Malaria in pregnancy: ultrasound studies of fetal growth

Marcus Rijken

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Malaria in pregnancy: ultrasound studies of fetal growth

Malaria in de zwangerschap: echografische studies van de groei van de foetus.
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op

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door

Marijnus Johannes Rijken

geboren op 15 april 1978 te Ede

Promotoren:	Prof.dr. G. H. A. Visser Prof.dr. F. H. Nosten
Co-promotoren:	Dr. R. McGready Dr. A. T. Papageorghiou

For the Karen people of Burma

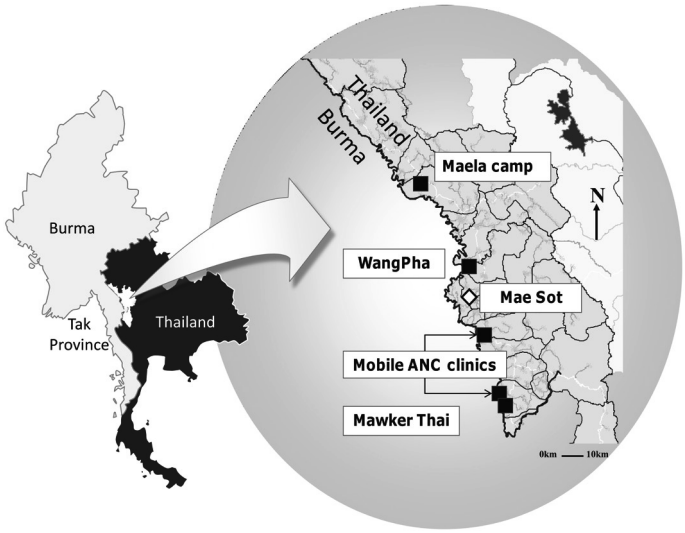
Contents

Part 1 Introduction

Chapter 1

Introduction

Figure 1.1 Geographical setting of the antenatal clinics of the Shoklo Malaria Research Unit



Location of the Shoklo Malaria Research Unit antenatal clinics and Mae Sot, the main town in the Thai province of Tak, bordering Burma. The locations of the antenatal clinics are represented by squares.

Malaria has been a plague for human mankind. The characteristic periodic fevers of malaria have been recorded from every civilized society from China in 2700 BC through the writings of Greek, Roman, Assyrian, Indian, Arabic and European physicians[1]. Hippocrates described the fevers in quite detail, but it was not until 1880 that Laveran identified the malaria parasite in the human blood. Until that time many theories have tried to explain the disease rising from the swamps; that is where malaria got its name from: "mala aria" which is Italian for "bad air". Malaria is caused by the protozoa in the genus Plasmodium. Five species of Plasmodium infect humans: *P.falciparum* is responsible for the most severe disease, as it sequesters in the deep vascular beds of organs, but *P.vivax* is not so benign as previously thought[2]. Infection with *P.ovale* and *P. malariae* result in variable morbidity, but minimal mortality, but *P.knowlesi*, the fifth human malaria parasite, is potentially life threatening[3].

Each year, roughly 125 million pregnancies occur in areas with malaria transmission[4]. Malaria infection in pregnancy is harmful for both the mother and the fetus[5-7]. Pregnant women are more susceptible for malaria, since they are more attractive to mosquitos[8, 9] due to hormonal, metabolic, or mechanical changes. Furthermore the placenta, which is the preferred place for plasmodium parasites to sequester in a pregnant woman, is the only source of oxygen and nutrients for unborn infants. *Plasmodium falciparum* parasites, and probably *P.vivax* parasites as well[10], bind to receptors in the placenta, causing local damage[11, 12]. Any damage to the placenta directly affects the development of the fetus.

The effects of malaria infections in pregnancy on the mother and the fetus, vary between endemic areas, prevention strategies, and timing and efficacy of treatment. For example, the dramatic effects of malaria of pregnant women and fetuses have been illustrated by Dr. Wickramasuriya, a visiting obstetrician to the Ceylon medical college, who described the great malaria epidemic in Sri Lanka (Ceylon) in 1934 and 1935 as the "greatest pestilence in the recorded history of the island"[13]. In women treated for malaria, he described a maternal mortality of 13.1% (47/358), which is double the rate of that in non pregnant women in the same period of time[13]. He observed that 82.2% (208/253) pregnancies complicated with malaria infections were premature interrupted, and explained: "in the ill-nourished, poverty-stricken, and hookworm-infested individual, even a mild attack of malaria is, as it were, the last straw"[13]. In the same series of women, intra-uterine fetal death had occurred in 32.4% (82/253) of pregnancies. Wickramasuriya wrote: "The localization of parasites in the capillaries of the uterus would appear to explain the frequent interruptions of pregnancy in malaria, whilst their concentration in the placenta accounts for the great frequency of intra-uterine fetal deaths"[13].

Prematurity and birthweight are strongly related to perinatal and infant mortality[14] and to morbidity and mortality in later life[15-17]. From epidemiological studies, mainly from high malaria transmission areas of the African continent, it is known that malaria reduces birthweight[5]. However, the exact mechanism is still not known; it may be due to intra uterine growth restriction (IUGR), preterm birth, or both[11]. To differentiate between these two, accurate birthweight measurement and assessment of gestational age

are pivotal. This is notoriously difficult in developing country settings and not available in most previously published malaria in pregnancy studies. In addition to the difficulties of accurate assessment of birthweight and gestation, it should be realized that the physiologically small for gestational age (SGA) neonate differs from the pathologic IUGR neonate[18, 19].

Birth weight is only one index of growth failure and babies of apparently appropriate birthweight may show signs of IUGR since they may not have achieved their full genetic potential[19, 20]. The diagnosis of IUGR can only be made by demonstrating significant deviation from the normal pattern of intra-uterine growth which requires accurate dating, a reference curve of fetal size, and a minimum of two antenatal biometry ultrasound measurements to be made[21, 22]. Several methods to estimate gestational age exists: the most commonly used is the first day of the last menstrual period, but this is often inaccurate and subject to recall; measurement of the symphysis fundal height is generally subject to large variations; neonatal neuro-developmental tests, such as Dubowitz tests, are applied retrospectively and have wide limits of agreement; finally, fetal biometry using early antenatal ultrasound is the gold standard. Since the 1990s, almost every pregnant woman in developed countries has had access to 1-4 routine scans during uncomplicated pregnancies[23]. However, in most developing countries antenatal ultrasound services are nonexistent or inadequate. Those that are available are usually limited to tertiary centers or private hospitals in urban regions [24-26].

Ultrasound suits developing countries by virtue of its versatility, relatively low cost and safety[27-29] compared with other imaging modalities. Recent literature highlights the usefulness of antenatal ultrasound in developing country settings[27, 30-32], but at the same time over-and misuse of ultrasound have been reported[33, 34], and in a district hospital in Botswana[35], ultrasound scanning has been associated with significant psychological stress and anxiety in pregnant women, especially when accompanied by minimal explanation by healthcare providers.

This thesis was carried out on the Thai-Burmese border, an area with malaria transmission. In order to be able to effectively explore the effect of malaria in pregnancy on fetal growth, several factors need to be in place, and operational development of some of these factors made this thesis possible. These included introduction and standardization of antenatal ultrasound to allow accurate dating of pregnancies by ultrasound and recognition of fetal growth restriction by locally trained staff; development of fetal size references for the local population, supporting a well functioning antenatal care system and building a safe delivery unit with standardized midwifery skills and newborn's anthropometric measurements.

This thesis summarizes the efforts and results that have been made on the Thai-Burmese border for a large project of: malaria in pregnancy – ultrasound studies of fetal growth.

Aim of this thesis

It was the aim of this thesis to explore the effects of malaria in pregnancy on fetal growth by antenatal ultrasound. Specifically, we:

1. review current evidence on malaria in pregnancy in the Asia-Pacific region and describe the methods used in previous malaria in pregnancy articles in relation to estimation of gestational age and measuring birthweight.
2. describe the challenges of fetal growth studies in a developing country setting, and the acceptance of antenatal ultrasound by the pregnant women in those countries.
3. demonstrate the feasibility of locally trained health workers to obtain accurate dating and biometry measurements which a required to create population fetal size equations and birthweight centiles.
4. describe the effects of malaria in pregnancy on fetal growth and development in accurately dated pregnancies.

Outline of the thesis

This thesis consists of five parts.

Part 1 Introduction

This part describes some methodological aspects of setting up an ultrasound study in a resource poor malaria endemic setting and puts the studies into the context of the Thai-Burmese border.

In **chapter 2** the Burmese and Karen migrant and refugee populations are introduced and the Shoklo Malaria Research Unit portrayed. The experience of setting up an ultrasound department, antenatal clinic and delivery unit in a migrant setting on the border with Burma is visualized.

Part 2 Review of the literature

This part presents background information on maternal malaria in the Asia-Pacific region and malaria in pregnancy studies in general. Although the articles are focused on malaria in pregnancy, the usefulness of antenatal ultrasound for pregnancy dating in malaria endemic areas is clearly highlighted.

In **chapter 3** the epidemiology, effect, treatment, and prevention of malaria in pregnancy in the Asia-Pacific region is reviewed.

In **chapter 4** the methodological difficulties of using birthweight as primary endpoint of previous malaria in pregnancy studies are described. A standardized method for the measurement and reporting of birthweight in future studies is proposed.

Part 3 Antenatal ultrasound on the Thai-Burmese border

This part describes the feasibility and results of ultrasound training of local health workers. It confirms the quality of antenatal ultrasound measurements performed by these health workers in the SMRU antenatal clinics. In this part the migrant and refugee pregnant women's views and acceptability of antenatal ultrasound is highlighted.

In **chapter 5** the encouraging results of intra- and inter-observer agreement assessments of fetal biometry for pregnancy dating by locally trained health workers on the Thai-Burmese border are described. This experience suggests that the training of local health workers in developing countries is possible and could allow effective use of obstetric ultrasound.

In **chapter 6** the accuracy of fetal biometry measurements performed by locally trained health workers is substantiated by comparing the z-scores and standard deviations of biometry equations created for this purpose to those from published equations of professional sonographers from Asian and European hospitals.

In **chapter 7** a mixed methods approach is used to understand how routine obstetric ultrasound is experienced in the displaced Burmese patient population.

Part 4 Malaria in pregnancy – ultrasound studies of fetal growth

In this part the effects of malaria in pregnancy on (early) fetal growth and birthweight centiles are described in retrospective and prospective studies with ultrasound dated pregnancies.

In **chapter 8** the effects of *P.falciparum* and *P.vivax* malaria infections during the first half of pregnancy on fetal head diameter are described.

In **chapter 9** birthweight for gestational age percentiles for the population on the Thai-Burmese border are introduced and compared to the global reference for birthweight percentiles. These percentiles were derived from accurately dated pregnancies, and the effect of malaria infection in pregnancy on low birth weight, premature labour and birthweight centiles is described.

In **chapter 10** the preliminary results of a prospective ultrasound cohort study are presented, in which 416 women living in a malaria endemic area on the Thai-Burmese border were followed throughout pregnancy, starting in the first trimester, with frequent ultrasound scans. This study was set up to study the mechanisms of IUGR due to malaria, and is still ongoing in 2012. This chapter forms the basis for future research plans; of which two have been completed already: chapter 11 and 12.

In **chapter 11** the placental volumes of malaria infected and uninfected women are compared. There has been no previously published article on the effect of malaria in pregnancy on placental volume measured by three-dimensional ultrasound.

Similarly, the first study of fetal cortex development in malaria infected pregnancies is presented in **chapter 12**.

Part 5 Summary, discussion and recommendations

In **chapter 13** all studies are summarized and discussed; the author draws conclusions from this thesis and provides suggestions for further studies.

In **chapter 14** each chapter is briefly summarized in Burmese and Dutch language.

In **chapter 15** some appendices are presented: article ultrasound on a bamboo floor, acknowledgements, list of publications and curriculum vitae.

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Chapter 2

Methodological aspects of ultrasound studies of fetal growth on the Thai-Burmese border

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The Shoklo Malaria Research Unit

The Shoklo Malaria Research Unit (SMRU; www.shoklo-unit.com) is a field station of the Faculty of Tropical Medicine, Mahidol University and part of the Mahidol Oxford Tropical Medicine Research Programme (MORU; http://www.tropmedres.ac/) funded by the Wellcome Trust of Great Britain. The SMRU was set up in 1986 and since then directed by professor François Nosten. One of the focuses of the SMRU is the study of the epidemiology[1, 2], prevention[3-6] and treatment[7] of *Plasmodium (P.) falciparum*[8] and *P.vivax*[9] malaria from early pregnancy onwards[10, 11].

The SMRU is based in Thailand's rugged, hilly, and mostly forested Tak province, which shares over 500 km of border with Myanmar (Burma). The climate is tropical with a 6-months rainy season (from May to early October). Mean annual rainfall varies between 1,400 mm in the southern and central areas and 2,300 mm in the northern district. The mean temperature ranges from 20.2 °C in December to 29.3 °C in April, and the annual relative humidity is above 75%[12]. Malaria transmission is unstable, low and seasonal in this area[8]. *P.falciparum* and *P.vivax* are the predominant species, while *P. malariae* and *P. ovale* are found occasionally. The entomological inoculation rate of *P.falciparum* was estimated as one infectious bite per person per year[2] and has declined dramatically in the last 15 years. As a result there is little or no natural immunity to malaria and infections are seen in all age groups.

The peak incidence of malaria cases occurs at the start and end of the rainy season. The predominant vectors are the forest and forest-fringe inhabiting *Anopheles (An) dirus*, and in the low hill areas *An. minimus* and *An. maculatus*, found at the margin of hilly forest zones and plantations[13]. These vectors have a preference for outdoor resting and feeding at a time when people are still active, which make them difficult to control. In addition, the efficacy of chloroquine in the treatment of *P.vivax* infections is declining[14, 15] and *P.falciparum* parasites are highly multi-drug resistant[16]. Consequently, there is a lack of effective prevention strategies. Hence, the SMRU runs an antenatal care (ANC) programme with weekly screening to detect and treat all parasitaemic episodes during pregnancy to prevent maternal deaths[8].

Population

The population in Tak province can be divided into three groups: (1) Thai citizens (approximately 300,000, of which half are Thai and the other half belong to ethnic minorities); (2) foreign nationals (FNs) (approximately 150,000, mainly migrant workers from Burma) and (3) displaced persons (65,000 mainly Karen displaced people) living in semi-open camps[12]. The Karen are one of the largest ethnic minority groups in South East Asia and the largest ethnic group in Burma (Myanmar) after the Burmans. There are over six million Karen in Burma, and more than 400,000 in Thailand. Most Karen are Animist, Buddhist, or Christians but, regardless of religion, the Karen hold

some deep animist beliefs. Traditionally, most Karen earned their livelihoods as subsistence rice cultivators or farmers. Men and women have equal access to education.
The Karen from Burma have been struggling for their independence since the end of Second World War. The armed conflict between the Burmese army and rebelled Karen factions, which is one of the longest civil wars on earth, has led many Karen people to flee and take refuge along the border with Thailand. Many more Burmese students, monks, and other dissidents fled to these areas after the student uprisings in 1988.
In 1984, the Thai-Burmese border was predominantly under the control of indigenous ethnic groups such as the Karen, Karenni, Mon and Shan (http://www.tbbc.org). For more than 35 years the Karen National Union (KNU) had been in rebellion against the Burmese government. Since the mid 1970s, the KNU had been under serious attack by the Burmese military, continuously being pushed back towards the Thai Border. However in 1984, the Burmese launched a massive attack which broke through the Karen front lines, sending 10 000 Karen refugees into Thailand. From 1984 to 1994, the Burmese Army continued to launch dry season offensives against the KNU, taking control of wider areas and forcing new refugees into Thailand. By 1994, the number of refugees had increased to approximately 80 000. In January 1995, a key KNU stronghold, Manerplaw, was lost to the Burmese. Since then, most of the previously controlled Karen territories have been taken by the Burmese army. There are 8 refugee camps with a total population estimated at 155 000. Since 1995 and the changes in the relations between Thailand and Burma, an increasing number of migrant workers have arrived in Thailand in search of a better living and work. This population of migrants quickly became larger than that of refugees but its health needs remained unattended by NGOs working in the refugee camps. SMRU set up clinics at the main border crossing points in an area that now stretches 200 km in the 5 border districts of Tak Province (see **Figure 1.1**).

Provision of Health Care

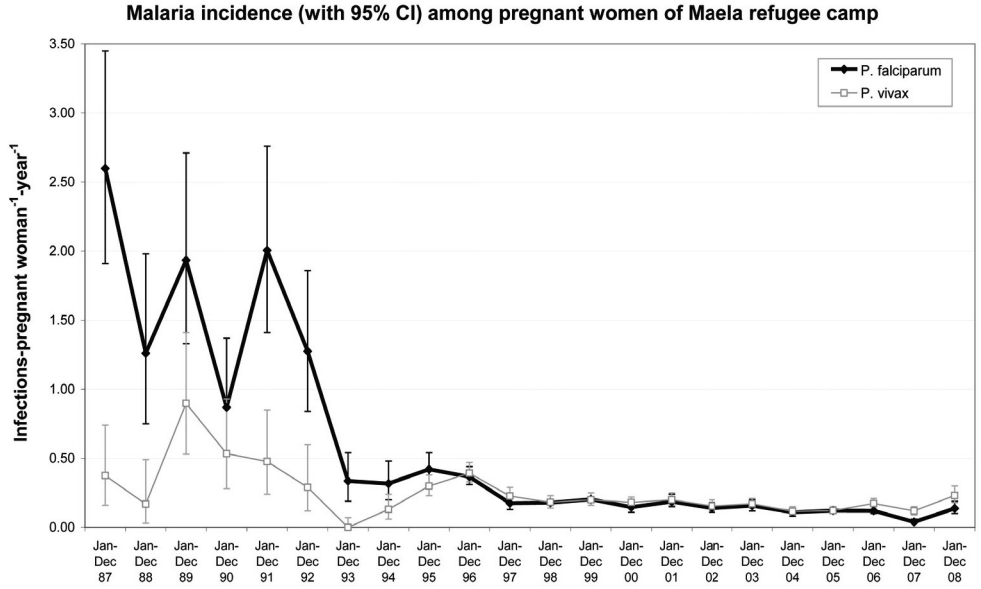
General organization of health care

Health care in the MaeLa camp is organized by international medical non-governmental organisations (NGOs), previously Médecins Sans Frontières (MSF), and since 2005 Aide Médicale Internationale (AMI). Food and other needs are provided by a consortium of charities (http://www.ccsdpt.org/). Patients who are suffering common diseases such as malaria, acute respiratory tract infections or diarrhea are treated in basic clinics located within the camp. Complicated cases such as surgical patients are transferred to the Thai hospital. The SMRU provides maternal health including obstetric care to the population of Maela Camp (50,000 inhabitants) and to the migrant worker population. Thai nationals have access to the Thai health care system. Care of foreign nationals and/or illegal workers is less organized, and for many illegal people it is difficult (for financial and security reasons) to travel to a health clinic. Health care is provided by the Mae Tao

Clinic (Dr Cynthia's Clinic) in Mae Sot, and by 5 SMRU migrant clinics scattered along the border (**Figure 2.1**) .
The migrant clinics are located in Thai villages (MawkerThai, Munruchai, Wang Pha, Mae Kon Ken and Tekaya) close to the Moei river, which is the natural border between Thailand and Burma in this area. Karen and Burmese migrant workers typically cross the river to seek medical treatment in these clinics. Antenatal care (ANC) is provided in 4 of these 5 clinics, and in Maela camp (see **Figure 1.1**).

Antenatal care in SMRU
All women are encouraged to attend the ANC as early as possible in pregnancy and to deliver at SMRU under the care of trained midwives and doctors. Those requiring Caesarean section are transferred to the nearest Thai hospital; these are a general Thai hospital (300 beds) in Mae Sot (1 hour drive from Maela camp) and two community hospitals (Mae Ramat, 30 minutes drive from WangPha and Poh Prah, 30 minutes drive from Mawker Thai).

Figure 2.1 Malaria Incidence (with 95% CI) among pregnant women in MaeLa refugee camp (V.Carrara created this figure)



Until 1995 most SMRU malaria in pregnancy studies took place in Shoklo, Bonoklo, Mae Salit, Maela and Wangka refugee camps and these camps were amalgamated into Maela in 1996. As a result of systematic screening and treatment of malaria during pregnancy, maternal mortality declined dramatically. Following the introduction of artemisinin based combination treatments (ACTs) in the population in 1991, the incidence

of malaria infections in pregnancy has reduced significantly[12, 17]. In 1998, antenatal care and studies were established in migrant sites including Mawker Thai (MKT), Mun Ru Chai (MRC) and Waley (WAL) and in 2004 in Wang Pha. These migrant sites were established in very old buildings and with the growth the clinics and a need for better hygiene and more space, new buildings were needed.
Consequently, between 2006 and 2012 Machteld and I contributed to the building of two new migrant clinics, in Wang Pha and Mawker Thai. These new clinics included a delivery room, operation room, ultrasound department, in-patient and out-patient departments, kitchen, pregnant mother waiting house (**Figure 2.2**). The Wang Pha staff number increased from 10 in 2006 to 86 in 2009, including the newly trained nurses, midwives, ultrasonographers, cleaners, and logistic staff.

Figure 2.2 Building of WangPha clinic

Antenatal ultrasound in the SMRU

In the antenatal clinics on the Thai-Burmese border less than a third of women are able to confirm their Last Menstrual Period. This is due to a number of reasons: a) GA is counted in months, and attempts to translate this to a LMP date are complicated and usually inaccurate, b) literacy levels in pregnant women in Maela Refugee camp are less than 50%[18] and c) there are several calendars in use: standard western, Thai, Karen, Burmese and Buddhist. Usually time and date were unknown among this ethnic group[19].

In response to a question concerning date of birth, the response is likely to have been 'when the lily flowers bloomed'[20]. Similarly, women have responded to questions concerning LMP as 'before planting the rice'. Nevertheless it has been observed that the Karen women can say how many months and days that they have been pregnant for with reasonable accuracy: +/- 1 to 3 weeks (Rose McGready, personal communication). From 1986 to 1992, gestational age was assessed by the symphysis-fundal height method. A formula was constructed and used: Gestational age in weeks = (fundal height in cm x 0.887) + 4.968 (95% of normal range predicted gestational age ± 6.26 weeks)[9]. In 1991, Dr Lilly Dubowitz visited the SMRU to train the local staff to perform the Dubowitz method of gestational age assessment by newborn tests[21]. From 1994, reliable Dubowitz assessment was available. A newer formula that allows gestational to be calculated from fundal height in this population, based on ultrasound dated pregnancies, has been refined by mathematical modeling work[22].

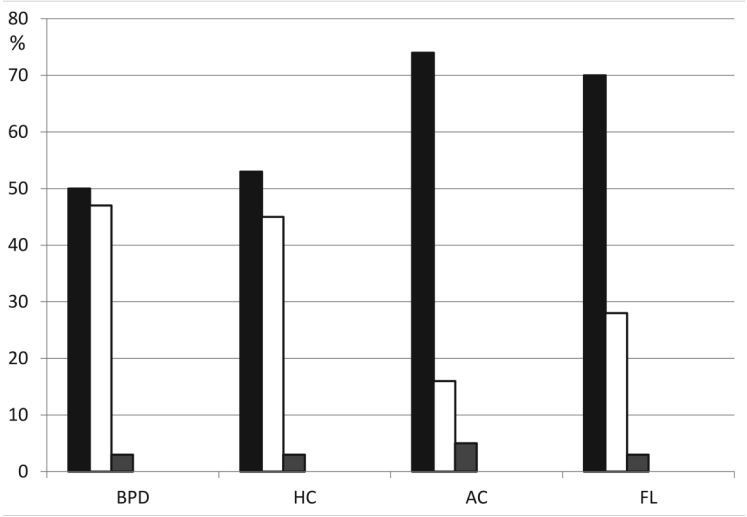
When Rose McGready introduced an ultrasound machine in the antenatal clinic of the Maela refugee camp in 2001, local Karen health workers (Saw Loo Mu Dwell and Ratree Arunjerdja), who were already skilled in Dubowitz assessment of gestational age, were trained in ultrasound scanning. From 2002, the Dubowitz method was only used in pregnancies with dating scan after 24 weeks' gestational age.

We (Rose McGready and I) developed a three month course of practical and theoretical training in obstetric ultrasound (see cover of this thesis) for newly employed staff, all of whom were chosen at interview on the basis of motivation, willingness to learn and proficiency in English. This course was based on World Health Organization (WHO) guidelines and British Medical Ultrasound Society (BMUS) recommendations[23, 24]. During three months all scans of the students were verified by the senior sonographer and by me. Only when we were satisfied with each person's scanning skills and written examination results, were they permitted to scan without supervision. Between 2006 and 2012, over twenty sonographers were trained (from different organizations), of which several left for resettlement to Australia and USA.

In SMRU, 16 locally trained health workers divided over 3 clinics obtain all ultrasound scans using Toshiba Powervision 7000 (since 2006) and Capasee (since 2010), Dynamic Imaging (since 2001), Fukuda Denshi UF 4100 (since 2002) and General Electronic Voluson i (since 2008) ultrasound machines. These staff work independently but, when needed, their practice is supervised by SMRU doctors certified in fetal ultrasonography. All women are offered two scans in pregnancy. The first scan occurs at the booking visit (between 8-14 weeks gestation) where ultrasound is used to determine viability, identify multiple pregnancies and estimate gestational age by Crown Rump Length (CRL) measurement. Based on this gestational age estimate women then return for a second scan performed at 18-24 weeks to re-assess viability, measure fetal biometry, identify major fetal abnormalities and determine placental location. Fetal biometry is routinely measured twice in each woman at each scan, as part of an existing quality control as described in this thesis. This group of sonographers was responsible for most of the

studies described in this thesis. The specific methods of each study are written in each chapter.
In 2008 the SMRU ultrasound studies were linked to "The international fetal and newborn growth consortium" in Oxford (INTERGROWTH-21st project, http://www.intergrowth21.org.uk/ and later INTERBIO-21st study). I spent 4 weeks in Oxford to be certified in antenatal ultrasound scanning and finalize the prospective fetal growth study protocol. Since then external quality control is done at Oxford University using a image-scoring method[25]. Six is the maximum score and the locally trained sonographers had a good score on the first quality control study (A.Papageorghiou, personal communication), see **Figure 2.3**.

Figure 2.3 Result of the image scoring method for quality control of the ultrasound images (n=76)



The colour of the bars indicate the scores: black = maximal score (6/6), white = maximal score minus 1 (5/6), grey = maximal score minus 3 (4/6)

Clinical context of ultrasound studies

Antenatal ultrasound has influenced clinical practice in this area. Ultrasound findings such as multiple pregnancies, placenta praevia, or intra-uterine growth restriction with absent end-diastolic flow can be diagnosed by local health workers with sufficient training. However the ultrasound finding is a part of a process not an end in itself: the medical and midwifery teams need to understand what this means, understand the limitations of ultrasound (e.g. dating in the third trimester is not reliable) and be prepared to act. SMRU has put a lot of effort in medical staff training. For example, in 2007 I was the medical editor of the fourth edition of a local medical guideline

book in Burmese and English language: the Burmese Border Guidelines (http://www.ibiblio.org/obl/docs4/BBG_2007-Eng.pdf). These guidelines are the reference for all treatments and medical training in the camps and migrant clinics along the border. In 2008 we developed a midwifery curriculum and updated the SMRU obstetric manual. Two large midwife trainings, in which over 50 midwives have been trained, who are working now in the SMRU delivery rooms. In 2009, we introduced the Advanced Life Support Obstetrics (ALSO) course on the Thai Burmese border. Since we were awarded faculty of this course, we are allowed to run ALSO courses with our 9 certified local instructors on the Thai Burmese border. Over 50 medical staff from 4 organizations did the course, and each SMRU delivery room practices the emergency drills. For malaria, the SMRU organizes a yearly malaria meeting for all health organizations in which the updated malaria guidelines are presented and the recent research findings summarized (www.shoklo-unit.com). Furthermore, the SMRU led the production of the British Royal College of Obstetricians and Gynecologists" (RCOG) Green-top guidelines on the prevention of malaria in pregnancy (Green-top 54A: http://www.rcog.org.uk/prevention-malaria-pregnancy-green-top-54a) and the diagnosis and treatment of malaria in pregnancy (Green-top 54B: http://www.rcog.org.uk/diagnosis-and-treatment-malaria-pregnancy-green-top-54b).

Support

On 13 March 2006 Machteld and I set up the "Stichting Malariadokters" (see www.malariadokters.nl), a foundation with the aim to improve healthcare and wellbeing of people in malaria endemic areas by providing medical, material and financial support, education and supporting research to fulfill this aim. Among many projects, more than 35, the foundation supported the transportation of the ultrasound machines to Thailand, the building of two clinics, purchasing equipment for delivery and operation rooms and special care baby units, providing teaching materials etc. Between 2006 and 2012, more than 200000 Euro has been transferred to projects on the Thai-Burmese border. Two Dutch hospital, the University Medical Centre Utrecht (2006) and Flevo Ziekenhuis Almere (2010), donated an ultrasound machine for the clinical care and research projects in the camp and migrant clinics (see appendix). Most equipment in Wang Pha and Mawker Thai delivery and operation rooms were purchased from "Stichting Medic" (http://www.medic.nl/). The delivery beds, operation table, newborn heater were developed locally with the creative design of Khun Tip. The Christophe and Rodolphe Mérieux Foundation supported the study through a 300000 Euro prize (2008) to François Nosten. The SMRU is part of the Mahidol Oxford University Research Unit, supported by the Wellcome Trust of Great Britain.

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Chapter 3

Malaria in pregnancy in the Asia-Pacific region

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Summary

Most pregnant women at risk for infection with *Plasmodium vivax* live in the Asia-Pacific region. However, malaria in pregnancy is not recognized as a priority by many governments, policymakers, and donors in this region. Robust data for the true burden of malaria throughout pregnancy are scarce. Nevertheless, when women have little immunity, each infection is potentially fatal to the mother, fetus, or both. WHO recommendations for the control of malaria in pregnancy are largely based on the situation in Africa, but strategies in the Asia-Pacific region are complicated by heterogeneous transmission settings, coexistence of multidrug-resistant *Plasmodium falciparum* and *Plasmodium vivax* parasites, and different vectors. Most knowledge of the epidemiology, effect, treatment, and prevention of malaria in pregnancy in the Asia-Pacific region comes from India, Papua New Guinea, and Thailand. Improved estimates of the morbidity and mortality of malaria in pregnancy are urgently needed. When malaria in pregnancy cannot be prevented, accurate diagnosis and prompt treatment are needed to avert dangerous symptomatic disease and to reduce the effects on fetuses.

Search strategy and selection criteria

Reports were identified through searches of PubMed those published before 10 January 2011, with the terms "malaria" and "pregnancy" combined with specific country names. The analysis was restricted to English language articles. The "Malaria in Pregnancy Consortium library", WHO regional websites the World Malaria Report[14] and "ClinicalTrials.gov" were searched for additional studies of malaria in pregnancy from included countries. We identified national malaria treatment and prevention policies for pregnant women on the websites of each country's Ministry of Health. All regional WHO representatives were approached to include available data from each National Malaria Program. Principal investigators of continuing trials in the region were invited to share unpublished data.

Introduction

Most pregnant women at risk for infection with *Plasmodium vivax* live in the Asia-Pacific region, which consists of the WHO South-East Asia and Western Pacific regions. These areas generally have low transmission of *Plasmodium falciparum* and women have little acquired immunity against malaria.[1] Many countries in the Asia-Pacific region have successfully reduced prevalence of malaria and several national malaria control programmes have adopted elimination of malaria as an objective. However, malaria infection in pregnancy is commonly not perceived as a major health problem. Pregnant women are a vulnerable population,[2] but in many areas their malaria-related morbidity and mortality are unknown.[3] Specific treatment guidelines or prevention strategies for malaria in pregnancy either do not exist or have only recently been introduced.[4-6] Interventions recommended by WHO for the control of *P.falciparum* malaria during pregnancy are largely based on findings from sub-Saharan Africa, where *P.falciparum* is the dominant species, transmission is high and *Anopheles gambiae* is the main vector.[7] Strategies for the Asia-Pacific region are complicated by several factors: diversity in malaria transmission and vector behaviour,[8] co-existence of *P.vivax*,[9] multidrug resistant parasites,[10, 11] substandard and counterfeit drugs,[12] cross-border movement of migrants[2], ethnic minorities that have little access to health care,[2] and the political climate in some countries.[2] In this review of the 20 countries in the Asia-Pacific region with malaria transmission,[13] we summarise both published (126 reports) and unpublished (six studies) data for malaria in pregnancy identified from various databases and the World Malaria Report[14] (**page 52, Web extra figure 3.2, Web extra table 3.1**), focussing on epidemiology, clinical presentation, treatment, and prevention of malaria in pregnancy. Additionally, we identify gaps in the evidence to assist identify priorities for future research in the Asia-Pacific region.

Epidemiology

Malaria Transmission

Malaria transmission in the Asia-Pacific region is highly heterogeneous. Generally, transmission is low (entomological inoculation rate of *P.falciparum* is less than one infective bite every year), unstable (with year-to-year variation and epidemics), highly focal, and seasonal, and it mostly occurs in rural areas.[15] Nonetheless, several countries have foci of entomological inoculation rates of more than ten infectious bites per year, indicating high transmission.[16] Of the total population at risk (more than 2·2 billion people), a quarter lives in areas of moderate or high *P.falciparum* malaria transmission.[1, 13, 17] Large variations in malaria transmission are present not only within countries, but also within regions. For example, malaria in Thailand occurs in forested pockets along international borders.[18, 19] Urban malaria is rare, except in India.[3, 20] All these variations have important effects on acquisition of natural immunity to malaria. *P.vivax*

could re-emerge in areas where it was eliminated[21] or become more prevalent where *P.falciparum* is controlled.[19, 22] The prevalence of *P.vivax* is difficult to estimate because *P.vivax* is often under-reported in co-infections with *P.falciparum* and relapses from the liver stages are unrelated to vector activity.[23, 24]

Pregnancies at risk

In 2007, roughly 75 million women became pregnant in the Asia-Pacific region,[1] but these women were mostly in India and China. Because of the focal nature of malaria transmission,[25] the substantial seasonality, and the variable strength of malaria control programmes, risk estimates have to be refined and combined with clinical data to be accurate and useful, and to minimise bias. In pregnancy, women and their babies are at risk of malaria's deleterious effect for the 40 weeks of gestation. However, longitudinal data collection is needed to estimate the true burden of malaria. Most investigators report rates of confirmed malaria infection at enrolment in the antenatal clinic (**Table 3.1)** or at delivery (**Table 3.2**); only a few report cumulative proportions or incidence rates.

Four studies[27-29, 32] that showed a high proportion of women with malaria (three from India and one from Laos) included only pregnant women with fever or a history of fever. After exclusion of these studies, malaria smears to detect parasites in maternal peripheral blood established that the median reported proportion of pregnant women infected with malaria was 15·3% (range 1·2-40·8) in antenatal clinics (nine studies; **Web extra table 3.2**) and 8·1% (range 1·6-18·5) in delivery rooms (12 studies; **Web extra table 3.4**). The median proportion of reported placental parasitaemia was 11·0% (range 1·4-29·1; 12 studies; **Web extra table 3.5**).

Screening surveys, however, are very prone to variation because they provide only an estimate of the prevalence of malaria infections in a short period. The wide range of positivity rates is a result not only of differences in transmission intensity, but also of discrepancies in methods or season when surveys are done. For example, at antenatal screenings every week at the Thai-Burmese border , which are essentially weekly cross-sectional surveys, the mean malaria prevalence in one clinic was 0.85% (95% CI 0.83-0.87; range 0-3.1) for *P.falciparum* and 3.18% (95% CI 3.14-3.22; range 0-8.3) for *P.vivax* in 2008 (unpublished). However, the cumulative proportions of infected women in the same population in the same year were 6.8% (50 of 733 women) for *P.falciparum* and 16% (119 of 733 women) for *P.vivax* (unpublished). In this setting in 2008, the median number of consultations per woman was 14 (IQR 8-20).

To establish the true burden of malaria in pregnancy, longitudinal follow-up of pregnant women is preferable. Investigators of 11 studies have followed up the same women during pregnancy, providing a cumulative proportion of malaria episodes (**Table 3.3**). In ten studies that showed the number of women with malaria, the median proportion of infected women was 36·5% (range 6·0-64·0).

Women can be infected in all trimesters[33, 47, 48] and in any pregnancy, although first-time mothers are at higher risk than are others in most studies.[20, 48] In five studies in India[27-29,

Table 3.1 Proportion of women with malaria in antenatal clinics by country in the Asia-Pacific region

	Year of study	Site	Timing of screening	Chemo-prophylaxis (% participants taking prophylaxis)	Screening method	Total women screened	Number with malaria	Infections by species			
								Plasmodium falciparum	Plasmodium vivax	Mixed	Other species
South East Asian region											
Bangladesh (Faiz, A, personal communication)	2004	South east	Any ANC visit	Chloroquine (2.4%)	MS	388	15 (3.9%)	n.a.	n.a.	n.a.	n.a.
Burma (2010)[26]	2006-08	East	Survey	No	RDT	850	100 (11.8%)	100 (100%)	n.a.	n.a.	n.a.
India (1993)[27]	1987-88	Gujarat Surat	Women with HOF	No	MS	322	186 (57.8%)	112 (60.2%)	70 (37.6%)	4 (2.2%)	0 (0%)
India (1995)[28]	1991-93	Jabalpur	Women with HOF	No	MS	831	145 (17.4%)	101 (69.7%)	41 (28.3%)	3 (2.1%)	0 (0%)
India (1999)[29]	1991-93	Jabalpur	Women with HOF	No	MS	2127	365 (17.2%)	244 (66.8%)	121 (33.2%)	0 (0%)	0 (0%)
India (2004)[30]	2001-02	9 sites	n.a.	No	MS	n.a.	215 (n.a.)	131 (60.9%)	69 (32.1%)	15 (7.0%)	1 (<1%)*
India (2009)[20]	2006-07	Jharkhand	Any ANC visit	Chloroquine (0.6%)	MS	2386	32 (1.3%)	n.a.	n.a.	n.a.	0 (0%)
					RDT	2386	43 (1.8%)	23 (53.5%)	16 (37.2%)	4 (9.3%)	0 (0%)
India (unpublished)	2007-08	Chhattisgarh	Booking at ANC	No	MS	2696	33 (1.2%)	n.a.	n.a.	n.a.	0 (0%)
					RDT	2696	35 (1.3%)	29 (82.9%)	6 (17.1%)	0 (0%)	0 (0%)
Indonesia (unpublished)	2008-10	Jayapura	Any ANC visit	No	MS	1551	238 (15.3%)	138 (58.0%)	68 (28.6%)	30 (12.6%)	0 (0%)
		Sumba		No	MS	1554	207 (13.3%)	139 (67.1%)	60 (29.0%)	8 (3.9%)	0 (0%)
Nepal (2000)[31]	1994-97	Sarlahi	Enrolment	No	MS	288	57 (19.8%)	0 (0%)	57 (100%)	0 (0%)	0 (0%)
Western Pacific region											
Laos (2000)[32]	1998	Vientiane	Women with HOF	No	MS	68	16 (23.5%)	16 (100%)	n.a.	0 (0%)	0 (0%)
PNG (1990)[33]	1985-87	Madang	First ANC	Chloroquine (23%)	MS	620	180 (29.0%)	170 (94.4%)	9 (5.0%)	n.a.	1 (<1%)#
PNG (unpublished)	2006-08	Madang	First ANC	No	MS	468	191 (40.8%)	142 (74.3%)	38 (20.0%)	n.a.	11 (2.4%)*
					PCR	468	307 (65.6%)	217 (70.7%)	n.a.	n.a.	52 (11.1%)*
Solomon (2008)[34]	2003	Marovo Lagoon	Any ANC visit	Very low coverage	MS	106	19 (17.9%)	15 (78.9%)	4 (21.1%)	0 (0%)	0 (0%)

Infections with *P.falciparum* and *P.vivax* were classed as mixed infections. ANC antenatal clinic, HOF history of fever, MS malaria smear, n.a. not available, PNG Papua New Guinea, RDT rapid diagnostic test. * Infection with Plasmodium ovale. # Infection with Plasmodium malariae

Table 3.2 Proportion of women with malaria at delivery by country in the Asia-Pacific region

	Year	Site	Chemo-prophylaxis (% participants taking prophylaxis)	Screening method	Total women	Number with malaria	Infections by species			
							Plasmodium falciparum	Plasmodium vivax	Mixed	Other species
South East Asian region										
India[29]	1992-95	Jabalpur	No	Maternal MS **	2127	365 (17.2%)	244 (66.8%)	121 (33.2%)	0 (0%)	0 (0%)
India[35]	2002-03	Mandla	No	Placental MS/RDT	182	53 (29.1%)	49 (95.5%)	1 (1.9%)	3 (5.7%)	0 (0%)
India[36]	2002-04	Mandla	No	Maternal MS	209	11 (5.3%)	7 (63.6%)	0 (0%)	4 (36.4%)	0 (0%)
		Mandla		Placental MS	209	30 (14.4%)	26 (86.7%)	0 (0%)	4 (13.3%)	0 (0%)
		Mandla		Placental RDT (PC)	209	56 (26.8%)	56 (100%)	n.a.	n.a.	n.a.
		Maihar		Maternal MS	590	41 (6.9%)	29 (70.7%)	10 (24.4%)	2 (4.9%)	0 (0%)
		Maihar		Placental MS	590	64 (10.8%)	54 (84.4%)	8 (12.5%)	2 (3.1%)	0 (0%)
		Maihar		Placental RDT (PH)	590	65 (11.0%)	65 (100%)	n.a.	n.a.	n.a.
India[20]	2006-07	Jharkhand	Chloroquine (0.3%)	Maternal MS / RDT	717	12 (1.7%)	9 (75.0%)	2 (16.7%)	1 (8.3%)	0 (0%)
		Jharkhand		PlacentalMS	712	10 (1.4%)	n.a.	n.a.	n.a.	n.a.
		Jharkhand		Placental RDT	712	17 (2.4%)	12 (70.6%)	2 (11.8%)	3 (17.6%)	0 (0%)
India (unpublished)	2007-08	Chhattisgarh	No	Maternal MS	1028	16 (1.5%)	n.a.	n.a.	n.a.	n.a.
		Chhattisgarh		Maternal RDT	1028	19 (1.8%)	13 (68.4%)	5 (26.3%)	1 (5.3%)	0 (0%)
		Chhattisgarh		Placental MS	1027	17 (1.7%)	n.a.	n.a.	n.a.	0 (0%)
		Chhattisgarh		Placental RDT	1027	33 (3.2%)	18 (54.5%)	12 (36.4%)	3 (9.1%)	0 (0%)
Indonesia[37]	2004-06	Timika	No	Maternal MS	2487	397 (16 .0%)	225 (56.7%)	141 (35.5%)	12 (3.0%)	18 (4.5%)# 1 (<1%)*
Indonesia (unpublished)	2008-10	Jayapura	n.a.	Maternal MS	830	90 (10.8%)	55 (61.1%)	29 (32.2%)	2 (2.2%)	4 (4.4%) *
		Jayapura		Placental MS	818	75 (9.2%)	46 (61.3%)	24 (32.0%)	3 (4.0%)	2 (2.7%) *
		Sumba		Maternal MS	981	112 (11.4%)	72 (64.3%)	35 (31.3%)	5 (4.5%)	0 (0%)
		Sumba		Placental MS	974	109 (11.2%)	68 (62.3%)	36 (33.0%)	5 (4.6%)	0 (0%)
Thailand[38]	2004-06	TBB	No	Maternal MS^	169	8 (4.7%)	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)

		TBB		Maternal PCR^	169	5 (3.0%)	5 (100%)	n.a.	n.a.	n.a.
		TBB		Placental MS^	156	7 (4.5%)	6 (85.7%)	1 (14.3%)	0 (0%)	0 (0%)
		TBB		Placental PCR^	168	6 (3.6%)	6 (100%)	n.a.	n.a.	n.a.
Thailand[39]	1995-2002	TBB	No	MaternalMS^	175	19 (10.9%)	12 (63.2%)	7 (36.8%)	0 (0%)	0 (0%)
		TBB		Placental MS^	173	12 (6.9%)	11 (91.7%)	1 (8.3%)	0 (0%)	0 (0%)
		TBB		Placental Histopath^	174	37 (21.3%)	n.a.	n.a.	n.a.	n.a.
Western Pacific region										
PNG[40]	1986- 88	East Sepik	No chloroquine past 2 weeks	Maternal MS	83	7 (8.4%)	6 (85.7%)	1 (14.3%)	n.a.	n.a.
		East Sepik		Placental MS	83	16 (19.3%)	16 (100%)	0 (0%)	n.a.	n.a.
PNG[41]	1994-96	Madang	Chloroquine (92.2%)	Maternal MS	987	183 (18.5%)	n.a.	n.a.	n.a.	n.a.
		Madang		Placental MS	860	206 (24.0%)	n.a.	n.a.	n.a.	n.a.
PNG[42]	2002-03	Madang	Chloroquine high compli-ance low efficacy	MaternalMS	402	63 (15.7%)	59 (93.7%)	4 (6.3%)	0 (0%)	0 (0%)
		Madang		Placental MS	402	66 (16.4%)	63 (95.5%)	3 (4.5%)	0 (0%)	0 (0%)
		Madang		Placental Histopath	192	81 (42.2%)	n.a.	n.a.	n.a.	n.a.
PNG (unpublished)	2006-08	Madang	SP + chloro-quine, chloro-quineweekly	Maternal MS	331	25 (7.6%)	20 (80.0%)	5 (20.0%)	0 (0%)	0 (0%)
		Madang		Maternal PCR	331	140 (42.3%)	102 (72.9%)	n.a.	n.a.	4 (2.9%)*
Solomon Islands[43]	1981	Malaita	Chloroquine (9%)	Maternal MS	180	14 (7.8%)	12 (85.7%)	2 (14.3%)	0 (0%)	0 (0%)
		Malaita		Placental MS	180	10 (5.6%)	9 (90.0%)	1 (10.0%)	0 (0%)	0 (0%)
Vanuatu[44]	1984-85	Malekula	n.a.	Placental MS	184	20 (10.9%)	10 (50.0%)	10 (50.0%)	0 (0%)	0 (0%)

Infections with *P.falciparum* and *P.vivax* were classed as mixed infections.
**History of fever. ^ All women had at least one malaria infection in pregnancy. * Infection with Plasmodium ovale. # Infection with Plasmodium malariae
MS malaria smear, n.a. not available, PC Paracheck, PH ParaHITf, PNG Papua New Guinea, RDT rapid diagnostic test, SP sulfadoxine-pyrimethamine, TBB Thai-Burmese border

[52, 53], pregnant women with fever or a history of fever (N=974) were infected (with *P.falciparum* or *P.vivax*) significantly more frequently, with significantly higher parasitaemia, than were non-pregnant women of child-bearing age with fever or a history of fever in the same area and study period. Women with a history of malaria in pregnancy are at increased risk for another episode during pregnancy.[37, 38]

Plasmodial species

In the reviewed studies, *P. vivax* caused a median of 28·5% (range 5-100) of malaria infections detected at antenatal clinics (13 studies, **Table 3.1)**. *Plasmodium ovale* and *Plasmodium malariae* were present in all sites, but studies with PCR diagnosis suggest that these species are probably under-reported when microscopy is used.[3, 54] *Plasmodium knowlesi* was not reported in any pregnancy studies. At the Thai-Burmese border, the incidence of malaria has fallen from more than three infections per pregnant woman-year to less than 0·5 in the past 20 years,[22] and distribution of malaria species has changed substantially (**Figure 3.1**, **Table 3.3**). Most malaria infections are now caused by *P.vivax*, [9, 48, 50, 51] and mixed infection is uncommon (**Table 3.1, Table 3.2, Table 3.3**). A protective effect of *P.vivax* infection against subsequent episodes and severity of P. falciparum malaria was noted in Thailand.[9, 55] However, an acute episode of *P.falciparum* could trigger a relapse of a previously acquired *P.vivax* infection.[56] Therefore, as the incidence of *P.falciparum* declines, the number of *P.vivax* episodes caused by relapses will eventually also decrease.

Clinical presentation

Diagnosis

The most common method used to detect malaria parasites is microscopy of stained blood smears (**Table 3.1, Table 3.2, Table 3.3**). A skilled and well-equipped microscopist can detect 15 parasites per μL of blood, which corresponds to a total biomass of 100 million parasites.[57] In field situations, however, equipment might not be ideal and experienced microscopists are often overloaded with work. No published data exist for quality control of malaria smears from pregnant women. Rapid diagnostic tests are practical because they do not demand long training, good infrastructure, or electricity, but they might not have the sensitivity needed in pregnancy.[36, 57] Studies done in Jharkhand and Chhattisgarh states in India showed that rapid diagnostic tests were more sensitive than were malaria smears for the detection of malaria in the peripheral blood of women in labour and in placental infection (**Table 3.2**).[36] However, these findings could be explained by the slow elimination of the histidine-rich-protein-2 detected by the "Paracheck-Pf" rapid diagnostic test used. PCR is also used for genotyping and detection of malaria parasites and is more sensitive than is microscopy.[57] At present, there is very little evidence for PCR diagnostic assessment in pregnancy in the Asia-Pacific region. In available studies, PCR detected more *P. falciparum* than did malaria smear

Table 3.3 Longitudinal follow-up of pregnant women with malaria by country in the Asia-Pacific region

	Year	Site	Frequency of malaria smear	Total women screened	Cumulative proportion with malaria	Total malaria episodes	Infections by species			
							Plasmodium falciparum	Plasmodium vivax	Mixed	Other species
India[45]	1995-96	Mandla	Every 2 weeks**	150	96 (64.0%)	n.a.	n.a.	n.a.	n.a.	n.a.
India (2000)[46]	n.a.	Orissa	Every 2 weeks	209	n.a.	92	n.a.	n.a.	n.a.	n.a.
India (2001)[47]	1997-98	Mandla	Every 2 weeks ^	274	151 (55.1%)	237	202 (85.2%)	27 (11.4%)	8 (3.4%)	0 (0%)
India (unpublished)	2007-08	Jabalpur	Every month	1742	105 (6.0%)	119	88 (73.9%)	31 (26.1%)	0 (0%)	0 (0%)
Thailand[48]	1986-89	TBB	Every week	1358	505 (37.2%)	888	712 (80.2%)	152 (17.1%)	24 (2.7%)	0 (0%)
Thailand[49]	1987-90	TBB	Every week	169	37 (21.9%)	89	62 (69.7%)	21 (23.6%)	5 (5.6%)	1 (1.1%)#
Thailand[9]	1986-97	TBB	Every week	9956	2509 (25.2%)	2509	1402 (55.9%)	634 (25.3%)	473 (18.8%)	0 (0%)
Thailand[50]	1993-96	TBB	Every week	1495	555 (37.1%)	1096	491 (44.8%)	570 (52.0%)	34 (3.1%)	0 (0%)
Thailand[51]	1998-2000	TBB	Every week ##	479	57 (11.9%)	163	78 (47.9%)	83 (50.9%)	n.a.	2 (<1%)#
Thailand (unpublished)	2007-10	TBB	Every week	824	319 (38.7%)	772	174 (22.5%)	592 (76.7%)	4 (0.5%)	1 (<1%)#; 1 (<1%)*
Thailand (unpublished)	2009-11	TBB	Every week	100	36 (36.0%)	97	10 (10.3%)	85 (87.6%)	2 (2.1%)	0 (0%)

Chemoprophylaxis was not used in these studies. Infections with *P.falciparum* and *P.vivax* were classed as mixed infections.
Placebo group in a randomised controlled trial, ** pregnant women with history of fever, ^ during a malaria epidemic
* Infection with Plasmodium ovale. # Infection with Plasmodium malariae. n.a. not available, TBB Thai-Burmese border.

(**Table 3.1, Table 3.2**). Continuing studies in India (Madhya Pradesh and Chhattisgarh states) and eastern Indonesia showed that PCR detected up to twice as many *P. vivax* and *P. falciparum* episodes as did malaria smears or rapid diagnostic tests (unpublished). In areas with early detection and treatment with artemisinin-based combination therapies, placental malaria is confined to pregnancies with concurrent maternal peripheral parasitaemia.[38, 39] Furthermore, sites at the Thai- Burmese border and Indonesia that use artemisinin-based combination therapies in pregnancy have a lower ratio of placental to peripheral smear positivity, than do sites in India and Papua New Guinea, where chloroquine or sulphadoxine-pyrimethamine, or both are used (**Figure 3.2**, **Table 3.2**). In India and Papua New Guinea, *P. falciparum* resistance to chloroquine and sulphadoxine-pyrimethamine is high[20, 29, 35, 36, 40-42]. Despite differences in transmission, this finding could be a result of increased clearance of parasites from the maternal and placental blood by the more effective artemisinin-based combination therapies.

Anaemia

The anaemia burden in pregnancy is profound: about 75% of women attending antenatal clinics in India,[20, 47] Nepal,[31] Papua New Guinea,[58] or at the Thai-Burmese border[50] develop anaemia at some stage of pregnancy. Malaria-induced destruction of red blood cell aggravates any underlying nutritional anaemia, intestinal parasitisation, or red-cell inherited disorders such as haemoglobinopathies.[15] In low-transmission areas, mild anaemia predominates, whereas in areas of high transmission, 10% of pregnant women might develop severe anaemia.[48, 50, 58, 59] Comparisons between studies are difficult because of differences in anaemia definitions, iron supplementation and use of chemoprophylaxis. Both falciparum and vivax malaria worsen anaemia, but *P.falciparum* has a stronger effect than does *P.vivax* (**Web extra table 3.6**).[9, 31, 37, 60] Even asymptomatic malaria episodes could cause anaemia,[37] and women during their second or subsequent pregnancies are more anaemic than are women in their first pregnancy.[37, 42, 48, 50] Severe maternal anaemia during pregnancy increases risk of premature labour,[37, 41] and stillbirth,[61, 62] and reduces birth weight[37, 48, 58]. Data from Thailand suggests that risk of *P.vivax* malaria increases after start of haematinic supplementation (iron and folate).[63] Deficiency of glucose-6-phosphate dehydrogenase is common in the Asia-Pacific region, but its interaction with risk of malaria in pregnant women and anaemia is unknown. Although southeast Asian ovalocytosis and α thalassaemia protect young children in Papua New Guinea against cerebral malaria,[64, 65] neither genetic trait seems to change the effects of malaria in pregnancy.[42, 59, 66] The risk of thrombocytopaenia is more than two times higher in pregnant women with malaria than in non-pregnant women infected with malaria, but this disorder is rarely severe (less than 10 000 platelets per μL).[60, 67] Prompt antimalarial treatment can normalise platelet counts within a week.[67] Malaria-induced thrombocytopaenia and anaemia could increase the risk of post-partum haemorrhage.[68]

Figure 3.1 Annual cumulative proportion of women infected with malaria during pregnancy at the Thai-Burmese border (N=48410)

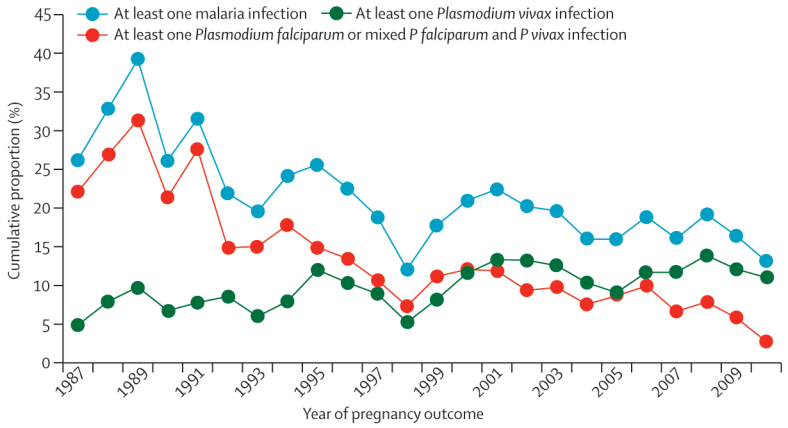
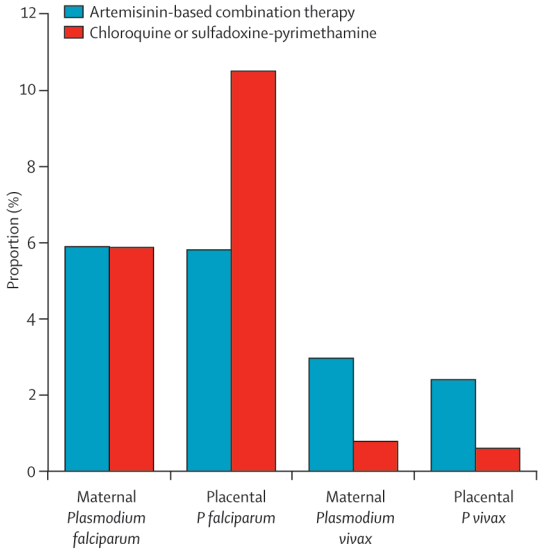


Figure 3.2 Proportion of maternal peripheral and placental positive smears at delivery in areas with artemisinin-based combination therapies, chloroquine, or sulfadoxine-pyrimethamine use.



Data are from **Table 3.2**.

Comorbidities

A relation between placental malaria and pre-eclampsia is suggested by studies from highly endemic areas,[69] but only one report is from the Asia-Pacific region after an epidemic in 1935.[70] In sub-Saharan Africa, the burden of malaria has been exacerbated

by HIV.[71] No reports of HIV and malaria in pregnancy come from the Asia-Pacific region. Investigators have recorded an association between low rates of *P.falciparum* or *P.vivax* infection in pregnant women and co-infection with Ascaris lumbricoides.[72] Hookworm infestation, however, is associated with an increased risk of *P.falciparum* infection and low birth weight, and high hookworm counts are linked with anaemia.[72-74] An integrated approach to malaria and helminth control has been promoted for pregnant women[75] which should be tailored according to the local prevalence and intensity of geohelminths and malaria, and anaemia burden.[72] About 10% of women with malaria had rickettsial co-infection in a cohort study done at the Thai-Burmese border.[76]

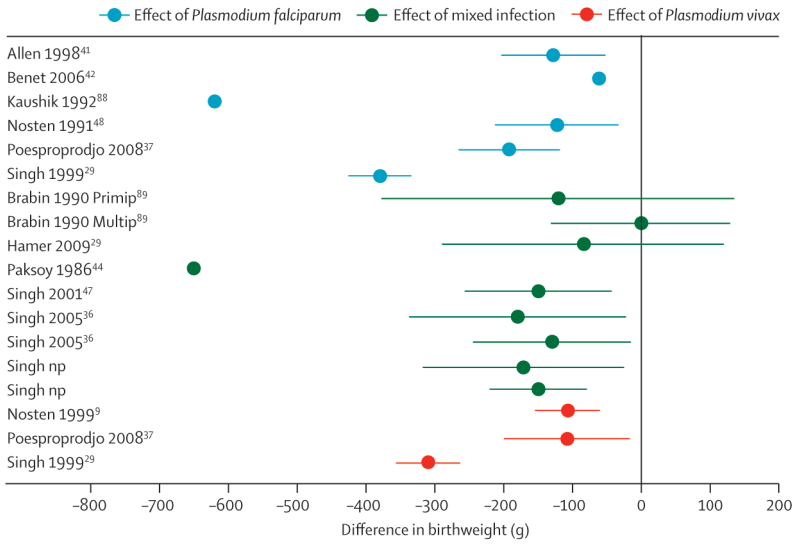
Severe malaria and mortality

Detailed malaria-attributable maternal mortality rates are rarely reported. However, in three districts in India, 22 (23%) of 95 maternal deaths were attributed to malaria between 2004 and 2006, which is a total mortality rate of 722 per 100 000 livebirths.[77] Malaria was the most common cause of maternal death during pregnancy (11 of 23 deaths; 48%) and was the cause of six (23%) of 26 post-partum maternal deaths. Before introduction of malaria-control programmes for pregnant women in western Thailand, five (1%) of 500 pregnant women died of malaria in 1 year.[48] Signs that are rare in childhood malaria—eg, acute renal failure, pulmonary oedema, and severe jaundice—are common manifestations of severe malaria in adults.[55, 78] Death during pregnancy or just after delivery occurs because of cerebral malaria, renal failure, hepatic impairment, severe anaemia, hypoglycaemia (worse with quinine treatment), uncontrollable post-partum haemorrhage, or acute respiratory distress syndrome.[30, 48, 70, 78-80]
Pregnant women have a three-times higher risk of severe malaria than do non-pregnant women[53, 55] In eight studies of severe malaria in pregnancy (n=227),[52, 78-84] the median maternal mortality was 39% (range 8-100). This wide range is related to the broad WHO definition of severe malaria: the lowest mortality was reported in pregnant women whose only sign of severity was hypoglycaemia.[79] By contrast, all women with renal failure died.[78] In an autopsy study of 277 women in India,[85] 10% of maternal mortality was attributable to infectious diseases, of which tuberculosis, malaria, and leptospirosis were most common. In reports from India,[35, 60, 86] women with severe vivax malaria had very poor pregnancy outcomes and maternal mortality was reported. An action-for-survival programme dedicated to care of pregnant women with malaria greatly reduced mortality in a regional hospital in Thailand, where malaria was the most common cause of maternal death during the 1980s.[87] Early detection and treatment at the Thai-Burmese border has eliminated maternal death and severe malaria in women who participate in weekly screening.[48]

Effect on fetuses and infants

The median reduction in birthweight in the reviewed studies was 150 g (range 1-650; **Figure 3.3**) for *P.falciparum* or mixed infections,[20, 29, 36, 37, 41, 42, 44, 47, 48, 88, 89] and 108 g (range 107-310) for *P.vivax* malaria.[9, 29, 37] Birthweight reduction occurs mainly in first pregnancies with *P.falciparum* malaria but also in subsequent pregnancies, and even with one episode of *P.vivax* or *P.falciparum* malaria.[9, 39, 48] Both symptomatic and asymptomatic malaria episodes increase the risk of low birthweight, although symptomatic infections in pregnancy might have a larger effect than does asymptomatic disease, particularly on premature delivery.[37, 48, 50] Malaria reduces birthweight independently of anaemia.[58] Macgregor[90] identified an exponential fall in the risk ratio for low birthweight in women during their first pregnancy after a reduction in malaria transmission in the Solomon Islands.[90] This review does not address how malaria reduces birthweight, particularly with *P.vivax*, which does not seem to cytoadhere in the placenta to the same extent as *P.falciparum*.

Figure 3.3 Reported mean difference in birthweight after malaria-infected and uninfected pregnancies.



Birth weight differences are shown as mean (95% CI when available). Primip primiparous women. Multip multiparous women. np not published

Low birthweight alone is not a reliable indicator of malaria's effect on fetal growth; other information is needed, such as parents' anthropometrics, gestational age, neonatal length and head circumference.[91] Gestational age estimation is notoriously difficult in resource-poor settings. In the studies shown in **Figure 3.3**, neither ultrasound dating

(early gestation ultrasound is the gold standard for gestational age estimation) nor fetal size charts to diagnose intrauterine growth restriction were available.[91]

Fetal distress (measured by cardiotocography or meconium staining of the amniotic fluid) is an important feature of symptomatic falciparum malaria and severe anaemia, both before and during labour.[62, 79] In areas where women attend antenatal clinics late in pregnancy or only for delivery, miscarriage rates are probably underestimated. Stillbirth and spontaneous abortion can result from infection with *P.falciparum* or *P.vivax*, and from severe anaemia.[27, 37, 58, 61, 62, 80] However, in an early report[9] from an intense antenatal malaria screening and prompt-treatment programme at the Thai-Burmese border, *P.vivax* infection was not associated with premature labour, miscarriage, or stillbirth. By contrast, of 25 unwell Indian pregnant women admitted to hospital because of *P.vivax* malaria, more than half of the pregnancies ended with these adverse outcomes.[60] This finding draws attention to the importance of early detection and treatment of any malaria in pregnancy to prevent the effects of symptomatic disease. Further studies into the effects of malaria infections in the first trimester are needed.

Congenital malaria occurs when malaria parasites cross the placenta either during pregnancy,[92] or at delivery.[93] Congenital *P.falciparum* or *P.vivax* malaria is a potentially serious complication of symptomatic and asymptomatic infection at any time in pregnancy, but neonatal symptoms are not typical for malaria and usually become evident only 10-30 days after birth.[92, 93] **Table 3.4** shows the prevalence of congenital malaria.

There are no WHO criteria for diagnosis or recommendations for drug dosing in the treatment of neonates with severe malaria. Severe disease in neonatal *P.falciparum* and *P.vivax* infections is common and can be characterized by severe anaemia and respiratory distress. All sick neonates in malaria endemic regions should have a malaria smear.[92, 96, 102]

Additionally, malaria in pregnancy directly affects infant survival. In Thailand, maternal infection (including malaria) in the week before delivery is the only risk factor for infant death in the first three months of life, after adjustment for birthweight and gestational age.[50] Anaemia was an independent risk for infant death in early studies in Thailand,[49, 106] but a 2001 investigation did not confirm this finding.[50] This difference might be explained by the multifactorial causes of anaemia in Thailand and co-deficiency of vitamin B1 in infants, a major cause of infant mortality.[107]

Although treatment[38] and prevention[51] for malaria had no adverse effects on infant growth and neurodevelopment at age 1 year in a study in Thailand, no studies of the interaction with nutritional status, child development, and the placental and fetal epigenome exist. However, high rates of childhood stunting were reported in Papua New Guinea in individuals exposed to malaria in utero during an epidemic.[108]

Table 3.4 Congenital Malaria by country in the Asia-Pacific region

	Study type	Number of neonates tested	Number with malaria at birth		Number with malaria at 1-7 days		Number with malaria at 8 days – 1 month		Number with malaria at 1 - 3 months		Maternal Malaria	Parasite discordance
			Pf	Pv	Pf	Pv	Pf	Pv	Pf	Pv		
South East Asian region												
Sri Lanka (1982)[94]	Case Report	1	-	-	-	-	-	-	-	1*	History of MIP	n.a.
India (1995)[95]	Case Report	1	-	-	-	-	-	-	-	1*	h.o.f. in first trimester	n.a.
India (1998)[45]	Prospective	100	-	-	-	-	1*	-	-	-	Persistent parasitaemia	None
Thailand (2004)[39]	Prospective	175	2*	-	-	-	-	1*	-	-	Placental malaria positive	None
Thailand (2006)[93]	Review	27	5#	20#			-	2*	-	-	All mothers history of MIP	n.a.
India (2007)[96]	Case Report	1	-	-	-	-	-	-	-	1*	Peripheral Pv and h.o.f.	None
Thailand (2007)[97]	Review	15	2#	-	1*	-	3*	3*	1*	5 (1*)	h.o.f. except in 1 case	n.a.
India (2010)[98]	Case Report	1	-	-	-	-	-	1*	-	-	History of MIP	n.a.
India (2010)[99]	Case Report	1	-	-	-	-	-	1*	-	-	Peripheral Pf and Pv	None
India (2010)[100]	Case Report	1	-	-	-	-	-	1*	-	-	History of MIP	n.a.
India (2010)[101]	Case Report	1	-	-	-	-	-	-	1*	-	History of MIP	n.a.
Indonesia (2010)[92, 102]	Cross Sectional	4884	29(1*)	6^#	-	-	-	-	-	-	29 pregnant women had parasitaemia	5 cases
Western Pacific region												
Malaysia (1980)[103]	Case Report	1	-	-	-	-	-	-	1*	-	Placental and peripheral Pf	None
Solomon Island (1983)[43]	Cross Sectional	180	1#	-	-	-	-	-	-	-	Peripheral Pf	None
PNG (1986)[104]	Case Report	1	-	-	-	-	-	1*	-	-	Peripheral Pv	None
PNG (1988)	Case Control	52	4#	-	-	-	-	-	-	-	Peripheral Pf	None

*symptomatic # asymptomatic ^Additionally, one Plasmodium ovale and 2 mixed cases of *P.falciparum* and *P.vivax*)
h.o.f. history of fever, MIP malaria in pregnancy, n.a. not available, Pf Plasmodium falciparum, PNG Papua New Guinea, Pv Plasmodium vivax

Treatment

The Asia-Pacific region has been affected by the emergence of antimalarial-drug resistance.[10, 11, 37, 109] Most countries have updated their national guidelines to match WHO recommendations of artemisinin-based combination therapies in the second and third trimester in pregnancy. Proactive operational guidelines include use of artemisinin-based combination therapies in pregnancy in parts of Melanesia for both *P.falciparum* and *P.vivax*.[37] Trials of antimalarials in pregnancy for infection with *P.falciparum* and *P.vivax* have been done (**Table 3.5**), but most occurred in one site only. No researchers have randomly assigned pregnant women in the first trimester.

No large recent studies of chloroquine efficacy in treatment of vivax malaria in pregnancy are available,[113, 121] but Indonesia, the Solomon Islands, Papua New Guinea, and Vanuatu have changed their national treatment to artemisinin-based combination therapies because of the high prevalence of chloroquine-resistant parasites in the general population.[122] *P.vivax* has liver stages (hypnozoites) that cause recurrent blood stage infections (relapses). Primaquine — the only drug effective against liver stages— is contraindicated in pregnant and lactating women because fetal red blood cells are susceptible to haemolysis.[123] Chloroquine remains the drug of choice for uncomplicated *P.knowlesi*, *P.malariae*, and *P.ovale*.[124]

In a small pharmacokinetic study in Papua New Guinea,[120, 121] 13 women were treated with a combination of sulphadoxine-pyrimethamine and chloroquine, which had a low cure rate of 62% (**Table 3.5**). Chloroquine had no effect against *P.falciparum* in pregnant women in India during a malaria epidemic in 1997-1998.[47] The mean cure rate of quinine monotherapy inseven studies (845 patients; **Table 3.5**) was 73·2% (SD 14·1), probably because of resistance and, in one study,[48] poor adherence to treatment. When clindamycin was added to quinine and treatment was supervised,[116] cure rate improved to 100%[112, 115]. However, the gametocyte carriage rate after treatment in women who did not have these sexual stages on admission was 13 times higher than it was in women given a 7 day course of artesunate monotherapy.[109] Mefloquine monotherapy had a cure rate of 72% in one investigation,[111] but in combination with artesunate it can reach 100%.[112, 115] In a study at the Thai-Burmese border,[38] 125 pregnant women treated with artemether-lumefantrine for three days had a cure rate of only 87%, which was inferior to artesunate monotherapy for seven days. Other artemisinin-based combination therapies (dihydroartemisinin piperaquine; artesunate-clindamycin; and artesunate, atovaquone, and proguanil) have cure rates of more than 90% (**Table 3.5**). Treatment of severe malaria in pregnant women has not been investigated, although these patients were not excluded from the SEAQUAMAT trial.[84] However, intravenous artesunate is the WHO recommended treatment of choice, irrespective of trimester.[84, 125]

In practice, health workers providing antimalarials either might not prescribe correct doses[126] or might be afraid to give drugs to pregnant women because of concerns about potential teratogenic effects or abortion.[126, 127] Recommendations about regimens to use in treatment of malaria during pregnancy are notably absent in many settings.[128]

Table 3.5 Efficacy studies of antimalarials in pregnancy in the Asia-Pacific region

	Year	Study type	Length of follow-up (days)	Drug	Sample size	Cure rate % *	Conclusion or concerns
Plasmodium falciparum							
Burma[110]	1985-86	RCT	7	Amodiaquine	19	100%	Very short follow up
India[47]	1997-98	Observational	35	Chloroquine	21	5% (0-13.9)	Efficacy <90%
TBB[48]	1997-98	Observational	28	Quinine	405	77.5%	Poor compliance to 7day treatment
TBB[111]	1992-96	Observational	42	Quinine	93	77%	Efficacy <90%
TBB[112]	1995-97	RCT	63	Quinine	43	67.0% (43.3-90.8)	Efficacy <90%, AE↑
TBB[113]	1995-2000	Observational	42	Quinine	209	71.3%	Efficacy <90%, Q safe in 1st trimester, AE↑
TBB[113]	2002	Observational	42	Quinine	25	56%	Treatment of repeat Pf, Efficacy <90%
TBB[114]	2001-03	RCT	63	Quinine	41	63.4% (46.9 - 77.4)	Unsatisfactory treatment response, AE↑
Thailand[115]	1995-98	RCT	28	Quinine	29	100%	Slower PCT and AE↑
TBB[116]	1997-2000	RCT	42	Quinine and clindamycin	65	100% (99.3-100)	Gametocytes ↑, AE ↑, cost ↑
TBB[117]	1992-96	Observational	42	Artesunate for 7days	53	90.6% (81.6-99.6)	Efficacy <90%
TBB[111]	1991-96	Observational	42	Mefloquine	194	72%	Efficacy <90%
TBB[112]	1995-97	RCT	63	Mefloquine and artesunate	65	98.2% (94.7-100)	Mefloquine and artesunate effective, gametocytes ↓
Thailand[115]	1995-98	RCT	28	Mefloquine and artesunate	28	100%	AE of Mefloquine and artesunate ↓, PCT↓, FCT↓
TBB[118]	2000-01	Observational	42	AAP	24	100%	Cost ↑↑
TBB[114]	2001-03	RCT	63	AAP	39	94.9% (81.4 - 99.1)	Well-tolerated, effective, practical, but cost ↑↑
TBB[119]	2006-07	Observational	63	DHAPPQ	50	92.2% (76.9–97.4)	Well tolerated, effective, no evidence of toxicity
TBB[38]	2004-06	RCT	42	Artesunate for 7days	128	94.9% (91.0-98.8)	Well tolerated, effective, no evidence of toxicity
TBB[38]	2004-06	RCT	42	AL	125	86.8% (80.5-93.1)	Efficacy low; unsatisfactory for deployment in pregnant women
TBB[38]	2004-06	Observational	42	Artesunate and clindamycin	88	95.4% (90.3-100)	Highly efficacious in MDR-Pf (unpublished)
PNG[120]	2006	Pharmacokinetic	28	SP and Chloroquine	13	62%	Small sample size
Plasmodium vivax							
TBB[113]	1995-2000	Observational	28	Chloroquine	111	95.5%	CQ safe in first trimester
PNG[121]	2006	Pharmacokinetic	28	SP and Chloroquine	2	100%	Small sample size

↑ increased, ↓ decreased, AAP artesunate, atovaquone, and proguanil, AE adverse effects, AL artemeter-lumefantrine, DHAPPQ dihydroartemisinin piperaquine, FCT fever clearance time; MDR multidrug resistant, PCT parasite clearance time; Pf Plasmodium falciparum, PNG Papua New Guinea, Pv Plasmodium vivax, RCT randomized controlled trial, SP sulfadoxine-pyrimethamine, TBB Thai-Burmese border. * (95%CI) if available,

A major disconnect has been identified between routine antenatal practices and known strategies to prevent and treat malaria in pregnancy.[128]
13 (62%) of 21 studies into antimalarial pharmacokinetics in pregnancy worldwide have been done in the Asia-Pacific region. Many drugs have been studied: quinine;[129] mefloquine;[130, 131] chloroquine;[132, 133] sulphadoxine-pyrimethamine;[134] artemether-lumefantrine;[135, 136] proguanil;[137, 138] artesunate;[139] –atovaquone and proguanil;[140] and azithromycin.[141] Several researchers report reduced drug concentrations in pregnant women's blood after standard dosing of antimalarials drugs and recommend the need for dose alterations, especially for lumefantrine, chloroquine, and proguanil.

Prevention

Unlike sub-Saharan Africa, prevention of malaria in pregnancy is not emphasised in national guidelines for malaria in pregnancy in the Asia-Pacific region. Long-lasting insecticide-treated bednets are distributed in all countries,[14] but reports in the past 3 years have shown low availability or use by pregnant women.[26, 34, 128] Case management is available everywhere, but in reality malaria smears are obtained from pregnant women only when fever or other malarial symptoms are present and these are often not checked by health care workers.[128] Papua New Guinea is the only country with a policy for intermittent preventive treatment in pregnancy. However, the policy has yet to be implemented in antenatal clinics. More than 10 years have passed since the reduction in incidence of *P.falciparum* in pregnant women at the Thai-Burmese border because of early detection and treatment of the general population with artemisinin-based combination therapies (e.g. mefloquine artesunate).[22] These factors have had a far greater effect than have chemoprophylaxis,[49] bednets,[106] or skin repellents[142] for pregnant women.
Vector-control measures aiming for total-population coverage are beneficial for pregnant women. Insecticide-treated bednets and indoor residual spraying reduce malaria transmission.[143] In the only vector-control investigation in pregnancy in the Asia-Pacific region region - a bednet study - fewer women in the experimental group had peripheral parasitaemia than in the group with untreated nets, but the difference was not significant.[106, 144] The parasite density and frequency of anaemia was lower in the pregnant women with an treated nets than in those with untreated nets. No effect on birthweight or premature labour was recorded.[106]
In India's Jharkhand state, most women report having untreated bednets in their homes, but only 3·3% had insecticide-treated bednets.[20] Several studies show that free treated nets for pregnant women have not been distributed despite government policy, that women in their first pregnancy are less likely to own treated bednets than are those in subsequent pregnancies, and that priority to sleep under the treated net is given to young children.[34, 127, 145, 146] Most mosquito vectors in the Asia-Pacific region have exophilic and exophagic behaviour. Moreover, these mosquitoes are most active in the early evening, when most pregnant women are not under nets, or at dawn, when they may already be

awake and active, potentially restricting the protective effect of treated nets.[147] Mosquito repellents with N,N-diethyl-meta-toluamide (DEET) are safe in pregnancy, [148, 149] can reduce exposure to insect bites,[148] and resulted in a non-significant reduction in the incidence of *P.falciparum* infection in pregnant women in one possibly underpowered study.[142]

Mefloquine chemoprophylaxis gave 86% protection against *P.falciparum* and complete protection against *P.vivax* infection in a double-blind, placebo-controlled study[49] at the Thai-Burmese border when mefloquine was still fully effective. A retrospective analysis of chloroquine chemoprophylaxis in pregnant women from Papua New Guinea showed that the drug did not reduce placental or maternal peripheral blood infection at delivery where resistance was high.[41, 150] In a double-blind, randomized controlled trial of chloroquine chemoprophylaxis at the Thai-Burmese border,[51] prophylaxis prevented *P.vivax* episodes, but had no effect on *P.falciparum* in pregnancy. In Papua New Guinea, prevalence and density of placental and maternal peripheral blood parasitaemia did not seem to be affected by chloroquine chemoprophylaxis during pregnancy, but haemoglobin concentration was higher after delivery in women who took chloroquine than in those who did not.[41] Weekly chloroquine prophylaxis resulted in poor parasite clearance despite good compliance in a longitudinal investigation in Papua New Guinea.[33] Few pregnant women use chloroquine chemoprophylaxis in India, Papua New Guinea, and the Solomon Islands.[20, 34, 151]

The only data for intermittent preventive treatment in pregnancy from the Asia-Pacific region are the pharmacokinetics of chloroquine and sulphadoxine-pyrimethamine (three chloroquine tablets every day for 3 days, and one sulphadoxine-pyrimethamine dose), which prevented all vivax episodes, but five of 13 women had another *P.falciparum* infection within 28 days of treatment.[120, 121]

The rationale of intermittent screening and treatment is to screen pregnant women frequently to detect and treat malaria parasitaemia at an early stage and use an effective drug to prevent the repercussions of symptomatic malaria in pregnancy. Despite elimination of severe malaria and mortality among women who attended weekly,[48] this strategy has not completely prevented the adverse effects of low birthweight and anaemia.

Discussion

Malaria in pregnancy in the Asia-Pacific region contrasts with that in Africa because many women are at risk in highly heterogeneous transmission settings. Irrespective of the number of children they have had, most pregnant women have little or no background immunity to malaria, so each infection is potentially fatal to mothers or fetuses. Prevention and treatment are complicated by different vectors, multidrug resistant parasites (*P.falciparum* and *P.vivax*) and suboptimal dosing of antimalarials, such as artemether-lumefantrine. *P.vivax* can relapse throughout pregnancy when primaquine is contra-indicated. The reduced birthweight of first-born babies is similar to that

recorded in Africa, but the effects of symptomatic malaria in pregnancy (maternal death, miscarriage, stillbirth, or premature labour) seem to be more prominent in the Asia-Pacific region. Although malaria control and the introduction of artemisinin-based combination therapies in the general population has resulted in a substantial decline in the prevalence of malaria, further efforts are needed from the national malaria control programmes and donors, because every infection in pregnancy is detrimental to mother and baby, with repercussions in infancy and childhood.

Methods of detection of parasitaemia (peripheral or placental malaria smear, rapid diagnostic tests, or histopathology) underestimate the burden of malaria in pregnancy. Prevalence surveys could show that few women are infected at one timepoint, but cumulative proportions of malaria infections are more useful indicators because of the duration of pregnancy. Of the 126 reports included in this review (**Web extra table 3.1**), 107 (85%) came from only three countries (Thailand, India, Papua New Guinea). In many regions of high malaria transmission, no study of malaria in pregnancy has been done or reported in English. Likewise, pharmacokinetic drug findings need confirmation in distinct populations.

Improved estimates of the morbidity and mortality of malaria in pregnancy calculated from longitudinal cohort data in the population at risk are urgently needed, as are drug-dose-optimisation studies in pregnant women, and implementation of strategies for prevention of malaria in pregnancy (Panel). When malaria in pregnancy cannot be readily prevented, accurate diagnosis and prompt treatment with efficient drugs for any detected parasitaemia are essential to avert dangerous symptomatic disease and to reduce effects on fetuses. Indeed, in a recent report of the largest study[152] into the effects of malaria and its treatment in the first trimester, both vivax and falciparum malaria contributed significantly to miscarriage. Risk of miscarriage was three-times higher in women with asymptomatic malaria, and four-times higher in those with symptomatic disease, compared with women who did not have malaria. No serious side-effects of antimalarial treatments were noted.[152]

However, even a safe and effective three day artemisinin-based combination treatment for women in the second and third trimester is still absent in most countries in the Asia-Pacific region. Evidence suggests that dihydroartemisinin-piperaquine is one of the best contenders. The best possible frequency, feasibility, and effectiveness of intermittent screening in the antenatal clinic in different settings should be assessed. For example, malaria screening at every antenatal visit could be provided to pregnant women at increased risk for malaria. More work is needed to address the difficult question of prevention and treatment of *P.vivax* during pregnancy.

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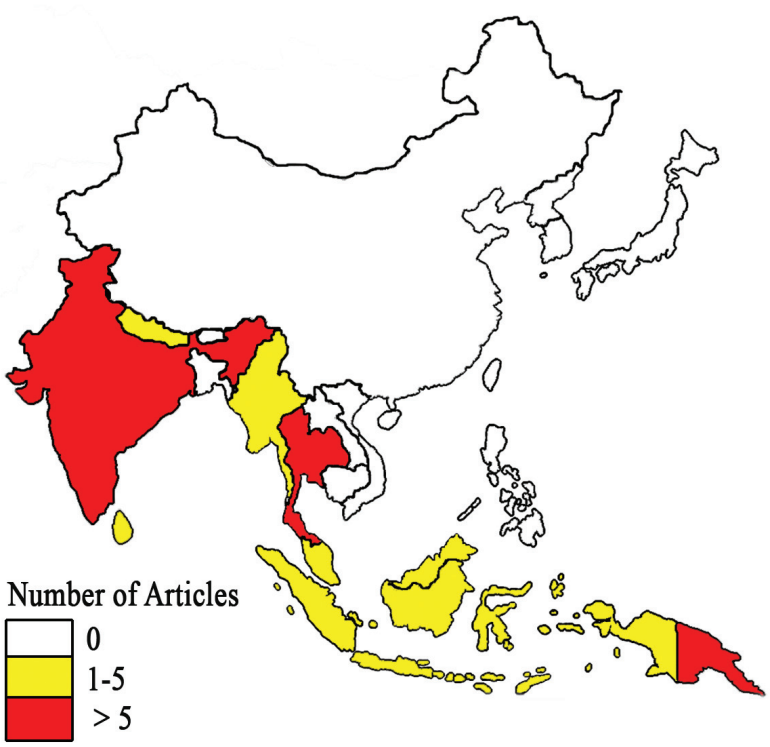
Panel

Research priorities in the Asia-Pacific region

- Regional clinical burden of malaria in pregnancy with longitudinal data collection
- Feasibility of early detection (frequent intermittent screening) and treatment with effective antimalarials
- Effect of submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections on maternal and fetal health
- Pharmacokinetic and drug-dose-optimisation studies in different populations, especially for artemether, lumefantrine, dihydroartemisinin, and piperaquine
- Efficacy of and compliance to quinine (and clindamycin) as first-trimester treatment for *P.falciparum* infection, and efficacy of chloroquine for *P.vivax* infections
- Effect of malaria in pregnancy and antimalarial exposure on growth, development and survival of the fetus, newborns, infants, and young children in longitudinal cohorts
- Interactions between malaria, red-cell inherited disorders, co-infections (e.g. helminths, HIV, or rickettsia) and nutrient supplementation
- Strategies to prevent malaria (*P.falciparum* and *P.vivax*) in at-risk groups such as migrants, or those exposed because of occupation or residential location
- Effect of malaria in pregnancy on post-partum morbidity and mortality

Web extra material: Malaria in pregnancy in the Asia-Pacific region

Web extra figure 3.1 Map of the Asia Pacific Region and the number of published articles included in this review.

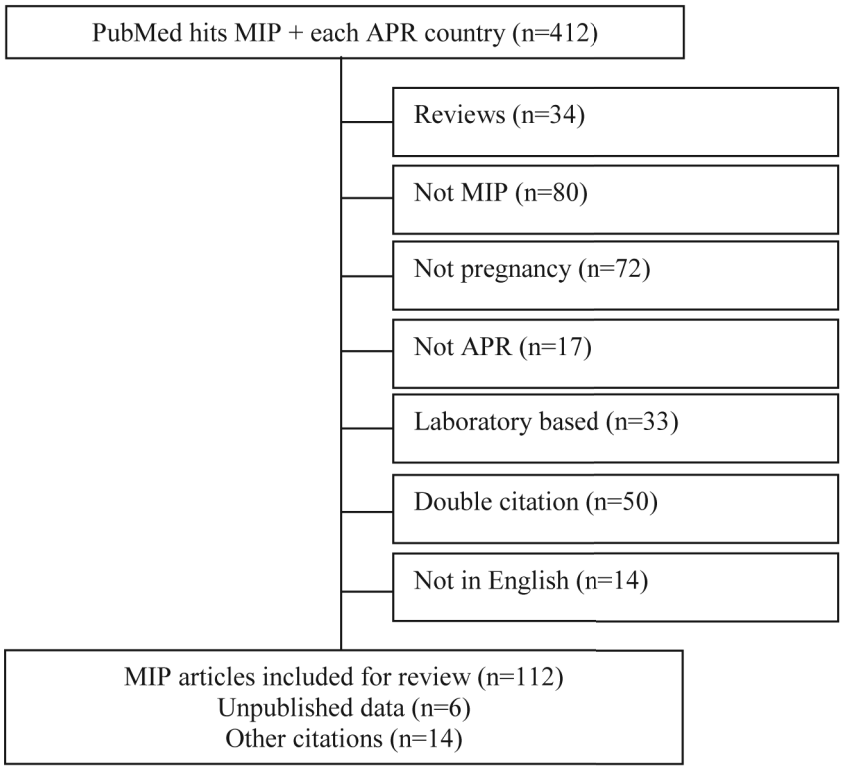


Solomon Islands and Vanuatu are not on this map due to their more easterly location but are included in the review; 4 and 1 articles respectively. (Figure created by T. Syrota)

Results

The PubMed search resulted in 412 hits published between 1965 and 2011 of which 112 articles were included for analysis (**Web extra figure 3.2**). Fourteen additional articles were found by the tracking of citations and six were not yet published at the time of review (**Web extra table 3.1**). Three of the 20 countries with malaria transmission provided 86% (96/112) of the published original studies or case reports on epidemiology, treatment or prevention of MIP: Thailand (n=46), India (n=30), and Papua New Guinea (PNG, n=20). The remaining articles were from Burma/Myanmar (n=3), Indonesia (n=2), Lao PDR (n=1), Malaysia (n=2), Nepal (n=1), Solomon Islands (n=4), Sri Lanka (n=2), and Vanuatu (n=1) (**Web extra figure 3.1** and **Web extra table 3.2**). Published review articles were used to confirm the findings of this study.

Web extra figure 3.2 Selection of articles



Abbreviations: MIP Malaria in pregnancy, APR Asia Pacific region

Web extra table 3.1 Summary of included articles

Author	Country	Year	Site	Sample size	Study design	Main finding related to MIP
South East Asia Region						
Mullany (2010)[26]	Burma	2006-08	East	5331	Health survey	Intervention: ↑screening and ↑ITN use
Kyi (1988)[153]	Burma	2002-03	Rangoon	7446	Observational	1 MD due to MIP
Naing (1988)[110]	Burma	1985-86	Shan State	42	RCT AMQ, Q	Q, AMQ clears parasites by day 7
Sholapurkar (1988)[53]	India	1984-85	Chandigarh	78	Observational	PW more severe illness than non-PW
Sholapurkar (1988)[154]	India	1984-85	Chandigarh	78	Observational	PW higher Pf Pv density than non-PW
Arya (1989)[83]	India	1988	Jhansi	3 severe	Case report	Cases of severe malaria in PW
Prasad (1990)[155]	India	1984-85	Chandigarh	78	Observational	Less antibody production PW
Kaushik (1992)[88]	India	1983-88	Jhansi	256 placentas	Observational	LBW associated with placenta malaria
Nair (1993)[27]	India	1987-88	Gujarat	322	Observational	PW more malaria than general population. Increased PTL, SB and AB in MIP.
Singh (1995)[28]	India	1991-93	Jabalpur	831	Observational	More Malaria in PW than non-PW (both h.o.f.) Pf more in primi, 2nd trim.
Singh (1996)[52]	India	1991-93	Jabalpur	22 severe	Observational	Severe PW higher parasitaemia
Singh (1998)[45]	India	1995-96	Mandla	456	Observational	CQ high failure: persistent parasites – asymptomatic MIP, all newborns LBW
Kochar (1998)[80]	India	1994-96	Bikaner	45	Observational (epidemic)	PW more severe malaria, mortality ↑ than non-PW. Increased PTL,SB,AB
Singh (1999)[29]	India	1992-95	Jabalpur	2127	Observational	PW higher Pf Pv density than non-PW. Both Pf and Pv ↓ BW
Das (2000)[46]	India	n.a.	Orissa	209	Observational	↑ Adverse birth outcomes MIP
Singh (2001)[47]	India	1997-98	Mandla	274	Observational (epidemic)	High prevalence of MIP, high reappearance of MIP infection
Singh (2002)[156]	India	1997	Jabalpur	155	Observational	Barriers to CQ prophylaxis
Kochar (2002)[82]	India	1992-98	Bikaner	56 severe	Observational	PW more MOF; adverse outcomes
Singh (2003)[35]	India	2002-03	Mandla	182 placentas	Observational	Placental Pv described
Munnur (2005)[157]	India	1992-2001	Mumbai	754	Observational	10% of PW Severe malaria

Singh (2005)[36]	India	2002-04	Mandla & Maihar	799	Observational	RDT useful detecting MIP
Aleyamma (2007)[158]	India	2006	Tamil Nadu	3 cases	Case report	2 of 3 PW died of Pf
Valecha (2007)[96]	India	2006	Delhi	1 case	Case report	Atypical presentation congenital Pv
Diamond-Smith (2009)[159]	India	2009	Madhya Pradesh	n.a.	Modeling	MIP is large health problem in India
Panchabhai (2009)[85]	India	1998-2006	Mumbai	277 autopsies	Audit of MD	10% of MD due to infectious disease
Thapa (2010)[101]	India	2008	West Bengal	1 case	Case report	Co infection TB and congenital Pf
Hamer (2009)[20]	India	2006-07	Jharkhand	3104	Observational	Low MIP point prevalence, but MIP responsible for ↑ hospitalizations
Recker (2009)[160]	India	1908-43	Punjab	n.a.	Modeling of historical data	MIP: high female mortality in epidemics, low transmission
Nayak (2009)[60]	India	2006-08	Bikaner	169	Observational	Pv major cause adverse effects PW
Sharma (2009)[3]	India	2009	New Dehli	n.a.	Opinion	MIP major hidden public health problem
Sabin (2010)[161]	India	2007	Jharkhand	73	FGD/ questionaire	PW misconception MIP preventive methods and treatment options
Wylie (2010)[128]	India	2006-08	Jharkhand Chhattisgarh	280	Cross sectional survey	MIP policy and practice diverge in ANC
Sankar (2010)[100]	India	2009	Kerala	1	Case report	Congenital Pv
Poespoprodjo (2008)[37]	Indonesia	2004-06	Timika	3046	Cross sectional	Multi drug resistant Pf Pv ↑AE
Poespoprodjo (2010)[92]	Indonesia	2009	Timika	1	Case report	Severe congenital malaria Pf
Dreyfuss (2000)[31]	Nepal	1994-97	Sarlahi	288	Observational	Pv associated with anemia
De Silva (1982)[94]	Sri Lanka	1980	Colombo	1	Case Report	Congenital Pv
Fernando (2009)[145]	Sri Lanka	2007-08	North	2467	Cross sectional	PW low utilization LLITN
Nosten (1990)[162]	Thailand	1989	TBB	20	MFQ dose find	MFQ effective without toxicity
Nosten (1991)[48]	Thailand	1986-89	TBB	1358	Observational	Pf ↓ BW, EDT prevented MD
Kietinun (1993)[87]	Thailand	1981-86	Chantaburi	n.a.	Report of intervention	Dedicated MIP care reduced MD
Nosten (1993)[163]	Thailand	1989-90	TBB	32	RCT spiramycin, plac	Spiramycin does not potentiate Q in Pf in PW
Dolan (1993)[106]	Thailand	1990-92	TBB	341	RCT ITN, NIBN, no net	ITN: ↓density ↓anaemia, (NS) reduction MIP. No effect BW, PTL
Nosten (1994)[49]	Thailand	1987-90	TBB	339	RCT MFQ, plac	MFQ safe and effective as prophylaxis

Na Bangchang (1994)[164]	Thailand	1993	Bangkok	9	PK MFQ	PW need larger dose of MFQ
Luxemburger (1997)[55]	Thailand	1990-92	TBB	4728	Observational	Severe malaria ↑3x in PW than non-PW
Lindsay (1998)[148]	Thailand	1996	TBB	n.a.	Observational	Thanaka good base for DEET repellent
McGready (1998)[117]	Thailand	1992-96	TBB	83	Observational	ACT ↓AE, ↑efficacy, ↓gametocyte compared with MFQ, Q
McGready (1998)[111]	Thailand	1991-96	TBB	372	Observational	Q and MFQ unsatisfactory efficacy
Nosten (1999)[9]	Thailand	1986-97	TBB	9956	Observational	Pv associated with anemia and LBW
Nosten (1999)[165]	Thailand	1991-94	TBB	3587	Retrospective comparison	MFQ treatment associated with risk of SB compared with other antimalarials
Nosten (2000)[22]	Thailand	1985-98	TBB	2195	Observational	Introduction ACTs: ↓MIP incidence
McGready (2000)[112]	Thailand	1995-97	TBB	108	RCT MFQ, Q	Efficacy MAS > Q
McGready (2001)[142]	Thailand	1995-96	TBB	897	RCT DEET, plac	DEET ↓Pf(NS), no effect anemia or BW
Luxemburger (2001)[50]	Thailand	1993-96	TBB	1495	Observational	Symptomatic MIP associated with PTL MIP increases neonatal mortality
McGready (2001)[149]	Thailand	1995-96	TBB	897	RCT DEET, plac	DEET appears safe 2nd, 3rd trimester
McGready (2001)[166]	Thailand	1992-2000	TBB	461	Observational	ACT effective, safe, well tolerated
Bounyasong (2001)[115]	Thailand	1995-98	Mae Hong Son	60	RCT MAs, Q	AE MAS3<Q
McGready (2001)[116]	Thailand	1997-2000	TBB	129	RCT QC, As	C ↑efficacy of Q, Gametocytes A7<QC7
McGready (2002)[113]	Thailand	1995-2000	TBB	300	Observational	CQ, Q appear safe in 1st trimester Q efficacy ↓ for Pf in 1st trimester
McGready (2003)[167]	Thailand	2000	TBB	87	PK A-P OCP, pregnancy	Pregnancy and OCP lower [(cyclo) (proguanil)]
McGready (2003)[168]	Thailand	2000-01	TBB	24	PK A-P	Lower [A-P] in PW
McGready (2004)[39]	Thailand	1995-2002	TBB	204	Observational Histopathology	Prompt treatment MIP limits placental pathology
Nacher (2003)[63]	Thailand	1993-99	TBB	2112	Observational	Fe/FA supplement ↑ Pv
McGready (2003)[118]	Thailand	1999-2001	TBB	27	Observational	AAP efficacious recurrent Pf, no ↑AE
Nacher (2005)[169]	Thailand	2002	TBB	1	Case report	Photo allergy in Q
Na-Bangchang (2005)[170]	Thailand	2004	Mae Sot	8	PK As-P	Lower [A-P] in PW (Zambia 18 PW)
McGready (2005)[171]	Thailand	2001-03	TBB	81	RCT AsA-P, Q	Efficacy AA-P > Q, gametocytes AA-P<Q

Stuetz (2006)[172]	Thailand	1998-2000	TBB	108	Observational	DDT present in blood of PW
McGready (2006)[173]	Thailand	2000-01	TBB	24	PK DHA	PW ↓ [DHA]
Carrara (2006)[19]	Thailand	1986-97	TBB	n.a.	Observational	EDT + treat ACT population: ↓ PW Pf
McGready (2006)[174]	Thailand	2004	TBB	13	PK AmL	PW ↓ [Am/DHA], ↓[L]
Villegas (2007)[51]	Thailand	1998-2000	TBB	1000	RCT CQ, placebo	CQ prevents Pv, no effect anemia BW GA
Coldren (2007)[124]	Thailand	2006	Bangkok	1	Case report	P.ovale in PW
Rijken (2008)[119]	Thailand	2006-07	TBB	45	Observational	DHAPPQ efficacious recurrent Pf, no ↑AE
Lee (2008)[175]	Thailand	1998	TBB	27	PK CQ	No change in [CQ] in PW
Sapbamrer (2008)[176]	Thailand	2003-04	Chiang Mai	80	Observational	DDT levels high in mother infant pairs
Tan (2008)[67]	Thailand	2004-06	TBB	974	Observational	PW risk thrombocytopenia, rarely severe
Newton (2008)[177]	Thailand	2000-01	TBB	44 shops	Observational	Ineffective OTC antimalarials for PW
Plewes (2008)[178]	Thailand	1997-2005	TBB	7792	Observational	Low HIV, Low Syphilis in PW
McGready (2008)[38]	Thailand	2004-06	TBB	252	RCT AmL, A7	Efficacy AmL too low for use in PW
Tarning (2009)[179]	Thailand	2004-06	TBB	103	PK L modeling	PW lower [L] in population PK, ↓[L] associated with recrudescent Pf (PCR)
Boel (2010)[72]	Thailand	1996 2007	TBB	829	Cross sectional	Ascaris less Pf, Pv; hookworm more Pf
McGready (2010)[76]	Thailand	2004-06	TBB	203	Observational	MIP ↑ febrile morbidity, 10% MIP co-infected with rickettsia
Western pacific Region						
Mayxay (2007)[126]	Laos	2000-01	Vientiane	60	Cross sectional	Health workers ↓ basic MIP knowledge
Thomas (1977)[180]	Malaysia	n.a.	Gombak	17 cord blood	Observational	No congenital malaria, 94% antibodies
Thomas (1980)[103]	Malaysia	1978	Gombak	1	Case Report	Congenital Pf
Sharp (1980)[108]	PNG	1978-79	Lagaip and Sau valley	n.a.	Cross sectional survey	Higher child stunting in those in utero at malaria epidemic
Schuurkamp (1986)[104]	PNG	1985	Tabubil	1	Case report	Congenital Pv
Mola (1987)[150]	PNG	1985	Raboul	150	Cross sectional	Failure of CQ prophylaxis for Pf in PW
Brabin (1988)[181]	PNG	1985-87	Madang	582	Observational	Splenomegaly in ↑multi, ↑CQ prophylaxis
Lehner (1988)[105]	PNG	1985	Madang	51 mother/ cord/ baby	Observational	Congenital malaria is common in PNG

Brabin (1990)[33]	PNG	1985-87	Madang	620	Observational	Failure of CQ prophylaxis for Pf in PW
Brabin (1990)[89]	PNG	1985-87	Madang	472	Observational	Primi, MIP, severe anemia lowest BW
Desowitz (1992)[40]	PNG	1986- 88	East Sepik	83	Observational	Relatively benign effect of MIP
Desowitz (1993)[182]	PNG	1986-88	East Sepik	46	Observational	Pf specific antibodies in mother and cord
Lalloo (1996)[78]	PNG	1990-92	Central	8 severe	Observational	Poor outcome of severe malaria in PW
Brabin (1997)[58]	PNG	1986-88	Goroka Madang	12181	Observational	MIP larger effect on LBW than anemia
Allen (1998)[41]	PNG	1994-96	Madang	987	Observational	MIP, smoking, malnutrition ↓ BW by IUGR and PTL
Mola (1999)[62]	PNG	1987-92	Port Moresby	27177	Observational	Severe anemia more stillbirths
Amoa (1998)[61]	PNG	1995-97	Port Moresby	315 SB	Case Control	MIP one of the risk factors for stillbirths
Piper (2001)[68]	PNG	1986-88	Goroka Madang	10702	Retrospective analysis	Higher risk of PPH in malarious areas
O'Donnel (2006)[66]	PNG	1994-96	Madang	913	Observational	☒ Thal: no effect on BW, PTL or MIP
Benet (2006)[42]	PNG	2002-03	Madang	402	Case control	Placenta sequestration SAO erythrocytes
O'Donnel (2007)[59]	PNG	1994-96	Madang	927	Observational	SAO no protection MIP
Karunajeewa (2009)[120]	PNG	2006	Madang	30	PK SP	PW lower [SDOX], [PYR]
Karunajeewa (2010)[121]	PNG	2006	Madang	30	PK CQ, DECQ	PW lower [CQ], [DECQ]
MacGregor (1974)[90]	Solomon islands	1969-72	Malaita	n.a.	Observational	↓LBW after reduction malaria transmission
Marshall (1983)[43]	Solomon islands	1981	Malaita	180	Observational	Placenta provides effective barrier for Pf
Dulhunty (2000)[127]	Solomon islands	1998-99	Malaita	n.a.	Interviews and FGD	Contradictory beliefs on threat of MIP and safety of CQ prophylaxis
Appleyard (2008)[34]	Solomon islands	2003	Marovo Lagoon	106	Observational	Prevalence of MIP; barriers to MIP control
Paksoy (1986)[44]	Vanuatu	1984-85	Malekula	248	Observational	10% of placenta's Pf or Pv
Other citations						
Wickramasuriya (1935)[70]	Sri Lanka	1930-34	Colombo	n.a.	Observational	Catastrophic consequences on pregnancy during malaria epidemic
Phillips (1986)[183]	Thailand	1985	Chantaburi	10	PK Q	PW ↓ [Q]
Wangboonskul (1993)[184]	Thailand	1991	TBB	10	PK P	PW ↓ [P]
Syracheun (2000)[32]	Laos	1998	Vientiane	68	Cross sectional	Pf ↓ haemoglobin in PW
Konar (2004)[30]	India	2001-02	9 centres	215	Observational	MIP varies significantly across regions
Looareesuwan (1985)[79]	Thailand	1982-83	East	12 severe	Observational	Hypoglycaemia in severe MIP

Barnett (2008)[77]	India	2004-06	Jharkland Keonjhar	13602	Observational Surveillance	Antepartum MIP causes MD
Hasan (2006)[81]	India	2003-05	Aligarh	32	Observational	MIP PW↑ MD vs non-PW, MIP ↑ SB
Kochar (2005)[86]	India	2003	Bikaner	3	Observational	Description severe Pv
Garner (1994)[185]	PNG	1984-87	Wosera	121	Observational	High prevalence LBW, <10% PTL
Dellicour (2010)[1]	n.a.	2007	Asia Pacific	n.a.	Risk estimation	77·4 million PW at risk in APR
Balatbat (1995)[95]	India	1995	Punjab	1	Case report	Congenital Pv in twin
Kashyap(2010)[98]	India	2009	Punjab	1	Case report	Congenital Pv
Mohan (2010)[99]	India	2009	Kanpur	1	Case report	Congenital Pv CQ resistant
Not yet published						
Faiz	Bangladesh	2004	Chittagong	388	Observational	n.a.
Singh	India	2007-08	Chhattisgarh	2696 ANC, 1028 delivery	Observational	n.a.
Syafruddin	Indonesia	2008-10	Jayapura Sumba	1551 1554	Observational	n.a.
Stanisic	PNG	2006-08	Madang	468	Observational	n.a.
Boel	Thailand	2007-10	TBB	824	Observational	n.a.
Rijken	Thailand	2009-11	TBB	100	Observational	n.a.

↓ decreased, ↑ increased, < less than, > more than, [ ] drug concentration;
A-P atovaquone-proguanil, AB abortion, ACT artemisinin combination therapy, AE adverse effects, Am Artemether, AMQ amodiaquine, As artesunate, BW birth weight, CQ chloroquine, DDT dichlorodiphenyltrichloroethane, DECQ monodesethyl chloroquine, DEET N, N-diethyl-m-toluamide, DHA dihydroartemisinin, FA folic acid, Fe ferrous sulphate, FGD focus group discussion, GA gestational age, h.o.f. history of fever, ITN Insecticide treated net, IUGR intra uterine growth restriction, LBW low birth weight, LLITN long lasting insecticide treated nets, MAS mefloquine artesunate, MD maternal death, MFQ mefloquine, MIP malaria in pregnancy, n.a. not available, MOF Multi Organ Failure, NIBN non impregnated bed nets, NNM neonatal mortality, NS not significant, OCP oral contraceptive pill, OTC Over the counter, Pf Plasmodium falciparum, PK pharmacokinetic, plac placebo, PNG Papua New Guinea, Po Plasmodium ovale, PPH post partum hemorrhage, PPQ piperaquine, PTL premature labour, Pv Plasmodium vivax, PW pregnant women, PYR pyrimethamine, Q quinine, RCT randomized controlled trial, RDT rapid diagnostic test, SB stillbirth, SDOX sulfadoxine, SP sulfadoxine-pyrimethamine, SAO South east asian ovalocytosis, TB Tuberculosis

Web extra table 3.2 Proportion of women with malaria detected by malaria smear in antenatal clinics by country in the Asia Pacific Region

	Year of study	Site	Timing of screening	Chemo-prophylaxis (type and % taken)	Screening method	Total women	Total women with malaria
						(N)	% (N)
Bangladesh (Faiz, A, personal communication)	2004	South east	Any ANC visit	Chloroquine (2·4%)	MS	388	3·9% (15)
India[20]	2006-07	Jharkhand	Any ANC visit	Chloroquine 0·6%	MS	2386	1·3% (32)
India (unpublished)	2007-08	Chhattisgarh	Booking at ANC	No	MS	2696	1·2% (33)
Indonesia (unpublished)	2008-10	Jayapura	Any ANC visit	No	MS	1551	15·3% (238)
Indonesia (unpublished)	2008-10	Sumba	Any ANC visit	No	MS	1554	13·3% (207)
Nepal[31]	1994-97	Sarlahi	Enrolment	No	MS	288	19·8% (57)
PNG[33]	1985-87	Madang	First ANC	Chloroquine 23%	MS	620	29·0% (180)
PNG (unpublished)	2006-08	Madang	First ANC	No	MS	468	40·8% (191)
Solomon Islands[34]	2003	Marovo Lagoon	Any ANC visit	Very low coverage	MS	106	17·9% (19)

ANC antenatal clinic, MS malaria smear, N number, PNG Papua New Guinea

Web extra table 3.3 Proportion of women with malaria detected by malaria smear, with a history of fever in antenatal clinics, by country in the Asia Pacific Region

	Year of study	Site	Timing of screening	Chemo-prophylaxis (type and % taken)	Screening method	Total women	Total women with malaria
						(N)	% (N)
India[27]	1987-88	Gujarat Surat	Women with HOF	No	MS	322	57·8% (186)
India[28]	1991-93	Jabalpur	Women with HOF	No	MS	831	17·4% (145)
India[29]	1991-93	Jabalpur	Women with HOF	No	MS	2127	17·2% (365)
Laos[32]	1998	Vientiane	Women with HOF	No	MS	68	23·5% (16)

HOF history of fever, MS malaria smear, N number

Web extra table 3.4 Proportion of women with peripheral blood smear malaria infection at delivery by country in the Asia Pacific region

	Year of study	Chemoprophylaxis (type and % taken)	Site	Screening method	Total women	Total women with malaria
					N	% (N)
India[36]	2002-04	No	Mandla	MS	209	5·3% (11)
India[36]	2002-04	No	Maihar	MS	590	6·9% (41)
India[20]	2006-07	Chloroquine (0·3%)	Jharkhand	MS / RDT	717	1·7% (12)
India (unpublished)	2007-08	No	Chhattisgarh	MS	1028	1·5% (16)
Indonesia[37]	2004-06	No	Timika	MS	2487	16·0% (397)
Indonesia (unpublished)	2008-10	n.a.	Jayapura	MS	830	10·8% (90)
Indonesia (unpublished)	2008-10	n.a.	Sumba	MS	981	11·4% (112)
PNG[40]	1986- 88	No Chloroquine past 2 weeks	East Sepik	MS	83	8·4% (7)
PNG[41]	1994-96	Chloroquine (92·2%)	Madang	MS	987	18·5% (183)
PNG[42]	2002-03	Chloroquine high compliance low efficacy	Madang	MS	402	15·7% (63)
PNG (unpublished)	2006-08	SP + Chloroquine, Chloroquine weekly	Madang	MS	331	7·6% (25)
Solomon Islands[43]	1981	Chloroquine (9%)	Malaita	MS	180	7·8% (14)

MS malaria smear, N number, PNG Papua New Guinea, RDT rapid diagnostic test, SP sulfadoxine-pyrimethamine

Web extra table 3.5 Proportion of women with placental blood smear malaria infection by country in the Asia Pacific region

Country (year)[ref]	Year of study	Chemoprophylaxis (type and % taken)	Site	Screening method		Total women	Total women with malaria
						N	% (N)
India (2003)[35]	2002-03	No	Mandla	MS / RDT	P	182	29·1% (53)
India (2005)[36]	2002-04	No	Mandla	MS	P	209	14·4% (30)
India (2005)[36]	2002-04	No	Maihar	MS	P	590	10·8% (64)
India (2009)[20]	2006-07	CQ 0·3%	Jharkhand	MS	P	712	1·4% (10)
India (unpublished)	2007-08	No	Chhattisgarh	MS	P	1027	1·7% (17)
Indonesia (unpublished)	2008-10	n.a.	Jayapura	MS	P	818	9·1% (75)
Indonesia (unpublished)	2008-10	n.a.	Sumba	MS	P	974	11·2% (109)
PNG (1992)[40]	1986- 88	No CQ last 2 weeks	East Sepik	MS	P	83	19·3% (16)
PNG (1998)[41]	1994-96	CQ 92·2%	Madang	MS	P	860	24·0% (206)
PNG (2006)[42]	2002-03	CQ high compliance low efficacy	Madang	MS	P	402	16·4% (66)
Sol Is (1983)[43]	1981	CQ 9%	Malaita	MS	P	180	5·6% (10)
Vanuatu (1986)[44]	1984-85	n.a.	Malekula	MS	P	184	10·9% (20)

CQ chloroquine, MS malaria smear, N number, n.a. not available, n.p. not published, P placenta, PNG Papua New Guinea, RDT rapid diagnostic test, ref reference, Sol Is Solomon Islands.

Web extra table 3.6 Summary of studies reporting anaemia related to malaria in pregnancy by country in the Asia Pacific region

Country	Year study	Site	Definition anaemia	Species	Anaemia during pregnancy*	Anaemia at delivery*	Remark
Thailand[48]	1986-89	TBB	Clinical diagnosis	Pf	OR 1·38 (1·05-1·81)	n.a.	Significantly more common in grandes MG
Thailand[9]	1991-94	TBB	Clinical diagnosis or Hct <30%	Pv	AOR 1·91 (1·42-2·56)	n.a.	Gravida 2-3: AOR 1·4 (1·1-1·78)
Thailand[50]	1993-96	TBB	Hct <30%	Pf & Pv	AOR 2·9 (2·1-3·9), PAF 27 (16-30)	AOR 1·6 (1·3-2·1) PAF 17 (9-23)	MG AOR 2·1 (1·6-2·7)
PNG[41]	1994-96	PNG	Different Hb methods	Pf & Pv	n.a.	Hb 101 (91-110) vs 107 (94-118) g/L (non mal).	No difference Hb PG or MG
PNG[89]	1985-87	Madang	No anaemia definition, raw Hb data	Pf & Pv	At booking: PG Hb 8·1±1·4 vs 8·8±1·9 (non mal). In MG Hb 8·7±1·2 vs 8·8±1·5 (non mal).	PG Hb 9·5±1·8 vs 9·6±1·8 (non mal) MG 9·1±1·8 vs 9·4±1·7 (non mal)	No difference Hb PG or MG
India[20]	2006-07	Jharkhand	Hb <110 g/L	Pf & Pv	OR 1·11 (0·54-2·45)	OR 4·2 (1·2 - 22·4)	Severe anaemia associated with parasitaemia, P=0·023
India (unpublished)	2007-08	Chhattisgarh	Hb<11g%	Pf & Pv	OR 2·92 (1·12-9·7)	OR 2·61 (0·91-10·3)	MG OR 1·23 (1·04-1·45)
India (unpublished)	2007-07	Jabalpur	Hb<11 g%	Pf & Pv	OR 1·66 (1·0-2·86)	n.a.	n.a.
India[29]	1992-95	Jabalpur	Hb <10 g/dL	Pf & Pv	Hb mal 9·05±1·39 (Pv), 6·42 ±1·98 (Pf) Non mal 10·03±1·11 g/dL	n.a.	PG with Pf more anemic than second (NS) and MG, p <0·005. In Pv anaemia ↑ with ↑gravidity (NS)
India[47]	1997-98	Mandla	Hb<10·0g/dL	Pf & Pv	all women were anaemic, whether or not they had malaria infection	PG: Hb 9·8 ± 1·21, Secund Hb 10·0 ± 1·26, MG 10·0±1·24, NS	
India[28]	1991-93	Mandla	Hb<9 g%	Pf & Pv	Mal 66% (95/145), (Non mal) 32% (51/157), p<0.01	n.a.	n.a.
India[27]	1987-88	Surat, Gujarat	Raw Hb data in g%	Pf & Pv	Hb 7·9 (malaria) vs 8·3 (non mal) p<0·05	n.a.	Hb Pf 7·6, Pv 8·2, p<0·05
Indonesia[37]	2004-06	Timika	Hb<11 g/dL Severe anaemia Hb <7 g/dL	Pf	Hb Pf women 1·1 g/dl lower than (non mal).	Severe anaemia OR=2·8 (2·0-4·0), p<0·001	Severe anaemia more prevalent in MG (10·5% 185/1764) than PG (7·6% 60/792): AOR 1·6 (1·2-2·3), p=0·005

Indonesia[37]	2004-06	Timika	Hb<11 g/dL Severe anaemia Hb <7 g/dL	Pv	Hb Pv women 0.4 g/dl lower than (non mal).	Moderate anaemia: OR=1.8 (1.2-2.9), p=0.01	
Indonesia (unpublished)	2008-10	Jayapura	n.a.	Pf	OR 1·18 (SD 0·10)	OR 1·35 (SD 0·20)	n.a.
Indonesia (unpublished)	2008-10	Jayapura	n.a.	Pv	Not increased	Not increased	n.a.
Indonesia (unpublished)	2008-10	South West Sumba	n.a.	Pf	OR 1·44 (SD 0·08)	OR1·27 (SD 0·20)	n.a.
Indonesia (unpublished)	2008-10	South West Sumba	n.a.	Pv	OR 1·08 (SD 0·24)	OR 1·23 (SD 0·49)	n.a.
Nepal[31]	1994-97		Hb<110 g/L	Pv	OR 2·24 (0·91-5·52). moderate to severe anaemia AOR 2·28	n.a	Anaemia in PG 62% (45/73), MG 76% (204/269), p<0·05). Prevalence of moderate to severe anaemia NS
Laos[32]	1998	Vientiane	Hb<11 g/dL	Pf	Hb in parasitaemic women 9·1±2·9 vs. 11·0±2·0 (non mal), p=0·007	n.a	n.a.
PNG[42]	2002-03	Madang	Severe anaemia Hb <7 g/dL	Pf	n.a.	Hb in Pf 0·25 g/dL lower	Mean Hb decreased with gravidity (P=0·012)

↑ increased, AOR: adjusted odds ratio, Hb: haemoglobin, Hct: haematocrit, mal: malaria, MG: multigravidae, NS not significant, PAF population attributable fraction; PG: primigravidae, Pf: *P.falciparum*, Pv: *P.vivax*, PNG: Papua New Guinea

* Odds ratios provided with 95%CI, or mean ± standard deviation, or %

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Chapter 4

Malaria in pregnancy: the difficulties of measuring birth weight

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Abstract

Recommendations for interventions to control malaria in pregnancy are often based on studies using birth weight as the primary endpoint. Differences in birth weight may be attributable partly to methodological difficulties. We performed a structured search of the literature using 'malaria', 'pregnancy' and 'birth weight' as search terms. Of the clinical trials reporting birth weight, only 33% (14/43) gave information about the timing of the measurement and details on the scales used. Seventy seven percent explained how gestational age was estimated. We propose a standardised method for the measurement and reporting of birthweight in future studies.

Introduction

Malaria in pregnancy has a major impact on the health of the mother and fetus. In endemic areas, malaria is estimated to be responsible for 20% of low-birthweight (LBW) infants, the greatest single risk factor for infant mortality[1-3]. However malaria can cause both intrauterine growth restriction (IUGR), related to sequestration of malaria parasites in the placenta, and preterm labour (PTL), which is associated with symptomatic maternal illness in the third trimester. IUGR and PTL can be distinguished only if the gestational age is known with some accuracy[4-6]. This can be difficult in resource-poor settings. Many published clinical trials on maternal malaria have recruited women at the time of delivery, and birth weight (but not IUGR or PTL) has been the major endpoint. Different types of intervention may have different impacts on IUGR and PTL.
In this article, we review the methods used to obtain and report birthweight in studies of malaria during pregnancy. We also propose a systematic method for the reporting of birth outcomes, with an emphasis on studies in resource-poor settings.

Methods

A Medline (PubMed) search was performed with the search terms 'malaria' AND 'pregnancy' AND 'birth weight' using a combination of MeSH headings and keywords. The search was not designed to identify all studies on malaria in pregnancy, but to analyse those that included birthweight and malaria as outcomes. Only trials that specifically used birthweight as the main outcome were included.
The search was limited to humans, clinical trials, randomised controlled trials (RCTs), case reports, and English language articles from 1 January 1966 to 23 July 2009. Full articles of all citations resulting from this search were obtained. We scrutinised all articles for details of the methodology used to obtain birth weight. Two investigators independently performed eligibility assessment and, if disagreements were not resolved by consensus, a decision was made by a third author. Data from included studies were extracted and entered onto an Excel spreadsheet for collation and analysis. Information on the type of scales, precision of scales, scale calibration, day of weight, inclusion criteria for birth weight analysis, proportion of pregnant women enrolled, proportion of infants weighed and studies reporting significant difference in birth weight were extracted. When available, the method of gestational age estimation and the potential confounders of birth weight measurements were also extracted.

Results

Sixty-three publications were identified. Three of these reports were excluded because one was a review article[7] and two did not report birth weight[8, 9]. There were 43 different

trials and three case reports described in the remaining articles. The case reports did not contain sufficient details on the methodology used to measure birth weight and were excluded[10-12]. Forty-three studies described in 59 publications were reviewed (**Table S1**)[13-72]. Most (56%, 24/43) were studies on the prevention of malaria by intermittent preventive treatment (IPTp) or chemoprophylaxis in the African subcontinent (**Table S1**).

Weighing scales: model and accuracy

Different types of weighing scales were described, varying from spring scales used in field situations to very precise digital scales in referral hospitals. Only 44% (19/43) of the articles reported the type of the scale (**Table S2**). Calibration of scales with standard weights was reported in three studies[46, 55, 68]. Scale precision varied from 1 to 100 g (**Table S2**).

In resource-limited, tropical, humid or dusty field conditions, electronic scales can break[56] or trained midwives can be too busy to measure all babies[15]; however, only two research teams reported such events. One study in Kenya confirmed birth weight measurement by a double reading and used the mean value for data analysis[68].

Table S2 shows the variation in the printed format of birth weight. Most articles provided the mean ± standard deviation (SD) birth weight in grams; those that reported a significant difference between groups (44% (19/43)) are highlighted bold in the table. One study reported a significant difference of 80 g between two groups, although 'birth weight at the two hospitals was recorded to the nearest 100 g' [59]. Three articles only reported the proportions of LBW, but not the actual mean birth weight[26, 55, 66]. The average percentage of newborns that were included for birth weight analysis was 71% (range 33-100%). In 58% (11/19) of the studies that reported a significant difference in birth weight, the result was based on the analysis of less than 80% of the participants.

Date of weighing the infant

Delay in the weighing of the infant has an effect on the reported result[73, 74]. Seventy per cent (30/43) of the publications reported the time interval between the delivery and the measurement of birthweight and 35% (15/43) included babies weighed within 24 hours of birth (**Table S2**). The percentage of babies that were included for birthweight analysis is shown in **Table S2**. Some studies had a low proportion (14%[53] or 15%[68]) of infants weighed within 24 hours. Eight manuscripts adjusted for the day of the weight using one of two formulae[35, 75]. However, the actual formulae were not provided, and different articles derived different percentage adjustments despite referencing the same formula. For example, some authors adjusted the weights by 1% for weight measured on day 4[33] and others, using the same formula[75], used a 3% correction[15], while a 5% adjustment was made using the formula described by Greenwood et al.[35].

Table S1 Included studies

Author	Study period	Continent	Trial Type	Malaria study type
Adam[13]	1998-2001	Africa	Prospective non-comparative	Treatment
Bounyasong[14]	1995-1998	Asia	RCT	Treatment
Browne[15]	1994-1995	Africa	RCT	ITN
Challis[16]	2001-2002	Africa	RCT	IPTp
Clerk[17]	2004 2007	Africa	RCT	IPTp
Cot[18,19]	1991-1993	Africa	RCT	Chemoprophylaxis
Cot[20, 21]	1987 1988	Africa	RCT	Chemoprophylaxis
Deen[22]	1999	Africa	Retrospective	Accidental exposure
Denoeud[23]	2004-2005	Africa	Observational	Chemoprophylaxis
Dolan[24]	1990-1992	Asia	RCT	ITN
Dorman[25, 26]	1996-1997	Africa	Observational within RCT	IPTp
Egwunyenga[27]	1992	Africa	Observational	Chemoprophylaxis
Ekejindu[28]	NA	Africa	Cross sectional	Not reported
Falade[29, 30]	2003-2004	Africa	Observational	IPTp
Filler[31]	2002-2005	Africa	RCT non blinded	IPTp
Fleming[32]	1980	Africa	RCT	Chemoprophylaxis
Gies[33, 34]	2004-2006	Africa	Observational	IPTp
Greenwood[35-37]	1984-1987	Africa	RCT	Chemoprophylaxis
Hamer[38, 39]	2003-2004	Africa	RCT	IPTp
Hamilton[40]	1965	Africa	RCT	Chemoprophylaxis
Kalanda[41-44]	1993-1994	Africa	Cross sectional	Non interventional
Kayentao[45]	1998-2001	Africa	RCT	IPTp + Chemoprophylaxis
Larocque[46]	2003-2004	S-America	RCT	Treatment
Mbaye[47]	2002-2004	Africa	RCT	IPTp
Mbonye[48,49]	NA	Africa	Community non RCT	IPTp
McGready[50]	2001-2003	Asia	RCT	Treatment
Menendez[51]	1987-1990	Africa	RCT	Chemoprophylaxis
Menendez[52, 53]	1987-1990	Africa	RCT	Treatment
Menendez[54]	2003-2005	Africa	RCT	IPTp + ITN
Msyamboza[55]	2002-2004	Africa	Community non RCT	IPTp
Mutabingwa[56, 57]	1988-1991	Africa	RCT	Chemoprophylaxis
Ndyomugyenyi[58]	1996-1998	Africa	RCT	Chemoprophylaxis
Ndyomugyenyi[59]	1997-1998	Africa	Retrospective	Labour record review
Nosten[60]	1987	Asia	RCT	Pharmacokinetic
Nosten[61]	1987-1990	Asia	RCT	Chemoprophylaxis
Rahimy[62]	1994	Africa	Prospective non-comparative	Non interventional
Shulman[63]	1992-1995	Africa	RCT	ITN
Steketee[64, 65]	1987-1990	Africa	RCT	Chemoprophylaxis
Taha[66]	1989-1990	Africa	Nested case control	Non interventional
Ter Kuile[67, 68]	1992-1999	Africa	RCT	ITN
Tukur[69]	2002	Africa	RCT	IPTp
Villamor[70, 71]	2000-2002	Africa	RCT	Treatment
Villegas[72]	1998-2000	Asia	RCT	Chemoprophylaxis

BW birth weight; IPTp Intermittent Preventive Treatment in pregnancy; ITN Insecticide Treated Net; NA not available; RCT Randomised controlled trial; S South.

Table S2 Reporting of weight measurements

	Author	Type scale	Reported precision scale (g)	Quotations of BW^	Included for BW			Day of weight	Who is included
					BW	Total	%		
Weight scales	Falade29	Digital	1	3204.3±487.2 (1500 – 4700)	980	983	99	NA	NA
	Kalanda41, 42, 44	Salter	10	2843±533	1344	4104	33	NA	Live singleton
	Menendez 54	Digital	1	3003.55±522.69	990	1030	96	NA	All
	Mutabingwa56	Hanging + Digital	10-100	2.79±0.42 kg	291	423	69	NA	Term singleton
	Ndyomugyenyi59	NA	100	2.930±0.54 kg	5991	5991	100	NA	All**
	Tukur69	NA	50	3.12±0.51 kg	351	500	70	NA	NA
Scales + Timing	Deen22	Seca 835	10	2.87 [% CI 2.77-2.961]	195	459	42	< 9	All
	Dolan24	Salter	10	2476±550	301	341	88	< 1	Live singleton
	Dorman25, 26	Digital	5	LBW Y/N	340	854	40	Birth	Singleton
	Filler31	Digital	1	2.74±0.56 kg	491	698	71	< 1	Live singleton
	Gies33, 34	UNICEF hanging	25	2563 [% CI 2420–2706]	1281	1883	68	< 1 + F	Singleton
	Kayentao45	Digital	NA	2676±435	1062	1163	91	< 1	Live singleton
	Larocque46	Seca 334 + 345$	10	3104±441.54	950	1042	91	< 2	Live singleton
	Mbonye49	Salter	50	3220 g [range 3186—3264]	1227	2785	44	< 5	Term singleton
	Msyamboza 55	Chasmor hanging-	100	LBW Y/N	1733	1752	99	< 7 +F	Singleton
	Ndyomugyenyi58	Spring	10-25	3007±4	472	860	55	< 7	Live singleton
	Steketee64, 65	Mettler°	1	2905 ± 461	1642	1835	89	< 1	Live singleton
	ter Kuile67	Salter 235,10S @	10	3.19 ± 0.02 kg	833	833	100	< 4 + F	Live singleton
	Villamor70	Beam balance	10	LBW Y/N	258	275	94	Birth	Live born
	Villegas72	Salter#	50	2777 ± 435 (1650–4200)	733	1000	73	<3	Live singleton

Timing	Bounyasong14	NA	NA	2785.00	57	60	95	< 1	NA
	Browne15	NA	NA	2.19 [95CI 2.05-2.33] kg	847	1961	43	< 7 + F	All**
	Challis16	NA	NA	3077±533	403	600	67	< 1	All
	Clerk17	NA	NA	2790±452	1133	3643	31	Birth + F	Singleton
	Cot18, 19	NA	NA	3069.8±56.8	122	266	46	Birth	Live singleton
	Cot20, 21	NA	NA	2937.8±452.5	1148	1464	78	Birth	Live singleton
	Fleming32	NA	NA	2723	89	200	45	Birth	All
	Denoeud23	NA	NA	2850 (range: 800–5000)	1087	1176	92	Birth	Live singleton
	Greenwood35	NA	NA	2790±400	730	1049	70	<7 + F	Singleton
	Mbaye47	NA	NA	3090	2002	2688	74	<7	NA
	McGready50	NA	NA	2930 ± 648 (1000–4100)	53	81	65	<1	Live singleton
	Menendez51	NA	NA	3028 ± 414	182	230	79	<1+F	Live singleton
	Menendez52, 53	NA	NA	3103	450	550	82	<7+F	Live singleton
	Rahimy62	NA	NA	2500 ± 540	89	108	82	Birth	Live singleton
	Shulman63	NA	NA	2.8 (SE 0.06) kg	130	503	26	Birth	Live singleton
	Taha66	NA	NA	LBW Y/N	2683	2683	100	Birth	Live singleton
None	Adam13	NA	NA	3.45 ± 0.76 kg	12	40	30	NA	Live singleton
	Egwunyenga27	NA	NA	2650 ± 240	284	656	43	NA	Live born
	Ekejindu28	NA	NA	NA*	NA	108	NA	NA	NA
	Hamer38, 39	NA	NA	2987 ± 40.77	387	456	85	NA	Live singleton
	Hamilton40	NA	NA	3020 ± 597	1137	1846	62	NA	NA
	Nosten60	NA	NA	3.4 ± 0.5 kg	20	20	100	NA	Live singleton
	Nosten61	NA	NA	2877 ± 433	311	339	92	NA	NA

F Formula to calculate BW if day of weight not within 1 day; NA not available, ^ Bold if significant effect on BW was reported, * There was no report of BW, but IUGR was reported in results section and LBW in abstract28, ** Unclear whether twins and stillborns were included for birth weight analysis15, 59, $ Seca corp., Baltimore, MD, USA, ~ Chasmor, London, UK, º Mettler, Rite weight, Inc, Duluth, GA, USA, @ Salter, Smethwick, UK, # Salter, Birmingham UK

Inclusion in birth weight analysis

Most of the included studies confined their analysis of birth weight to liveborn singletons (**Table S2**). Three publications included multiple deliveries (twins or triplets)[54, 59, 70]. Three teams specifically reported that twins and congenital abnormalities were excluded from further analysis[41, 43, 72]. The remaining publications did not mention whether twins, stillborn infants or those with congenital abnormalities were included in the analysis. Congenital abnormalities were reported in 15 of the publications[13, 14, 17, 22, 23, 29, 43, 45, 46, 50, 54, 60-62, 72]. Eight studies (18%) reported length of the infant at birth and, of these, four reported IUGR[41, 50, 64, 72].

Method of gestational age estimation

Gestational age is defined as the time elapsed from the first day of the last menstrual period (LMP), if known, to the day of delivery[76]. Gestational age can then be divided into blocks depending largely on neonatal viability (see **Figure 4.1**). The World Health Organization (WHO) uses a 22-week threshold to define miscarriage, but different definitions continue to be used. In the articles reviewed, the method of estimating gestational age was described in 77% (33/43) of the publications. **Table 4.1** shows that symphysis-fundal height (SFH) was the most commonly used method.

Table 4.1 Reporting of estimation of gestational age during pregnancy or in the post partum period

		Pregnancy dating					Total
		None	LMP	SFH	LMP / SFH	US	
Post Natal dating method	No postnatal test	10[19, 20, 23, 27, 35, 47, 49, 58, 59, 69]	3[16, 22, 70]	8[28, 32, 33, 40, 51, 53, 55, 60]	3[15, 46, 63]	4**[13, 14, 26, 62]	28
	Ballard	1[31]	0	1[45]	2[41, 67]	0	4
	Capurro	0	1[66]	0	0	0	1
	Dubowitz	1[17]	1[64]	4[24, 54, 61, 72]	1^[38]	1[50]	8
	Lubchenko	1*[29]	0	0	0	0	1
	Other	0	0	1#[56]	0	0	1
Total		13	5	14	6	5	43

LMP, Last Menstrual Period; SFH, Symphysis-Fundal Height, US, Ultrasound

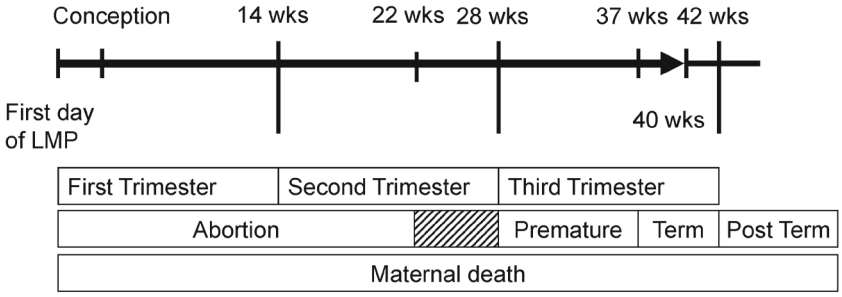
* The gestational age was determined using the Ballard score. Anthropometric parameters and gestational age were used to classify the babies using a Lubchenco chart as pre-term, term and post-date[29, 30].

"Midwives and MCH aids recorded newborns as being full term or premature using personal experiences and based on the indicators for rapid assessment of maturity[56].

^If the discrepancy between the LMP- and Dubowitz-derived gestational age was >14 days, the Dubowitz score was used[38].

**Ultrasound-derived gestational age assessment was used when menstrual dates were unknown (31%) or when measurement of fetal size fell more than 2 SD above or below the mean for gestation calculated from menstrual history (22%)[26]. Only women who could afford to pay had an ultrasound dating scan in Benin[62].

Figure 4.1 Definitions of pregnancy partitions



Maternal mortality can occur while pregnant or until 42 days after termination of pregnancy

SFH

This measurement enables gestational age to be calculated, but the formulae differ between populations[72, 77, 78]. Several publications report SFH to be inaccurate[34, 43, 68]; however, it was the most common single method used to describe gestational age in this review (**Table 4.1**)[24, 28, 32, 34, 40, 45, 51, 52, 54-56, 60, 61, 72].

LMP

Use of the LMP was reported to be inaccurate in studies in which another method of gestational age estimation was available[26, 41, 43, 64, 65]. Examples of such inaccuracies were because the women could not recall their LMP[26, 50], or there was no menstruation between two consecutive pregnancies[43]. One study reported 22% of pregnancies in which the ultrasound measurement of fetal size was more that two SDs above or below the mean for gestation calculated from menstrual history[26]. In another study, estimation of gestational age was based on the identification of "quickening", which was interpreted as an indication that gestational age was more than 18 weeks[42]. A combination of LMP and SFH was used to estimate gestational age in six studies[15, 38, 41, 46, 63, 67]. One study that compared postnatal tests and LMP/ SFH reported, 'an unacceptably high number of women had pregnancies of more than 44 weeks, which makes the usefulness of LMP for assessment of gestational age doubtful' and 'gestational age derived from SFH is not reliable, with a range of 20-50 weeks'[43].

Newborn gestational age assessment

The number of physical and neuromuscular maturity criteria examined in standardised neonatal scoring systems is critical to the accuracy of the test[79]. However, a number of modifications to the methods of Ballard[43] and Dubowitz[80,81] have been used in resource-poor settings. Newborn tests were reported to be less accurate when performed after 12-20 h of age[22], and to be time consuming[56].

Ultrasound gestational age assessment
Ultrasound measurement to estimate gestational age was reported from Kenya[26], Sudan[13] and Thailand[14, 50]. In Benin, ultrasound scans were limited to patients who could afford to pay, but it was not reported how many women were scanned[62].

Confounding factors
Several factors were reported to have an impact on birthweight, including maternal smoking (n=2)[66, 72], high blood pressure or pre-eclampsia (n=9)[17, 23, 26, 32, 60-62, 66, 72], maternal infections (e.g. chorioamnionitis) as risk factors for premature labour (n=3)[29, 45, 61], parity, height and nutritional status of the mother; number of antenatal clinic visits, rainy season and sex of the baby[17, 23, 24, 29, 45, 61, 63-66, 72]. The sex of the newborn was reported in 40% (17/43) of studies. A few authors make reference to the problem of 'infants that were not weighed', attributed to highly mobile rural populations and large numbers of home deliveries resulting in missing delivery information[34, 55, 69]. These missing data may introduce bias, and one study showed that the loss of contact with subjects during the follow-up was more prevalent in the control group than in the treated group[20].

Discussion

Considerable effort by researchers and the pregnant women themselves has been devoted to determine the impact of antimalarial interventions on birthweight, often under difficult conditions. Important information regarding the methodology and reporting of birth outcome data is often missing or inaccurately reported. Journal space restrictions may not allow authors to describe completely what they actually did and this is a potential limitation of our review. This article does not question the association between malaria and birthweight reduction, but highlights that the conclusions drawn about the effects of interventions based on differences in birthweight could partly be explained by inaccuracy in measurement methods.

Gestational age estimation
When designing and conducting perinatal research studies, careful selection of the method of gestational age estimation is necessary, as findings can differ considerably according to the method[82]. When differences in birthweight are found, a bias caused by the selection of a particular method must be considered as an alternative explanation for any association found[82].
An error in gestational age estimation of even 1 week has major implications on birthweight. The weight gain of a fetus in the late third trimester can be as much as 250 g per week[83]: this value is similar to the reduction in weight attributable to malaria[1-3]. Ideally, gestational age should be estimated by fetal crown-rump length (CRL) or early second-trimester ultrasound, which is the standard in resource-rich countries[84-86] and becoming available in developing countries[87]. When no reliable LMP, SFH or ultrasound

measurements are available, postnatal examination of the newborn, with clinical scoring for external and/or neurological characteristics, can be used. These methods can be performed by locally trained paramedical health workers or nurses[43, 88]. The Dubowitz[80] examination for estimation of gestational age is recommended from 6-72 hours of life, which can make it difficult to include home births.

Weighing scales and reporting of birth weight data

All that is needed to measure newborn weight is a scale. As a result, birthweight is frequently used as the only item to describe birth outcome. To describe the type of growth restriction caused by malaria, additional parameters, such as gestational age, newborn length and/or head circumference measurements, are required.

The accuracy of the equipment used to measure birth weight is paramount[89]. It is preferable to use scales that have been registered for medical use, and the name, model and accuracy should be reported, especially if newborns are weighed at home. In the articles included here, none compared birthweight of home- and hospital-delivered babies, but this has potential for large differences in measurements. Although some studies reported weight to the nearest gram[29, 31, 54, 64], the reported accuracy by the manufacturer is usually in the order of 10 g, even though some digital scales may provide readouts to the nearest gram.

Research teams should be adequately trained in obtaining and reporting measurements. Ideally, research measurements should be taken by two different trained observers who are blind to the results obtained by the other, with measurements repeated that exceed preset maximum allowable differences[90]. A standard method of calibrating scales should also take place at least once a week. With a sufficient sample size of newborns, birth weight is a normally distributed continuous variable, so presentation of such data would be expected to include the mean ± SD, as well as the minimum and maximum (range).

Date of weight

Normal birthweight reduction can be as much as 10% by day 3[73, 74], and, in a 3000- g baby, this would result in a weight reduction of 300 g; this is within the order of magnitude of the effect described for malaria in pregnancy. Consequently, the day the newborn is weighed has important implications for research, particularly as a large proportion of women in resource-poor settings deliver at home and delays in recording birth weight are expected. Ideally, birthweight should be obtained within 24 hours of birth or, if taken after 24 hours, a correcting formula should be applied. However, blanket correction factors do not account for the differences in postnatal weight loss as a result of birthweight categories[91], gravidity/parity[92], race, asphyxia[92] and age at the initiation of breastfeeding[92]. In the context of RCTs, the proportion of infants weighed on different days should not be significantly different between the groups.

Birth weight analysis
The inclusion of multiple pregnancy, stillbirths or infants with congenital abnormalities will have an impact on birthweight analysis. While such pregnancies need to be reported, they should not be included in any analysis of birthweight. Whether minor congenital abnormalities have an impact on birth weight is debatable but, for the sake of consistency, they should probably be excluded from any birthweight analysis in the context of clinical trials. Nevertheless, it is important to highlight that congenital abnormalities will not be reliably reported without an adequate examination of the newborn by a trained observer and a standardised method of classifying abnormalities. WHO has established a pregnancy registry (http://apps.who.int/tdr/svc/grants/calls/call-contributions). that will include the prevalence of birth defects in Asia, Africa and Latin-America, and classification using International Classification of Diseases (ICD10).

Confounders
Most potential factors confounding birthweight can be detected by adequate antenatal and intrapartum care. Inclusion of gender in the analysis could be used as an internal validity check, as female newborns are lighter than males[94, 95]. Although clinical trials often exclude women with known medical or obstetric problems, some could arise after inclusion and should be controlled for, for example hypertension (eclampsia, pre-eclampsia, pregnancy induced hypertension), infections (pyelonephritis, sexually transmitted infections, local area-specific infections, e.g. typhoid or scrub typhus), obstetric problems, such as preterm labour and whether there is a recognised trigger, for example symptomatic malaria or pyelonephritis. RCTs could minimize the effect of potential confounders but they should be included in any (multivariate) analysis.
Birthweight may not necessarily be the best method to evaluate the efficacy of interventions against malaria in pregnancy. The study of IUGR and the type of growth restriction (symmetrical or asymmetrical) requires additional parameters, including gestational age, newborn length and/or head circumference measurements.

Conclusion
Differences in birthweight are often used to compare the efficacy of interventions aimed to reduce the impact of malaria during pregnancy[96-99]. Such differences can clearly be affected by inaccuracies in measurement methods, and confounders such as those affecting gestational age or time of weighing. The reporting of birthweight and gestational age in maternal malaria studies can be improved. Simple methodological guidelines for reporting birth outcome, with an emphasis on studies on malaria in pregnancy, are provided (Box).

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Recommendations

Birthweight:

- Measure birthweight on all newborns (alive or stillborn) as soon as possible after birth, preferably before 24 hours.
- Only live born singletons without congenital abnormality should be included in birth weight analysis.
- Report the actual number of newborn babies included in the birth weight analysis.
- Report the time interval (in days) between birth and measurement of birthweight.
- Certified medical scales should be used.
- Report the name, model and accuracy of the weight scale and state whether the scale was sufficiently sensitive to detect any difference identified. Scales should be calibrated on a weekly basis.
- Head circumference and length of the newborn should be measured.
- Regular standardisation sessions and quality control checks of the measurers are required[90].
- The sex of the baby should be recorded.

Gestational age assessment

- Report the method of estimating gestational age.
- A suggested algorithm to obtain the best estimate for the woman is, in order of priority: (1) ultrasound at <24 weeks by measuring, preferably, crown rump length (8-14 weeks) or head circumference (15-24 weeks); (2) if ultrasound is not available, validated newborn gestational age assessment; (3) if (1) and (2) are not available, date of last menstrual period or symphyseal-fundal height.
- Analysis using birthweight or low birth weight should be controlled for gestational age.
- Methods used to estimate gestational age should have regular (yearly) standardization sessions and ongoing quality control (http://apps.who.int/tdr/svc/grants/calls/call-contributions).
- Bias due to the selection of a particular dating method – or no dating method – should always be considered as an alternative explanation for any identified associations[65].

Confounders

- Potential confounders should be diagnosed and included in the birthweight analysis.

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Part 3 Antenatal ultrasound on the Thai-Burmese border

Chapter 5

Obstetric ultrasound scanning by local health workers in a refugee camp on the Thai-Burmese border

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Keywords :
Ultrasound; Fetal biometry; Accuracy; Reproducibility; Developing country

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Abstract

Objectives:
Ultrasound examination of the fetus is a powerful tool for assessing gestational age and detecting obstetric problems but is rarely available in developing countries. The aim of this study was to assess the intraobserver and interobserver agreement of fetal biometry by locally trained health workers in a developing country.

Methods:
One expatriate doctor and four local health workers participated in the study, which included examinations performed on every fifth pregnant woman with a singleton pregnancy between 16 and 40 weeks' gestation, and who had undergone an early dating ultrasound, attending the antenatal clinic in Maela Refugee camp. At each examination, two examiners independently measured biparietal diameter (BPD), head
circumference (HC), abdominal circumference (AC) and femur length (FL), with one of the examiners obtaining duplicate measurements of each parameter. Intraobserver measurement error was assessed using the intra-class correlation coefficient (ICC) and interobserver error was assessed by the Bland and Altman 95% limits of agreement method.

Results:
A total of 4188 ultrasound measurements (12 per woman) were obtained in 349 pregnancies at a median gestational age of 27 (range 16-40) weeks in 2008. The ICC for BPD, HC, AC and FL was greater than 0.99 for all four trainees and the doctor (range 0.996-0.998). For gestational ages between 18 and 24 weeks, interobserver
95% limits of agreement corresponding to differences in estimated gestational age of less than ±1 week were calculated for BPD, HC, AC and FL. Measurements by local health workers showed high levels of agreement with those of the expatriate doctor.

Conclusions:
Locally trained health workers working in a well organized unit with ongoing quality control can obtain accurate fetal biometry measurements for gestational age estimation. This experience suggests that training of local health workers in developing countries is possible and could allow effective use of obstetric ultrasound imaging.

Introduction

Ultrasound examination of the fetus is a powerful tool for assessing gestational age, detecting multiple pregnancy, intra-uterine growth restriction and determining placental location[1-5]. Since the 1990s, almost every pregnant woman in developed countries has had access to between one and four routine scans during uncomplicated pregnancies[6]. However, in most developing countries antenatal ultrasound services are non-existent or inadequate. Those that are available are usually limited to tertiary centers or private hospitals in urban regions[7-9].

A lack of qualified sonographers and a shortage of ultrasound machines, most likely due to their high cost and maintenance difficulties, have been barriers to the implementation of routine ultrasound examination in many antenatal clinics in resource-poor settings. Recently, assistant medical officers, clinical officers, midwives or local radiographers have been identified as potential sonographers[7, 10, 11]. Given that ultrasound imaging has no value if the ultrasonographer is inadequately trained or inexperienced, recent efforts have concentrated on training[8]. Some African countries have reported promising results from starting ultrasound teaching programs[10], but as more developing countries introduce such programs, studies to ensure the quality and consistency of locally trained sonographers will be required.

At the Shoklo Malaria Research Unit (SMRU) local health workers (schooled until 16 years of age) have been trained in basic ultrasound since 2001. They have performed approximately 3000 obstetric ultrasound scans per year in Maela refugee camp over the past 5 years. The aim of this study was to assess the intraobserver and interobserver agreement of fetal biometric measurements performed by these health workers.

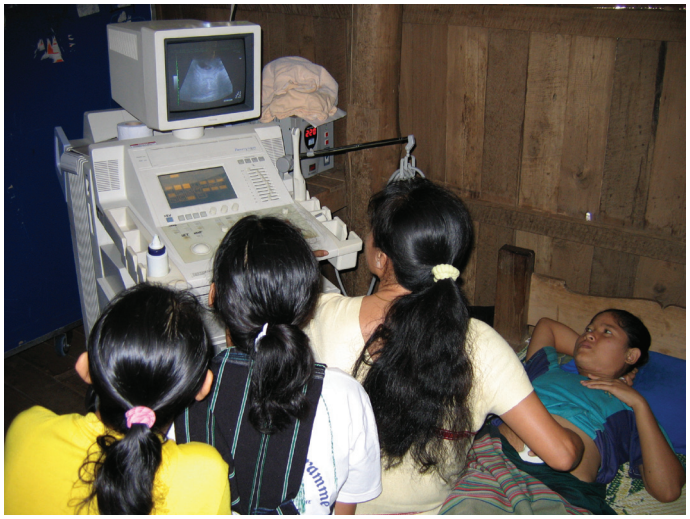
Methods

The SMRU is located on the Thai-Burmese border and has studied the epidemiology, prevention and treatment of malaria in pregnancy since 1986. It has five established clinics, one of which is based in refugee camp Maela, where Karen people (a minority group in Burma) are the principal inhabitants. In all its clinics SMRU runs a program of antenatal care (ANC) to detect and treat all parasitaemic episodes during pregnancy through weekly malaria screening in order to prevent maternal death[12]. Since the inception of this ANC program, all pregnant women have been encouraged to attend as early as possible in pregnancy. At the first visit (usually between 8 and 13 weeks' gestation), ultrasound imaging is used to determine viability, detect multiple pregnancy and estimate gestational age. A second scan is performed at 18-24 weeks to confirm gestation, viability and placental position. In women who do not have an early scan, gestational age assessment is based on fetal biometry scans between 18 and 24 weeks' gestation, or using the Dubowitz gestational age examination at birth if no such scan is available[13].

SMRU ultrasound department

The SMRU introduced ultrasound examination for gestational age assessment owing to the low proportion of women who could reliably provide the date of their last menstrual period (LMP). In the past 3 years only 31% (994/3184) of women in Maela refugee camp provided a reliable LMP. When the ultrasound department in the antenatal clinic of the Maela refugee camp opened in 2001, one of the co-authors (SLMD), a local Karen health worker who was already skilled in Dubowitz assessment of gestational age, was trained in ultrasound gestational age assessment. A 3-month course of practical and theoretical training in obstetric ultrasound was then developed (**Figure 5.1**) for newly employed staff, all of whom were chosen at interview on the basis of motivation, willingness to learn and proficiency in English. The course was based on World Health Organization (WHO) guidelines and British Medical Ultrasound Society (BMUS) recommendations[14, 15]. During the next 3 months all scans were verified by a senior sonographer. Only when the head of the department was satisfied with each person's scanning skills and written examination results, were they permitted to scan alone.

Figure 5.1 Photograph showing training in the ultrasound room Maela Refugee Camp, 2008



Procedures

As part of a larger fetal growth study, quality control evaluation (interobserver and intraobserver variability) was performed between four local sonographers and one expatriate doctor (MJR), certified and experienced in obstetric ultrasound scanning. The Mahidol-Bangkok and Oxford University ethics committees approved both the main and quality control studies.

Every fifth pregnant woman attending the ANC was invited to participate in the study if she had an early (8-13 week) dating scan at the SMRU ANC, a singleton pregnancy, and

a gestational age of between 16-40 weeks. A maximum of 15 women for each gestational week were invited. After obtaining written informed consent, an abdominal ultrasound examination was performed. At each examination, two examiners independently measured biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) in millimeters (mm). Each image was acquired according to BMUS guidelines, ensuring that the image filled at least a thrid of the monitor screen. The machine automatically calculated the gestational age (weeks and days) from each measurement using Hadlock's charts.[16] Each examiner was blinded to his own results and the results of the other examiner. All measurements were obtained twice by one examiner (Examiner 1) to assess intraobserver variability, and once by another examiner (Examiner 2) to assess interobserver variability, resulting in 12 measurements per woman (i.e., three sets of four measurements).
All scans were performed using a Toshiba Powervision 7000 machine (Toshiba, Tokyo, Japan) with a 3.75-MHz convex probe, which was donated by the University of Utrecht, The Netherlands. Owing to electrical surges in the refugee camp, a voltage stabilizer was used to operate the ultrasound scanner.

Statistical analysis.
The extent to which measurements agree between two sonographers is limited by the amount of variation in repeated measurements made on the same subject by the same individual. This measurement error was assessed using the intraclass correlation coefficient (ICC), with a value of 1 (possible range 0 to 1) indicating no measurement error17. However, as the ICC was calculated for each parameter will be artificially inflated owing to the large range of gestational ages included, summary measures (mean, minimum and maximum differences and SD) for each sonographer are also reported.
The agreement between the mean of the two measurements made by the Examiner 1 and the measurement made by Examiner 2 was then estimated, using the 95% limits of agreement method proposed by Bland and Altman[18, 19]. Data were initially plotted, with a line of equality, to gauge the degree of agreement between measurements. All points would lie on the line of equality if the two examiners reported exactly the same measurements. The assumptions that the standard deviation of repeated measures was not related to the magnitude of the ultrasound measurements and that the differences between the measurements followed a normal distribution were then checked visually using scatter plots and histograms, respectively. If the assumptions were not met, calculations were carried out on log transformed values and the antilog was taken to obtain limits of agreement that could be related to the original scale of measurement[18, 20]. As gestational age assessment in clinical practice normally occurs between 18 and 24 weeks, interobserver variation was calculated for this subgroup of measurements, as well as for the entire dataset. Biometry measurements in the second trimester have an accuracy of ± 1 week in estimating gestational age, whereas the accuracy decreases to ± 2 weeks in the third trimester[21-25]. A difference in measurements (in millimeters) between the two examiners that corresponded to a difference in gestational age of ±1 week or less

was considered to be very good agreement[21-25]. Clinically acceptable and unacceptable findings were gestational age differences that were ±1-2 weeks and more than ±2 weeks, respectively.
We used the mean of the repeated measurements taken by the Examiner 1 against the measurements made by Examiner 2 to assess interobserver variation. One could expect the estimate of standard deviation to be smaller (owing to removal of repeated measurement error) by using the mean[18]. When compared with using only one set of measurements from Examiner 1, however, the results differed by less than 1mm for all parameters, regardless of whether the first or second set of repeated measurements was used (results not shown). Therefore, only the results using the mean of Examiner 1's measurements are reported here, and no adjustments to the standard deviation were made.
All analyses were carried out using STATA/SE, version 9.2 for Windows (StataCorp LP, College Station, Texas, USA).

Results

Between April and September 2008, 349 pregnant women consented to the ultrasound examination. The median gestational age was 27 (range 16-40) weeks. It was possible to complete the examination and obtain all 12 measurements in all women, and so a total of 4188 measurements were obtained.

Education level of local health workers
All four local health workers involved in the obstetric ultrasound course agreed to participate in this quality control study. One had completed 3 years of training as a nurse at a recognized institution in Burma. The others did not have any tertiary education but had completed school to grade 10 (16 years old). At the start of the study, they had a median of 20 (range 12 – 62) months of work experience.

Intra-observer variation
Table 5.1 shows the summary of the repeated measurements of all examiners. The ICC for all four parameters (BPD, HC, AC, FL) was greater than 0.99 for all four trainees and the doctor (range 0.996-0.998), indicating that almost all the variation observed was due to differences between patients rather than differences in the repeated measurements taken by one examiner on any one patient.

Interobserver variation
The agreement between the mean of the two measurements made by Examiner 1 was compared with the single measurement made by Examiner 2 to assess interobserver variation. This was done for the complete dataset (**Table 5.2**) as for a subset of measurements obtained for pregnancies at 18–24 weeks (**Table 5.3**). The distribution of mean

differences was approximately normal for each of the four parameters but the standard deviations and differences for BPD, HC and AC increased with the magnitude of the measurements (**Figure 5.2 and Figure 5.3**). These parameters were log-transformed for analysis and the back transformed values used to estimate the 'V-shaped' 95% limits of agreement (i.e., the range within which measurements were expected to agree 95% of the time increased as the size of the measurement increased)[18, 20].

Table 5.1 Mean, minimum and maximum differences for each pair of measurements obtained by the same locally trained sonographer (A-D) or the doctor.

	Biparietal diameter		Head circumference		Abdominal circumference		Femur length	
Sonographer (N)	Mean difference (SD)	Min, max	Mean difference (SD)	Min, max	Mean difference (SD)	Min, max	Mean difference (SD)	Min, max
A (157)	0.15 (1.62)	-5.5, 7.7	0.43 (6.52)	-26, 20	0.01 (8.54)	-24, 36	-0.28 (1.56)	-5.4, 4.3
B (70)	-0.11 (1.05)	-2.3, 4.9	0.90 (4.86)	-14, 16	0.51 (5.90)	-14, 24	0.03 (1.27)	-3.3, 5.1
C (67)	-0.26 (1.43)	-3.7, 2.9	-0.64 (6.62)	-22, 15	-0.93 (7.97)	-33, 14	0.14 (1.59)	-3.6, 8.7
D (18)	0.08 (1.46)	-2.0, 3.3	-1.44 (4.51)	-9, 5	0.39 (10.6)	-30, 27	0.21 (1.53)	-2.5, 3.2
Doctor (37)	0.05 (1.16)	-3.2, 3.3	1.27 (4.56)	-6, 19	0.41 (5.45)	-13,12	-0.02 (0.91)	-1.9, 2.1

SD = standard deviation, Min = Minimum difference, Max = Maximum difference N = number of pairs of measurements obtained by each examiner

Table 5.2 Mean difference in ultrasound measurements obtained by two different examiners on 349 women at 16 – 40 weeks' gestation.

Parameter	N	Mean difference (mm (95% CI)
BPD	349	-0.12 (-0.30 to 0.06)
HC	349	-0.11 (-0.77 to 0.54)
AC	349	-0.09 (-0.98 to 0.80)
FL	349	-0.13 (-0.28 to 0.03)

AC = abdominal circumference; BPD = biparietal diameter; FL = femur length; HC = head circumference

Femur Length

The only parameter for which the standard deviations and the differences were constant throughout the range of measurements was FL (**Figure 5.2 and Figure 5.3**). The mean difference between the ultrasound measurements for each fetus obtained by the two different examiners is presented in **Table 5.2**. On average, the measurement of femur length by the Examiner 1 differed from the measurement made by Examiner 2 by -0.13mm (95% CI -0.28 to 0.03) (**Table 5.2**). The 95% limits of agreement ranged

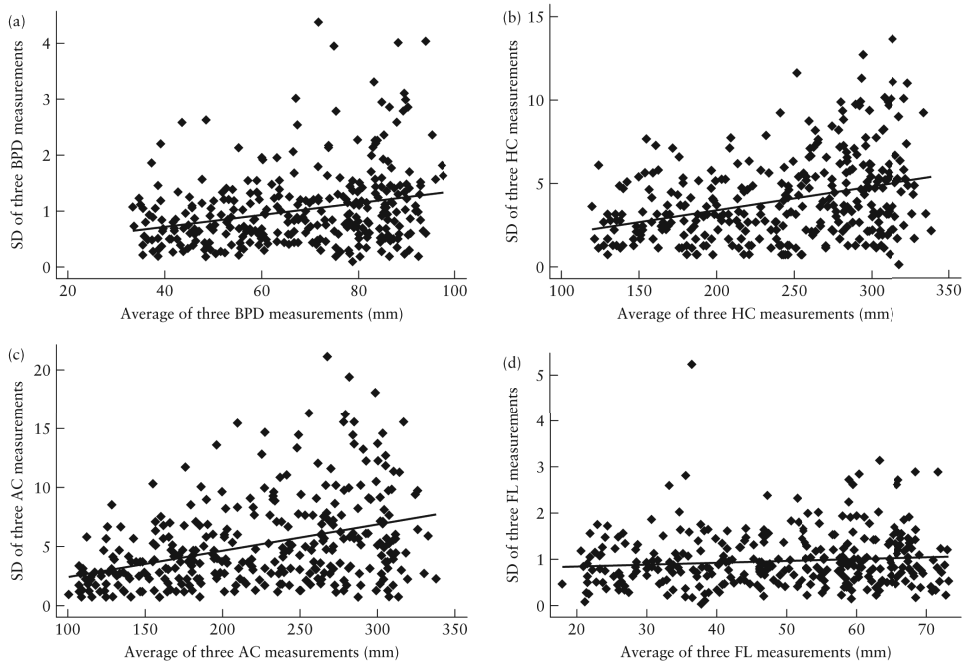
from -3.03 to 2.78mm. This meant that the measurements of Examiner 2 were likely to be within -3.03 to 2.78 mm of Examiner 1's measurements 95% of the time, a difference that corresponds to ±1-1.5 week variation in gestational age estimation.

Table 5.3 Mean difference and 95% limits of agreement (LOA) by measurement and corresponding estimated gestational age of ultrasound measurements obtained by two different examiners on 90 women at 18 - 24 weeks' gestation.

	N	Measurement			Gestational age	
		Mean difference (mm (95% CI))	SD (mm)	95% LOA (mm)	Mean difference (weeks (95% CI))	SD (weeks)
BPD	90	-0.43 (-0.68 to -0.17)	1.21	-2.80 to 1.94	-0.12 (-0.19 to -0.04)	0.36
HC	90	-1.63 (-2.63 to -0.62)	4.80	-11.0 to 7.77	-0.12 (-0.20 to -0.03)	0.42
AC	90	-0.59 (-1.77 to 0.60)	5.65	-11.7 to 10.45	-0.03 (-0.13 to 0.07)	0.50
FL	90	-0.35 (-0.64 to -0.07)	1.37	-3.03 to 2.33	-0.11 (-0.21 to 0)	0.50

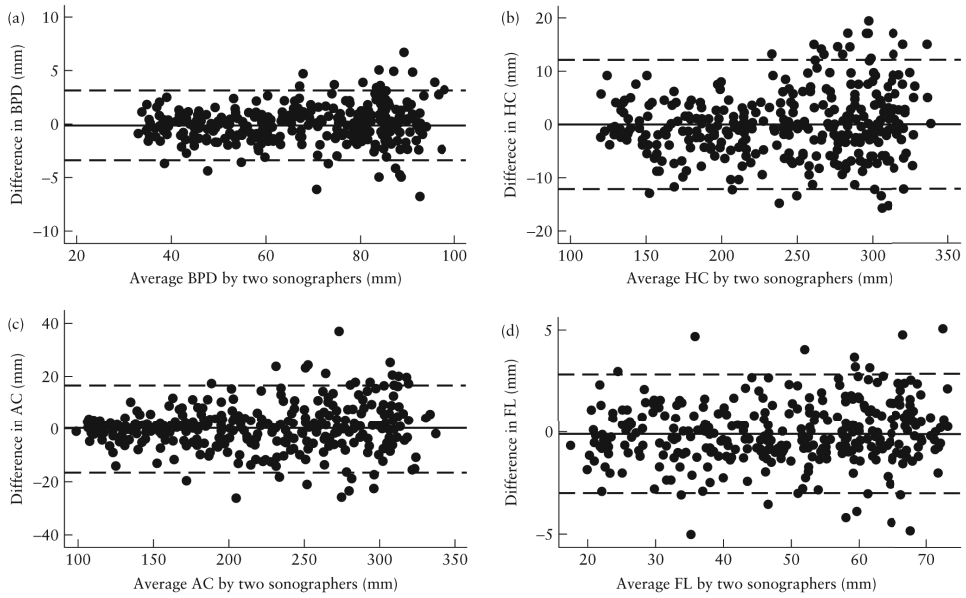
AC = abdominal circumference; BPD = biparietal diameter; FL = femur length; HC = head circumference

Figure 5.2 Scatter plots of standard deviation against the average of the three measurements for biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) in each fetus.



For each graph, the solid line represents the regression line.

Figure 5.3 Bland-Altman plots of the interobserver differences in the measurement of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL), showing the variation increased with the magnitude of the measurements for BPD, HC and AC.



For each graph, the solid line represents the mean difference and the dashed lines are the mean difference ± 2SD.

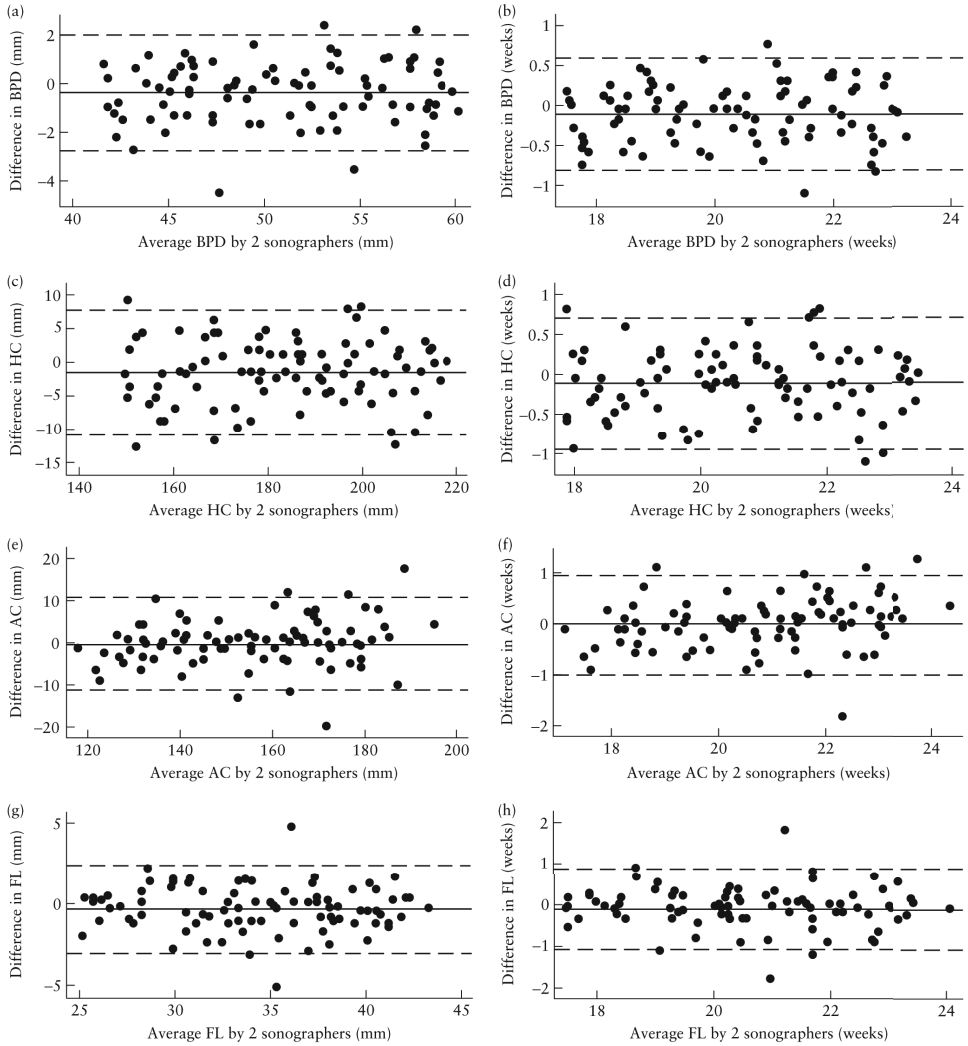
Biparietal diameter, head circumference and abdominal circumference

To account for the increase in variation that occurred with the increase in magnitude of the measurements, the values for BPD, HC, and AC were log-transformed to calculate the limits of agreement. These were then backtransformed so they could be related to the original scale of measurement. For HC and BPD, 95% of measurements by Examiner 2 could be expected to be between 0.95 to 1.05 times the measurement by Examiner 1. This meant that the measurements by Examiner 2 could differ by 5%, above or below, that of Examiner 1. If the HC measurement by Examiner 1 was 116mm (minimum HC), corresponding to a gestational age of 15+3 wks, we would expect the measurement by Examiner 2 to be ±5.8mm, 95% of the time. This corresponds to an acceptable variation in estimated gestational age of ±3 days (15+0 and 15+6 wks). The variation increased with the size of the measurement. Therefore, if the HC measurement obtained by Examiner 1 was 284mm, we would expect the measurement by Examiner 2 to be ±14.2mm 95% of the time. An HC of 284mm corresponds to a gestational age of 30+0 wks, and ±14.2mm to a possible difference of 1.5 weeks; which was considered as just clinically acceptable.

Similarly for a measurement of 344 mm (the maximum HC, corresponding to 39+3 weeks) we would expect a possible difference of 4 weeks, larger than clinically acceptable. Performing similar calculations for BPD, there was a variation of ±0.5 weeks for the minimum BPD and ±2.5 weeks for the maximum BPD.
The largest variation between examiners was seen for AC measurements. Those made by Examiner 2 differed from the measurements of Examiner 1 by ±7, 95% of the time.

Gestational age assessment in clinical practice
Between 18 and 24 weeks (when biometry scans are used to assess gestational age if no first trimester crown rump length measurement is available), the variation in measurements was constant throughout the range of measurements (**Figure 5.4**): mean differences (95% CI) and limits of agreement of this subgroup were therefore calculated without log transformation (**Table 5.3**).
The largest mean difference was for HC measurements (**Table 5.3** and **Figure 5.4**), indicating that the measurements by Examiner 2 differed from those made by the first examiner by 1.63 mm (95% CI, 0.62 - 2.63mm). The 95% limits of agreement indicated that the measurements made by Examiner 2 could be expected to be within 11.0 mm lower to 7.8 mm higher than the measurements made by Examiner 1, 95% of the time (Table 3). This corresponded to a possible difference in estimation of gestational age of less than ±1week. Similarly, differences of less than ±1 week were estimated for BPD, AC, and FL.

Figure 5.4 Bland-Altman plots of the interobserver differences in the measurement of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) at 18-24 weeks' gestation, expressed as the measurement itself (left column) and the corresponding estimated gestational age (right column).

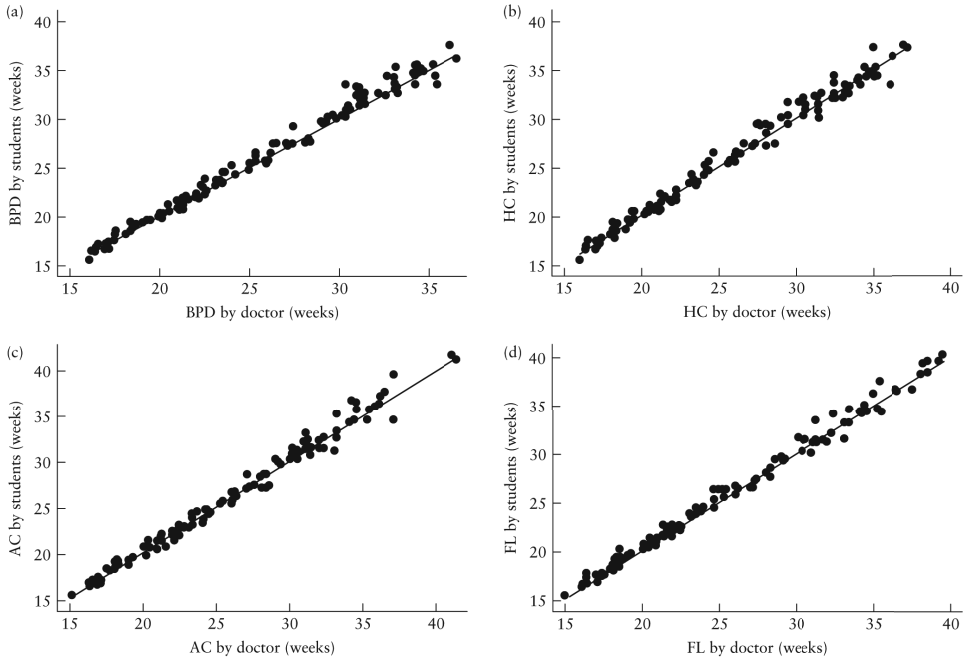


For each graph, the solid line represents the mean difference and the dashed lines are the mean difference ± 2SD (see **Table 5.3**).

Comparison between doctor and local trainees

The expatriate doctor took at least one set of measurements on 124 women. Scatter plots between his measurements and those made by the trained health workers showed that all points were tightly clustered around the line of equality for all four parameters (**Figure 5.5**), indicating a high degree of agreement in ultrasound use by both teacher and students.

Figure 5.5 Scatter plots of fetal biometry measurements (n=124) made by the students against those made by the doctor for biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL), with the line of equality shown for each.



Discussion

In this study we found that local health workers can be trained to use ultrasound imaging reliably and consistently to assess gestational age. The intraobserver variation (ICC all greater than 0.99) demonstrated that measurements are made consistently by the same sonographer. For gestational ages between 18 and 24 weeks, there was a difference of less than one week in gestational age estimated using the measurements made by different examiners. In addition, when compared with the skill of an experienced doctor, the local trainees demonstrated a high level of agreement in measuring all four parameters. These findings reassured us that the criteria for selecting the trainees were adequate.

In developed countries it has long been established that fetal biometry at between 14 and 22 weeks' of gestation can accurately predict gestational age within 7 days (±2SD)[21-25]. The variation in measurements between examiners in this study falls within this period. Our overall findings, therefore, strengthen the argument that obstetric ultrasound imaging can be introduced in developing countries for gestational age assessment, particularly when one considers the unreliability of LMP recall in such settings[26].

It is essential to maintain quality control in any antenatal ultrasound service to ensure that the data obtained are clinically meaningful, e.g. by estimating the accuracy and reproducibility of the fetal biometry measurements taken[18, 27-29].

In SMRU clinics, quality control is achieved by routinely taking all ultrasound measurements twice to assess intraobserver variability, and by an expatriate doctor qualified in ultrasound imaging annually checking the skills of all sonographers. Reassuringly, therefore, the measurement errors for gestational age estimation in this study were comparable to those obtained by highly experienced sonographers[29].

Every measurement in clinical science is associated with error and, unsurprisingly, the variation increased as BPD, HC and AC sizes increased. This was not the case for FL, perhaps because the clearly defined landmarks of the FL (two edges of the femur bone, which are not affected by fetal breathing as in AC measurements) might have contributed to reducing the variation between measurements.

Apart from trained health workers, robust ultrasound machines are needed to make obstetric ultrasound imaging available in remote areas. Unfortunately, as observed by Kurjak and Breyer "many developing countries cannot afford to buy good quality ultrasound diagnostic instruments and do not have enough trained specialists who can devote a large fraction of their active time to the science and art of ultrasound diagnosis[7]. However, ultrasound imaging has become more feasible in developing countries as machines become less expensive and require less servicing[6, 10, 30, 31].

To solve the problem of the lack of trained sonographers, the WHO has recognized the urgent need to raise education levels in ultrasound scanning in developing nations[32]. Hence, in 1998, it published a report concerning the essentials, principles and standards of training for both physicians and allied health professionals in diagnostic ultrasound imaging[14]. In reality, however, physicians in developing countries are heavily overloaded with work, resulting in inadequate use of available ultrasound machines[7, 8]. Thus, other health workers have been identified as potential sonographers[7, 10, 11].

Several reports of international ultrasound training programs have been published previously[10, 32-34]. Some of these were based in district hospitals in developing countries, where local health workers were trained successfully in theoretical and practical scanning skills. In this study, we have shown that candidates with limited or no tertiary education and limited English in a refugee camp can acquire good quality basic ultrasound skills for gestational age estimation with a short training course, a period of on-the-job training and ongoing quality control measures.

To our knowledge, this is the first report on quality assurance of gestational age estimation of locally trained sonographers in a refugeecamp. Our results show that adequately

trained health workers, working in a well organized unit with ongoing quality control can obtain accurate fetal biometry measurements, whether the scan is performed by the same sonographer or by different sonographers. Given the importance of gestational age assessment in obstetric management, we recommend that ultrasound machines are made available and that ultrasound training is provided for local health workers in developing countries.

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Chapter 6

Quality of ultrasound biometry obtained by local health workers in a refugee camp on the Thai-Burmese Border

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Keywords:
Fetal biometry, reference equations, Thai-Burmese border, ultrasound, quality, z-score, standard deviation, developing country, health workers

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Abstract

Objectives
In a refugee camp on the Thai-Burmese border, accurate dating of pregnancy relies on ultrasound measurements obtained by locally trained health workers. The aim of this study is to substantiate the accuracy of fetal biometry measurements performed by locally trained health workers by comparing the z-scores and standard deviations of biometry equations created for this purpose to those from published equations of professional sonographers from Asian and European hospitals.

Methods
This prospective observational study included 1,090 women who had a dating crown rump length scan and one study appointed ultrasound biometry scan between 16 and 40 weeks of gestation. The average of two measurements of biparietal diameter, head circumference, abdominal circumference and femur length was used in a polynomial regression model for the mean and standard deviation against gestational age.

Results
Reference equations of biometry parameters were reported as a cubic polynomial model. The standard deviations and z-scores of measurements from locally trained health workers were well within those from centers with professional sonographers.

Conclusion
Locally trained health workers in a refugee camp on the Thai-Burmese border can obtain measurements that are associated with low standard deviations and within the normal limit of published Asian and European equations. The fact that the standard deviations were lower than other studies may be explained by using the average of two measurements, crown rump length dating or motivation of the locally trained sonographers.

Introduction

Assessment of fetal growth is an important component of antenatal care, and precise dating is crucial for detection subsequent growth restriction. Although accurate gestational age (GA) assessment is not a problem unique to resource-poor settings[1-3], a large proportion of women in such settings is unable to give reliable last menstrual period (LMP) dates[4]. For example, in the antenatal clinics on the Thai-Burmese border less than a third of women are able to confirm their LMP[5]. This is due to a number of reasons: a) GA is counted in months, and attempts to translate this to a LMP date are complicated and usually inaccurate, b) literacy levels in pregnant women in Maela Refugee camp are less than 50%[6] and c) there are several calendars in use: standard western, Thai, Karen, Burmese and Buddhist[5]. Accordingly, dating of pregnancy relies on ultrasound measurements, ideally in the first trimester.
Local health workers are trained as sonographers. We have shown that these staff, with limited or no tertiary education, can achieve high levels of accuracy in GA assessment with a 3-month training course, including on the job training and ongoing quality control (QC) measures[5]. This ongoing QC-system is in place for dating scans at first trimester (Crown Rump Length (CRL)) and second trimester biometry scans. The aim of this study is to substantiate the accuracy of the health workers' fetal biometry measurements by comparing the z-scores and associated standard deviations (SD) of biometry equations created for this purpose to those from published equations of professional ultra sonographers from Asian and European hospitals.

Methods

Study site and population

The Shoklo Malaria Research Unit (SMRU) is located on the Thai–Burmese border and has five established clinics, one of which is based in Maela refugee camp. The main population in this camp belongs to the Karen ethnic group. Details of SMRU antenatal clinics are described in full elsewhere[5, 7]. In short, the antenatal clinics were commenced in 1986 and ultrasound scanning was introduced in 2001. All pregnant women have been encouraged to attend as early as possible in the first trimester of pregnancy. At the first visit, ultrasound imaging is used to determine viability, detect multiple pregnancy and estimate GA. Routinely, a second scan is performed at 18–24 weeks to re-assess viability, measure fetal biometry, identify major fetal abnormalities and determine placental position.

Study procedures

Women who attended the SMRU antenatal clinic in Maela refugee camp were invited to consent to have an additional biometry scan done at a specific GA in order to assess image quality at any time in pregnancy as part of the ongoing QC of local

sonographers[5]. The data for this study was collected from this QC program of the same group of sonographers. Two sonographers left the refugee camp in 2009 for resettlement into a western country and were replaced with two newly trained sonographers. One of the sonographers had completed three years of training as a nurse at a recognized institution in Burma. The others did not have any tertiary education but had completed school to grade 10 (16 years old). All sonographers had at least 12 months of work experience before participating in this data collection and each month in 2010, they scanned a median of 439 (range 340-492) women. The focus on image and measurement quality for sonographers was part of preparation and training for a fetal growth study (ClinicalTrials.gov Identifier: NCT00840502), approved by Oxford University (OxTREC (14-08)) and Mahidol University (TMEC 2008-028) Ethics Committees.
Women with a live singleton fetus, who had an early dating ultrasound (defined as CRL measurement between 8 mm and 79 mm, which corresponds to 7 and 14 weeks of gestation), were assigned to return for a study scan between 16 and 40 weeks at which biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) were measured. This study scan was in addition to their routine second trimester scan. When a woman did not attend for the appointed study scan, the data of the routine biometry measurements were used. Pregnancies complicated by serious infectious diseases (e.g. malaria) before the scan and pregnancies that had an unknown outcome or resulted in stillbirth were excluded. No fetuses were excluded on the basis of abnormal biometry, birth weight, preterm delivery, or congenital abnormality, which included six cases (sacrococcygeal teratoma, two cases with skin tags, Down syndrome, syndactyly and cleft palate).
The training manual and protocol for obtaining trans-abdominal CRL and biometry measurements were from the British Medical Ultrasound Society recommendation[8],
the BPD is measured locally in the plane of the HC by placing the calipers on the outer border of the upper and the inner border of the lower parietal bones ('outer to inner', BPD) across the widest part of the skull. All scans were performed with no time constraints, in a room on a reinforced bamboo floor by four locally trained sonographers[5] using a Toshiba Powervision 7000 machine (Toshiba, Tokyo, Japan) with a 3.75-MHz convex probe, which was donated by the University of Utrecht, the Netherlands. Owing to electrical surges in the refugee camp, a voltage stabilizer was used to operate the ultrasound scanner. At each scan, the measurements, recorded in mm, were obtained twice and the examiners were blinded to the expected GA and results of the examinations.

Statistical analysis

Data were entered into a Microsoft Access database and statistical analysis was performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago Ill, USA) and Microsoft Excel. The mean of the two first trimester CRL measurements was used to define GA[9]. Each woman provided just one designated biometry examination, and the mean of two measurements for each biometric parameter was included for analysis.

In order to obtain reference ranges for fetal measurements a polynomial regression model was used as recommended previously[10, 11]. Least square regression analysis was used to model the mean, by fitting a polynomial equation including a linear, quadratic, and cubic component for all measurements. The variability in measurements was modeled by computing the SD at each week of gestation and the SD values were regressed on GA using a linear equation. From the predictive mean and SD equations centiles were calculated using the formula:

$$\text{centile} = \text{mean} + K \times SD$$

where K is the corresponding centile of the standard normal distribution: ±1.88 for 3rd and 97th centiles, and ±1.28 for 10th and 90th centiles. Charts were computed by plotting predicted means and 3rd, 10th, 50th, 90th, and 97th centiles against GA.
For each GA between 16 and 40 weeks this study's biometric measurements were compared to published equations from Asian[12, 13] and European[14-18] hospitals using the z-score method[19-21]. The data were expressed as z-scores using the formula:

$$\text{z-score} = (X_{GA} - M_{GA})/SD_{GA}$$

where X_{GA} is the mean value from other populations at a known GA, M_{GA} is the mean value for the study population calculated from our equation at this GA, and SD_{GA} is the SD associated with the mean value at the same GA from our population. The z-scores and the published SD of each equation are presented graphically across the different GA to allow visual comparison.

Results

Between April 2007 and October 2010, 1090 women with a live singleton newborn were included in this prospective observational study. Median (inter quartile range) age, gravidity and mid upper arm circumference (MUAC) were 25 (20 – 30) years, one (1-4), and 24.0 (23.0 - 26.0) respectively. The mean (SD) birth weight in this group was 3017 (428 grams) and gestational age 39.1 (1.6) weeks. The median number of examinations performed at each week of gestation was 39 (interquartile range 32-45, **Supplementary table S5**)
Raw data fitted satisfactorily with a cubic polynomial model for all biometric parameters as follows (all measurements in mm and GA in exact weeks):

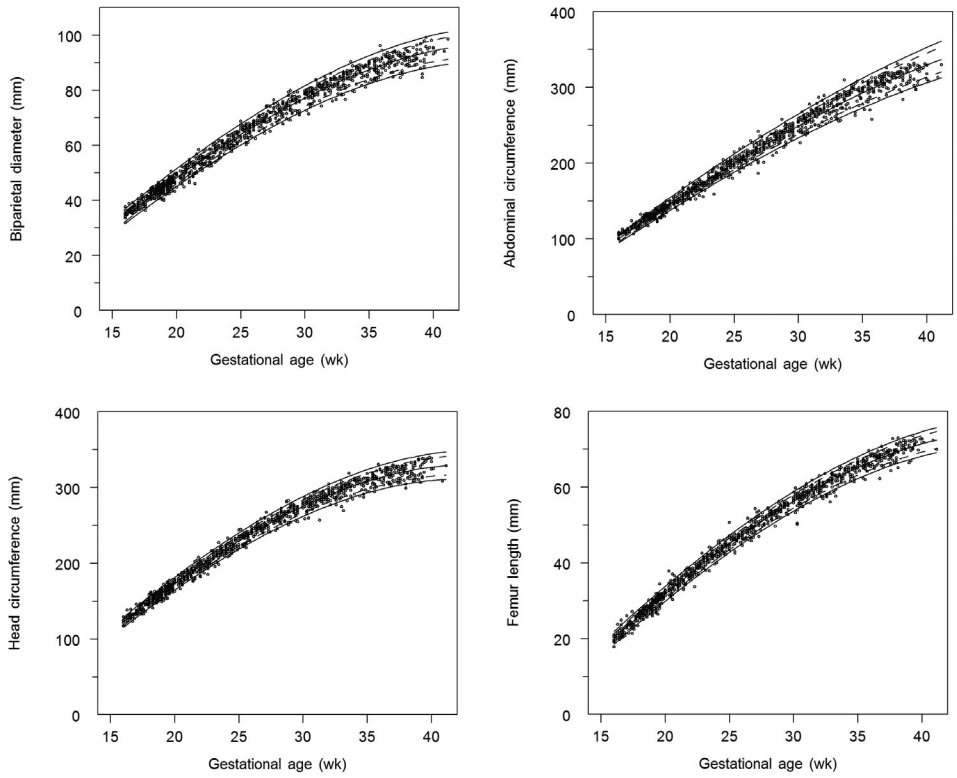
$$BPD = -29.179 + 4.136 \times GA - 0.00065571 \times GA^3 \quad (R^2=0.986)$$
$$HC = -114.263 + 15.493 \times GA - 0.002786989 \times GA^3 \quad (R^2=0.990)$$
$$AC = -98.125 + 12.722 \times GA - 0.00126688 \times GA^3 \quad (R^2=0.988)$$
$$FL = -32.294 + 3.413 \times GA - 0.00051213985 \times GA^3 \quad (R^2=0.992)$$

Data analysis showed that the addition of a quadratic component did not improve the fit of the curves.
The corresponding equations for the SD fitted in a linear equation:

BPD SD = 0.37 + 0.067 × GA
HC SD = -0.363 + 0.244 × GA
AC SD = -3.466 + 0.397 × GA
FL SD = 0.353 + 0.034 × GA

The goodness of fit of the model by was assessed using raw data for each measurement (**Figure 6.1**). **Supplementary figures S1-S4** and **supplementary tables S1-S4** present the charts and tables for clinical use with the 3rd, 10th, 50th, 90th and 97th percentiles of BPD, HC, AC and FL.

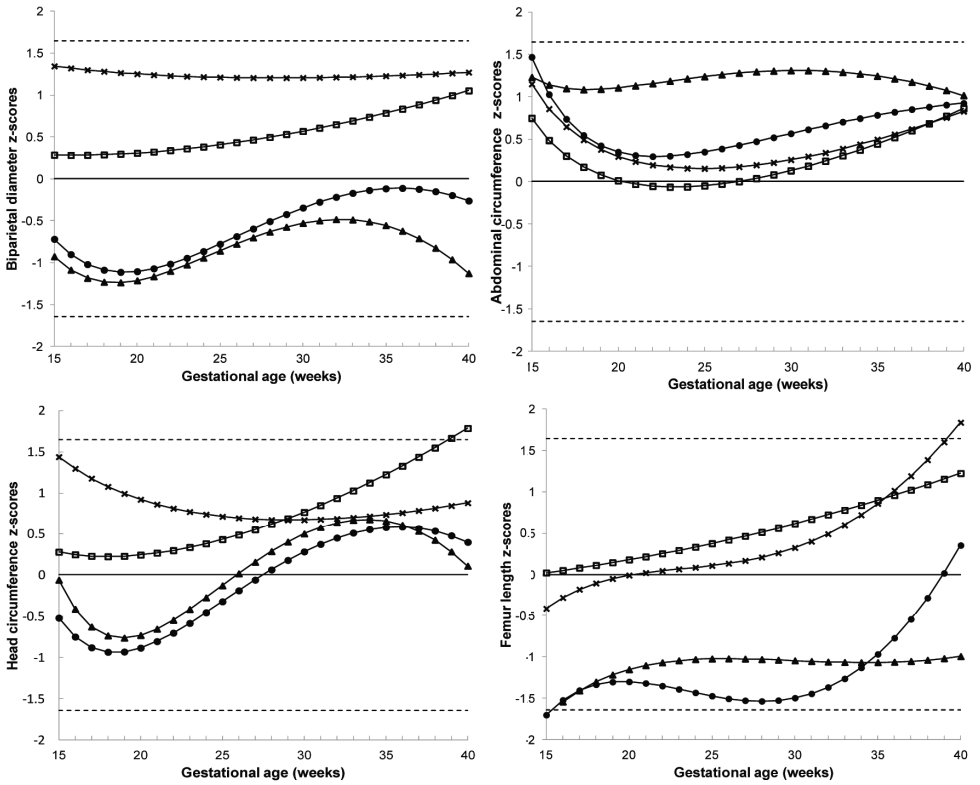
Figure 6.1 Raw data with 3rd, 10th, 50th, 90th and 97th fitted centiles (n=1090) for BPD, HC, AC and FL.



The x-axis shows the gestational age in weeks, the y-axis the biometry measurement in millimeters.

Figure 6.2 shows the 50th centiles of each biometric parameter from previously published equations expressed as z-scores based on our equations[12-18]. If the measurements from all populations were identical **Figure 6.2** would show one line at y=0. For nearly the entire GA range the mean values of the Asian and European equations for all four biometric measurements were within the 5% (mean – 1.645 SD) and 95% (mean + 1.645 SD) range of our equations. The mean AC of fetuses in this study was smaller throughout the pregnancy than any of the other equations, and the mean HC and FL were smaller in the second half of pregnancy.

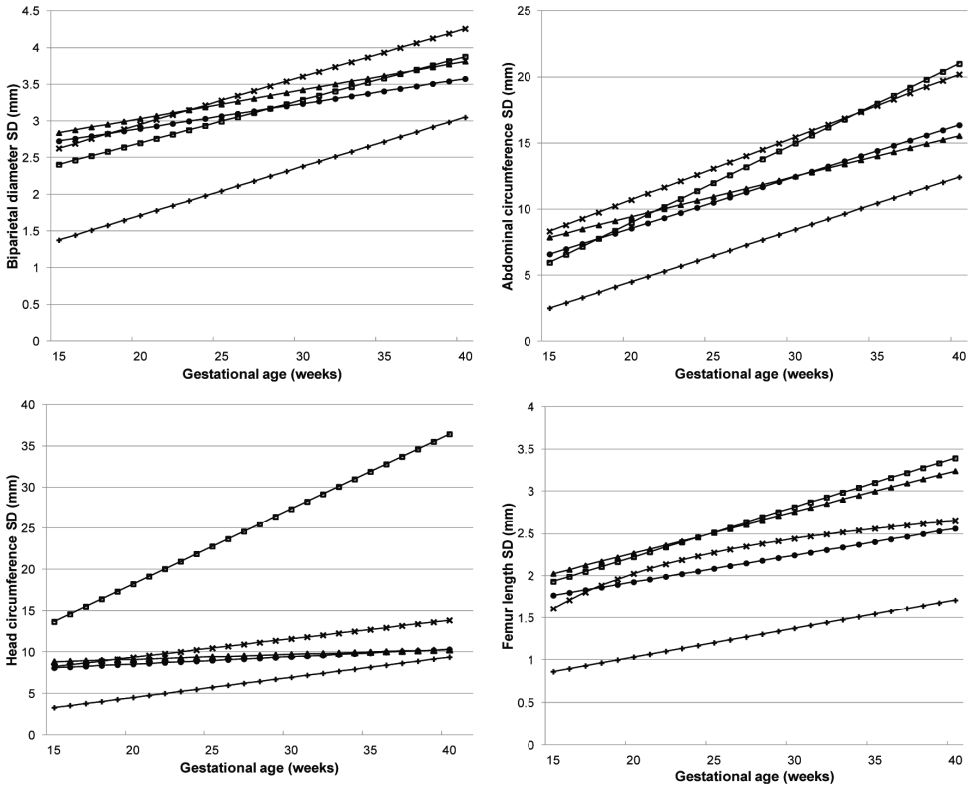
Figure 6.2 Z-scores for comparison of fetal biometry with Asian and European equations



Z-score comparison of the present study's equations with Asian: China[13] (●), Korea[12] (▲) and European: Switzerland[14, 15] (×) and UK[16-18] (□) equations for mean BPD, HC, AC and FL. Mean expected z-score, or 50th percentile is shown as straight black line, dashes lines represent the expected z-scores for the 5th and 95th centiles, i.e. -1.645 and 1.645 respectively. The x-axis shows the gestational age in weeks, the y-axis the z-score.

When comparing the SD for this population with SD generated from the equations of Asian[12, 13] and European[14-18] studies, it can be observed that - for any GA - the SD was significantly smaller in this study population (**Figure 6.3**).

Figure 6.3 Standard deviations for comparison of fetal biometry with Asian and European equations



Comparison of the present study's standard deviations (+) with Asian: China[13] (●), Korea[12] (▲) and European: Switzerland[14, 15] (×) and UK[16-18] (□) standard deviation equations of BPD, HC, AC and FL. The x-axis shows gestational age in weeks, the y-axis the SD in mm.

Discussion

Antenatal ultrasound is increasingly seen as a useful adjunct to obstetric care in resource poor settings: ultrasound machines are becoming available[22-24] and locally trained health workers can obtain can obtain accurate fetal biometry measurements for GA estimation[5, 25]. Accurate pregnancy dating is one of the obvious benefits in populations where LMP is not available or cannot reliably be obtained. However, the value of antenatal ultrasound depends on appropriate use by adequately trained sonographers with ongoing QC support in settings with the necessary infrastructure[5, 24].

This study confirms that very motivated locally trained health workers can successfully obtain biometry measurements between 16 and 40 weeks of GA. The low SDs derived from the reference equations suggest that the quality of measurements are associated with a low random error.

The choice of reference charts and equations for fetal size has an impact on the quality of fetal biometry in clinical practise[11, 19]. There have been concerns about incorrect methods that are being used to estimate age-specific reference intervals ("normal ranges") for fetal measurements[11]. Country specific differences in e.g. caliper placement have been highlighted to explain differences between reference charts and equations within European studies[20]. Midwives had a greater tendency than physicians to normalize biometry data[26]. In this study the examiners were blinded to the estimated GA and their measurement, so such a normalization of data is unlikely to have occurred. Furthermore, all sonographers received the same training and followed the strict guidelines for ultrasound examination, including the plane of the measurement and caliper placement.

When z-scores were used to compare the reference equations with previously published equations from two Asian (China[13] and Korea[12]) and two European (Switzerland[14, 15] and UK[16-18]) studies that provided BPD outer-to-inner measurements as well, several points can be made (**Figure 6.2**). The fact that the z-scores of the other equations were within the 5% and 95% range of our equation, suggests that the locally trained sonographers are able to obtain measurements that are comparable with expert sonographers. Generally the z-scores of Asian fetuses appear smaller than European scores. The smaller AC of Karen fetuses throughout pregnancy compared to the other populations may reflect the socio-economic conditions in the refugee camp. In this study, the FL was smaller compared to European FL, which is in agreement with previously published articles, where it was explained by racial differences[12, 13].

From **Figure 6.3**, the most striking difference is the smaller SD at any GA for this study's equations. By definition, SD shows the variation or "dispersion" from the mean (or expected value) and depends on measurement error as well as the sample size. Our sample size (n=1090) was larger than the Chinese (n=709) and UK (n=633) studies, but smaller than the Korean (n=10455) and Swiss studies (n= 6557), so sample size alone cannot explain the difference in SD. One explanation could be that in this study the equations were based on the mean of two measurements for both the CRL and the biometry measurements, which reduces variation from the expected value. Secondly in

this study dating was based on first trimester CRL, which results in less variation of GA than when LMP is used for pregnancy dating[2, 27]. In the four other studies GA was only corrected to the ultrasound value if the difference between the CRL estimated GA and the menstrual gestational age exceeded four[13], five[15] or ten[12, 18] days. On the other hand this may also be seen as a limitation of our study as the use of a CRL measurement at a single time point for dating does not account for first trimester growth restriction[28-30]. Nevertheless, in the absence of reliable LMP dates in our population dating by CRL is the most appropriate method.

The SD, being the denominator of the formula, has an important impact on magnitude of a z-score. When the z-scores were calculated based on the SD of another equation[16-18], the z-scores were all closer to zero (see **Table 6.1**). To put z-scores into clinical context, a mathematical example was created. When an examiner measures a fetus at exact 20 weeks GA, the mean, SD and 95% CI in mm and weeks of the five equations are shown in **Table 6.2**. The 95% CI varies from less than one week to more than three weeks between equations.

In conclusion, SD and z-score comparison can be used to assess QC, and this study suggests that locally trained health workers can obtain measurements that are associated with low SDs and within the normal limit of Asian and European equations. The fact that the SDs were even lower than other studies may be explained by various possibilities, and these include that a) the average of two measurements was used for both CRL and biometry, b) CRL dating and not LMP method was used for pregnancy dating, and c) locally trained sonographers were particularly motivated. Further research is needed to assess the relative impact of these possible factors in other populations.

Table 6.1 Number of observations according to gestational age (GA) in completed weeks in singleton pregnancies on the Thai-Burmese border

GA	Number of women scanned	GA	Number of women scanned
16	39	29	30
17	37	30	46
18	134	31	38
19	123	32	39
20	41	33	39
21	50	34	30
22	47	35	33
23	42	36	38
24	45	37	30
25	45	38	26
26	42	39	17
27	32	40	6
28	41		

Table 6.2 Corresponding mean, standard deviation and 95% confidence interval for a head circumference measurement at 20^{+0} weeks for different equations.

Equation Sample size of study	GA (weeks)	Mean ± SD (mm)	95% CI (mm)	95% CI (weeks)
Present study N= 1090	20^{+0}	173.3 ± 4.5	164.5 - 182.1	19^{+2} – 20^{+5}
Switzerland[15] N= 6557	$20^{+}0$	177.5 ± 9.4	159.1 - 195.9	18^{+6} – 21^{+6}
UK[18] N= 633	20^{+0}	174.4 ± 18.2	138.7 - 210.1	17^{+2} – 23^{+1}
China[13] N= 709	20^{+0}	169.3 ± 8.5	152.6 - 186.0	18^{+2} – 21^{+0}
Korea[12] N= 10455	20^{+0}	170.0 ± 9.1	152.2 - 187.8	18^{+2} – 21^{+1}

CI: confidence interval, GA gestational age, mm millimeter, SD standard deviation, UK United Kingdom

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Supplementary Figures and Tables S1, S2, S3, S4

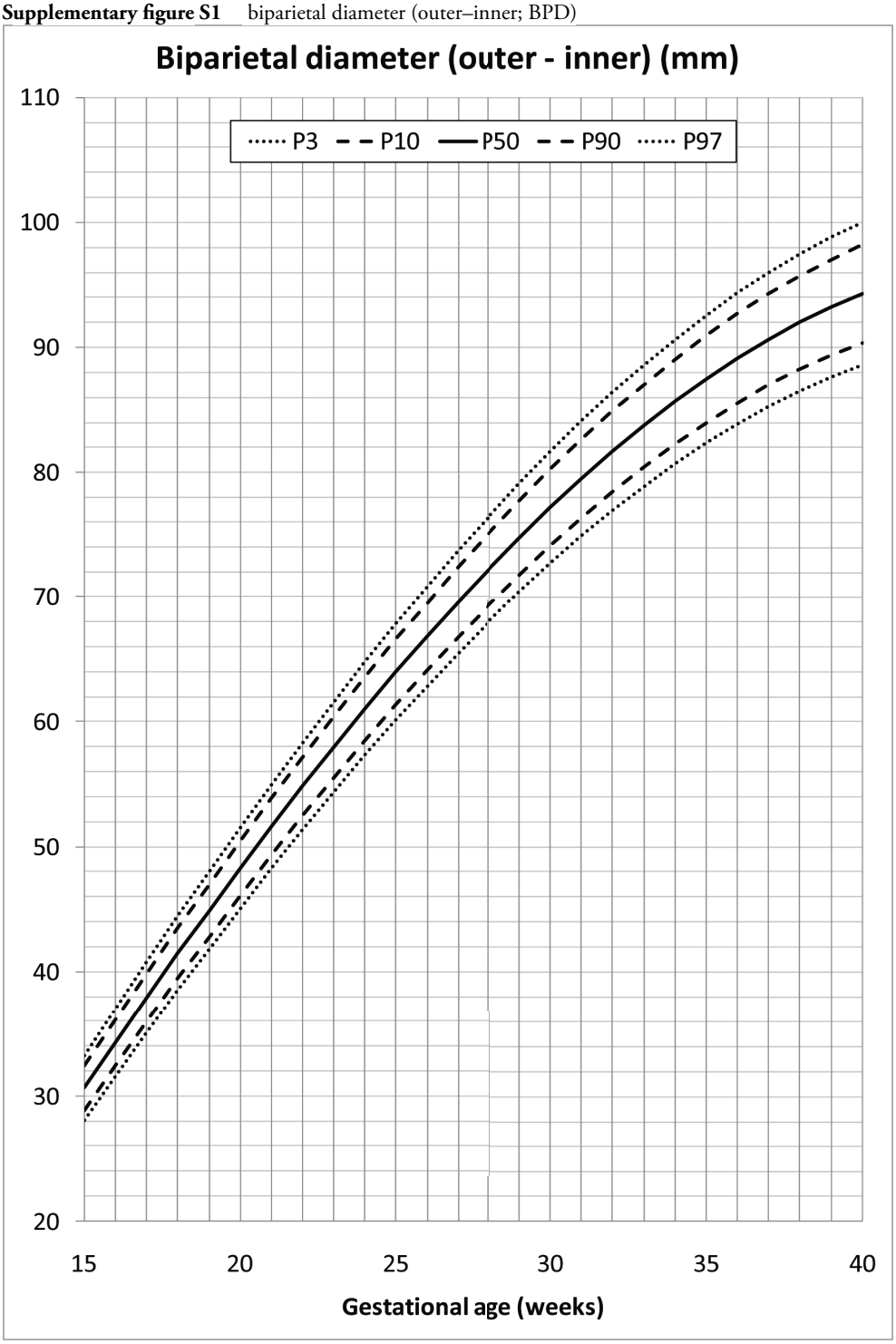
Fetal biometric charts with 3rd, 10th, 50th, 90th, and 97th fitted centiles in a Karen population in a refugee camp on the Thai-Burmese Border:

Table S1 biparietal diameter (outer–inner; BPD)
Figure S1 biparietal diameter (outer–inner; BPD)
Table S2 head circumference (HC)
Figure S2 head circumference (HC)
Table S3 abdominal circumference (AC)
Figure S3 abdominal circumference (AC)
Table S4 femur length (FL)
Figure S4 femur length (FL)

Supplementary table S1 Biparietal diameter (outer to inner) in mm

Weeks of gestation	Percentiles										
	P2.3	P3	P5	P10	P25	P50	P75	P90	P95	P97	P97.7
15	28.0	28.1	28.4	28.9	29.7	30.6	31.6	32.4	32.9	33.2	33.3
16	31.5	31.6	31.9	32.5	33.3	34.3	35.3	36.2	36.7	37.0	37.1
17	35.0	35.1	35.4	36.0	36.9	37.9	38.9	39.8	40.4	40.7	40.9
18	38.4	38.5	38.8	39.4	40.4	41.4	42.5	43.5	44.0	44.4	44.5
19	41.7	41.8	42.2	42.8	43.8	44.9	46.0	47.0	47.6	48.0	48.1
20	44.9	45.1	45.5	46.1	47.1	48.3	49.4	50.5	51.1	51.5	51.6
21	48.1	48.3	48.7	49.3	50.4	51.6	52.8	53.9	54.5	54.9	55.1
22	51.2	51.4	51.8	52.5	53.6	54.8	56.1	57.2	57.9	58.3	58.4
23	54.2	54.4	54.8	55.5	56.7	58.0	59.3	60.4	61.1	61.6	61.7
24	57.1	57.3	57.8	58.5	59.7	61.0	62.3	63.6	64.3	64.7	64.9
25	60.0	60.1	60.6	61.4	62.6	64.0	65.3	66.6	67.3	67.8	68.0
26	62.7	62.9	63.3	64.1	65.4	66.8	68.2	69.5	70.3	70.8	71.0
27	65.3	65.5	66.0	66.8	68.1	69.6	71.0	72.4	73.2	73.7	73.9
28	67.8	68.0	68.5	69.4	70.7	72.2	73.7	75.1	75.9	76.5	76.6
29	70.2	70.4	71.0	71.8	73.2	74.8	76.3	77.7	78.6	79.1	79.3
30	72.5	72.7	73.3	74.2	75.6	77.2	78.8	80.2	81.1	81.7	81.9
31	74.7	74.9	75.5	76.4	77.9	79.5	81.1	82.6	83.5	84.1	84.3
32	76.8	77.0	77.5	78.5	80.0	81.7	83.4	84.9	85.8	86.4	86.6
33	78.7	78.9	79.5	80.4	82.0	83.7	85.5	87.0	88.0	88.6	88.8
34	80.5	80.7	81.3	82.3	83.9	85.7	87.4	89.1	90.0	90.7	90.9
35	82.1	82.4	83.0	84.0	85.6	87.5	89.3	90.9	91.9	92.6	92.8
36	83.7	83.9	84.5	85.6	87.3	89.1	91.0	92.7	93.7	94.4	94.6
37	85.1	85.3	85.9	87.0	88.7	90.6	92.5	94.3	95.3	96.0	96.2
38	86.3	86.5	87.2	88.3	90.1	92.0	94.0	95.7	96.8	97.5	97.7
39	87.4	87.6	88.3	89.4	91.2	93.2	95.2	97.0	98.2	98.8	99.1
40	88.3	88.6	89.3	90.4	92.3	94.3	96.3	98.2	99.3	100.0	100.3

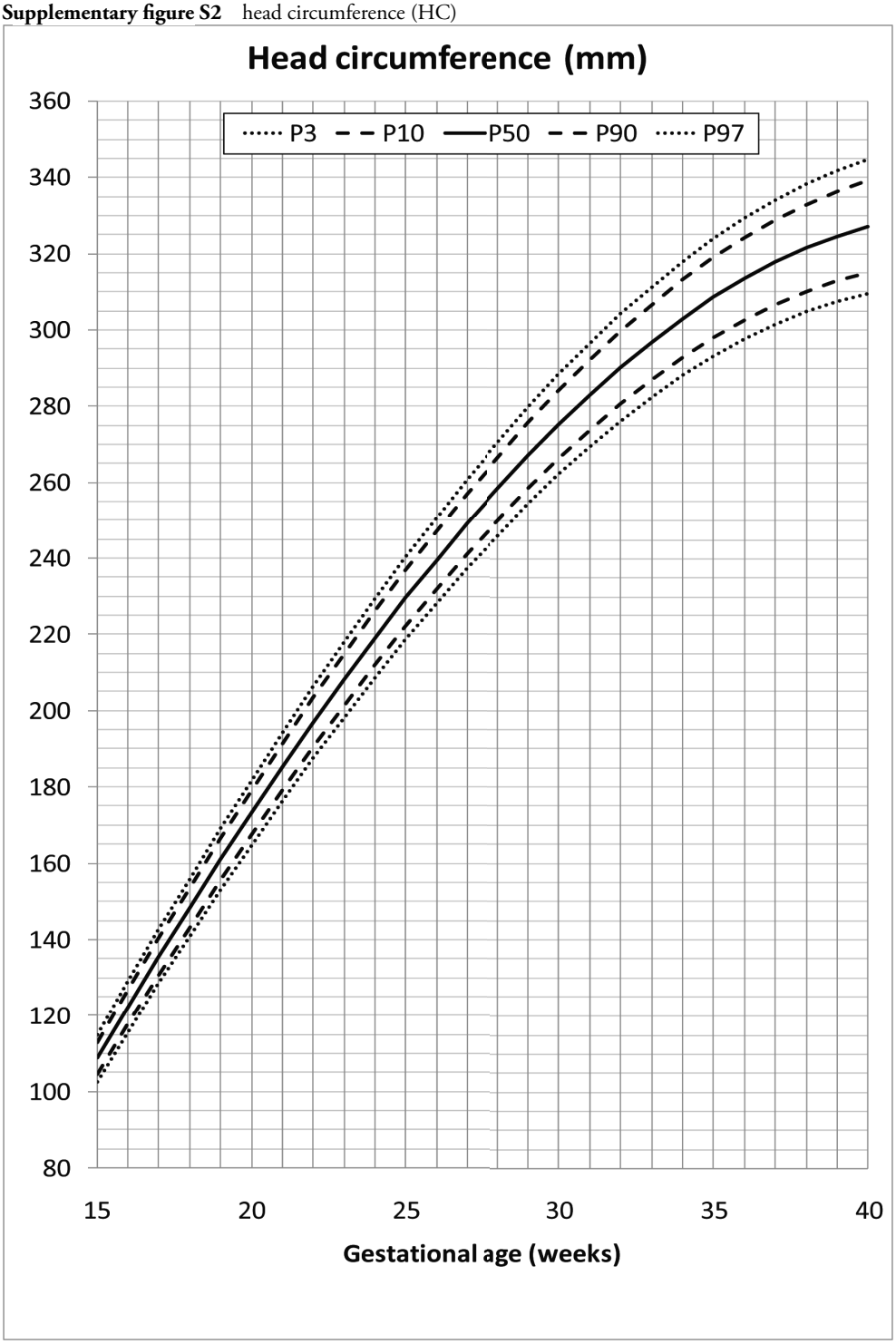
Supplementary figure S1 biparietal diameter (outer–inner; BPD)



Supplementary table S2 Head circumference in mm

Weeks of gestation	Percentiles										
	P2.3	P3	P5	P10	P25	P50	P75	P90	P95	P97	P97.7
15	102.3	102.5	103.3	104.5	106.5	108.7	110.9	112.9	114.2	114.9	115.2
16	115.3	115.6	116.4	117.7	119.8	122.2	124.6	126.7	128.1	128.9	129.1
17	128.0	128.3	129.2	130.6	132.9	135.4	138.0	140.3	141.7	142.5	142.8
18	140.5	140.8	141.7	143.2	145.7	148.4	151.1	153.5	155.0	155.9	156.3
19	152.6	153.0	153.9	155.5	158.1	161.0	163.9	166.5	168.0	169.0	169.4
20	164.4	164.8	165.8	167.5	170.3	173.3	176.3	179.1	180.8	181.8	182.2
21	175.9	176.3	177.4	179.2	182.1	185.3	188.5	191.4	193.1	194.2	194.6
22	187.1	187.5	188.6	190.5	193.6	196.9	200.3	203.3	205.2	206.3	206.7
23	197.9	198.3	199.5	201.4	204.6	208.2	211.7	214.9	216.8	218.0	218.5
24	208.3	208.7	210.0	212.0	215.4	219.0	222.7	226.1	228.1	229.4	229.8
25	218.3	218.7	220.0	222.2	225.7	229.5	233.4	236.9	239.0	240.3	240.8
26	227.8	228.3	229.7	231.9	235.6	239.6	243.6	247.2	249.4	250.8	251.3
27	237.0	237.5	238.9	241.2	245.0	249.2	253.4	257.2	259.5	260.9	261.4
28	245.7	246.2	247.7	250.1	254.0	258.4	262.7	266.6	269.0	270.5	271.0
29	253.9	254.4	256.0	258.5	262.6	267.1	271.6	275.7	278.1	279.7	280.2
30	261.6	262.2	263.8	266.4	270.6	275.3	279.9	284.2	286.8	288.4	288.9
31	268.9	269.5	271.1	273.8	278.2	283.0	287.8	292.2	294.9	296.5	297.1
32	275.6	276.2	277.9	280.7	285.2	290.2	295.2	299.7	302.5	304.2	304.8
33	281.8	282.4	284.2	287.0	291.7	296.8	302.0	306.7	309.5	311.3	311.9
34	287.4	288.0	289.9	292.8	297.6	303.0	308.3	313.1	316.0	317.9	318.5
35	292.5	293.1	295.0	298.0	303.0	308.5	314.0	319.0	322.0	323.9	324.5
36	297.0	297.6	299.6	302.7	307.8	313.5	319.1	324.2	327.3	329.3	330.0
37	300.8	301.5	303.5	306.7	312.0	317.8	323.6	328.9	332.1	334.1	334.8
38	304.1	304.8	306.8	310.1	315.6	321.5	327.5	332.9	336.2	338.3	339.0
39	306.7	307.4	309.5	312.9	318.5	324.6	330.8	336.4	339.7	341.9	342.6
40	308.7	309.4	311.6	315.1	320.8	327.1	333.4	339.1	342.6	344.8	345.5

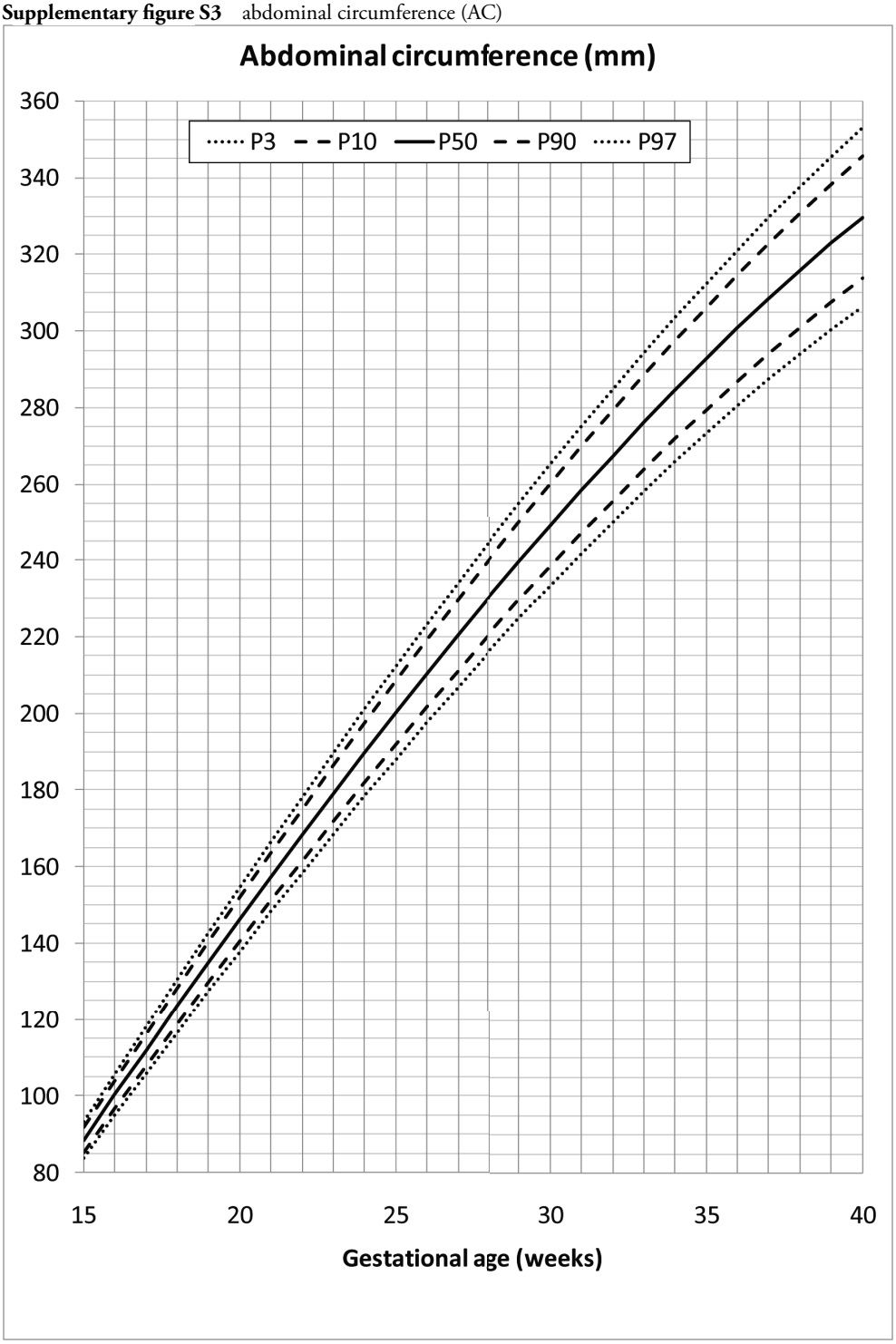
Supplementary figure S2 head circumference (HC)



Supplementary table S3 Abdominal circumference in mm

Weeks of gestation	Percentiles										
	P2.3	P3	P5	P10	P25	P50	P75	P90	P95	P97	P97.7
15	83.6	83.7	84.3	85.2	86.8	88.4	90.1	91.6	92.5	93.1	93.3
16	94.6	94.8	95.5	96.5	98.3	100.2	102.2	103.9	105.0	105.7	105.9
17	105.5	105.8	106.5	107.7	109.7	111.9	114.1	116.1	117.3	118.1	118.4
18	116.3	116.6	117.4	118.8	121.0	123.5	125.9	128.2	129.6	130.4	130.7
19	126.9	127.2	128.2	129.7	132.2	134.9	137.6	140.1	141.6	142.6	142.9
20	137.4	137.8	138.8	140.5	143.2	146.2	149.2	151.9	153.6	154.6	154.9
21	147.8	148.1	149.3	151.1	154.0	157.3	160.6	163.5	165.3	166.5	166.9
22	157.9	158.4	159.6	161.5	164.7	168.3	171.8	175.0	177.0	178.2	178.6
23	168.0	168.4	169.7	171.8	175.3	179.1	182.9	186.3	188.4	189.7	190.2
24	177.8	178.3	179.7	181.9	185.6	189.7	193.8	197.4	199.7	201.1	201.6
25	187.5	188.0	189.5	191.9	195.8	200.1	204.5	208.4	210.8	212.3	212.8
26	196.9	197.5	199.1	201.6	205.8	210.4	215.0	219.2	221.7	223.3	223.8
27	206.2	206.8	208.5	211.1	215.6	220.4	225.3	229.7	232.4	234.1	234.6
28	215.3	215.9	217.7	220.5	225.2	230.3	235.4	240.1	242.9	244.7	245.3
29	224.1	224.8	226.6	229.6	234.5	239.9	245.3	250.2	253.2	255.0	255.7
30	232.8	233.5	235.4	238.5	243.7	249.3	255.0	260.1	263.3	265.2	265.9
31	241.2	241.9	243.9	247.2	252.6	258.5	264.4	269.8	273.1	275.1	275.8
32	249.4	250.1	252.2	255.6	261.3	267.5	273.7	279.3	282.7	284.8	285.6
33	257.3	258.1	260.3	263.8	269.7	276.2	282.6	288.5	292.1	294.3	295.1
34	265.0	265.8	268.1	271.8	277.9	284.6	291.4	297.5	301.2	303.5	304.3
35	272.4	273.2	275.6	279.5	285.8	292.8	299.8	306.2	310.0	312.4	313.3
36	279.5	280.4	282.9	286.9	293.5	300.8	308.0	314.6	318.6	321.1	322.0
37	286.4	287.3	289.9	294.1	300.9	308.4	315.9	322.8	326.9	329.5	330.4
38	293.0	293.9	296.6	300.9	308.0	315.8	323.6	330.7	335.0	337.6	338.6
39	299.3	300.3	303.1	307.5	314.8	322.9	330.9	338.3	342.7	345.5	346.4
40	305.3	306.3	309.2	313.8	321.4	329.7	338.0	345.6	350.2	353.0	354.0

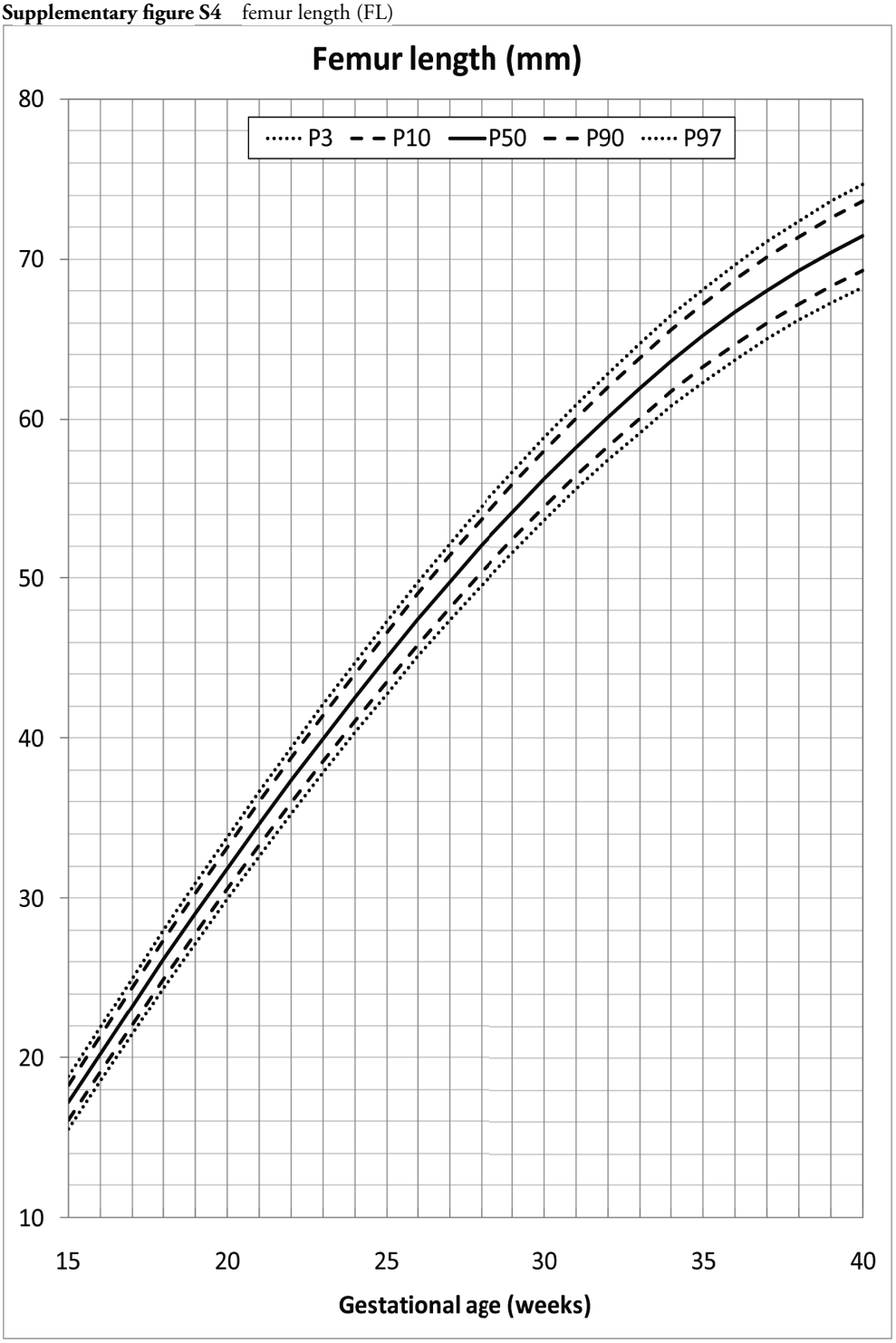
Supplementary figure S3 abdominal circumference (AC)



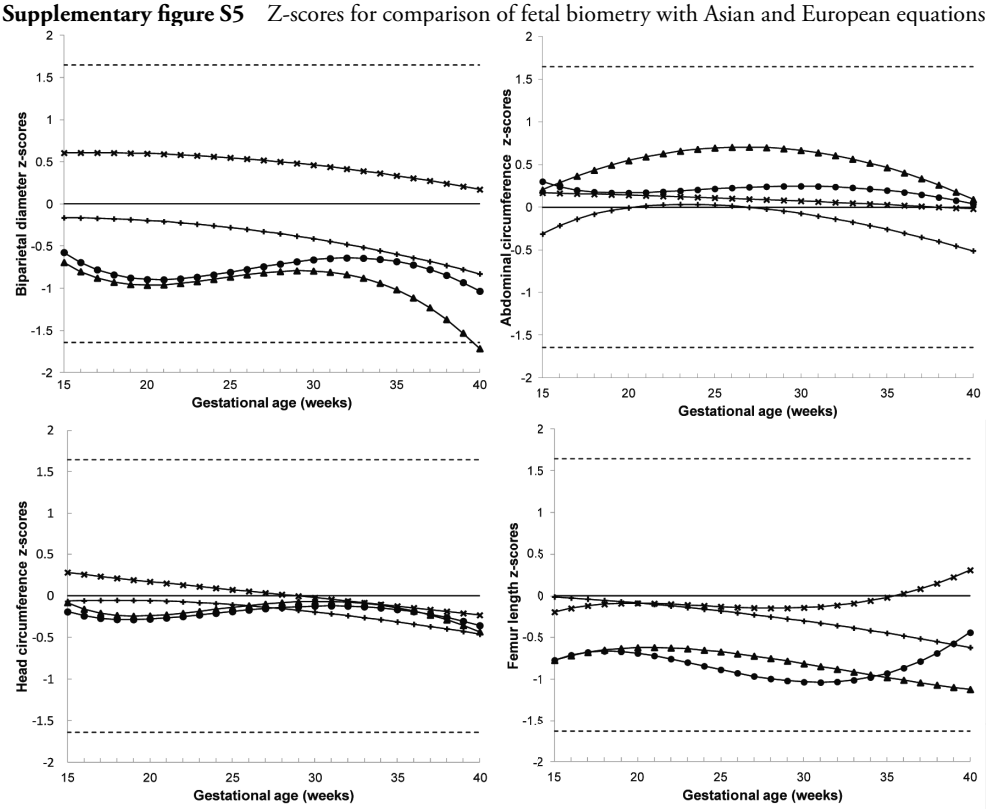
Supplementary table S4 Femur length in mm

Weeks of gestation	Percentiles										
	P2.3	P3	P5	P10	P25	P50	P75	P90	P95	P97	P97.7
15	15.5	15.6	15.7	16.1	16.6	17.2	17.8	18.3	18.6	18.8	18.9
16	18.5	18.5	18.7	19.1	19.6	20.2	20.8	21.4	21.7	21.9	22.0
17	21.4	21.5	21.7	22.0	22.6	23.2	23.8	24.4	24.7	25.0	25.0
18	24.3	24.3	24.6	24.9	25.5	26.2	26.8	27.4	27.7	28.0	28.0
19	27.1	27.2	27.4	27.8	28.4	29.0	29.7	30.3	30.7	30.9	31.0
20	29.8	29.9	30.2	30.5	31.2	31.9	32.6	33.2	33.6	33.8	33.9
21	32.5	32.6	32.9	33.3	33.9	34.6	35.4	36.0	36.4	36.6	36.7
22	35.2	35.3	35.5	35.9	36.6	37.3	38.1	38.7	39.2	39.4	39.5
23	37.7	37.8	38.1	38.5	39.2	40.0	40.7	41.4	41.8	42.1	42.2
24	40.2	40.3	40.6	41.0	41.8	42.5	43.3	44.0	44.5	44.7	44.8
25	42.7	42.8	43.0	43.5	44.2	45.0	45.8	46.6	47.0	47.3	47.4
26	45.0	45.1	45.4	45.9	46.6	47.4	48.3	49.0	49.5	49.8	49.9
27	47.3	47.4	47.7	48.1	48.9	49.8	50.6	51.4	51.9	52.2	52.3
28	49.5	49.6	49.9	50.4	51.2	52.0	52.9	53.7	54.2	54.5	54.6
29	51.6	51.7	52.0	52.5	53.3	54.2	55.1	55.9	56.4	56.7	56.8
30	53.6	53.7	54.0	54.5	55.3	56.3	57.2	58.0	58.5	58.8	59.0
31	55.5	55.6	55.9	56.5	57.3	58.3	59.2	60.1	60.6	60.9	61.0
32	57.3	57.4	57.8	58.3	59.2	60.1	61.1	62.0	62.5	62.8	63.0
33	59.0	59.2	59.5	60.0	60.9	61.9	62.9	63.8	64.4	64.7	64.8
34	60.7	60.8	61.1	61.7	62.6	63.6	64.6	65.6	66.1	66.5	66.6
35	62.2	62.3	62.7	63.2	64.2	65.2	66.2	67.2	67.7	68.1	68.2
36	63.6	63.7	64.1	64.7	65.6	66.7	67.7	68.7	69.3	69.6	69.8
37	64.9	65.0	65.4	66.0	67.0	68.0	69.1	70.1	70.7	71.1	71.2
38	66.1	66.2	66.6	67.2	68.2	69.3	70.4	71.4	72.0	72.4	72.5
39	67.1	67.3	67.7	68.3	69.3	70.4	71.6	72.6	73.2	73.6	73.7
40	68.1	68.2	68.6	69.3	70.3	71.4	72.6	73.6	74.3	74.7	74.8

Supplementary figure S4 femur length (FL)



Supplementary figure S5 Z-scores for comparison of fetal biometry with Asian and European equations



Comparison of the Switzerland[1, 2] (×), China[3] (●), Korea[4] (▲) and present study (+) equations with the UK[5-7] equations for mean BPD, HC, AC and FL.
Mean expected z-score, or 50th percentile is shown as straight black line, dashes lines represent the expected z-scores for the 5th and 95th centiles, i.e. -1.645 and 1.645 respectively. The x-axis shows the gestational age in weeks, the y-axis the z-score

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Chapter 7

Refugee and Migrant Women's Views of Antenatal Ultrasound on the Thai-Burmese border: a Mixed Methods Study

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Keywords
Ultrasound; Antenatal; Pregnancy; Refugee; Migrant; Communication; Thailand; Burma

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Abstract

Background

Antenatal ultrasound suits developing countries by virtue of its versatility, relatively low cost and safety, but little is known about women's or local provider's perspectives of this upcoming technology in such settings. This study was undertaken to better understand how routine obstetric ultrasound is experienced in a displaced Burmese population and identify barriers to its acceptance by local patients and providers.

Methodology/Principal Findings

Qualitative (30 observations, 19 interviews, seven focus group discussions) and quantitative methods (questionnaire survey with 644 pregnant women) were used to provide a comprehensive understanding along four major themes: safety, emotions, information and communication, and unintended consequences of antenatal ultrasound in refugee and migrant clinics on the Thai-Burmese border. One of the main concerns expressed by women was the danger of childbirth which they mainly attributed to fetal malposition. Both providers and patients recognized ultrasound as a technology improving the safety of pregnancy and delivery. A minority of patients experienced transitory shyness or anxiety before the ultrasound, but reported that these feelings could be ameliorated with improved patient information and staff communication. Unintended consequences of overuse and gender selective abortions in this population were not common.

Conclusions/Significance

The results of this study are being used to improve local practice and allow development of explanatory materials for this population with low literacy. We strongly encourage facilities introducing new technology in resource poor settings to assess acceptability through similar inquiry.

Introduction

Antenatal ultrasound has become part of standard antenatal care in the developed world1. This technology equally suits developing countries as well by virtue of its versatility, relatively low cost and safety[2-4] compared with other imaging modalities. In clinics in western Thailand, serving migrant workers and refugees from Burma, obstetric ultrasound has been adopted as part of routine antenatal care since 20015. Yet it is not known how this technology is viewed by pregnant women, or by the local providers implementing the system. Recent literature highlights the usefulness of antenatal ultrasound in developing country settings[2, 6-8], but at the same time over-and misuse of ultrasound have been reported[9, 10].

Globally, not much is known about women's or provider's perspectives of obstetric ultrasound in low income settings. A systematic review of the literature on women's views of pregnancy ultrasound[11] identified one district hospital in Botswana[12], where ultrasound scanning was associated with significant psychological stress and anxiety in pregnant women, especially when accompanied by minimal explanation by healthcare providers. In different settings in Nigeria women were satisfied with most aspects of antenatal ultrasound experience[13], but incorrect determination of fetal sex had an important negative impact on women's psychosocial health and general acceptance of antenatal ultrasound[14].

By contrast, in industrialized countries ultrasound scanning is associated with positive emotion: hope, reassurance and a sense of enhanced connection with the fetus[11, 15-18]. Most women appreciate seeing the image of the fetus and hearing verbal reassurance from the ultrasonographer[19]. This social component is so prominent that women may be unaware of the medical indications for the procedure and potentially unprepared for adverse findings[20].

This study was undertaken to better understand how routine obstetric ultrasound is experienced in a developing country setting, in particular in a displaced Burmese patient population. The results of this study are being used to improve local practice and allow development of explanatory materials for this population with low literacy[5, 21].

Methods

Background and study population:

This investigation took place in the Shoklo Malaria Research Unit (SMRU) antenatal clinics (ANC) of Maela refugee camp (MLA), Mawker Thai (MKT), and WangPha (WPA), as well as two mobile clinics under supervision of MKT (see **Figure 1.1 on page 11**). The SMRU is located on the Thai-Burmese border and has conducted research focused on the epidemiology, prevention and treatment of malaria in pregnancy since 1986. This has included provision of free obstetric and medical care for the local Burmese population, mostly of the Karen ethnic minority. The border population in this

area consists of a mixture of Buddhist and Christian groups, with Muslims constituting a significant minority, more in the refugee than migrant communities. The refugee situation is one of the oldest in the world. As a low proportion of women could reliably provide the date of their last menstrual period[21], antenatal ultrasound was introduced in 2001 to improve gestational age estimation. Furthermore, ultrasound examination of the fetus is a powerful tool to detect multiple pregnancy, placental localization and intra-uterine growth restriction. Ten locally trained health workers perform ultrasound scans at all sites free of charge, supervised by doctors certified in ultrasound scanning[5].

Ethics

This investigation was part of a larger fetal growth study (ClinicalTrials.gov Identifier: NCT00840502), and was approved by Oxford University (OxTREC (14-08)) and Mahidol University (TMEC 2008-028) Ethics Committees.

Data collection

Qualitative (observations, interviews, focus group discussions (FGD)[22]) and quantitative methods (questionnaire survey) were used to provide a comprehensive understanding of the subject. The techniques were employed iteratively, with the results from one method feeding into the development of subsequent data collection tools, focused on four major themes: safety, emotions, information and communication, and unintended consequences of antenatal ultrasound.

Observations of ultrasound scans were used to develop a topic guide for semi-structured interviews with a selection of pregnant women. Native speakers (including authors MM and KML) conducted the interviews, which were recorded with the participants' permission. The recordings were transcribed into English language and confirmed by a second interpreter. One author (MEG) interviewed experienced midwives who worked in the ANCs since before 2001 to elicit information on the impact of the introduction of ultrasound on midwifery practice. Subsequently, FGDs with providers (one group) and pregnant women (six groups stratified by language and religion) were organized to further investigate issues raised during the individual interviews. These were analyzed within the framework of the four themes. Finally, a questionnaire was designed to investigate whether the interviews and FGDs reflected pregnant women using the ANC services as a whole. Due to low literacy in this population[21], these were facilitated by local staff trained to obtain information anonymously and confidentially without suggesting responses. All women presenting to the ANC clinics over the course of a month were invited to complete the survey once, and women involved in the FGDs and interviews were excluded.

Statistical analysis

The results of the questionnaires were entered into a Microsoft Access database and analyzed using SPSS version 18 (SPSS Inc., Chicago Ill, USA). Student's t-test and Mann-Whitney test were used for comparison of means and ranks respectively.

Categorical data were compared using the chi-squared test or the Fisher's exact test, as appropriate, with Bonferroni correction in case of multiple comparisons.

Results

Between November 2010 and February 2011, 30 ultrasound scans were observed and 19 interviews were conducted; 17 with pregnant women and two with senior midwives. The seven FGDs included one with four sonographers, three with six Christian, Buddhist and Muslim women each, two with Karen (six women) and Burmese (seven women) from mixed religious backgrounds, and a mixed group of six participants. The discussions lasted from 30 minutes to an hour. The questionnaire (See **Supplementary file S1**) was completed by 67% (644/964) women who attended the ANC and were eligible (**Table 7.1**).

Table 7.1 Demographics of women participating in the questionnaire

		N=644
Woman's age, years		26.0 [15-47]
Woman's marriage number		1 [1-3]
Husband's age, years		28.5 [17-65]
Husbands marriage, number		1 [1-4]
Number of pregnancies		2 [1-12]
Parity (number of delivered infants)		1 [1-9]
Residence on the Thai-Burmese Border, months		48 [0-576]
Schooling, years		4 [0-16]
Previous ultrasound scans		2 [0-13]
Location	Maela refugee camp	53.7 (346)
	Mawker Thai	15.7 (101)
	Wang Pha	30.6 (197)
Teenager		14.8 (95)
Reports ability to read		64.4 (415)
Reports ability to write		64.0 (412)
Religion	Buddhist	69.6 (448)
	Christian	21.0 (135)
	Muslim	9.3 (60)

Data are in median [range], or percentage (number)

Safety

Safe Pregnancy and Delivery
Forty-one percent of the interviewed women (7/17) highlighted the danger of pregnancy, when asked about the usefulness of ultrasound. Women were primarily concerned about how antenatal care and the use of ultrasound could increase the safety of what they see as a potentially life threatening event of childbirth.

"I came to SMRU because pregnancy is dangerous… I came for safety and deliver here. Home delivery is not safe. Before ultrasounds, women would deliver in the village and they wouldn't know the baby's position. Because they might try to deliver a baby that was in the wrong position in the village, they would have serious problems with bleeding and other things" [23 yo Karen G1 at WPA]

The most common concern noted in the interviews and FGDs was the position of the fetus. Other safety concerns mentioned included bleeding, premature delivery, multiple pregnancies (twins), and miscarriage. A 38-year old woman in MLA stated:

"I have had many pregnancies so I am afraid of complications. If tharamu [word of respect for someone knowledgeable e.g teacher, midwife] ***does the ultrasound then she can detect problems ahead of time, and maybe she can even save my life."***

In addition to fetal position, the experienced midwives highlighted early pregnancy bleeding and antepartum hemorrhage as examples of potential obstetric emergencies, where ultrasound had improved practice safety and decreased the need for referral:

"Before the ultrasound, if someone came in with early pregnancy bleeding we could not do a dilatation and curettage because we did not know about if there was a fetal heartbeat or not. With ultrasound now we can know the presentation, the location of the placenta, about any fetal abnormalities, and about the fluid level. Before ultrasound we estimated based on the clinical exam but we can know more with ultrasound. For example, before if there was antepartum hemorrhage we might not be sure if just close to delivery, or if it was placenta praevia. Before, we would refer all women with antepartum haemorrhage to the hospital, but with the ultrasound we can check and only refer if there is an indication." [45 year old midwife with 25 years experience]

The greater certainty in diagnosis and therefore improved safety for patients convinced both midwives that antenatal ultrasound is beneficial. In the group discussion with the sonographers, who are mostly unmarried and younger women, safety was not raised as a primary concern. They expressed increased personal interest, but also some distress, when abnormal findings were found.

In an open ended question in the survey, determination of fetal position was the most commonly named reason for ultrasound (**Table 7.2**). These results, however, differed somewhat by site, with the majority of patients at MLA and one of the mobile sites reporting gender determination most frequently as the reason for performing the ultrasound.

Table 7.2 Responses to open questions in a questionnaire among 644 pregnant women on the Thai-Burmese border

Why does SMRU do ultrasound scanning for pregnant women?	1696 answers % (N)
Position of the baby	22.1 (375)
Confirmation of pregnancy	20.2 (343)
Health of the baby	18.8 (318)
Sex of the baby	17.3 (293)
Normal hands and feet	8.7 (148)
Baby's breathing	6.8 (116)
At your most recent ultrasound, what did the staff explain to you before they started?	1414 answers % (N)
I would need to lie down	40.2 (569)
I would need to open my sarong	23.1 (326)
A machine would be used to check the baby	14.8 (209)
During or after the most recent ultrasound, what did they tell you?	1224 answers % (N)
Everything is okay	24.5 (300)
They told me the sex of the baby	19.9 (243)
They told me the position	16.3 (200)
They told me that I am pregnant	10.5 (129)
Nothing	8.6 (105)

This table refers to questions 6-8 of the questionnaire in **Supplementary file S1**

Abnormal findings

The interviewed midwives raised the concern that women may discontinue antenatal care after abnormal results found by ultrasound are given to them. Such women sometimes go to traditional birth attendants (TBAs) for treatments or to seek unsafe abortions. One example is that of a woman who learns that the fetus is in breech position. If there were no contraindications she would routinely be scheduled for an external cephalic version – a process of rotating the near-term fetus using external pressure on the abdomen, while monitoring the fetal wellbeing with ultrasound. In the clinic this is always performed by a physician and only if there is an emergency car available for transport to a referral hospital in case of complications. However, TBAs in the community

also provide this service, sometimes with tragic results. In the surveys, 6.2% (40/644) of respondents reported they would seek care with a TBA in addition to continuing care at the SMRU clinic if told the fetus was breech. These responses were independent of parity but were more common among Buddhist patients (8.3%) compared to Muslim (3.3%) and Christian (0.7%), the latter being significantly different, p=0.021 (**Table 7.3**). There was a trend toward higher frequency in TBA visits in illiterate patients; of concern one illiterate Buddhist multiparous woman reported that she would seek care with a TBA only in this situation, and not with the SMRU clinic. If the fetus was found to be "abnormal" by ultrasound 3.1% (20/644) of women reported they would seek care with a TBA in addition to SMRU, and 1.7% (11/644) would do so if there were no fetal heartbeat found (**Table 7.3**). There was no deeper questioning about why these choices would be made.

Safety of the Ultrasound scan

Women were almost unanimous in reporting that they felt ultrasound scanning was safe to them and their babies. This confidence was attributed both in the interviews and the FGDs to faith in the providers at the clinic:

"If tharamu says there is no problem, then I think there is no problem. If there were a problem, she would tell me. So I am not worried" [29 yo Burmese G1]**.**

After the official discussion in one FGD, a pregnant medic asked if there were any risks to repeated scans. She referred to a rumor in MLA that ultrasound could damage the fetal brain, but was not sure whether the ultrasound scan were performed because of a brain abnormality. The sonographers reported that Burmese patients expressed more concern about safety of the ultrasound than Karen patients, but that all patients appeared satisfied with some reassurance.

"Some patients think if we do the ultrasound frequently then there will be some danger to the baby." [23 year old sonographer, 4 years experience]

In the surveys, 5.1% (33/644) of respondents reported that they believed it could be dangerous, with no differences between gravidity or religion.

Emotions: Shyness and anxiety

Experiences of shyness and anxiety were noted during the observations and were themes that emerged in the interviews. In each room one sonographer and two other staff members engaged in interviewing patients were present with the pregnant women, who occasionally brought small children into the room. Usually another pregnant woman was already waiting inside the room as well. In ultrasound rooms that were not fully enclosed and private, women showed body language consistent with discomfort – squirming, attempting to cover the belly etc. In this community, where breastfeeding in

public is accepted, the abdomen is treated with particular modesty. "Showing the belly" was commonly mentioned in the interviews and FGDs as a notable part of prenatal care. The sonographers reported that some women try to cover their abdomen before the scan is completed, and that this embarrassment was more common in younger women. On the other hand, pregnant women that received multiple scans appeared relaxed, even bored.

Table 7.3 Responses to "what would you do if?" questions in a questionnaire among 644 pregnant women on the Thai-Burmese border

	Multi (n=440)	Primi (n=203)	Cannot read (n=217)	Can Read (n=415)	Many US (n=441)	First US (n=202)	Buddhist (n=447)	Christian (n=135)	Muslim (n=60)
What would do you if the ultrasound tells you your baby is in the breech presentation?									
Continue normal ANC	411	191	201	390	412	190	409	134	58
ANC + TBA	28	12	15	25	28	12	37	1	2
TBA	1	0	1	0	1	0	1	0	0
I do not know	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0
What would do you if the ultrasound tells you your baby is abnormal?									
Continue normal ANC	427	196	212	400	428	195	430	132	60
ANC + TBA	13	7	5	15	13	7	17	3	0
TBA	0	0	0	0	0	0	0	0	0
I do not know	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0
What would do you if the ultrasound tells you your baby has no Fetal Heart Beat?									
Continue normal ANC	433	196	213	405	434	195	433	135	60
ANC + TBA	6	5	4	7	6	5	11	0	0
TBA	0	0	0	0	0	0	0	0	0
I do not know	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0
What would do you if the ultrasound tells you are pregnant, but you do not want this pregnancy?									
Continue normal ANC	403	188	201	379	405	186	410	127	53
ANC + TBA	29	8	13	24	25	12	34	2	1
TBA	7	4	3	8	8	3	1	4	6
I do not know	1	1	0	2	2	0	1	1	0
Other	0	0	0	0	0	0	0	0	0

This table refers to questions 14-17 of the questionnaire in **Supplementary file S1**

When the emotional impact of the ultrasound was initially probed in the interviews by asking the women to describe what happened at their first ANC visit, 71% (12/17) of the women did not mention the ultrasound at all. Positive and neutral feelings by far

exceeded negative feelings and many seemed to include it as part of the routine obstetric exam. Women used terms conveying, "It was no big deal, it was no problem." Women expressed that they were "happy", often in the context of relief to know that the pregnancy is confirmed, that the fetal position was correct and that the baby appeared alive and normal. A minority of women expressed negative emotions that seemed to reflect the discomfort noted in the ultrasound observation.

"I am a little embarrassed about the pregnancy because they uncovered my belly. So I am a little shy" [29 yo Burmese G1 from MKT]**.**

Another stated she felt

"Ashamed because it was in front of all the other pregnant women" [28 yo Burmese G3P2]

when presenting for her first ultrasound at a migrant clinic where the scan is done in the general waiting area. However, when probed further she said she would have still felt shy even if the scan was done in private. Women in the FGDs reported that this shyness or shame disappeared completely by the second ultrasound. All agreed that it was not a problem for a male healthcare provider to enter the room when needed. In the FGDs, embarrassment was reported most strongly in the Muslim group (4/6), followed by the Buddhist (2/6), and Christian group (0/6). The questionnaire showed a significantly higher prevalence of embarrassment among primiparous women (18.2% (37/203)) compared to multigravidae (11.6% (51/440)), p=0.023, those experiencing their first ultrasound (17.8 (36/202)) vs many ultrasound scans (11.8% (52/441)), p=0.039, and Muslim religion (28.3% (17/60) vs Christian (6.7% (9/135)), p<0.001, but there was no significant difference in shyness based on education or literacy (supplementary table 1). Women reported a greater degree of shyness at WPA (19.7% (39/197)), where scans were done in the semi-private room, than in MLA (11.3% (39/345)), where the ultrasound room is private (closed wooden door, high walls), p=0.032.

The second negative emotion expressed was a sense of anxious anticipation:

"My heart was racing… because I have never had an experience with this machine before" [19 yo G1 Karen at MKT]**.**

In the individual interviews, this was exclusively expressed by women presenting for their first ultrasound. Women in the FGD and the individual interviews stated that this feeling disappeared immediately after the ultrasound scan started and was not present for the second ultrasound. In the questionnaire, this anxiety was more common at MLA with the private ultrasound room, where women cannot see what happens to the women who went ahead of her (26.1% (90/345) compared to WPA (17.8% (35/197)),

p=0.027. Women in the FGD stated that provision of further information prior to the ultrasound would greatly reduce this anxiety.
Though the questionnaire confirmed a decrease in embarrassment and anxiety with greater experience, it did not corroborate the consensus of the FGDs and individual interviews that these emotions were confined to the initial ultrasound experience. Among veteran ultrasound users, 11.8% (52/441) reported shyness at their most recent ultrasound and 20.6% (91/441) reported anxiety. As with embarrassment, anxiety levels differed by religious group: most commonly reported by Muslim women (50.0% (30/60)), followed by Christian (26.7% (36/135)) and Buddhist (17.7% (79/447)), p<0.001.

Information and Communication
During the ultrasound observations, minimal sonographer communication with patients was noted. All sonographers were bi- or tri-lingual (Karen, Burmese and in most cases English language) but they frequently chatted in their primary language with one another. Patients who shared the same primary language sometimes joined these conversations. Women of other language groups lay in silence or, rarely, asked a question.
Overall, the counseling varied depending on the indication for the ultrasound. For all patients presenting for their first ultrasound, prior history and risk factors were reviewed as routine practice. No major differences were observed among the sonographers. Minimal explanation was given to women having their dating or routine biometry scan, although such scans could take 30 minutes or more. At the other extreme, scans for placenta position lasted less than five minutes but, in cases of low-lying placenta (two of 30 scans observed), were accompanied by concurrent counseling that exceeded the time spent performing the scan. In the one case observed in which there were catastrophic findings – no fetal heart beat at term – minimal explanation was given to the patient until a midwife was asked for help.

Any counseling about ultrasound process or results, reported in the interviews and FGDs, was minimal, and generally restricted to friendly spoken directives: "lie down, open your sarong" etc:

"They told me that the baby is well and the position is okay, and then counseled me about what to avoid in pregnancy and other things" [29 yo G1 Burmese at MKT]**.**

This was supported by the 1,414 answers to the question "At your most recent US, what did the staff explain to you before they started to scan?". The most frequent answers in all sites were: "I would need to lie down" (88.4% (569/644)), "I would need to open my sarong" (50.6 % (326/644)) and "a machine would be used to check the baby" (32.5% (209/644)). The third most common response at WPA was "it is safe" (30.5% (60/197))

and more than 20% of the women at the two mobile ANC sites reported they were told "nothing" before the test.
As noted above, women in the interviews and FGDs expressed knowledge that the ultrasound was used to detect potential problems for delivery (malpresentation, twins), confirmation of pregnancy, checking for fetal health ("breathing", movement, if the baby is strong or not), ruling out abnormal development (normal hands and feet) and determining gender. The questionnaire showed a variety of responses both for content of post ultrasound counseling and understanding of the reasons for the test (**Table 7.2**). Women at MLA reported most often being counseled about fetal sex and reported this most often as the reason for the scan, while women at WPA reported fetal position as the primary reason for the test. This suggested that, even in this setting of minimal counseling, patients did internalize as significant the information they received.

Sonographers felt that time pressure, due to patient volume, limits their ability to give counseling beyond the essentials. They noted that women rarely ask questions. When they do, the most common questions are about gender, position, fetal heart beat and whether or not the infant was normal.

Patient satisfaction with levels of communication was probed in the FGDs. Most women expressed receiving some feedback about the scan, often "everything is okay", and this was felt to be sufficient. General statements from the sonographer about fetal health, position and gender were most commonly reported. A minority of women reported asking about these topics. Several women reported not receiving any counseling at all. Though some were content with this, others expressed continued curiosity and desire for further counseling. Others expressed having received detailed counseling about one scan, which they appreciated and this single episode of education seemed to satisfy them for future scans as well.

All women in the FGDs said that they would have liked to see the fetal image on the screen, with the exception of the Muslim group and one teenager in the mixed group. Most women in the interviews and FGDs stated that they could not see, or didn't know what they were looking at.

"I saw small spots running around the screen" [28 yo Burmese G3P2 at WPA]**.**

Only two women in the FGDs reported that the sonographer showed her images of fetus and explained what was happening with it. Both women expressed very positive feelings about that experience, even though, for one it was in the setting of a miscarriage. One other woman reported that, though she couldn't see her own fetus on the screen, she recognized someone else's fetus while she was waiting in the room.

Overall, 90.4% (582/644) of women answered that they wished to see the screen, and 39.4% (254/644) reported that they were able to see it (**Supplementary table 7.1**). Due to space considerations, the ease with which patients are able to see the screen differs significantly by site. The desire to see the screen was slightly higher in non-Buddhists, experienced patients and those who were literate. Interest was lowest in MKT (64.9 (24/37)) and the mobile sites (51.2 (21/41)), but above 90% in the larger clinics (MLA 98.0%, WPA 94.9%).

Another special topic of information sharing was sex determination. As in the developed world, many women in this community enjoy knowing the gender of their unborn child.

"I think it is good to know the gender so you can prepare in advance; so you can dream for the future." [23 yo Karen primigravid WPA]

The ultrasonographers noted that gender was the most commonly asked question, and that they told patients, "when we remember". Some sonographers admitted to sometimes rescanning women who were really curious about gender later in the day, after the regular scans were complete. Desire to know gender was reported by almost all participants (98.4% (634/643)) but disclosure differed markedly by site: 22.8% (45/197) in WPA and 53.5% (185/345) in MLA, p<0.001.

Unintended Consequences: Gender Selection and Overuse

Located in Asia where gender selective practices are common[23, 24], questions were raised at all levels of the study to assess the risk of unintentionally facilitating gender selective abortion by introducing ultrasound. Unlike other populations, a preference for males is not as strongly held in this community, so the inquiry included termination of any pregnancy due to non-desired gender. As noted above, almost all participants expressed a desire to know the gender of the fetus. When asked directly in the interview setting, none of the participants expressed an intention to seek an abortion if told that they are carrying the less-desired gender.

"If it is a girl, I want a girl. If it is a boy, I want a boy" [21 yo Karen G2P1 at MKT]**.**

"No, I would not think of [an abortion], it is my own flesh and blood" [25 yo Karen G3P2 at MLA].

Three women responded that they had heard of gender selective abortion in their communities. The experienced midwives expressed that they had seen many women present for care after unsafe abortion, but had not heard of this practice for gender selection. All FGDs reported knowing of abortions in their community, but that these were almost always for unwanted pregnancies in general, regardless of gender, and usually occurred before gender was known. While disapproving abortion in general, and gender

selection specifically, women in the FGDs reported that this is an uncommon occurrence. Only 0.6% (4/644) respondents reported that they had heard of women seeking abortion after learning from an SMRU ultrasound that they are carrying a child of the undesired gender. Both in the FGD and in the questionnaire, Muslim women reported that no abortions are attempted for any reason in their community, which does not reflect clinical experience (unpublished data).

Special attention was paid to determine what impact the presence of antenatal ultrasound has on care-seeking behavior in this patient population. None of the women in the individual interviews named the ultrasound as a primary reason for seeking care at SMRU's ANCs. The report by the sonographers that they would occasionally repeat or extend a scan to look for gender, suggests that there may be a risk for patient demand for ultrasounds, but at this point such demand appears to be low.

Discussion

Qualitative studies on obstetric ultrasound in the developed world have focused on feelings of expectation, possibility, enhanced bonding (both maternal and paternal) with the fetus, and concern about the possibility of fetal anomaly[19]. One of the main concerns of women in this study was the danger of childbirth which they mainly attributed to fetal position. This correlates with the objective risk of pregnancy in this area: maternal and neonatal mortality in developing countries may be over hundred times higher than in western countries[25] (McGready, submitted). One of the top priorities of the Millennium Development Goals is to reduce maternal mortality. A large number of maternal deaths are caused by conditions that could be prevented or managed with the assistance of ultrasound, such as fetal malposition[7].

Although happiness was an emotion frequently endorsed by patients, transient embarrassment or shame on exposing the abdomen (a part not normally exposed in public by local women in this culture) was noted by primigravids or teenagers. Anxiety and "racing heart" was also reported in a few cases and appeared to be related to not knowing what kind of examination would occur and how it would be done. Further training in counseling for ANC and ultrasound staff and provision of simple tools to help them with patient education has the potential to alleviate distress and improve patient health knowledge.

In contrast with the profound fears about harm from the ultrasound scan due to factors as a dark examining room, foreign technicians and almost total language barriers as reported in Botswana[12], in this study no woman reported fears that an ultrasound scan was dangerous to themselves or to the fetus. This may be due to several reasons, firstly in the SMRU clinics these factors are not present, though at times a foreign doctor may perform part of a complicated scan, or the sonographers may discuss results in a language that the patient does not understand (eg. Karen for a Burmese patient).

However, in this multilingual area, this is typical of daily life and not confined to the clinics. Secondly, pregnant women expressed an immense trust in the health providers, which may be due to the long existence of the SMRU ANC (25 years) or the method of frequently intermittent screening for malaria in which women are invited to come every week, and this inevitably results in a personal friendly attitude towards women who come regularly. Similar to the Botswana site was the paucity of patient counseling, frequently limited to "everything is okay".

The participants and medical staff in this study overwhelmingly reported that they believe antenatal ultrasound improves patient safety and they would not want to have ultrasound services stopped. On the other hand, provider-driven overuse is unlikely to happen, mainly since there is no financial incentive for the providers to increase the number of scans[20].

Given the prevalence of gender selection in nearby countries[23]Induced, this study investigated carefully the potential unintended harm of antenatal ultrasound by determining gender prior to delivery. Though several women reported that they had heard of gender selective abortion, and women do seek unsafe abortions after confirming pregnancy by ultrasound, all denied any intentions of selecting for gender. There are many challenges to gathering this sensitive information, but these were minimized as much as possible by the mixed methods approach of our study. Seeking abortion after pregnancy confirmation occurred before the introduction of ultrasound and abortion rates did not show a significant change before and after ultrasound (unpublished data). Protective factors in the local culture may include a kind of fatalism rooted in the animist beliefs that pervade most peoples' world views.

Limitations and future research

This study benefits from a mixed methods approach, drawing from both quantitative and qualitative techniques. However, more could be done in either of these research traditions – both filling out the qualitative description of the reception of ultrasound among the local cultural groups, and widening the scope of the quantitative investigations to include more aspects of the ultrasound experience. A more systematic observational study might be able to better quantify what counseling is routinely given, without relying on the participants' memory. Due to time constrains in the busy antenatal clinics the local staff was able to complete the questionnaire in 67% of the eligible women, and this may have introduced some selection bias.

The disinterest in viewing the screen expressed by the participants in the Muslim FGD was anomalous and contradicted by the 96.7% of Muslim survey participants who reported interest in seeing the screen. This confirmed the impression held by those conducting the discussion that the results of that particular FGD were skewed by one outspoken older women whose voice dominated parts of the discussion. This dynamic was not observed in the other focus groups, where most of the opinions expressed were confirmed by the survey results.

While the main interviewer and leader of FGD (MM) is not an obstetric provider, she is a SMRU employee, and this may have affected the information the women were willing to reveal or the way in which they responded to questions. The SMRU has a long relationship with the communities in which it works, and they may be hesitant to give negative reports of their care. Respect for authority was evident in various answers we received, and this is a strong current in Burmese and Karen culture. Nevertheless, participants did report negative experiences in both group and private sampling settings, a fact that implies that these cultural barriers were not insurmountable.

Implications for clinical practice

Changes within the clinic have already occurred based on these results including a brief explanation to all women about their first and future ANC visits by the enrolling midwife and including antenatal ultrasound in a health promotion video for pregnant women. The ultrasound rooms have been modified to allow more easy vision of the screen by the woman. The sonographers have had a workshop including role plays and focusing on greeting the woman and explaining what they will do, as well as inviting the woman to ask questions. However there are cultural and educational limits to what can be overcome.

The small number of women who reported they would see a TBA if there a problem reported is of concern and efforts have been made to counsel women receiving abnormal results about the dangers of such treatments. Patients are also encouraged to bring TBAs to the clinic for joint discussion of care, and collaboration, rather than seeking independent treatments concurrently. Another limitation of these data is that the setting of the questionnaire in the clinic could have introduced bias. It is unknown what percentage of women routinely seeks "double care" with TBAs in addition to SMRU but it has been normal for centuries to deliver with a TBA in this area.

Implementation of technological innovations in a resource poor setting is often initiated by outsiders and patient mistrust or discomfort can compromise otherwise well designed programs. Because of this, we would strongly advocate inquiry along similar lines to be done in other settings concurrent with the introduction of ultrasound in order to facilitate development of effective and acceptable programs.

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Supplementary table 7.1 Responses to defined questions (yes/no) in a questionnaire among 644 pregnant women on the Thai-Burmese border

Question number and topic		Gravidity		Literacy		How many ultrasound scans?		Religion		
		Multi (n=440)	Primi (n=203)	Cannot read (n=217)	Can Read (n=415)	Many US (n=441)	First US (n=202)	Buddhist (n=447)	Christian (n=135)	Muslim (n=60)
9. Shy?	Yes	11.6 (51)	18.2 (37)	14.7 (32)	13.0 (54)	11.8 (52)	17.8 (36)	13.9 (62)	6.7 (9)	28.3 (17)
	No	88.4 (389)	81.8 (166)	85.3 (185)	87.0 (361)	88.2 (389)	82.2 (166)	86.1(385)	93.3 (126)	71.7 (43)
10. Anxiety?	Yes	21.4 (94)	25.6 (52)	18.9 (41)	24.3 (101)	20.6 (91)	27.2 (55)	17.7 (79)	26.7 (36)	50.0 (30)
	No	78.6 (346)	74.4 (151)	81.1 (176)	75.7 (314)	79.4 (350)	72.8 (147)	82.3 (368)	73.3 (99)	50.0 (30)
11. Dangerous?	Yes	4.8 (21)	5.9 (12)	5.1 (11)	5.3 (22)	4.8 (21)	5.9 (12)	5.6 (25)	4.4 (6)	3.3 (2)
	No	94.5 (413)	91.1 (185)	93.5 (202)	93.5 (386)	94.3 (413)	91.6 (185)	92.4 (413)	93.3 (126)	96.7 (58)
12a. See the screen?	Yes	38.4 (169)	41.9 (85)	35.5 (77)	41.2 (171)	42.6 (188)	32.7 (66)	31.5 (141)	60.0 (81)	53.3 (32)
	No	61.6 (271)	57.6 (117)	64.5 (140)	58.6 (243)	57.4 (253)	66.8 (135)	68.2 (305)	40.0 (54)	46.7 (28)
12b. Want to see screen?	Yes	90.6 (397)	91.1 (185)	88.9 (192)	91.5 (379)	92.7 (407)	86.6 (175)	88.1 (394)	96.3 (130)	96.7 (58)
	No	9.1 (40)	8.9 (18)	11.1 (24)	8.2 (34)	7.3 (32)	12.9 (26)	11.4 (51)	3.0 (4)	3.3 (2)
13a. Tell the sex?	Yes	40.2 (177)	43.3 (88)	33.5 (73)	44.8 (186)	46.3 (204)	30.2 (61)	34.7 (155)	59.3 (80)	50.0 (30)
	No	58.9 (259)	56.2 (114)	65.9 (143)	54.5 (226)	53.1 (234)	68.8 (139)	64.7 (289)	40.0 (54)	48.3 (29)
13b. Want to know sex?	Yes	98.9 (435)	98 (199)	98.2 (213)	99.0 (411)	98.4 (434)	99.0 (200)	98.7 (442)	29.5 (132)	98.3 (59)
	No	0.9 (4)	2.0 (4)	1.4 (3)	1.0 (4)	1.4 (6)	1.0 (2)	1.1 (5)	1.5 (2)	1.7 (1)
18. Wrong sex: abort?	Yes	0.7 (3)	0.5 (1)	0.5 (1)	0.7 (3)	0.7 (3)	0.5 (1)	0.7 (3)	0.7 (1)	0 (0)
	No	99.3 (437)	99.5 (1)	99.5 (216)	99.3 (412)	99.3 (438)	99.5 (201)	99.3 (444)	99.3 (134)	100 (60)
19. US useful?	Yes	98.0 (431)	98.5 (200)	98.2 (213)	98.1 (407)	98.2 (433)	98.0 (198)	97.5 (436)	99.3 (134)	100 (60)
	No	1.6 (7)	1.5 (3)	1.4 (3)	1.7 (7)	1.6 (7)	1.5 (3)	2.0 (9)	0.7 (1)	0 (0)
20. Pay money?	Yes	24.5 (108)	30.5 (62)	20.3 (44)	29.4 (122)	27.0 (119)	25.2 (51)	24.8 (111)	36.3 (49)	16.7 (10)
	No	(75.2) 331	69.5 (141)	79.7 (173)	70.4 (292)	73.0 (322)	74.3 (150)	74.9 (335)	65.2 (88)	83.3 (50)
21. Continue?	Yes	98.9 (435)	99.0 (201)	98.6 (214)	99.0 (411)	99.3 (438)	98.0 (198)	99.3 (440)	100 (135)	100 (60)
	No	0.9 (4)	0.0 (0)	0.9 (2)	0.5 (2)	0.5 (2)	1.0 (2)	0.9 (4)	0 (0)	0 (0)

Data shown as % (n). The question number refers to the questionnaire (**Supplementary file S1**). Abbrevisations: N number, US ultrasound

Supplementary file S1

Ultrasound Questionnaire Date: |__|__|-|__|__|-|__|__| ANC code: |__|__|__|- |__|__|__|__|__|

1. Religion[circle]: Buddhist / Christian / Muslim / Other: _____
2. How many ultrasounds (US) have you had? |__|__|
3. Years at school |__|__| years
4. Grade |__|__|
5. Other _____

6. Why do we do US for pregnant women? More answers are possible for question 6-8. If you need more space to write please turn page.

|__| **Position**[1]
|__| **Sex**[2]
|__| **Normal hands and feet**[3]
|__| **Health of the baby**[4]
|__| **Baby's breathing**[5]
|__| **Confirm pregnancy**[6]
|__| **How many babies**[7]
|__| **Other**[8]: _____
|__| **I don't know**[9]

7. At your most recent US, what did the staff explain to you before they started to scan?

|__| **Nothing**[1]
|__| **A machine would be used to check the baby**[2]
|__| **I would need to lie down**[3]
|__| **I would need to open my sarong**[4]
|__| **Not to worry**[5]
|__| **It is safe**[6]
|__| **The reasons for the US**[7]
|__| **Other**[8]: _____
|__| **I don't know/I cannot remember**[9]

8. During or after the most recent US scan, what did they tell you?

|__| **Nothing**[1]
|__| **They explained what they were looking at**[2]
|__| **They said "Everything is okay"**[3]
|__| **They told me that I am pregnant**[4]
|__| **They told me the sex of the baby**[5]
|__| **They told me the position**[6]
|__| **They told me about a problem**[7]
|__| **Other**[8]: _____
|__| **I don't know/I cannot remember**[9]

During your last scan: Please enter 1 answer only for question 9-21. If you need more space to write please turn page.

9. Did you feel shy? |__| **Yes**[1] |__| **No**[2]
10. Did you feel your heart racing or anxiety? |__| **Yes**[1] |__| **No**[2]
11. Did you think it was dangerous? |__| **Yes**[1] |__| **No**[2] |__| **I don't know**[3] |__| **No answer**[4]
12a. Did you see the screen? |__| **Yes**[1] |__| **No**[2] |__| **No answer**[3]
12b. Did you want? |__| **Yes**[1] |__| **No**[2] |__| **I don't care**[3]
13a. Did they tell the sex? |__| **Yes**[1] |__| **No**[2] |__| **No answer**[3]
13b. Did you want? |__| **Yes**[1] |__| **No**[2] |__| **I don't care**[3]

Write down 1,2,3 or 4 in the box to answer questions 14-17:

|_1_| **Continue with normal antenatal care**[1]
|_2_| **Continue with normal ANC but also go to TBA**[2]
|_3_| **Stop coming for antenatal care; only go to TBA**[3]
|_4_| **I don't know**[4]

14. What will you do if the US finds the baby is breech? |__| other[5]_____
15. What will you do if the US finds the baby is not normal? |__| other[5]_____
16. What will you do if the US finds the baby has no heart beat? |__| other[5]_____
17. What will you do if the US confirms you are pregnant but you do not want to be pregnant? |__| other[5]_____

18. Have you ever heard of someone coming to SMRU for US and learning from the staff that the baby will be the sex that they do *not* want, and trying to get an abortion for that reason? |__| **Yes**[1] |__| **No**[2] |__| **No answer**[3]
19. Do you think US for pregnant women is useful? |__| **Yes**[1] |__| **No**[2] |__| **No answer**[3]
20. In SMRU US is free, at other hospitals it costs 500 baht. Could you pay that? |__| **Yes**[1] |__| **No**[2] |__| **No answer**[3]

Part 4 Malaria in pregnancy – ultrasound studies of fetal growth

Chapter 8

Ultrasound Evidence of Early Fetal Growth Restriction after Maternal Malaria Infection

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Keywords
Ultrasound; malaria; pregnancy; intra uterine growth restriction; dating; gestational age; biparietal diameter; crown rump length; P.falciparum; P.vivax; asymptomatic; Thai-Burmese border

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Abstract

Background

Intermittent preventive treatment (IPT), the main strategy to prevent malaria reduce anaemia and low birthweight, focuses on the second half of pregnancy. However, intrauterine growth restriction may occur earlier in pregnancy. The aim of this study was to measure the effects of malaria in the first half of pregnancy by comparing the fetal biparietal diameter (BPD) of infected and uninfected women whose pregnancies had been accurately dated by crown rump length (CRL) before 14 weeks of gestation.

Methodology/Principal Findings

In 3,779 women living on the Thai-Myanmar border who delivered a normal singleton live born baby between 2001-10 and who had gestational age estimated by CRL measurement < 14 weeks, the observed and expected BPD z-scores (<24 weeks) in pregnancies that were (n=336) and were not (n=3,443) complicated by malaria between the two scans were compared. The mean (standard deviation) fetal BPD z-scores in women with *Plasmodium (P) falciparum* and/or *P.vivax* malaria infections were significantly lower than in non-infected pregnancies; -0.57 (1.13) versus -0.10 (1.17), p< 0.001. Even a single or an asymptomatic malaria episode resulted in a significantly lower z-score. Fetal female sex (p<0.001) and low body mass index (p=0.01) were also independently associated with a smaller BPD in multivariate analysis.

Conclusions/Significance

Despite early treatment in all positive women, one or more (a)symptomatic *P.falciparum* or *P.vivax* malaria infections in the first half of pregnancy result in a smaller than expected mid-trimester fetal head diameter. Strategies to prevent malaria in pregnancy should include early pregnancy.

Introduction

Malaria remains one of the most common parasitic infection of human pregnancy[1-4], and it lowers birthweight whether or not maternal symptoms are present[5]. Even a single episode of treated *Plasmodium (P.) falciparum* or *P.vivax* malaria during pregnancy has a negative effect on birthweight[6,7]. The mechanisms of this reduction in birthweight include placental insufficiency by sequestration of malaria parasites leading to intrauterine growth restriction (IUGR), premature labour or a combination of the two[8,9]. The evidence is less clear in *P.vivax* infected pregnancies where placental sequestration is probably limited[10]. Difficulties in estimating gestational age (GA) accurately and diagnosing malaria infection in early pregnancy have complicated the interpretation of previous malaria studies on fetal growth[9,11]. IUGR may start in the first trimester and influence late pregnancy outcomes[12]. Early antenatal ultrasound - which is essential to date pregnancy accurately[13] - is becoming available in developing countries[14-16]. The aim of this study was to assess whether malaria infection affects early fetal growth by comparing the fetal biparietal diameter (BPD) before 24 weeks gestation in infected and uninfected women whose pregnancies had been accurately dated by crown rump length (CRL) measurement before 14 weeks.

Methods

Study site and population

The Shoklo Malaria Research Unit (SMRU) is located on the border between Thailand and Burma in Tak province where the majority of people belongs to the Karen ethnic group[17]. *P.falciparum*[18] and *P.vivax*[7] transmissions are low and seasonal[19]. Since 1986 the SMRU has offered free antenatal care (ANC) to refugees and later (1998) to migrant women, including weekly malaria screening to detect and treat all parasitaemic episodes during pregnancy in order to prevent maternal death[18]. There is no presumptive treatment of malaria, chemoprophylaxis or intermittent preventive treatment in pregnancy due to drug resistance. Since the inception of this ANC program, all pregnant women have been encouraged to attend as early as possible in the first trimester. In 2001 antenatal ultrasound was introduced to improve pregnancy dating in this population because of low literacy rates and poor recall of the date of the last menstrual period[14,20]. At enrolment in the antenatal clinic all women are interviewed for demographics and have anthropometric measurements taken. At every visit ferrous sulphate (200 mg daily) and folic acid (5 mg weekly) for anemia prophylaxis and thiamine (Vitamin B1, 100 mg daily) to prevent infant mortality from beri-beri[21] are offered to all women. Anaemic women receive treatment doses of ferrous sulphate (200 mg three times daily) and folic acid (5 mg daily). All medical and obstetric problems in pregnancy are investigated and treated free of charge by locally trained health workers working in SMRU facilities [22,23].

Pregnancy ultrasound

Locally trained health workers (10 sonographers at 3 clinics) obtain all ultrasound scans using Toshiba Powervision 7000 (since 2006), Dynamic Imaging (since 2001), Fukuda Denshi UF 4100 (since 2002) ultrasound machines. Their practice is supervised by doctors certified in fetal ultrasonography. All women are offered two scans in pregnancy. The 1st scan occurs at the booking visit (between 8-14 weeks gestation) where ultrasound is used to determine viability, identify multiple pregnancy and estimate GA by CRL measurement. Based on this GA estimate women then return for a 2nd scan performed at 18-24 weeks to re-assess viability, measure fetal biometry, identify major fetal abnormalities and determine placental location. Fetal biometry is routinely measured twice in each woman at each scan, as part of an existing quality control[14].

The training manual and protocol for obtaining trans-abdominal CRL and biometry measurements were from the British Medical Ultrasound Society recommendation[24].

The BPD is measured at the cross-sectional view of the fetal head at the level of the ventricles by placing the calipers on the outer border of the upper and the inner border of the lower parietal bones ('outer to inner', BPD) across the widest part of the skull. Importantly for this study, operators taking fetal measurements were not aware of the maternal malaria status.

Outcomes and SMRU delivery rooms

All women are encouraged to deliver in the SMRU facilities under supervision of locally trained midwives who speak their language. Women requiring Caesarean section are referred to the nearest Thai hospital; in this study the Caesarean section rate was 3.4% (129/3,779). Each baby is weighed within 24 hours on electronic SECA (Model 336 or 376, accuracy = 10g) digital newborn scales.

Ethics statement

This is a retrospective hospital record analysis. For those patients in trials a written informed consent was obtained including storing of data and samples. For the women seen in the ANC the routine clinical records were anonymized and these pregnancy records have been routinely entered into a database since 1987. Permission was granted by Oxford Tropical Research Ethics Committee (reference: OXTREC 28–09) to use these records for analysis.

Diagnosis of malaria

Malaria is diagnosed by Giemsa stained thick and thin blood films; 200 fields on the thick film are read before being declared negative. All parasite densities are counted per 500 white blood cells or per 1000 red blood cells. *P. vivax* or *P. falciparum* malaria infection is defined by the presence of asexual stages of the respective parasite in the peripheral blood.

Definitions

Severe malaria is defined as per WHO treatment guidelines[25] and hyperparasitaemic malaria by the presence of at least 4% infected red blood cells in the absence of other signs of severity. Anaemia is defined by a haematocrit less than 30%. Symptomatic malaria is defined by a temperature ≥37.5 °C or a history of fever[25]. When a women had at least one symptomatic episode between the 1st and the 2nd scan she was classified as symptomatic. Mid Upper Arm Circumference (MUAC) was measured at the first ANC consultation on an unclothed left arm with a SECA measuring tape (model 212) accurate to one mm and low MUAC is defined as < 21.0 cm[26]. Maternal height is measured at the first ANC consultation and short stature is defined as < 145 cm. Maternal weight of women wearing the lightest possible clothing, is measured at the first consultation and at the time of the biometry ultrasound scan on mechanical SECA weight scales (model 762) with graduation of 500 grams. Weight gain is defined as the difference in maternal weight between the two scans. The weight in the first trimester is used to calculate the body mass index (BMI): a BMI of <18.5 kg/m2 is considered underweight[27]. Pregnancy duration is defined as 280 days post menstruation. Miscarriage is a pregnancy ending before 28 weeks GA and stillbirth a delivery from 28 weeks or ≥ 800 g birthweight in which the infant displayed no sign of life (gasping, muscular activity, cardiac activity). The 28-week GA, rather than the current WHO 22-week GA cut-off was chosen, as no infant ventilatory support is available in the clinics. This cut-off has been in place since SMRU was established as the lower limit of viability in this area. Congenital abnormality is considered if any major abnormality was present at birth by staff trained in examination of the newborn.

Inclusion and Exclusion criteria

All women who had GA estimated by CRL measurement < 14 weeks (1st scan) and BPD measured < 24 weeks (2nd scan), were included in the analysis. Twin pregnancies, pregnancies that were complicated by miscarriage, stillbirth or fetal structural abnormalities and pregnancies with an unknown outcome were excluded (**Figure 8.1**. Women who had their first malaria episode before or at the time of the 1st scan or after the 2nd scan were also excluded. Therefore in this analysis all malaria infected women had their first malaria episode between the first and the second ultrasound scans. For the analysis of birth outcomes women who had another episode after the second scan were excluded to avoid the confounding effects of malaria outside the window of measurement (14^{+0}-24^{+0} weeks).

Statistical Analysis

Clinical data and the results of the two ultrasound scans were entered into a Microsoft Access database and analyzed using SPSS version 18 for Windows. Student's t-test and Mann-Whitney test were used for comparison of means and ranks respectively. Categorical data were compared using the chi-squared test or the Fisher's exact test, as appropriate. The mean of two CRL measurements was used to calculate GA in days

using a well established formula[28]. The average of two BPD measurements was recorded as 'BPD observed'. For the purpose of this study the 'expected BPD size' for each GA was calculated using a reference equation for the Karen population[29]. For validation purposes all analyses were repeated for z-scores derived from a chart based on an ethnic Chinese population[30].
Each observed BPD was then converted into a z-score using the following formula:

z-score = (BPD observed – BPD expected) / SD,

where SD is the standard deviation of the BPD at that GA. The advantage of converting measurements into z scores is that it eliminates variability by GA allowing measurements to be compared[31,32]. A positive z-score indicates a larger and a negative value a smaller than expected BPD.
In order to assess the impact of malaria infection on fetal growth during early pregnancy, the mean BPD z-scores were compared in women who did and did not have peripheral parasitaemia in the window between the 1st and 2nd scans. Other factors associated with BPD were evaluated by univariate analysis; variables with a significance of $P<0.1$ were kept in the multivariate regression model. A secondary endpoint was the effect of malaria infection between the 1st and 2nd scan only, on GA and birthweight at delivery. For this purpose a population birthweight centiles chart (per gestational age) was computed from all pregnancies in SMRU with accurate ultrasound dating. Only newborns who were weighed within the first 24 hours after delivery were included[11].

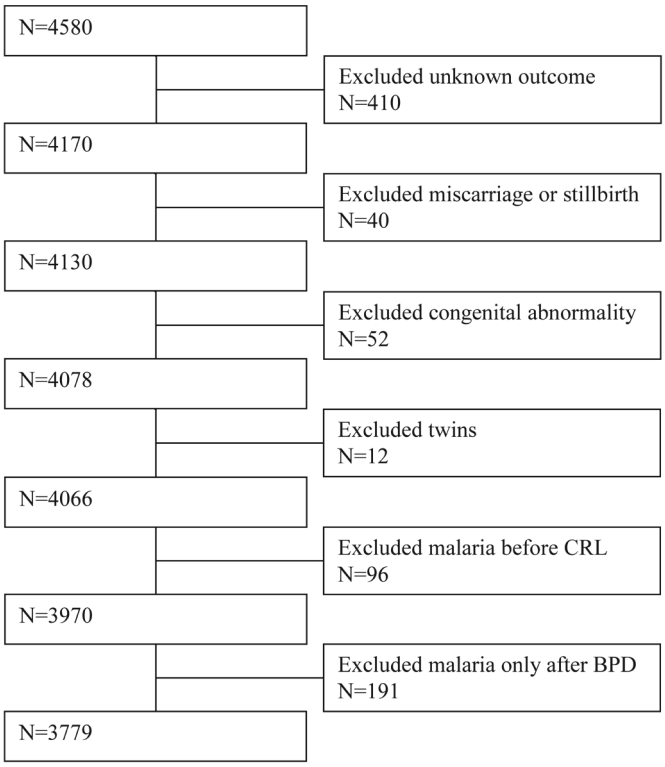
Results

Between September 2001 and January 2010, 4,580 women had a 1st (CRL measurement < 14 weeks) and a 2nd scan (BPD measurement < 24 weeks). Women with an unknown pregnancy outcome (410, 9.0%) were excluded from further analysis; they were more likely to be younger and primigravid, to book at a lower GA and have malaria (data not shown). A further 391 women (8.5%) were excluded for miscarriage/stillbirth (n = 40), congenital abnormality (n = 52), twin pregnancy (n = 12), first malaria before the CRL dating scan (n = 96) or first malaria infection after the BPD scan (n = 191) (**Figure 8.1**). There were three maternal deaths: two women died from post-partum hemorrhage after delivering stillborn infants (excluded from analysis due to stillbirth); one woman died of severe malaria five weeks post-partum and was included as the infant was live born. Therefore, a total of 3,779 women remained for analysis.

Frequent intermittent malaria screening in the ANC
The median number of antenatal visits was 23 [range 3-38]. At each visit a malaria smear was obtained and malaria parasites were detected in 930 (1.1%) of the available 86,416

blood slides; these were from 336/3,779 (8.9%) women. The median number of malaria episodes per woman was 2 [range 1-8] for the duration of pregnancy.

Figure 8.1 Selection of pregnant women



Timing of Malaria episodes

The 336 women who had their first malaria infection in the window between the two scans had a lower BMI, a lower Hct and were younger and more likely to smoke, than the 3,443 uninfected women (**Table 8.1**). Of the 336 women with malaria, 240 (71.4%) had only *P.vivax* infections and 96 (28.6%) had *P.falciparum* or both infections (**Table 8.2**). In 233 (69.3%) there was a single malaria infection in the window between the two scans; of these 80.3% (187/233) were *P.vivax*. Multiple malaria infections [range 2-4] in the window were diagnosed in 103 women (30.7%). There were only six women (1.8%) with a hyperparasitaemic *P.falciparum* infection in the window and no severe malaria infections (**Table 8.2**). Only 114 (33.9%) women had all their episodes of malaria between the two scans and none after the second scan.

Table 8.1 Demographics of the refugee and migrant women from Thai-Burmese border, 2001-2010.

	Malaria +	No Malaria	P value
	n = 336	n = 3,443	
Age, years	24.0 [20-30]	26.0 [21-31]	**0.006**
Gravida	3 [1-4]	3 [2-4]	0.187
Parity	1 [0-3]	1 [0-3]	**0.036**
Nulliparous, % (n)	28.3 (95)	24.3 (836)	0.105
Height, cm $	151 [148 – 155]	151 [147 – 155]	0.349
BMI, kg/m2 $	20.0 [18.6 – 21.5]	20.5 [19.0 – 22.4]	**<0.001**
Weight gain, kg*	2.0 [1.0-3.0]	2.0 [1.0 – 3.0]	0.265
Hct, %	28 (26 – 30)	30 (28 – 32)	<0.001
MUAC, cm#	24.3 [23.0 – 26.0]	24.0 [22.8 – 26.0]	0.483
Smoker, % (n)	30.7 (103)	22.5 (773)	**0.001**
NOC during pregnancy	23 [19 - 28]	23 [17 – 29]	0.485

Median [IQR], or as indicated
BMI body mass index, Hct Haematocrit at first consultation, MUAC middle upper arm circumference, NOC number of consultations
+ between the 1st and 2nd scans.
* Weight gain from the first to the second scan; available from 301 in malaria and 2,677 in no malaria group.
# Available from 292 in malaria and 2,626 in no malaria group.
$ Available from 314 in malaria and 3,044 in no malaria group

Table 8.2 Species, episodes and severity of malaria and mean BPD z-score of women infected between the first and second scan.

	Total malaria episodes	*P.falciparum* or mixed	*P.vivax*	Proportion of women with one episode	Proportion women at least one symptomatic episode	Proportion at least one hyper/ severe episode
	N=336	N=96	N=240	N=336	N=334#	N=336
Between 1st and 2nd scan	1 [1-4]	1 [1-3]	1[1-4]	69.3% (233)	49.1% (165)	1.8% (6)
Mean BPD z-score	-0.57 (1.1)	-0.45 (1.2)	-0.62 (1.1)	-0.51 (1.2)	-0.59 (1.1)	n.a.

Median [min-max], or mean (SD). BPD biparietal diameter; hyper = hyperparasitaemia (≥4% red blood cells infected), n.a. not applicable, P plasmodium.
Missing data n=2

Table 8.3 Risk factors associated with mean BPD z-score as a measure of early fetal growth restriction

		Frequency (%)	Univariate		Multivariate (n=2972)	
			Coefficient (95% CI)	P-value	Coefficient (95%CI)	P-value
Teenager	No	3,210 (84.9)	-0.02 (-0.13, 0.08)	0.66	*	NS
	Yes	569 (15.1)				
Primigravida	No	2,848 (75.4)	0.07 (-0.02, 0.16)	0.10	*	NS
	Yes	931 (24.6)				
Smoking	No	2,893 (76.8)	0.04 (-0.05, 0.12)	0.43	*	NS
	Yes	876 (23.2)				
Low MUAC	No	2,801 (96.0)	0.07 (-0.14, 0.29)	0.51	*	NS
	Yes	116 (4.0)				
Short	No	2,995 (89.2)	0.04 (-0.09, 0.16)	0.58	*	NS
	Yes	362 (10.8)				
Low BMI	No	2,738 (81.6)	0.18 (0.08, 0.28)	**0.001**	0.15 (0.04, 0.26)	**0.005**
	Yes	619 (18.4)				
Weight loss	No	2,396 (80.5)	0.09 (-0.02, 0.19)	0.10	0.11 (0.00, 0.21)	**0.05**
	Yes	581 (19.5)				
Anaemia	No	2152 (56.9)	0.16 (0.09, 0.24)	**<0.001**	$	
	Yes	1627 (43.1)				
Malaria	No	3,443 (91.1)	0.47 (0.34, 0.60)	**<0.001**	0.50 (0.36, 0.63)	**<0.001**
	Yes	336 (8.9)				
Symptomatic malaria	No	169 (50.6)	0.05 (-0.19, 0.30)	0.68	*	NS
	Yes	165 (49.4)				
Newborn gender	M	1,936 (51.2)	0.25 (0.18, 0.33)	**<0.001**	0.25 (0.17,0.34)	**<0.001**
	F	1,843 (48.8)				

Significant differences are shown in bold.
BMI body mass index, MUAC middle upper arm circumference, NS not significant
* Not included in the multivariate analysis
$ Anaemia was highly collinear with malaria, and the co-variates in the multivariate model were unchanged when anaemia was adjusted for or not

Effect of malaria on BPD

The BPD measurements of malaria infected and uninfected women were superimposed on the 2.5th, 50th, and 97.5th centiles of the reference equation[29] and presented graphically between 16 and 24 weeks GA to allow visual comparison (**Figure 8.2**). Most of the BPD measurements of women with malaria (red diamonds) were below the 50th centile (**Figure 8.2** and insert, which shows the BPD measurements between 17 and 20 weeks GA in which 90% (302/336) of the measurements were obtained). The BPD measurements were then converted into z-scores that were normally distributed. There was a statistically significant difference between the mean (SD) BPD z-scores of the fetuses of infected and uninfected women (-0.57 (1.13) versus -0.10 (1.17), $p < 0.001$) (**Figure 8.3**, **Table 8.3**). Both *P.falciparum* (or mixed) (n = 96) and *P.vivax* (n = 240)

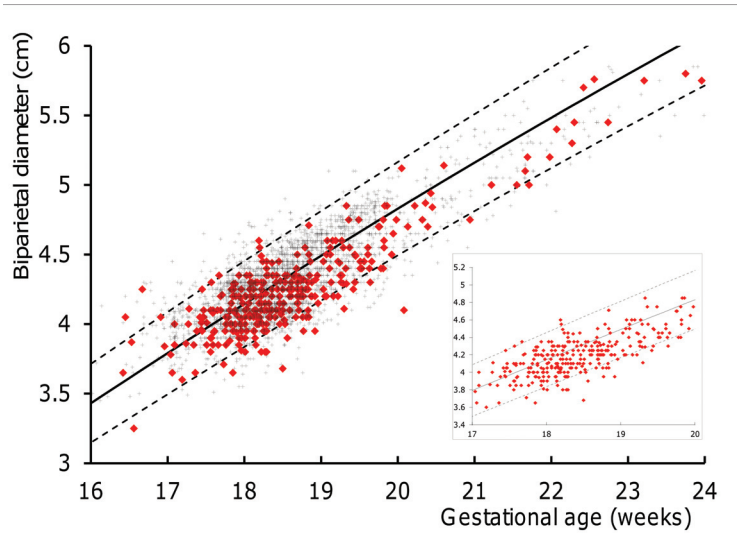
were associated with a lower mean z-score than uninfected women (-0.45 (1.2), p = 0.006 and -0.62 (1.1) respectively, p < 0.001). There was no significant difference in mean (SD) z-scores between women with symptomatic (n = 165) or asymptomatic malaria (n = 169) episodes: -0.59 (1.1) versus -0.54 (1.2) respectively, p = 0.68. Women with multiple malaria infections (n=103) had the lowest mean (SD) z-scores (-0.72 (1.0), p < 0.001) but even a single malaria episode between the 2 scans (n=233) resulted in a significantly lower mean (SD) BPD z-score: -0.51 (1.2), p < 0.001). There was no significant difference in mean z-scores between women with uncomplicated and hyperparasitaemic malaria, but the number of hyperparasitaemic women was too small for analysis (**Table 8.2**).

Aside from malaria, female sex of the fetus, maternal anaemia and low maternal BMI were also significantly associated with a lower mean z-score in the univariate analysis. These remained independent risk factors in the linear regression model which included 2972 women, the remainder being excluded because of missing values mainly maternal weight at the second scan (**Table 8.3**). This significantly lower mean BPD z-score (difference in z-score of 0.47 SD) means that malaria during the period between the two scans reduces the diameter of the fetal head by approximately 1 mm when measured at a GA of 22 weeks. The same analysis was repeated for z-scores derived from a Chinese population and except for a small shift in mean z-score (as expected for different populations) the magnitude and significance of the differences were similar (**Supporting File S1** and **Supporting Table S1**).

Effect of malaria on birth outcomes

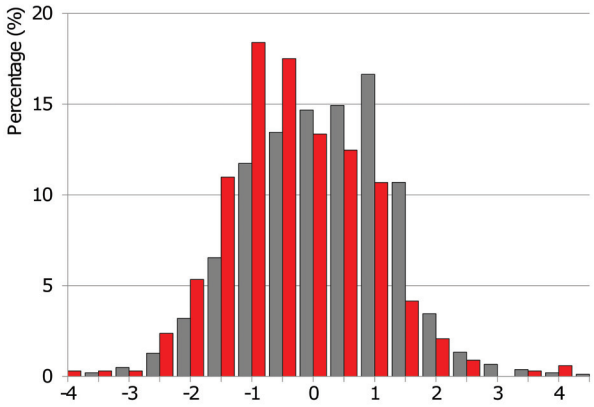
Of the 3,083 live born congenitally normal singleton infants weighed within 24 hours of delivery, the z-score for BPD was positively correlated with birthweight centile: B = 0.168 (95% CI 0.128 – 0.209), p<0.001. Seventy two of the 114 women (63.2%) who had all their malaria infections between the two scans only, had their baby weighted within 24 hours. Neonates from these pregnancies had similar birth outcomes as the ones from uninfected pregnancies (n=3,011): mean (SD) birthweight 2919 (SD 533) g versus 2973 (SD 433) g, p=0.40, mean GA 272 (SD 13) days (or 38.9 (SD 1.9) weeks) versus 273 (SD 11) days (or 39.0 (SD 1.6) weeks), p=0.22 and birthweight centile 0.00 (SD 1.2) versus 0.03 (SD 1.0) respectively. Within this small malaria sub-group (n=72) there was no association between BPD z-score and birthweight centile.

Figure 8.2 Fetal biparietal diameter measurements in Burmese and Karen pregnant women with and without malaria.



The x-axis shows the gestational age (GA) in weeks, based on first trimester dated pregnancies on the Thai-Burmese border from 2001 to 2010. The y-axis depicts the fetal biparietal diameter measurement (BPD) in centimeters. The fetal BPD in pregnant women with malaria (red diamonds, n=336) and in women without malaria (+, n=3,443) between 16 and 24 GA weeks were superimposed on the 2.5th, 50th and 97.5th centiles of a reference equation for this population[29]. Note that the majority of fetal BPD measurements in malaria infected women lie below the 50th centile in both the main figure (16 to 24 GA weeks) and in the inset (17 to 20 GA weeks, where 90% (302/336) of the measurements in malaria infected women were obtained).

Figure 8.3 Z-scores of fetal biparietal diameter in Burmese and Karen pregnant women with and without malaria.



The x-axis shows the z-score and the y-axis depicts the distribution in percentages. The distribution of z-scores of fetal biparietal diameter in pregnant women with malaria (n=336, red bars) is significantly lower than in women without malaria (n=3,443, grey bars) on the Thai-Burmese border from 2001 to 2010.

Discussion

In this study a significantly smaller fetal BPD was observed by ultrasound when malaria infection occurred in the first half of pregnancy, compared to pregnancies unaffected by malaria. Previous studies have shown that malaria early in pregnancy has an impact on birthweight, but they were limited by small numbers of first trimester exposures[33] or by inaccuracy in the dating of gestation[34]. In this analysis the women had a documented and treated episode of malaria during a specific period between two ultrasound scans. By studying this selected group the effects of malaria on fetal growth could be quantified in-utero. Even a single infection of treated *P.vivax* or *P.falciparum* was associated with reduced BPD irrespective of whether the woman was symptomatic or not. The mechanisms underlying the adverse effects of malaria in pregnancy are not fully understood[7,35,36]. *P.falciparum* is thought to sequester in the placenta and interfere with materno-fetal exchanges but other mechanisms may also be involved [8,36]. Systemic or hormonal mechanisms may play a role in *P. vivax* related growth restriction, as there is little evidence that *P.vivax* sequesters in the placenta, like *P.falciparum* does[7,10,35,36]. In non-malaria endemic areas, early pregnancy growth restriction has been associated with miscarriage[37], maternal physical characteristics and lifestyle habits related to early fetal growth[38], and low BPD growth rates between the first and second trimester are associated with increased perinatal mortality and IUGR[39,40]. In malaria endemic areas ultrasound studies have related malaria in pregnancy to changes in maternal and fetal blood flow [41,42] and associated malnutrition and malaria in pregnancy with IUGR[43]. So the results presented here are not entirely unexpected but show for the first time the direct evidence of the effect of malaria (both falciparum and vivax) on fetal growth. More surprising perhaps is that low BPD growth was observed after a single (even asymptomatic) infection and despite early treatment.

Studies on the impact of malaria in pregnancy have almost always focused on birthweight. However, for infections that occur in early pregnancy, the size of the fetal head may be a more appropriate indicator of growth restriction. It has been shown that the growth velocity of the fetal head (in mm/day) is maximal during the second trimester [44-46]. In contrast the fetal "weight velocity" (in g/week) peaks in the third trimester. This weight gain velocity curve has often been cited wrongly as a fetal "growth velocity" curve[47,48]. The characteristics of the "fetal head size" and "fetal weight" growth velocity curves are similar but the timing is different. One of the strengths of this study is that the timing of the BPD measurement coincided with the maximal growth velocity of the fetal head, making it a better marker of the effect of malaria in early pregnancy than birthweight.

The reduction in BPD size occurred despite prompt treatment with effective antimalarials in this setting, which highlights the importance of prevention in pregnancy. Multiple episodes of *P.vivax* are most likely from liver stage relapses instead of newly acquired infections. There is no treatment available in pregnancy for liver stages. Furthermore, in this setting of multidrug resistant parasites there are no safe and effective drugs available

to prevent malaria from the start of pregnancy. To protect the developing fetus from growth restriction from both symptomatic and asymptomatic *P.vivax* and *P.falciparum* infections, prevention strategies from early pregnancy onwards or even pre-pregnancy interventions should be considered [33,34,49,50].

There are some limitations to this analysis. Firstly, although dating by CRL is generally considered to be the most accurate method of estimating GA, some factors that may have had an impact on CRL dating, for example maternal age [51] or haematocrit[38]. Such factors are difficult to control for in this type of population because the date of the last menstrual period is often not available. The second limitation is that the observed difference in BPD is small and within the range of error for most ultrasound machines and sonographers. The scans were obtained by locally trained technicians who were previously reported to have a mean difference of 0.43 (SD 1.21) mm in their BPD measurements in scans between 18 and 24 weeks, corresponding to 0.12 (SD 0.36) weeks[14]. The observed reduction in BPD in malaria infected pregnancies at 22 weeks is within the range of this measurement error. However the data of this retrospective analysis were derived from the entire population of women attending ANC for their routine ultrasound scans, which minimizes selection bias. In addition the sonographers were not aware whether women had malaria or not during pregnancy and therefore observer bias is unlikely. If there was no true effect of malaria infection on BPD any observed difference would likely be occulted by the expected measurement error of the examiners. In contrast, within this large population of routine ultrasound scans, malaria in the late first and/or second trimester was the largest risk factor for a smaller BPD. Thirdly, in order to examine the effect of the malaria between the scans on birth outcomes, a highly selected group of women was studied for this analysis. This small group (N=72) had their malaria infections only between the two scans, and was malaria free in the time period where weight gain velocity is highest. Therefore no firm conclusions on the relationship between malaria in early pregnancy, its impact on BPD and birth outcome can be drawn from this analysis. Finally, other biometric parameters, such as fetal head circumference, were not available for most women.

Intermittent preventive treatment, one of the main WHO recommended strategies for malaria prevention and control during pregnancy in areas of stable malaria transmission, aims to provide two or three treatment doses after quickening (around 20 weeks GA) at least one month apart[48]. This approach fails to protect women in the gestational weeks of the highest fetal head growth velocity. Fetal growth has been postulated to be a dynamic system where pulsatile characteristics of saltatory growth events are able to change throughout pregnancy[52-55]. Such pulsatile growth events all the way through pregnancy stress the importance of protecting each fetus from the effects of malaria parasites starting as early as possible in pregnancy.

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Supporting Table S1

Factors associated with mean BPD z-score when calculated from the Chinese equation[1]

		Frequency (%)	Univariate		Multivariate (n=2972)	
			Coefficient (95%CI)	P-value	Coefficient (95%CI)	P-value
Teenager	No	3,210 (84.9)	-0.01 (-0.07, 0.05)	0.67	*	NS
	Yes	569 (15.1)				
Primigravidae	No	2,848 (75.4)	0.04 (-0.01, 0.09)	0.09	*	NS
	Yes	931 (24.6)				
Smoking	No	2,893 (76.8)	0.02 (-0.03, 0.07)	0.42	*	NS
	Yes	876 (23.2)				
Low MUAC	No	2,801 (96.0)	0.04 (-0.09, 0.16)	0.60	*	NS
	Yes	116 (4.0)				
Short	No	2,995 (89.2)	0.02 (-0.05, 0.09)	0.55	*	NS
	Yes	362 (10.8)				
Low BMI	No	2,738 (81.6)	0.10 (0.05, 0.16)	**<0.001**	0.09 (0.03 – 0.15)	**0.004**
	Yes	619 (18.4)				
Weight loss	No	2,396 (80.5)	0.05 (-0.01, 0.11)	0.08	0.06 (0.00, 0.12)	**0.04**
	Yes	581 (19.5)				
Anaemia	No	2152 (56.9)	0.09 (0.05, 0.14)	**<0.001**	$	
	Yes	1627 (43.1)				
Malaria	No	3,443 (91.1)	0.27 (0.20, 0.35)	**<0.001**	0.29 (0.210.37)	**<0.001**
	Yes	336 (8.9)				
Symptomatic malaria	No	169 (50.6)	0.03 (-0.11, 0.16)	0.71	*	NS
	Yes	165 (49.4)				
Newborn gender	M	1,936 (51.2)	1.42 (0.10, 0.18)	**<0.001**	0.144 (0.10 – 0.19)	**<0.001**
	F	1,843 (48.8)				

Significant differences are shown in bold.
BMI body mass index, MUAC middle upper arm circumference, NS not significant
* Not included in the multivariate analysis
$ Anaemia was highly collinear with malaria, and the co-variates in the multivariate model were unchanged when anaemia was adjusted for or not

Supporting File S1

Calculations derived from the equations based on a Chinese population[1].

The equations for this population are:

Expected BPD(oi) = −1.295192 + 0.197042 × GA + 0.008247 × GA2 − 0.000163 × GA3.

SD = 1.253 × (0.176837 + 0.002714 × GA).

In this equation BPD is in cm and Gestational age (GA) in weeks.
The z-scores were normally distributed. There was a statistically significant difference between the mean (SD) BPD z-scores of the fetuses of infected and uninfected women (0.29 (0.63) versus 0.56 (0.66), p < 0.001) (**Supporting Table S1**). Both *P.falciparum* (or mixed) (n=96) and *P.vivax* (n=240) were associated with a lower z-score than for uninfected women (0.35 (0.69), P=0.004 and 0.26 (0.61), p<0.001, respectively). There was no significant difference in z-score between symptomatic (n=165) and asymptomatic malaria (N=169) episodes, 0.27 (0.63) versus 0.30 (0.64) respectively, p=0.71. Women with multiple malaria infections (n=103) showed the lowest z-scores (0.20 (0.58), P<0.001), but even a single malaria episode (n=233) resulted in a significantly lower BPD z-score: 0.33 (0.65), P<0.001). There was no significant difference in z-score between women with uncomplicated or hyperparasitaemic malaria, but the number of hyperparasitaemic women was too small for further analysis.

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Chapter 9

Accurately dated pregnancies: malaria is associated with birthweight reduction across all gestational ages

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Keywords
Malaria; pregnancy; ultrasound; centiles; z-scores; birthweight; gestational age

SUBMITTED

Abstract

Introduction
Malaria in pregnancy is associated with low birthweight. Despite the enormous burden of malaria in pregnancy in Africa, Asia and South America the contribution of malaria to preterm births and intrauterine growth restriction remains unclear.

Methods
Birth weight and gestational age of all accurately ultrasound dated pregnancies in live-born, normal singletons weighed in the first hour of life and born from 28+0 to 41+6 weeks were entered into a database. Least-squares regression analysis was used to determine mean birthweight and standard deviation as a polynomial function of gestational age. The proportion of low birthweight (birthweight less than 2500 g) and small for gestational age (birthweight below the 10th centile for the gestational age) infants and mean z-scores were calculated for women with malaria. Malaria was diagnosed by active weekly screening by microscopy. The proportion of small for gestational age for the recently introduced global reference curve and local population centile were compared.

Results
In 10,264 newborns with a mean birth weight of 2953 grams (SD 454; range 790 – 5080) and GA of 38+6 weeks (SD 1+1; range 28+2 to 41+6), the best fitting curve was found to be a first degree cubic polynomial equation (birthweight in grams and GA in days): BW = 41007.32 - (536.419 * GA) + (2.323342 * GA * GA) – (0.0031821* GA* GA* GA). In pregnancies affected by malaria, the z-scores were reduced across nearly all gestations. Low birth weight was a poor predictor of small for gestational age: positive predictive value 58.2%. The global birthweight percentiles were within the 5% mean and 95% range of the local population centile but the proportion of small for gestational age was 1.7 (95% CI 1.53 to 1.86) fold higher using the global reference curve.

Conclusion:
To understand the impact of malaria in pregnancy on infant birthweight, accurate estimates of gestational age assessment measured by ultrasound and population centiles are needed to differentiate low birthweight newborns in preterm labor and small for gestational age. Low birthweight is a poor predictor of small for gestational age newborns. Malaria in pregnancy was associated with a lower birthweight for gestational age for nearly all gestational ages in this population and not with a significant reduction in gestational age at birth.

Introduction

Each year more than hundred million pregnant women are at risk from *P.falciparum* and *P.vivax* malaria[1] and their infants are at increased risk of low birth weight (LBW)[2]. Several decades ago, obstetricians and paediatricians recognized that infants with LBW (birth weight less than 2500 grams) were an assortment of preterm births and small for gestational age (SGA) infants[3].

These infants have different short and long term needs and outcomes[4, 5]. SGA infants can be further subdivided into constitutionally small for gestational age (SGA) and intrauterine growth restricted (IUGR) infants[3, 6]. Despite the enormous burden of malaria in pregnancy, progress on differentiating the relative contribution of preterm births and SGA to LBW has been very slow.

To distinguish between preterm birth and SGA, accurate ultrasound dating is required[7]. Due to the difficulty of this in resource poor malaria endemic areas, dating has relied on gestational age (GA) estimations by the first day of the last menstrual period, symphysis fundal height[8], or from newborn examinations using the Ballard or Dubowitz test[9], which are all subject to wider intervals of accuracy than early ultrasound. Recently published trials[10-14] suggest accurate dating by early ultrasound (the current gold standard), is becoming available in malarious areas[7, 15-17]. Accurate pregnancy dating allows population-specific percentiles to be developed, whereby birthweight analysis can be performed relative to GA. A method to shortcut the collection of population data to establish birth weight norms has been proposed recently[18] and an ultrasound-based generic global reference for fetal weight and birthweight centiles, applicable to low-income and middle-income countries has been developed. The authors suggested that at least 100 accurately dated pregnancies with delivery outcomes at GA 40 weeks were sufficient to create centiles using a web-based formula[18]. Asian women from Thailand (9,334 women), Vietnam (13,167 women) and Cambodia (5,362 women) were included in the development but these women tended to come from the main cities of the countries where the standard of living differs from the rural areas. The objective of this study was a) to create a formula for birthweight percentiles from ultrasound dated pregnancies for a rural population on the Thai-Burmese border; b) to study the effect of malaria in pregnancy on LBW, preterm labour and SGA across all GA; c) to test the sensitivity and specificity of using LBW to identify SGA babies in pregnancies affected by malaria; and d) to evaluate the global reference in this context[18].

Methods

Study site and population

The Shoklo Malaria Research Unit (SMRU) is located on the border between Thailand and Myanmar, a malaria endemic area where *P.falciparum*[19] and *P.vivax*[20] transmission is low and seasonal[21]. Briefly, since the inception of the ANC programme in 1986, all

pregnant women have been encouraged to attend as early as possible in the first trimester and return for weekly malaria screening and routine obstetric care. In 2001 antenatal ultrasound was introduced to reduce the problem of GA estimation in this population with low literacy rates and poor recall of the date of the last menstrual period[22, 23]. Locally trained health workers (10 sonographers) obtain all ultrasound scans using Toshiba Powervision 7000 (since 2006), Dynamic Imaging (since 2001), Fukuda Denshi UF 4100 (since 2002) ultrasound machines. Their practice is supervised by doctors certified in fetal ultrasound. At the booking visit, ultrasound is used to determine viability, identify multiple pregnancies, and estimate GA by CRL (before 14 weeks GA) and fetal head (biparietal diameter (BPD) or head circumference (HC)) measurement (between 14 – 24 weeks GA). Fetal biometry is routinely measured twice in each woman, as part of an existing quality control system[22]. The training manual and protocol for obtaining trans-abdominal CRL and biometry measurements were based on recommendations from the British Medical Ultrasound Society[24].

Outcomes and SMRU delivery rooms

All women are encouraged to deliver in the SMRU facilities under supervision of locally trained midwives. Each labour is monitored on a WHO partogram. All medical and obstetric problems are investigated and treated by locally trained health workers in SMRU facilities[25, 26] and supported by doctors. Women requiring Caesarean section are referred to the nearest Thai hospital. Each baby is weighed within 1 hours on an electronic SECA (Model 336 or 376, accuracy = 10 g) digital newborn scale.

Ethics statement

This is a retrospective cohort analysis based on hospital records, which was approved by the Oxford Tropical Research Ethics Committee (reference: OXTREC 28–09). Clinical records from Sept-2001 to Oct-2010 were anonymized and ultrasound dated pregnancy records were entered into a database.

Diagnosis of malaria

Malaria is diagnosed by Giemsa stained thick and thin blood films; 200 fields on the thick film are read before being declared negative. Malaria infection is defined by the presence of asexual stages of, mainly, *Plasmodium (P.) vivax* or *P.falciparum* in the peripheral blood irrespective of maternal symptoms. There is no presumptive treatment of malaria or chemoprophylaxis.

Definitions

Gestational age (GA) is calculated from the mean of two first trimester CRL measurements at the first antenatal visit using a well established formula[27] or the mean of two BPD measurements using a Chinese formula[28]. Miscarriage was defined as a pregnancy ending before 28 weeks GA and stillbirth a delivery from 28 weeks or ≥ 800 g birthweight in which the infant displayed no sign of life (gasping, muscular activity, cardiac

activity). The 28-week GA, rather than the current WHO 22-week GA cut-off was chosen, as no infant ventilatory support was available in the clinics. This cut-off has been in place as the lower limit of viability since SMRU was established in this area. Preterm birth was defined as delivery before a GA of 37+0 weeks. Congenital abnormality was defined as any major abnormality present at birth. All newborns were systematically examined by staff trained in this examination. Four dichotomous variables included in analysis were: low birthweight (LBW) (defined as a birthweight below 2500 grams), small for gestational age (SGA) (defined as a birthweight below the 10th centile for the GA) and SGA-global (defined as a birthweight below the 10th centile for the GA as predicted by the global reference for birthweight centiles) and preterm birth (PTB, defined as a GA at birth below 37 weeks).

Inclusion and Exclusion criteria

All women who attended SMRU ANC between 2001 and 2010 and had a GA estimated by CRL or BPD measured < 24 weeks, and delivered a live born congenitally normal singleton at SMRU, with a gestation from 28+0 to 41+6 weeks, who was weighed within the first hour after birth, were included.

Statistical Analysis

Clinical data and the results of the ultrasound scans were entered into a Microsoft Access database and analyzed using SPSS version 18 for Windows and STATA version 11.2 (StataCorp LP, College Station, TX, USA). Categorical data were compared using the chi-squared test, means with the student's t test.

Least-squares regression analysis was used to determine mean birthweight and standard deviation as a polynomial function of GA using the procedure described by Royston and Wright[29] for first degree polynomials and using the fracpoly command in STATA for second degree polynomials[30]. The appropriate polynomial curve was identified as the best fitting model for both the mean and SD. In this large sample size absolute adherence to p-values to decide best model fit was avoided[31]. Rather, terms were included when their value was significantly different from zero (i.e., the coefficient was at least twice the value of its standard error)[32] and "best fit" was assessed using Akaike's Information Criterion (AIC) and visual inspection of the residuals and of the fitted values to the actual values. For the standard deviation (SD) curve, the 'scaled absolute residuals' from the model for the mean were regressed against GA after checking and accounting for any trends in variation. Good fit of the model was confirmed by a straight line of the probability plot of the z-scores (actual value-fitted value/fitted SD) and the Shapiro-Francia W' test. A plot of the z-scores against gestational age was verified to have random scatter about zero before the final model was chosen.

Centiles
Centile curves were calculated using the formula: centile = predicted mean + K × predicted SD, where K was the corresponding centile of the standard normal distribution: ±1.88 for 3rd and 97th centiles, and ±1.28 for the 10th and 90th centiles.

Global reference for birthweight percentiles
In the study by Mikolajczyk et al.[18], it was suggested that the mean birthweight for at least 100 infants without risk factors for either growth restriction (e.g. smoking) or macrosomia (poorly controlled diabetes) born at 40 completed weeks of gestation (40 weeks + 0 days to 40 weeks + 6 days) in the local population should be used. For the purpose of establishing the population birthweight for gestational age curves we selected all "optimal pregnancies" defined as mothers who were not primigravidae, did not smoke or have hypertension and had no infection (malaria and non-malaria febrile illness) detected during prospective pregnancy follow-up. In this study, the birthweights of 847 optimal pregnancies were entered in the formula.

Z-scores
The birthweight of each newborn was converted into a z-score using the following formula:
z-score = (actual BW– fitted BW) / fitted SD. A positive z-score indicates a larger, and a negative value a smaller than expected birthweight within this population.

Results

There were 10,264 women who attended SMRU ANC between 2001 and 2010 and met the inclusion criteria. They had a mean age of 26 years (SD 7; range 14-48) years and 27.1% were primigravida. They delivered at an average GA of 38+6 weeks (SD 1+1; range 28+2 to 41+6) and mean birth weight was 2953 g (SD 454 ; range 790-5080g). In 65% (6673/10265) the GA was determined by CRL and in the remaining 35% (3592) of women by the BPD.

Local population centiles
The best fitting curve was found to be a first degree cubic polynomial equation (birthweight in grams and GA in days): BW = 41007.32 - (536.419 * GA) + (2.323342 * GA * GA) – (0.0031821* GA* GA* GA). The addition of another component or a fractional cubic polynomial (second degree) model did not significantly improve the fit of the curve. The corresponding SD curve was best fit with a linear equation: SD = (2.368685 * GA) - 289.9445. The fit of the model against the actual values is shown together with centiles for clinical use (**Figure 9.1** and **Supporting Table S1**).

Figure 9.1 Raw data with the fitted mean and 3rd, 10th, 90th and 97th centiles (n= 10265) for birthweight against gestational age, Thai-Burmese border 2001-2010.

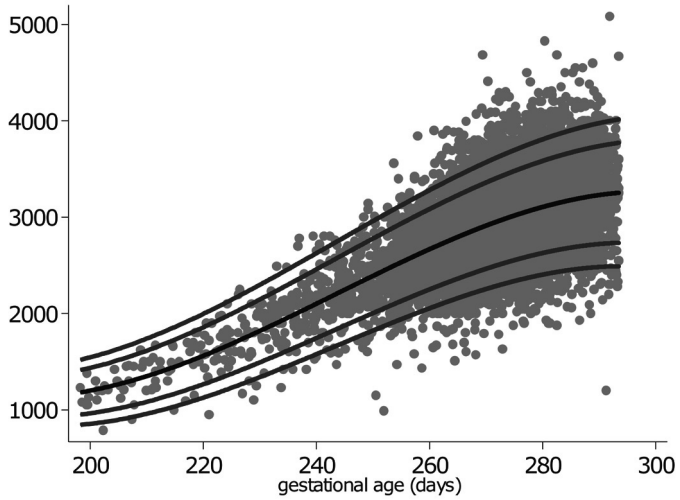


Table 9.1 The characteristics of newborns classified by malaria infection in pregnancy on the Thai-Myanmar border 2001-2010 (n=10264)

	Malaria	Non Malaria	P value
Frequency	1292 (12.6)	8972 (87.4)	
LBW	225 (17.4)	1072 (11.9)	<0.001
SGA	177 (13.7)	808 (9.0)	<0.001
PTB	119 (9.2)	670 (7.5)	0.032
SGA-global	297 (23.0)	1421 (15.8)	<0.001
Z-score, SD	-0.197 (1.03)	0.031 (1.02)	<0.001
Gestational age (days)	273 (13)	273 (11)	0.052

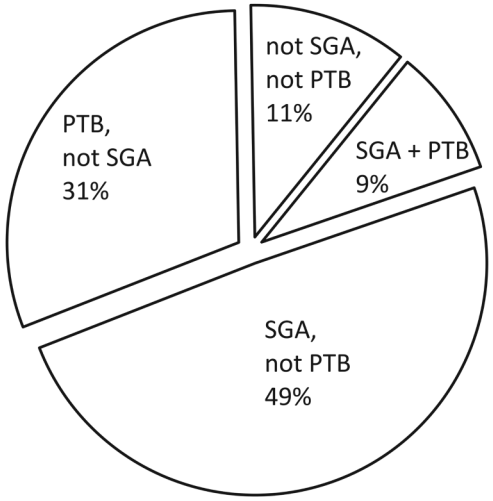
Data shown as N (%) or mean (SD)
LBW: low birth weight (birth weight < 2500 grams, regardless of gestation)
SGA: small for gestational age (birth weight <10th percentile for a given gestational age)
SGA-global: the proportion of newborns identified as SGA according to the global reference18, when completed for optimal pregnancies.
PTB preterm birth (gestational age at delivery less than 37 weeks)

Malaria

The proportions for the dichotomous variables LBW, SGA, SGA-global and PTB, gestational age at birth and birthweight z-scores calculated from the population centiles, were tabulated for pregnancies complicated with malaria and non-infected pregnancies (**Table 9.1**). Malaria infection in pregnancy was associated with a significantly smaller birthweight z-score, but had no effect on gestational age measured in days. The newborns

with LBW in the malaria infected group were mostly due to SGA (**Figure 9.2**). Malaria in pregnancy resulted in a lower mean z-score for birthweight across nearly all gestational ages (**Figure 9.3**). The ability of LBW to predict SGA in infants born to mothers who had malaria was low: sensitivity 74% (131/177; 95% CI 67.2 – 80.1), specificity 91.6% (1021/1115; 95% CI 89.8 – 93.1) with a positive predictive value 58.2% (131/225; 95% CI 51.7 – 64.5) and negative predictive value of 95.6% (1021/1067; 95% CI 94.3 – 96.8). Similar characteristics were present when calculated for the whole population (**Table 9.2**).

Figure 9.2 Proportion of small for gestational age and preterm in low birth weight newborns from malaria infected pregnancies, Thai-Burmese border 2001-2010 (n=1292)

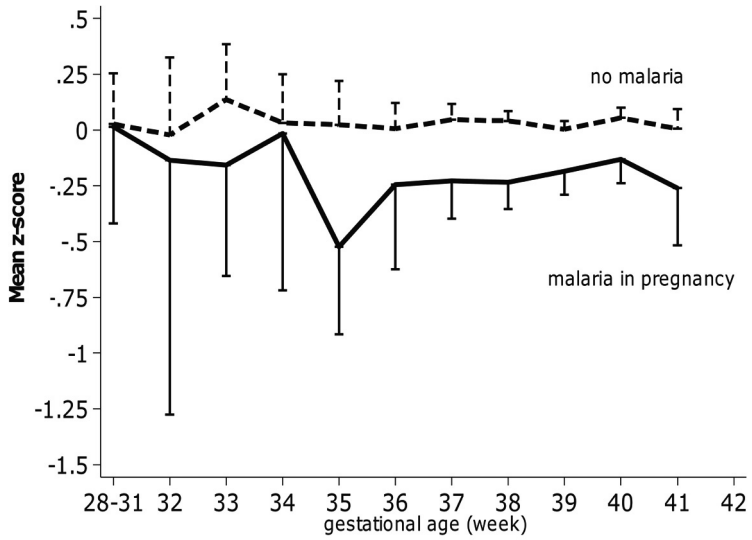


SGA: small for gestational age (birth weight <10th percentile for a given gestational age). PTB: preterm birth (delivery before 37 weeks of gestational age)

The global reference curve

In **Table 9.1**, the proportion of SGA-global[18] is 1.7 fold higher (95% CI 1.53 to 1.86) than the proportion of SGA predicted from the local centiles. In **Figure 9.4**, the mean birthweight as predicted by the global reference[18] of "optimal pregnancies" (blue lines, mean birthweight 3233 g), are expressed as z-score curves and compared to the present equation. If the results from both equations were identical, the mean z-score lines in **Figure 9.4** would show one line at y=0. The 10th and the 90th centiles (z-score +/- 1.282) are illustrated by dotted lines. For the entire GA range, the mean values of the global birthweight percentiles were at approximately +0.5 SD of the studied population. This is within the 5% (mean – 1.645 SD) and 95% (mean + 1.645 SD) range of our equation, indicating no significant difference. However the 10th centiles of the global reference (optimal group) equations are within one SD from the mean across all gestational ages.

Figure 9.3 Mean z-scores of malaria (continuous line) and non-malaria (dashed line) with 95%CI by gestational age on the Thai-Burmese border 2001-2010.



The x-axis demonstrates the gestational age in weeks, the y-axis the mean z-score of birthweight for gestational age in this population. Weeks 28 to 31 have been combined due to small numbers.

Number of malaria cases

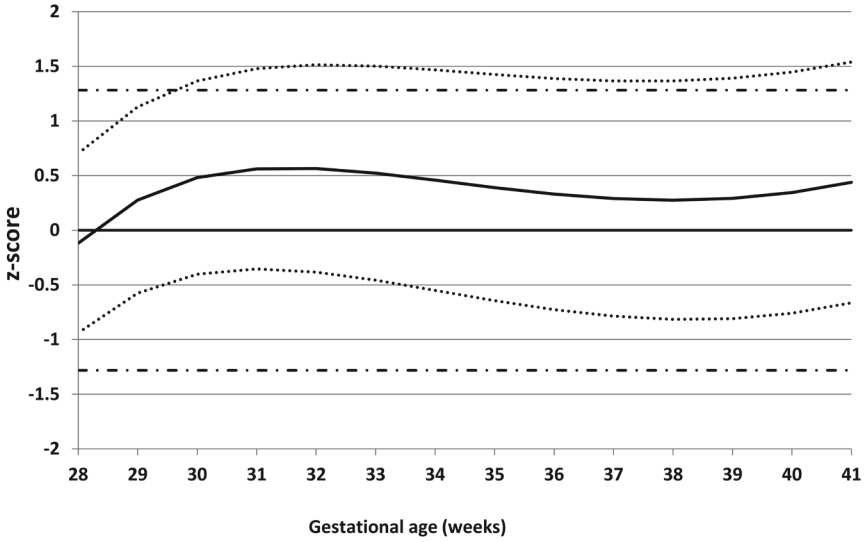
GA wks	28	29	30	31	32	33	34	35	36	37	38	39	40	41
n	3	5	4	5	9	12	10	29	49	126	300	362	292	86

Table 9.2 Characteristics of LBW as a test to identify SGA babies (n=10264)

	SGA	Not SGA	Total
LBW	693	604	1297
No LBW	292	8675	8967
Total	985	9279	10264

Sensitivity = 693 / 985 = 70.4 % (95% CI 67.5 – 73.2)
Specificity = 8675 / 9279 = 93.5 % (95% CI 93.0 – 94.0)
Positive Predictive value = 693 / 1297 = 53.4% (95% CI 50.7 – 56.1)
Negative Predictive value = 8675 /8967 = 96.7 % (95% CI 96.4 – 97.1)

Figure 9.4 Z-scores for comparison of the global reference with the present equation.



The x-axis indicates the gestational age in weeks, the y-axis the z-score of birthweight for gestational age in this population. The mean expected z-score, or 50th percentile from the present equation is shown as a straight black line, the black dashed lines represent the expected z-scores for the 10th and 90th centiles, i.e. -1.282 and 1.282 SD respectively. The z-score comparison of the present equation with the global reference mean is demonstrated with the curved lines for mean birth weights, 10th, and 90th percentiles (dotted lines) per gestational age for optimal pregnancies (n=847).

Discussion

In this study, population specific birthweight centiles from accurate ultrasound dated pregnancies were created for a rural population on the Thai-Myanmar border. The resulting population specific centiles are clinically relevant for obstetric and midwifery care along the Thai-Myanmar border, as well as a reference for the resettled population in countries as USA, Australia, New Zealand, Canada, Denmark, Finland, Sweden, Norway, the Netherlands and the United Kingdom, that have accepted more than 75,000 individuals from the Thai-Myanmar border since 2005.

The local centiles demonstrate that malaria impacts on birthweight across all nearly all gestations and that LBW, while specific, was poorly predictive of SGA infants. LBW is a poor indicator of SGA and remains one of the most frequent primary outcomes of studies on malaria and pregnancy (see www.clinicaltrials.gov; search terms “malaria” AND “pregnancy”). Ultrasound dating, precise methods of birthweight measurements and population centiles along with repeated measurements of growth within the same

pregnancy are required to understand exactly what constitutes LBW in malaria affected pregnancies.

In pregnancies affected by malaria the proportion of SGA observed by applying the global reference[18] was 1.7 fold higher than the proportion using the local centiles.

The development of the local birthweight centiles was based on data collected over 10 years. As many developing country settings lack the resources or infrastructure to accomplish such a large sample size, the idea of a global reference is appealing. However in a population that has no routine ultrasonography (most malaria endemic areas), achieving at least 100 accurately dated pregnancies with a gestation of 40 weeks is also a major undertaking. Since nobody can predict who will deliver in the 40 week interval, a much larger number than 100 would need to be scanned. In our setting it took 523 deliveries and 15 months from the first scan to the last delivery to reach the first 100 such pregnancy outcomes.

Although the predicted mean birthweights from the global reference were slightly higher than the predicted mean birthweight of the studied population, this difference was around 0.5 SD and thus well within the limits of normality. This confirms that the recently developed global reference centiles for mean birthweight are valid for refugee and migrant populations in resource poor settings as well. However, the 10th centile of the global reference was close to the mean (50th centile) of the population equation for GA 28-34 weeks (**Figure 9.4**). This may explain the 1.7 fold increase of neonates that would have been marked as SGA by using the global reference.

A limitation of using SGA is that it still encompasses neonates that are constitutionally small and those that are pathologically small due to IUGR. The diagnosis of IUGR can only be made by demonstrating significant deviation from the normal pattern of intra uterine growth which requires a minimum of two antenatal ultrasound measurements to be made[33-35]. Alterations in the patterns of growth at different stages in pregnancy may lead to different anthropometric phenotypes at birth[36]. Early pregnancy growth failure might be associated with a proportional reduction in length, weight and head size at birth. Late pregnancy growth failure might be associated with a reduction in weight, fat mass, and perhaps length and weight with "sparing" of brain growth[37, 38]. Ideally such anthropometric measurements should be taken into account for future malaria in pregnancy studies.

The data presented here supports the findings of previous work from this area when pregnancy dating relied on the Dubowitz gestational age examination; not malaria per se, but febrile illness close to delivery was associated with preterm birth[39]. Prevention of malaria would be expected to have a major effect in reducing SGA newborns and a smaller effect in reducing the mortality attributable to preterm births. In this community, malaria in pregnancy was observed to increase neonatal mortality indirectly by reducing birth weight[39]. Here, we have shown that malaria in pregnancy was associated with a lower birthweight for gestational age for nearly all gestational ages. Malaria in pregnancy needs to be prevented, because this will increase infant survival.

Acknowledgments

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Supporting Table S1 Birthweight (g) percentiles per completed gestational week for the refugee and migrant population on the Thai-Myanmar border

	P2.3	P3	P5	P10	P25	P50	P75	P90	P95	P97	P97.7
28	821.3	835.2	875	940	1046	1163	1280	1386	1451	1491	1505
29	863.1	878.3	922	993	1109	1237	1365	1482	1552	1596	1611
30	942.6	959.2	1007	1084	1210	1349	1488	1615	1692	1739	1756
31	1053.4	1071.3	1123	1206	1342	1493	1643	1779	1862	1914	1932
32	1188.8	1208.1	1263	1352	1499	1661	1822	1969	2058	2113	2132
33	1342.4	1363.0	1422	1517	1674	1847	2019	2176	2271	2330	2351
34	1507.6	1529.5	1592	1694	1861	2044	2228	2395	2496	2559	2581
35	1677.8	1701.0	1768	1875	2052	2247	2441	2619	2726	2793	2816
36	1846.4	1871.0	1942	2055	2242	2448	2654	2841	2955	3025	3050
37	2007.0	2032.9	2107	2227	2424	2641	2858	3055	3175	3249	3275
38	2153.0	2180.2	2258	2384	2592	2820	3048	3255	3381	3459	3486
39	2277.8	2306.3	2388	2520	2738	2977	3216	3434	3566	3648	3676
40	2374.9	2404.8	2491	2629	2856	3107	3357	3584	3722	3808	3838
41	2437.7	2468.9	2559	2703	2941	3202	3463	3701	3845	3935	3966
42	2459.8	2492.3	2586	2736	2984	3256	3529	3777	3927	4021	4053

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Chapter 10

Malaria in pregnancy: fetal growth

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Keywords
Malaria; pregnancy; ultrasound; centiles; z-scores; birthweight; gestational age

MANUSCRIPT IN PREPARATION

Introduction

In all malarious areas infection by *Plasmodium (P.) falciparum* or *P.vivax* during pregnancy is detrimental to the mother and the fetus[1-3]. Babies born to mothers infected with malaria parasites have a lower birth weight and are therefore at increased risk of death in infancy[4] or morbidity in later life[5]. The reduced birth weight is thought to result from placental insufficiency by sequestration of malaria parasites, leading to intra uterine growth restriction (IUGR) and possibly also to an increased incidence of preterm delivery, although the exact mechanisms remain unclear[6-8]. However, the negative effects of malaria on fetuses have often been documented from highly malaria endemic areas, where infections may have been left untreated or treated with ineffective drugs[9], where low birthweight was used as a primary endpoint, and where (early) ultrasound, the gold standard for pregnancy dating, was often not available[10]. IUGR is a difficult parameter to measure; it requires accurate dating of pregnancies and evidence of a reduction in growth, which requires at least two measurements. Malaria in pregnancy studies have been complicated by difficulties to follow women longitudinally during pregnancy, difficulties in diagnosis of malaria in (early) pregnancy and estimation of gestational age and differences in anthropometric measurements[10]. As a consequence, studies describing in-utero fetal growth in malaria endemic areas are limited, partly due to a lack of ultrasound resources necessary to diagnose IUGR[11-13]. In published articles, a significant independent effect of malaria on small for gestational age (SGA) fetuses was only seen among women with three of more infections in pregnancy in Congo, and this was related to maternal nutritional status[11]. On the Thai-Burmese border, malaria infection in early pregnancy was associated with smaller mid pregnancy fetal head diameters in a retrospective study[13], and there was evidence that early *P.falciparum* infections may be related to smaller mid pregnancy placental volumes[14]. Besides malaria, other factors are related to fetal growth such as parental anthropometrics[15], socio-economic status, maternal behaviour in pregnancy, smoking, red blood cell abnormalities[16-18] and morbidities during pregnancy[19].

The aim of this observational longitudinal study from the first trimester onwards, was to relate fetal growth to treated malaria episodes detected by weekly screening, taking into account haemoglobinopathies, blood cell indices, maternal and paternal anthropometrics, socio-economic status and compare fetal anthropometrics to that of uninfected pregnancies.

Although the study is still continuing at the time of the printing of this thesis, the aim of this chapter is to provide a general overview of the ongoing study, and describe the available data and highlight some preliminary results to guide future detailed analysis.

Methods

The women who participated in this longitudinal prospective study were attending two antenatal clinics (ANC) at the Shoklo Malaria Research Unit (SMRU): Wangpha and MawkerThai. The SMRU is located on the Thai-Burmese border, a malaria endemic area where *P.falciparum*[20] and *P.vivax*[21] transmission is low and seasonal. The majority of the people belong to the Karen ethnic group[22]. Due to a lack of effective prevention strategies (insecticide treated bed nets are insufficiently protective[23] and the malaria parasites are multi-drug resistant[2]) SMRU runs an active program of antenatal care (ANC) without charge to the refugee (since 1986) and migrant populations (since 1998)[20]. This includes a service of frequent (weekly) malaria screening, tympanic temperature and malaria smear, to detect and treat all parasitaemic episodes during pregnancy to prevent maternal death. At each visit the woman receives health education, for example through a video in her own language. In addition, routine obstetric evaluation including weight and symphysis fundal height measurement, blood pressure, fetal heart beat, Leopold's Maneuvers, and when indicated urine check, take place during the first consultation, and then at week 18, 24, 28, 32, 34, 36, and weekly until delivery. At the first antenatal visit, each woman has a base-line demographic interview, including tobacco and drug use, medical and obstetric history. Each woman has a full medical examination; height, and Mid Upper Arm Circumference (MUAC) are measured and an ultrasound examination for accurate pregnancy dating. Since the inception of this ANC program, all pregnant women have been encouraged to attend as early as possible in pregnancy. Women routinely receive ferrous sulphate, folic acid and vitamin B1[24] supplementation from the first consultation until delivery and tetanus vaccination is provided. All women are screened for HIV[25] and receive treatment if needed. All medical and obstetric problems in pregnancy are investigated and treated by locally trained medics working in SMRU facilities supervised by physicians and obstetricians[26, 27].

Inclusion criteria

All women aged ≥18 years with singleton viable pregnancies who registered in the antenatal clinic in the first trimester were invited to participate in the study. Exclusion criteria were: evidence of major congenital abnormality detected at the first trimester scan, known chronic maternal illness that could influence fetal growth, and unable to speak Burmese or Karen or English language.

Study procedures

Women who provided informed consent to comply with the study protocol were recruited in the study when their gestational age (GA) was between 9+0 and 13+6 weeks determined by fetal CRL on the study ultrasound machine. Briefly, using three separate images, the average of three blinded CRL measurements was used to determine the GA using Robinson's charts[28] as the first day of the last menstrual period is largely unknown in this population[12]. At baseline, a blood sample for detailed information

about haemoglobinopathies, G6PD status, red blood cell indices (complete blood count and red blood cell deformability) was obtained. Thereafter, women were invited to attend for a fetal biometry scan and uterine, umbilical and middle cerebral artery bloodflow measurements every 5 weeks. If a woman did not attend a scheduled scan, she was scanned at the next available opportunity. At each ultrasound appointment a complete blood count was taken and red blood cell deformability was measured. All pregnant women and their partners were invited to participate in a socio-economic questionnaire. Every woman with a malaria episode detected by peripheral blood smear was immediately treated irrespective of species parasite count or symptoms according to WHO protocols[29]. Additional ultrasound scans were performed at the time of a positive smear and two weeks later.

All women were encouraged to deliver in the SMRU facilities under supervision of locally trained and Advance Life Support Obstetrics© certified midwives who speak their language. Each labour was monitored on a WHO partograph. Complicated deliveries requiring Cesarean section were referred to the nearest Thai hospital (in a recent study the Cesarean section rate was 3.4% (129/3779)[13]). Maternal, cord and placenta blood, and placental biopsies were obtained at delivery. All newborns were examined for congenital abnormalities by staff trained in a standardized assessment. Birth weight and placental weight were measured twice by the anthropometry team on electronic Seca baby scales (Model 376, accuracy = 10 grams). Infant length and head circumference were measured twice by the same team on a Harpenden Infantometer with digital counter readings and Seca Head Circumference Tape, both accurate to one mm, respectively. The anthropometry team underwent specific training and ongoing quality control, and scales and infantometers were calibrated twice a week.

Ultrasonography

All ultrasound scans were performed trans abdominally using two General Electric (GE) Voluson i ultrasound machines with a RAB2-5-RS; 2-5MHz / Real time 4D probe purchased for the purpose of the study. The first machine was available since the start of the study in WangPha antenatal clinic and the second machine arrived the next year and was placed in MawkerThai antenatal clinic (which is 72 kilometers south of WangPha). Study ultrasound rooms were created with air conditioning and a voltage stabilizer installed. The protocol and operating manual for obtaining Crown Rump Length (CRL), and biometry measurements of the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) were similar to those used in the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) Project[30]. After each scan three of the following BPD, HC, AC, FL, left and right uterine artery, umbilical artery and middle cerebral artery Dopplers (in total 24) two-dimensional images were stored on the hard disk of the machine and the measurements tabulated automatically. In addition, at each scan a three dimensional sweep through the fetal head, abdomen, femur and placenta was acquired and stored. All scans were obtained by sonographers specifically trained in fetal

growth scanning, with regular internal quality control in the SMRU; in addition 10% of images were regularly sent for external quality control to the INTERGROWTH-21st Project team at the University of Oxford[30]. The Color pulsed-wave Doppler ultrasound was used to measure bloodflow characteristics in the maternal uterine arteries, and fetal umbilical and middle cerebral arteries using standard techniques.

Partner

All partners were invited to complete the socio-economic questionnaire (see **Supplementary file S1**). From each partner height, weight and MUAC were measured. Short stature was defined as height < 160 cm, for MUAC and BMI the same definitions were used as for the women[31].

Analysis and definitions

Clinical data were entered using Microsoft Access, and analyzed using SPSS version 15.0 for Windows (SPSS Inc., Chicago Ill, USA) and STATA version 11. Student's t-test and Mann-Whitney test were used for comparisons of the means and ranks respectively. Categorical data were compared used the chi-squared test. Associations with biometry and birthweight z-scores were assessed by univariate and multiple linear and logistic regression.

Each ultrasound measurement was converted into a z-score (z-score = (observed measurement – expected measurement) / standard deviation) based on a Chinese formula for biometry[32], for which the mean biometry measurements were not significantly different from this population[33]. This formula was chosen instead of our own[33], because it included measurements from 12 weeks onwards and outer-to-outer BPD measurements. The advantage of converting measurements into z- scores is that it eliminates variability by GA allowing measurements to be compared throughout pregnancy[34].

A positive z-score indicates a larger and a negative value a smaller than expected ultrasound measurement of fetal size or birth weight within the population. A dichotomous variable was created for each biometry measurement: ever small or never small, the latter indicating that none of the measurements during pregnancy was below the 10th centile. "Ever small" indicates that there was at least one measurement below the 10th percentile. The weight of each newborn was converted similarly into a z-score derived from the population reference curves for birthweight by gestational age (n=10264), see **chapter 9**). Each newborn was classified as small for gestational age (SGA) or normal, based on the local population centile formula: SGA was defined as a birthweight for gestational age below the 10th centile (z-score <1.28) of this population. IUGR is defined as a significant change in z-score during gestation compared to the reference population.

The "before-malaria"- episode scan is defined as the last ultrasound scan before parasites were detected, and the "after-malaria-episode" scan as the first ultrasound scan after a malaria episode, routinely 2 weeks later. There were four z-score analysis performed, see **Figure 10.2**. For z-score study 2 and 3, each women was only included once for the second trimester analysis and only once for the third trimester analysis. When there were

multiple second trimester infections available (5 women in the second and 3 women in the third trimester), the malaria episode with the highest parasitaemia was selected.
Malaria diagnosis was made by Giemsa stained thick and thin blood films; 200 fields on the tick film were read before declared negative. All parasite densities were counted per 500 white blood cells or per 1000 red blood cells and expressed as parasitaemia per microliter. A case of *Plasmodium (P).vivax* or *P.falciparum* malaria was defined by the presence of asexual stages of the respective parasite in the peripheral blood. All microscopists of SMRU had continuous quality control assessments. Anaemia was assessed from the complete blood count and was defined by a haematocrit less than 30%. Symptomatic malaria was defined by a tympanic temperature ≥ 37.5°C and/or a history of recent fever[29].
At the first consultation the Mid Upper Arm Circumference (MUAC) was measured on the unclothed left arm hanging loosely with a SECA tape measure (model 212) accurate to one mm and maternal height, in bare feet and with no head cover, was measured to the nearest 0.1 centimeter with a SECA stadiometer (model 242). Low MUAC was defined as <23.0 cm (previously a cut-off of 21.0 cm was used[13], but in this cohort only 3.3% of women had a MUAC <21.0[35]) and short stature as a height<145 cm. Maternal weight was measured at the first consultation as well as at the time of each ultrasound scan on an adult scale (model SECA 877) to the nearest 100 gram. Change in maternal weight gain was calculated every 5 weeks and categorized as low (<1.5 kg/5 wks) or adequate (≥ 1.5 kg/5 wks) weight gain. The body mass index (BMI) at the first consultation in the first trimester (as a proxy for pre-pregnancy anthropometric status) was calculated and a BMI of <18.5 kg/m^2 was considered low. Low socio economic status was defined as a binary composite variable, with women meeting all of the following criteria: no possession of a pig nor a television, and family income less than 2000 THB (49 EURO) per month.
Red blood cell deformability was measured with a laser-assisted optical rotational cell analyzer (LORCA)[36]which was on loan during the entire study period, except during the 2009 rainy season (16 May to 22 September 2009) and housed in one antenatal clinic. At a sheer stress of 1.96 Pascal, low red blood cell deformability was defined as an elongation index <0.16. At recruitment G6PD phenotype was analyzed in the clinic by fluorescent spot test, a CBC sample was taken and analyzed at the SMRU's hematology laboratory using a quality checked Sysmex pocH-100i automated haematology analyzer and a sample was send to Mae Sot Hospital for hemoglobin characterization by HPLC. Filter paper was used by hematology laboratory to extract human DNA by Saponin-Chelex method, and genotyping of G6PD Mahidol mutation was performed using primers as described in ref[37]. For the purpose of analysis the genotyping result was used to define G6PD deficiency.

Histopathology

Specimen Preparation
Placental biopsy specimens were fixed in 10% neutral buffered formalin, then subsequently cut and processed using standard embedding and processing for paraffin embedded sections. Paraffin embedded blocks were then used to cut 4 micron sections and stained with hematoxylin and eosin (H&E), or Giemsa staining. Slides were examined using an Olympus BX40 microscope with objectives including magnification of x100 (for general histopathological examination), x200 (for examination and quantitation of hemazoin malaria pigment), x400 (for assessment of inflammatory cells) and x1000 oil (for detection of parasitized erythrocytes).

Histopathological scoring system
As well as a general histological examination, the sections were examined for a number of pathological features which have been reported as occurring in placental malaria, although are not entirely specific to the disease, such as intervillous fibrin deposition, focal villous necrosis, excessive syncitiotrophoblastic knotting, and trophoblastic basement membrane thickening. Other more specific features which have been reported in a number of studies[38-44] include the presence of intervillous inflammation, malaria-parasitised red blood cells and the deposition of hemazoin pogment in fibrin clot. These three features were quantitated using the scheme of Muehlenbachs[45]. This allows semi-quantitative scoring of the degree of parasitisation, inflammation and hemazoin deposition according to the following scheme. The slides were examined blinded to clinical details by two independent pathologists and consensus reached by a third examination where there was any disparity in scoring.

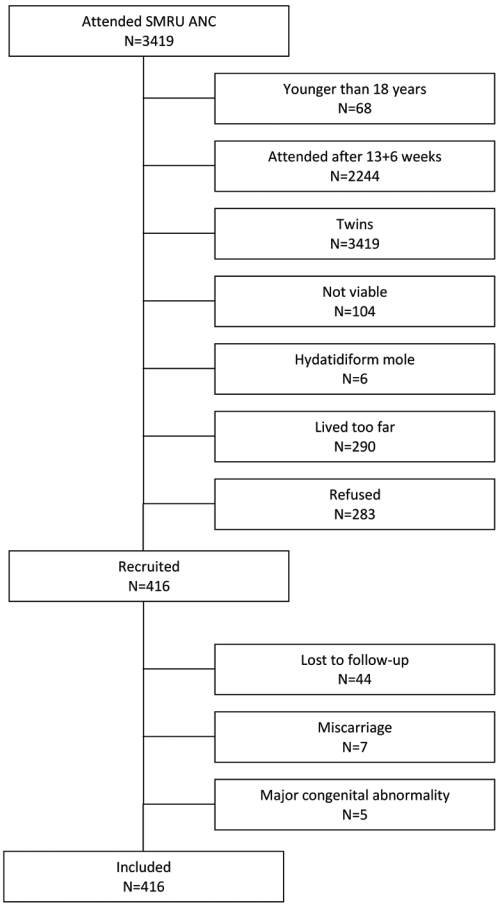
Ethics
All participants to this study (ClinicalTrials.gov Identifier: NCT00840502) provided written informed consent and the study protocol was yearly approved by Oxford University (OxTREC (14-08)) and Mahidol University (TMEC 2008-028) Ethics Committees.

Results

Between 10 February 2009 and 10 May 2011, 3419 new women attended SMRU ANC (the study started in Wangpha 10 February 2009 and in Mawker Thai 6 July 2010). The selection of pregnant women is illustrated in **Figure 10.1**; 416 women provided informed consent and were recruited. The characteristics of the women who were lost to follow up (n=44), miscarried (n=7) or delivered a newborn with a major congenital abnormality (n=5) were not significantly different from the 360 women who were followed throughout pregnancy and delivery (data not shown). These women attended

the ANC at a median gestational age of 9^{+2} (IQR 7^{+4} – 11^{+3}) weeks and were enrolled in the study at a median gestational age of 11^{+5} (10^{+3} – 12^{+5}) weeks. There were no stillbirths, but there was one maternal death; a homicide victim two weeks postpartum.

Figure 10.1 Selection of pregnant women



Malaria

The 360 women had 7485 antenatal visits and the same number of malaria smears; a mean of 21 per woman (SD 6) in pregnancy. Ninety-seven of 360 women (26.9%) were infected at least once with malaria during pregnancy, the baseline characteristics of infected and uninfected women are shown in **Table 10.1**. Women with malaria were more likely to chew betel nut, and to use fermented fish paste (Nya Oo Htee), their socio-economic status was lower than uninfected women, and they had less ANC visits and were more likely to be anaemic at any point in pregnancy. Their husbands were shorter and thinner, and had less schooling than the husbands of the non-infected women.

Table 10.1 Characteristics of pregnant women with and without malaria infections in pregnancy, and their partners

		Malaria (n=97)	Non malaria (n=263)	P value
Age		25 (21 – 29)	26 (21 – 30)	0.47
Age category				0.39
	18-24	44 (45.4)	118 (44.9)	
	25-29	30 (30.9)	66 (25.1)	
	>=30	23 (23.7)	79 (30.0)	
Primigravidae		31 (32.0)	82 (31.2)	0.90
Ethnic group pregnant woman				0.12
	Burmese	35/94 (37.2)	126/259 (48.6)	
	Karen	52/94 (55.3)	122/259 (47.1)	
	Other	7/94 (7.4)	11/259 (4.2)	
School		77/94 (81.9)	199/259 (76.8)	0.38
Can Read		61 (62.9)	174 (66.2)	0.61
Smoke		18 (18.6)	33 (12.5)	0.17
Alcohol		6/94 (6.4)	12/259 (4.6)	0.58
Betel Nut		45/94 (47.9)	89/259 (34.4)	**0.025**
Nya Oo Htee (fish paste)		76/94 (80.9)	231/259 (89.2)	**0.049**
Pill use before pregnancy		48/93 (51.6)	143/254 (56.3)	0.47
Low Socio economic status[$]		40/94 (42.6)	46/259 (17.8)	**<0.0001**
Height		152 (148 – 155)	153 (149 – 156)	0.40
Weight, first trimester		45.0 (42.0 – 50.5)	46.0 (43.0 -53.0)	0.20
BMI		20.2 (18.7 – 21.5)	20.3 (18.7 – 22.3)	0.35
MUAC		24.6 (23.5 – 26.3)	25.0 (23.2 – 27.0)	0.45
First trimester Diastolic BP		65 (60 – 70)	60 (60 – 70)	0.89
Thalassaemia		21/96 (21.6)	48/254 (18.9)	0.55
G6PD Mahidol variant		18/95 (18.9)	61/260 (23.5)	0.39
Paternal ethnic group				0.22
	Burmese	33/85 (38.8)	116/233 (49.8)	
	Karen	45/85 (52.9)	102/233 (43.8)	
	Other	7/85 (8.2)	15/233 (6.4)	
Paternal age*		28 (24.5 – 35)	29 (25 - 34)	0.86
Paternal height*		161 (157 – 165)	163 (159 – 167)	**0.014**
Paternal weight*		52.2 (48.2 – 56.0)	55.0 (50.4 – 59.0)	**0.001**
Paternal BMI*		20.1 (19.0 – 21.2)	20.7 (19.3-22.0)	**0.044**
Paternal MUAC*		26.2 (25.1 – 27.5)	27.0 (25.8 – 28.2)	**0.006**
Paternal schooling		52/85 (61.2)	178/233 (76.4)	**0.010**
Paternal smoking		60/85 (70.6)	148/233 (63.5)	0.29
ANC visits		20 (16 – 23)	22 (16 – 26)	**0.016**
EGA first consultation		62 (52-78)	66 (54-82)	0.07
Anaemia at any time		41 (42.3)	61 (23.2)	**0.001**
Morbidity at any time		27 (27.8)	67 (25.5)	0.69

Data shown as N (%) or median (IQR)
Paternal information available in 85 and 233 Partners of Malaria and Non malaria women respectively.
$ Low Socio economic status; women meeting all of the following criteria: no pig, no television, family income per month less than 2000 THB.

The 97 infected women had 243 malaria episodes (median 2, range 1-8 per woman) during pregnancy. The number and timing of episodes (**Table 10.2**) highlights that nearly half of the number of women with malaria (43/97) had their first malaria episode in the first trimester, before recruitment in the study (total 46 episodes). Three of these women were infected twice in the first trimester, but in four episodes there were no details available about symptoms or parasitaemia (3, 4, 5 and 12 weeks GA) as they were treated for malaria in an out-patient department in Burma, based on a rapid diagnostic test. There were in total 26 women with 30 *P.falciparum* malaria infections; two recurrent infections were genotypically different and seen as new infections, and from the other two no genotyping was possible, as the first episodes had occurred before the women were enrolled in the ANC. Three women had mixed *P.falciparum* and *P.vivax* infections and four women had two *P.falciparum* episodes.
Thirty seven percent (36/97) of the women had a single malaria infection in pregnancy: nine women with *P.falciparum* and 27 women with *P.vivax* infections. The remainder had multiple malaria episodes in pregnancy: 23 women (24%) had 2 infections, 38 (39%) had three or more. The geometric mean parasite density of *P.falciparum* (or mixed) and *P.vivax* was: 4050 (range 32 – 1051272) parasites / µl and 181 (range 16 – 51119) parasites / µl, respectively. The highest parasitaemia occurred in a woman who was absent from the weekly consultation for 19 days and arrived in labour with severe malaria (GA 37+4 weeks). Thirty-eight percent (92/243) of the episodes were symptomatic: 75.0% (18/24) of the *P.falciparum* episodes, 100% (3/3) of the mixed *P.falciparum* and *P.vivax* infections and 33.6% (71/211) of the *P.vivax* episodes. The geometric mean parasitaemias (/uL) in the asymptomatic episodes for *P.falciparum* (524) and *P.vivax* (88) were significantly lower than they were in symptomatic episodes; 7263 and 771, both $p<0.001$, respectively..

Red blood cells
Of the 360 women, there were 72 (20.0%) women with haemoglobinopathies, mainly HBE trait (48.6%, 35/72). Five (1.4%) women were G6PD deficient by rapid diagnostic test, and 79 by genotyping, mainly the Mahidol mutation (21.9%). Three hundred forty-five (95.8%) of 360 women had their blood group confirmed; the most common blood group was O (42.9%), followed by B (25.5%), A (24.9%) and AB (6.7%). There were 1574 red blood cell deformability measurements in 315 women (median 5, IQR 3-6) (all women of WangPha ANC). Twenty-three percent (72/315) of them had at least one measurement during pregnancy with a reduced deformability. Women with haemoglobinopathies (55.7%) had a higher proportion of reduced deformability than had women with normal haemoglobin (44.3%), $p<0.001$.

Morbidity
There were 94 (26.1%) women with 99 morbidity episodes other than malaria requiring medical therapy. The most common were infectious diseases (71.7%, n=71), high blood pressure (13.1% n=13) and pre-eclampsia (10.1%, 10). Of the infectious diseases,

symptomatic soil transmitted worms (18%, n=13), urinary tract infection (18%, n=13), scrub typhus (13%, n=9), diarrhea (11%, n=8) and pyelonephritis (10%, n=7) were the most common.

Table 10.2 Timing and numbers of malaria episodes, Thai-Burmese border 2009-2011

	First infection Number of women	Number of episodes	Number *P.falciparum* or mixed	Number of symptomatic episodes	Number of high parasites
Before CRL	43 (44.3)	46	15/46 (32.6)	21/42 (50.0)$	25/42 (59.5)$
Between CRL – biometry	26 (26.8)	45	1/45 (2.2)	20/45 (44.4)	29/45 (64.4)
Between first and last biometry	25 (25.8)	145	13/145 (9.0)	48/145 (33.1)	82/145 (56.6)
After last biometry scan	3 (3.1)	7	1/7 (14.3)	2/7 (28.6)	4/6 (66.7)#
Total	97 (26.9)	243	30/243 (12.3)*	91/239 (38.1)	140/238 (58.8)

$ No clinical information available for 4 women, who had their infections treated before enrolment in ANC
* Four women had another P.falciparum infection in pregnancy; of which 2 were new by PCR genotyping. From the other 2 there was no PCR spot available as they were before enrolment in the ANC.
# 1 smear (during labour) parasite count not available

Socio economic questionnaire

The socio-economic questionnaire was completed by 98.1% (353) of the 360 pregnant women and 88.3% (318/360) of their husbands. Most women and their husbands belonged to the Karen ethnic group, 49.3% (174/353) and 46.2% (147/318) respectively. More women than their husbands went to school: 78.2 % (276/353) versus 72.3% (233/318). Nearly one quarter of women (86/353) fitted the criteria for low socio-economic status.

Anthropometrics

In the first trimester the mean BMI was 20.7 kg/m2 (SD 2.9) and of the partners this was 20.7 kg/m2 (SD 2.4). Twenty-two percent of women (79/360) and 14.2% (45/317) of the partners had a low BMI. For low MUAC this was 20.0% (72/360) and 1.3% (4/318); the mean MUAC was 25.2 cm (SD 2.9) and 26.9 (SD 2.1), respectively. The mean height was 152 (SD 5.5) for the pregnant women and 162 (SD 5.7) for their partners.

Ultrasound

Apart from the CRL dating scan, the 360 women had in total 2039 ultrasound scans; a median of 5 scans per woman (IQR 5-6). The proportion of women with at least one biometry measurement below the 10th percentile (defined as SGA) and the percentage of these women infected with malaria are shown in **Table 10.3**. Thirty percent of the

women had at least one measurement below the 10th centile, and one third of the number of these women were infected with malaria (**Table 10.3**). For women with all biometry measurements larger than the 10th centile the percentage of malaria infected women was always lower; for BPD 22.6% (58/257), p=0.004, for HC 23.6% (61/258) p=0.034, for AC 23.8% (59/248) p=0.054, and for FL 26.0% (73/281), p=0.47.

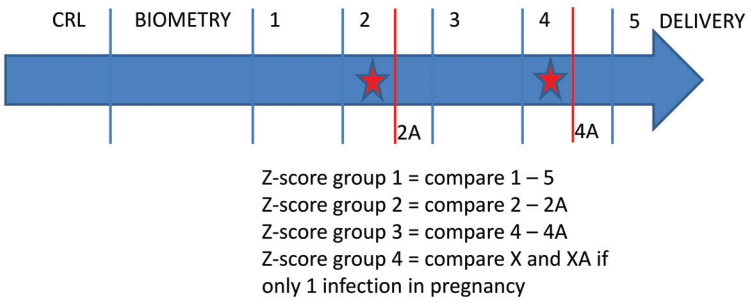
Table 10.3 The proportion of women with at least one biometry measurement below the 10th percentile (z-score < -1.28), Thai-Burmese border 2009-2011.

	N (%)	Single observation N (%)	Median (IQR) GA first observation	Proportion with malaria in pregnancy N (%)	Median (IQR) GA first malaria
Small BPD	103 (28.6)	53 (51.5)	26+6 (23+1, 35+0)	39 (37.9)	13+3 (10+3, 19+3)
Small HC	102 (28.3)	56 (54.9)	32+4(26+5, 35+1)	36 (35.5)	13+3 (11+2, 18+4)
Small AC	112 (31.1)	52 (46.4)	27+4 (23+5, 32+2)	38 (33.9)	13+0 (9+5, 18+0)
Small FL	79 (21.9)	44 (55.7)	33+6 (24+5, 37+5)	24 (30.4)	13+4 (9+1, 18+6)

GA gestational age, BPD biparietal diameter, HC Head circumference, AC Abdominal circumference, FL Femur length

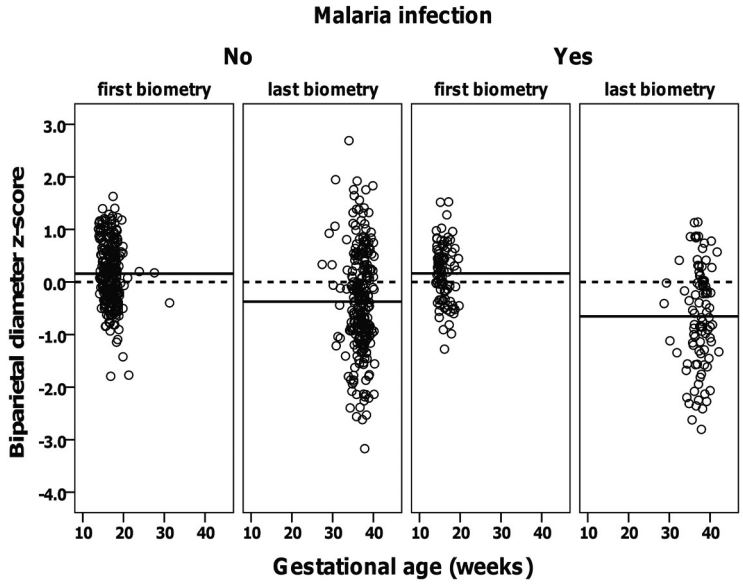
Four different steps of preliminary analysis of fetal growth were iteratively conducted (**Figure 10.2**). In z-score study group 1, the differences in z-scores between the baseline biometry in the early second trimester and the last available biometry measurement in the third trimester were compared between all women infected and not infected by malaria between these measurements. At the last biometry scan, the BPD and HC were significantly lower in women with at least one malaria infection in the second or third trimester (N=76) than in women who did not have malaria (n=263) (**Figure 10.3**). This was not the case for AC or FL (data not shown).

Figure 10.2 Preliminary analysis plan for z-scores comparisons.



The horizontal arrow indicates the pregnancy duration; the vertical blue lines represent 5 weekly ultrasound scans, which are numbered consecutively. The red stars show hypothetical malaria infections. The red vertical line indicates an extra scan performed 2 weeks after each malaria infection, which is labeled by a number (corresponding to the prior routine scan number) and a letter (A). X indicates that this infection can happen at any moment in pregnancy after 14 weeks gestational age.

Figure 10.3 The individual biparietal diameter z-scores at the first biometry scan and the last biometry scan in all women infected with malaria in between (n=76, right columns) and women without malaria (n=263, left columns).

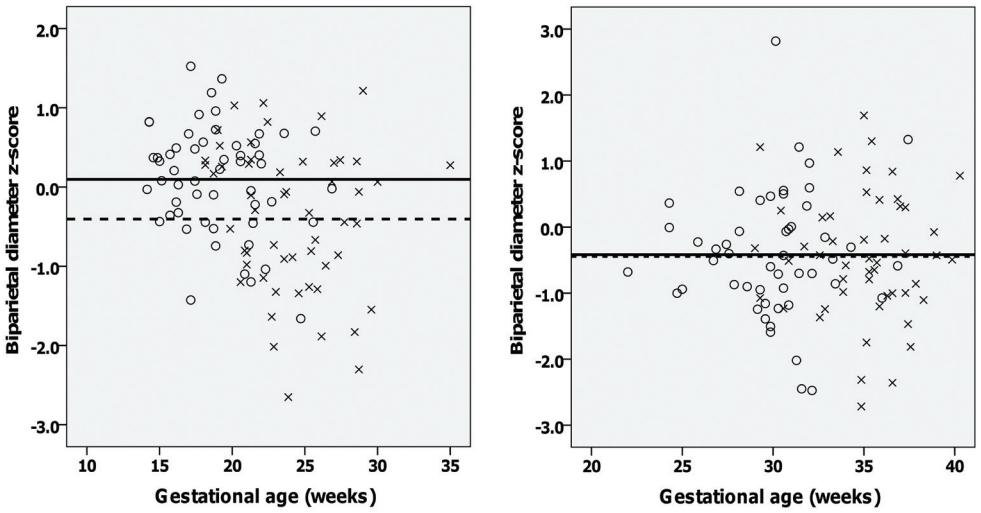


The mean z-scores are shown as black lines. The dotted line indicates the z-score = 0.

For z-score study group 2 and 3, seventy-two percent (n=104) of the 145 malaria infections after the first biometry scan were included: 54 in the second and 50 in the third trimester. Reasons for not including were: two malaria episodes within the 5 week window (n=19 (12.5%)) or no biometry scan after the malaria infection available (all late third trimester infections, n=20 (13.2%)), or already included (n=10 (6.6%)). Malaria infections in the second trimester resulted in smaller BPD (difference 0.502 SD, p=0.002) and HC (difference 0.429 SD, p=0.003), but this was not the case in the third trimester infections (**Figure 10.4**). There were no statistically significant differences for AC or FL measured. Of the 54 malaria infections in the second trimester, there were 25 (42.4%) symptomatic, 35 (59.3%) high parasitaemic, and eight (13.6%) *P.falciparum* episodes; but none of these were significantly related to lower BPD z-score. However, a higher parasitaemia as significantly associated with a lower HC z-score (B= -0.488, 95% CI -0.962, -0.015, p=0.043) in univariate analysis.

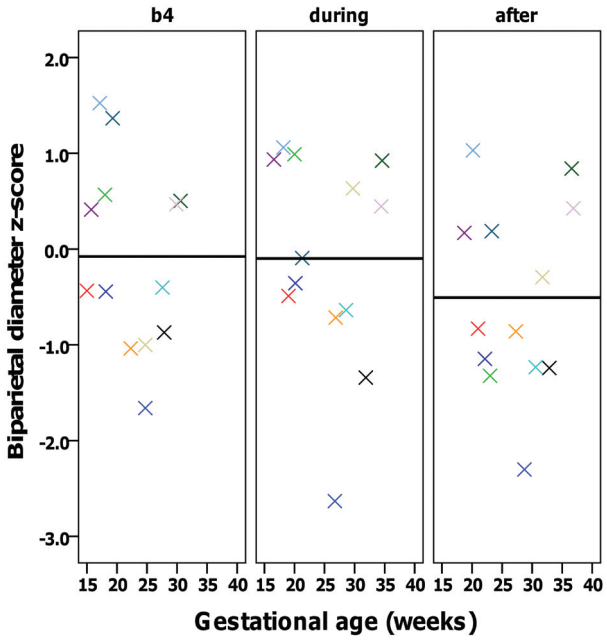
For z-score study group 4 only women with a single infection in pregnancy, and two consecutive scans before and after that malaria episode were included (n=13). Two weeks after a malaria infection, the z-scores of BPD (-0.51) and HC (-0.32) were lower than they were before and during the malaria episode; respectively -0.08 and -0.10 for BPD and 0.09 and 0.13 for HC, but these differences were not significant, p=0.27 and p=0.32 respectively (**Figure 10.5**).

Figure 10.4 Individual z-scores of biparietal diameter before (circles) and after (crosses) malaria infection in the second (left figure, n=54) and third trimester (right figure, n=50).



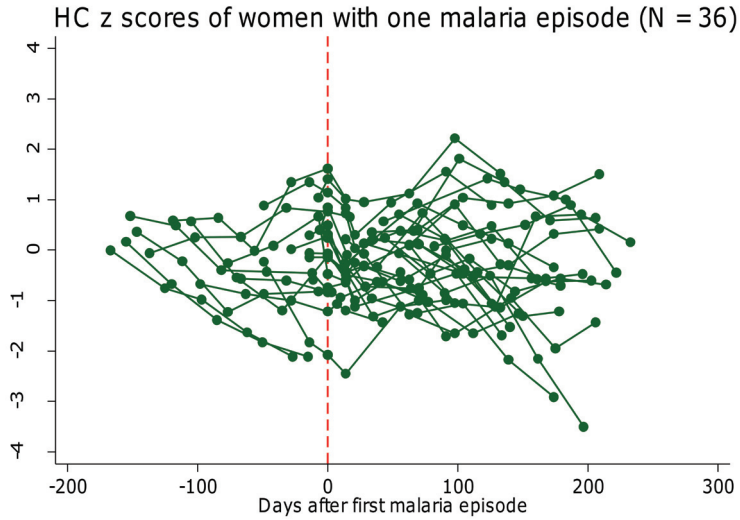
The horizontal lines indicate the mean z-score before (straight lines) and after malaria infections (dotted lines).

Figure 10.5 Individual z-scores of BPD before (left column), during (middle column) and after (right column) a single malaria episode in pregnancy.



The black lines represent the mean of the groups.

Figure 10.6 Head circumference z-score for women who had only one malaria episode before and after that malaria episode.



The individual z-scores for all 36 women with a single malaria episode in pregnancy are illustrated in **Figure 10.6**. Each line indicates the longitudinal HC z-scores a single woman. The red vertical line indicates the malaria episode. The lines left from the malaria episode are the HC z-scores before the woman was infected with malaria. The lines on the right size of the figure indicate the HC z-scores after the malaria episode. One may speculate that there may be a reduction in z-score immediately after the malaria episode followed by a more random behaviour of z-scores. Detailed modeling of the effect of malaria on z-scores at several time points will be performed in the future detailed analysis. The risk factors for a small biometry measurement (below the 10th centile (z-score < -1.28)) at any moment in pregnancy are shown in **Table 10.4**.
Risk factors for a small BPD at any time were a lower socio-economic status, female fetal gender, short height, and malaria (*P.falciparum* and higher parasitaemia). Anaemia was an additional risk factor for small HC. In the multivariate analysis, only socio-economic status and female gender remained independent risk factors, p=0.011 and p=0.046 respectively. The relation of malaria infection and timing of first appearance of the small biometry measurement will be performed in future analysis.

Birth outcomes
There were no stillbirths, but one perinatal death in an infant born preterm who died five days after birth from neonatal sepsis (GA 35+2, birthweight 2120, z-score birthweight -0.92). One hundred ninety nine newborns were male (54.5%). Most infants were born through normal vaginal delivery (92.3% 337/365); three were vaginal breech deliveries (0.8%), 12 instrumental vaginal births (11 vacuums and one forceps) (3.3%)

and 13 caesarean sections (3.6%). Ten percent (36/360) were born at home. There were nine (2.5%) newborns with congenital abnormalities: four minor (pre auricular skin tag (twice) and skin pit, small posterior encephalocele and five major: congenital heart disease, Down's syndrome, unilateral microtia, (symptomatic) hydronephrosis, duodenal atresia with double bubble sign). The newborns with major congenital abnormalities were excluded from all analysis; for the birthweight analysis only neonates, who were weighed within 24 hours, were included (89%, 327/365).

Table 10.4 Univariate analysis of risk factors for a biometry measurement below the 10th centile (z-score < -1.28) at any moment in pregnancy, Thai-Burmese border 2009-2011.

		Number (%)	BPD <10th centile		HC <10th centile	
			RR	95% CI	RR	95% CI
Socio-economic status	Low	86/353 (24.4)	**1.75**	**1.26-2.43**	**1.53**	**1.09-2.15**
	High	267/353 (75.6)		Ref		Ref
Gender	F	165 /360 (45.8)	**1.41**	**1.02-1.95**	**1.91**	**1.36-2.69**
	M	195/360 (54.2)		Ref		Ref
Height	Small	31/360 (8.6)	**1.70**	**1.09-2.56**	1.55	0.99-2.43
	Tall	329/360 (91.4)		Ref		Ref
Anaemia	Y	102/360 (28.3)	1.04	0.73-1.49	**1.44**	**1.03-2.01**
	N	258/360 (71.7)		Ref		Ref
Malaria	Y	97/360 (26.9)	**1.65**	**1.20-2.28**	**1.48**	**1.06-2.06**
	N	263/360 (73.1)		Ref		Ref
P.falciparum	Y	26/289 (9.0)	**1.74**	**1.06-2.86**	1.38	0.78-2.43
	N	263/289 (91.0)		Ref		Ref
High parasitaemia	Y	61/324 (18.8)	**1.68**	**1.17-2.44**	**1.50**	**1.02-2.21**
	N	263 /324 (81.2)		Ref		Ref

Not significantly associated with a small BPD or head circumference at any time in pregnancy were: Age, number of previous pregnancies, MUAC, BMI, literacy, smoking, alcohol, betel nut, or fish paste use, G6PD or haemoglobinopathy status, MCV and red blood cell deformability in the first trimester, BMI, weight, height and age of the partner.

Having at least one biometry measurement below the 10th centile was strongly associated with a smaller birthweight, head circumference, arm circumference, length, ponderal index and placental weight, but not on GA at birth (**Table 10.5**, data shown for HC). There was no difference in any birth outcome measure between women infected and uninfected with malaria (**Table 10.6**). However, subgroup analysis showed that women with *P.falciparum* (n=22), multiple malaria infections (n=51), symptomatic malaria (n=49), and higher parasite counts (n=52) had infants with significantly smaller birth-weight for gestational age z-scores than non infected women: -0.77 (p=0.034); -0.55 (p=0.039); -0.60 (p=0.028) and -0.63 (p=0.009) respectively.
Risk factors for a smaller birthweight for gestational age z-score in the univariate analysis were: primigravidae, smoking, low MUAC, short, low BMI, low SES, symptomatic

malaria, female sex, young father, father low BMI, having anaemia. However, with multivariate regression only primigravida, smoking, lower socio-economic status and female gender remained independent significant risk factors, but not malaria (**Table 10.7**).

Histopathological evidence for malaria parasites in the placenta was only present for one (1.0%) of the 97 women, and this was in the woman with concurrent severe malaria.

Table 10.5 Associations between head circumference measurements below 10th percentile and birth outcomes, Thai-Burmese border 2009-2011.

Head circumference	Z score < -1.28 (10th centile)		P value
	Ever small N=94	Never small N=230	
Low Birth Weight	20 (21.3)	23 (10.0)	**0.011**
Small for Gestational age	20 (21.3)	20 (8.7)	**0.003**
Preterm birth	5 (5.3)	12 (5.2)	1.0
Gestational age (weeks)	39.5 (1.7)	39.4 (1.7)	0.88
Birth weight	2701 (446)	2972 (450)	**<0.001**
z-score BW/GA	-0.84 (1.0)	-0.10 (0.9)	**<0.001**
Length	48.7 (2.4)	49.8 (2.5)	**<0.001**
Newborn head circumference	31.5 (1.3)	32.6 (1.7)	**<0.001**
Newborn arm circumference	10.3 (1.1)	10.7 (1.0)	**0.001**
Ponderal Index	23.2 (2.4)	23.9 (2.8)	**0.028**
Placental weight	470 (102)	531 (113)	**<0.001**

BW/GA = birthweight for gestational age

Table 10.6 Pregnancy outcomes in women infected with malaria (n=86) and non-infected women (n=238), Thai-Burmese border 2009-2011.

	Malaria	No Malaria	P
	n=86	n=238	
Low Birth Weight	13 (15.1)	30 (12.6)	0.58
Small for Gestational age	14 (16.3)	26 (10.9)	0.25
Preterm labour	4 (4.7)	13 (5.5)	1.0
Gestational age (weeks)	277 (12)	276 (11)	0.44
Birth weight	2859 (461)	2906 (466)	0.42
z-score BW/GA	-0.44 (0.99)	-0.27 (1.0)	0.16
Length	32.4 (1.6)	32.2 (1.7)	0.34
Newborn head circumference	10.6 (1.0)	10.6 (1.1)	0.90
Newborn arm circumference	49.4 (2.5)	49.5 (2.5)	0.74
Ponderal Index	23.6 (2.6)	23.8 (2.8)	0.51
Placental weight	506 (103)	515 (117)	0.53

BW/GA = birthweight for gestational age

Table 10.7 Risk factor for smaller z-score birthweight for gestational age, Thai-Burmese border 2009-2011.

		Frequency (%)	Univariate Coefficient (95% CI)	P-value	Multivariate (n=Coefficient (95%CI)	P-value
Primigravida	No	220 (67.9)	0.43 (0.20, 0.66)	**<0.001**	0.37 (0.10, 0.65)	**0.008**
	Yes	104 (32.1)				
Smoking	No	286 (88.3)	0.34 (0.03, 0.68)	**0.048**	0.39 (0.03, 0.75)	**0.036**
	Yes	38 (11.7)				
Low MUAC	No	263 (81.2)	0.29 (0.01, 0.57)	**0.043**	-0.08 (-0.42, 0.29)	0.66
	Yes	61 (18.8)				
Short	No	298 (92.0)	0.52 (0.11, 0.91)	**0.012**	0.41 (-0.09, 0.90)	0.11
	Yes	26 (8.0)				
Low BMI	No	252 (78.0)	0.37 (0.11, 0.63)	**0.006**	0.29 (-0.04, 0.61)	0.08
	Yes	71 (22.0)				
Low SES	No	243 (75.9)	0.32 (0.06, 0.58)	**0.014**	0.27 (-0.02, 0.56)	**0.066**
	Yes	77 (24.1)				
Betel Nut	No	206 (64.4)	0.05 (-0.19, 0.28)	0.70	*	NS
	Yes	114 (35.6)				
Nya Oo Htee	No	43 (13.4)	-0.12 (-0.44, 0.21)	0.48	*	NS
	Yes	277 (86.6)				
Alcohol	No	305 (95.3)	-0.28 (-0.81, 0.24)	0.29	*	NS
	Yes	15 (4.7)				
Illiterate	No	103 (32.0)	-0.02 (-0.24, 0.23)	0.99	*	NS
	Yes	219 (68.0)				
Malaria	No	238 (73.5)	0.18 (-0.07, 0.42)	0.16	*	NS
	Yes	86 (26.5)				
Symptomatic malaria	No mal	238 (82.9)	0.34 (0.03, 0.63)	**0.032**	0.12 (-0.20, 0.45)	0.45
	Yes	49 (17.1)				
Morbidity	No	239 (73.8)	0.08 (-0.17, 0.33)	0.54		
	Yes	85 (26.2)				
Sex	M	176 (54.3)	0.30 (0.09, 0.52)	**0.006**	0.24 (0.01, 0.47)	**0.045**
	F	148 (45.7)				
Father Low MUAC	No	4 (1.4)	0.31 (-0.66, 1.28)	0.53	0.10 (-0.19, 0.40)	0.50
	Yes	288 (98.6)				
Father Short	No	209 (71.6)	0.12 (-0.13, 0.36)	0.34	*	NS
	Yes	83 (28.4)				
Father Low weight	No	232 (80.0)	0.22 (-0.06, 0.50)	0.13	*	NS
	Yes	58 (20.0)				
Father young	No	220 (75.3)	0.29 (0.04, 0.55)	**0.026**	0.14 (-0.16, 0.42)	0.37
	Yes	72 (24.7)				
Father Low BMI	No	250 (85.6)	0.41 (0.09, 0.72)	**0.012**	0.02 (-0.03, 0.07)	0.45
	Yes	42 (14.4)				
Anaemia	No	235 (72.5)	0.37 (0.13, 0.61)	**0.003**	0.22 (-0.05,0.49)	0.11
	Yes	89 (27.5)				
G6PD	No	247 (76.2)	-0.06 (-0.32, 0.20)	0.64		
	deficient	72 (22.2)				
Thalassaemia	No	250 (77.2)	-0.02 (-0.30, 0.25)	0.87	*	NS
	Yes	64 (19.8)				
RBC deformability	>0.16	215 (76.0)	-0.23 (-0.49, 0.04)	0.10	*	NS
	<0.16	68 (24.0)				

* Not included in the multivariate analysis

Discussion

This is the first prospective longitudinal study on ultrasound assessed fetal growth in a malaria endemic area, which included all women in the first trimester of pregnancy. In this chapter the preliminary analysis of fetal growth in a low-transmission malaria endemic area is described. In this setting, each malaria infection is potentially fatal to the mother and the fetus. An effective system to prevent maternal death from malaria on the Thai-Burmese border has been frequent (weekly) intermittent screening (McGready, submitted). This is confirmed by the present study, as there was only one woman with severe malaria and she failed to attend her weekly screening for nearly 3 weeks.

In this study, the majority of malaria infections were *P.vivax* (87.7%), which is now the prominent parasite on the Thai-Burmese border[2]. Two third of the women had more than one infection in pregnancy. More than 60% of the infections were asymptomatic; more than 40% had a parasitaemia less than 80 parasites per microliter. The ANC system of early detection and treatment with effective drugs eliminated parasites in early stage of the infection before they could cause symptoms or multiply rapidly and therefore may have prevented sequestration of parasites of *P.falciparum* and probably *P.vivax* in the placenta. Similar to previously reported[44] there was no histopathological evidence of sequestration of parasites in the placenta in women who were infected in pregnancy, but not at the time of the delivery. Often it is proposed that IUGR is caused by a local effect of *P.falciparum*-infected erythrocytes in the placental intervillous space[7, 8], detailed future histopathological analyses of the placenta biopsies from this study should focus on placental changes other that just parasitaemias.

In this area, the mean reduction in birth weight of *P.falciparum* (317 infants) and *P.vivax* (525 infants) infected women compared to non-infected women has been reported as -123 (95% CI 34-212)[20] and -107 g (95% CI 61–154) grams[21], respectively. The present study demonstrates that early detection and treatment with efficacious drugs has a positive effect on fetal growth, but does not prevent IUGR completely. Clearly, although a quarter of women were infected with malaria at least once during pregnancy in this cohort, there was no effect of malaria on birthweight centiles or gestational age. However, at least one episode of *P.falciparum*, a symptomatic episode, an episode with a higher parasitaemia or multiple malaria infections in pregnancy were associated with a smaller birthweight for gestational age in the univariate analysis. By studying SGA, instead of LBW or birth weight as an outcome, the effect of malaria in pregnancy could be assessed with more precision. No increase in incidence of preterm birth was found in the malaria infected pregnancies, which confirmed a previous finding[4]. This study was not powered to detect a malaria associated difference in birthweight, but was set up to document the changes in fetal growth around malaria episodes.

Previous studies conducted in areas of high *P.falciparum* transmission have reported associations between SGA and both antenatal[46-48] and placental malaria infection[46-50]. However in none of these studies ultrasound was used for dating pregnancy and the definition of SGA was often based on 10th centiles for populations in developed countries,

which may have over-estimated the proportions of IUGR fetuses in developing countries. Our study findings are in line with those of the only other published longitudinal study of IUGR[51], where dating was mainly done in the second trimester (mean GA at enrolment 18 (SD 3) weeks). In that study a significant independent effect of malaria was only seen in women with three or more infections during pregnancy and the highest risks of IUGR were among the most undernourished women[51]. The authors explained this finding by the frequent (monthly) malaria screening and treatment of all positive antenatal parasitaemia and two presumptive doses of sulfadoxine-pyrimethamine[51].
The ultimate proof that malaria causes IUGR is to detect growth restriction in-utero, while controlling for other factors that affect growth. In these preliminary findings, the only effect of malaria infection on ultrasound assessed fetal growth was a smaller BPD and HC z-score in the second trimester two weeks after a malaria infection. This confirms the findings of a large retrospective study where malaria infections in the first half of pregnancy resulted in a smaller than expected mid-trimester fetal head diameter[13]. The growth velocity of the fetal head is maximal during the second trimester[52-54] and malaria infection during this time period had the most effect of fetal head growth. All other measurements did not differ between infected and non-infected cases. However, such a reduction in growth need to be controlled for timing and type of (multiple) malaria infections and other factors that affect growth, such as anaemia, socio-economic status and timing of infection requiring complex statistical modeling.
The final analysis of this data will have several challenges: firstly, nearly half of the infected women (44.3%) in this study had their first infection very early in pregnancy, before the CRL dating scan. Such infections may cause first trimester IUGR, as shown for smoking, maternal age[55] or anaemia[56]. Such factors are difficult to control for in this setting because the date of last menstrual period was not available, but may potentially have an impact on the CRL size, and thus on the dating of the pregnancy. An error in dating has an effect on birth weight for GA z-scores, but may not be problematic for the intra-uterine comparisons of two z-scores in the same women. While the dramatic effects of malaria infections in the first trimester have been reported[57], ultrasound studies of fetal growth in the first trimester after malaria infection do not exist. Furthermore, all ultrasound biometry measurements were converted into z-scores based on a Chinese population, which had comparable, but not exactly similar fetal size characteristics[33]. The fact that the z-scores of BPD and HC are reduced with advanced gestational age in the non malaria group as well, needs further explorations. Thirdly, although newborns who had at least one measurement of small BPD or HC in-utero had a smaller HC at birth, this was not seen for women infected with malaria. This may suggest that highly effective treatment of malaria allows some recovery of placental damage and perhaps a catch up in growth. A future longitudinal modeling approach where the details of individual growth velocity can be analyzed, may provide information about the possibility of recovery of growth after efficacious treatment of malaria infections. Lastly, fetuses whose mother had a lower socio-economic status, smokers, or anaemic, or whose father had a low BMI were at risk for being SGA in-utero. In contrast to previous studies

thalassaemia was not significantly related to adverse birth outcomes[16-18]. The ANC with frequent blood smears and haematocrit checks and weekly provision of supplements may have prevented the adverse effects of thalassaemia.

The fact that there were no stillbirths in this study, may be related to the intensive follow up of all pregnancies by antenatal ultrasound. All fetuses were closely monitored, and there were four labour inductions because of abnormal ultrasound findings. Had this information not been available these women might have suffered a stillbirth. The value of antenatal ultrasound in developing settings countries on clinical outcomes such as stillbirth or neonatal outcome needs assessment.

This study included a large range of assessments of risk factors for IUGR, such as socio-economic status, paternal anthropometrics, and other morbidities. The aim of this preliminary report was to summarize the available data so far, and to generate hypothesis for more detailed, complicated statistical analysis. Data still to analyze include: uterine, umbilical, and middle cerebral artery dopplers, placental and fetal organ volumes throughout pregnancy, first trimester placental hormones, placental histopathology, utero-placental hemodynamics in relation to RBC indices and RBC deformability, sensitive detection of parasites by PCR during pregnancy to account for sub-microscopic malaria infections, and analysis of placenta specific biomarkers in the maternal peripheral blood before, during and after antenatal malaria.

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Supplementary file S1

ULTRASOUND STUDY: QUESTIONNAIRE MOTHER

CHECK OPD LEMMA with CRF

Study Code: UPS- ☐☐☐
ANC code: ___- ☐☐☐☐
EGA: ☐☐ wks

Address: ☐☐☐☐☐☐ House number ☐☐☐ Section ☐☐☐
Ethnic Group _____

Status:(R=residence; W=worker; V=visitor) ☐ Age ☐☐ year
MUAC ☐☐☐ Height ☐☐☐ cm Weight ☐☐ kg

1. When did you arrive On the Thai Burmese border? ☐☐ years ☐☐ months

2. Where did you live before you arrived on the Thai Burmese border? ☐
1. Village in Karen side
2. Township or big city in Karen side
3. Refugee camp
4. Other _____

3. Which activity did you do before arriving here? ☐
1. Farmer
2. Worker: describe _____
3. Housework
4. Student
5. Shopkeeper
6. Other _____

4. Did you go to school? Yes ☐ No ☐

5. Standard level: How many years did you go to school ☐☐ years
e.g. grades 4: Can read and write; primary school ☐☐ grade
5, 6 or 7: middle school
8, 9 or 10: high or secondary school
Other _____

6. What languages do you speak? (ask her to speak) mark each box as yes or no

1. Sgaw Karen	Yes ☐	No ☐
2. Pao Karen	Yes ☐	No ☐
3. Burmese	Yes ☐	No ☐
4. Thai	Yes ☐	No ☐
5. Arabic	Yes ☐	No ☐
6. Urudoo	Yes ☐	No ☐
7. English	Yes ☐	No ☐
8. Other _____	Yes ☐	No ☐

7. What languages do you read? (pick one of the language cards at random)

0. Cannot read at all	Yes ☐	
1 Sgaw Karen	Yes ☐	No ☐
2. Pao Karen	Yes ☐	No ☐
3. Burmese	Yes ☐	No ☐
4. Thai	Yes ☐	No ☐
5. Arabic	Yes ☐	No ☐
6. Urudoo	Yes ☐	No ☐
7. English	Yes ☐	No ☐
8. Other _____	Yes ☐	No ☐

ETHICS COMMITTEE
FACULTY OF TROPICAL MEDICINE
MAHIDOL UNIVERSITY
VALID UNTIL: 08 JAN 2013

version 18.07.09

Questionnaire p.2 **Study Code: UPS-** ☐☐☐☐
ANC code: _ _ _- ☐☐☐☐

8A. Since your pregnancy, did you work?

0. No/stay home	No ☐	Yes ☐
1. House-cleaner / maid	No ☐	Yes ☐
2. Teacher / healthworker	No ☐	Yes ☐
3. Farmwork	No ☐	Yes ☐
4. Forest work	No ☐	Yes ☐
5. Factory_____	No ☐	Yes ☐
6. Shopkeeper	No ☐	Yes ☐
7. Other _____	No ☐	Yes ☐

8B. What did you get for this work?

☐ Nothing
☐ Money
☐ Food
☐ Other_____
☐ No answer

9. What is your religion? ☐

1. Buddhist
2. Muslim
3. Christian
4. Other_____

10. Do you have?

1. Chicken or duck	Yes ☐	No ☐
2. Pig or goat	Yes ☐	No ☐
3. Cow	Yes ☐	No ☐
4. Radio	Yes ☐	No ☐
5. Television	Yes ☐	No ☐
6. Bicycle	Yes ☐	No ☐
7. Motorbike	Yes ☐	No ☐
8. Your own house	Yes ☐	No ☐
9. Mobile phone	Yes ☐	No ☐

11. In one month, how much money do your family/household earn (in average)? ☐

1. Nothing (=nobody works for money in the family)
2. Less than 500 baht / month
3. 500-2,000 baht / month
4. More than 2,000 baht /month
5. Other: _____

If she cannot answer question 11, ask:

11a. How many people in your family? _____
11b. How many of them have a job? _____
11c. How many months of work per year in average? _____
11d. How many days of work per month in average? _____
11e. What is the average daily salary for your family? _____

12. Do you grow your own fruits or vegetables? Yes ☐ No ☐

13. How many people normally live in your house (including yourself and babies)? ☐

14. How many times do you take betel nut? ☐

1. Never use it
2. Not every day, only some days
3. Everyday but normally only once
4. Many times a day

If answer is "never", go directly to question 15

14a. When do you take betel nut? ☐

1. On an empty stomach - before meals
2. After meals
3. Between meals
4. After and between meals
5. Other _____

version 18.07.09

Questionnaire p.3 Study Code: UPS-☐☐☐
ANC code: ___-☐☐☐☐

15. Do you take your pregnant women vitamin supplements? ☐
1. No *If answer is "no", go directly to question 16*
2. Sometimes
3. Yes

15a. When you take your pregnant woman vitamin supplements? ☐
1. On an empty stomach - before meals
2. After meals
3. Between meals
4. Other _____

16. How many meals per day do you have nya oo htee? ☐
1. Never
2. Only some days
3. 1 meal per day
4. 2 meals per day
5. 3 - 4 meals per day
6. Other _____

17. Do you drink alcohol/happy water? Yes☐ No☐

If answer is "never", go directly to question 18

17a. How many times do you have happy water? ☐
1. Only some days
2. Once a week
3. 1 time per day
4. 2-3 times per day
5. More than 3 times per day

18. Did you smoke last week: Yes☐ No☐

If the answer is no, go directly to question 19

18a. how many cigarettes ☐ cigarettes

18b. how many cheroots ☐ cheroots

18c. other _____, how many ☐

19a. Last week, did you sleep under a mosquito bednet? Yes☐ No☐

If yes, how many nights (give a number between 0 days and 7 nights) ☐

19b. How many persons sleep under the same mosquito bednet (including you) ? ☐
(example: husband + 1 child + pregnant woman = 3 persons under mosquito net)

19c. Is your mosquito bednet impregnated? Yes☐ No☐ I don't know☐

19d. Where did you get your bednet from? ☐
1. Buy; where _____
2. Gift; from where _____
3. Other: _____

20. Did you use the pill or depot before this pregnancy?
Yes☐ No☐ I don't know☐

If yes; when did you take the last one? ☐☐☐☐☐☐

Name of the person who asked the questions: _____

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ULTRASOUND STUDY: QUESTIONNAIRE PARTNER

CHECK OPD LEMMA

Study Code: UPS-
ANC code Wife: ___-
Date dd/mm/yy

Address: House number Section

Ethnic Group

Status:(R=residence; W=worker; V=visitor) Age

MUAC Height cm Weight kg

1. When did you arrive on the Thai Burmese border? years months

2a. Most of the time you:

1. Stay in the same house as your wife	Yes	No
2. Stay and work outside the village (max 1 day/week)	Yes	No
3. Stay and work outside the village (>1 day/week)	Yes	No
4. Are working as a soldier	Yes	No
5. Other	Yes	No

2b. What work do you do?

1. Stay home	No	Yes
2. Teacher/healthworker	No	Yes
3. Forest work	No	Yes
4. Farmwork	No	Yes
5. Factory	No	Yes
6. Shopkeeper	No	Yes
7. Mechanical/Electric repair	No	Yes
8. Daily labor	No	Yes
9. Soldier	No	Yes
10. Other	No	Yes

2c. What do you get for this work?

- Nothing
- Money
- Food/soldier's ration
- Other
- No answer

3. Where were you born?

1. Burma
2. Thailand
3. Other

4. What job did **you do before** you arrived here?

1. No job
2. Student
3. Soldier
4. Farmer
5. Other

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5. How many years did **you** go to school?

6. Standard level: Grade

7. What languages do you speak? (ask him to speak) mark each box as yes or no

1 Sgaw Karen	Yes	No
2. Pao Karen	Yes	No
3. Burmese	Yes	No
4. Thai	Yes	No
5. Arabic	Yes	No
6. Urudoo	Yes	No
7. English	Yes	No
8. Other	Yes	No

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Questionnaire p.2 **Study Code: UPS-** ☐☐☐
ANC code: ___- ☐☐☐☐

8. What languages do you read? (pick one of the language cards at random)

0. Cannot read at all	Yes ☐	
1 Sgaw Karen	Yes ☐	No ☐
2. Pao Karen	Yes ☐	No ☐
3. Burmese	Yes ☐	No ☐
4. Thai	Yes ☐	No ☐
5. Arabic	Yes ☐	No ☐
6. Urudoo	Yes ☐	No ☐
7. English	Yes ☐	No ☐
8. Other _____	Yes ☐	No ☐

9. What is your religion? ☐
1. Buddhist
2. Muslim
3. Christian
4. Other _____

10. Do you smoke in your house: Yes ☐ No ☐

If the answer is no, go directly to question 11

10a.how many cigarettes ☐ cigarettes

how many cheroots ☐ cheroots

other _____, how many ☐

11. Did you ever have malaria? Yes ☐ No ☐

When was the last episode _____

12. Did you ever take medicine for a long time? Yes ☐ No ☐
if yes, what disease

_____ year _____

_____ year _____

13. In one month, how much money do your family/household earn (in average)? ☐
1. Nothing (=nobody works for money in the family)
2. Less than 500 baht / month
3. 500-2,000 baht / month
4. More than 2,000 baht /month
5. Other: _____

If he cannot answer question 11, ask:
11a. How many people in your family? _____
11b. How many of them have a job? _____
11c. How many months of work per year in average? _____
11d. How many days of work per month in average? _____
11e. What is the average daily salary for your family? _____

Name of the person who asked the questions: _____

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Chapter 11

Effect of malaria on placental volume measured using three-dimensional ultrasound: a pilot study

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Keywords
Malaria, pregnancy, three-dimensional ultrasound, placenta volume, IUGR

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Abstract

Background

The presence of malaria parasites and histopathological changes in the placenta are associated with a reduction in birth weight, principally due to intrauterine growth restriction. The aim of this study was to examine the feasibility of studying early pregnancy placental volumes using three-dimensional (3D) ultrasound in a malaria endemic area, as a small volume in the second trimester may be an indicator of intra-uterine growth restriction and placental insufficiency.

Methods

Placenta volumes were acquired using a portable ultrasound machine and a 3D ultrasound transducer and estimated using the Virtual Organ Computer-aided AnaLysis (VOCAL) image analysis software package. Intra-observer reliability and limits of agreement of the placenta volume measurements were calculated. Polynomial regression models for the mean and standard deviation as a function of gestational age for the placental volumes of uninfected women were created and tested. Based on these equations each measurement was converted into a z-score. The z-scores of the placental volumes of malaria infected and uninfected women were then compared.

Results

Eighty-four women (uninfected = 65; infected = 19) with a posterior placenta delivered congenitally normal, live born, single babies. The mean placental volumes in the uninfected women were modeled to fit 5th, 10th, 50th, 90th and 95th centiles for 14-24 weeks' gestation. Most placenta volumes in the infected women were below the 50th centile for gestational age; most of those with Plasmodium falciparum were below the 10th centile. The 95% intra-observer limits of agreement for first and second measurements were ± 37.0 mL and ± 25.4 mL at 30 degrees and 15 degrees rotation respectively.

Conclusion

The new technique of 3D ultrasound volumetry of the placenta may be useful to improve our understanding of the pathophysiological constraints on fetal growth caused by malaria infection in early pregnancy.

Background

Falciparum and vivax malaria have a markedly negative impact on mothers and babies[1, 2]. For example, malaria infection in pregnancy is associated with a reduction in birth weight, principally due to intrauterine growth restriction (IUGR)[3, 4]. Although the mechanisms responsible for IUGR are not fully understood, histopathological changes, such as thickening of basal membranes of the placenta[5], intervillositis[6-9], hypoxia[10, 11] and syncytial destruction[12, 13] have all been implicated.

The introduction of three-dimensional (3D) ultrasound has made it possible to assess intrauterine growth more accurately by allowing fetal organ and placental volumes to be measured[14, 15]. For example, a small placental volume in the second trimester may be an indicator of IUGR and placental insufficiency[16, 17]. Virtual Organ Computer-aided AnaLysis (VOCAL™, General Electric (GE) Healthcare, Austria) is a software analysis package used for calculating volumetry of different organs, such as the placenta[18], fetal volume[19] and organs including the fetal brain[20], thigh[21], spleen[22] and lungs[23].

This relatively new technique has never been used to study placenta volumes in pregnancies complicated by malaria. The aim of this study was to examine the feasibility of studying early pregnancy placental volumes in a malaria endemic area.

Methods

The participants in the present study were attending the antenatal clinic (ANC) at Shoklo Malaria Research Unit (SMRU), which is located on the Thai-Burmese border. SMRU has focused on the epidemiology, prevention and treatment of malaria in pregnancy since 1986. The epidemiology of Plasmodium (P.) falciparum[24] and P. vivax[25] malaria in pregnancy is well described. There is a lack of effective prevention strategies and the malaria parasites are multi-drug resistant. Hence, SMRU runs an ANC programme with weekly screening to detect and treat all parasitaemic episodes during pregnancy to prevent maternal deaths[24]. All women are encouraged to attend the ANC as early as possible in pregnancy and to deliver at SMRU under the care of Advance Life Support in Obstetrics (ALSO) trained midwives and doctors; those requiring Caesarean section are transferred to the nearest Thai hospital.

The study was part of a larger fetal growth project (ClinicalTrials.gov Identifier: NCT00840502), approved by the Ethics Committees of Oxford (OxTREC (14-08)) and Mahidol (TMEC 2008-028) Universities.

Ultrasonography

Ultrasound scans were performed trans-abdominally using a Voluson i (GE Healthcare, Austria) with a RAB2-5-RS; 2-5MHz / Real time 3D probe. The machine was housed

in a dedicated air-conditioned room equipped with a voltage stabilizer. All scans were obtained by sonographers specifically trained in fetal growth scanning[26], with regular internal quality control at SMRU; in addition, images were regularly sent for external quality control to the INTERGROWTH-21st Project team at the University of Oxford[27].

Only women with singleton pregnancies were recruited. As the last menstrual period is largely unknown in this population[26], gestational age (GA) was determined by measuring the fetal Crown Rump Length (CRL) between 9+0 and 13+6 weeks. The protocol and operating manual for obtaining CRL measurements were similar to those used in the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) Project[27]. Briefly, using three separate images, three blinded measurements were taken of each CRL; the mean of the three values was then used to estimate GA using Robinson's charts[28].

Thereafter, women were invited to attend for a fetal growth scan every five weeks until delivery to take standard 2D fetal biometric measurements. If a woman did not attend a scheduled scan then she was scanned at the next available opportunity. Every woman with a malaria episode detected by peripheral smear was treated using WHO protocols[29] and had fetal growth scans (in addition to those planned) at the time of a positive smear and two weeks later.

After standard 2D measurements were taken, a 3D sweep was obtained through the placenta. The 3D placental volume was acquired keeping the probe perpendicular to the placental plate and the size of the volume box was adapted to include the entire placenta. The sweep angle was set at 85° so as to visualize the maximum amount of placenta possible. Care was taken to minimize movement artifact. Once the scan was complete, volume data were stored on the system's hard drive for later analysis.

Analysis

Only 3D ultrasound images of the placenta on the posterior uterine wall taken between 14+0 to 23+6 weeks (two sets of five weeks) were analysed. This approach was chosen because most placentas (especially on the anterior wall) are too large to fit in a single 3D sweep after 20 weeks. Based on published recommendations for cross-sectional studies, only one measurement per woman was included in the analysis[30, 31]; if more than one good quality volume scan was available, the last one was analysed. Women who miscarried, and those with early onset pre-eclampsia or who left the study area before birth, were excluded. Scans were excluded from the analysis if the image quality was poor or a single continuous outline could not be drawn around the placenta as accurate volume data could not be obtained in these circumstances.

One author (WEM), blinded to clinical data, performed the volume measurements using the VOCAL™ method as described by Wegrzyn et al[32]. A sequence of sections of the placenta was obtained with the VOCAL™ method, which is based on the rotation of

an object along its axis, using a predefined angle. Each placental volume was calculated with fixed angles of 30° (six sections) and 15° (12 sections). All volume measurements were taken twice for both rotation angles and an average of the two volume measurements was computed for each rotation to be used in the analysis.
Intra-observer reliability and limits of agreement (mean difference ± 1.96 SD) of the placenta volume measurements were calculated as described by Bland and Altman[33]. Systemic bias was determined by calculating the 95% confidence intervals (CI) for the mean difference between the measurements. If zero was included within this interval, no bias was assumed.
From the 15° measurements, separate polynomial regression models were fitted for the mean and standard deviation (SD), each as a function of GA[30], of the placenta volumes of women who had not been infected with malaria prior to the volume scan. This approach assumes that the distribution of measurements at each GA is Gaussian. The models were examined under the family of fractional polynomials utilizing its wide range of possibilities and flexibility[34]. An iterative procedure implemented in STATA version 11 statistical software (StataCorp, TX, USA) was used, whereby the model for the mean is weighted by the reciprocal of the square of the fitted SD[35]. A goodness-of-fit test of the models was also assessed by a likelihood ratio test by comparing the deviances. The estimated equations for the mean and the SD of these placenta volumes were of the form y=a+b*GA (where GA is in days). From these predictive mean and SD equations the corresponding centiles were calculated using the formula:

centile = mean + K * SD,

where K is the corresponding centile from the theoretical Gaussian distribution (e.g. determination of the 10th and the 90th centiles requires K to be ± 1.28, and ± 1.645 for the fifth and 95th centiles).
Based on these equations the placental measurements of all women were converted into a z-score:

z-score = (measured volume - expected volume)/ SD volume.

The advantage of using z-scores is that it eliminates the variability of measurements by GA, which allows for direct comparability of measurements. A negative z-score denotes a placental volume that is smaller than the expected mean for the population at a given GA. The z-scores of women who were or were not infected with malaria before the scan were compared.
Clinical data were entered using Microsoft Access, and analysed using SPSS version 15.0 for Windows (SPSS Inc., Chicago Ill, USA) and STATA version 11. The student's t-test was used to compare means, the Shapiro-Wilk test-to-test normality.

Results

Between 10 February 2009 and 30 April 2010, a total of 250 women were recruited at a mean gestational age of 80 ± 10 (SD) days. Of these women, 84 (33.6%) were eligible for placental volume analysis as defined in the Methods section (**Figure 11.1**). Their baseline characteristics, e.g. age, parity, smoking status, etc., did not differ from the non-included women (data not shown). Of these 84 women, 19 (22.6%) had a malaria episode before the volume scan; the other 65 women remained malaria free (**Table 11.1**). All women delivered congenitally normal, live born, single babies.

Figure 11.1 Inclusion of placental scans

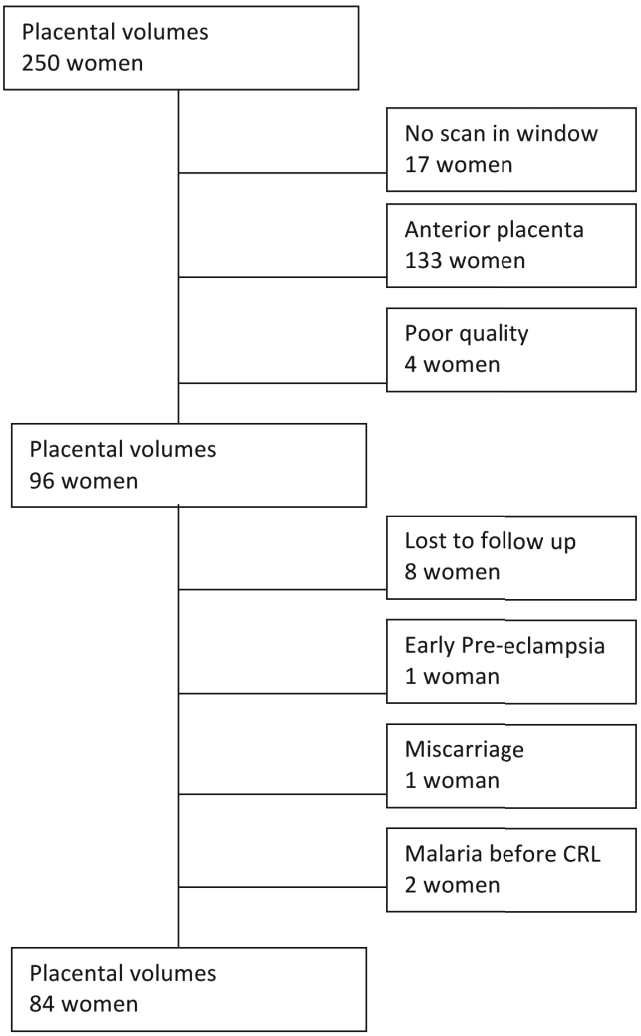


Table 11.1 Table 1 Baseline characteristics of women

	Malaria (N=19)	Non malaria (N=65)
Age (years)	25.1 ± 5.3	27.6 ± 7.0
Teenager, n (%)	3 (16)	7 (11)
Gravida	2 [1-7]	3 [1-14]
Parity	1 [0-5]	1 [0-13]
Primigravida	6 (32)	25 (29)
Smoking	4 (21)	11 (17)
Height (cm)	152 ± 5	153 ± 4
Hct at time of ultrasound (%)	31.2 ± 2.7	32.6 ± 2.8
HBP or PE	3 (16)	5 (8)
Non-malarial infection	3 (16)*	6 (9)#

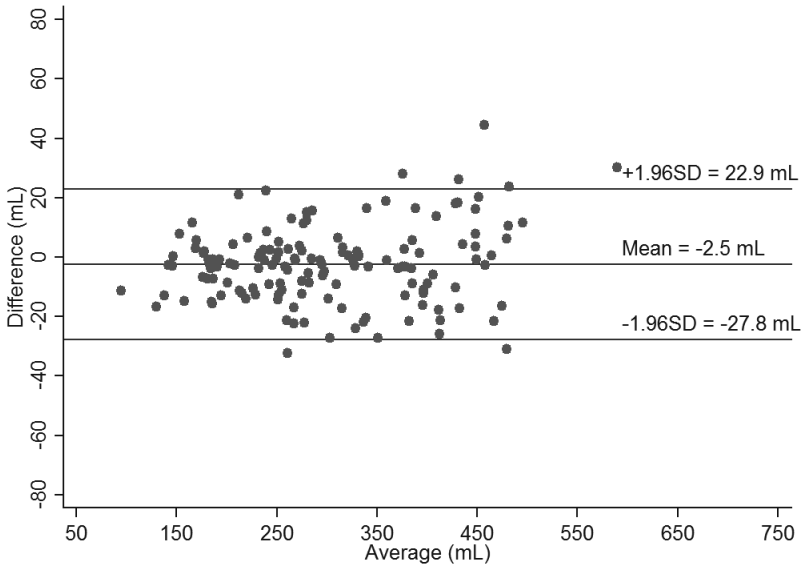
Data are presented as median [range], mean ± SD, or n (%)
HBP high blood pressure, Hct Haematocrit, , PE pre-eclampsia, SD standard deviation
* dysentery (32 weeks), pneumonia (24 weeks), suspected scrub typhus (8 weeks)
# pneumonia (39 weeks), pyelonephritis (20 weeks), suspected scrub typhus (3 cases; 25, 26 and 40 weeks), unspecified sepsis (36 weeks)

Measurement variability

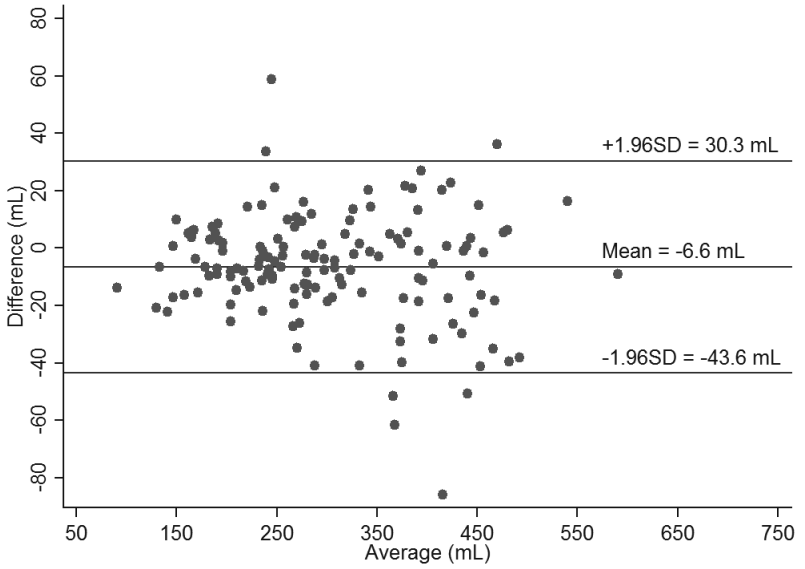
For this part of the analysis the volume of all placentas (n = 96 women, see **Figure 11.1**) were included irrespective of birth outcome or malaria status. The difference between the first and second measurements and the different rotation angles on the same 3D volume were normally distributed. There was no significant difference between the mean placental volumes for a single measurement at 30° and 15°, p = 0.27. The mean difference between the two measurements was 6.6 (95% CI: 3.6 – 9.70) mL at 30°, and 2.5 (95% CI: 0.3 – 4.6) mL at 15°. The 95% intra-observer limits of agreement for first and second measurements were ± 37.0 mL at 30° and ± 25.4 mL at 15° (**Figure 11.2**).

Figure 11.2 Intra-observer variability of placental volume measurements.

B)



B)



Plot of difference against mean for intra-observer variability of 145 placental volume measurements in 96 women using VOCAL™ at 15° (A) and 30° (B) rotational angles, with mean difference and 95% limits of agreement indicated

Placental volumes in uninfected women

The fractional polynomial linear regression model yielded the following formulae:

Mean volume = – 162.83 + 3.60 × GA

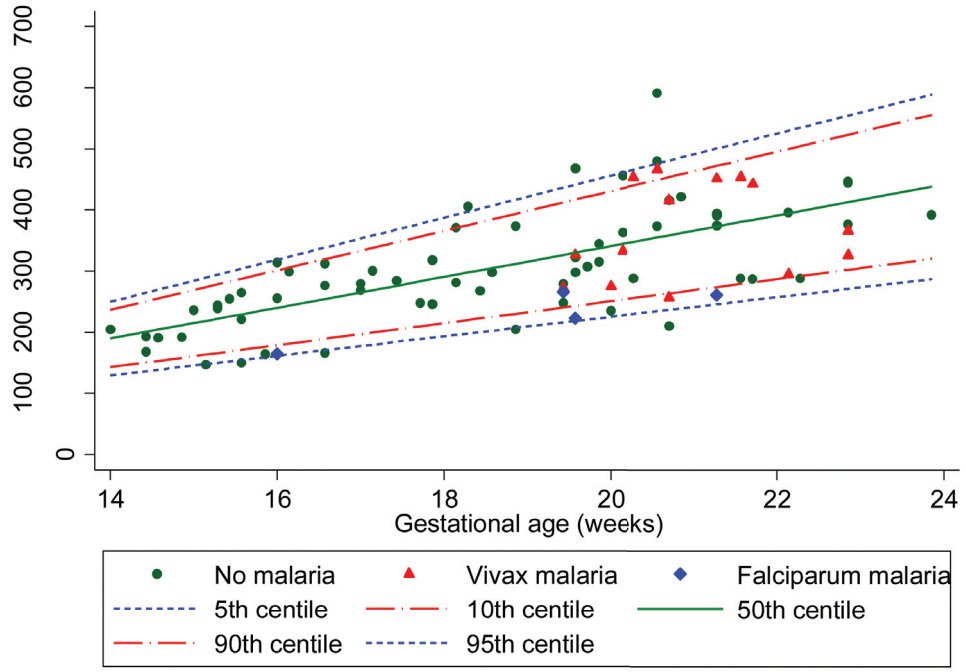
SD = – 41.55 + 0.80 × GA

A simple linear model with no quadratic or cubic terms for both the mean and SD provided the best fit for the model with a deviance of 717.98. The z-scores from the model did not suggest any deviation from normality using the Shapiro-Wilk test, p = 0.36.

Malaria

Nineteen women were infected with malaria between the dating and placental volume scans: four (21%) with P. falciparum and 15 (79%) with P. vivax. The baseline characteristics of infected and uninfected women were similar, except for a lower mean haematocrit at the time of the volume scan, as expected following infection with malaria: 31.2 ± 2.7% versus 32.6 ± 2.8% respectively, p=0.05 (**Table 11.1**). The median number of malaria episodes was one (range 1-4). Four women had more than one malaria episode in this period: three women had multiple *P. vivax* episodes (four, two and two episodes), and one woman had one *P. vivax* and one *P. falciparum* episode and was classified as *P. falciparum*. The median time between the last episode of malaria and the volume scan was 29 (IQR 14-42, range 0-59) days and this was not different for the *P.falciparum* malaria or P.vivax malaria group. **Figure 11.3** illustrates the model fit to the raw data for each measurement with the fifth, 10^{th}, 50^{th}, 90^{th} and 95^{th} centiles for placenta volumes between 14^{+0} and 23^{+6} weeks obtained from the uninfected women (green dots). Most of the placental volumes of the infected women as a whole were below the 50^{th} centile; most of those with *P. falciparum* were below the 10^{th} centile (**Figure 11.3**).

Figure 11.3 Placental volume centiles with placental volumes of women with falciparum and vivax malaria.



Fitted the fifth and 95th (blue lines), 10th and 90th (red lines) and 50th (green line) centile curves on the placental volumes of women without malaria or adverse birth outcomes (green dots) and superimposed the placental volumes of women with Plasmodium falciparum malaria (blue diamonds) or Plasmodium vivax malaria (red triangles)

Placental volume z-scores

The mean z-scores were similar in women when compared for smoking status, gravidity, age, anaemia (haematocrit < 30%) and those who had other infections in pregnancy (**Table 11.2**). However, the difference was significant for women with *P. falciparum* infections, p = 0.003 (**Table 11.2** and **Figure 11.3**). The z-scores could not be related to the number of malaria episodes or symptoms because of the small numbers involved.

Table 11.2 Risk factors associated with placental volume: z-scores

		N (%)	Z-score Mean ± SD	P -value
Smoking	Yes	15 (18)	0.00 ± 1.1	0.73
	No	69 (82)	-0.11 ± 1.0	
Primigravida	Yes	31 (37)	-0.14 ± 1.1	0.72
	No	53 (63)	-0.06 ± 1.0	
Non-malarial infection	Yes	9 (11)	-0.14 ± 0.55	0.80
	No	75 (89)	-0.08 ± 1.1	
Anaemia	Yes	16 (19)	-0.38 ± 0.86	0.16
	No	68 (81)	-0.02 ± 1.1	
Teenager	Yes	10 (12)	0.09 ± 1.5	0.70
	No	74 (88)	-0.11 ± 1.0	
Malaria*	Yes	19 (23)	-0.38 ± 1.0	0.17
	No	65 (77)	0.00 ± 1.0	
*P. falciparum**	Yes	4 (6)	-1.30 ± 0.4	**0.003**
	No	65 (94)	0.00 ± 1.0	
*P. vivax**	Yes	15 (19)	-0.13 ± 1.0	0.66
	No	65 (81)	0.00 ± 1.0	

*occurring between the CRL dating and volume scans.

Discussion

This pilot study demonstrates the feasibility of a) relating placental volume to gestational age between 14 and 24 weeks in a malaria endemic area, and b) comparing volumes between women with and without malaria. The findings are important because measurement of placental volume in the first or second trimester may predict which pregnancies are at high-risk of adverse outcomes[17, 36-38].

A variety of 2D and 3D ultrasound methods to measure placental volume have been reported[32, 36, 39-41] but, in clinical practice, 3D may be more accurate than 2D methods[36]. VOCAL™, one commercially available method of analysing 3D images, is considered relatively fast and reproducible. It allows the borders of the target organ to be modified after volumetric calculations and it is superior to other methods in evaluating very irregularly shaped structures, such as the placenta[42]. However, accurate 3D volume measurement of the whole placenta is only possible in the first half of pregnancy, because the volume seen is limited by the transducer footprint[36].

Accuracy is also influenced by the rotation angle of the analysis method. In this study, a small error was observed between the volume measurements when comparing the two rotation methods, 30° and 15° (**Figure 11.2**). The 30° rotation method resulted in a wider range of intra-observer 95% limits of agreement than the 15° method. Differing

reproducibility data have been reported for 3D measurements and analyses of placental volume, varying from relatively poor to highly similar intra- and inter-observer agreement[39, 42-46]. The results of this study are similar to those of Cheong et al, who reported that measurements made with VOCAL 30°, in an ex-vivo experiment, were faster to complete, but associated with significantly higher variability than those made with VOCAL 12°[18].

As in studies of fetal organ volumetry[19], placental studies show wide discrepancies in reference ranges[36]. The volumes reported in this study seem larger than previously published data[17, 36, 38, 40, 41, 45]. Rather than indicating true biological differences between populations, the discrepancies are more likely due to methodological differences; for example, in defining the placental border. Hence, there is a clear need to standardize 3D volumetric methods, and definitions of imaging planes and anatomical landmarks in particular. In the absence of standardized methods, this dataset was not compared with previously published placental volumes; rather, the focus was on studying the effect of malaria within the same population using a single set of well-defined methods.

In this preliminary investigation, infection with *P. falciparum* before 24 weeks' gestation appears to be associated with smaller placental volumes. In other studies, principally in developed countries, early placental volumetry has been shown to predict IUGR and adverse pregnancy outcomes[47, 48], due probably to impaired trophoblast invasion; in addition, small placentas in the first trimester are associated with high resistance uterine perfusion in the second trimester[49]. All these factors have previously been related to malaria in pregnancy as well[1, 50, 51].

Infection with *P.vivax* did not seem to be related to placenta volume. The mechanisms underlying the adverse effects of *P.vivax* malaria in pregnancy are not fully understood[25, 52, 53]. Systemic or hormonal mechanisms may play a role in *P.vivax* related growth restriction, as there is very little evidence that *P.vivax* sequesters in the placenta, like *P.falciparum* does[53].

There was a borderline difference in haematocrit at the time of the placental volume scan between the groups (**Table 11.1**), however anaemia was not related to placenta volume in the logistic regression model (**Table 11.2**). The timing of anaemia in pregnancy and the effect on placental weight and volume[1] needs further investigation.

This study, therefore, suggests that 3D placental volumetry is worthy of further investigation, in order to assess whether IUGR related to malaria is mediated via a smaller placental volume.

The data presented here involve small numbers and should be interpreted cautiously. Another limitation of the study was the initial decision to include only placentas on the posterior wall to maximize the likelihood of capturing the whole target organ in the volume sweep. This enabled the inclusion of complete placental volumes up until 24 weeks' gestation, but resulted in a large group of women with an anterior placenta being excluded (n = 133, **Figure 11.1**). The data used in this study may not be comparable with other populations because of the methodological differences described above. Lastly, no inter-observer variability analysis was available[19] but, for the purpose of this analysis,

which compared volumes measured by a single observer using the same methods, this was not a vital constraint.
Generally, ultrasound machines with 3D measurement capacity are delicate, expensive, and require a high level of technical skills and are therefore not available in most malaria endemic areas. Nevertheless, the suggestion that malaria in early pregnancy reduces placental volume in the second trimester should be confirmed by prospective studies evaluating volumes in relation to fetal growth and adverse pregnancy outcomes. Such studies should also allow for better assessment of potential confounders (such as maternal anaemia[1, 54], smoking[55] and parental anthropometry[56]). These are essential steps in unravelling the sequence of events from maternal malaria infection to IUGR.
In conclusion, the new technique of 3D ultrasound volumetry of the placenta may be useful to improve our understanding of the pathophysiological constraints on fetal growth caused by malaria infection in early pregnancy.

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Chapter 12

Effect of malaria in pregnancy on fetal cortical brain development: a longitudinal observational study

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Malaria; pregnancy; ultrasonography; prenatal; brain; fetus; cerebral cortex

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Abstract

Background

Malaria in pregnancy has a negative impact on fetal growth, but it is not known whether this also affects the fetal nervous system. The aim of this study was to examine the effects of malaria on fetal cortex development by three-dimensional ultrasound.

Methods

Brain images were acquired using a portable ultrasound machine and a 3D ultrasound transducer. All recordings were analyzed, blinded to clinical data, using the 4D view software package. The fetal supra-tentorial brain volume was determined and cortical development was qualitatively followed by scoring the appearance and development of six sulci. Multilevel analysis was used to study brain volume and cortical development in individual foetuses,

Results

Cortical grading was possible in 161 out of 223 (72%) serial fetal brain images in pregnant women living in a malaria endemic area. There was no difference between fetal cortical development or brain volumes at any time in pregnancy between women with immediately treated malaria infections and non-infected pregnancies.

Conclusion

The percentage of images that could be graded was similar to other studies. Maternal malaria does not have a gross effect on fetal brain development, at least in this population which had access to early detection and effective treatment of malaria.

Background

Both *Plasmodium (P.) vivax* and *P.falciparum* malaria are associated with maternal and fetal morbidity and mortality[1, 2]. Malaria in pregnancy causes a decrease in birthweight by intrauterine growth restriction (IUGR), preterm delivery, or both[3]. Malaria infection in the first half of pregnancy is associated with a reduction in fetal head diameter[4] and maternal *P.falciparum* malaria changes utero-placental hemodynamics[5]. Whether in-utero exposure to malaria has an effect on the growth and development of the fetal central nervous system is not known.

Studying the pathophysiological consequences of malaria in pregnancy on the fetus has been complicated by unreliable diagnosis of (early) pregnancy infections, difficulties in gestational age (GA) estimation and use of various methods to measure newborn anthropometrics[6]. The primary cortical folding process in the newborn is an early marker for functional neuro-development[7]. Growth restricted foetuses show a faster cortical folding process in comparison to normal foetuses, when measured with magnetic resonance imaging[7] or three-dimensional (3D) ultrasound (C. Businelli, unpublished data). Such increased cortical maturation might be related to the functional disturbances (e.g. lower IQ, attention deficit hyperactivity disorder) found in children affected by IUGR[8, 9]. The relatively new technique of 3D-ultrasound imaging of the fetal brain has rarely before been available in malaria endemic areas. The aim of this pilot study was to examine the effects of malaria in pregnancy on fetal brain development. The hypothesis was that foetuses affected by maternal malaria experience growth restriction, including smaller fetal brain volume and an accelerated cortical folding process.

Methods

Population

The participants in this study were attending the antenatal clinic (ANC) at Shoklo Malaria Research Unit (SMRU), which is located on the Thai-Burmese border where malaria transmission is low and seasonal[10]. Since 1986, the SMRU runs an ANC programme including weekly malaria screening to detect and treat all parasitaemic episodes during pregnancy to prevent maternal deaths[10]. There is no presumptive treatment of malaria or chemoprophylaxis. Every woman with a malaria episode detected by peripheral smear is immediately treated using WHO protocols[11]. Routine antenatal ultrasound performed by locally trained health workers commenced in 2001[12]. All women are encouraged to attend the ANC as early as possible in pregnancy and to deliver at SMRU under the care of Advance Life Support in Obstetrics (ALSO) trained midwives and doctors; those requiring Caesarean section are transferred to the nearest Thai hospital.

In this study, pregnant women with documented *P. falciparum* or *P. vivax* parasitaemia were included. They were compared with uninfected women matched for fetal gender, parity, and maternal age (within 5 years). Ultrasound scans were performed

trans-abdominally using a General Electric Voluson i (GE Healthcare, Austria) with a RAB2-5-RS, 2-5 MHz real-time 4D probe. The machine was housed in a dedicated air-conditioned room. All scans were obtained by locally trained sonographers specifically skilled in advanced fetal growth scanning (SK and NK) or a resident in obstetrics certified in antenatal ultrasound scanning (MJR), with regular internal quality control at SMRU. In addition, all images were sent for external quality control to the INTERGROWTH-21st Project team at the University of Oxford[13]. Fetal crown rump length (CRL) between 9^{+0} and 13^{+6} weeks was used to define gestational age (GA). Thereafter, women were invited to attend a fetal growth scan every five weeks until delivery to take 2D fetal biometric measurements and 3D sweeps, including the fetal head at the mid-ventricular plane. The volume box size and sweep angle were adapted to include the entire head. Care was taken to minimize movement artifacts. Once the scan was complete, volume data were stored for later analysis.

Malaria was diagnosed by Giemsa stained thick and thin blood films; 200 fields on the thick film were read before being declared negative. Severe malaria was defined according to WHO treatment guidelines[11]. Birth weight analysis was confined to life born, congenitally normal, singleton infants weighed in the first 24 hrs of life. Prematurity was defined as delivery before 37+0 weeks' gestation. Birth weight, length, and head circumference were measured twice by the trained and quality controlled anthropometry team on electronic Seca baby scales (Model 376, accuracy 10 grams), a Harpenden Infantometer with digital counter readings to one mm, or Seca Head Circumference Tape accurate to one mm, respectively, the first two being calibrated twice a week[6]. All ultrasound and anthropometric methods were identical to the INTERGROWTH-21st study protocol[13].

One author (MCW), blinded to any clinical data, analyzed all recorded volumes using the 4D view software package, version 9.1 (GE healthcare). The fetal supra-tentorial brain volume was determined and cortical development was qualitatively followed by scoring the appearance and development of six sulci (the Sylvian fissure and the superior temporal, central, parieto-occipital, calcarine and cingulate sulcus)[14]. A grading system was used to systematically assess the development of every sulcus independently. The depth and ramification of the specific sulcus was scored in a range from zero to five, where zero equals "not visible" and five "fully developed", as described previously[14]. Sulci on both the left and right side of the brain were graded, and the mean grade was used for analysis.

Statistical Analysis

Clinical data and the results of the ultrasound scans were entered into a Microsoft Access database and analyzed using SPSS version 17 for Windows. The Mann-Whitney, Chi-square or Fisher's exact test were used for comparison of ranks or categorical data, as appropriate. Multilevel analysis was used to study brain volume and cortical development in individual fetuses, with grades considered as a continuous variable[14]. The significance level was set at α=0.05.

Ethical approval
This study was part of a larger fetal growth project (ClinicalTrials.gov Identifier: NCT00840502), approved and yearly renewed by the Ethics Committees of Oxford (OxTREC (14-08)) and Mahidol (TMEC 2008-028) universities. All women provided written informed consent.

Results

In total 215 women were recruited between February 2009 and August 2010. Of these 22 women were diagnosed with malaria infections: 14 were infected with *P.falciparum* malaria and eight women with *P. vivax*. The characteristics of both infected and non-infected women are shown in **Table 12.1**. The frequency and timing of the infections are shown in **Figure 12.1**. Most malaria episodes were uncomplicated (77/78; 98.7%). One pregnant woman had severe malaria during labour. There were more anaemic women in the malaria group, as expected. Three of these women required a blood transfusion. Other morbidities in pregnancy are summarized in **Table 12.1**.

Cortical maturation and supra-tentorial brain volume

In total, 223 brain images were analysed: 113 in the malaria group and 110 in the non-infected group.
The median number of brain images per fetus was five (range 4-6) and did not differ between both groups. In the malaria affected group, 73.4% (83/113) of images could be visualised well enough to grade them reliably. In the non-infected group this percentage was similar (70.9%; 78/110), p=0.78. The presence of reverberation artefacts was the most important reason for the inability to visualize a sulcus in the hemisphere closest to the abdominal wall. Furthermore, in early pregnancies, fetal motion artefacts often troubled visualisation. The head circumference and brain volumes between malaria infected and uninfected pregnancies, which were not significantly different, are illustrated in **Figure 12.2**. **Table 12.2** shows the mean GA at first appearance and full fetal development per sulcus. All six sulci developed similarly between the two groups (**Figure 12.3**, **Table 12.2**). The median number of days between the first appearance of any sulcus and the first fully matured sulcus in the same fetus was 105 for both malaria [range 79-133] and non-malaria [range 70-139] groups (p=0.21). Only the cingulate sulcus initially matured significantly faster in foetuses affected by maternal malaria in pregnancy in comparison to foetuses of malaria-free pregnancies (**Figure 12.3**).

Table 12.1 Maternal and newborn characteristics

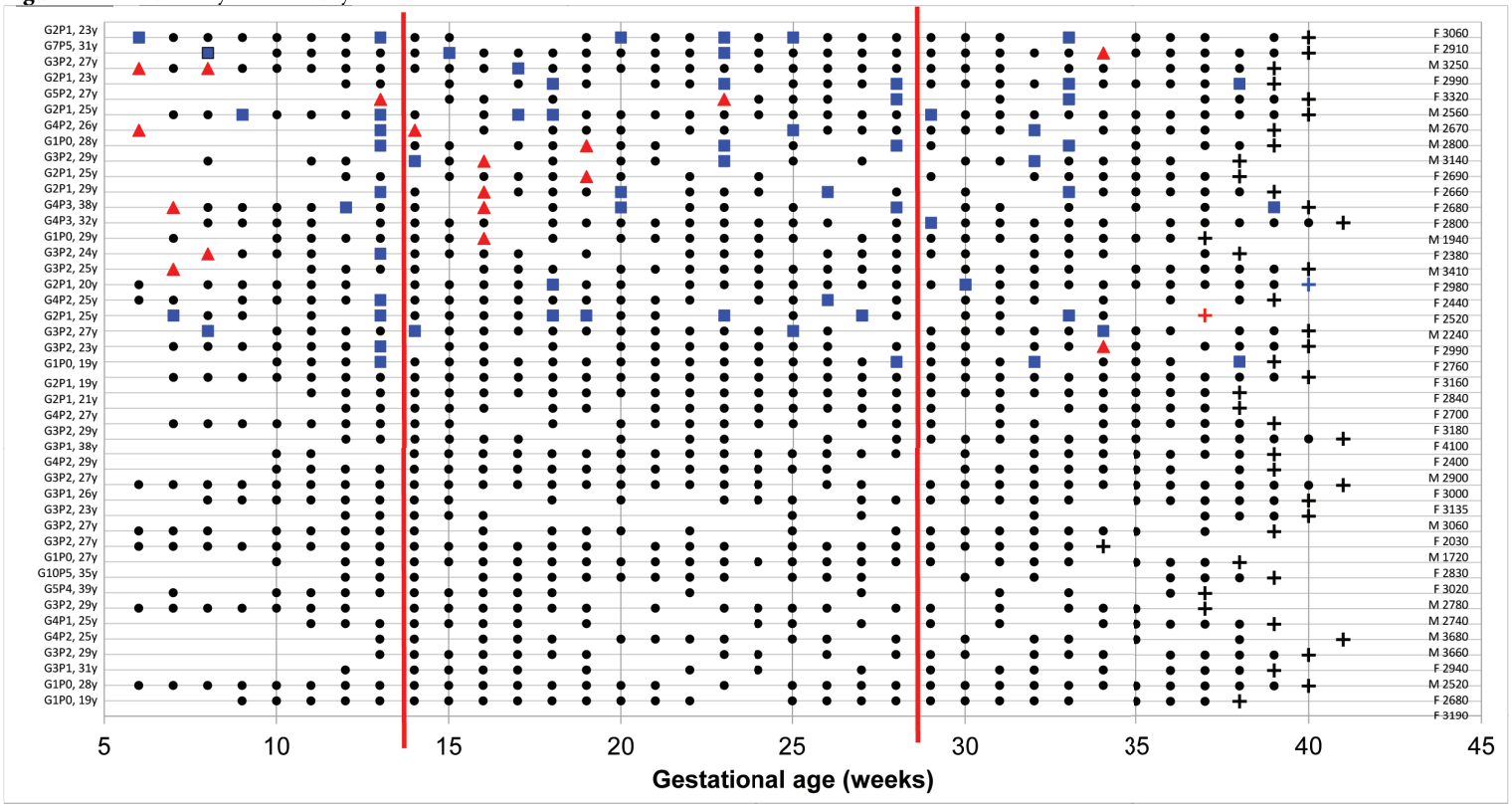
	No malaria (n=22)	Malaria (n=22)	P
Pregnant women			
Age (yrs)	27.0 (19 – 39)	25.5 (19 – 38)	0.23
Nulliparous	3 (14)	3 (14)	1.0
Gravida	3 (1 – 10)	3 (1 – 7)	0.36
Parity	2 (0 – 5)	2 (0 – 5)	0.82
Height (m)	1.53 (1.46 – 1.64)	1.53 (1.44 – 1.61)	0.71
Weight (kg)	50.5 (39 – 70)	46.5 (39 – 61)	0.20
Weight gain (kg)	7.5 (-2 – 17)	9 (4 – 15)	0.76
BMI (kg m^{-2})	21.5 (17.8 – 30.2)	20.1 (17.1 – 27.6)	0.15
MUAC (cm)	25.6 (14.5 – 32.0)	25.0 (20.6 – 30.6)	0.81
Smoking	3 (14)	7 (32)	0.28
NOC	25.5 (12-34)	26.5 (15-33)	0.48
Father's age	29.5 (19 -43)	29.5 (24 – 38)	0.80
Other infections	3 (14)	5 (23)	0.70
Anaemia	7 (32)	14 (64)	0.07
Severe anaemia	0 (0)	3 (14)	0.23
Early pre-eclampsia	2 (9)	2 (9)	1.0
Newborns			
Gestational Age (days)	277 (241 – 295)	279 (261 – 293)	0.58
Sex (% boys)	36.4%	36.4%	1.0
Weight (grams)*	2840 (1720 – 3660)	2685 (1940 – 3410)	0.18
Length (cm)*	49.0 (44.6 – 51.0)	49.4 (45.0 – 51.3)	0.88
Head circumference (cm)*	32.0 (29.5 – 39.3)	32.0 (31.0 – 35.1)	0.53

Data are presented as median (range) or as number (%). MUAC mid upper arm circumference, NOC number of consultations. * Data shown of newborns weighed within 24 hours of delivery; 17 and 18 in the no malaria and malaria group, respectively.

Newborns

Eleven (25%) women delivered at home, one (2%) underwent a caesarean section because of prolonged labour and the remaining 32 (73%) delivered in the SMRU clinic. There were no stillbirths, and all newborns appeared congenitally normal, although one infant from the non- malaria group was diagnosed with congenital heart disease later in life. Overall, the median birth weight was 2780 [range 1720 - 3660] grams in newborns weighted within 24 hours after delivery (n=35), and the gestational age at delivery was 39^{+3} [range 33^{+3} – 41^{+2}] weeks, including one premature neonate. All anthropometric measurements in the newborn were similar between the two groups (**Table 12.1**).

Figure 12.1 Summary of morbidity



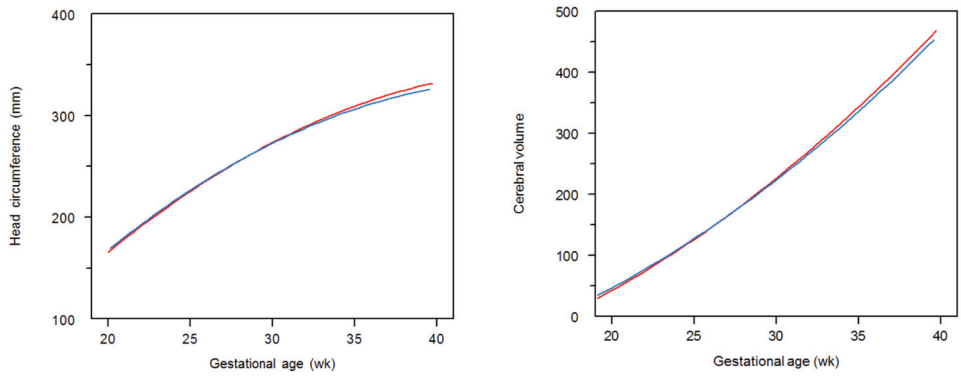
The x-axis is the gestational age in weeks. Each pregnant woman is shown as a horizontal line; the upper 22 horizontal lines represent women with malaria infections. The left column indicates the parity and the age of each pregnant woman, the right column shows the sex and weight of the newborn. The vertical red lines mark the first, second and third trimester. Blue boxes are *P.vivax* infections, and red triangles *P.falciparum* infections. Each black dot is an antenatal clinic consultation including a malaria screening, a cross (+) is a delivery, and could have a blue or red colour indicating *P.vivax* or *P.falciparum* infection at delivery, respectively.
F=Female, G=Gravida, M=Male, P=Parity, Y=Year

Table 12.2 Time (in days) of first appearance, interval and full development of cortical sulci

Sulcus	Group	First appearance	p	Maximal grade	p
Sylvian	Malaria	115 (99 – 131)	0.539	230 (204 – 268)	0.772
	Control	117 (101 – 134)		231 (204 – 272)	
Superior Temporal	Malaria	145 (118 – 179)	0.362	243 (211 -268)	0.455
	Control	150 (112 – 177)		238 (172 – 263)	
Parieto-occipital	Malaria	123 (99 – 147)	0.422	240 (204-277)	0.455
	Control	120 (98 – 146)		244 (216 – 274)	
Central	Malaria	145 (104 – 167)	0.429	221 (201 – 268)	0.586
	Control	150 (112 – 169)		219 (191 – 241)	
Calcarine	Malaria	126 (99 – 151)	0.282	226 (204 – 226)	0.706
	Control	133 (109 – 172)		224 (202 – 263)	
Cingulate	Malaria	145 (106 – 179)	0.156	248 (217 – 277)	0.600
	Control	154 (134 – 179)		244 (211 - 274)	

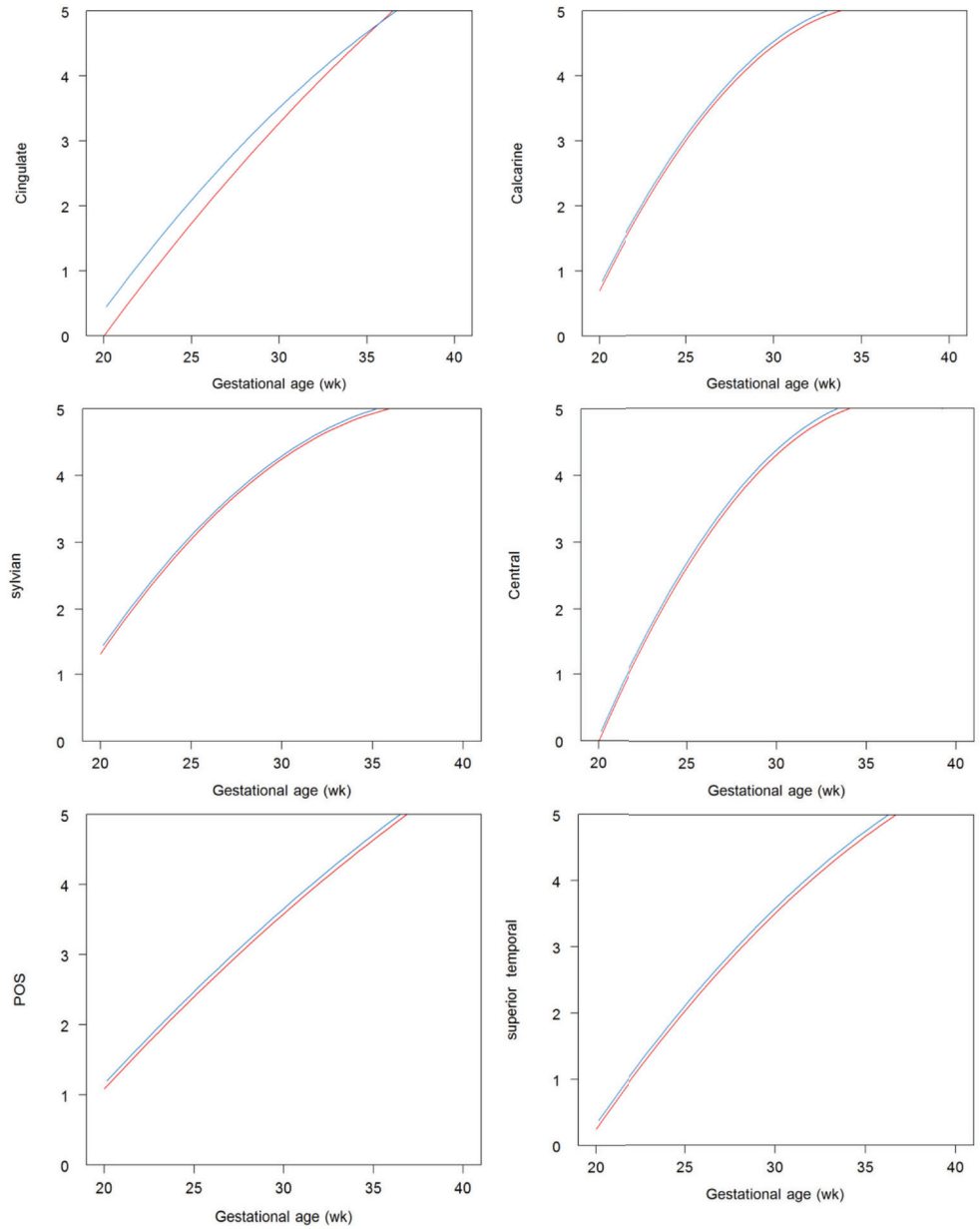
Data are presented as median (range).

Figure 12.2 Head circumference and cerebral volume



The x-axis is the gestational age in weeks; the y-axis represents the head circumference in millimetres or cerebral volume in centilitres. The blue lines represent foetuses from women with malaria infections (n=22) and the red lines show foetuses from women without malaria infections (n=22) in pregnancy.

Figure 12.3 Development of fetal cortex (Sylvian fissure, superior temporal, central, parieto-occipital, calcarine and cingulate sulcus)



The x-axis is the gestational age in weeks; the y-axis represents the grading of the Sylvian fissure, superior temporal, central, parieto-occipital, calcarine and cingulate sulcus.
The blue lines represent foetuses from women with malaria infections (n=22) and the red lines show foetuses from women without malaria infections (n=22) in pregnancy.

Discussion

In this study the fetal brain volumes and fetal cortex development were compared between women with and without malaria. Although the images of this study were not primarily obtained for neuro-sonographic evaluations and 3D ultrasound exposure time was limited, seventy percent of sulci could be visualized well enough to be graded. This percentage is similar to a previous study in healthy volunteers, where ultrasound scans were performed by a neuro-sonography expert[14]. In the busy malaria clinics, there was no possibility for real-time evaluation of the quality of obtained images. This is the first study to address the effect of maternal malaria on fetal cortical development or supra-tentorial volume. No difference in brain volume expansion or fetal cortical folding in general at any time in pregnancy between women with immediately treated malaria infections and non-infected pregnancies was detected. Of all graded sulci, only the cingulate sulcus matured faster in fetuses of women affected by malaria during pregnancy. The significance of a difference in maturation of a single sulcus has to be interpreted cautiously in the analysis of the general cortical development. Although the sample size of this pilot study is small, the blinding of the sonographers and observers to the malaria status of the mother, and the well matched groups may allow preliminary conclusions that maternal malaria does not have a gross effect on fetal brain development, at least in this population which had access to early detection and effective treatment of malaria.

The timing of malaria infection has an impact on the growth and development of the fetus[15]. The small sample size did not allow sub-analysis of groups infected in certain trimesters in pregnancy, nor of effect of symptoms or malaria species separately, but most women (73%, 16/22) were infected as early as the first trimester and most women had malaria infections throughout pregnancy. Similar studies with larger sample sizes in different malaria endemic settings may be needed to confirm these findings. Although three dimensional ultrasound machines are delicate and expensive and not available in most malaria endemic settings, this tool may be helpful in determining the impact of malaria on the fetal nervous system and indicating the newborns neurodevelopment.

In conclusion, maternal malaria does not have a gross effect on fetal brain development, at least in this population which had access to early detection and effective treatment of malaria.

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Part 5 Summary, discussion and recommendations

Chapter 13

Summary, discussion and recommendations

In one of the few textbooks devoted entirely to the subject of malaria in pregnancy[1], it was mentioned that the first reports of the impact of malaria on the infant's birth weight came from Góth in 1881 and Laveran in 1898. A lower birth weight has been repeatedly documented in Asia (**chapter 3**) and other malaria endemic areas of the world over the past century[2]. In previous studies, the impact of malaria in pregnancy to the newborn is principally reported as low birth weight, but also includes abortion[3], preterm birth, stillbirth, anaemia and congenital malaria which may vary with the local entomological inoculation rate and maternal immunity (**chapter 3**). The median reduction in birthweight in the reviewed studies was 150 g (range 1–650) for *P.falciparum* or mixed infections, and 108 g (107–310) for *P.vivax* malaria (**chapter 3**). Birthweight reduction occurs mainly in first pregnancies with *P.falciparum* malaria but also in subsequent pregnancies, and even with one episode of *P.vivax* or *P.falciparum* malaria. Both symptomatic and asymptomatic malaria episodes increase the risk of low birthweight, although symptomatic infections in pregnancy generally have a larger effect than has asymptomatic disease, particularly on preterm delivery (**chapter 3**).

There are two possible mechanisms by which malaria could produce low birthweight. The first of these is by reducing the gestational age at birth: as there is a close correlation between the length of gestation and birthweight, babies born at an earlier gestation will also be lighter. The second is through reducing fetal growth, thus causing a lower birthweight for the same gestational age than a peer who has not had malaria. The only way to discriminate between these two mechanisms for any individual pregnancy is to know the gestational age. The contribution of malaria induced preterm delivery to low birth weight has only been described in a limited number of sites in the world where the gestational age of pregnancy could be assessed (**chapter 4**). In the reviewed articles the method of estimating gestational age had been described in 77% (33/43) of the publications and was usually by symphysis-pubis fundal height measurement and/or last menstrual period which have a large window of confidence (**chapter 4**). Only 11.6% (5/43) of malaria in pregnancy studies which included the keyword birthweight utilized ultrasound (**chapter 4**), which is the most accurate method for pregnancy dating. It should be noted that an error in gestational age estimation of just 1 week may have important implications in assessing whether birthweight is optimal for that gestation or not. This is because weight gain of a fetus in the late third trimester can be as much as 250 g per week, which is similar to the reduction in weight attributable to malaria (**chapter 4**).

Accurate dating of pregnancies is notoriously difficult in resource poor settings, but is not a problem of the developing world alone[4, 5] (**chapter 5**). Ideally, gestational age should be estimated by fetal crown–rump length (CRL) or early second-trimester ultrasound, which is the standard in resource rich countries[6] (**chapter 4-6**). This thesis is not the first to emphasize that birth weight is only one index of growth and should always be considered in relation to accurate dated gestational age[7] (**chapter 4**). Birthweight may be normal despite abnormal growth, and newborns with a weight less than the 10th percentile are not necessary growth restricted[7-9]. For example, babies of apparently

appropriate birth weight may be exhibiting signs of intrauterine growth restriction (IUGR) since they have not achieved their full genetic potential[10, 11]. Normal fetal growth is one of the best indicators of good health and nutrition, while abnormal fetal growth (e.g. intrauterine growth restriction) is associated with an increased likelihood of perinatal complications and infant death[7]. Accurate information on gestational age is also important to avoid unnecessary obstetric interventions regarding the timing of delivery[12]. This holds true for both developed and developing countries. While obstetricians and paediatricians have acknowledged and acted upon these differences for approximately three decades[13], these concepts are in some ways just being discovered in relation to malaria in pregnancy. The question is why?

A lack of qualified sonographers and a shortage of ultrasound machines, most likely due to their cost and maintenance difficulties, may have been barriers to the implementation of routine ultrasound examination in malaria in pregnancy studies and antenatal clinics in resource-poor settings[14, 15] (**chapter 5**). However, local health workers with schooling up to age 16 and a 3-month ultrasonography course achieved high levels of accuracy in gestational age assessment (**chapter 5**). This experience suggests that training of local health workers in developing countries is possible and may allow effective use of obstetric ultrasound imaging. Indeed, in a ultrasound teaching program for emergency medicine in Rwanda, ultrasound particularly benefitted women's health and obstetrical care[16]. Recently, assistant medical officers, clinical officers, midwives or local radiographers have been identified as potential sonographers[17-19].

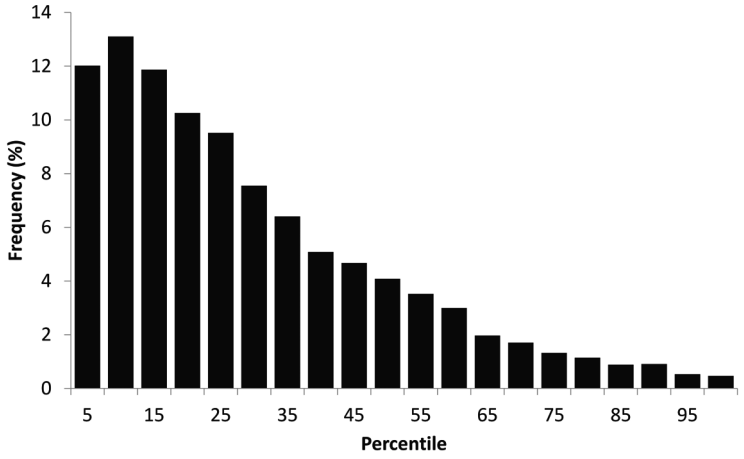
Some African countries have reported promising results from starting ultrasound teaching programs[18] and the International Society for Ultrasound in Obstetrics and Gynecology has introduced an "outreach program", in which local staff receive ultrasound training from western experts (http://www.isuog.org/Outreach/). Although such initiatives are promising, a major problem concerns training of existing staff in local hospitals. Skilled midwives should continue to save lives in antenatal clinics and delivery rooms and not use their time dating pregnancies. Introduction of antenatal ultrasound in developing countries is a strong aid to antenatal care, but should never disrupt the existing structure. In the SMRU clinics the ultrasound team is separate from the midwifery team. The last training session (October –December 2011) at SMRU included two local staff members who were trained as trainers. Ongoing quality control (**chapter 6**) will be required to monitor the usefulness of this step.

Apart from trained health workers, robust ultrasound machines, and safety measures such as voltage stabilizers are needed to make obstetric ultrasound imaging available in remote areas. Unfortunately, as observed by Kurjak and Breyer[17] "many developing countries cannot afford to buy good quality ultrasound diagnostic instruments and do not have enough trained specialists who can devote a large fraction of their active time to the science and art of ultrasound diagnosis". However, recently ultrasound imaging has become more feasible in developing countries as machines become less expensive, portable and require less servicing. Governments should be encouraged to add ultrasound machines and training of health workers into their overall plans for investment in health

services (**chapter 5**). Obviously as ultrasound is a just a diagnostic tool, it should always be embedded within and coupled with clinical management infrastructures.
Although the recent literature highlights the usefulness of antenatal ultrasound in developing countries, at the same time over-and misuse of ultrasound have been reported (**chapter 7**). Health care providers and pregnant women may overestimate the diagnostic capabilities and therapeutic possibilities, and their history taking and physical examination may weaken (**chapter 7**)[20]. In Botswana[21], ultrasound scanning was associated with significant psychological stress and anxiety in pregnant women, especially when accompanied by minimal explanation by healthcare providers. In the clinics of the Shoklo Malaria Research Unit (SMRU) pregnant women expressed an immense trust in the health providers, which may be due to the long existence of the SMRU antenatal clinic (25 years) or the method of frequent intermittent screening for malaria in which women are invited to come every week (**chapter 7**). The participants and medical staff in this study overwhelmingly reported that they think that antenatal ultrasound improves patient safety and that they would not want to have ultrasound services stopped (**chapter 7**). In low-income countries, additional clinical benefits of antenatal ultrasound include: diagnosis of multiple pregnancies, differentiation between types of vaginal bleeding at any stage of pregnancy, confirmation of fetal presentation, confirmation of viability of the fetus and detection of major congenital abnormalities (**chapter 7**).
When reliable antenatal ultrasound and accurate pregnancy dating is available in malaria endemic settings like the Thai-Burmese border, population specific reference charts for fetal biometry measurements (**chapter 6**) and birthweight for gestational age (**chapter 9**) can be created. Apart from the epidemiological value of such advances, antenatal ultrasound has also a strong clinical value as it may allow the detection of growth restricted fetuses in the hope that early recognition, appropriate surveillance and possible intervention will optimize both perinatal and long term outcome[22]. The definition of small for gestational age (SGA), a birthweight for gestational age below the 10th centile, is highly dependent of the reference population. When, for example, the gestational ages and birth weights of 3000 randomly chosen pregnancies from the SMRU's database of accurately dated pregnancies from the Thai-Burmese border were converted into centiles with a reference formula from a population in Oxford, UK,[23] the left shift in distribution of centiles became obvious (**Figure 13.1**, data not published). A large proportion, 25%, of these babies would be classified as SGA by using the UK data. The local birthweight for gestational age centiles have demonstrated that malaria has an impact on birthweight across all gestations and that LBW, while specific, was poorly predictive of SGA infants (**chapter 9**). Malaria infection in early pregnancy is associated with smaller mid trimester fetal head diameters (**chapter 8**), a reduction in growth of fetal biparietal diameter and head circumference in the second trimester (**chapter 10**) and smaller placentas before 24 weeks of gestation (**chapter 11**). However, the preliminary results of the cohort of 360 women where more than a quarter had malaria, early detection and treatment of malaria episodes at all times in pregnancy were very

promising; newborn characteristics (such as head circumference, length, or ponderal index (**chapter 10**) or fetal cortex development (**chapter 12**)) were not significantly different from those of fetuses not exposed to malaria. However, when such an early detection and treatment system is not available the effects on fetal growth could be worse, perhaps because malaria parasites are able to multiply and sequester in the placenta (**chapter 8 and 10**). Relatively close monitoring of pregnant women in an African setting with provision of preventive medicine prevented third trimester *P.falciparum* infections to have a positive effect on birthweight[24]. Of concern is that the preventive effect of Intermittent Preventive Treatment with sulfadoxine pyrimethamine (IPTP-SP) is rapidly declining due to resistance[25]. *Plasmodium falciparum* (and probably *P.vivax*[26]) infection in pregnancy is characterized by the sequestration of infected erythrocytes in the maternal placental vascular space, in which materno-fetal exchanges occur[27]. This sequestration causes an infiltration of inflammatory cells and elevation of cytokine levels in the infected placenta. Systemic or hormonal mechanisms may play a role in *P.vivax* related growth restriction, as there is little evidence that *P.vivax* sequesters in the placenta, like *P.falciparum* does[26, 28-30] Early malaria infection could alter the process of placentation, similar to what occurs in pre-eclampsia [31].

Figure 13.1 Birthweight for gestational age percentiles of 3000 neonates of randomly selected accurately dated pregnancies from the Thai-Burmese border in an equation from Oxford, UK.

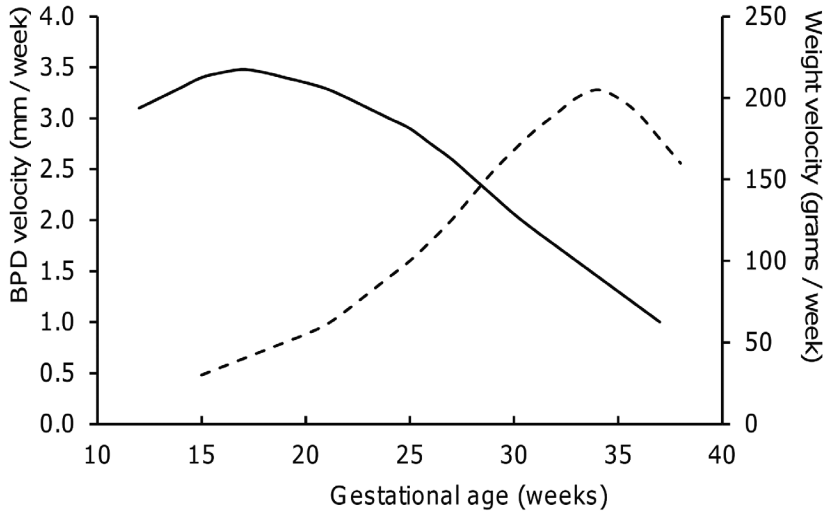


Our malaria in pregnancy studies are unique because women received a detailed surveillance programme during pregnancy. They were accurately dated in the first trimester and followed to delivery with regular two-and three-dimensional ultrasound scans; in addition each malaria episode was promptly treated with efficacious medicine. In the longitudinal fetal growth study (**chapter 10**), malaria infection was not associated with any detectable adverse effects on neonatal anthropometric parameter (birthweight, head circumference, arm circumference, length, ponderal index), but sub-analysis revealed

that more precise measurements, such as birthweight percentiles, could still detect an impact of malaria in univariate analysis (**chapter 10**). With the introduction of the global reference for fetal weight and birthweight percentiles[32], identification of small for gestational age infants has come a step closer for low-income countries. Nevertheless, there are still challenges to make such references available in all settings: even the small requirement of 100 accurately dated pregnancies that deliver at 40 weeks is a major undertaking in an area that lacks ultrasound capacity, and this includes most of the malarious tropics (**chapter 9**).

Our longitudinal studies allowed detection of the effects of malaria on fetal growth; malaria infection in the second trimester was associated with a significant reduction in the z-score of the fetal biparietal diameter (**chapter 8 and 10**) and head circumference (**chapter 10**), two weeks after a malaria infection. The infections in the second trimester coincide with the maximal growth velocity of the fetal head (**chapter 8, Figure 13.2**). These findings need to be balanced against the fact that at birth the head circumferences in both studies were not smaller; this could be explained by a recovery of fetal growth after prompt and efficacious treatment Indeed, the preliminary results of histopathological examination indicate that no placental malaria induced damage was present in any of these pregnancies. This is in line with previous findings[33].

Figure 13.2 Fetal biparietal diameter growth velocity and fetal weight velocity curves



Schematic presentation of fetal biparietal diameter growth velocity (continuous line, in mm per week) and fetal weight velocity (dotted line, in grams per week) curves in pregnancy from previous studies[40, 41].

A malaria episode may have an impact on the woman's general wellbeing, and the general illness may have an impact on fetal growth. In order to try to tease out what placental effects are attributable to malaria, a holistic approach is being taken, by studying the

volume growth velocity of placentas, markers of placental damage, (first trimester) placental hormones, and detailed histopathology.
Based on the present data, future details of fetal growth velocity of individual organs (by two and three dimensional ultrasound data) around each malaria episode should also be studied and the effect of a malaria event should be modeled. Our data show that although smaller heads were measured after second trimester malaria infections, immediately treated malaria episodes did not have a gross effect on fetal cortex development and cerebral volumes (**chapter 11**). This is encouraging, but such a study should be repeated in different malaria endemic settings and linked to infant neurological developmental milestones. Of concern, although not significantly different, all sulci showed a faster development in the malaria infected pregnancies (**chapter 11**). In this population, which had access to early detection and effective treatment of malaria, the sample size may have been too small to detect a significant effect on fetal brain development. Similar studies in different malaria endemic settings are needed to confirm these findings.
When malaria in pregnancy cannot be prevented, the negative effects of malaria on birthweight may be reduced to a minimum by early detection and treatment of each malaria episode with efficacious drugs. This may reduce placental inflammation or allow the placenta to recover from malaria induced damage. However, multiple episodes of *P.vivax* are most likely to result from liver stage relapses instead of newly acquired infections. There is no treatment available in pregnancy for liver stages. Furthermore, in this setting of multidrug resistant parasites there are no studies on safe and effective drugs available to prevent malaria from the start of pregnancy. Dihydroartemisinin-piperaquine may be a promising candidate[34-36]. To protect the developing fetus from growth restriction from both symptomatic and asymptomatic *P.vivax* and *P.falciparum* infections, prevention strategies from early pregnancy or pre-pregnancy interventions should be considered[24, 37-39].
In conclusion, our results indicate that even promptly treated malaria infections in early pregnancy may have an effect on fetal head size in the second trimester. In the past 3 years a huge amount of longitudinal data, including fetal organ volumes, utero-placental hemodynamics, red blood cell indices, paternal and maternal characteristics have been collected in a standardized fashion with careful quality control. The planned statistical analysis of these data means that further elucidation of how malaria effects fetal growth is possible, and may also establish the potential for recovery. This thesis has demonstrated the usefulness of antenatal ultrasound as a tool for broadening our understanding of adverse fetal growth effects associated with malaria in pregnancy. Pregnant women and local staff found antenatal ultrasound an acceptable tool and described the benefits they perceived, in this setting. Close monitoring of pregnant women and prompt treatment of any malaria episode with efficacious drugs minimizes the negative effects of malaria on fetal growth. Governments should encourage the value of antenatal care, early detection and treatment of malaria in pregnancy and correct use of antenatal ultrasound in malaria endemic countries.

Summary of the most important findings of this research:

- Every malaria infection in pregnancy in the Asia-Pacific region is detrimental to mother and fetus.
- Malaria infection in pregnancy is associated with a reduction in fetal growth
- In assessing the impact of malaria on fetal growth, low birth weight is a coarse indicator.
- In malaria in pregnancy studies, differences in birthweight can clearly be affected by inaccuracies in measurement methods, and confounders such as gestational age or time of weighing.
- Estimation of gestational age is difficult in low income settings, but ultrasound machines for accurate dating are becoming available.
- Locally trained health workers in a resource poor setting, can obtain accurate fetal biometry measurements for gestational age estimation.
- Both providers and pregnant women on the Thai-Burmese border recognized antenatal ultrasound as a technology improving the safety of pregnancy and delivery.
- Despite early treatment one or more (a)symptomatic *P.falciparum* or *P.vivax* malaria infections in the first half of pregnancy result in a smaller than expected mid-trimester fetal head diameter.
- Population specific reference percentiles for fetal biometry and birthweight are valuable indicators of fetal size and useful for both research and clinical practice.
- The new technique of 3D ultrasound volumetry of the placenta and fetal organs, such as the fetal brain, is a useful and achievable tool to improve our understanding of the pathophysiological constraints on fetal growth caused by malaria infection in (early) pregnancy.
- In a pregnant population with access to early detection and prompt treatment of malaria, no gross effect on fetal brain development was observed following maternal malaria infection.

Recommendations:

- Improved estimates of the morbidity and mortality of malaria in pregnancy calculated from longitudinal cohort data in the population at risk are urgently needed, as are drug-dose-optimisation studies in pregnant women, and implementation of strategies for prevention of malaria in pregnancy.
- When malaria in pregnancy cannot be readily prevented, accurate diagnosis and prompt treatment with efficient drugs for any detected parasitaemia in pregnancy are essential to avert dangerous symptomatic disease and to reduce effects on fetuses
- Researchers designing malaria in pregnancy studies should follow the simple methodological guidelines for reporting birth outcomes as provided in this thesis.
- Ultrasound dating, precise methods of birthweight measurements and population centiles along with repeated measurements of growth within the same pregnancy are required to understand exactly what constitutes LBW in malaria affected pregnancies.
- Governments, policy makers, hospital directors, doctors and midwives should invest in antenatal care
- Robust, cheap and low-maintenance ultrasound machines should become available all over the world
- Local health workers could be trained as sonographers and qualify in basic obstetric ultrasound scanning
- Implementation of technological innovations in a resource poor setting is often initiated by outsiders and patient mistrust or discomfort can compromise otherwise well designed programs. Because of this, we would strongly advocate inquiry along lines described in this thesis to be done in other settings concurrent with the introduction of ultrasound in order to facilitate development of effective and acceptable programs.
- The new technique of 3D ultrasound volumetry and ultrasound Doppler should be considered in future fetal growth studies determining the impact of malaria infection in (early) pregnancy.
- To protect the developing fetus from growth restriction from both symptomatic and asymptomatic *P.vivax* and *P.falciparum* infections, prevention strategies from early pregnancy onwards or even pre-pregnancy interventions should be considered

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Chapter 14

Summary in Burmese and Dutch language

Introductie De geschiedenis door heeft malaria de mens achtervolgt. Ook nu nog lopen er zo'n 125 miljoen zwangere vrouwen per jaar risico om met malaria geinfecteerd te worden. Zo'n infectie in de zwangerschap kan leiden tot een spontane miskraam, vroeggeboorte, foetale groeivertraging, maar ook tot ernstig zieke moeders of zelfs moedersterfte. Opvallend is dat in de meeste studies over malaria in de zwangerschap er geen zwangerschapsduur bekend is, en er grote verschillen zijn in de gevolgde methodologie om het geboortegewicht nauwkeurig te bepalen. Dit komt doordat vroege echografie, de gouden standaard voor het bepalen van de zwangerschapsduur, niet voorhanden is in de meeste malaria gebieden. Het doel van dit proefschrift is om de mogelijkheid van echografie in de zwangerschap in een ontwikkelings land (Thai-Birmese grensgebieden) te onderzoeken. Indien dit mogelijk blijkt, kan in zwangerschappen met een bekende (echografisch gedocumenteerde) termijn, het effect van malaria infectie op de zwangerschapsduur en op de groei van het kind nauwkeurig bepaald worden.
In **hoofdstuk 2** worden de Karen, een ethnische groep uit Birma, en andere Birmese migranten geintroduceerd. Sinds 1986 voorziet de Shoklo Malaria Research Unit (SMRU) deze groepen van medische zorg. Alle studies die in dit proefschrift beschreven staan, werden uitgevoerd onder de verantwoordelijkheid van de SMRU, als onderdeel van het Mahidol Oxford Tropical Medicine Research programma. De SMRU heeft momenteel verschillende poliklinieken waar lokaal opgeleide verloskungen zorg bieden aan zwangere vrouwen, dit onder supervisie van dokters. Tijdens het opzetten van de grote "malaria in de zwangerschap" studies, werden er met steun van de "Stichting Malariadokters" twee verloskamers gebouwd, en nieuwe verloskundigen, echografistes, en medische staf opgeleid. Er kwamen nieuwe echo-apparaten en er werd lesmateriaal gemaakt. Het uiteindelijke resultaat was dat lokaal opgeleide meisjes iedere zwangere vrouw konden scannen, zodat van iedere zwangerschap op de juiste manier de termijn bepaald kon worden, en dat er voor alle vrouwen een veilige plek gecreëerd werd om te bevallen, waarbij ieder pasgeboren kind nauwkeurig onderzocht werd.

De Shoklo Malaria Research Unit
Een van de vele speerpunten van de SMRU is malaria in de zwangerschap. In 1986 heeft professor François Nosten, de oprichter van de SMRU, een zwangerschaps controle systeem opgezet, waarbij zwangere vrouwen iedere week gescreend worden op malaria. Dit systeem is opgezet omdat in die tijd op de Thai-Birmese grens 1% van de zwangere vrouwen dood ging aan malaria. In dit gebied is de malaria parasiet resistent tegen alle gangbare antimalaria middelen, en werken muskietennetten niet zo effectief als in Afrika. Het wekelijkse screening systeem waaraan in de vluchtelingenkampen meer dan 90% van de vrouwen meedoet, is arbeidsintensief maar zeer effectief. Sindsdien is er bij vrouwen die wekelijks komen geen moedersterfte door malaria meer voorgekomen.

De populatie
Sinds de 2e wereldoorlog is er een burgeroorlog gaande in Birma tussen de Birmese junta en etnische minderheden, die strijden voor onafhankelijkheid. Deze guerrillastrijd wordt

met name gevoerd in de bergen in de grensstreken; in de loop der jaren zijn duizenden dorpsbewoners hun land ontvlucht en opgevangen in vluchtelingenkampen in Thailand. Ook zijn er veel "internally displaced people" (IDPs), en economische migranten. De gezondheidszorg in grote delen van Birma is slecht, met name in de gebieden waar de burgeroorlog heerst. De SMRU heeft één kliniek in een groot vluchtelingenkamp (50.000 mensen), en vijf klinieken langs de grensrivier, waar mensen uit Birma alleen de rivier hoeven over te steken om medische zorg te krijgen. De SMRU richt zich met name op malaria in de hele populatie, en doet zwangerschapscontroles met onder andere wekelijkse screening voor malaria. De auteur heeft met zijn gezin bijna zes jaar op de Thai-Birmese grens gewoont en gewerkt.

Zowel *Plasmodium (P.) falciparum* als *P.vivax* zijn gevaarlijk voor de moeder en de baby. De Wereld gezondheidsorganizatie heeft het malaria in de zwangerschap programma met name gebaseerd en gericht op gebieden in Afrika, waar erg veel malaria voorkomt. In vergelijking met deze gebieden komt malaria in de Asia-Pacific regio meer op specifieke plekken voor, zodat niet alle mensen weerstand tegen malaria hebben opgebouwd, en elke infectie gevaarlijk is. Toch is het aantal zwangere vrouwen dat risico loopt op vivax en/of falciparum malaria erg groot. Daarnaast is de malaria parasiet resistent tegen veel antimalaria medicijnen. **Hoofdstuk 3** vormt een overzicht van alle tot nu toe gepubliceerde artikelen over malaria in de zwangerschap uit de Asia-Pacific regio, geschreven door dokters en wetenschappers die in dit deel van de wereld werken. Dit overzicht werd in april 2011 door de autheur gepresenteerd in Genève tijdens de jaarlijkse malaria in de zwangerschap werkgroep vergadering van de wereldgezondheids organizatie.

Er zijn specifieke interventies beschikbaar om de gevolgen van malaria in de zwangerschap te beperken. Om zulke interventies te beoordelen, werd er vaak alleen gekeken naar verschillen in geboortegewicht van de pasgeboren babies. Zulke verschillen zijn erg afhankelijk van de methodes van wegen, en van verstorende factoren zoals zwangerschapsduur of de leeftijd van het kind op het moment dat het gewogen wordt. In **hoofdstuk 4** worden alle gepubliceerde artikelen in de literatuur die rapporteren over malaria in de zwangerschap en het geboortegewicht tegen het licht gehouden en er valt op dat er in alle artikelen erg verschillende methoden gebruikt worden om het geboortegewicht te meten en te beschrijven. Aan het eind van hoofdstuk 4 zijn er aanbevelingen geschreven om in het vervolg op een eenvoudige, maar systematische wijze geboortegewicht te bepalen in studies in ontwikkelingslanden.

De figuren en tabellen in **hoofdstuk 5** laten zien dat lokaal opgeleide medewerkers goed echografische metingen van het ongeboren kind kunnen maken, deze nauwkeurig kunnen herhalen, en dat hun metingen overeenkomen met die van de dokter. Deze medwerkers hebben alleen de middelbare school (tot 16 jaar oud), en een speciaal voor hen opgezette echografie cursus van 3 maanden, gevolgd. Het is dus mogelijk om lokale staf in ontwikkelingslanden zo op te leiden dat ze betrouwbare termijn echo's kunnen maken tot en met 24 weken zwangerschapsduur. In **hoofdstuk 6** worden deze resultaten bevestigd, en nu ook voor foetale echografische metingen gedurende de gehele zwangerschap (16 tot 40 weken). Met deze metingen werden referentie grafieken gemaakt van

de specifieke foetale eigenschappen (foetale hoofdomtrek en diameter, buikomtrek en lengte van het bovenbeentje) voor de lokale populatie. Deze grafieken kunnen gebruikt worden in de klinische praktijk en voor wetenschappelijke doeleinden.
De dokters van de SMRU zijn overtuigd dat het invoeren van echografie de kwaliteit van zorg in een ontwikkelingsland kan verbeteren en dat zwangere vrouwen en hun babies er voordeel bij hebben. Maar wat denken zwangere vrouwen en de lokale medewerkers daar eigenlijk zelf van? In een breed opgezette studie (30 observaties, 19 interviews, 7 focus groep discussies en 644 vragenlijsten), beschreven in **hoofdstuk 7**, gaven zwangere vrouwen en lokale stafleden aan dat zij echografie inderdaad zien als een middel dat de veiligheid rondom de zwangerschap en de bevalling vergoot. Sommige vrouwen hadden kortdurend gevoelens van schaamte of angst tijdens de scan, maar gaven aan dat met goede voorlichting zulke gevoelens meteen verdwenen. Wij hebben geen aanwijzingen gevonden in dit gebied dat er sprake zou zijn van overmatig of onjuist gebruik van echografie, bijvoorbeeld om het geslacht te bepalen om sexe specifieke zwangerschaps-beëindiging na te streven.
Ondanks vroege behandeling van alle malaria infecties, werd er halfverwege de zwangerschap een verschil gevonden in de diameter van foetale hoofdjes tussen vrouwen die wel (344 vrouwen) en niet (3779 vrouwen) een malaria infectie doorgemaakt hebben gedurende het eerste deel van de zwangerschap (**hoofdstuk 8**): malaria was gerelateerd aan kleinere hoofdjes. Zelfs één infectie met vivax en/of falciparum malaria was genoeg om een significant verschil aan te tonen. Dit onderzoek benadrukt het belang van het voorkomen van alle malaria infecties, zelfs in de vroege zwangerschap.
Sinds het invoeren van termijn bepaling door middel van vroege echografie in de klinieken op de Thai-burmese grens is het mogelijk de geboorteuitkomsten te analyseren. Van 10264 zwangerschappen waarvan de termijn nauwkeurig bekend was en de baby binnen 24 uur gewogen, werden populatie referentie curven gecreëerd voor geboortegewicht per zwangerschapsweek (**hoofdstuk 9**). Opvallend was dat de geboortepercentielen van de babies van vrouwen die één of meerdere malaria infecties hadden doorgemaakt in de zwangerschap duidelijk lager waren dan van niet geïnfecteerde zwangerschappen in bijna alle zwangerschaps weken. Verder geeft laag geboortegwicht (geboortegewicht kleiner dan 2500 gram) op zichzelf (los van zwangerschapsduur) geen goede indicatie van het aantal te kleine kinderen voor de zwangerschapsduur in deze populatie.
Hoofdstuk 10 vormt de basis of fungeert als springplank voor veel nieuwe analyses. In dit hoofdstuk worden de methodes beschreven van een grote longitudinale echo studie die nog steeds doorgaat. Van de driehonderdzestig vrouwen die elke vijf weken echografisch gevolgd werden werden er 97 (26.9%) geinfecteerd met malaria op tenminste één tijdstip in de zwangerschap. Voorlopige conclusies van dit onderzoek zijn dat er in deze heel zorgvuldig echografisch gevolgde groep geen babies werden doodgeboren na 28 weken zwangerschapsduur, dat er slechts één geval was van ernstige cerebrale malaria, dat malaria infectie in de tweede trimester een groeivertraging laat zien van de foetale hoofdomtrek, maar dat er geen verschil in geboortegewicht gevonden wordt. Een noodzakelijke volgende stap is de longitudinale analyse van de individuale groei patronen

van alle foetussen om zo herstel van groeisnelheid na vroeg en effectief behandelde malaria infecties vast te stellen. Deze analyses zullen in de komende jaren plaatsvinden. Van 65 vrouwen met een uiterlijk normaal, levendgeboren kind bij wie de placenta op de achterwand van de baarmoeder lag, werd een formule beschreven die het volume van de moederkoek tijdens de vroege zwangerschap weergeeft (**hoofdstuk 11**). Bij de vrouwen die een *P.falciparum* infectie doormaakten in de vroege zwangerschap werden kleinere placenta's gevonden. De nieuwe techniek van driedimensionale echografie zou een bijdrage kunnen leveren in het begrijpen van het negatieve effect van malaria op de groei van foetale organen inclusief de placenta.
In **hoofdstuk 12** wordt de hersenontwikkeling van 22 foetussen, van wie de moeder malaria infecties doormaakte, vergeleken met die van 22 vergelijkbare foetussen zonder malaria infecties. Tussen deze kleine groepen, waarbij alle malaria infecties onmiddelijk werden behandeld, werden geen duidelijke verschillen gevonden. Dit benadrukt het belang van vroege diagnose en behandeling van malaria in de zwangerschap.
In **hoofdstuk 13** worden de resultaten van dit proefschrift samengevat en de belangrijkste conclusies and aanbevelingen benadrukt en besproken. Malaria infectie in de zwangerschap kan gevaarlijk zijn voor de moeder en het kind. Om de gevolgen van malaria in de zwangerschap te bestuderen is het nodig om de zwangerschapstermijn precies vast te stellen. Echografische termijnbepaling vroeg in de zwangerschap is de gouden standaard en nauwkeurige informatie over de zwangerschapsduur leidt tot minder interventies rondom de bevalling. In het algemeen wordt aangenomen dat malaria infectie in de zwangerschap leidt tot meer kinderen met een laag geboortegewicht, maar of dit door vroeggeboorte komt of door groeivertraging is niet duidelijk. Echografie is niet meer weg te denken uit de westerse verloskunde, maar is niet voorhanden in de meeste malaria endemische gebieden. Dat komt doordat er geen betaalbare, robuste machines beschikbaar zijn. Daarnaast zijn er erg weinig mensen die echo's kunnen maken beschikbaar. De afgelopen jaren komen er steeds meer goedkope, onderhoudsvriendelijke echomachines. Uit dit proefschrift blijkt dat lokaal opgeleide mensen in een vluchtelingenkamp uitstekend in staat zijn termijn- en groei echo's te maken, mits ze een goede training krijgen en er regelmatig kwaliteitscontroles plaatsvinden. Zwangerschapsechografie is dus mogelijk in onwikkelingslanden voor en door lokale mensen. Stafleden en zwangere vrouwen in de ziekenhuizen op de Thai-Birmese grens denken dat echografie de veiligheid van de zwangerschap bevordert. Met de beschikbaarheid van betrouwbare echografisch vastgestelde zwangerschapsduur werden foetale en geboortegewichte percentielen gemaakt specifiek voor deze populatie. Deze percentielen werden gebruikt voor onderzoeken naar het effect van malaria op de groei van de foetus. Malaria in de vroege zwangerschap leidt tot kleinere diameters van foetale hoofdjes, maar heeft geen duidelijk effect op de hersenontwikkeling. Er zijn aanwijzingen dat falciparum malaria in de vroege zwangerschap geassocieerd is met kleinere placenta volumes. Ongeacht de zwangerschapsduur krijgen vrouwen die een malaria infectie doormaakten tijdens de zwangerschap kinderen met een lager geboortegewicht. Verdere, longitudinaal uitgevoerde analyses van de beschikbare gegevens zijn nodig om de mogelijkheden van inhaal

groei na een malaria infectie te bepalen. Overheden in ontwikkelingslanden moeten investeren in zorg rondom de zwangerschap, inclusief de training van echografisten en het beschikbaar maken van echomachines. Tijdens zulke zwangerschaps controles is vroege herkenning en behandeling van malaria in de zwangerschap mogelijk, zodat de negatieve gevolgen van zo'n malaria infectie beperkt blijven.

ပါရဂူ ဘွဲ့ဆိုင်ရာ သုတေသန စာတမ်း အနှစ်ချုပ် (မြန်မာဘာသာ)

ကိုယ်ဝန်ဆောင်များ၏ဂှက်ဖျားရောဂါ။ ။သန္ဓေသား၏ ဖွံ့ဖြိုးမှုအား တီဗီဓာတ်မှန်ဖြင့် လေ့လာခြင်း

ဂှက်ဖျားရောဂါသည် လူသားတို့အတွက် ကပ်တွယ်ညီနေသော ရောဂါဖြစ်ပါသည်။ နှစ်စဉ် ၁၂၅သန်း မျှသော ကိုယ်ဝန်ဆောင် များသည် ဂှက်ဖျားရောဂါ ကူးစက်နိုင်သောအန္တရာယ်နှင့် ကြုံတွေ့နေရပါသည်။ ကိုယ်ဝန်ဆောင်စဉ် ဂှက်ဖျားရောဂါ ဖြစ်ပွားခြင်းသည် ကိုယ်ဝန် ပျက်ခြင်း၊ လမစေ့ဘဲ ကလေးမွေးခြင်း၊ သန္ဓေသား ကြီးထွားမှု နှေးကွေးခြင်း၊ မိခင်၏ ကျန်းမာရေး ချို့ယွင်းခြင်း၊ အပြင် မိခင် သေဆုံးမှုပါ ဖြစ်စေနိုင်ပါသည်။သို့သော် ကိုယ်ဝန်ဆောင် ဂှက်ဖျား လေ့လာမှုအများစုတွင် မီးဖွားချိန်တွင်(gestational age) မရရှိနိုင်သလို (birth outcomes) တိုင်းတာမှု များသည် လည်း လေ့လာမှု တခုနှင့်တခု ကွဲပြားမှု ရှိနေပါသည်။ကိုယ်ဝန်ဆောင်ကာလအစောပိုင်း၌ တီဗီ ဓာတ်မှန်ဖြင့် စစ်ဆေးခြင်းသည် ကိုယ်ဝန်ဆောင် ကာလခန့်မှန်းခြင်းအတွက် အကောင်းဆုံး နည်းလမ်း ဖြစ်သော်လည်း ဂှက်ဖျား ဖြစ်ပွားလေ့ရှိသော ဒေသ များတွင် စစ်ဆေး နိုင်မှု မရှိသေးပါ။ ဤစာတမ်း၏ ရည်ရွယ်ချက်မှာ တီဗီဓာတ်မှန် စစ်ဆေး ခြင်း အား ထိုင်းမြန်မာ နယ်စပ် ကဲ့သို့ (low income setting) များတွင် ကိုယ်ဝန်ဆောင် များအကြား လုပ်နိုင်စွမ်း ရှိ၊မရှိနှင့် ကိုယ်ဝန်ဆောင် သက်တမ်းမှတ်တမ်းပြု ကိုယ်ဝန်ဆောင် များအတွင်း ဂှက်ဖျားရောဂါကူးစက်ခြင်း၏ သန္ဓေသား ဖွံ့ဖြိုးမှု အပေါ် အကျိုးသက်ရောက်မှု လေ့လာရန် ဖြစ်ပါသည်။

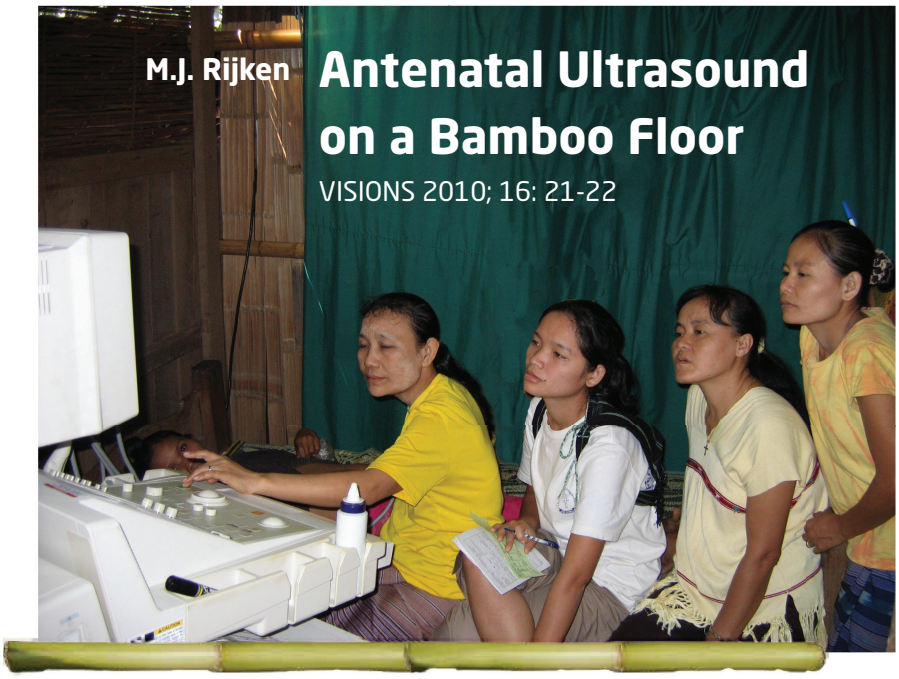
အခန်း ၂၊ ရှိကလို ဂှက်ဖျားသုတေသနအဖွဲ့သည် ၁၉၈၆ ခုနှစ်မှ စ၍ မြန်မာနိုင်ငံရှိ ကရင် လူမျိုးများ၊ မြန်မာရွှေ့ပြောင်း အလုပ် သမားများအတွက် ကျန်းမာရေး စောင့်ရှောက်မှုများကို ဆောင်ရွက်ပေးနေပါသည်။ ဤစာတမ်း၌ညွှန်းဆိုသော လေ့လာမှုများသည် Mahidol Oxford Tropical Medicine Research programme ၏ တစိတ်တဒေသအဖြစ် SMRU မှ ဆောင်ရွက်ခဲ့သော လေ့လာမှုများဖြစ်ကြပါသည်။SMRU သည် ဆရာဝန်များ၏ အနီးကပ် ကြီးကြပ်မှု အောက်တွင် သင်တန်း ပေးထား သော နယ်ခံကျန်းမာရေး ဝန်ထမ်းများဖြင့် ကိုယ်ဝန်ဆောင် စောင့်ရှောက်ရေး ဆေးခန်း ၅ ခုကို ဖွင့်လှစ် ဆောင်ရွက်လျှက် ရှိပါသည်။ ကိုယ်ဝန်ဆောင် ဂှက်ဖျားလေ့လာမှု သုတေသနကြီး၏ ကြီးတင်ပြင်ဆင်မှု အစိတ်အပိုင်းတခု အနေဖြင့် ဆေးခန်း အသစ် ၂ခုကို ဆောက်လုပ်ခြင်း၊ သားဖွားဆရာမ၊ တီဗီဓာတ်မှန် ကျွမ်းကျင်ဝန်ထမ်းများ မွေးထုတ်ခြင်း၊ ဆေးမှူးများ သင်တန်းပေးခြင်းတို့ကို ပြုလုပ်ခဲ့ပါသည်။ တီဗီဓာတ်မှန် စက်များ တပ်ဆင်ခြင်း၊ သင်တန် ပေးခြင်းများ ပြုလုပ်ခဲ့ပါသည်။ ဤသို့ ပြုလုပ်မှုများသည် ကိုယ်ဝန်ဆောင် သက်တမ်းကို တိကျစွာ တွက်ချက်ပေးနိုင်ခြင်း၊ သေးအွန္တရာယ် ကင်းရှင်းသော မီးဖွားရန်နေရာ ဖြစ်ပေါ်စေခြင်း၊ birth outcomes ကို တိကျစွာတိုင်းတာပေးနိုင်ခြင်းတို့အတွက် အထောက်အကူဖြစ်စေ ပါသည်။ ဖယ်ဆီပရမ် နှင့် ဗိုင်ဗတ် ဂှက်ဖျား ၂ မျိုးစလုံးသည် ကိုယ်ဝန်၏ နောက်ဆုံးရလဒ်ကို ပြောင်းလဲ ပစ်စေနိုင် ပါသည်။ သို့သော် ဗိုင်ဗတ် ဂှက်ဖျား ထိန်းချုပ်ရေးအတွက်မှာ ခက်ခဲဆဲ ဖြစ်ပါသည်။ ဂှက်ဖျားကူးစက်မှုအများဆုံးဖြစ်သော ဆာဟာရ-အာဖရိကဒေသနှင့် ယှဉ်ပြီး ကမ္ဘာ့ကျန်မာရေးအဖွဲ့သည် ကိုယ်ဝန်ဆောင် ဂှက်ဖျားထိန်းချုပ်ရေး နည်းပျူဟာများကို စီစဉ်ချမှတ်ခဲ့ပါသည်။ အာရှ-ပစိဖိတ်ဒေသ ဂှက်ဖျားများမှာ ဒေသတခုခြင်းဆီ နေရာကွက်၍ ကွဲပြားခြားနားစွာ ရှိနေနိုင်သော်လည်း ကိုယ်ဝန်ဆောင်များအတွက် ဖယ်ဆီပရမ် နှင့် ဗိုင်ဗတ် ဂှက်ဖျားကူစက်နိုင်မှု အန္တရာယ်မှာ မြင့်မားပါသည်။ ဆေးယဉ်ပါးမှု အန္တရာယ်မှာလည်း မြင့်မားပါသည်။ အခန်း ၃ တွင် ၂၀၁၁ ကမ္ဘာ့ကျန်မာရေးအဖွဲ့ ကိုယ်ဝန်ဆောင် ဂှက်ဖျား လုပ်ငန်း အဖွဲ့၏ နှစ်ပတ်လည် စည်းဝေးတွင် အခြားလူများ တင်ပြသော အာရှ-ပစိဖိတ်ဒေသတွင်းကိုယ်ဝန်ဆောင် ဂှက်ဖျားဆိုင်ရာ ပြုစုထားသော စာတမ်းများအကြောင်း ဖြစ်ပါသည်။ ဂှက်ဖျားကြောင့် ကိုယ်ဝန်ဆောင်မိခင်တွင် ပေါ်ပေါက်နိုင်သော ဆိုးကျိုးများ လျော့ချရေး ဆောင်ရွက်မှုများ၏ အကျိုးသက်ရောက်မှုကို မီးဖွားစဉ် ကလေး၏ကိုယ်အလေးချိန် ပြောင်းလဲမှုများအား ဖြင့် တိုင်းတာလေ့ရှိပါသည်။ သို့သော် တိုင်းတာမှု နည်းစံနစ် မတိကျမှု၊ မသေချာ မှုကြောင့် ကွဲပြားမှု ရှိနိုင်ပြီး အခြား အချက်အလက်များဖြစ်သော တိုင်းတာချိန် မတူညီမှု၊ ကိုယ်ဝန်ဆောင်သက်တမ်း အခြေအနေထို့သည် မီးဖွားစဉ် ကလေး၏ကိုယ်အလေးချိန် ပြောင်းလဲမှုများကို သက်မှတ်ရာတွင် အကျိုးသက်ရောက်မှု ရှုနိုင်ပါသည်။မီးဖွားစဉ် ကလေး၏ကိုယ်အလေးချိန်၊ gestational age တွက်ချက် သတ်မှတ်၊ တင်ပြရာတွင် အခန်း ၄ တွင် ဖေါ်ပြပါရှိသလို တိုးတက်အောင် ဆောင်ရွက်ရန်လိုအပ်ပါသည်။ ရိုးရှင်းသော နည်းစနစ် လမ်းညွှန် ဖြစ်လျှင် ပို၍ကောင်းပါသည်။ အခန်း ၅ တွင် fetal biometric measurements အတွက် ၂ဦး၂ဖက်(အတွင်းပြင်ပလေ့လာသူကြား) သဘောတူညီမှု ဖြစ်ပါသည်။ ဇယားစာရင်းတွင် ပါရှိသည်မှာ သင်တန်းရရှိပြီး လေ့လာမှုတွင်ပါဝင်ဆောင်ရွက်ခဲ့သော ဒေသခံဝန်ထမ်းများ၏စာရင်း ဖြစ်ပါသည်။ထိုဝန်ထမ်းများသည် အသက် ၁၆ နှစ် အထိ ကျောင်းတက်ခဲ့ကာ တီဗွီဓာတ်မှန်သင်တန်း ၃လ ရရှိခဲ့သူများဖြစ်ပြီး gestational age ခန့်မှန်းရာတွင် တိကျမှန်ကန်စွာ ဆောင်ရွက်နိုင်သူများ ဖြစ်ပါသည်။

ရရှိလာခဲ့သော အတွေ့အကြုံများအရ ဖွံ့ဖြိုးဆဲ နိုင်ငံများတွင် ဒေသခံများအား သင်တန်းပေး၍ တီဝီဓာတ်မှန်ဖြင့် ကိုယ်ဝန်ဆောင်များအား pregnancy datingဆောင်ရွက် နိုင်ခြေရှိကြောင်း သိရပါသည်။ အခန်း ၆ တွင် တီဝီဓာတ်မှန်ဖြင့်ကိုယ်ဝန်သက် ၁၆ ပတ်မှ ၄၀ ပတ်အတွင်း သန္ဓေသား၏ အရွယ်အစား တိုင်းတာခြင်းအား ဆောင်ရွက်နိုင်ကြောင်း သက်သေပြချက်များ ပါရှိပါသည်။ သန္ဓေသား၏ အရွယ်အစား တိုင်းတာချက် ပုံသေနည်းများ ဖေါ်ထုတ်ထား ပါသည်။(biparietal diameter, head circumference, abdominal circumference and femur length)၄င်းတို့သည် ထိုင်းမြန်မာနယ်စပ်တွင် သန္ဓေသား၏ အရွယ်အစားတိုင်းတာရေး၌ အသုံးပြု ရ ကောင်း ရ နိုင်ပါသည်။ SMRU ဆရာဝန်များအနေဖြင့် တီဝီဓာတ်မှန်သည် ကိုယ်ဝန်ဆောင်များ၊ သန္ဓေသား(ကလေး) နှင့် ကိုယ်ဝန်ဆောင် စောင့်ရှောက်မှုလုပ်ငန်းများအတွက် အသုံးဝင်သည်ဟု ခံယူပါသည်။သို့သော် ဒေသခံဝန်ထမ်းများ၊ ကိုယ်ဝန်ဆောင်များအနေဖြင့် မည်သို့ ခံယူပါသနည်း။ နည်းလမ်းပေါင်းများစွာ (ဇေ့လာစောင့်ကြည့်မှု ၃၀၊ လူတွေ့မေးမြန်းမှု ၁၉ခု၊ အုပ်စုဖွဲ့ဆွေးနွေးမှု ၇ခု၊ မေးခွန်းဖြင့် ဆန်းစစ်မှု ၆၄၄ ခု)အရ (အခန်း ရ တွင် ဖေါ်ပြ) ဘေးကင်းလုံခြုံသော ကိုယ်ဝန်ဆောင် ဘဝ၊ မီးဖွားရေးတွင် တီဝီဓာတ်မှန်သည် လိုအပ်သော နည်းပညာတခုအဖြစ် တည်ရှိသည်ဟု ဒေသခံဝန်ထမ်းများ၊ ကိုယ်ဝန်ဆောင်များအနေဖြင့် ခံယူကြောင်း တွေ့ရှိရပါသည်။ လူနာအချို့တွင် တီဗီဓာတ်မှန် မရိုက်မီ ခဏတာ ရုက်ရွံ့ခြင်း၊ စိတ်လှုပ်ရှားမှု ကြုံခဲ့ရကြောင်း လေ့လာတွေ့ရှိရပါသည်။သို့သော် ဝန်ထမ်းများ၏ဆက်ဆံရေး၊ အဖြေကို ကြားရချိန်တို့တွင် ထိုခံစားရမှုများ ပျောက်ကွယ်သွားကြောင်းလေ့လာသိရှိရပါသည်။ အလွန်အဂျွံသုံးစွဲမှုကြောင့် မမျှော်လင့်သော ဆိုးကျိုးများ၊ ဖြစ်ပေါ်မှု၊ ကျား၊ မခွဲခြား ကြိုတင် သိရှိရခြင်းကြောင့် ကိုယ်ဝန်ဖျက်ချမှု ရှိသည်ဟု မတွေ့ရှိခဲ့ရပါ။ အခန်း ၆ တွင် ဖေါ်ပြပါရှိသော ပုံသေနည်းများကို သုံးပြီး တွက်ချက်မှုများ အရ ၄က်ဖျားဖ၏ သန္ဓေသား ကြီးထွားမှု အပေါ် သက်ရောက်ပုံကို အခန်း ၈ တွင် ဖေါ်ပြထားပါသည်။ ကိုယ်ဝန်သက် ဒုတိယ ၃ လတွင် ၄က်ဖျားဖြစ်သော ကိုယ်ဝန် ၃၄၄ ၏ သန္ဓေသား အရွယ် အစားသည် ၄က်ဖျားမဖြစ်သော ကိုယ်ဝန်ဆောင် ၃၇၇၉၏ သန္ဓေသား များထက် သေးငယ်ပါသည်။စောစီးစွာကုသမှု ပေးသော်လည်း လက္ခဏာ ရှိသည်ဖြစ်စေ၊မရှိသည်ဖြစ်စေ ကိုယ်ဝန်နစ်ဝဖယ်ဆီပရမ်၊ ဗိုင်ဗတ်၄က်ဖျားဖြစ်သော မိခင်၏ သန္ဓေသား ဦးခေါင်း အတိုင်းအတာ သည် ကိုယ်ဝန်သက်တမ်း အလယ် (ကိုယ်ဝန်သက် ကြားချိန်)ထက်သေးငယ်ပါသည်။ထို့ကြောင့် ကိုယ်ဝန်နစ်၌ ၄က်ဖျားမဖြစ်အောင် ကာကွယ် သင့်ပါသည်။ ကိုယ်ဝန်ဆောင်သက် မှတ်ထားသော ၁၀၂၆၄ ကိုယ်ဝန်ဆောင်များ၏ မွေးစဉ် ကလေး အလေးချိန်များကို အခန်း၉ တွင် ဖေါ်ပြထားပါသည်။ ၄က်ဖျားဖြစ် မိခင်များ မွေးစဉ် ကလေး အလေးချိန်များသည် ရောဂါမဖြစ်သူများနှင့်ယှဉ်လျှင် gestational ages အားလုံးတွင် လျော့နည်းကြောင်းတွေ့ရှိရပါသည်။ မွေးစဉ် ကလေး အလေးချိန်လျော့ကျခြင်းတခုတည်းဖြင့် gestational age အရ သေးငယ်သည်ဟုပြောရန်မှာမူ မခိုင်လုံသော တင်ပြချက်ဖြစ်ပါသည်။ အခန်း ၁၀ တွင် a prospective ultrasound cohort study ၏ အဖြေများကို တင်ပြထားပါသည်။ ၅ ပတ်တကြိမ် ကိုယ်ဝန်ဆောင် ၃၆၀အား တီဝီဓာတ်မှန်ဖြင့် စစ်ဆေး ပါသည်။ ၉၇ (၂၆.၉%)သည် အနည်းဆုံး တကြိမ်(ကိုယ်ဝန်သက်တမ်းအတွင်း)၄က်ဖျားကျစက်ခံခဲ့ကြရပါသည်။ ကနဦးတွေ့ရှိချက်များအရ stillbirths မရှိပါ။ ပြင်းထန်၄က်ဖျားဖြစ်သူ တဦးရှိပါသည်။ ကိုယ်ဝန်သက်တမ်း ဒုတိယ အပိုင်းအခြားတွင် ၄က်ဖျားဖြစ်လျှင် သန္ဓေသား ၏ဦးခေါင်းဖွံ့ဖြိုးမှု လျော့နည်းခြင်းနှင့် ဆက်စပ်မှု ရှိသော်လည်း birthweight တွင် ၄က်ဖျား ဖြစ်ခြင်း၊ မဖြစ်ခြင်း ဆက်စပ်မှု မရှိပါ။ကြီးထွားမှု ပြန်လည်ကောင်းမွန် လာမှုအခြေအနေအတွက် အချိန်ကာလကြာကြာ စောင့်ကြည့် လေ့လာဖို့လိုသည် ကို အထူးပြု ပြောကြားလိုပါသည်။ အချင်း ပမာဏတိုင်းတာခြင်းပုံသေနည်းကို သန္ဓေသား တယောက်သာလွယ်၊မွေးသော၊ ပုံမှန်(ချို့ယွင်းမှု မပါပဲမွေးလာသော) ကိုယ်ဝန်ဆောင် ၆၅ ဦး၏ အချင်း နောက်ဘက်ကို သုံးပြီးဖေါ်ထုတ်ထားပါသည်။(အခန်း ၁၁) ဖယ်ဆီပရမ်ပိုးကူးစက်ခံရသော အချင်းများ၏ ပမာဏသည် ပုံမှန်ထက် 10th centile လျော့နည်းပါသည်။ သုံးဘက်မြင် တီဝီဓာတ်မှန်ဖြင့် အချင်းပမာဏ တိုင်းတာမှုသည် ကိုယ်ဝန်နစ်၌ ၄က်ဖျားကူစက်ခံရမှု နှင့် သန္ဓေသားကြီးထွားမှု အကြားရောဂါဗေဒ၊ ဇီဝကမ္မဆိုင်ရာဆက်စပ်မှုများကို ပိုမိုနားလည် စေပါသည်။ စောလျှင်စွာ ၄က်ဖျားရောဂါရှာဖွေနိုင်ခြင်း၊ ထက်မြက်သော ဆေးဝါးများနှင့်ကုသခံရနိုင်ခြင်း ရှိနေသော လူအတွင်း ၄က်ဖျားဖြစ်သော ကိုယ်ဝန်ဆောင် ၂၂ ဦးနှင့် မဖြစ်သော ၂၂ ဦး၏ သန္ဓေသားဦးနှောက် ဖွံ့ဖြိုးမှုနှိုင်းယှဉ်ရာတွင် ထူးခြားသော ကွာဟချက်မတွေ့ရပါ။(အခန်း၁၂)

Translator: Dr Khin Maung Lwin

Chapter 15

Appendices



So much has changed. Twenty years ago, medical literature noted that "the use of ultrasound in developing countries is problematic because there are no machines or radiologists available." Meanwhile, it is inconceivable that in Western countries an OB / GYN clinic could function without ultrasound equipment. Even in primary antenatal care ultrasound has emerged as a valuable technology. Every woman in Europe receives at least one or two ultrasound scans during her pregnancy to determine for example the date of delivery and to monitor the fetus' health.

In Thailand, along the Burmese border, pregnant women are in need of the same kind of monitoring. They live under difficult circumstances and are at greater risk of complications in pregnancy, including infectious diseases such as malaria. The Shoklo Malaria Research Unit (SMRU, www.shoklo-unit.com) has five clinics along this border to provide assistance to migrants and refugees. In addition, the SMRU is investigating the optimal treatment of multi-resistant malaria in this area. The SMRU is a field station of the Tropical Medicine Department of the University of Oxford (UK) and Mahidol University, Bangkok. Together with my wife Machteld, I have been working in the SMRU since 2006.

Around 4,000 pregnant women visit our ANC (Antenatal Clinics) each year. Half of these women cannot read or write, or both. No one knows exactly the date of their last menstruation. Here, women measure pregnancy duration in "waxing moons". What do we do in Europe if the pregnant woman's tummy is far too small? How do we determine how far along the pregnancy is? How can we tell if the baby is still alive? We use ultrasound. The same is true for heavily bleeding pregnant women, a threatening miscarriage, or an oversized tummy. Ultra- sound technology has proved to play a vital role during pregnancy for many years. The SMRU believes that ultrasound in pregnancy can be very useful, ESPECIALLY in developing countries. For example pregnancy duration and location of the placenta are difficult to determine "manually" as is the assessment of multiple pregnancies. But this is vital information. Fortunately, the UMCU (University Medical Center Utrecht) and Toshiba agree! In 2006, they donated a PowerVision plus box to transport the device from the Netherlands to Maela refugee camp on the Thai-Burmese border. At that time, Machteld and I had just started as doctors in the refugee camp and had begun conducting research on malaria in pregnancy. Three years later we have learnt so much. We have written about some of our experiences on the website www.malariadokters.nl.

The donated ultrasound machine is located in our hospital in the refugee camp on a

Marcus Rijken, MD
Shoklo Malaria Research Unit Thailand

bamboo wooden floor, under a canopy and has been in use every day for more than three years. Recently, Toshiba once again supported this project with a new transducer! Meanwhile, I have taught eight young women to make echoes and we are busy with a large study measuring fetal growth. Most of these local health workers have no medical training or experience; however, they are eager to learn and are very enthusiastic. After three months of training they sit an ex- am testing them on their ultrasound expertise. Our

"Ultrasound Department" is professionally organized and there is always a doctor available for difficult cases. We have also established a continuous quality control system (intra- and inter-observer). Recently, we have published an article about these locally trained health workers as we found that they are able to carry out biometrics/growth scans of an adequate quality (see Rijken et al, Ultrasound Obstetrics Gynecology 2009; 34: 395-403).

Is ultrasound in developing countries a problem? In a well organized unit with continuous quality control and supervision, it is possible for locally trained staff to carry out good fetal growth scans. Expensive, highly trained radiologists are therefore not necessary. And the machines? They are becoming cheaper and more compact making it increasingly possible for ultrasound to be available in the developing world. In addition, old written-off ma- chines from one of the many OB / GYN clinics in the West become available on a regular basis. Problematic? What we then need is people or companies prepared to give such a machine a "second life", in the jungle, on a bamboo wooden floor for example. Toshiba is one such company. Thank you!

Acknowledgements

Six years ago, a journey started that changed my life. A journey to "Kawthoolei". Before 2006, I knew very little about this "land without evil" on the eastern border of Burma. Older Karen generations remember this beautiful land of mountains, trees, flowers and butterflies and for many younger generations this is the land of their dreams. But what has happened in Burma (for example civil war resulting in hundred thousands of refugees) is in contrast to all that. Furthermore, multidrug resistant malaria parasites are an additional threat for the populations on the Thai-Burmese border.

In March 2006, Machteld and I left the Netherlands with words of Shalom from Numbers 26: 22-27.

When we arrived in Mae Sot, we were warmly welcomed by the Karen and Burmese staff of the Shoklo Malaria Research Unit (SMRU). Many of these people have already left to a "third country" (e.g. Australia, USA, or Europe), after they fled to Thailand. Being refugees themselves, they took care of us. HehWah, Chaw Chaw, Slight and Annie, Noble, Titus, Carrit and Magno, Thida Saw Loo, Eh Hti Kaw, and so many others; thank you for your patience and trust during those days.

Many other Karen people decided to stay close to their homeland to continue helping their own people. We became friends over the years. As if we became part of the SMRU family. Below I have written the names of all present SMRU family members. I will never forget your dedication in caring for other people, your eagerness to learn, and your wish to improve. For example the young midwifery team of WangPha (with Tharamu pado Ohmu): you grew from giggling schoolgirls to mature confident midwives performing vacuum assisted deliveries and managing serious obstetric emergencies. Or the ultrasound team with their teachers; professor Hser Gay War and doctor Umbrella. Many people got excited, when I told your story during international conferences: that you were able to perform all those 2D and 3D ultrasound scans so well. Aye Kyi Win; when you graduated as a SMRU midwife, you told me that you left your village and family deep inside Burma. As a school girl you noticed too many young women died in their village during childbirth. You wanted to learn how to save those lives and help your own people. Your motivation and dedication kept us going!

Some people I would like to thank especially. In the first place all women on the Thai-Burmese border who agreed to participate in one of the studies of SMRU. The life of the women on the Thai-Burmese border is not easy; every day is a struggle to survive. I hope and pray that one day life will become better for them and their families, without being afraid for violence, for diseases like malaria, or for the risks of pregnancy and childbirth.

Professor dr. Piet Kager introduced us to the SMRU. Daan Kuipers, dr. Michèle van Vugt, professor Bernard Brabin and professor Feiko ter Kuile shared their experiences

from Shoklo and Maela refugee camps and made us extremely motivated to move. Although rather idealistic, we did not fully realize yet that we would join a top malaria research unit in the world. Thank you!

Thara pado, professor François Nosten, from the first moment we met you on a terrace in Paris, until now, you have been a mentor and an inspiration (Science 2010, 329; 1142) for me. After my first experience of a tragic maternal death due to severe malaria, you told APP and me: "Today, you have met malaria. From this moment, you know and feel that malaria is lethal. This experience will make you different from other people in a congress room, who only know malaria from their computer, the books or the laboratory bench. And now you understand why I have spent my life fighting this parasite…".

Dr. Rose McGready, tharamu pado, I do not know anyone else with so much energy and so many clever ideas as you have. Thank you for your patience and allowing us to become a strong team over the years! You have taught me to write papers and review manuscripts precisely. And I will never forget your philosophy that anything could and should be studied, rather than following others. There are so many things to do, looking forward continuing working together!

Professor Nick White, thank you for letting us be the "guinea pigs" listening to one of the lectures you were preparing. Such teaching hours were the most inspiring malaria moments in my life. You have introduced me to professor Stephen Kennedy in Oxford, which was the first step to a very fruitful collaboration. Thank you for your participation in the sack-race earlier this year, when we all needed support from each other.

Professor Stephen Kennedy, only a few months ago we were walking through the hills in Oxfordshire and we talked about life, fetal growth, malaria and research in obstetrics; unforgettable moments. You made me feel home. I would like to thank your department for all exciting moments in Oxford and in Mae Sot. We are proud to be part of the Interbio-21ST study!

Dr. Aris Papageorghiou, during the many hours of skype meetings, when you taught me z-scores and how to focus, we became friends! I cannot believe, we have met only a few times in real life (Hamburg, London, Oxford, Los Angeles, Mae Sot). Looking forward to many more skype and real meetings, and one day; working as colleagues?

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One day we will meet in Kawthoolei!
TABLU
PADO
MMJD

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Curriculum vitae

In 1978, Marcus Rijken was born in the Streekziekenhuis in Bennekom, the Netherlands. He grew up in Veenendaal, were his mother was a pastor and his father a general practitioner. He completed primary and secondary education at "Het Baken" and "Ichthus-College", respectively. He studied medicine at the University of Utrecht from 1996 and graduated in 2003. This included a one month nursing-elective in the ALYN- Orthopaedic Hospital for Handicapped Children, Jerusalem, Israel; a 6-months laboratory research project (gene therapy) at the Leiden University Medical Centre (dr. A.A.F. de Vries) and a 2-months surgical internship in Techiman, Ghana (Dr. H. Wegdam). After his residency in Surgery in Amersfoort (Dr. R.Voorhoeve) and Obstetrics & Gynaecologie in Apeldoorn (Dr. P. Van de Weijer) and the Netherlands course in Tropical Medicine and Hygiene in Amsterdam, he graduated as Dutch tropical doctor in 2005. Together with his wife, he was the initiator of the foundation: "Stichting Malariadokters" (www.malariadokters.nl). From 2006 to 2012 he worked with the Shoklo Malaria Research Unit, a field station of the Mahidol-Oxford tropical medicine Research Unit (prof. F. Nosten and prof. N. White) in a refugee-camp and migrant clinics on the Thai-Myanmar border. He did consultancies for the International Rescue Committee and World Health Organization and was one of the initiators and instructors of the locally adapted, but recognized, Advanced Life Support in Obstetrics (ALSO) course. He was certified in basic obstetric ultrasonography in the J. Radcliffe Hospital, Oxford, UK (prof. S. Kennedy). Since then, he closely worked together with this group towards the international Interbio-21st study (http://www.intergrowth21.org.uk).
In March 2012, he returned back to the Netherlands and started his residency in Obstetrics & Gynaecology under supervision of dr. J. Schagen van Leeuwen at the St. Antonius Ziekenhuis in Nieuwegein and prof. dr. A. Franx at the University Medical Centre Utrecht, the Netherlands. Marcus is married to Machteld Boel and they have two children: Janne (2007) and Daniël (2009).