
Summary

Since the British doctor Ronald Ross received the 1902 Nobel Prize in medicine for his work on malaria, more people have died from the disease than all world wars combined. This is in spite of the fact that the French chemists Pierre Joseph Pelletier and Joseph Bienaimé Caventou made quinine available from as early as 1820. According to the World Health Organisation (WHO), there were about 219 million cases of malaria in 2010 and an estimated 660,000 deaths majority of which (80% of cases and 90% of deaths) were in Africa.

Of almost two billion people who are anaemic globally, 41.8% are pregnant women. Iron supplements are used to prevent anaemia. There are concerns that iron given in high doses may increase malaria rates. Uncertainties regarding the safety of iron supplementation in malaria endemic regions were propelled by a randomized controlled trial that evaluated the effects of iron and folic acid supplementation in 32,155 children in Pemba, Tanzania. This study found that children who received iron and folic acid supplements were more likely to die or to need hospitalisation for an adverse event. At the same time, malaria is known to exacerbate anaemia; an almost inevitable consequence of malarial infection. As such, the safety of daily oral use of iron supplements by pregnant women, as a public health intervention is still not clearly established; at least not until publication of our main findings.

This thesis assessed the effects of iron supplementation on safety indicators, and on iron status, in pregnant women and their neonates. Several preparatory activities were carried out, including a census of the population in the study area and a pilot study to check the operability of the study protocols. A main study was designed with the hypothesis that consumption of food products fortified with iron combined with intake of iron supplements especially in pregnancy, would be detrimental to the health of pregnant women and their neonates.

The objectives of the study were: 1) to compare the presence of malarial infection in parturient women who received a combination of iron-fortified foods with iron supplements versus iron-fortified foods only; 2) to assess intervention effects on the maternal prevalence of iron deficiency anaemia at 1 month after delivery; 3) to assess intervention effects on neonatal iron stores at 1 month of age; 4) to assess the diagnostic utility of Zinc protoporphyrin (ZPP) in diagnosing iron deficiency in malaria endemic regions; 5) to identify baseline factors that are prognostic for the Non-Transferrin Bound Iron (NTBI) response to consumption of a single iron supplement; 6) to determine the factors that predict *Plasmodium* infection in pregnancy; 7) to identify factors associated with birth weight; 8) to develop a methodology to predict cases of low birth weight, using a single prognostic score that is based on prognostic variables collected at the second trimester of

pregnancy; and 9) to develop methods for community-based flour fortification with iron.

Most countries have enacted, or are in the process of enacting legislation for mandatory fortification of flour with iron. Thus pregnant women may receive iron from fortified foods and from universal iron supplementation programmes. This thesis provides answers to pertinent questions regarding the safety and efficacy of iron supplementation by comparing daily high-dose iron (i.e. iron-fortified foods plus iron supplements) versus low-dose iron (i.e. iron-fortified foods only) during pregnancy. The main outcome measure was the presence of maternal *Plasmodium* infection at birth, regardless of species. Chapter 1 is a detailed introduction of the background to the study and the design of the study.

This thesis presents concrete evidence that *iron supplementation to pregnant women in a highly malaria endemic region does not result in increased risk of malarial infection; percent difference (95%CI) = 0.0% (−9.3% to 9.3%)*. Programme implementers and governments in malaria endemic regions should not be held back by previous recommendations that cautioned against issuing iron supplements to pregnant women. In light of these findings, there is no need to first screen for malaria before giving iron supplements.

Iron supplementation had major benefits for mothers and their neonates (chapter 2). The findings reported in this thesis showed a mean increase in birth weight of 143 g relative to the low-dose iron group. The effects of iron were influenced by the participants initial iron status. Correction of iron deficiency increased birth weight by 249 g, even though we cannot exclude the possibility that this may have increased malarial infection by 10%. There was no evidence that effects of iron on birth weight were influenced by intermittent preventive treatment against malaria. Iron supplementation also increased fetal growth by 0.27 SD, 95%CI: (0.04 to 0.50) probably as a result of gains in length and weight for gestation age.

We also showed improved neonatal iron stores one month post-partum as indicated by a 17.1 % (95% CI: 2.0% to 34.3%) increase in plasma ferritin concentration in neonates of mothers who received high-dose iron compared to those who received low-dose iron. This provides more impetus to the need to offer iron supplements to pregnant women with the aim of boosting infant iron stores (chapter 2).

ZPP was found to be of unreliable diagnostic utility when discriminating between pregnant women with and without iron deficiency in regions where chronic diseases are prevalent (chapter 3). The current conventional cut off points for whole blood ZPP e.g. >70 $\mu\text{mol/mol}$ heme, can result in gross estimates of the prevalence of iron deficiency especially if the true prevalence is low.

The appearance of non-transferrin bound iron (NTBI) in circulation after oral ingestion of iron supplements has been thought to aid the growth and multiplication of *Plasmodium*

parasites thereby increasing malaria induced morbidity and mortality. We did not observe any increase in NTBI concentrations three hours after oral ingestion of 60 mg of ferrous fumarate (Chapter 4). We cannot exclude the possibility that iron supplementation leads to NTBI production when supplements are not consumed with food, because the lunch meal consumed by majority of our participants during the 3-hour waiting period probably contained natural compounds (phytates) that may have limited an NTBI response.

In chapter 5 of this thesis, we aimed to develop a field friendly tool that can be used to predict asymptomatic *Plasmodium* infection. This was motivated by the fact that most point-of-care dipstick tests used to detect *Plasmodium* infection are not able to detect 100% of all the infection present yet asymptomatic infections are increasingly associated with adverse maternal and neonatal outcomes. Although many likely predictive factors were assessed individually or in combination with others, we did not succeed in developing a reliable tool that is easy to apply in resource-poor malaria endemic settings.

In Chapter 6, we aimed to identify factors associated with birth weight and to develop a methodology to predict cases of low birth weight using a single prognostic score that is based on prognostic variables collected at the second trimester of pregnancy. Factors that were found to be independently associated with reduced birth weight were being never married, inflammation, being a girl, and iron deficiency. Being overweight was associated with increased birth weight. The results indicate that we can use variables collected rapidly and at relatively low cost and ease to identify with fair accuracy women in the second trimester of pregnancy who are at high risk of giving birth to a neonate with low birth weight.

The various aspects of the work presented in this thesis including the implications for policy makers are discussed in chapter 7. For policy makers, the findings of this thesis are a welcome relief. The findings therein eliminate all doubt that has hitherto been associated with antenatal iron supplementation in malaria endemic areas. Most countries already have iron supplementation policies that are well aligned to the World Health Organisation policies. Efforts to widen the coverage of antenatal iron supplementation especially in malaria endemic regions should be urgently scaled up. However, the evidence provided in this thesis is only applicable to pregnant women and cannot be extrapolated to children in malaria endemic regions. For this population, the current WHO policy must be used thus before iron supplementation, children must first be screened for malaria.

Although this thesis provides answers to key scientific questions that have hitherto baffled the scientific community, there are still research questions that can be clarified further. The effects of a high iron dose in pregnancy (as per national and international guidelines, the daily supplementation dose for pregnant women should be doubled to 120 mg iron if they are anaemic or if 6 months duration cannot be achieved in pregnancy (Chapter 2)) on maternal and neonatal outcomes need to be elucidated. Further research is needed in order to describe fully, the NTBI response to consumption different types and amounts of oral

iron supplements. There is urgent need for diagnostic tools that can be used in resource-poor settings to diagnose asymptomatic infections. Further research in children is needed to provide evidence of the safety and efficacy of iron supplementation in malaria endemic regions and to assess the diagnostic performance of zinc protoporphyrin in children. In addition, since our studies suggest that screening based on Hb concentration as now practiced in many countries, is inaccurate in discriminating between women at high and low risk of delivering neonates with low birth weight, further studies are needed to identify appropriate markers and cut-off points that are suited to this purpose.

In conclusion, this thesis has shown that there is no evidence that antenatal iron supplementation increases *Plasmodium* infection. Antenatal iron supplementation leads to large improvements in birth weight, fetal growth and infant iron stores, with potentially immense benefits for infant survival and health that should outweigh any possible concerns about risks of malaria. Epidemiological calculations indicate that if our results are applied to all women in developing countries in order to eliminate iron deficiency, we could avoid 3 million births with low birth weight annually and save the lives of more than half a million neonates. Scaling up universal iron supplementation in pregnancy in developing countries will generate major public health gains.