09)	0.457	-0.07 (-0.22, 0.08)	0.374
Two weeks after PV	146	-0.09 (-0.25, -0.07)	0.289	-0.10 (-0.26, 0.06)	0.226
Three weeks after PV	37	-0.11 (-0.36, 0.14)	0.393	-0.12 (-0.37, 0.13)	0.331
Four weeks after PV	40	0.11 (-0.13, 0.35)	0.355	0.10 (-0.14, 0.35)	0.407
Five weeks after PV	42	0.08 (-0.16, 0.32)	0.525	0.06 (-0.18, 0.31)	0.614
Six weeks after PV	31	-0.14 (-0.41, 0.13)	0.295	-0.19 (-0.47, 0.08)	0.166
Seven weeks or more after PV	214	0.06 (-0.09, 0.22)	0.449	0.04 (-0.12, 0.21)	0.595

*In multivariable analysis, the cofounders identified by DAG, such as age, gravida, and calendar year were adjusted.

Abbreviation: BPD Biparietal diameter; BMI body mass index; CI confidence interval; PE pre-eclampsia; PF plasmodium falciparum; PV plasmodium vivax

Table A 5-9: Univariable and multivariable analysis of FL z-score and malaria infection in pregnancy stratified by species.

		Univariable analysis		Multivariable analysis*	
	Scans	**Coefficient (95% CI)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Falciparum malaria infection (PF)					
No malaria infection before	6,371	Reference		Reference	
One week after PF	18	-0.07 (-0.31, 0.18)	0.595	-0.02 (-0.28, 0.24)	0.889
Two weeks after PF	8	-0.27 (-0.61, 0.70)	0.119	-0.29 (-0.66, 0.06)	0.106
Three weeks after PF	6	0.03 (-0.38, 0.44)	0.906	0.01 (-0.40, 0.42)	0.959
Four weeks after PF	5	-0.32 (-0.73, 0.09)	0.123	-0.26 (-0.71, 0.18)	0.249
Five weeks after PF	8	-0.30 (-0.67, 0.08)	0.117	-0.31 (-0.69, 0.07)	0.107
Six weeks after PF	6	-0.33 (-0.73, 0.63)	0.1	-0.19 (-0.62, 0.07)	0.398
Seven weeks or more after PF	116	-0.26 (-0.50, -0.02)	0.033	-0.25 (-0.50, -0.01)	0.045*
Vivax malaria infection (PV)					
No malaria infection before	5,846	Reference		Reference	
One week after PV	182	-0.08 (-0.20, 0.04)	0.189	-0.06 (-0.18, 0.06)	0.358

Two weeks after PV	146	-0.16 (-0.29, -0.03)	0.013	-0.13 (-0.26, -0.01)	0.040*
Three weeks after PV	37	-0.15 (-0.33, 0.03)	0.11	-0.13 (-0.31, 0.05)	0.162
Four weeks after PV	40	-0.09 (-0.26, 0.09)	0.336	-0.08 (-0.26, 0.10)	0.388
Five weeks after PV	42	-0.13 (-0.31, 0.05)	0.147	-0.11 (-0.29, 0.07)	0.229
Six weeks after PV	31	-0.07 (-0.26, 0.13)	0.509	-0.04 (-0.24, 0.16)	0.688
Seven weeks or more after PV	214	-0.10 (-0.23, 0.04)	0.149	-0.07 (-0.21, 0.06)	0.288

*In multivariable analysis, the cofounders identified by DAG, such as age, gravida, and calendar year were adjusted.

Abbreviation: BMI body mass index; CI confidence interval; FL femur length; PE pre-eclampsia; PF *plasmodium falciparum*; PV *plasmodium vivax*