1 Introduction and Study Objectives
INTRODUCTION

More than 500,000 women die each year in childbirth, 99% of them in developing countries. This amounts to one woman dying every minute, from mainly preventable causes. Many of these deaths are related directly to childbirth and many are due to indirect causes such as severe anaemia and malaria.

For every maternal death there are 14 perinatal deaths (7 million per year). More than one third of fetal and newborn deaths are related to the mother’s health and nutrition. Low birth weight is the most important risk factor for infant mortality, and twenty four of the twenty five million low birth weight babies born each year are from developing countries. Malaria and anaemia are both risk factors for low birth weight and perinatal mortality.

SEVERE ANAEMIA AND MATERNAL AND PERINATAL HEALTH

The World Health Organisation has defined anaemia in pregnancy as a haemoglobin below 11g/dl, and severe anaemia as a haemoglobin below 7 g/dl. In sub-Saharan Africa, it is estimated that between 50 - 70% of pregnant women are anaemic with 5-15% being severely anaemic. In Sub-Saharan Africa, this anaemia is more frequent in primigravidae, during the rainy season when malaria transmission is high and during the second and third trimesters.

Severe anaemia is associated with an increased risk of maternal mortality from cardiac failure and shock, progressing through the following stages:
1) compensation, with breathlessness on exertion only;
2) decompensation, with breathlessness at rest. This becomes likely when haemoglobin (Hb) is less than 7 g/dl (packed cell volume (PCV) < 24);
3) cardiac failure. There is a high risk with Hb below 4 g/dl (PCV < 13).

Blood volume increases in pregnancy raising cardiac output. In severe anaemia, the heart has to increase this output even further in response to hypoxaemia. Commonly women with severe anaemia develop cardiac failure for the first time during labour or in the first few hours following delivery, and are highly likely to die if not within easy reach of good health facilities. Anaemia also renders women less able to withstand blood loss at delivery. Women who are not anaemic can usually lose over a litre of blood with little risk of collapse, whereas in women who are severely anaemic much lower blood loss may prove fatal. Anaemia may also contribute to deaths from sepsis.

Severe anaemia has been reported as the main cause of 8%-20% of maternal deaths in some hospital series and of 11%-13% in community-based studies. The association between severe anaemia and maternal mortality is difficult to quantify, because haemoglobin (Hb) estimations are often not performed systematically in settings where severe anaemia is highly prevalent. The retrospective diagnosis of severe anaemia as a primary factor contributing to a death is difficult. Community surveys may under-estimate the association because they do not take into account the contribution that severe anaemia makes towards deaths from haemorrhage and sepsis. All cause anaemia attributable maternal mortality has been estimated to be 6.37% of maternal deaths for Africa, with the relative risk of mortality associated with severe anaemia (defined as <4.7 g/dl) being 3.51 (95% CI 2.05, 6.00). As well as contributing to maternal mortality, it is likely that severe anaemia causes considerable morbidity, such as tiredness, lassitude, breathlessness, and also decreases pro-
ductivity and ability to work. There has been very little research on the morbidity or effect on quality of life due to anaemia in pregnancy.

Severe anaemia is also an important cause of low birthweight (LBW) and fetal anaemia, and the risk of perinatal death has been shown to increase significantly in severely anaemic women.

**CAUSES OF ANAEMIA IN PREGNANCY**

Normal physiological changes of pregnancy are such that there is always a progressive fall in haemoglobin / haematocrit from the end of the 12th week of pregnancy until about the 34-36th week, with a return to normal levels 6-8 weeks post-partum. This "dilutional anaemia" is due to an increased blood volume of 50%, with an increased red cell mass of only 18%. Haemoglobin is a measure of the ratio of red cell mass to plasma volume, so consequently always decreases during pregnancy due to sero-dilution in the presence or absence of anaemia.

In Sub-Saharan Africa a major preventable cause of severe anaemia in pregnancy is malaria infection, with the aetiology of anaemia in primigravidae often being dominated by haemolysis due to malaria. Other important causes of anaemia in pregnancy are iron, folate and vitamin A deficiencies, hookworm infections, haemoglobinopathies and advanced HIV infection.

Before discussing malaria in pregnancy in greater depth, other causes of anaemia in pregnancy that should be preventable through antenatal services are discussed briefly below.

**Iron deficiency**

Iron deficiency is common where diet is poor, and is exacerbated by the increased demands of pregnancy. Iron deficiency produces an anaemia characterised by microcytic hypochromic red blood cells. Iron requirements are considerably increased during pregnancy, because of increase in red cell mass (about 500mg of iron); active iron transport to the fetus (about 250mg) and constitution of the placenta (about 25mg). Including basal losses, total requirements are greater than 1000mg for the duration of pregnancy, averaging out at more than 3.5mg/day, though with the requirements more concentrated during the second and third trimesters. By comparison, iron requirements in men are approximately 1mg/day. In addition to the daily requirements, iron is lost through the blood loss at delivery, the mean loss being between 150 and 350mls. Iron requirements are increased further if there is any pathological cause of iron loss, such as parasitic infestation.

Hookworm infection is one of the principal causes of iron deficiency anaemia in developing countries. Hookworm parasites attach themselves to the mucosa of the intestinal tract and suck blood from the submucosal vessels. Each worm of Necator Americanus (the commonest in Tropical Africa) causes 0.05ml blood loss/day. As there is frequently infestation with many parasites, it would not be uncommon to lose 2ml/day (1mg iron/day). Similarly, with a heavy trichuris infection blood loss can be equivalent to at least 1mg iron /day. Schistosoma haematobium causes haematuria, and urinary blood loss has been estimated to range from 0.5 to 125 ml/day (0.25 to 60mg iron/day). Schistosoma mansoni, by causing inflammation and polyps in the large bowel can cause 1-6mg iron loss/day.
Despite this high iron need, the diet of women of reproductive age in developing countries is frequently more iron deficient than that of men, at a given level of poverty\textsuperscript{20}. Women often enter pregnancy already iron deficient for a mixture of reasons including dietary inadequacy, short birth interval, prolonged lactation, heavy menstrual losses and parasitic infestation.

Even in well-nourished communities, the iron requirements in pregnancy are usually too high to be supplied by diet alone, despite increased absorption. The extra iron requirements normally have to be met by iron stores. However, many women in developing countries have minimal or absent iron stores when they enter pregnancy. In well-nourished women from industrialised countries, iron stores are significantly reduced from early pregnancy onwards\textsuperscript{21}, and were absent in 16% of French women in the third month of pregnancy\textsuperscript{19}. It has been estimated that in the absence of iron supplementation it can take two years to regain pre-pregnancy ferritin levels. This is of particular relevance where there are short birth intervals\textsuperscript{19}.

**Vitamin A deficiency**
Dietary vitamin A deficiency is associated with anaemia in pregnancy. A trial conducted in pregnant women in Indonesia found that simultaneous administration of vitamin A along with iron supplementation produced a better haematological response than iron or vitamin A alone\textsuperscript{22}. Improvement in vitamin A status is believed to improve the absorption and mobilisation of iron, and may enhance immune responsiveness and decrease infections contributing to anaemia\textsuperscript{23}.

**Folate deficiency**
As with iron, folate deficiency is also common where diet is poor, and is exacerbated by the increased demands of pregnancy – though there are marked regional variations in deficiency. Folate deficiency produces an anaemia characterised by unusually large red blood cells (megaloblastic anaemia). Folate requirements are approximately doubled during pregnancy, especially during the last trimester and during the puerperium. Requirements for folate are also increased by disease processes associated with haemolysis such as malaria or sickle cell disease. Folate levels were higher in primigravidae taking antimalarial prophylaxis in Ibadan, Nigeria, than in those unprotected from malaria\textsuperscript{24}.

**Malaria in pregnancy**
Malaria is a potentially preventable cause of both severe anaemia in pregnancy and also perinatal morbidity and mortality.

The maternal anaemia which develops in association with *falciparum* malaria infection is mediated through haemolysis of both infected and uninfected red cells. It is thought that the haemolysis of uninfected cells is in part immune complex mediated. Dyserythropoiesis may also contribute to the development of anaemia during and after malaria infection. In addition, folate deficiency may develop secondary to haemolysis and the already increased demands for folate in pregnancy\textsuperscript{17}.

The clinical features of *falciparum* malaria in pregnancy depend to a large extent on the immune status of the woman, which in turn is determined by her previous and continued exposure to malaria.
Low or unstable transmission areas

In areas with low or unstable transmission of malaria, exposure is not constant enough to result in effective immunity in the population. People of all ages are at risk of severe disease if exposed to infection. In these settings, pregnant women of all parities are at 2-3 times greater risk of developing severe disease than non-pregnant women and at approximately 3 times greater risk of dying if they do develop severe disease.25,26 Severe disease in pregnant women has been associated with 20-30% maternal mortality and a very high risk of miscarriage, premature delivery or neonatal death.27 Particular dangers of malaria in pregnancy in women with absent or low levels of immunity are hyperpyrexia, hypoglycaemia, severe haemolytic anaemia, cerebral malaria and pulmonary oedema.25 Women of all parities are affected.

Moderate or high transmission areas

In most of sub-Saharan Africa and some parts of Asia, malaria transmission is moderate or high. Malaria is present every year, commonly with seasonal peaks. The work that is included in this thesis was undertaken in an area of moderate transmission. Immunity to malarial disease takes a number of years to develop, so in these settings, children under five are at particular risk of severe disease and death. With continued exposure, older children and adults still get infected with malaria, may have a low-grade fever in association with infection, but rarely go on to develop severe disease.

During pregnancy this immunity to malaria is altered. Pregnant women have a higher prevalence of parasitaemia and density of parasitaemia than non-pregnant women. Primigravidae are affected most, with the risk of malaria decreasing with each successive pregnancy50. Severe disease is uncommon, though placental parasitisation is frequent. Infection is frequently asymptomatic. Consequently, malaria may go unsuspected and undetected. The main clinical problems of malaria in pregnancy in these settings are the development of maternal anaemia, which is often severe16, and low birth weight deliveries (<2500g)13. The severe anaemia may develop insidiously and may therefore be overlooked until very severe. Peripheral parasitaemia may be absent, although placental parasitaemia is present, so investigations may fail to reveal malaria as the cause of the anaemia.

The increased low birthweight prevalence in primiparous resulting from malaria is substantial, and may be over 50% in some areas. In The Gambia reduction of low birthweight by chemoprophylaxis was estimated, using data on the relationship between birthweight and risk of death, to reduce the neonatal death rate by 42% and infant mortality by 18% among children of primigravidae, and by 6% and 4% respectively among children of multigravidae28. The low birth weight results from a combination of intra-uterine growth restriction (IUGR) and prematurity41. The mechanisms through which it occurs include an effect of maternal anaemia; haemodynamic disturbance of utero-placental circulation51; placental damage leading to impaired nutrient supply; and possibly an association with pre-eclampsia (PET) or a PET like process29.

Impact of HIV on malaria in pregnancy

A number of studies have shown that P. falciparum parasitaemia occurs more frequently in HIV infected pregnant women30,31. HIV infection appears to impair malarial immunity, such that HIV infected multigravidae may show higher malaria prevalence than HIV uninfected primigravidae. The interaction may be synergistic in increasing the risk of
maternal anaemia and low birthweight, and consequently reducing child survival. It is also possible that placental malaria increases the risk of mother-to-child HIV transmission.

Pathophysiology of falciparum malaria in pregnancy

A unique feature of Plasmodium falciparum is its ability to sequester in deep capillary beds during the asexual stages of parasite replication, thereby avoiding host immune surveillance and splenic clearance. Infected erythrocytes adhere to a variety of ligands on vascular endothelium. It is this feature which is thought to result in P. falciparum being responsible for most of the severe disease and almost all of the mortality associated with malaria worldwide.

What makes malaria in pregnancy unique is that parasites sequester in the placenta, where infection is often extremely heavy. Parasites are seen in maternal erythrocytes in the intervillous space in active / acute infection. If there is longer-standing infection, haemozoin (malaria pigment) is seen in peri-villous fibrin deposits in the placenta. Thickening of the syncytiotrophoblast basement membrane in association with placental malaria infection is a consistent feature and an intervillous inflammatory response often occurs with infiltration of mononuclear inflammatory cells.

Until recently, the mechanisms through which placental parasite sequestration occurs have been unclear. Studies in Malawi and Kenya, however, have identified strains of parasite which are pregnancy specific and may be selected by their ability to adhere to chondroitin sulphate A (CSA) on the syncytiotrophoblast.

Prevention of falciparum malaria

Chemoprophylaxis

Though most countries in malaria endemic areas of sub-Saharan Africa have had policies for the prevention of malaria in pregnancy, they have often been poorly implemented. Historically, the mainstay of this prevention has been with weekly chloroquine prophylaxis, but with increasing levels of resistance and poor adherence, this is inadequate in the majority of countries. In parts of West Africa, weekly pyrimethamine prophylaxis has been used for many years. Though initially highly effective, high levels of parasite resistance have rendered this approach ineffective. Proguanil is safe in pregnancy but needs to be given daily, and must be preceded by effective parasite clearance. FORTnightly pyrimethamine-dapsone (Maloprim), has been shown to be effective in increasing birth-weight and reducing anaemia in primigravidae in The Gambia. In South-East Asia and in Malawi, weekly prophylaxis with mefloquine has been effectively used.

Problems with the available chemoprophylaxis

At the time when this work commenced, there were many unresolved issues regarding the efficacy and effectiveness of antimalarial chemoprophylaxis:

1) It was argued that there was inadequate evidence on which to base policies for preventing malaria in pregnancy, and there had been a call for large scale placebo controlled intervention studies addressing the effectiveness of different antimalarial regimes on important maternal and perinatal outcomes.

2) Lack of an appropriate drug: the main-stay of prevention of malaria in pregnancy had been with weekly chloroquine. However, the increase in chloroquine resistance in
many areas had reduced its prophylactic efficacy. The only other drug that was recommended as prophylaxis during pregnancy was proguanil, which is expensive and needs to be taken daily.

3) Problems of delivery of regular antimalarials and poor adherence: even in areas where there was sensitivity to chloroquine, many problems remained regarding the effectiveness of prophylaxis. The antimalarials need to be given and taken regularly. In many parts of sub-Saharan Africa women only attend antenatal care once or twice. Even when they attend and supplies are available, adherence is not guaranteed. In Malawi, only 36% of women being given chloroquine chemoprophylaxis had evidence of chloroquine in the urine, implying very low adherence.

**Potential alternative interventions**

**Preventative intermittent treatment**

Most of the drugs used for the treatment of chloroquine resistant malaria have too many side effects if given as weekly prophylaxis. If an antimalarial is effective when taken periodically in doses sufficient to clear parasitaemia, and needs only be given twice during pregnancy it would be a safer alternative to regular weekly prophylaxis. This would also mean it could be given when women come to the antenatal clinic, thereby improving adherence. It had been shown in 1994 in Malawi, that Sulfadoxine-pyrimethamine (SP) given twice during pregnancy (one dose in the second trimester and one dose in the third trimester) had a significant effect on reducing placental parasitaemia. It was considered necessary to determine if this regime of preventative intermittent treatment is effective in preventing severe maternal anaemia. Malaria control advisors at WHO were supportive of a further study to be undertaken, evaluating this regime.

**Insecticide treated bed-nets**

Insecticide treated bed-nets are effective at reducing exposure to biting mosquitoes, and so can substantially reduce the number of infective bites received. Whether this reduction in the number of sporozoite inoculations results in less morbidity depends on many factors, including the endemicity of the malaria and the individuals' pre-existing immunity. Insecticide treated bed nets (ITBN) can reduce severe morbidity and mortality from malaria in children. However, it was still not known whether this form of malaria control would be effective in pregnant women in sub-Saharan Africa. A study in a mesoendemic malarious area of the Thai-Burmese border had found that insecticide treated bed nets resulted in a significant reduction in maternal anaemia, despite a marginal effect on peripheral parasitaemia.

**DETECTION OF SEVERE ANAEMIA IN PREGNANCY**

In addition to preventing severe anaemia, it is important that there is improved detection and treatment. Currently, accurate diagnosis of anaemia depends on blood testing. Many health facilities are unable to perform blood testing on all women attending for antenatal care. It is therefore important to develop an instrument that is non-invasive and relatively low cost that can detect women at risk of severe anaemia. Many health facilities rely on pallor testing for diagnosing anaemia. However, there are very few published studies in which the sensitivity and specificity of this have been investigated in large numbers of pregnant women in a developing country setting. In addition, there has been little work
done to investigate the possible use of other non-invasive screening tools, based on, for example, a combination of history and examination.

**CONCLUSIONS**

Prior to the start of this work, there remained fundamental inadequacies in our understanding of severe anaemia and malaria in pregnancy. These related to the following main areas:

1) The importance of malaria as a cause of severe anaemia in pregnancy in women of different gravidities.
2) The identification of effective ways of preventing severe anaemia secondary to malaria in pregnancy.
3) The inter-relationship between maternal malaria, severe anaemia and birth weight.
4) The contribution of severe anaemia to poor health.
5) Ways to diagnose of severe anaemia in pregnancy in areas where blood testing was not available.

**STUDY OBJECTIVES**

The studies described in this thesis aimed to:

1) identify / confirm whether malaria is an important cause of severe anaemia in pregnancy in women attending for antenatal care at Kilifi District Hospital,
2) measure in primigravidae the impact of the use of insecticide treated bed-nets (ITBN) on
   a) prevalence of severe anaemia in the third trimester of pregnancy
   b) prevalence of peripheral parasitaemia in the third trimester of pregnancy
   c) prevalence of placental malaria infection
3) measure in primigravidae the impact of between one and three doses of sulfadoxine-pyrimethamine compared with placebo, in a population with ITBN and a population without ITBN on:
   a) prevalence of severe anaemia in the third trimester of pregnancy
   b) prevalence of peripheral parasitaemia in the third trimester of pregnancy
   c) prevalence of placental malaria infection
4) determine the contribution of placental malaria to severe maternal anaemia and low birth weight across all gravidities in order to evaluate the potential benefits of routine preventive strategies for malaria in pregnancy,
5) investigate the interrelationships between maternal haemoglobin, birth weight, placental malaria infection in primigravidae and multigravidae,
6) describe the consequences of severe maternal anaemia in terms of morbidity and women’s quality of life, enabling other potential benefits of preventing malaria and severe anaemia to be evaluated,
7) develop a non-invasive screening tool based on women’s history and basic examination that can be used in the absence of routine antenatal haemoglobin estimation, to diagnose women at high risk of being severely anaemic,
8) disseminate the findings of the intervention trials to those working in Reproductive Health and Safe Motherhood as well as those working in Malaria Control.
STUDY SITE
The studies were all conducted in Kilifi District, on the Kenyan Coast, situated between Mombasa and Malindi. This area of Kenya has perennial transmission of *Plasmodium falciparum* with two seasonal peaks in the principal vectors, *An. gambiae* and *An. funestus*, between June-August and during January, coinciding with the 2 rainy seasons. On average individuals can expect to receive 10 infective bites per person per annum\textsuperscript{46,47}.

The population of Kilifi district is made up of Mijikenda people of whom 90% are the Giriama\textsuperscript{48}.

All of the antenatal clinic based components of the study are conducted from Kilifi District hospital. This hospital has a busy antenatal clinic with an average of 900 women from a predominately rural population attending each month. In addition, 2 of the studies (the study of intermittent SP and the screening study for severe anaemia) also recruited women attending for care at Vipingo Health Centre, half way between Kilifi town and Mombasa. It is estimated that at least 90% of women attend for antenatal care at least once during their pregnancy in Kilifi district\textsuperscript{49}.

The standard antenatal care when the study was planned was to give women ferrous and folate supplements. Chloroquine prophylaxis for pregnant women had been stopped in the Province the year before this study commenced, as it was believed to be no longer effective.

KENYA MEDICAL RESEARCH INSTITUTE / WELLCOME-TRUST RESEARCH UNIT
There is a well-established research unit based at the site of Kilifi District Hospital, undertaking research on malaria under the scientific leadership of Professor Kevin Marsh. There was a large community randomised study assessing the impact of insecticide treated bednets on childhood severe morbidity and deaths, which commenced at the time this work was being proposed under the leadership of Professor Bob Snow. This presented an ideal opportunity for collaborating to look at the impact of ITBN on pregnancy outcome. Most other work taking place in the KEMRI unit at that time related to the management of severe malaria in children.

ETHICAL APPROVAL
All of the studies have ethical approval from both the London School of Hygiene and Tropical Medicine Ethics committee and the Kenya Medical Research Institute National ethical review committee.
THE THESIS IS DIVIDED INTO THE FOLLOWING CHAPTERS:

Chapter 1: provides background on the importance of malaria and severe anaemia in pregnancy, the preventable causes of severe anaemia and potential ways of preventing malaria in pregnancy. It also outlines the objectives of the study in Kilifi, Kenya.

Chapter 2: summarises the findings of a preliminary study on the aetiologies of anaemia in pregnancy in Kilifi Kenya. Malaria was found to be an important cause of severe anaemia in primigravidae.

Chapter 3: summarises the findings of a community randomised controlled trial of insecticide treated bednets on the prevention of malaria and severe anaemia among primigravidae women.

Chapter 4: summarises the findings of a double blind randomised placebo controlled trial of preventative intermittent treatment with sulfadoxine-pyrimethamine on the prevalence of severe anaemia and malaria in pregnancy.

Chapter 5: investigates the relationship between placental malaria, low birth weight (< 2500g) and maternal anaemia in women of all parities in order to determine whether multigravidae are likely to benefit from antimalarial interventions as well as primigravidae.

Chapter 6: describes the psychological, social and physical morbidity associated with severe anaemia in pregnancy.

Chapter 7: investigates and compares alternative non-invasive ways of screening for severe anaemia in pregnancy of potential use in areas where there are limited facilities for testing blood.

Chapter 8: summarises and discusses the findings and policy implications of the studies.
References


