

IRON SUPPLEMENTATION AND MALARIA

A Randomized Placebo-Controlled Field Trial

On Women and Children in Rural Ethiopia

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**A Randomised, Placebo-controlled Field Trial
in Rural Ethiopia**

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Summary

The health situation in Sub-Saharan Africa is complex and multifactorial. Along with its poorly developed economy and lack of basic health services, vector-borne diseases and nutritional disorders are major contributors to the high morbidity and mortality seen today. Malaria and anaemia due to iron deficiency and/or to infectious diseases are rampant in the area affecting its population in general women and children in particular. Although the problem of iron deficiency and anaemia was dealt with varying strategies in different places iron supplementation is widely used in many developing countries and has become a routine procedure in maternal and child health programmes. In recent years, however, there has been a growing concern regarding this approach due to the emergence of conflicting evidence on the health outcome of iron supplementation. In addition to the beneficial effect of iron supplementation, a possible adverse effect on health, particularly on the risk to malaria, has been suggested. The continued controversy has raised doubts as to whether to implement iron supplementation programmes in malaria endemic areas.

A randomised, placebo-controlled field trial was conducted in a malaria endemic area in the north-western part of Ethiopia in May 1993-October 1995 to determine the haematological response to oral iron supplementation and measure the risk of malarial illness associated with iron. The study involved 776 women and 841 children with low haemoglobin (HB) level who were randomly allocated to receive oral iron or a look alike placebo for a period of 12 weeks.

The results of this study showed that anaemia is common in the study population with prevalences of 72.3% and 84.6% among women and children respectively. The content of the staple diet in the area was generally iron insufficient. The dietary iron insufficiency was further realised to be both due to inadequate intake and poor absorption of the iron ingested. The supplementation for 12 weeks was completed by 729 (93.9%) women and 740 (88%) children in the study. After supplementation more women (82.6%) and children (93.5%) in the iron group showed an improvement in their HB compared with those women (54.6%) and children (43.7%) in the placebo respectively. The mean HB rise was also significantly higher in women and children in the iron than in the placebo group, 1.43 vs 0.28 g/dL, ($t=12.6$; $p<0.0001$) for women and 1.38 vs 0.22 g/dL ($t=21.3$; $p<0.0001$) for children respectively. After supplementation women and children with severe anaemia were fewer in the iron (5.1% and 0.3%; $\chi^2=17.2$; $p<0.001$) than in the placebo group (13.5% and 8.0%; $\chi^2=27.9$; $p<0.001$). After supplementation more women (9.6%) and children (18.2%) in the iron group reached the HB cut-off point showing no anaemia than women (1.4%) and children (9.6%) who received placebo ($\chi^2=23.9$; $p<0.001$ and $\chi^2=34.3$; $p<0.001$ for women and children respectively).

Post-supplementation prevalences of clinical malaria among women in the iron and placebo groups were 24.2% and 18.3% respectively (RR=1.3, CI, 1.0-1.8). The parasite rate was also higher in the iron supplemented women than those in the placebo, 29.4% and 20.2% respectively (RR=1.47, CI, 1.4-1.9). During supplementation, women in the iron group experienced more frequent episodes of fever and spent more days with fever than women in the placebo group, (RR=1.16, CI, 1.03-1.30). Similarly, clinical malaria was diagnosed in 19.7% and 13.2% of children in the iron and placebo

treatment groups respectively, (RR=1.49, CI, 1.07-2.08). Splenomegaly in children was detected in 27.3% of those in the iron 19.1% of those in the placebo, (RR=1.43, CI, 1.1-1.9). The parasite rates for iron and placebo supplemented children were 34.5% and 27.1% respectively, (RR=1.28, CI, 1.0-1.6). Febrile episodes were experienced by 68.9% and 58.9% of children in the iron and placebo groups respectively, (RR=1.17; CI, 1.05-1.30).

The results indicated not only that the dietary iron intake of the population was inadequate but also was poorly bioavailable due to lack of foods which enhance absorption and due to a high intake of food substances which interfere absorption all of which may have contributed to the anaemia. Study women and children who received iron supplementation have shown a substantial haematological response (HB rise) and significantly improved their anaemia. The study has also demonstrated that there is a considerable risk of uncomplicated malaria associated with oral iron supplementation as malarionometric indices used in this study were all significantly higher among women and children in the iron than in the placebo group.

Intervention programmes involving iron supplementation in malaria endemic areas in the future need to weigh the health benefits and the malarial risk attributable to iron. In the present study area, where nearly all women and children suffer from anaemia and its consequences on health, the haematological response to iron supplementation outweighs the associated drawbacks. In such areas oral iron supplementation should therefore continue to be used to prevent and control iron deficiency and anaemia. Public health activities pertaining to the control and prevention of malaria and/or anaemia should, however, protect the population most at risk from malaria when implementing iron supplementation during the malaria transmission seasons.

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Chapter One

A. BACKGROUND

1.1. INTRODUCTION

The poor quality of life in terms of high morbidity and mortality seen today in the developing world, particularly in Sub-Saharan Africa, is increasingly unacceptable. Causes are multifactorial and complex. Along with prevailing under-development and poverty, vector-borne diseases, nutritional disorders and lack of basic health services are major contributors to this situation.

Vector-borne and other endemic tropical diseases are rampant, malaria being the most important. It is estimated that about 2200 million people in the world are exposed to malaria infection and that about 300-500 million clinical cases occur each year. In Africa alone, over 275 million people are infected with a total of over 100 million clinical cases annually. In malaria endemic areas, about 10% of deaths of infants and children below 14 years of age is attributed to malaria. Women and children are the groups most at risk of developing and dying from malaria.

Besides malaria, women and children are at risk of many other health problems, of which deficiency of nutrients, including iron, is one of the most important. Anaemia in women and children can result from various causes, but nutritional deficiency and infectious diseases are the most important. Apart from dealing with underlying causes, combating iron deficiency and anaemia requires supplementation with elemental iron and food fortification; the former approach is a relatively feasible strategy in developing countries.

It has been asserted that iron deficiency and anaemia impairs the optimal functioning of the immune system thus predisposing the individual to an increased risk of infections including malaria but this view has been questioned in recent years by new evidence to the contrary. Reports of an increased risk of malaria and other infections in association with iron supplementation continue to appear. As a result, the iron-malaria controversy continues to divide the scientific community and presumably, affects the implementation of public health interventions against iron deficiency and anaemia. The issue of whether iron supplementation is beneficial or harmful in areas of high malaria endemicity needs to be re-examined. It is particularly important in countries where malaria is endemic, anaemia (nutritional and/or infectious) prevalent and where strategies to control and prevent anaemia rely mainly on oral iron supplementation.

The present investigation was conducted in a rural community in north-western Ethiopia to investigate the malarial risk among women and children given supplementation with oral iron.

The design of the study was a randomised, placebo controlled, double-blind field trial. Women and children with anaemia were allocated randomly into oral iron or placebo supplementation groups and followed for a total of 12 weeks in order to monitor their malaria experience. In the study area, transmission of malaria is seasonal and follows the short and long rains, during March/April and June-September respectively. The supplementation trial was conducted during the rainy season of July-October 1994.

This study was conducted to provide information on the possible antagonism between iron and malaria and to contribute to the continued effort to narrow the division in opinion on this subject.

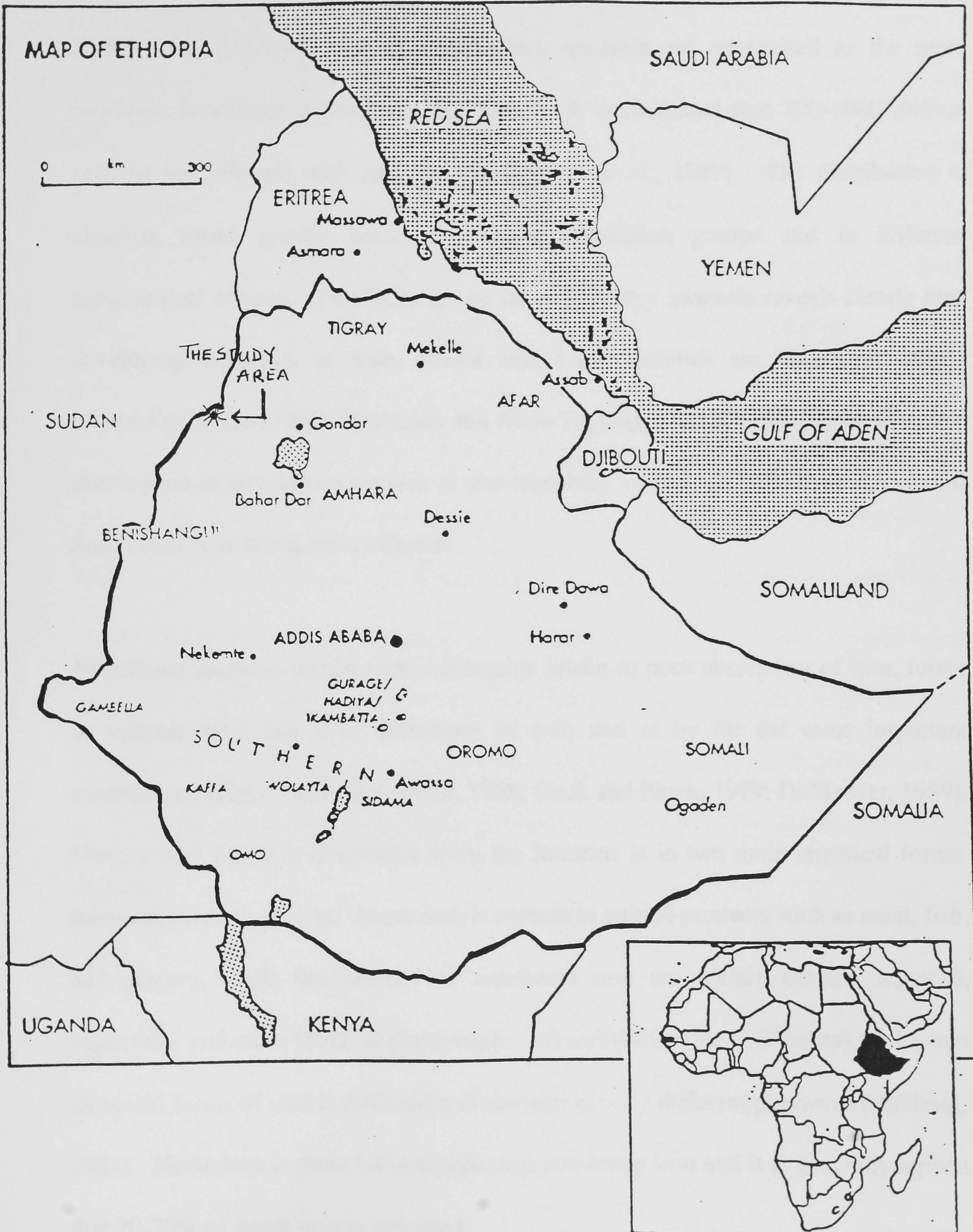
1.2. BACKGROUND INFORMATION ON ETHIOPIA

Ethiopia is one of the oldest nations in Africa, with a history and culture identified with ancient civilisation and with contrasting chapters of prosperity and poverty, war and peace, drought and famine. Ethiopia is situated in the north-eastern part of Africa (known as the Horn) extending between latitudes of 3-18° north and longitudes of 33-48° east. The country shares international borders with the Sudan in the west, Kenya, Somalia and Djibouti in the south and east, and since very recently, with Eritrea in the north (Figure 1). The massive high plateau in the central and northern parts of the country is surrounded by the hot semi-arid lowlands in the west, east, and south and is bisected by the Rift Valley. It is estimated that about 50% of the land above 2000 m in Africa is in Ethiopia (Kloos, 1993). The varied topography and landscape of the country provides a mixture of tropical and temperate climatic zones which is of immense importance to the pattern of disease in different areas.

Numerous rivers originate from the highlands and flow outwards in all directions through deeply incised valleys and gorges. The river basins, lakes and other water bodies in the country are of enormous agro-economic and public health importance. Ethiopia has an area of 1.25 million km² and is the third most populated country in Africa with a population of 51 million (projected from the 1984 census. CSA,1990).

Although a few ethnic groups (the Amhara, Oromo, Gurage and Tigre) form the great majority of the population, the country is home to nearly a hundred other minority groups. The population is largely rural and agriculture is the basis of the economy. In spite of the vast geographical area of the country, population settlement is very uneven with densely populated areas in the central highlands and sparsely populated regions in the lowlands.

Figure 1. Map of Ethiopia



1.3. IRON DEFICIENCY AND ANAEMIA

Today, iron deficiency and iron deficiency anaemia are recognised as the most prevalent nutritional problems in the world. It is estimated that 700-1000 million persons are affected with anaemia (DeMaeyer *et al.*, 1989). The distribution of anaemia varies greatly between different population groups and in different geographical regions. The literature on iron deficiency anaemia reveals clearly that developing countries in Asia, Africa, and Latin America are disproportionately affected (Royston, 1982; DeMaeyer and Aiels-Tegman, 1985; DeMaeyer, 1989). The distribution in developing regions is also markedly variable, sub-Saharan Africa and South East Asia being most affected.

Nutritional anaemia results from inadequate intake or poor absorption of iron, folate or vitamin B12, but it is deficiency of iron that is by far the most important contributor (WHO, 1959; Hofvander, 1968; Cook and Finch, 1979; DeMaeyer, 1989). Dietary iron which is absorbable from the intestine is in two main chemical forms, *haem* and *non-haem* iron. *Haem* iron is present in animal products such as meat, fish, and poultry, while the sources of *non-haem* iron are mainly cereals, legumes, vegetables and other foods of plant origin. Absorbability (bioavailability) of the two chemical forms of iron is different and absorption is by different pathways (Hallberg, 1981). *Haem* iron is more bioavailable than non-haem iron and it is generally agreed that 20-30% of *haem* iron is absorbed.

In contrast, as little as 1-8% of *non-haem* iron in plant diets, even those rich in iron content is absorbable (Monsen *et al.*, 1978; Hallberg, 1981). In most rural parts of developing countries, green leafy vegetables, legumes and cereals form the main part of the diet. Such diets contain insufficient iron and consequently absorption of dietary iron is very low. A cereal and legume-based diet also contains substances capable of chelating dietary iron and diminishes its bioavailability. These substances such as phytic (phytates) and phosphoric acids which interfere with the bioavailability of minerals are sometimes referred to as antinutrient substances (WHO, 1959; Hofvander, 1968; Hallberg, 1981). In many cases, African children and women whose cereal diets contain these inhibiting substances are victims of iron deficiency anaemia not only due to their high physiological demand but also due to inadequate intake and poor absorption of the dietary iron available. Under such circumstances in developing countries, iron deficiency can start early in life because of the adoption of plant-based diets immediately after weaning, although iron in breast milk is highly bioavailable and provides considerable protection during infancy.

Anaemia secondary to infectious diseases is also prevalent in most areas in the tropics and lowland areas. Malaria contributes to anaemia in endemic areas. Another common parasitic disease which causes blood loss and anaemia is hookworm infection which generally shares a geographic distribution with malaria.

There is an inverse relationship between hookworm infections and altitude, with a higher prevalence of hookworm infection in lowlands with an altitude of <2000 m. below sea level (Jemaneh and Tedla, 1984; Tedla and Jemaneh, 1985). There is an overlap in the geographical distribution of malaria and hookworm which together may lead to severe anaemia.

Many studies have shown that iron deficiency and anaemia are more prevalent among women of reproductive age and children, due to the extra physiological demand for elemental iron in these subjects. A world wide review (DeMayer and Adiels-Tegman, 1985) showed that, on average, the proportion of non-pregnant women with anaemia in the world is 40% against a figure of 15% among men. This global review documents that the prevalence of anaemia among women and men is 37% and 27% in Africa, 23% and 11% in Latin America, and 47% and 17% in Southern Asia. Even in the developed world, the distribution pattern between women and adult males remains similar (14% and 2% anaemic respectively). During their reproductive period women experience a regular cycle of menstrual blood loss, an average of 40 ml per period, (Jacobs and Butler, 1965; Hallberg *et al.*, 1966). This suggests that a normal menstruating woman needs on average an extra 0.6-0.8 mg per day of iron which is about a 75% increase over the usual daily requirement of 1 mg. An even higher intake is needed in about 10% of women who have an abnormally high loss of blood at menstruation.

Contraceptive use can influence menstrual blood loss. Hormonal preparations (pills) are known to decrease blood loss by 50% (Guilleband *et al.*, 1976) and their use can reduce the risk of iron deficiency anaemia. On the other hand, intra-uterine devices increase average blood loss by 35%-150% (Guilleband *et al.*, 1976). However, contraceptive usage in areas where iron deficiency is likely is very low. For example, contraceptive usage by women in Ethiopia is less than 2-5% (MOH unpublished document, 1989).

During pregnancy, a woman's demand for iron increases greatly due to foetal and placental development. Overall the demand for iron during and a little after pregnancy increases by an extra 35-70% with a dramatical increase during the last trimester when iron requirements are more than six times greater than the requirement of a non-pregnant women of the same age (Royston, 1982; INACG, 1981). The global prevalence of anaemia among women during pregnancy was found to be 51.0%, ranging from 3-100% and prevalences in South Asia and Africa were 65% and 63% respectively (DeMayer and Adiels-Tegman, 1985). Following blood loss during birth, lactating women continue to require additional iron to replace this loss and to compensate for losses to the infant through breast milk. The prevalence of anaemia among lactating women, although lower than that found in menstruating women, is still higher than that found in adult men or in non-lactating and non-pregnant women.

1.3.1. Iron deficiency and anaemia in Ethiopia

In Ethiopia, information on anaemia is generally lacking. The available data are from either scanty focal prevalence studies or from data collected in health facilities. An extensive description on dietary intake of highland Ethiopians (Hofvander, 1968) indicated that in Ethiopia, dietary iron intake per person was among the highest in the world, largely due to consumption of the iron rich cereal *Eragrostis Abyssinica*, locally known as *teff*, which is a staple food in most parts of the country. Hofvander estimated the average iron intake to be 433 (98-1400) mg per day, which is very much higher than the recommended daily allowance of 15-30 mg per day (WHO, 1959). As mentioned earlier, absorption of iron depends on factors which influence its bioavailability. Bioavailability (absorbability) of *non-haem* iron from plant sources is estimated to be about 2-8% of the total ingested (Monsen *et al.*, 1978), so only 9-35 mg of the estimated 433 mg dietary iron ingested every day by the *teff* consuming population of Ethiopia is likely to be absorbed. This estimate of iron absorption suggests that the average Ethiopian's dietary iron intake is adequate, or even in excess of the recommended daily allowance and sufficient to prevent and control iron deficiency and nutritional anaemia. Thus, it would be anticipated that iron deficiency and anaemia would be unlikely to be a problem of public health concern in areas where *teff* is a dietary staple.

The very low prevalence of anaemia found in the highland study undertaken by Hofvander (1968) was attributed to the consumption of iron rich grain and other investigators have supported this view (Zewdie *et al.*, 1993). On the other hand, investigations on the biochemical content of *teff* (Besrat *et al.*, 1980) indicated that the high iron content claimed for this particular grain derives much from soil contamination during traditional threshing rather than from the seed itself and highlights the overemphasis given to this grain in relation to iron sufficiency in *teff* consuming areas. Studies on the prevalence of anaemia in the north-western part of the country, where *teff* is a staple diet (Zein and Mekonen, 1987; Zein, 1991), have shown a higher prevalence of iron deficiency anaemia in the lowland compared with highland regions, and have found a prevalence of 40.0% in areas where *teff* is a staple diet, suggesting that consumption of this grain alone is not adequate to prevent the population from developing anaemia.

In the lowland parts of Ethiopia such as the present study area, other cereals such as millet and sorghum are grown widely and form the major source of dietary iron; production and consumption of *teff* is on only a small scale and *teff* is grown mostly as a cash crop. The problem of iron deficiency and anaemia is more widely distributed in the lowlands, compared with the highlands, due to additional causes such as malaria and hookworm infections which are more prevalent in the lowlands.

In some parts of the Ethiopian lowlands, the magnitude of anaemia and its overall prevalence, adjusted for altitude, was estimated by different investigators to be between 55.2% and 59.0% (Hofvander, 1968; Zein and Mekonen, 1987).

Further data on anaemia in Ethiopia are provided by reports from health institutions. In a recent report from the Ministry of Health (MOH, 1991), anaemia was shown to be the 14th most important cause of out patient visits to health institutions at all levels and it was the 11th most important cause of hospital deaths. It is apparent that hospital statistics reflect only the tip of the iceberg of anaemia, particularly of severe anaemia, as there are only limited health facilities where anaemic patients can obtain treatment. In addition, most patients with mild to moderate anaemia, who feel generally healthy and fit enough to engage in their routine daily work do not necessarily seek medical help and are unlikely to be detected by health institutions.

1.3.2. Consequences of iron deficiency and anaemia for health

The effect of anaemia on health is dependent on its magnitude but is also related to the underlying cause. Iron deficiency anaemia and its ill effects on health have been documented by many researchers. Infants and children with iron deficiency and low levels of haemoglobin suffer from impaired growth and have retarded development.

Young children with anaemia have poor motor co-ordination, delayed language development and impaired scholastic achievement (Soemantri *et al.*, 1985; Pollitt *et al.*, 1986; Pollitt *et al.*, 1989).

Undesired consequences of anaemia in women, particularly during their reproductive age, are well identified. Major health problems which affect women include an increased risk of maternal mortality and morbidity, foetal morbidity and mortality and an increased risk of low-birth weight (Oppenheimer and Hendrickse, 1983; Brabin *et al.*, 1990). Maternal morbidity is believed to increase when the haemoglobin level falls below 7.0 g/dL and when it falls below 4.9 g/dL cardiac failure which is often fatal may develop (Oppenheimer and Hendrickse, 1983). Foetal mortality is also increased among women when maternal haemoglobin falls below <7 g/dL and in cases of untreated severe anaemia during pregnancy up to 30% of infants may die of hypoxia (Fleming, 1989). The risk of low birth weight was found to be significantly increased in primigravidae whose haemoglobin level was below 8.0 g/dL (Brabin *et al.*, 1990).

In anaemia, there is an impairment of the delivery of oxygen to tissues for which the human body compensates through increased ventilation and cardiac output. However, the compensatory mechanism can only be effective up to a certain degree and cannot overcome the deficit completely.

Adult populations of either sex with a low haemoglobin have a decreased capacity for physical work and experience fatigue more easily than people with normal haemoglobin levels. A positive correlation between maximum physical work and haemoglobin level has been shown, and work capacity increased by 83% after patients with severe anaemia were transfused (Edgerton *et al.*, 1979; Edgerton *et al.*, 1981). In the developing world, the way of life (manual labour in the field and walking long distances) relies very much on physical fitness. Impaired work performance of adults has a major effect on the health of the individual and on the well-being of the family and the community at large.

1.3.3 Treatment, control, and prevention of iron deficiency and anaemia

The treatment of choice for confirmed or suspected cases of iron deficiency anaemia, is based on iron repletion, mainly in the form of medicinal iron. While individual patients are handled in this way, the problem of anaemia in the community is much more difficult to address due to the wide spectrum of iron deficiency and anaemia and also due to lack of health facilities for proper diagnosis and treatment. Under these circumstances alternative approaches which are feasible and cost effective are needed. These are usually based on either fortification of certain food items with iron or supplementation of populations most at risk with medicinal iron.

Many attempts to implement food fortification or to administer supplementation with elemental iron have been made. Iron supplementation has often been tried along with other primary health care programmes to control iron deficiency and anaemia in developing countries. Nowadays supplementation of pregnant women and infants with low birth weight with iron is often carried out through maternal and child health programmes and is promoted by international agencies, such as the World Health Organisation (WHO) and the United Nations Children's Fund (UNICEF).

Prevention of iron deficiency anaemia in poor countries has not been easy and may be impossible to achieve under existing circumstances. In the developed world, the major causes of anaemia, including malaria, schistosomiasis and hookworm, have either been eradicated or controlled and as a result, the problem of anaemia, nutritional and/or infectious, has fallen sharply. In addition, because the life style and eating habits in the developed world allow fortification of food with iron, the overall improvement of the dietary intake of both *haem* and *non-haem* iron, has been successfully implemented and this has contributed to the low level of iron deficiency seen in these areas.

The success in the developed world will be difficult to attain in the developing world. Firstly, it is nearly impossible to eradicate the nutritional deficiency and infectious diseases which are major causes of anaemia. Secondly, conditions are less favourable to food fortification programmes in developing countries and these may be impractical. Finally, due to various socio-economic and cultural reasons, it is very difficult to improve and/or modify the dietary habits of rural communities towards an improved and balanced dietary iron consumption. As the problems associated with iron deficiency and anaemia do not generally lend themselves to easy solutions, it seems that poorer countries are left with the limited option of relying on supplementation of iron to prevent iron deficiency and to control anaemia.

1.4. MALARIA IN ETHIOPIA

As the health information system in Ethiopia is inadequate, accurate and comprehensive morbidity and mortality data, including that relating to malaria, is lacking. Over 60% of the population inhabit areas below an altitude of 2000 metres above sea level (CSA, 1990), the altitude previously believed to be the level above which malaria was no longer a health threat. However, the distribution of the overall population of Ethiopia indicates that two-thirds inhabit areas where malarial transmission exists. It is presumed that fear of malaria has prevented population movement into the more fertile, but malarious, areas and has resulted in the dense settlement found in many of the malaria-free highlands of the country.

In recent years, however, the demographic and settlement pattern has changed due to the over-cultivation and soil erosion in the densely populated highlands, and by forced re-settlement programmes instituted by the government.

Malaria is accepted as a major public health problem in Ethiopia. It remains the single most important communicable disease with a considerable impact on the nation's health, economy and development. It is only in this century that useful information on the epidemiology of malaria in Ethiopia became available, in part as a result of the work of Italian and British investigators, but the overall pattern of malaria in the country is now more or less known. Pilot control projects were under way when, in 1958, a large scale epidemic hit most of the country resulting in three million cases and 150,000 deaths (Fontaine, 1961). Since then, cyclic waves of epidemics and outbreaks have been noted every few years (Negussie, 1988). Following the devastating epidemic in 1958, malaria control in Ethiopia has gone through various stages of organisational development. An eradication programme established in 1959 gave way to a well organised, national control programme operating more or less vertically within the Ministry of Health. This has recently been restructured and decentralised into regions, reducing the national control programme to a core expert team in the Ministry.

Assessments of malaria morbidity and evaluation of control activities in selected parts of the country indicated that malaria is a major public health problem in Ethiopia. Even though country wide information is unavailable, the magnitude of the malaria problem is reflected in an annual parasite index of 41.7 per thousand and a disease incidence of 21 per thousand population in 1989 (Negussie, 1988; Tulu, 1989). Hospital and health centre data for 1989 (MOH, 1991) showed that malaria was the eighth leading cause of outpatient visits in health facilities at all levels and that it was the sixth most important cause of hospitalisation. Nearly 5% of all hospital deaths were attributed to malaria.

During the past few decades, frequent outbreaks of malaria have been experienced in lowland, urban centres and rural areas at an altitude above 2000 m above sea level, the previous geographic barrier. Changes of climate in terms of rainfall, humidity, and temperature and the varied topography of the country have favoured the present disease pattern (Tulu, 1996). The social and political instability of the past two decades, particularly the unpopular mass resettlement programmes in which hundreds of thousands of highlanders were forcibly mobilised to malarious lowlands resulted in epidemics and high morbidity and mortality from malaria. Cyclic famine and drought in the country have also led to population displacements and these large scale migrations have also contributed substantially to the change in the epidemiology of malaria.

Development activities with the construction of irrigation schemes and state farm programmes, which demanded migration of labour from the malaria free highlands to endemic areas, are also believed to have influenced host resistance and vector behaviour in favour of malaria.

Earlier studies on vectors of malaria in Ethiopia started by Italian and British malariologists have been continued by the National Control Programme. So far, forty-two Anopheline species have been identified (Negussie, 1988), of which only four, *An. gambiae, s.l.*, *An. funestus*, *An. pharoensis*, and *An. nili* are of public health importance. Because of its wide distribution in the country, *An. gambiae s.l.* is the most important vector. Among the species in the *An. gambiae* complex, *An. arabiensis* is the most important vector due to its breeding capacity in temporary small water collections around human dwellings and its resting and feeding characteristics (Tulu, 1989).

The four species of plasmodium causing human malaria are known to exist in Ethiopia. However, the distribution and importance of each species varies within the different geographical regions of the country.

Overall, *Plasmodium falciparum* and *P. vivax* are the two most important species in Ethiopia comprising about 60% and 40% of all infections respectively (Tulu, 1989). Although *P. ovale* is rarely reported, *P. malariae* is frequently reported in the southwestern tip of the country and both are reported in < 1% of all malaria cases. *P. falciparum* is widely distributed in much of the country being responsible for the malarial epidemics which result in many cases of severe and complicated malaria. *P. vivax* is the second most important species in terms of overall malarial morbidity, but it rarely causes epidemics or death from malaria.

The malarial situation in Ethiopia has been aggravated by the emergence of *P. falciparum* resistant to chloroquine. Until recently, the problem of drug resistant malaria was observed only in the peripheral lowland areas bordering Kenya, Somalia and the Sudan, (Teklehaimanot, 1986). However, recent reports of chloroquine treatment failures in different parts of the country suggest that the problem is progressively moving inland (Alene and Bennet, 1996; Tulu, 1996).

Host factors contributing to malaria in Ethiopia have not been well studied. As malaria is by and large seasonal and unstable, all population groups living in endemic areas are potentially at risk irrespective of their age, sex or ethnicity. However, it is well known that women and young children are the groups most affected from malarial morbidity and mortality compared with other population groups in areas

with moderate or high levels of transmission. In a study in the Gambia, cell mediated immunity against malaria among pregnant women, particularly among primigravidae, was shown to be suppressed (Riley *et al.*, 1989). Pregnant women in Kenya have shown an increased prevalence of clinical malaria, with a higher parasite rate and parasite density, which was more marked among primigravid women than multigravida women (Vleugels *et al.*, 1989). Complications of malaria such as anaemia, low birth weight, and an increased risk of abortion and stillbirth have all been documented among pregnant women (Brabin *et al.*, 1990).

Other host factors which influence susceptibility to malaria include genetic polymorphisms. Studies have pointed out that the pattern of malarial illness varies within various population groups exposed to the same malarial endemicity and similar environmental factors. Greenwood (1989) pointed out that both genetic and environmental factors are likely to contribute to variations in the prevalence of malaria between neighbouring villages and within different parts of the same village. Similarly, in a study in Papua New Guinea, Brabin (1988) identified a high spleen rate in a subpopulation of women who lived under the same conditions of malaria endemicity and suggested that genetic factors could have played a major role in explaining the differences observed. Ethiopia is home to many ethnic populations, and genetic variations of interest to health and disease are expected to exist.

However, genetic factors related to the epidemiology of malaria and other diseases in the country have been little studied. A study done in the south-western parts of the country described the presence of considerable resistance to *P. vivax* infection among people of Nilotic origin when compared to Hamito-Semites. As measured by slide positivity, prevalences of *P. vivax* infections in populations of Nilotic origin and among Hamito-semitcs was 0.7% and 4.6% respectively (Armstrong, 1978). Further investigations in the same area and people revealed that people of Nilotic origin lack the *Duffy*-antigen with positivity for this antigen of 8.0% compared with 70.0% among the Hamito-Semitic group. The prevalence of *P. vivax* among the Nilotics was 2.4% compared with 27.3% among the Hamito-Semites respectively (Mathews & Armstrong, 1981). Other genetic factors relevant to malaria susceptibility are deficiency of glucose-6-phosphate dehydrogenase (G-6-P-D) and presence of sickle cell trait (Hb S). In an earlier survey, Perine and TesfaMichael (1974) showed that both conditions were absent in Ethiopia except in a small tribe in the south western part of the country where they found two cases of G-6-P-D deficiency but no carrier of the sickle cell gene. In view of the vastness of the country and the diversity of the population it is impossible to make any firm conclusions about the likely role of genetic factors in predisposing to malaria in Ethiopia.

1.5. MALARIA AND ANAEMIA

Anaemia is a common presenting feature of malaria which is sometimes life threatening, depending on the severity and duration of the infection and the intensity of parasitaemia (Weatherall, 1988, WHO, 1990a). Even though the mechanism by which malaria causes anaemia is not well defined, several studies have shown that malaria is an important cause of anaemia. Chronic anaemia may result from dyserythropoetic changes in the bone marrow due to repeated attacks of uncomplicated malaria while in patients with a previously normal haemoglobin, severe falciparum malaria can cause the rapid development of anaemia as a result of massive haemolysis (McGregor *et al.*, 1956; Abdalla *et al.*, 1980). It has been suggested that the pathogenesis of malarial anaemia is the result of destruction of parasitised red cells and enhanced erythrophagocytosis of normal red cells (Gilles *et al.*, 1969; McGregor *et al.*, 1966; Greenwood, 1987a; Abdalla *et al.*, 1980; Royston, 1982).

As the level of malaria parasitaemia is usually higher in young children and women during pregnancy, malarial anaemia is also more common and severe in these population groups, (Abdalla *et al.*, 1980).

Anaemia during pregnancy is multifactorial and varies in cause with geographical locations, but malaria is often the dominant cause in malaria endemic areas (Brabin, 1983; Fleming, 1989; WHO, 1990b). In areas where both malaria and anaemia are prevalent, treatment and chemoprophylaxis against malaria has usually been observed to improve the haemoglobin level and to reduce the incidence of anaemia. Children in Nigeria and pregnant women in Papua New Guinea who were protected with antimalarial chemoprophylaxis showed a higher mean haemoglobin level and higher packed cell volume compared with the control subjects (Bradley-Moore *et al.*, 1985; Brabin *et al.*, 1990).

In malaria endemic areas, schistosomiasis and hookworm, which both cause prolonged blood loss and anaemia tend to be prevalent and are considered among the major public health problems in such areas (Roche and Layrisse, 1966; Variyam and Banwell, 1982; Latham *et al.*, 1983). Frequently these disease conditions coexist with malaria in the same person, each playing a role in causing their anaemia. It has been shown that in children with a high schistosome egg counts, heavy hookworm infection and malaria positivity are all associated with a low haemoglobin (Stephenson *et al.*, 1985) although it may be difficult to establish the importance of each in the causation of the anaemia, posing practical problems for treatment and control programmes.

1.6. THE EFFECT OF IRON SUPPLEMENTATION ON INFECTIONS AND MALARIA

Treatment and control of iron deficiency and anaemia has depended largely on food fortification, on treatment of individual cases or on supplementation of groups at risk with medicinal iron. Although both fortification and supplementation are advocated as solutions to the control and prevention of iron deficiency and anaemia, the effectiveness and efficacy of each varies greatly, depending on the circumstances of the place where it is implemented.

Over the years, particularly during the last two decades, the role of iron and its effect on health in general, and its role in infections in particular, has been the subject of substantial scientific scrutiny involving experts in epidemiology, nutrition and public health. Although a considerable effort has been made to investigate the role of iron in health and disease, results from *in vivo* and *in vitro* studies have so far been inconsistent. The beneficial haematological effect of iron, given either by supplementation or fortification, has long been accepted and backed by the medical and public health professions. However, clinical and epidemiological evidence for a beneficial effect of iron on morbidity is rather scanty. Some studies have shown that iron has a protective effect against infections while others suggest that iron supplementation results in an increased risk of infections.

An early prospective study, (Andelman *et al.*, 1966) compared morbidity patterns among infants given iron fortified or unfortified cow's milk. The authors suggested that the incidence of respiratory infections was lower among the group on iron fortified milk compared with the group without iron. In another study, adult male plantation workers in Indonesia were supplemented with oral iron for 60 days; men in the iron group had decreased morbidity scores and the prevalences of influenza, bronchitis and diarrhoea were significantly higher among those in the placebo group (Basta *et al.*, 1979). Although both studies provided useful evidence on the beneficial effect of iron on morbidity, the reliability of the morbidity data is open to question due to lack of diagnostic criteria and other issues in the study design.

Other studies have shown neither a protective nor a deleterious effect of iron supplementation on morbidity. In a study in India, 300 children aged <6 years received either oral iron or placebo for 1 year. Morbidity follow up showed that there was no difference in the average number of diarrhoeal attacks and respiratory infections among children in the two supplementation groups (Damodaran, *et al.*, 1979). A study from the Gambia investigated the relationship between nutritional status, as determined by anthropometric and measurements of iron status, and susceptibility to malaria among children (Snow *et al.*, 1991).

The incidence of clinical and asymptomatic malaria were not significantly different between children with different levels of protein calorie malnutrition and/or iron deficiency. It was noted, however, that children who developed a clinical attack of malaria accompanied by a high level of parasitaemia tended to have a higher mean *weight-for-age* and a higher mean serum ferritin level. The authors concluded that neither protein calorie malnutrition nor iron deficiency have a protective effect against clinical or asymptomatic malarial infections.

A similar study was conducted in the Gambia to examine the combined effects of iron, thiamine, riboflavin, and vitamin C supplementation on the incidence of malaria among 5-14 year old children (Bates *et al.*, 1987). No difference in the incidence of malarial episodes was found between the supplemented and the control groups. However, among children who developed parasitaemia, those who received supplementation had higher parasite counts compared with the placebo group implying the possibility of adverse effects of micronutrient interventions.

A carefully designed randomised, placebo-controlled study in Papua New Guinea was carried out to investigate the effect of iron therapy on malarial infections (Harvey *et al.*, 1989). The study was undertaken in 318 school children, aged 8-12 years, matched by haemoglobin levels and age who were randomised to receive either 200 mg of iron or a look alike placebo. Children were followed for 16 weeks with thick and thin smears, serological assessment for antimalarial antibody and morbidity recall on children absent from school. No evidence was found to support an increased incidence of malaria among the children who received iron and the authors suggested that oral iron for iron deficiency can be given safely to school children in malaria endemic areas. The absence of adverse effects of iron supplementation on malaria observed in Papua New Guinea was substantiated further by a relatively recent iron supplementation trial in Togo, West Africa, which was conducted on 241 children aged 6-36 months (Chippaux *et al.*, 1991) to determine the risk of malaria associated with iron supplementation. Results of this study revealed that the frequency of parasitaemia was identical in both the iron and placebo groups and the prevalence of *P. falciparum* was identical in all three surveys, i.e. before, during and after supplementation of iron. It was also noted that the mean parasite density decreased during and after the rainy season which was in contrary to expectations and the authors concluded that iron supplementation did not modify the susceptibility to malaria in children aged 6-36 months.

Because iron is an essential nutrient for all living cells, including pathogens, it has been postulated that increasing circulating iron, following treatment or supplementation, will give invading organisms the opportunity to compete for iron and to grow in a way which would have not been possible in a state of iron deficiency. Thus, iron deficiency in the host could play a protective role against infection (Pearson and Robinson, 1976; Stockman, 1981).

An early study investigated adult patients with iron deficiency anaemia admitted into a Tanzanian hospital (Massawe *et al.*, 1974) and showed that patients with anaemia experienced more malarial infections following iron treatment. On the other hand, bacterial infections were relatively infrequent among the group receiving iron. Though ambiguity in case definitions of malaria, anaemia and other infections were all evident and the sequence of disease occurrence during admission to the hospital was unclear, the results of this study were among the earliest to raise the issue on the possible adversity of elemental iron on malarial and other infection.

Another trial carried out among 137 Somali nomads in south-eastern lowlands in Ethiopia who received 900 mg of ferrous sulphate or placebo for 30 days showed an increased rate of disease episodes and reactivation of pre-existing malaria, brucellosis and tuberculosis among those anaemic subjects supplemented with iron compared with those given placebo (Murray *et al.*, 1978).

The authors of this study on Somali nomads did not clarify whether their observation of increased malaria among iron supplemented nomads was a direct effect of iron or as has been suggested, was due to improvement in T cell function following the repletion of iron leading to an enhanced immune response to a pre-existing infection (Tomkins and Watson, 1989). This study also had methodological problems and lacked measures against confounders. The size of the study population was determined by the availability of ferrous sulphate rather than by conventional statistical and other objective considerations. However this study was among the earliest to alert the scientific community on the possible health hazards associated with iron supplementation.

A more recent controlled randomised study was conducted in Papua New Guinea (Oppenheimer *et al.*, 1986a) to test the hypothesis that iron deficiency increases the susceptibility to infections and that administration of prophylactic iron to actual or potential iron deficient subjects diminishes this risk. Infants aged two months were given 3 ml of intramuscular iron dextran and followed up for a period of one year. The prevalence of malaria, as measured by parasite and spleen rates, was higher in the first year of life among infants who received prophylactic iron dextran, a finding contrary to the hypothesis on which the study was based. The results revealed that iron dextran had a deleterious effect on all infections, particularly on respiratory infections. More admissions and a longer duration of hospital stay were noted among

infants in the iron dextran group compared with those in the placebo group. The observation that higher haemoglobin levels at birth were correlated with the risk of admission and that the deleterious effect of iron dextran was greatest among infants with high birth haemoglobin strengthens the conclusions of this study. Although this study was better designed and more carefully conducted than many of the earlier studies, there were some issues which may have influenced its outcome. The administration of parenteral iron to infants with undetermined baseline iron status poses a problem in interpreting outcomes and the appropriateness of the subjects recruited for the study. Clinical and laboratory data collection to determine outcome measures were conducted at intervals of 8, 24, and 52 weeks after the single injection of iron dextran so the time between the exposure to iron treatment and the outcome measurements was perhaps too long to relate morbidity experiences to the treatment alone. The study population comprised infants with a low degree of exposure to malaria, so it is difficult to generalise the results of this study to other populations with varying status of exposure to malaria.

A more controlled trial was conducted among Gambian children aged 6 months to 6 years to determine their susceptibility to malaria following oral iron supplementation (Smith *et al.*, 1989). Two hundred and thirteen children were randomly assigned to oral iron (107) or placebo (106) treatment and followed for twelve weeks. Results of

this study demonstrated that the incidence of fever associated with parasitaemia was higher among the anaemic group receiving iron compared with children treated with placebo. This effect was significant in those with 10 or more high power fields positive for malaria parasites on microscopy. Cross sectional results, failed to show statistically significant differences between groups although parasite and spleen rates were higher among the iron supplemented than among the placebo group. The design of this study in the Gambia was sound and carefully conducted and its results were in agreement with the previous study in Papua New Guinea (Oppenheimer *et al.*, 1986a) which suggested the need to reassess the role of iron in malaria endemic areas.

Studies of iron supplementation given together with antimalarial treatments have also shown conflicting results. A recent study of iron supplementation given with chloroquine or pyrimethamine-sulfadoxine for Gambian children with uncomplicated malaria showed that iron was not associated with a delay in the response to anti-malarial treatment and found no evidence of any harmful parasitological consequences (van-Hensbroek *et al.*, 1995). On the other hand a contrasting result was shown in another study in Malawi where children treated with pyrimethamine-sulfadoxine together with iron failed to clear their parasitaemias compared with those given the antimalarial alone (Nwanyanwu *et al.*, 1996).

Review of previous studies on this subject generally indicates that the evidence regarding the protective or the adverse effects of iron supplementation is inconclusive. Explanations on the proposed mechanism of interaction between iron in the host and the pathogenic agent remain inadequate to fully support either side of the argument. Past studies showed major differences in design characteristics and outcome measures. Many of them were methodologically dissimilar and they showed lack of uniformity in the type and size of the study population, drug formulations and dose, duration and route of administrations and above all in selection of outcome measures which are all potential factors likely to influence the results. A summary of previous works on iron and infection are presented in Table 1.

Table 1. Summary table of selected studies on the effect of Iron supplementation on malaria and other infections.

Investigator, Country (year)	Study Population (N)	dose	Iron supplementation route	duration.	Design	Outcome (morbidity)
Damodaran <i>et al.</i> , India (1970)	children under 6 yrs. (383)	20 mg/d	PO	1 year	NS	no change in ARI & diarrhoea
Massawe <i>et al.</i> , Tanzania (1974)	adults (110)	NS	NS	NS	not randomised	no change in bacterial infections
Murray <i>et al.</i> , Ethiopia (1978)	adults (137)	900 mg/d	PO	1 month	not randomised	increased malaria
Oppenheimer <i>et al.</i> , PNG (1986)	infants (486)	150 mg,	IM	stat.	RCT	increased malaria & ARI
Harvey <i>et al.</i> , PNG (1989)	school children (318)	400 mg/d	PO	16 wks	RCT	no change in malaria
Smith <i>et al.</i> , Gambia (1989)	children under 5 yrs. (213)	3 mg/kg	PO	3 mth	RCT	increased risk of malaria
Chippaux <i>et al.</i> , Togo (1991)	children under 3 yrs. (241)	NS	PO	3 mth	RCT	no change in malaria

NB: NS=not specified; IM=intramuscular; PO=per os (orally); RCT= randomised controlled trial; PNG= Papua New Guinea; stat= single dose

B. PURPOSE AND AIMS OF THE PRESENT STUDY

1.1. BROAD OBJECTIVES

Because clinical and epidemiological evidence linking malaria and iron supplementation is still inconclusive, the need to further work on the subject is apparent. The need is most urgent in developing countries, particularly in Africa, where anaemia is highly prevalent, iron supplementation widely used and malaria an important public health problem. The present study was conceived and planned within this framework of public health and was carried out among populations where iron deficiency and anaemia are prevalent and in an area where malaria is endemic.

Following a quick census and environmental assessment, a cross sectional survey was conducted to determine the level and distribution of iron deficiency anaemia and of malaria. Iron deficient and mild to moderately anaemic persons were randomly allocated to iron supplementation or placebo groups and followed for the development of malaria. This study was intended to provide information to policy makers and programme managers to help them in formulating new strategies for the treatment and/or control of anaemia and malaria.

1.2. SPECIFIC OBJECTIVES OF THE STUDY

1. To determine the prevalence of anaemia among Ethiopian women 15-49 years and children 6-84 months of age by measuring haemoglobin concentration, packed cell volume and serum ferritin concentration.
2. To assess the determinants of iron deficiency anaemia in the community by identifying actual and potential sources of dietary iron and by studying the iron consumption behaviour of community members.
3. To assess the haematologic response to oral iron supplementation and measure the effect on women and children with different levels of anaemia.
4. To measure the risk of malarial illness among anaemic women of 15-49 years and children 6-84 months during and following a 12 week-period of oral iron supplementation.
5. To investigate morbidity experiences by measuring the frequency of probable episodes of diarrhoea, acute respiratory and helminthic infections among children and common maternal illnesses during the iron supplementation period.

Chapter Two

STUDY METHODS

2.1. Study area

2.1.1. Geography & Climate:

The study was carried out in two rural communities, one rural town and a small village situated in the north-western part of Ethiopia, about 900 kms north of the capital Addis Ababa, and 180 kms west of the historical town of Gondar. It was initially planned to conduct the study in the south-western part of Ethiopia. However, due to a change in the government and other political reasons it was necessary to change the study site in consultation with the Malaria Control Programme office.

Once it had been decided that the study should be moved to the north-western part of the country, one town and one village twelve kilometres apart, were chosen using similar criteria to those that had been used to identify the original site. The town, Shehdi, is relatively new one, which in the last decade, has become the administrative capital of the surrounding area. Many people had been attracted to this town as it has become an important trading centre between people in the border areas of Ethiopia and the Sudan. In contrast to the highland terrain to its east, which has an altitude of over 4500 metres, the study area is warm and only 900 meters above sea level. The area is covered with forests and bushes, although increasing agricultural activity is intensifying deforestation. The climate is generally influenced by the highlands in the east and the Sudanese desert in the west. The area is dry and hot for most of the year. There are two rainy seasons which have major importance for agriculture, nutrition and public health. The ‘short’ rains during March-April and the ‘long’ rains in June-September, locally known as *belg* and *kiremt* respectively, dictate agricultural productivity and the availability of food. When compared with the central highlands rainfall in this particular region is scanty but, if timely, it is normally sufficient for agricultural purposes. Although annual rainfall fluctuates from year to year, in 1994/95 it was 82.8 mm. Similarly, the annual temperature varies greatly from season to season, ranging from the lower twenties in July/August to the high forties in April/May. During the same period of 1994/95, the annual mean maximum and minimum temperatures were 35.1° C and 14.7°C respectively.

2.1.2. Socio-economic characteristics:

The study site is situated within an area of substantial economic and agricultural potential as the soil is fertile and the climate favourable for agricultural crops. However, as for the region in general, traditional and labour intensive farming methods including the use of oxen and bare hands dominate and fertilisers and improved seed are unknown. Recently, efforts have been made to introduce large scale and mechanised farming by the state and private corporate groups. Large and small rivers cut through the area and flow westwards to join the Blue Nile. Despite the generous water resources, agricultural activities is predominantly rain dependent and irrigation is not practised. The local economy is largely dependent on the production of the principal cash crops such as cotton and sesame but also on millet, sorghum and maize which are produced for local consumption and for sale. There are many cattle in the area. Small scale production of dairy products and honey adds a little to the income of farmers.

There are two primary schools in the study area, one in the town and another in the village. Most children in the town go to school but fewer children in the village do so. There are two radio communication installations, one in the hospital and one in another government institution but these are not open to the public. A postal service is available, but there is no telephone.

The study area is accessible by surface or air. There is an all weather gravel road leading to the area but access is difficult during the rainy seasons during which the road and bridges are flooded and covered with mud. There are flights to and from Gondar three times a week. At the start of the study no electricity was available for public use in the town, but a generator was installed during the study period. The local hospital has its own generator which runs only six to eight hours a day depending on the availability of fuel. Water is obtained, from a protected, deep well and distributed through a few stand pipes. As water supply from this source is neither adequate nor available at all times, residents use water from the nearby river to supplement their need.

2.1.3. Health and disease in the study area:

The overall health situation of the study area has not been defined. As it has long been an area of armed conflict between opposition forces and the previous government, development of health services has been severely impeded. The area and its people have suffered negligence from central governments and have experienced waves of displacement and migration due to war and its consequences. The one rural hospital has about 60 beds and is staffed with five doctors, fifteen nurses and technicians of various disciplines. The hospital provides basic diagnostic services except for x-rays as the machine has been out of order for more than two years.

As was true for the country as a whole, major problems facing the health service in the study area at the time of the survey were an acute shortage of manpower and supplies which was reflected in the organisational and managerial disarray observed during the study. There is one health centre and about four satellite health stations scattered within a diameter of 30-50 kilometres to the town. The hospital and its satellite health posts provide their respective communities with services such as ante-natal care, epidemic control and other primary health care activities.

Hospital and health post records indicate that malaria, malnutrition, tuberculosis and onchocerciasis are the most frequent causes of morbidity and mortality in the study area. Review of hospital data for the year 1994/95 revealed that 12,290 patients from the town and its surrounding areas visited the out-patient department. A third (4001) of patients presented with malaria alone constituting 32.6% of all ambulatory visits. More than 65% of malaria cases during that year were among the population between the ages of 15-44 years and about 21% were among children below the age of 4 years. Of the total malaria cases seen during the year, 182 admissions (4.5%) were classified as cases of severe malaria and 24 (< 1%) died in hospital.

The hospital's annual report indicated that malaria was the single most common cause for ambulatory visits and hospital admissions and the second most important cause of hospital deaths next to tuberculosis (Annual Report, Shehdi Hospital, 1994).

A team comprising field technicians and surveillance personnel, who work closely but independently from the hospital, are responsible for malaria control activities which include passive and active case detection, control of epidemics and mass drug administration. During the year 1994/95, the malaria team examined a total of 20,642 blood slides of which 7374 (54.0%) were positive for malarial parasites. *P. falciparum* was the predominant species, being found in 77.3% of positive slides. The age and sex distribution of positive cases showed that more than 80% were identified among those aged 10 years or above whilst children below 9 years old comprised about 20% of all positive cases. The ratio of affected males to females was approximately 2:1. The malaria team collects and sends vectors to the regional office though information on the local vectors was not available. Seasonal spraying of insecticides is also run by this team, usually in collaboration with the hospital. Except for some focal breeding sites along the big rivers, mosquito breeding and outbreaks normally follow the rainy seasons.

Traditional medical practices and local healers contribute to the health services in the study area. As people live scattered across large areas where transport and communication systems are unavailable, the meagre health service in the area is not accessible to many. It is not uncommon for the local people to seek formal medical help only after they have exhausted all local means available to them. For this reason many patients present late to hospital with either a terminal illness or severe complications.

Until recently, accessibility to health services was difficult for the general population as military activities in the area were intense and whatever care was available was largely for the military or civilians participating in the war. As the civil war and political conflict in the country have subsided, the situation has improved and by the end of the study improvements in the service and working conditions of the hospital were being observed, new building blocks were being erected and new diagnostic facilities were being installed.

2.2. Study populations

The target populations for this study were women aged 15-49 years and children aged 6-84 months of age. All women and children in the two study sites were identified from a census which had been conducted a little earlier. Women and the parents of children of the specified age groups who were potentially eligible for the study were approached individually with a brief explanation of the purpose of the study and were invited to participate. Those who accepted the invitation to take part in the first cross-sectional survey underwent a comprehensive health assessment including determination of their iron status. From the survey results, it was possible to identify those women and children whose iron status fell below the normal cut-off values and for whom iron supplementation was indicated. A set of inclusion and exclusion criteria were prepared separately for women and children as indicated below.

Inclusion criteria

Women and children who satisfied the following criteria were considered eligible for the study:

inclusion criteria for women

- *age:* 15- 49 years
- *anaemia & iron status:* Hb \geq 6.0 and $<$ 12 g/dL for non pregnant women

- Hb ≥ 8.0 - ≤ 11.0 for pregnant women *and/or*
haematocrit of $\leq 36\%$ *and/or* serum ferritin ≤ 12.0 $\mu\text{g/l}$
- consent: informed consent

inclusion criteria for children

- age: 6-84 months
- anaemia & iron status: Hb ≥ 6.0 and ≤ 11.0 gm/dl *and/or*
haematocrit of $\leq 36\%$ *and/or* serum ferritin ≤ 12.0 $\mu\text{g/l}$
- consent: verbal from parents or guardian

exclusion criteria for women and children :

- pregnancy: pregnant women in their third trimester at the time of
the first survey and those reaching the third trimester
after starting treatment
- health status: women and children who at the first assessment were
found to have debilitating chronic diseases or with
acute infectious diseases
- residence: subjects who were newly resident (< 3 months) in the
study area and those unlikely to stay for the trial period.
- Hb level: subjects with very severe anaemia, Hb < 6 g/dL
(< 8.0 g/dL for pregnant women)

2.3. TYPE OF THE STUDY

The study comprised a randomised, placebo-controlled, double-blind field trial (RCT), preceded by a cross sectional (prevalence) survey. The census and the cross-sectional survey was conducted to identify and screen subjects in line with the selection criteria. A schematic diagram of the study design is shown in Figure 2.

2.3.1. Census & Cross-sectional Survey

The selected areas were first mapped and all residential houses were identified. To simplify the census and the collection of follow-up data, the town was divided into eight temporary zones (A-H) and the small village was similarly divided into two, (zones A-B). All residential houses were identified and given a 4-digit house number, unique to each house (e.g. A098, B142, F121 and in some cases B234a and B234b etc.). Eight enumerators were recruited from the hospital and the malaria team and trained on how to conduct the census using the sketch map of both sites. Enumerators were assigned to specified zones and allocated a certain number of houses for which they were expected to conduct a *de jure* census using a structured census forms. The primary objective of the census was to determine the size, age and sex distribution of the population. It was also intended to identify houses with women aged 15-49 years and/or children aged between 6-84 months.

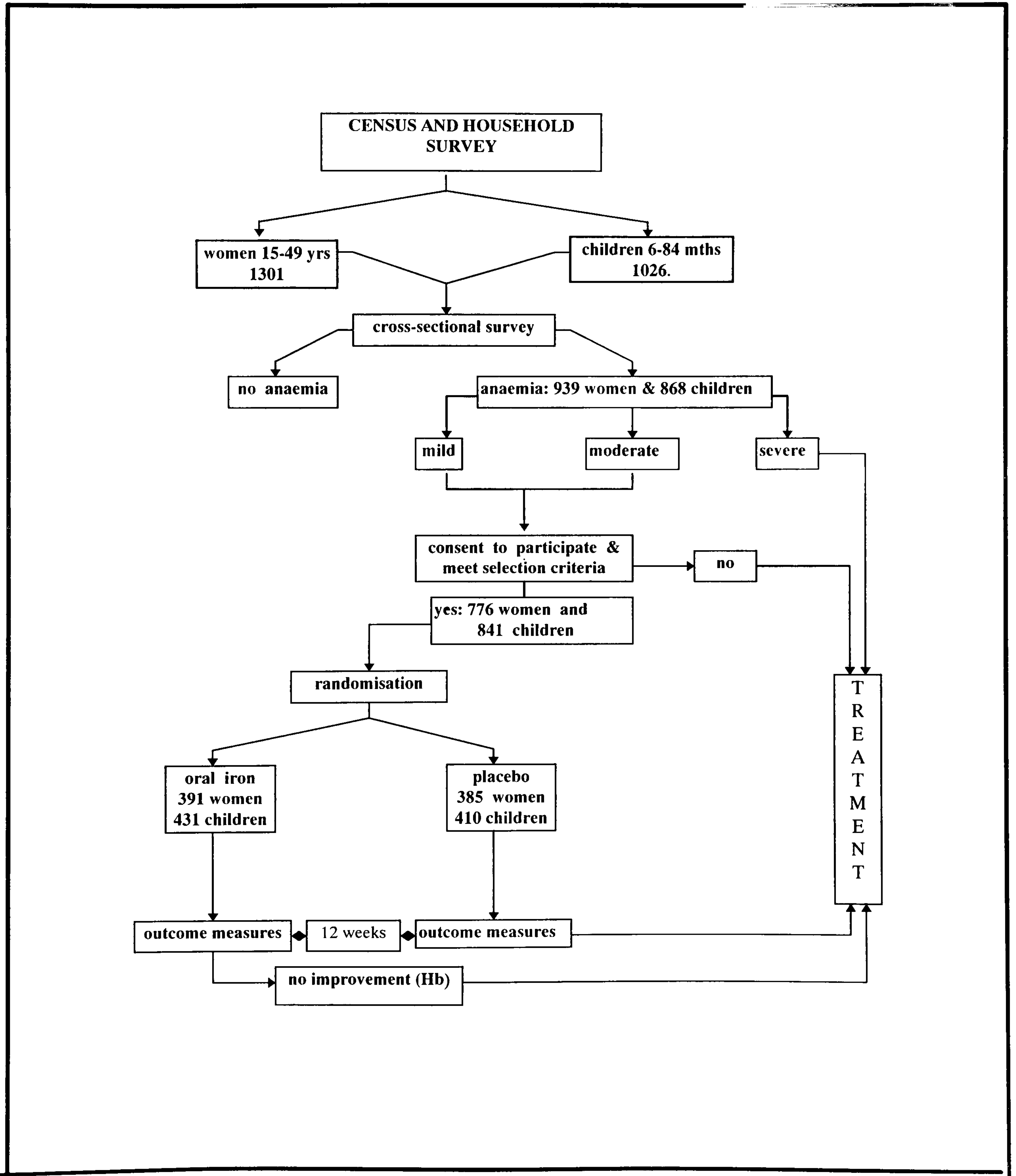
Each household with women and/or children within the specified age groups was selected from the census form and a list was prepared separately for women and children. During a house to house visit women and the parents of eligible children were briefed on the purpose of the study and were invited to take part in a cross-sectional survey to determine their eligibility for the randomised supplementation trial. Those women and children who accepted the invitation and consented to participate were assigned a unique identification number. During the cross-sectional survey, data on general socio-economic and living conditions, diet and general health of the population were collected. The health status assessment included anthropometric measurements, determination of the magnitude and distributions of anaemia and the presence of malaria. The sampling frame for the randomised trial was based on the information obtained during this survey.

2.3.2. Randomised placebo-controlled double blind trial (RCT)

To determine the risk of malaria in relation to oral iron supplementation, a double blind, placebo-controlled trial was designed which constituted the core of this study. All subjects who satisfied the inclusion criteria were identified and two sampling frames, one for women and another for children, were prepared. Though the actual units of the study were individual women and children, sampling units for both frames were households.

The decision to use households as sampling unit was made to ensure that women and children in the same household were in the same supplementation group so that confusion with mixing of iron or placebo preparations would be avoided. To achieve a balanced allocation, households were first stratified according to the size and number of eligible members in each house. The sampling frame was again stratified into blocks of four houses and within each block, houses were assigned to either iron or placebo groups. Details on randomisation and allocation to treatments are presented later in section 2.3.6.

Figure 2. Study Design



2.3.3. Treatment schedule

Drug formulation & dosages.

Iron supplementation for women was in the form of coated ferrous sulphate tablets while an elixir preparation of ferrous sulphate was given to children. Look-alike tablet and elixir placebo preparations, identical in all physical characteristics, except their iron content, were used. Tablets of 300 mg ferrous sulphate (60 mg base elemental iron) were given to women to be taken orally once a day for a period of 3 months. Placebo tablets were also given out with similar instructions. Tablets were supplied in screw top plastic containers on which the name, house number and identification numbers of the subject were recorded.

Children were given $\frac{1}{2}$ -1 teaspoonful (2.5-5.0 millilitres) of iron suspension (25 mg/millilitre) which represents a dose of approximately 3 mg/kg, administered orally by their mothers once a day for three months. Exactly similar procedures were applied to the distribution and administration of the placebo. Syrup was supplied in bottles of 100 ml labelled with the name, house number and identification number of each child.

Supply and reissuing of medication was done every four weeks for women and every two weeks for children. Supplementation was generally unsupervised, but use of medications was monitored by interviewers on their scheduled visit every two weeks. Reminders on the importance of taking the medications were given by field workers and supervisors at all possible times and during treatment supply schedules. Replacements were made at times of spoilage of tablets or spillage of suspension. Possible side effects were recorded and reasons for loss to follow-up and dropouts were recorded.

The groups who received iron or placebo and those who supplied the medication were kept blind to which group a subject was allocated. The hospital pharmacist, who possessed the code, was responsible for preparing and refilling containers and bottles without any contact with the subjects. The issue of bottles and containers was undertaken by a pharmacy technician or the nurse on duty on the day of the supply or re-supply.

After the trial all women and children who received placebo were given iron for twelve weeks. In addition, iron was continued for those subjects in the iron group whose haemoglobin level did not reach the normal cut-off level. Similarly, pregnant women allocated to placebo who reached their third trimester before the conclusion of the trial were switched to ferrous sulphate.

2.3.4. Longitudinal observation

After 12 weeks of supplementation, women and children who completed the supplementation trial were observed for a further nine months. Visits were made every other week to each household, focusing on the outcome measures of the trial. House to house interviews by the same interviewers continued until the end of the twelfth month follow up period.

2.3.5. Outcome measures and operational definitions

To measure the difference in risk of malaria among anaemic women and children given oral iron compared with those who received placebo, indices were chosen to measure exposure to the risk factor (oral iron) and its outcome (malariometric indices and other diseases). Measurement of exposure to oral iron was relatively objective and straight forward. Characteristics such as dosage, duration and route of supplementation were known before the trial, so that status of exposure was easily quantifiable.

On the other hand, measurement of the outcome, mainly the disease malaria, was more difficult. The wide spectrum and complexity of the disease makes simple and precise quantification difficult. Though demonstration of parasites in the blood

establishes the presence of infection, ascertaining disease in those who are parasite positive remains a major problem. This is especially difficult in malaria endemic areas where the entire population is exposed to the risk of infection and likely to host the parasite. Although malaria in the study area is generally seasonal, there is all year round transmission and infection is likely to take place early in life. In such a situation parasitaemia was expected to be prevalent.

From epidemiological research and clinical studies, disease status can be characterised using selected criteria and disease indicators which enable quantification and comparison between groups or studies. In this particular study, indicators which could best elicit the disease and define malarial status were chosen. A set of the commonest symptoms and signs of clinical malaria, (splenomegaly due to malaria and identification of parasites in the blood) were used to determine the disease status. Quantification of these measures was done to generate indices which in turn allowed comparisons between the iron supplementation and placebo groups. The chosen measures were believed to be adequate to pick up differentials between subjects and groups and to show the relative effect of iron supplementation compared with that of placebo. The outcome measures and their definitions chosen for the study are shown below.

2.3.5.1. Malaria

A study subject was considered to have experienced a malarial illness only when one or more of the following were demonstrated during the study period:

- The subject (mother of the subject in the case of a child) presented during the clinical assessment sessions, with signs and symptoms suggestive of clinical malaria and on the basis of clinical judgement a doctor diagnosed clinical malaria and recorded this on the individual clinical assessment form.
- When during clinical assessment, a doctor detected an enlarged spleen in a child, which in the doctor's judgement, was due to infection with malaria.
- When during a blood film examination, the laboratory technicians detected plasmodia on a Giemsa stained thin or thick blood smear and report these findings on the form provided.
- When during the biweekly recall interview, a subject or the mother/guardian of a child, reported one or more symptoms and signs of clinical malaria as per the list on the structured questionnaire.

On the basis of these definitions the following indices were used to compare findings in the iron and placebo groups.

- Point prevalence of clinical malaria - the proportion of women and children diagnosed as having clinical malaria on the day of clinical assessment.
- Parasite rate and parasite density - the proportion of women and children whose blood smear was positive for one or more species of *Plasmodium* parasites and in those who were positive, the mean parasite count per μl of blood.
- Spleen rate - the proportion of children with a palpable, enlarged spleen.
- Incidence and duration of malarial episodes - the proportion of women and children with reported episodes of malaria as defined by fever and other common malarial symptoms and signs but in whom no blood film was done.

2.3.5.2. Iron deficiency and anaemia

The spectrum of iron deficiency ranges from asymptomatic depletion of iron in storage sites to an advanced form of severe anaemia. Various methods of measuring of iron levels relevant to the different stages of deficiency have been developed.

Choice of method depends primarily on the purpose of the measurement of the level of iron deficiency which it is necessary to detect and the feasibility of each methodology.

The first stage of iron deficiency is the asymptomatic stage when body iron is depleted from the iron stores but when the haemoglobin remains normal. Measuring the serum ferritin concentration has proved to be a specific and relatively practical method of measuring this kind of iron deficiency in population surveys. A serum ferritin level of $<12 \mu\text{g/L}$ is normally taken as the cut-off value below which deficiency of stored iron is considered to exist. Serum ferritin concentration can, however, be influenced by other clinical conditions such as malignancy, liver diseases and infections such as malaria which falsely elevate the serum ferritin concentration.

The second stage of iron deficiency is the one that occurs when there is a restricted supply of iron for haemoglobin synthesis. This second stage is characterised by a fall in the plasma iron concentration and an increase in the serum level of the iron binding capacity. Evaluation of this serum iron level indicates the status of the circulating iron supply to the erythroid marrow. Change in the concentration of iron transport parameters begins as soon as the stored iron is completely exhausted, and among several techniques to measure iron deficiency at this stage,

detection of a serum iron concentration of $<60 \mu\text{g/dL}$ of blood is used commonly. However, serum iron levels are depressed during acute phase reactions such as those induced by infections. The haemoglobin concentration and the red cell mass remain within normal range at this stage.

The third stage is that of advanced stage of iron deficiency associated with overt anaemia. At this stage the haemoglobin concentration falls below the cut-off level that defines anaemia. Though it is seemingly arbitrary, anaemia has been defined by the World Health Organisation (WHO, 1968) for field applications as a haemoglobin of $<11.0 \text{ g/dL}$ for children and pregnant women and $<12.0 \text{ g/dL}$ for all other women.

As there is no one single specific and sensitive indicator to determine the exact iron status at any of these stages, a combination of two or more parameters is frequently used. In this study, taking into account logistic and other practical issues as well as the recommendations from the World Health Organisation (WHO, 1968) and the Centres of Disease Control in Atlanta (CDC, 1989), the cut-off values of haemoglobin concentration shown in Table 2, were used to define anaemia.

Table 2- cut-off levels of haemoglobin to define anaemia

Age/sex group	Haemoglobin level (g/dL)
Children 0.5-7 yr	<11.0
Adult females(pregnant)	<11.0
Adult females(non-preg.)	<12.0

Source: WHO, 1968

Other parameters used to define iron deficiency and anaemia were:-

iron deficiency and anaemia in women

- serum ferritin level $\leq 12.0 \mu\text{g/L}$
- haemoglobin concentration $\leq 11.9 \text{ g/dl}$ (≤ 11.0 for pregnant women) *and/or*
- haematocrit level of $<36\%$

iron deficiency and anaemia in children

- serum ferritin level of $\leq 12.0 \mu\text{g/L}$
- haemoglobin concentration of $\leq 11.0 \text{ g/dL}$ *and/or*
- haematocrit level of $<36\%$

2.3.5.4. Severity and degree of Anaemia

For the purpose of this study the severity of anaemia was categorised into three levels - mild, moderate or severe. This classification was based on percentiles of haemoglobin and haematocrit cut-off values of the recommended standard.

Anaemia was considered to be mild if the haemoglobin fell between 80.0-100.0% of the standard, moderate if between 60.0-80.0%, and severe if <60.0% of the standard.

Grades of severity for various age and sex groups are shown in Table 3 below.

Table 3- severity of anaemia for different age and sex groups

age/sex group	mild		moderate		severe	
	Hb	Hct	Hb	Hct	Hb	Hct
<i>Children 6-84 months</i>	8.8-10.9	28.9-36	6.6-8.7	21.7-28.8	≤6.5	≤21.6
<i>Pregnant women</i>	8.8-10.9	28.9-36	6.6-9.5	21.7-28.8	≤6.5	≤21.6
<i>Non-pregnant women.</i>	9.6-11.9	28.9-36	7.2-9.5	21.7-28.8	≤7.1	≤21.6

Hb=haemoglobin in g/dL

Hct=haematocrit in %

2.3.5.4. Other Variables

Although the main aim of this study was to investigate the development of malaria in relation to oral iron supplementation in women and children, data on other conditions that might influence maternal or child health were collected. These included respiratory conditions, diarrhoea, helminthic infections and nutritional status. Definition of these conditions, as used in this study, are given below.

2.3.5.4.1. Acute respiratory infections (ARI)

A child was said to have experienced an episode of ARI when during the clinical assessment or biweekly morbidity interview he/she presented with one or more of the following:

- fever, running nose, cough, wheezes, fast and difficult breathing, chest indrawing or ear pain and discharge from one or both ears.
- If the child was reported to have experienced fever, fast & difficult breathing and chest indrawing the episode was considered to be case of severe ARI.

2.3.5.4.2. Childhood diarrhoea & other gastrointestinal disorders

A diarrhoeal episode was recorded when a child was reported to have experienced :

- loose stools 3 times or more during a day and/or a bloody stool with tenesmus.

2.3.5.4.3. Intestinal Parasitic Infections (Helminthic infections) in children

A child was considered to have been infected with intestinal parasites when the subject :

- was reported to have passed worms visible with the naked eye and/or when eggs or cystic form of a pathogenic intestinal parasite(s) had been noted during a microscopic stool examination.

2.3.5.4.4. Obstetric/gynaecological & other genitourinary outcomes

When a woman, (whether pregnant or not) reported one or more of the following conditions she was considered to have an obstetric/gynaecological problem.

- lower abdominal pain and frequent and painful micturition
- vaginal discharge of unusual odour & colour, vaginal bleeding,
- miscarriages, still births and complicated livebirths

2.3.5.4.5. Nutritional status

Anthropometric data on women and children obtained during the study has been analysed in different ways to allow nutritional comparisons between the two treatment groups to be made. Measures of weight and height, hip and waist circumferences, generate different ratio measures including body mass index (BMI), waist and hip circumference ratio (WHR). BMI is defined as the ratio between the weight in kilograms and the square of height in metres. WHR is defined as the ratio between circumference measurements of the waist and the hip. Nutritional status among children was assessed using various indices generated from measurements of weight and height. *Height-for-age* (HA), *weight-for-age* (WA) and *weight-for-height* (WH) have been expressed in either percentile, z-score (-2.0 S.D), or median values in relation to the standard growth curves. WHO growth curves recommended for international use were used (WHO, 1986).

2.3.6. SAMPLING AND RANDOMISATION

2.3.6.1. Sampling and sample size determination

Separate sampling frames for women and children were prepared on the basis of the findings of the census and cross-sectional survey. Sampling units for both frames were individual women and children. However, allocating women and children of the same household into different supplementation groups could have resulted in the mixing and exchanging of treatments distorting the effect of supplementation and complicating the analysis and interpretation of the outcome measures. Thus, households rather than individual subjects were selected as the sampling unit to overcome this problem. Because households vary in the number of women and children, and to maintain the equal distribution of subjects into each supplementation groups, households in the frame were listed in the order of the size of eligible subjects in each house.

To ensure a fair and balanced process of allocation into treatment groups, randomisation was by the technique of random permuted blocks of 4 households. To apply this technique, the treatment groups of Iron (**I**) and Placebo(**P**) were arranged in blocks of four.

There are six possible ways of permuting the two supplementation groups into blocks of four, **IIPP**, **PPII**, **IPIP**, **PIPI**, **PIIP** and **IPPI**. In order to match the reading of random table numbers with these six combinations, numbers **1-6** were assigned to each block while numbers **7, 8, 9** and **0** were left out and passed if they appeared during reading of the random numbers table. Assignment of numbers were as follow:

1 = IIPP **2 = IPIP** **3 = IPPI**

4 = PPII **5 = PIPI** **6 = PIIP**

Numbers 7, 8, 9 and 0 disregarded.

During reading of the random numbers table, if a number between **1** and **6** was picked, four households were assigned into either iron (**I**) or placebo (**P**) supplementation groups in accordance with the sequence corresponding to the picked number. If numbers **7, 8, 9**, or **0** were picked they were ignored. For example, if the number **4** was picked from the random table, the permutation corresponding to number **4**, i.e. **PPII** was selected and the next four houses in the frame were assigned into the placebo group (the first two households) and into the iron group (the next two households). The assignment process continued in this manner until all households in the sampling frame were assigned. All women and children were allocated to their supplementation group according to the designation of the household to which they belonged.

The required size of the study population was determined using a statistical formula, (Pocock, 1991), which took into consideration the prevalence of malaria (parasite rate) at baseline.

In the cross-sectional survey at baseline, parasite rates for women and children were 17.3% and 21.6% respectively. A difference in the incidence of malaria of 10% among women and children in the iron supplementation (exposed) group compared to those in the placebo (unexposed) group, was considered to be a clinically significant difference and sample size was calculated on this basis.

Thus, a risk difference of 0.10 ($d = 0.10$) between the two groups, which applies equally to women and children was set. The study was planned to have a power of 90% ($1-\beta=0.9$) and a significance level of 5% ($\alpha=0.05$).

To calculate the required size (N) of women and children for each treatment group, the following mathematical formula was used;

$$N = \frac{2p^*(1-p)^*}{d^2} * f(a,b)$$

where

p = the proportion of those with malaria in the group without oral iron supplementation (unexposed) which, in this case, was 0.173. for women and 0.216 for children

d = the difference between proportions in the two supplementation groups, i.e. between those supplemented with oral iron (p_i) and those supplemented with placebo (p_p) beyond which a true difference is considered to exist. For this particular study, a difference of $d=(p_i-p_p)=0.10$ was used for both women and children.

$f(a, b)$ = a function at significance level of $\alpha = 0.05$, and degree of certainty of $\beta = 0.10$ to detect real difference, which in this case is $f = 10.5$.

According to the calculation, the total number of required women and children were 620 (310x2) and 720 (360x2) respectively. To allow for possible withdrawals and losses to follow up, a further 10% of the calculated size for each category and treatment group was added to make the study size 682 women and 792 children.

Although a total of 2029 women and 1784 children aged 15-49 and 6-84 months were identified from the census, only 1301 women and 1026 children accepted the invitation to take part in the cross-sectional survey. At the first survey, 939 (72.3%) of the women and 868 (84.6%) of the children had a haemoglobin concentration below the defined normal cut-off point. After applying the full inclusion criteria, the number of women and children who qualified for the study were 776 and 841 respectively. At this stage the number of women and children in excess of the required number of women (682) and children (792) were 94 and 49 respectively. In view of this small difference, it was decided to include all these women and children to give a study with a final size of 776 women and 841 children.

2.3.7. Limitations of the study design

Ideally, study of such a complex subject as the interaction between iron supplementation and malaria would have been addressed best by studies of a more complex design that incorporated clinical, epidemiological, experimental and biochemical tests to explain the iron-malaria relationships and the mechanisms involved. Planning and execution of such a study demands an enormous amount of financial and logistic resources and, to carry it out in the field, in a developing country such as Ethiopia is not possible at present.

The population included in the present study constituted anaemic women and children in a malarial endemic rural part of Ethiopia and they may not represent women and children who live in other non-malarious areas of Ethiopia. Results of the present study, could however, still be useful in planning and executing intervention programmes for women and children under similar circumstances in the rest of country and elsewhere.

In designing and conducting epidemiological research of this type, there is always a chance of biases which can distort the results. In this study every effort was made to control and guard against possible confounders and the most common biases.

2.3.7.1. Selection bias

Selection of study subjects (women and children) was made on the basis of a set of inclusion and exclusion criteria. Allocation of subjects into either of the treatment groups was also based on randomisation which ensured comparability of treatment groups in all baseline characteristics. The study also ensured that none of the selected subjects in the placebo groups was on iron treatment and that exposure to iron was in control of the investigators. As subjects were followed prospectively outcome measures were preceded by exposure to iron supplementation. The possibility of the introduction of potential selection bias was therefore controlled and any distortion on effect estimation was unlikely.

2.3.7.2. Information bias

Follow up and data collection among both the exposed (iron) and non-exposed (placebo) groups used identical methods and procedures. In addition, the study was double blind for neither the investigators nor subjects were aware of who was in which group. Therefore, information bias should have been controlled or absent. The need for women and children receiving placebo to continue with treatment for 12 weeks without any effect on their anaemia was expected to lead to withdrawals and a compliance problem. Similarly, it was anticipated that some subjects in the group under iron supplementation would experience side effects leading to more drop outs than in the placebo group. A continuous effort was made to encourage every subject to remain in the follow up during the entire study period to prevent withdrawals and loss to follow up.

In general, a randomised, placebo-controlled, double blind trial will by design take care of many of the distortions that may result from biases and confounders. Though dilution of true effect measurement, particularly in measuring malarial illness, was anticipated, the randomized design allowed such error to be randomly distributed among subjects and supplementation groups.

2.3.8. Ethical considerations

Epidemiological and clinical trials involving human subjects have to be ethically justified and must meet ethical requirements. The following ethical issues were considered during the present study.

- At present, the controversy on the role of iron and its alleged association with an increased risk of malaria is still unresolved. Thus, there is a need for more information on this topic. An epidemiological study to investigate such a controversial relationship is relevant because the results from such studies will help in the design of the control and prevention strategies of iron deficiency anaemia.
- The choice of women and children as the study population was made because it is these groups who are most at risk of developing malaria and who are the main victims of anaemia and its sequelae. Their inclusion in the study was, therefore, appropriate.
- Data collection methods and procedures used in the study were strictly in line with its stated objectives. The application of these methods was in accordance with the required standards for such procedures. Due to the nature of the study, subjects underwent multiple finger pricks and venipuncture to obtain blood specimens; maximum precaution was taken to reduce the risk of infections such as HIV.

Throughout the study, blood specimen collection was under aseptic conditions using sterile and disposable needles, syringes, lancets and other equipment.

- In the process of conducting this study, the rights of individual subjects were fully respected. Enrolment into the study was by an initial communal consent following education on the purpose and nature of the study, the immediate and ultimate health benefits and on possible unwanted effects that might follow iron supplementation. Informed, verbal consent was secured from individual women and parents of all children who took part in the whole study. The right of individuals to withdraw from the study at any stage was fully respected.
- It might be considered unethical to withhold or delay iron supplementation and/or put under placebo half of the subjects already known to have iron deficiency and anaemia. This was considered justified as there is substantial evidence to indicate that any haematological benefit these women might achieved could be offset by an increased risk of infection. To ensure that no severely anaemic women or children were put at risk subjects with severe anaemia were not included in the trial but were given iron treatment. In addition, pregnant women in their last trimester, irrespective of their haemoglobin level, were treated with iron in order to avoid any risk of endangering the health of either the mother or the new-born.

In addition, all those who were assigned to placebo supplementation, were switched to iron supplementation after the trial for a period of 12 weeks.

- Every subject in whom malaria or any other disease was detected during the study was given appropriate treatment depending on the availability of drugs. None of the subjects was denied treatment because of their participation in the study. The research activities had little or no interference on the delivery of local health service and did not have a rebound effect on the health services after the study has been completed. Involvement of hospital staff and the use of hospital facilities whenever these were needed was with prior arrangement and did not affect the normal duties of hospital staff.
- Minor effects known to occur following oral ferrous sulphate include gastrointestinal irritations, which usually subside on treatment with antacids or on discontinuing the supplementation. Subjects were given free treatment for complaints such as gastric irritation and vomiting, diarrhoea or constipation.
- The study was approved by the Ethical Committees of the London School of Hygiene and Tropical Medicine and the Faculty of Medicine at Addis Ababa University in Ethiopia.

2.3.9. DATA COLLECTION

Methods, Instruments and Procedures

Data was collected using multiple methods and techniques considered appropriate to the study objectives.

2.3.9.1 Interview method

The face to face interview method was used extensively to collect data. This method was applied first in the collection of data on the demographic and household characteristics of the study population. Data on morbidity experiences of women and children during the entire study period were also collected using this method. For the census and the initial household survey, heads of households served as the source of information. Data on perceived morbidity among women was collected using the women themselves as respondents. For children, mothers were used as respondents. On rare occasions, when the mother of a child in the study was permanently or temporarily absent, the nearest care taker or guardian of the child was used as the primary source of data.

Structured questionnaires geared towards the objectives of the study were the primary instruments used during interviews. The design and development of questionnaires was based on an effort to maintain clarity, simplicity and precision. Questions were made unambiguous to minimise difficulties for interviewers and were kept short and precise to make them acceptable to the respondents. Expert advice and comment was sought and obtained on the contents and construction of questions. Questionnaires were field tested in the study area before being finally used. Data on morbidity during and after the trial were obtained by a fortnightly house to house visit.

Two types of questionnaires were used. The first was used only once to collect data on the socio-demographic and dietary habits of the study population and was administered during the census and household survey. The second consisted of the clinical assessment and the morbidity survey questionnaires developed to collect data on health conditions of study subjects during the supplementation trial and throughout the study period.

The census and the general household data were collected by a temporary team recruited from the hospital staff and members of the malaria control team. Though most of them had previous experience in carrying out census and cross-sectional surveys, they were all given a short period of training specific to the objectives of this study. To assess the study population and to generate data on their health profile at baseline and at the end of the trial required more skilled personnel and procedures

Medical doctors and other technical staff working in the hospital were hired to collect clinical data. These were collected at two time points, at entry and at exit from the supplementation trial. Additional data were collected on women and children who, during the supplementation period, developed any illness for which they were examined and treated.

Perceived morbidity data among women and children were collected every other week by field interviewers who were recruited and trained for this purpose. After the first comprehensive household survey, each household with a study subject(s), was visited 6 times during the trial and for a total of 26 visits throughout the entire study, including the post-supplementation follow-up.

Criteria used in the selection of interviewers included completion of high school education, residence in the study area, social acceptance and similar experience in the past. It was estimated that for a single visit of a house with a study woman and a child, an interviewer would need an average of 25 minutes to complete both questionnaires. In the hot and humid weather conditions prevalent in the study area one interviewer could work effectively a maximum of only six hours. On these assumptions, and making allowances for unforeseen circumstances such as absence because of sickness and other social obligations, the work output of interviewers was calculated in order to decide on the required number of interviewers.

The number of interviewers was set at six, and three female and three male candidates were finally chosen. They were given a three day period of training on the purpose of the study and their role and responsibilities. Training included practical field exercises using the actual questionnaires employed in the study.

The interview method was applied uniformly throughout the study period. Five of the interviewers were stationed in one of the study sites where there were 777 households. The sixth interviewer was placed in the smaller village with only 120 houses. The number of houses allocated to each interviewer was more or less the same with slight variations depending on the area and how houses were scattered. As morbidity data collection was on a fortnightly basis, the interviewer systematically divided houses he/she was assigned to visit into 14 so that each house was visited every 14 days. In case of short absences of respondents or closure of houses, the interviewer made arrangements for another visit without affecting their regular schedule.

During each visit, the interviewer presented a series of questions to each woman or mother of the child on health conditions during the 14 days preceding each visit. Questions were based on the most commonest symptoms and signs which might best elicit the presence or absence of a malarial illness, acute respiratory or gastrointestinal tract infections.

Questions on pregnancy and related diseases including other genitourinary tract disorders were incorporated into the questionnaire for women in the study. Responses were recorded in the space provided on the questionnaire. After each round of visits, i.e. every two weeks, completed questionnaires were handed to the field supervisor stationed in the hospital who checked the completeness of forms.

2.3.9.2. Clinical examination

The second method of data collection was clinical evaluation of women and children in the study before, during and immediately after the supplementation trial. Cross-sectional baseline information on their medical history and a general physical examination of all women and children was undertaken before supplementation was started. During the course of supplementation, clinical data collection was limited to those who were sick or reported to have been sick. Those who were ill were referred to the hospital to be examined and treated. On the completion of twelve weeks of supplementation, clinical data were again collected from all subjects.

Clinical evaluations of subjects were carried out in the hospital, where prior arrangements had been made so as not to interfere with routine hospital activity. In co-operation with the hospital administration, separate rooms for waiting and examination of subjects were organised.

As women and children in the first site, *Shehdi*, were too many to handle in a single day, they were grouped into a manageable size for one clinical session, and appointments issued to every participant. On arrival, women were met by nurses or health assistants who registered them and verified their identification numbers and then they were led to the examination room. The doctor undertook a full evaluation of the study subject and put his diagnostic remarks on the personal record of the subject.

In the clinical evaluation, the instruments of data collection were the doctor's skill and basic diagnostic equipment such as stethoscope, sphygmomanometer and ophthalmoscope. A structured, individual medical record form specific to study objectives was designed on which doctor's findings and diagnostic remarks were recorded.

For the second village, separate dates, (usually weekends) were chosen for clinical evaluation and a mobile team of doctors and other staff was organised. A convenient site in the village was sought and the school compound was found to be suitable and central for women and children in the village. All subjects were informed in advance of the time and place of the session and this was further reinforced by house to house reminders on the day of the visit.

2.3.9.3. Laboratory investigations

Most of the laboratory tests required for the study were investigations on blood specimens. A major effort was made to co-ordinate blood specimen collection for the various tests so as to ensure minimum discomfort and harm to study subjects. Whenever women or children had their finger pricked, a specimen for haemoglobin, haematocrit and blood film was taken at the same time. Three technicians for three tests were arranged side by side, so that one immediately followed the other in collecting the specimen for their respective test before the blood flow from the finger dried up.

2.3.9.3.1. Determination of iron status and anaemia

Iron deficiency and anaemia among women and children were estimated by measuring serum ferritin concentration, haemoglobin concentration and packed cell volume (haematocrit).

(a) Serum Ferritin

Baseline serum ferritin concentrations among women and children were estimated at the National Institute of Health Research in Addis Ababa using an enzyme-linked immunoassay method (ELISA). This laboratory was better equipped to do this test than the laboratory in Gondar College of Medical Sciences.

Venous blood: Large veins of the forearm were selected as the venipuncture site and a tourniquet applied high on the arm. A convenient site was cleaned with 70% alcohol and dried with sterile gauze. Vacutainer tubes were labelled with identification code numbers specific to each subject before blood was drawn. Experienced technicians and nurses using assembled vacutainers inserted the needle into the vein to automatically draw about 6-7 ml of blood. The needle was removed and test tubes with the blood sample were kept in an ice box. Serum was separated the same day and transferred into screw topped vials labelled with the same code number as that on the test tube. The serum was then transported to Gondar Hospital for storage at -20.0 °C until it was finally transported to the central laboratory in Addis Ababa.

Determination of serum ferritin concentration: This was performed by a senior technician in the laboratory in Addis Ababa with automated equipment using ELISA (Boehringer Mannheim GmbH, Diagnostica, Germany).

(b) *Haemoglobin concentration*

The Sahli method was used to measure haemoglobin. The Sahli method only requires capillary blood and was thus more acceptable to study subjects than other methods requiring venipuncture. Two rounds of haemoglobin measurements were conducted. In the first round, haemoglobin was measured on all women and children in order to select those who qualified for the supplementation trial.

Secondly, it was with data from this round that the baseline information was generated with which post treatment measurements were compared. Measurement in the second round was only on subjects who completed the twelve weeks of supplementation. Procedures and methods of haemoglobin data collection were the same in both rounds.

Blood specimen: Specimens from women and older children were obtained by pricking the third left finger. For younger children the big toe was preferred. The chosen pricking site was cleaned with 70% alcohol and allowed to dry before it was pricked with a sterile lancet. A sample of 0.02 ml of blood was drawn slowly into a graduated pipette.

Determination of haemoglobin: The pipette with the blood was then dipped into the graduated glass tube containing 0.01% Hydrochloric acid (HCL) solution into which the blood was slowly released. The pipette was rinsed repeatedly with the reagent while it was in the tube to make sure that no blood was left in the pipette. Blood in the solution was stirred gently with a glass rod to ensure that the blood had been mixed thoroughly with the solution. After 3 minutes, distilled water was added drop by drop with a rubber-topped dropper until the colour of the mixture matched the standard comparator on both sides of the tube. The level of haemoglobin concentration was then read from the graduated tube and recorded against the identification of the subject.

(c) Packed cell volume (haematocrit)

The other laboratory test conducted on women and children was the Packed Cell Volume (Haematocrit) which provides a quick and convenient estimate of the degree of anaemia as the ratio between the volumes of red blood cells and whole blood.

Blood specimen: The blood specimen for packed cell volume was collected immediately after the specimen for haemoglobin was obtained, without need to re-puncture the subject. After the specimen was collected, the capillary tube was sealed with a clay sealant compound by placing its other end perpendicularly and slightly rotating to ensure that the tube was well plugged.

Determination of haematocrit: The sealed capillary tube with the specimen was placed in a microhaematocrit centrifuge with the sealed end towards the periphery. Position numbers, shown on the centrifuge where each sample was placed, were recorded on the data sheet against the identification details of that subject from whom the specimen was taken. Loaded with 24 specimens at a time, the centrifuge was run at 3000 rpm for 5 minutes. After centrifugation, each capillary tube was placed on a special reading device attached to the centrifuge. Reading was done by adjusting the plasma and red cells boundary against a calibrated reading device. Sample readings were recorded onto the record form adjacent to that subject's identification. Measurements of haematocrit was made during each round.

2.3.9.3.2. Haemoparasites (malaria)

The malarial status of women and children before, during and after supplementation was assessed.

Specimen collection: Specimens for blood film examination were obtained on the same day that specimens for other procedures were collected. Experienced technicians from the Malaria Control office prepared thick and thin blood smears on the same slide. Code numbers were written on the thin film with lead pencils. Dry slides were carefully wrapped in paper and transported to the Regional Malaria Control laboratory by the next day. Slides were stained with Giemsa stain and examined under a microscope.

Determination of malarial parasites: Stained slides were examined by senior technicians in the regional malaria laboratory who screened 100 microscopic fields under oil immersion. Slides found to be positive were examined further to identify the species of *Plasmodium* present. For positive slides, parasite density was estimated by counting the number of parasites in the thick film until a total of 300 white blood cells had been counted and calculation was done on the basis of an assumed WBC of 8000. Slides were reported negative for malaria parasites only after 200 microscopic fields of thick film had been examined. Results were entered into a data summary sheet prepared especially for this.

2.3.9.3.3 Intestinal parasites

In order to identify children infested with intestinal parasites, stool examination was done once, using the ether-formalin concentration (RITCHE) method (WHO, 1991).

Specimen collection: Mothers or guardians of all children were invited to bring their children to the hospital. On their arrival, they were provided with waxed paper cups and wooden applicators with instructions on how to collect faecal samples from their children. Stool samples of about 5-10 grams were then transferred into tight-fitting screw-topped glass vials containing 4 ml of 10% formaldehyde solution. Formaldehyde solution was used to preserve the faecal material which may have contained eggs, larva and cyst of parasites until it was examined finally. Identification code numbers of children were attached to each bottle. All stool samples were transported to the Gondar College of Medical Sciences laboratory where they were processed and examined.

Microscopic stool examination: A gauze filter was fitted into a funnel placed on top of a centrifuge tube on which the faecal material was passed. After the filter was removed, about 3 ml of ether was added and the tube was centrifuged for 1 minute. After the debris was removed, a drop of the sediment was drained on a glass slide and covered with a cover slide. The slide was then mounted on the microscope and examined by moving systematically up and down until the entire coverslip area was examined with a low power objective (x 10).

2.3.9.4. Anthropometric measurements

Anthropometric data of women and children were collected at baseline and at the end of the supplementation trial. Data collected on women included weight, height, hip circumference and waist circumference. Waist measurement was made by a tape measure applied horizontally around the abdomen at the mid-point between the lowest rib margin and the iliac crest on the mid-axillary line. Women were asked to breathe normally preventing contraction of the abdominal muscle and the reading was taken to the nearest 0.1 centimetres. Hip circumference was measured on women standing erect by applying the tape measure around the buttock and noting the measurement that gave the maximum circumference. Reading was to the nearest 0.1 centimetre. Weight measurement on each woman was carried out by two health assistants using a beam balance. The subject was instructed to remove heavy clothing and shoes and stand on a beam balance. Weight was recorded to the nearest 0.1 kilo gram. Height was measured to the nearest 0.5 centimetres using a graduated bar with sliding head piece attached to the beam scale.

Anthropometric measurements among children included weight, height and mid-arm circumference. For older children, weight and height measurements and other procedures were similar to those used for women. Mid arm circumference was measured using a plastic measuring tape to the nearest 0.1 centimetre. The tape was put around the left arm of the child which was supported at the finger tip by the mother. Data from children who were too young to stand on their own were measured using a different set of measuring equipment. A hanging spring balance was used for measuring weight to the nearest 1.0 gram. Length was measured with a calibrated horizontal board with a sliding foot piece. The child was put in a supine position, the head against the fixed head piece and the sliding foot piece adjusted to the heel of the child. Length was recorded to the nearest 0.1 centimetre.

2.3.9.5. Body temperature recording

Women and children who complained of fever during biweekly morbidity visits had their axillary temperature measured with a digital electronic thermometer. Interviewers were trained how to measure and record body temperature. Temperature was measured only when subjects complained of fever. Temperature readings $\geq 37.5^{\circ}\text{C}$ were recorded as fever.

2.3.10. DATA QUALITY CONTROL

Throughout the study period, a continuous effort was made to maintain the quality of data collection. Training of field workers was considered the most important way of achieving this objective. Permanently assigned interviewers and staff temporarily hired were trained on the specific roles required of them during the study. Detailed training sessions were carried out on all data collection methods and procedures before the actual work was started. Regular and random spot checks were used to control the quality of the data collection. A full-time field supervisor was available on the site for field workers to consult and he monitored their day to day activity. The principal investigator was also regularly available during the supplementation trial and at intervals of 2-4 weeks afterwards. Specific measures taken included the re-examination of blood slides by the same microscopists on about 10% of all the slides selected at random. Iron and placebo preparations were also inspected at two different times by hospital nurses who were invited to distinguish colour, taste and smell of preparations.

2.3.11. DATA PROCESSING AND ANALYSIS

Data collected in the field were transferred to summary forms designed to simplify data entry. Data on each woman and child was entered into a personal computer using EPINFO versions 5/6 package. Data cleaning, including range and consistency checks, was conducted. Preliminary analysis was made in the University in Ethiopia, while detailed analysis was carried out at London School of Hygiene in the UK. Generally, the strategy of data analysis was a stage by stage approach. Assembling and summarising the information on villages, households and their immediate environment preceded the more detailed manipulation of socio-demographic and dietary variables. Characteristics and useful indices are expected to evolve at this stage to provide an overall picture of the population and study subjects.

Following the cross-sectional survey, the iron status and prevalence of anaemia at baseline was determined. The frequency of iron deficiency, anaemia and malaria was worked out in order to plan the following components of the study. Iron deficient and anaemic populations were stratified by the degree of deficiency and severity of anaemia. Data was then examined further by cross tabulating selected socio-economic variables against one another, to identify possible correlates.

The second stage of analysis was focused mainly on calculating different disease measures to make comparisons between treatment groups. The episodic point and period prevalence, i.e. the proportion of clinically ill before, during and after treatment and the incidence and duration of episodes of illness during the trial period were all computed in relation to oral iron and placebo supplementation. Age and sex specific indices, when appropriate, were also calculated to make comparisons between treatment and placebo groups, and between different levels of anaemia.

Proportions of clinical malaria, parasite rate and spleen rates before and after treatment were calculated and relative risk and confidence intervals for each disease outcome in the iron treatment and placebo groups were compared. Data were further analysed by sub-grouping the treatment and placebo subjects by socio-demographic variables to see whether differences interacted between them with the iron/placebo effect. Chi-squared and student *t*-tests were applied to test differences for any statistical significance. The effect of supplementation and response to iron treatment was assessed by comparing the mean change in haemoglobin concentration and PCV in the oral iron and placebo groups. For each study the haematological change (difference between levels before and after treatment) was calculated and the overall mean difference for each group was determined. The overall effect was thus estimated by comparing mean estimates between the two treatment groups, and testing the difference for significance using student's '*t*' test.

Chapter Three

RESULTS

3.1. CENSUS AND HOUSEHOLD SURVEY

3.1.1. Socio-demographic characteristics of the population

3.1.1.1. Demographic characteristics

In the two study sites of *Shehdi* town and *Aftit* village, a *de jure* census revealed a total population of 8865 living in 2118 houses. The population distribution is typical of a rural developing country, where the young population forms the majority. Nearly half (47.3%) of the total population was below the age of 20 years. The population above the age of 55 was only 2.1% suggesting that the average life expectancy of the population in the study area is short.

A male to female sex ratio of 0.95 was found. This ratio, however, varied with age. Up to the age of 9 years the male to female ratio was almost 1:1. This balance started to change towards a higher proportion of females after the age of 10 (Table 4). For example, in the age group of 20-29 years the female population was nearly twice the size of the male, with 100 females to every 56 males. The distribution reversed again over the age of 30 years when the male population outnumbered the female in each age group.

TABLE 4. Age and sex distribution of the general population

age gp.(yr)	males (%)	females (%)	total (%)	sex ratio
0-4	677 (15.7)	618 (13.6)	1295 (14.6)	1.09
5-9	545 (12.6)	544 (11.9)	1089 (12.3)	1.00
10-19	770 (17.9)	1041 (22.9)	1811 (20.4)	0.74
20-29	654 (15.1)	1161 (25.5)	1820 (20.5)	0.56
30-39	918 (21.3)	743 (16.3)	1661 (18.7)	1.23
40-49	471 (10.9)	310 (6.8)	781 (8.8)	1.52
50 & above	276 (6.4)	133 (2.9)	409 (4.6)	2.07
ALL AGES	4311 (100)	4550 (100)	8865 (100)	0.95

It is generally assumed that in developing countries the population between 15-54 years of age comprises the relatively active and economically productive population group and that they are thus responsible to support the entire population of a given area. In the study area, the dependent population of children <15 years and those aged >55 years comprised 40.1% of the total population, giving a dependency ratio area of 1.67, suggesting that every 100 people of the productive age group support 67 additional people.

During the census questions on births and deaths were asked. In the 12 months preceding the census, a total of 231 livebirths and 90 deaths were reported suggesting crude birth and death rates of 26 per thousand and 10.5 per thousand respectively. Taking these crude rates, the rate of natural population increase in the area was calculated to be about 1.6%. Seventy-seven percent of all deaths reported to have occurred during the past year were among those under 15 years. Forty-one percent of all children who died in the past year were below 1 year of age. Deaths were reported more frequently among females than among males (62.5% and 37.5% respectively). According to the respondents' beliefs, causes of deaths were various ranging from superstitious to organic causes. However, the most frequently mentioned probable causes of deaths were malaria (35.4%), diarrhoea and tuberculosis (8.3% each) and other respiratory causes (6.3%).

3.1.1.2. Socio-economic characteristics

Following the census, a cross-sectional household survey was undertaken to investigate the general living conditions and dietary history of the population. After excluding commercial institutions and non-residential houses, a sample of 896 households, approximately 50% of all households, were selected for the survey. Family size in the study area varied greatly, ranging from 1-21 persons per household. On average a household consisted of 4.18 persons. Most heads of households and residents seemed to have moved into the study area relatively recently, with a mean duration of stay of only 9.3 years.

The ethnic mixture of the study area is not as varied as that seen in the country as a whole. Highland *Amharas* and *Tigreans* who, through time, have moved down to the lowlands form the large majority of the population, constituting 84.7% and 8.6% respectively. Both groups are said to be of Semitic origin while the native *Gumz*, with only 6.8% of the total, are of Hamito-Nilotic origin. By and large the population follow Orthodox Christianity or Islam (77.7% and 22.0% respectively). Less than 1.0 % of the *Gumz* community identified themselves as having no religion.

Illiteracy in the area is high, but not as high as in other remote parts of Ethiopia. It was found that 27.8% of the adult population can read and write despite the fact that only 2.7% had had some kind of formal education. The household and socio-economic characteristics of the study population, shown in Table 5, are generally similar to the pattern of rural populations in the rest of the country. Most people (73.3%), live in small houses which are single-room, rounded tukuls, where walls are made from wood with mud structure and thatched roofing. A minority of the population live in properly constructed houses with one or more windows and corrugated iron roofs.

Houses are owned mostly by the people themselves and only 14.5% of households are rented. Though the study area is rural and agricultural, only 39.9% of the population have land of their own and the majority of heads of households are engaged in hired farm labour, unskilled manual labour and small scale trade. Most families have cattle of their own which play an important role in agricultural activities, and are a source of income through the sale of dairy products. Sanitation facilities are generally very poor and the majority of house-holds use the open field for defecation and other dry waste disposal. Only 12.8% of the households were found to have pit latrines. On the other hand, the supply of water in the area is adequate and easily accessible but only 52.1% of the households obtain their supply from a protected, deep well from which water is distributed through stand pipes. The remaining 33.8% of households fetch water from nearby rivers and other unprotected sources.

Table 5. Household characteristics of the study population

Household Characteristics(N = 896)	Percent
Ethnicity:	
Amhara	84.7%
Tigre	8.6%
Gumz	6.8%
Religion:	
Christian	77.7%
Muslim	22.2%
Education:	
Illiterate	72.2%
Literate, read &/or write only	25.1%
Literate, formal schooling	2.7%
Marital status	
Married	77.6%
Single	12.7%
Divorced	9.8%
Occupation:	
farming	43.5%
unskilled manual work	23.0%
local trade and small businesses	19.1%
government & NGO (salaried)	14.2%
all others	11.6%
Housing:	
owned	85.5%
rented	14.5%
thatched roofing	73.3%
roof with corrugated iron	26.7%
with windows of 1 or more	22.1%
without any window	77.9%
with latrine of any kind	12.8%
Water supply	
deep well (stand pipes)	52.1%
river water	33.8%
spring (unprotected)	14.2%
Land ownership (owned)	39.9%
Mean duration of residence	9.3 years
Mean number of persons in the house	4.1 persons

3.2. DIETARY HISTORY IN THE STUDY AREA

As part of the household survey, an assessment of the dietary history and eating habits of the community was made. Households were asked to provide details of the food eaten during the day prior to the interview and the frequency of consumption of selected groups of food items on all days other than holidays and feast occasions was recorded. Results of the survey showed that the overall diet of the study population is typical of most communities in Ethiopia and other parts of the developing world - a thin pancake known as “*enjera*” eaten with a spicy and hot stew known locally as “*wot*”. *Enjera* can be made from flours of a range of cereals, but most commonly from fermented millet, sorghum, and maize paste. In the highlands and most urban centres in the country, *enjera* is made from the iron rich cereal, *teff* (*Eragrostis Abyssinica*). However, in the study area *teff* is neither grown nor easily available in the local market so meals are generally based on cereals with limited iron content. Less than 1.0% of households respondents said that they had eaten *enjera* made from *teff* during their meals the previous day. As shown in Table 6, the three main meals of the large majority of households (85.0-99.4%) in the previous day of the survey comprised foods which were based on millet and sorghum.

Table 6. Composition of meals during the day prior to the survey.

	Breakfast	Lunch	Dinner
food groups (composition)	(percentage of households)		
Cereals (millet, maize, sorghum)	99.4	95.6	85.0
Legumes (beans, pea, lentils)	28.8	36.4	33.1
Vegetable (cabbage, pumpkin, potato)	9.4	11.0	15.0
Meat (beef, lamb, chicken)	1.8	1.3	2.6
Dairy products(milk, cheese, yoghurt)	2.2	0.8	0.7

Meat and dairy products were eaten rarely and fresh vegetables infrequently. As the population is mainly Orthodox Christian they adhere strictly to fasting with abstention from all food items of animal products, including fish, milk and butter for up to 220 days (about 60.0%) of the year. The dietary survey was conducted during the non-fasting period but nevertheless only 1-3 % of households consumed meat in any one of their meals during the day before the interview. As shown in Table 7, 54.1% of households reported that, on average, they ate meat only once a week even during non-fasting seasons.

During the dietary survey, women were asked to identify particular food items considered good or bad for pregnant women and children. Most women knew what was good for women during pregnancy. Over 60% of respondents identified meat and other animal products as useful during pregnancy. Liver, an excellent source of *haem* iron, was identified by 20.0% of women as the single most important source needed to replace blood losses during pregnancy and birth. Fruits and vegetables were also mentioned by 20.6% and 27.8% of the respondents as good foods for pregnancy. Foods reported to be harmful for pregnant women were hot and spicy foods, alcohol and too much salt, which were considered bad for pregnancy and bad for the foetus by 43.5%, 3.7% and 9.9% of respondents respectively.

Nearly all mothers mentioned meat, eggs, and milk as good food for children, while 71.1% said breast feeding is good for infants. Bottle feeding was mentioned by 20.3% of mothers as being good for their children, a surprisingly high percentage. About 30.2% of mothers said they liked to feed their new-born child with raw butter, a traditional feeding practice in Ethiopia which is known to be harmful.

Table 7. Frequency of consumption of selected categories of diet

Food items	frequency of intake of selected food items (percentage of households)		
	1/ wk	2/ wk	≥3 per wk
Meat/chicken	54.1	30.6	14.2
Milk & other dairy product	19.0	27.9	51.6
Fat (mainly veg.oil)	3.6	0.5	95.0
Vegetables	32.6	26.0	40.5
Legumes	2.7	8.5	86.3

3.3. SCREENING AND SELECTION OF SUBJECTS

Following the census, a cross-sectional survey was conducted in April 1994 to determine the iron status of women aged 15-49 and of children aged 6-84 months. This survey was necessary to identify potentially eligible subjects for the trial and to form the sampling frame for the study. Baseline information on several variables was also collected at this stage, for comparison with the results obtained during the randomised, placebo-controlled supplementation trial that followed.

A total of 1301 women aged 15-49 years and 1026 children aged 6-84 months resident in the two study sites of *Aftit* and *Shehdi* responded to the invitation and gave their verbal consent to take part in the first cross-sectional survey. Assessment of the iron status of the study subjects relied on the measurement of haemoglobin, haematocrit and serum ferritin concentration. The first two measurements were made on all women and children while serum ferritin was determined in only 660 women and 310 children. This was mainly because the procedure used for serum ferritin determination required 5-10 millilitres of venous blood, and collection of that much blood by venipuncture was not acceptable to many participants, particularly children and their parents.

The magnitude of the problem of anaemia among both women and children varied according to the method used to define anaemia. As shown in Table 8, 72.3% of the women and 84.6% of children were classified as anaemic using haemoglobin level, while using their haematocrit level as a criterion, only 48.6% women and 57.2% children were anaemic. Measurement of serum ferritin concentration, on the other hand, categorised very few women or children as iron deficient. The geometric mean (s.d) serum ferritin concentration was found to be 46.(1.98) $\mu\text{g/L}$ for women and 42.9 (2.0) $\mu\text{g/L}$ for children. When a serum ferritin level of $<12 \mu\text{g/L}$ was used as a cut off value to define iron deficiency, only 4.4% and 7.3% of women and children respectively were found to be iron deficient. It was revealed that all women and children with low haematocrit and/or ferritin levels had low haemoglobin, but not all subjects with low haemoglobin showed lower values in the other two assays.

Table 8. Prevalence of anaemia among women and children.

haematological indices	women (n=1299) * children (n=1026)	
	number (%)	number (%)
Haemoglobin <11.9 gm/dL	939 (72.3)	--- ---
Haemoglobin <11.0 gm/dL	--- ---	868 (84.6)
Haematocrit <36 %	632 (48.6)	587 (57.2)
Serum Ferritin <12 µg/L**	29 (4.4)	22 (7.3)

* two blood samples were discarded.

** n=660 women; & 310 children

3.3.1. Selection of subjects & allocation to treatment**3.3.1.1. Selection of women**

Based on the screening results, all women and children whose haemoglobin levels fell below the respective cut-off values were considered eligible for the study. Accordingly, 939 women were initially identified for enrolment. Applying the selection criteria, a total of 163 of these women were excluded; 43 were pregnant (37 in the third trimester and 6 with Hb <8.0 g/dL), 37 had severe anaemia with a haemoglobin below 6.0 g/dL, 36 women were unwilling to take part, 31 women were

unsuitable due to either chronic or acute illnesses and a further 16 were unavailable or left the area before randomisation. Thus, 776 women were available for the supplementation trial; 391 and 385 women were randomly allocated into iron and placebo treatment groups respectively. The age distributions of women allocated to the two treatment groups are shown in Table 9. Women in both treatment groups were relatively young, nearly two thirds were below the age of 30 years. The mean ages of women in the iron and placebo groups were 27.6 and 27.0 years respectively. Women in both groups were generally comparable in their age structure.

Table 9. Age distribution of women by treatment group

Age group(yrs)	Treatment Group		TOTAL
	IRON(%)	PLACEBO(%)	
15-19	69 (17.6)	54 (14.0)	123 (15.8)
20-29	152 (38.9)	168 (43.6)	320 (41.2)
30-39	131 (33.5)	122 (31.7)	253 (32.6)
40-49	39 (9.9)	41 (10.6)	80 (10.2)
ALL AGES	391 (100.0)	385 (100.0)	776(100.0)

Following randomisation, women in the two treatment groups were further compared for their similarity in terms of selected variables, particularly those related to their reproductive characteristics (Table 10). All but 15.7% of the women had experienced pregnancy at least once, with a mean number of 3.1 pregnancies. Of those women who gave a history of previous conception, 14.4% reported that they had been pregnant once. During the survey, 115 women (11.9%) were found to be pregnant while 502 (64.7%) of women said they were lactating.

The proportion of women in the area using modern contraceptives was extremely low. The proportion of all women who reported using oral contraception was 4.0%. When women in the two groups were compared, those allocated to the placebo group had a higher use of contraceptives (5.5%) than their counterparts in the iron group (2.8%). and this difference is statistically significant, ($\chi^2=7.0, p=0.007$). With the exception of contraception, women in both treatment arms were comparable in all other variables.

Table 10. Reproductive characteristics of women by treatment group

Characteristics	Treatment Group	
	Iron (N=391)	Placebo(N=385)
Mean Gravida	3.03	3.42
Mean Para	2.64	3.04
Mean number of live children	2.34	2.44
Mean number of abortions	1.30	1.50
Mean age of last child(yrs)	2.70	2.90
Proportion of women lactating	63.5%	65.1%
Proportion of women pregnant	12.5%	10.9%
Proportion of women on contraceptive	2.8%	5.5%

3.3.1.2. Selection of children

Selection and allocation of children into iron and placebo treatment groups was conducted in a similar way as that used for women. Out of 1026 children who were screened for haematological parameters, the haemoglobin of 868 children fell below the cut-off point of 11.0 gm/dL and these children were identified as potentially eligible for selection.

However, 27 children failed to meet all the required selection criteria; 13 had severe malnutrition and severe anaemia (Hb < 6.0 g/dL), 9 children had acute illnesses and the parents of 5 children would not allow their children to take part. A total of 841 children, 452 males and 389 females, with a mean age of 45.2 months were finally available for selection and randomisation; 431 and 410 children were allocated into iron and placebo treatment groups respectively. The age and sex distributions of children in the two treatment groups are shown in Table 11. Children assigned to each treatment group were very comparable in their age and sex structure.

Table 11. Age-sex distribution of children by treatment group

Age group (months)	Treatment Group				TOTAL(%)
	IRON (%)		PLACEBO(%)		
	males	females	males	females	
6-11	31(3.7)	24(2.8)	24(2.8)	23(2.7)	102 (12.1)
12-23	40(4.9)	31(4.6)	42(5.0)	30(3.6)	143 (17.0)
24-35	27(3.2)	33(3.9)	40(4.7)	30(3.6)	130 (15.4)
36-47	29(3.4)	37(4.4)	28(3.3)	34(4.0)	128 (15.2)
48-59	32(3.8)	30(3.6)	29(3.4)	28(3.3)	119 (14.1)
60-71	30(3.6)	23(2.7)	30(3.6)	26(3.1)	109 (12.8)
72-83	36(4.3)	28(3.3)	34(4.0)	12(1.4)	110 (13.1)
ALL AGES	225(52.2)	206(47.8)	227(55.4)	183(44.6)	841 (100)

3.4. CROSS SECTIONAL SURVEY RESULTS AT BASELINE

3.4.1. Haematological parameters (iron status)

Haematological indices used to define anaemia and to determine the degree of severity of anaemia were those recommended by the World Health Organisation (WHO). Accordingly, measured values of haemoglobin and haematocrit were used to define the iron status and anaemia of study subjects as either *normal* ($\geq 100\%$), or as having *mild* (81-99%), *moderate* (61-80%) or *severe* ($\leq 60\%$) anaemia. A serum ferritin concentration, of 12 $\mu\text{l/L}$ was used to define iron deficiency in both women and children.

3.4.1.1. Haematological indices of women

Haematological measurements were made in study women to determine their iron status and degree of anaemia before they started supplementation. The distribution of haemoglobin and the level of anaemia showed a similar pattern among women assigned to either of the supplementation groups (shown in Table 12) with means (standard deviation) haemoglobin levels of 8.52 (1.3) and 8.55 (1.4) g/dL among women in the iron and placebo groups respectively. Baseline measurements of haematocrit showed a similar picture. The mean haematocrit level in women in each group was 29.2% (Table 13).

The geometric mean serum ferritin concentration of all women was 46.03 $\mu\text{g/L}$, and less than 5.0% of women had a serum ferritin below the cut-off value of 12 $\mu\text{g/L}$. The mean (s.d.) serum ferritin levels of women assigned to receive either iron or placebo were very similar, 45.5 (1.9) $\mu\text{g/L}$ and 46.5 (2.0) $\mu\text{g/L}$ respectively.

Table 12 **Distribution of anaemia among women as measured by Hb at baseline**

haemoglobin g/dL (degree of anaemia)	Treatment group	
	Iron (N=391) number (%)	placebo (N=385) number (%)
< 7.1 (severe)	70 (17.9)	63 (16.4)
7.2-9.5 (moderate)	234 (59.8)	239 (62.1)
9.6-11.9 (mild)	87 (22.3)	83 (21.6)
> 12.0 (normal)	0 (00.0)	0 (00.0)
Mean (sd) Hb in g/dL	8.52 (1.3)	8.55 (1.4)
	<i>t=0.3, p=0.75</i>	

Table 13. Distribution of anaemia among women as measured by haematocrit at baseline

haematocrit (%) (level of anaemia)	Treatment group	
	Iron (n=391) number (%)	Placebo (N=385) number (%)
≤21.6 (severe)	23 (5.9)	35 (9.0)
21.7-28.8 (moderate)	168 (42.6)	138 (35.8)
28.9-36 (mild)	158 (40.4)	180 (46.8)
> 36 (normal)	42 (10.7)	32 (8.3)
Mean (sd) Hct. %	29.2 (5.4)	29.2 (5.4)

3.4.1.2. Haematological indices among children

Specimens from 41 of the 841 children in the study (21 from the iron and 20 from placebo group) were either inadequate or unavailable and it was possible to estimate iron status in only 800 children. Distributions of haemoglobin and haematocrit measurements are shown in Tables 14 and 15. The means (s.d.) haemoglobin at baseline was 8.27 (1.2) g/dL for children in the iron group and 8.27 (1.3) g/dL for those in the placebo group. Mean haematocrit levels were 26.4 (4.3) % and 26.4 (4.7) respectively.

Using haemoglobin as a measure of anaemia, 63.4% of children in the iron and 61.8% of those allocated to placebo treatment were moderately to severely anaemic, compared with figure of 70% and 69.2% using haematocrit values to define anaemia. In addition, 1.5-2.3% of children with anaemia, defined by haemoglobin, fell into the normal category (Hct >36%) when haematocrit was used as a measure of anaemia (Table 15).

Only 7.4% of the 310 children assessed had a serum ferritin value below the cut-off point of $< 12 \mu\text{g/L}$. The geometric mean (s.d) serum concentration among children was $42.0 (2.2) \mu\text{g/L}$ with values ranging from 2.2 to $196.3 \mu\text{g/L}$. Mean (s.d.) values were similar in iron and placebo supplementation groups, $42.7 (1.9) \mu\text{g/L}$ and $40.5 (2.2) \mu\text{g/L}$ respectively.

Table 14 Distribution of anaemia among children defined by haemoglobin.

haemoglobin (g/dL) & degree of anaemia	Treatment group	
	Iron (N=410) number (%)	placebo (N=390) number (%)
≤ 6.5 (severe)	49 (12.0)	50 (12.8)
6.6-8.7 (moderate)	211 (51.4)	191 (49.0)
8.8-10.9 (mild)	150 (36.6)	149 (38.2)
≥ 11 (normal)	0 (0.0)	0 (0.0)
Mean (sd) Hb.	8.27 (1.2)	8.27 (1.3)

Although there was a slight variation between groups in the distribution by severity of anaemia there was no statistically significant difference among children in the two supplementation groups.

Table 15 Baseline distribution of anaemia among children as measured by haematocrit levels.

haematocrit (%) & degree of anaemia	Treatment group	
	Iron (N=410) number (%)	Placebo (N=390) number (%)
≤ 21.6 (severe)	58 (14.1)	71 (18.2)
21.7-28.8 (moderate)	229 (55.9)	199 (51.0)
28.9-36 (mild)	117 (28.5)	111 (28.5)
> 36 (normal)	6 (1.5)	9 (2.3)
Mean (sd) Hct.	26.4 (4.3)	26.4 (4.7)

3.4.2. Anthropometric status of women and children at baseline

3.4.2.1. Nutritional status of women

As an indirect indicator of the adequacy of dietary intake, height, weight, hip and waist circumferences were measured in study women during the baseline survey. Nutritional indices of body mass index (BMI), and waist-hip ratio (WHR) were computed from the anthropometric data. The results of anthropometric measurements on study women are summarised in Table 16. As anticipated, study women were, in general, under weight and short in stature, with a mean (s.d) weight of 47.8 (6.3) kgs and mean (s.d) height of 157 (6.0) cms. The prevalence of a low BMI (≤ 18.5) among all women was 36.7%. The mean ratio between waist and hip circumferences (WHR) was 0.826, which suggests that study women had little adipose tissue. The overall picture was that of a marked degree of malnutrition implying that there had been food shortages in the past. When the baseline nutritional status of women assigned to either supplementation group was compared, no significant differences between them were found (Table 16).

Table 16 . Anthropometric status of women before treatment

Anthropometric indices	Treatment Group		<i>p</i>
	Iron (N=391)	Placebo (N=385)	
Mean weight(sd) Kgs	48.2 (6.6)	47.4 (6.1)	0.08
Mean height(sd) cms	157.0 (6.0)	157.0 (6.1)	0.90
Mean waist circumference(cm)	73.3 (5.7)	72.2 (5.9)	0.08
Mean hip circumference (cm)	88.1 (5.9)	88.1 (5.7)	0.90
Mean Body Mass Index (sd)*	19.5 (2.3)	19.2 (2.3)	0.06
Mean Waist-Hip ratio **	0.831	0.821	0.08

Waist- Hip Ratio (WHR) is the ratio between waist and hip circumferences in centimetres.

Body Mass Index (BMI) is weight in kgs divided by the square of height in meters.

3.4.2.2. Nutritional status of children

At baseline all children (841) were measured for height, weight and mid-arm circumference. The mean (s.d) weight and height of the children in the study were 12.5 (4.0) kgs and 91.5 (15.8) cms respectively. Anthropometric indices of *height-for-weight (hw)*, *height-for-age (ha)* and *weight-for-age (wa)* were also computed on the basis of the standard deviation (z-score) method in reference to *-2.0 SD* of the standard

as recommended by WHO. The prevalences of low ($< -2.0 SD$) **hw**, **ha** and **wa** among study children at baseline were 19.6%, 45.0% and 47.8% respectively. Though each measure indicates a different form of under nutrition, it is obvious that all the three indices show a high degree of malnutrition among children in the study. Results of the baseline measures suggested that children in the study had nutritional deficiencies that reflected chronic food shortages in the past as well as a poor diet at the time of the survey. Slow skeletal growth and development for age, often associated with nutritional problems of long duration, were indicated by the high prevalence of low *height-for-age* of 44.9%. Male children were slightly shorter for their age than females, particularly between the age of 6-12 months, with sex-specific prevalences of low *height-for-age* 46.0% and 43.7% for males and females respectively. However, the difference is not statistically different, ($\chi^2=0.41$, $p=0.51$). Undernutrition among children was further assessed using Waterlow's (1977) classification, by cross-classifying the *z*-score values of *weight-for-height* and *weight-for-age*, into four broad categories of nutritional status as shown in Figure 3.

Figure 3 Cross-tabulation of anthropometric z-scores of children and classification of malnutrition *

z-score	<i>height-for-age</i> ≥-2 SD	<i>height-for-age</i> <-2 SD
<i>weight-for-height</i> ≥-2 SD	normal to mildly malnourished	stunting
<i>weight-for-height</i> <-2 SD	wasting	wasting & stunting

* Source: Waterlow, 1977

Summary estimates of each of these categories of malnutrition are shown in Table 17; over a third of all children were found to suffer from one or other form of malnutrition. Children assigned to the iron group appear to have suffered more from the chronic form of undernutrition compared to those in the placebo, as prevalences of stunting were 17.6% and 22.6% respectively, but the difference between groups is not statistically significant.

On the other hand, wasting and stunting combined, which is the severest form of undernutrition, was seen in 3.4% and 3.9% of the children in the iron and placebo groups respectively. Mid arm circumference measurements were similar in the two groups with mean circumferences of 14.3 and 14.0 cms among children in the iron and placebo groups respectively.

Table 17 Anthropometric status of children before supplementation

Nutritional status	Treatment Groups		χ^2	<i>p</i>
	Iron (N=431)	Placebo (N=410)		
Mean (s.d.) weight in kgs	12.5 (4.0)	12.4 (3.9)		<i>t</i> =0.4 0.7
Mean (s.d) height in cms.	91.7 (15.0)	91.3 (15.7)		<i>t</i> =0.4 0.7
Normal to moderately malnourished (%)	64.6	60.8	1.28	0.25
Wasting (%)	14.4	12.7	0.52	0.47
Stunting (%)	17.6	22.6	3.34	0.06
Wasting & stunting(%)	3.4	3.9	0.11	0.74
Mean arm cf. in cm (sd)	14.3 (3.6)	14.0 (1.3)		<i>t</i> =2.53 0.11

3.4.3. General health status of women and children at baseline

A cross-sectional health assessment was undertaken for all the 776 women in the study before they started their respective supplementation. On the day of clinical assessment a total of 262 (33.8%) women presented themselves with some kind of illness indicating that there was a high degree of ill health among women in the study area. The number of women with selected categories of illness is presented in Table 18. Although all women were known to have varying degrees of anaemia, as determined by laboratory measurements, only 90 (11.5%) women were considered to be anaemic using clinical signs and symptoms as defining criteria. The prevalences of clinically diagnosed anaemia among women in the iron and placebo groups were 14.1% and 9.1% respectively, a statistically significant difference ($\chi^2=5.15, p=0.02$). Clinical malaria was diagnosed in 12.8% and 12.5% of women in the iron and placebo groups respectively. The distribution of all other categories of illness was fairly similar in both groups. Women assigned to the iron and placebo supplementation did not show any significant difference in the overall burden of morbidity at baseline (34.5% and 33.0% respectively) ($\chi^2=0.21, p=0.70$).

Table 18 Health status of women for selected diseases at baseline

Diseases	Iron (n=391)		Placebo (n=385)		χ^2	<i>p-value</i>
	no.	%	no.	%		
all illness on examination day	135	34.5	127	33.0	0.06	0.79
anaemia	55	14.1	35	9.1	5.15	0.02
malaria	50	12.8	48	12.5	0.00	0.99
GUTI*	13	3.3	6	1.6	2.63	0.11

* GUTI= genito urinary tract infection

The health status of children was also assessed clinically in all except for 23 study children who were not available on the examination day. On the day of examination, 423 children (51.7%) were found to have one or more illnesses of varying severity. Numbers of cases of selected diseases among children assigned to the two treatment groups are shown in Table 19. The overall prevalence of all illnesses was similar in iron and placebo groups, 52.4% and 51.0% respectively. Individual diseases were also found to be distributed similarly in children assigned to receive iron or placebo.

Table 19. General health status of children at baseline.

Diseases	Iron(N=422)		Placebo(N=396)		χ^2	<i>p</i>
	number	(%)	number	(%)		
children with illness	221	52.4	202	51.0	0.10	0.74
anaemia	83	(19.7%)	70	(17.7%)	0.41	0.52
malaria	48	(11.4%)	53	(13.4%)	0.59	0.44
splenomegaly	82	(19.4%)	78	(19.7%)	0.0	0.99
ARI*	82	(19.4%)	83	(21.0%)	0.21	0.64
diarrhoea	69	(16.4%)	69	(17.4%)	0.10	0.75

* ARI = acute respiratory infection

3.4.4. Intestinal parasites among children

At baseline, stool specimens were examined to determine the prevalence of intestinal parasitic infections among children. Specimens were available from only 493 (58.6%). Samples from the remaining 348 children were not examined as they were; specimen not available (287), insufficient (39) or because containers broke during transportation (22). Of the 493 stool samples examined, 157 (31.8%) showed one or more pathogenic intestinal ova or parasites.

Hook worm and *Hymenolepis nana* infections were the commonest parasites found affecting 13.4% and 7.7% of children respectively. Thirty-six stools contained more than one parasite. The distribution of intestinal parasitic infections is shown in Table 20.

Table 20 Percentage distribution of intestinal parasites among children (N=493)

Total negative	336 (68.2 %)
Total positive	157 (31.8 %)
distribution of intestinal parasites among those tested positive	
parasite	number (%)
hookworm	66 (13.4)
<i>Hymenolepis nana</i>	38 (7.7)
<i>Ascaris lumbricoides</i>	32 (6.5)
<i>Strongyloidis stercoralis</i>	28 (5.7)
<i>Giardia lamblia</i>	11 (2.2)
<i>Scistosoma mansoni</i>	9 (1.8)
all others *	9 (1.8)

* all other includes: 5 *E. hystolitica*, 4 *Trichuris tricurria*

3.5. GENERAL HEALTH STATUS DURING SUPPLEMENTATION

The health of women and children during the supplementation period was followed in two ways. Firstly, study subjects were visited every other week and asked about symptoms and signs suggestive of malaria and other common maternal and childhood diseases since the last visit. Secondly, hospital data on study subjects who sought and obtained medical assistance during the supplementation period were secured. At the biweekly morbidity assessment details on any morbid experiences which had occurred during the period between visits were recorded as present or absent and if present, the duration of that specific episode was also recorded. Except for episodes of fever, which in most instances were verified by measuring axillary temperature, other illnesses were recorded as perceived and described by the subject (or by the parent of a child). From the morbidity data set, the frequency and mean number of episodes of illness per subject and the mean duration per an episode were calculated for women and children in each of the two supplementation groups. The frequency of hospital visits and proportions of visits by diagnosis were calculated for subjects in each group. Morbidity data for women and children are presented separately.

3.5.1. Health status of women during the period of supplementation (biweekly morbidity)

Seven hundred and fifty-three of the 776 women enrolled (382 receiving iron and 371 receiving placebo) were visited six times during the supplementation period. Twenty-three women (9 in the iron and 14 in the placebo) were lost to follow up. Fifteen women either moved out of the area or were unavailable on consecutive visits and 8 women refused to continue to participate for personal reasons such as work-related inconvenience or death of a family member. The frequency of episodes of malarial symptoms and signs (fever, headache, chills and rigors, back and joint pains) and the duration of each episode was recorded and results are summarised in Table 21. The follow-up period for all women was a total of 9034 person-weeks (753 women x 12), comprising 4584 person-weeks for the iron group and 4452 person weeks for those in the placebo group. The incidence rates of illness episodes among women in both groups are shown in Table 22.

A total of 449 (59.6%) women in both groups reported at least one episode of fever during the 12-week period of follow-up. An episode of fever was reported by 244 (63.9%) women in the iron and 205 (55.3%) women in the placebo group, RR=1.16, (95% CI:1.03-1.30), ($\chi^2=5.8$, $p=0.01$). The distribution of fever episodes among women in the two groups revealed a similar picture, higher in the iron group.

Six hundred and fifty-two episodes were reported by women who were receiving iron and 514 episodes by those in the placebo group, with a mean number of 1.71 and 1.38 febrile episodes per women in the iron and placebo groups respectively, RR= 1.23, (95% CI: 1.11-1.37), ($\chi^2=14.2$, $p<0.001$). When the distribution of fever episodes were categorised, as shown in Table 23, a linear trend was observed indicating that febrile episodes were more likely to be experienced by women receiving iron supplementation than those who received the placebo, ($\chi^2=10.7$; $df=4$, $p=0.029$).

Analysis of the duration of febrile episodes showed that women in the iron group spent 2645 days with fever in contrast to those in the placebo who had 1917 days of fever. The mean durations per episode of fever among women in the iron and placebo groups were thus 4.06 and 3.72 days respectively. Analysis of durations of febrile episodes (Table 24) showed no statistically significant difference between women in the iron supplementation and placebo groups ($\chi^2=10.3$; $df=6$, $p=0.123$).

Table 21 **Distribution of women with at least 1 episode of illness suggestive of malaria during 12 weeks of supplementation**

	TREATMENT GROUP				RR (95%CI)	χ^2	<i>p</i>
	IRON (N=382)		PLACEBO (N=371)				
malarial episodes	no.	%	no.	%			
fever	244	63.9	205	55.3	1.16 (1.03-1.30)	5.80	0.01
headache	299	78.3	256	69.0	1.13 (1.04-1.24)	8.33	0.003
chills &/or rigor	239	62.6	200	53.9	1.16 (1.03-1.31)	5.79	0.01
back/joint pain	267	69.9	227	61.2	1.14 (1.03-1.27)	6.32	0.01

Table 22 Incidence rates for episodes of illness suggestive of malaria per person-week among women during 12 weeks of supplementation

Malarial episodes	TREATMENT GROUP				RR(95%CI)	χ^2	<i>p</i>
	IRON (N=382)		PLACEBO (N=371)				
	no.	rate/pw	no.	rate/pw			
febrile episodes	652	0.14	514	0.12	1.23(1.11-1.37)	14.2	<0.01
headache	1140	0.25	964	0.22	1.15(1.07-1.24)	12.9	<0.01
chills &/or rigor	523	0.11	413	0.09	1.23(1.09-1.39)	10.8	<0.01
back/joint pain	735	0.16	609	0.14	1.17(1.06-1.29)	9.7	<0.01

Table 23 **Distribution of reported fever episodes among women during 12 weeks of supplementation**

number of episodes	IRON (n=382)		PLACEBO (n=372)	
	number	%	number	%
0	137	36.0	169	45.2
1	67	17.5	70	18.9
2	58	15.2	50	13.5
3	51	13.4	30	8.0
≥4	68	17.8	55	14.7

$\chi^2 = 10.76$ (df=4); $p = 0.029$

Table. 24 Mean duration (days) of febrile episodes among women during the supplementation period by treatment group

(N=244 in the iron group and 205 in the placebo)

Treatment group	Mean duration in days							TOTAL
	1-3	4-6	7-9	10-12	13-15	16-18	≥19	
IRON	54	44	31	29	22	23	41	244
	(22.1)	(18.0)	(12.7)	(11.9)	(9.0)	(9.4)	(16.8)	100.0
PLACEBO	63	26	37	22	18	15	24	205
	(30.7)	(12.7)	(18.0)	(10.7)	(8.7)	(7.3)	(11.7)	(100.0)
All women	117	70	68	51	40	38	67	449
	(26.0)	(15.6)	(15.1)	(11.4)	(08.9)	(08.4)	(14.9)	(100.0)

$\chi^2=10.3, (df=6) p=0.123$

3.5.2. Health status of children during the supplementation period (fortnightly morbidity)

Morbidity data were obtained for all study children except for 54 children (26 from iron and 28 from placebo) who were lost to follow-up. The frequency of probable malarial episodes among children in the two groups is shown in Table 25. A total of 279 (68.9%) children in the iron group and 225 (58.9%) children in the placebo group were reported to have experienced at least one episode of fever, RR=1.17 (95% CI: 1.05-1.30); ($\chi^2=8.09, p=0.004$).

Table 25 Distribution of children with at least 1 episode of illness suggestive of malaria during 12 weeks of supplementation

	TREATMENT GROUP		RR(95% CI)	χ^2	<i>p</i>
	IRON (N=405)	PLACEBO (N=382)			
malarial episodes	no. (%)	no. (%)			
Fever	279 (68.9)	225 (58.9)	1.17(1.05-1.30)	8.09	0.004
Ill feeling	215 (53.1)	171 (44.8)	1.19(1.03-1.37)	5.44	0.019
Chill/rigor	154 (38.0)	114 (29.9)	1.27(1.04-1.55)	5.73	0.016
Generalised pain	139 (34.4)	98 (25.7)	1.32(1.06-1.64)	6.51	0.010

The incidence rates of episodes suggesting malarial illness are shown in Table 26. As indicated in the table, a total of 708 and 611 fever episodes occurred among children in the iron and placebo groups in a follow up period of 4860 and 4584 person-weeks respectively. Thus, the calculated incidence rates for febrile episodes among children in the two groups were 14.6% and 13.3% respectively with a rate ratio of 1.09 (95%CI: 0.99-1.21), ($\chi^2=2.91$; $p=0.08$).

Table 26 Incidence rates for episodes of suspected malaria per person-week (pw) among children during supplementation

	TREATMENT GROUP				RR	95%CI	χ^2	p
	IRON (N=405)	PLACEBO (N=382)	no.	rate /pw				
malarial episodes								
Fever	708	0.15	611	0.13	1.09	(0.99-1.21)	2.9	0.08
Ill feeling	431	0.09	339	0.74	1.20	(1.05-1.37)	6.6	0.01
Chill/rigor	288	0.06	209	0.05	1.30	(1.09-1.55)	8.6	<0.01
Generalised pain	203	0.04	137	0.03	1.40	(1.13-1.73)	9.3	<0.01

The distribution of febrile episodes (Table 27) among the two groups of children showed that those in the iron group experienced more episodes compared with those in the placebo, ($\chi^2=10.3$; $p=0.029$).

When the duration of fever was investigated it was noted that iron supplemented children had experienced a total duration of 2841 days of fever against 2168 days among placebo supplemented children. Thus, the mean duration of illness was 4.01 and 3.54 days per an episode among iron and placebo groups respectively. Analysis of durations of febrile episodes by grouping into 3-days showed no significant difference between children in the two groups, ($\chi^2=2.32$; $p=0.88$) (Table 28).

Table 27 **Distribution of reported fever episodes among children during 12 weeks of supplementation**

number of episodes	IRON (n=405)		PLACEBO (n=382)	
	number	%	number	%
0	126	(31.1)	156	(40.8)
1	81	(20.0)	57	(14.9)
2	82	(20.2)	59	(15.4)
3	50	(12.3)	46	(12.0)
≥4	64	(15.8)	64	(16.7)
$\chi^2=10.73$; ($df=4$) $p=0.029$				

TABLE. 28 Distribution of children by mean duration (days) of febrile episodes over the supplementation period

(N=279 in the iron group and 225 in the placebo)

Treatment group	Mean duration of fever in days							TOTAL
	1-3	4-6	7-9	10-12	13-15	16-18	≥19	
IRON	63	55	47	25	25	22	42	279
	(22.6)	(19.7)	(16.8)	(8.9)	(8.9)	(07.9)	(15.0)	(100.0)
PLACEBO	52	40	40	26	22	14	28	225
	(23.1)	(17.8)	(17.8)	(11.6)	(09.8)	(06.2)	(12.4)	(100.0)
All children	115	95	87	51	47	36	70	504
	(22.8)	(18.8)	(17.3)	(10.2)	(9.3)	(7.1)	(13.9)	(100.0)
$\chi^2=2.32; (df=6) p=0.88$								

3.5.2. Hospital visits and medical help during supplementation

During the twelve weeks of supplementation a total of 94 women, 46 from the iron and 48 from the placebo group sought medical help from the hospital for a variety of health conditions and diseases. Similarly, 73 children, of whom 41 were from the iron and 32 from the placebo group made visits to the hospital. The distribution of diseases and categories of conditions diagnosed among women and children who attended the hospital is summarised in Table 29. The proportions of women in the iron and placebo group who obtained medical help from the hospital were 12.0 % and 12.9% respectively ($\chi^2=0.07$; $p=0.79$). The usage of hospital services by children was also very similar among those in the iron and placebo groups (10.1% and 8.4% respectively), ($\chi^2=0.52$; $p=0.47$). The morbidity pattern among women and children who visited the hospital during the supplementation period was similar in the two groups. The distribution of diseases (Table 29) showed no significant difference between supplementation groups.

Table 29. Distribution of diseases diagnosed in the hospital during the trial

	women		children	
	iron (n=382)	placebo (372)	iron (n=405)	placebo (n=382)
no. (%) of hospital visitors	46 (12.0)	48 (12.9)	41 (10.1)	32 (8.4)
Reason for visit (Diagnosis)				
malaria	24	24	41	32
PUO*	8	6	--	--
ARI	-	-	20	16
other respiratory	3	3	20	16
diarrhoea	5	6	37	31
GUTI**	24	10	--	--
other GI***	20	14	36	36
ob/gy ****	13	9	--	--
all other illnesses	39	36	22	15

* PUO= fever of unknown origin

** GUTI=genito urinary tract infections

*** other Gi= gastro intestinal problems including intestinal parasitic infections

**** ob/gy- obstetric and gynaecological disorders

3.6. POST SUPPLEMENTATION RESULTS

3.6.1. Haematological measurements

Women and children who successfully completed their respective treatment schedule were re-evaluated to measure changes in haematological parameters that may have resulted from supplementation with iron. Results for women and children are presented separately.

3.6.1.1. Haematological status of women after supplementation

A total of 737 women completed supplementation, an overall response rate of 94.9%. Three hundred and seventy-four (95.7%) of the iron and three hundred and sixty-three (94.3%) of the placebo treated women had their haemoglobin and haematocrit levels measured following supplementation.

Haematological findings after supplementation are shown in Tables 30 and 32. More women in the iron than in the placebo group had an improvement in their haematological status. Mean haemoglobin (sd) were 9.93 (1.52) g/dL and 8.82 (1.46) gm/dL in the two groups respectively ($t=10.1$, $p<0.0001$). Haematocrit measurement showed a similar pattern of improvement with means (sd) of 32.6 (5.4) % for women in the iron group and 29.9 (4.9)% for those who received placebo ($t=7.9$, $p<0.0001$).

Prevalences of severe anaemia were 5.1% and 13.5% in the iron and placebo groups respectively, ($\chi^2=14.6$; $p<0.001$). More women in the iron group fell into the category of mild anaemia (Hb 9.6-11.9 g/dL) or normal haematological status (Hb ≥ 12.0 g/dL) than did women in the placebo group. Women with mild anaemia (Hb of ≥ 9.6 g/dL) improved significantly from 22.3% at baseline to 52.9% after supplementation, compared with women in the placebo among whom there was little improvement, an increase from 21.6% to 29.2%. There was a highly statistically significant difference between the proportion of women in the two supplementation groups who had either mild anaemia or normal Hb level, 62.5% in the iron group and 30.6% in the placebo group, ($\chi^2=75.7$; $p<0.0001$). The improvement of anaemia following iron supplementation was further demonstrated by the haematocrit levels shown in Table 31. When the haematocrit level was used to examine the response to iron, the pattern emerged very similar to that shown when haemoglobin level was used.

Table 30 Haemoglobin level of women before & after supplementation

Haemoglobin (g/dL) severity	IRON		PLACEBO	
	before (N=391)	after (N=374)	before (N=385)	after (N=363)
< 7.1 severe	70 (17.9)	19 (5.1)	63 (16.4)	49 (13.5)
7.2-9.5 moderate	234 (59.8)	121 (32.4)	239 (62.1)	203 (55.9)
9.6-11.9 mild	87 (22.3)	198 (52.9)	83 (21.6)	106 (29.2)
>12 normal	0 (0.0)	36 (9.6)	0 (0.0)	5 (1.4)
Mean Hb(sd)	8.5 (1.3)	9.9 (1.5)	8.6 (1.4)	8.8 (1.5)
	<i>t=13.8 p<0.0001*</i>		<i>t=1.89 p=0.06**</i>	

* comparison between baseline and post supplementation Hb in the iron group

** comparison between baseline and post supplementation Hb in the placebo group

Table 31 Haematocrit levels of women before and after treatment

Haematocrit (%) severity	IRON		PLACEBO	
	before (N=391)	after (N=374)	before (N=385)	after (N=363)
<21.6 severe	24 (6.1)	6 (1.6)	35 (9.0)	15 (4.1)
21.7-28.8 moderate	168 (43.0)	82 (21.9)	138 (48.8)	131 (36.1)
28.9-36.0 mild	157 (40.1)	198 (52.9)	180 (46.8)	195 (53.7)
>36.0 normal	42 (10.7)	88 (23.5)	32 (8.3)	22 (6.1)
Mean Hct(sd)	29.2 (5.4)	32.6 (5.4)	29.2 (5.4)	29.6 (4.9)
	<i>t=8.7 p<0.0001*</i>		<i>t=1.05 p=0.28**</i>	

* comparison between baseline and post supplementation Hct in the iron group

** comparison between baseline and post supplementation Hct in the placebo group

The effect of iron supplementation on individual women was assessed further by comparing values measured at baseline with values at the end of supplementation. Marked changes in haemoglobin were seen in favour of women supplemented with iron compared with the changes obtained in those supplemented with placebo (Table 33). Nearly half, 45.5%, of placebo treated women did not show any improvement, compared to only 17.4% of women in the iron group who did not show a positive change after supplementation. The overall gain in haemoglobin was markedly higher among the iron supplemented women than among those in the placebo group, ($\chi^2=115$, $p<0.0001$). Women who were supplemented with iron gained on average 1.43 g/dL of haemoglobin compared to an average of only 0.28 g/dL gained by those who received placebo, ($t=12.5$, $p<0.001$). The benefit of iron treatment can also be seen from the marked difference of the proportion of women whose haemoglobin level increased by ≥ 2.1 g/dL, 28.3% and 5% among those in the iron and placebo treatment groups respectively. Haematocrit levels after supplementation showed a similar picture (Table 31).

Table 32 Gain in haemoglobin among women after supplementation

haemoglobin	Iron (N=374)	placebo (N=363)
gain (g/dL)	no. %	no. .%
no change	65 17.4	165 45.4
0.1-1.0	75 20.0	106 29.2
1.1-2.0	119 31.8	94 20.4
≥2.1	115 28.3	18 5.0
Mean Hb change (g/dL)	1.43	0.28
<i>t=12.6, p<0.001</i>		

The degree of anaemia observed at baseline also changed dramatically among women in the iron group compared with those treated with placebo. At baseline, severe anaemia (Hb < 7.1 g/dL) was of similar magnitude among women in the iron and placebo treatment groups, 17.9% and 16.4% respectively. After supplementation, however, the proportion of iron treated women with severe anaemia decreased to 5.1% while the placebo group remained with slight decrease at 13.5% (Tables 31 and 33).

Table 33 Distribution of women and change in the status of their anaemia after supplementation

Anaemia at baseline	Anaemia after supplementation				Total
	severe (Hb <7.1)	moderate (7.2-9.5)	mild (9.6-11.9)	normal (≥12.0)	
severe (Hb <7.1)					
iron	10 (2.7)	45 (12.0)	14 (3.7)	0 (0.0)	69 (18.4)
placebo	28 (7.7)	30 (8.3)	0 (0.0)	0 (.0.0)	58 (16.0)
moderate (7.2-9.5)					
iron	9 (2.4)	74 (19.8)	127 (34.0)	15 (4.0)	225 (60.2)
placebo	21 (5.8)	146 (40.2)	59 (16.2)	0 (0.0)	226 (62.3)
mild (9.6-11.9)					
iron	0 (0.0)	2 (0.5)	57 (15.2)	21 (5.6)	80 (21.4)
placebo	0 (0.0)	27 (7.4)	47 (13.2)	5 (1.4)	79 (21.8)
normal (≥12.0)					
iron	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
placebo	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total					
iron	19 (5.0)	121 (32.4)	198 (52.9)	36 (9.6)	374 (100)
placebo	49 (13.5)	203 (55.9)	106 (29.2)	5 (1.4)	363 (100)

The response to iron supplementation by women with severe anaemia at baseline was shown to be significantly higher than that seen in those who had either moderate or mild anaemia. The mean haemoglobin rise in women with severe anaemia at baseline was almost 2.0 g/dL compared with rises of 1.662 g/dL in those with moderately severe anaemia and 1.087 g/dL in those who were mildly anaemic. When response was examined by severity (Table 33), it clearly shows that those who had severe anaemia before supplementation showed the highest response than those who had either moderate or mild degree of anaemia. Furthermore, those with moderate anaemia responded better than those with mild anaemia at baseline. The distribution of the mean haemoglobin rise in relation to the severity of anaemia at baseline among women in the two supplementation groups is shown in Table 34.

Table 34. Mean haemoglobin gain among women with different levels of anaemia

mean haemoglobin gain in g/dL		
anaemia at baseline	iron	placebo
severe	1.99	0.76
moderate	1.66	0.73
mild	1.09	0.32
Overall Hb gain(g/dL)	1.43	0.28

3.6.1.2. Pregnant women

A total of 91 pregnant women were studied and were allocated to receive supplementation, (49 on iron and 42 on placebo), and 85 (46 on iron and 39 on placebo) completed their respective treatment. Pregnant women had a relatively high level of haemoglobin (Hb 8.0-11.0 g/dL) at entry to the supplementation trial as the criteria, particularly the haemoglobin level, used to select pregnant women was slightly different from that used in non-pregnant women.

The response to iron supplementation among pregnant women, examined separately, showed that those in the iron group achieved higher haemoglobin levels and improved their anaemia compared with those in the placebo group ($\chi^2 = 6.46$; $p < 0.05$). However, because the number of pregnant women allocated into the two treatment groups was small, it was not possible to compare the level of response with that of non-pregnant. The distribution of pregnant women by severity of anaemia and supplementation group is shown in Table 35.

Table 35 Haematological response of pregnant women compared with values at baseline

Haemoglobin (g/dL) severity	baseline	post-supplementation	
	all pregnant (N=91)	iron (N=46)	placebo (N=39)
8.0-8.9	24 (26.4)	3 (6.5)	6 (15.4)
9.0-9.9	61 (67.0)	14 (30.4)	19 (48.7)
10.0-10.9	6 (6.6)	27 (58.7)	14 (35.9)
≥11.0	0 (0.0)	2 (4.3)	0 (0.0)
		$(\chi^2 = 6.46; p < 0.05)$	

3.6.1.3. Haematological status of children after supplementation

Although children aged 6-84 months assigned to the iron and placebo supplementation groups had a similar iron status and degree of anaemia at baseline, the picture changed markedly after supplementation. Children who were supplemented with iron showed a pronounced gain in their haemoglobin compared with those in the placebo group.

Following supplementation, the mean (sd) Hb of iron supplemented children was 9.68 g/dL (1.2) compared with a mean haemoglobin of 8.52 g/dL (1.2) among those in the placebo group. The distribution of children in each supplementation group by severity of anaemia is shown in Table 36.

Children on iron supplementation improved their haemoglobin by an average of 1.38 gm/dL compared to the 0.22 g/dL gained by children in the placebo group (Table 37). The proportion of children in the placebo group whose haemoglobin did not improve was 56.1% compared with only 6.5% of children supplemented with iron ($\chi^2=209$; $p < 0.0001$). The proportions of children in the iron and placebo groups who gained a haemoglobin concentration of more than 1.0 g/dL were 67.9% and 13.7% respectively ($\chi^2=224$; $p < 0.0001$).

The mean Hb rise was also higher in those children who had severe anaemia at baseline compared with those with moderate or mild anaemia. For iron supplemented children the mean Hb rise was 1.73, 1.50 and 1.10 g/dL in those with severe, moderate and mild anaemia respectively. The distribution of children by severity of anaemia and supplementation group shown in Table 39 demonstrates further that the response to iron was relatively higher in those who were severely anaemic at baseline compared with those who were either moderately or mildly anaemic.

Table 36. Distribution of children by degrees of anaemia before & after supplementation

degree of anaemia(Hb g/dL)	TREATMENT GROUP			
	IRON		PLACEBO	
	before (N=410)	after (N=368)	before (N=391)	after (N=374)
severe (< 6.5)	49 (12.0)	1 (0.3)	50 (12.8)	28 (7.5)
moderate(6.6-8.7)	211 (51.5)	82 (22.3)	191 (49.0)	179 (47.9)
mild(8.8-10.9)	150 (36.6)	218 (59.2)	149 (38.2)	150 (40.1)
normal(> 11.0)	0 (0.0)	67 (18.2)	0 (0.0)	17 (4.5)
Mean Hb(sd)	8.3 (1.2)	9.7 (1.2)	8.3 (1.3)	8.5 (1.3)
	<i>t=16.1 p<0.0001*</i>		<i>t=2.1 p=0.03**</i>	

* comparison between baseline and post supplementation values in the iron group

** comparison between baseline and post supplementation values in the placebo group

Table 37 Gain in haemoglobin among children after supplementation

haemoglobin (g/dl)	Treatment group	
	Iron (N=368) no. %	placebo (N=374) no. %
no change	24 6.5	210 56.1
0.1-1.0	94 25.5	113 30.2
1.1-1.9	170 46.2	47 12.6
≥2.0	80 21.7	4 1.1
Mean Hb change (g/dL)	1.38	0.22
	<i>t=21.3 p=<0.0001</i>	

The distribution of haematocrit levels (Table 38) also indicated that a significantly higher level was achieved by those in the iron group from a mean haematocrit level of 26.4% at baseline to 31.6% after supplementation ($t=13.8$; $p<0.0001$) compared with 26.4% to 28.6% in those in the placebo group ($t=1.95$; $p=0.06$). The overall pattern of response of children to oral iron supplementation was very similar to that observed in women.

Table 38. Haematocrit levels of children before and after supplementation

Haematocrit (%) severity	IRON		PLACEBO	
	before (N=410)	after (N=368)	before (N=390)	after (N=374)
<21.6 severe	58 (14.1)	6 (1.6)	71 (18.2)	28 (7.5)
21.7-28.8 moderate	229 (55.9)	89 (24.2)	199 (51.0)	152 (40.6)
28.9-36.0 mild	117 (28.5)	231 (62.8)	111 (28.5)	173 (46.3)
>36.0 normal	6 (1.5)	42 (11.4)	9 (2.3)	21 (5.6)
Mean Hct(sd)	26.4 (4.3)	31.6 (4.6)	26.4 (4.7)	28.6 (4.9)
	<i>t=16.3 p<0.0001*</i>		<i>t=6.3 p=0.06**</i>	

* comparison between baseline and post supplementation Hct in the iron group

** comparison between baseline and post supplementation Hct in the placebo group

Table 39 **Distribution of children and change in their status of anaemia after supplementation**

Anaemia at baseline	Anaemia after supplementation				Total
	severe (Hb <6.5)	moder. (6.6-8.7)	mild (8.8-10.9)	normal (≥11.0)	
severe (Hb <6.5)					
iron	1 (0.3)	40 (10.9)	2 (0.5)	0 (0.0)	43 (11.7)
placebo	23 (6.1)	23 (6.1)	0 (0.0)	0 (0.0)	46 (12.3)
moderate ((Hb 6.6-8.7)					
iron	0 (0.0)	40 (10.9)	139 (37.8)	7 (1.9)	186 (50.5)
placebo	4 (1.1)	143 (38.2)	32 (8.6)	0 (0.0)	179 (47.9)
mild (Hb 8.8-10.9)					
iron	0 (0.0)	2 (0.5)	77 (20.9)	60 (16.9)	139 (37.8)
placebo	1 (0.3)	13 (3.4)	118 (31.6)	17 (4.5)	149 (39.8)
normal (Hb ≥ 11.0)					
iron	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
placebo	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total					
iron	1 (0.3)	82 (22.3)	218 (59.2)	67 (18.2)	368 (100)
placebo	28 (7.5)	179 (47.9)	150 (40.1)	17 (4.5)	374 (100)

3.6.2. Post-supplementation anthropometric status

After 12 weeks of supplementation, second anthropometric measurements were made in women and children to see whether nutritional status had changed during the supplementation period.

3.6.2.1. Anthropometric status of women after supplementation

A summary of anthropometric measures and selected indices before and after supplementation is shown in Table 40. Women in each treatment group did not show any significant changes in either weight or height or other anthropometric indices such as the BMI or WHR.

Table 40 Anthropometric indices of women before & after treatment

Anthropometric indices	Treatment Groups			
	Iron before (n=391)	Iron after (n=374)	Placebo before (n=385)	Placebo after (n=363)
Mean weight (kg)	48.2	48.5	47.4	48.1
Mean height (cm)	157.1	157.3	157.0	157.0
Mean waist circumference	73.1	73.3	72.2	73.1
Mean hip circumference	88.1	88.1	88.1	87.9
Mean Waist-Hip ratio*	0.831	0.832	0.821	0.850
Mean Body Mass Index**	19.5	19.6	19.2	19.7

* ratio between waist & hip circumferences in cm

** weight in kg divided by the square of height in meters

3.6.2.2. Anthropometric status of children

The nutritional status of 758 children (369 iron and 389 placebo) was assessed by anthropometry following completion of treatment. Children treated with iron or placebo had mean weight and height measurement of 13.4 kgs and 95.0 cms and 13.3 kgs and 93.6 cms respectively. Weight and height measurements of each child were adjusted for z-score values to obtain *weight-for-height*, *weight-for-age* and *height-for-age* indices. Post supplementation values for children in iron and placebo supplementation groups are shown in Table 41. No significant differences in any anthropometric measurement were found between treatment groups.

Table 41 Distribution of low weight-for-age and low height-for-age among children by supplementation group at the end of the supplementation

Z-score	IRON GROUP		PLACEBO GROUP	
	wt-for-age(%)	ht-for-age(%)	wt-for-age(%)	ht-for-age(%)
≥ -2 S.D.	245 (66.2)	280 (75.7)	269 (69.2)	280 (72.0)
< -2 S.D.	125 (33.8)	90 (24.3)	120 (30.8)	109 (28.0)
Total	369 (100.0)	369 (100.0)	389 (100.0)	389 (100.0)

3.6.3. HEALTH STATUS AFTER SUPPLEMENTATION

Following completion of supplementation with either iron or placebo, clinical assessment sessions similar to those undertaken at baseline were conducted to determine the health status of women and children.

3.6.3.1. General Health status after supplementation (Clinical Assessment)

3.6.3.1.1. *Women*

All women who completed treatment were assessed at the end of the period of supplementation. On the day of examination, 282(38.5%) women were identified as suffering from one or more illnesses of varying degree and severity. Those in the iron group were ill significantly more than those in the placebo treatment group (Table 42). The proportions of women with illness were 43.5% (158/363) and 33.9% (124/366) among the iron and placebo treatment groups respectively; RR=1.28, (95% CI:1.07-1.55), ($\chi^2=7.14$; $p=0.007$).

**Table 42 Post treatment status of women for selected diseases
(clinical assessment at the end of supplementation)**

Diseases	Iron (n=363)		Placebo (n=366)		RR (95% CI)	χ^2	p
	no.	(%)	no.	(%)			
Illness present	158	(43.5)	124	(33.9)	1.28 (1.1-1.5)	6.75	<0.001
anaemia	52	14.3	74	20.2	0.72 (0.5-1.0)	4.03	<0.05
malaria	88	24.2	67	18.3	1.32 (1.0-1.8)	3.49	0.06
GUTI*	13	4.1	10	2.1	1.31 (0.6-2.9)	0.20	0.65
All others**	65	17.9	50	13.7	1.31 (0.9-1.8)	2.16	0.14

*=Genito urinary tract infection

**=all sorts of diseases:

3.6.3.1.2. Children

A total of 747 children completed their 12 weeks of supplementation and were available for post-treatment evaluation. The overall response rate and compliance to treatment for children was 88.8%(747/841). At the end of supplementation 368(85.3%) children in the iron group and 372 (90.2%) children in the placebo group respectively were available for the clinical assessment.

Of those children who underwent clinical assessment, 386(52.3%) children were diagnosed as having one or more ailments. The distribution of children in the two supplementation groups by diagnosis of selected diseases is presented in Table 43; 63.4% of children in the iron group and 41.4% in the placebo group were diagnosed as having one or more diseases ($\chi^2=33.8$; $p<0.001$). Prevalences of anaemia, malaria, splenomegaly, acute respiratory diseases (ARI), diarrhoea and intestinal parasitic infections were compared. Malaria and splenomegaly but none of the other conditions were found significantly more frequently in children who had been supplemented with iron.

Table 43 General Health status of children after supplementation

Diseases	Treatment Group		RR (95% CI)	χ^2	<i>p</i>
	iron (n=366) no. (%)	Placebo (n=372) no. (%)			
Illness present	232 (63.4)	154 (41.4)	1.53 (1.3-1.8)	33.8	<0.001
anaemia	39 (10.7)	54 (14.5)	0.73 (0.5-1.1)	2.16	0.14
malaria	72 (19.7)	49 (13.2)	1.49 (1.1-2.1)	5.07	0.02
splenomegaly	100 (27.3)	71 (19.1)	1.43 (1.1-1.9)	6.36	0.01
ARI	47 (12.8)	44 (11.8)	1.09 (0.7-1.6)	0.08	0.72
Diarrhoea	45 (12.3)	50 (13.4)	0.91 (0.6-1.3)	0.15	0.72
intest. parasite	26 (07.1)	25 (06.7)	1.06 (0.6-1.8)	0.01	0.95
All other diseases	71 (19.4)	36 (09.7)	2.00 (1.4-2.9)	13.0	0.001

3.6.3.2. Clinical Malaria And Parasitaemia

3.6.3.2.1. Malaria in women after supplementation

On the assessment day, clinically apparent malaria was diagnosed among 155(21.3%) of all women, of whom 88(56.8%) were women who had received iron supplementation. Rates of malarial illness among women in the iron and placebo were 24.2%(88/363) and 18.3%(67/366) respectively, RR=1.32, 95% CI=1.0-1.76, ($\chi^2=3.83$, $p=0.05$). Estimates of clinical illness from malaria and other malarimetric indicators are shown in Table 44.

One hundred and ninety of a total of 737 thin and thick film examined were positive for one or more species of malaria parasite, an overall parasite rate of 25.7%. Nine slides from women who stopped treatment early were excluded and the parasite rate among 729 women who completed treatment was 24.8%(181/737). The parasite rate among women in the iron group was 29.4% (107/363), while it was 20.2%(76/366) among those in the placebo group, RR=1.47, 95% CI=1.14-1.90, ($\chi^2=9.21$; $p=0.002$). Mean parasite density was also calculated in both groups and shown to be 6,145 and 4,512 parasites per μl of blood among women treated with iron and placebo respectively but this difference is not statistically significant.

Table 44 **Distribution of malarimetric indices among women after supplementation (clinical assessment)**

Malarimetric measures	TREATMENT GROUP		RR (95%CI)	<i>p</i>
	Iron (n=363)	Placebo (n=366)		
Malarial symptoms & signs on examination day				
fever $\geq 37.5^{\circ}\text{C}$	103 (27.1)	67 (18.1)	1.7 (1.2,2.5)	0.001
headache	89 (23.8)	79 (21.8)	1.1 (0.8,1.6)	0.39
chills/rigors	56 (14.9)	43 (11.8)	1.3 (0.8,2.0)	0.17
back/joint pain	70 (18.7)	70 (19.3)	0.9 (0.7,1.4)	0.96
nausea/vomiting	103 (26.3)	88 (22.9)	1.2 (0.9,1.5)	0.21
Doctor's final diagnosis				
clinical malaria	88 (24.2)	67 (18.3)	1.3 (1.0,1.8)	0.06
Parasitological				
positive for parasite	107 (29.4)	74 (20.2)	1.5 (1.4,1.9)	0.04
parasite count / μl (GM)*	6,145	4,512	$t=1.37$, $p=0.17$	

* GM =geometric mean

3.6.3.2.2. Malaria in children after supplementation

Malaria was diagnosed in 72 children (19.7%) in the iron group and in 49(13.2%) in the placebo group on assessment at the end of the supplementation period, RR=1.49, 95% CI:1.07-2.08, ($\chi^2 =7.02$; $p=0.01$). Similarly, splenomegaly was detected more frequently among the iron supplemented than among the placebo supplemented children, 27.3% versus 19.1% respectively, RR=1.43, (95% CI:1.10-1.87), ($\chi^2=6.6$; $p=0.011$). A total of 776 blood films were collected. Thirty-six slides were either broken or found inappropriate for examination and were excluded. A total of 740 slides were examined and 228 were positive for one or more malaria parasites, an overall positivity of 30.8%. Positivity in 368 slides from children in the iron group was 34.5% against 27.1% positivity among 372 children in the placebo group, a rate ratio of RR=1.28 (95%CI: 1.03-1.58) ($\chi^2=5.01$; $p=0.02$). The geometric mean parasite count was higher among children in the iron group than among those in the placebo group, 15,059 and 8,224 parasites per μl of blood respectively and this difference was statistically significant ($t=2.4$, $p=0.01$). A summary of malariometric indices in children in the two supplementation groups is shown in Table 45.

Table. 45 Malarionetric indices among children after supplementation

Malarionetric measures	(N=368) freq.(%)	TREATMENT GROUP		χ^2	<i>p</i>
		Iron (N=372) freq.(%)	Placebo RR (95% CI)		
Symptoms and signs					
fever $\geq 37.5^\circ\text{C}$	97 (26.5)	67 (18.0)	1.43 (1.1,2.3)	7.01	0.008
Doctor's final diagnosis					
clinical malaria	72 (19.6)	49 (13.2)	1.49 (1.1,2.1)	5.07	0.022
splenomegaly	100 (27.2)	71 (19.1)	1.43(1.1,1.9)	6.57	0.011
Parasitological					
positive for parasite	127 (34.5)	101 (27.1)	1.28(1.0,1.6)	3.76	0.052
parasite count / μl (GM)*	15,059	8224	<i>t</i> =2.4, <i>p</i> =0.01		

* GM =geometric mean

Chapter Four

DISCUSSION

4.1 Introduction

As one of the universally accepted strategies to prevent iron deficiency and anaemia, iron supplementation has long been advocated and used widely, particularly in developing countries, where alternative approaches are either not available or difficult to implement. However, in recent years studies have indicated potential adverse effects of iron supplementation and underlined the need to reconsider intervention programmes which involve iron. The suspicion that iron supplementation can increase the risk of malaria in particular has led to a growing concern which laid the basis for the present study.

The present study showed that anaemia due to nutritional deficiency and secondary to malaria was highly prevalent among women aged 15-49 years and among children between the age of 6-84 months in a rural area of Ethiopia. After twelve weeks of oral iron supplementation, anaemic women and children significantly improved their haematological status.

The results of this study have also demonstrated that iron supplemented women and children had a higher malarial experience compared with those supplemented with placebo. The discussion presented in this chapter focuses on three main issues. Firstly, it discusses the nutritional history and eating habits of the study population in order to highlight the actual and potential sources of dietary iron in the study community. It also reassesses the prevailing view that iron deficiency is not a major cause of anaemia in Ethiopia. Secondly, the haematological response to iron supplementation by women and children is discussed and factors which may potentially determine the outcome of intervention programmes are identified. Finally, the adverse effects of oral iron supplementation on the health of women and children, particularly on morbidity from malaria are discussed. Lastly, the discussion describes briefly the continuing uncertainty about the explanations for the interaction between oral iron supplementation and increased malarial morbidity in order to stimulate further investigations.

4.2. Dietary iron, iron deficiency and anaemia

In Ethiopia anaemia has not been investigated thoroughly and the magnitude of the problem, its impact and causes are incompletely known. However, there are strong indications that anaemia is one of the major public health problems in the country (MOH, 1991; Zein and Assefa, 1987; Zein, 1991). The aetiology of anaemia is multifactorial, but iron deficiency due to an inadequate supply to an increased demand or to both has been recognised as the most common cause in developing countries (DeMaeyer, 1989; Royston, 1982; Cook and Finch, 1979) and this is likely to be the case in Ethiopia. Unlike other regions where genetic defects of red blood cells contribute to the aetiology of anaemia, such as thalassaemia in north Africa and the Middle East, and sickle cell trait in western Africa (DeMaeyer and Adiels-Tegman, 1985; Fleming, 1989; Facer and Jenkins, 1989), little is known about the contribution of these conditions to anaemia in Ethiopia. Although a few hospital based studies indicated the rarity or absence of genetic traits as causes for anaemia in the country (Perine and Tesfamichael, 1974; Abdulkadir, 1977; Shamebo, 1987), there is not enough evidence to exclude a role for genetic factors entirely.

In view of the wide distribution of parasitic diseases such as malaria and hookworm, and because the staple diet of most Ethiopians is usually considered to be rich in iron, there has been a long standing impression that anaemia in Ethiopia is likely to be secondary to infectious diseases. Hofvander (1968) considered repeated acute or chronic infections of various kinds to be the main causative factors for the generally low haemoglobin values that he observed. Zewdie (1993) stated that nutritional anaemia of iron deficiency was not a problem of public health significance and even suggested that to control anaemia attention should be paid to programmes for the control of malaria and hookworm and other parasites through personal hygiene and environmental sanitation.

Other investigators have taken a similar stand on the grounds that dietary iron intake in Ethiopia is higher than the recommended daily allowance (Gebremedhin *et al.*, 1976; Ross *et al.*, 1972). However, the allegedly high iron intake by Ethiopians has not been biochemically validated, and seems to have been based mainly on the fact that one of the staple foods in the country, the cereal *teff*, has a very high iron content (Hofvander, 1968; Besrat *et al.*, 1980). This view remains influential even today and dominates the formulation of policies and public health activities regarding the control of anaemia.

The amount of iron found in the cereal *teff* coupled with contamination of the seed with iron rich soil during threshing (Besrat *et. al.*, 1980) is undeniably very high. On the other hand the assertion that *teff* is a staple diet for the general population seems to be an over generalisation, at least until qualified by dietary studies. The cereal is staple for the urban population but there is little or no evidence whether this applies also to rural communities. In urban areas, *teff* often commands very high prices and this encourages farmers to produce it for the market rather than for their own consumption. In the present study area, a negligible proportion of households were found to have used this cereal in their regular diet because it is neither grown locally nor available at a price affordable by the population. The generalisation that equates the high iron content of *teff* with an adequate supply of dietary iron is perhaps too simple.

The adequacy of dietary iron depends upon its bioavailability: that is the amount of iron absorbed from the digestive tract rather than the content of iron in the diet. Because the efficient absorption of dietary iron depends on its chemical form, a higher iron content of the diet alone does not necessarily result in adequate absorption. Unlike *haem* iron, which is found in diets of animal origin, the bioavailability of *non-haem* iron in cereals such as *teff* is low (Hallberg, 1981). In addition other dietary factors are known to influence the absorption of *non-haem* iron in both directions. Absorption of *non-haem* iron is influenced markedly by individual and dietary factors; more iron is absorbed by iron-deficient and pregnant women and

also absorption of *non-haem* iron is also enhanced if eaten with meat, fish and foods rich in ascorbic acid (Hallberg, 1981). In spite of the ample livestock resources and the marked potential to produce fruits and vegetables, consumption of such food items was found to be extremely low in the study area.

The poor consumption of meat and other animal products has a serious implications not only because it is the source of an important form of *haem* iron, but also because it is a good enhancer of *non-haem* iron absorption. The main reason for the low consumption of animal based food items is mainly due to the absolute abstention from all food of animal origin observed for up to 150-200 days a year by Coptic Christians (Knutsson. and Selinus, 1970). In addition people rarely kill their own animals for consumption unless for occasions such as weddings and holiday festivals, because owning cattle and other domestic animals reflect prestige and status in the community.

The diet in the present study area and in most developing countries is by and large based on cereals and legumes. Cereals and legumes are not only poor sources of dietary iron but they also contain nutrients which inhibit the absorption of *non-haem* iron. Substance such as phytates, found commonly in cereals and legumes, are known to interfere with the absorption of *non-haem* iron (Monsen *et al.*, 1978; Hallberg, 1981).

Iron deficiency and anaemia was highly prevalent among populations in Iran whose average dietary iron intake far exceeded the recommended daily allowance (Haghshenass *et al.*, 1972). This was attributed to the presence of a considerable amount of phytates in their diet (wholemeal wheat bread). Even though the majority of the population from the highland regions and urban centers in Ethiopia base their diet on the iron rich cereal (*teff*), its high iron content does not ensure high iron intake and does not entail the absorption of the iron proportional to the amount ingested. The dietary pattern in the present study area was such that dietary iron intake was unlikely to be sufficient to reach the desired level due to both the lack of nutrients to enhance iron absorption and the high intake of foods which inhibit iron absorption. This may also be the reason for the finding of a high prevalence of iron deficiency anaemia in the *teff* growing areas of north-west highland regions of Ethiopia where the main diet of the population was *teff* (Zein and Mekonen 1987; Zein, 1991).

Haemoglobin and haematocrit measurements at baseline showed that anaemia was highly prevalent in the study population in general. Detection of a low haemoglobin or haematocrit level does not by itself distinguish anaemia of iron deficiency from other causes. The biochemical measurement which is often regarded as the one most specific for iron deficiency is measurement of the serum ferritin concentration (INACG, 1985). In the present study, serum ferritin measurements did not show the picture of iron status that would have been expected on the basis of the haemoglobin and haematocrit levels. Fewer women and children in the study had a ferritin level

below the cut off values of $<12 \mu\text{g/L}$ set to define iron deficiency. The reasons for this discrepancy could be that the anaemia was due to causes other than iron deficiency or that serum ferritin level were raised for some other reason. Several studies have shown that elevation of ferritin concentration can be induced by acute infections including malaria (Lipschitz *et al*, 1974; Adelekan and Thurnham, 1990).

Although not all subjects had a malarial illness or parasitaemia at the time that ferritin was measured, they may have been exposed to previous attacks or even have had undetectable parasitaemia. The likely explanation for the high level of ferritin found in the subjects in this study was malaria. In addition at the time of the baseline haematological and clinical assessment there was a high degree of morbidity due to a variety of causes among women and children which may have distorted ferritin values. Thus, relatively high ferritin values falling within the normal margin did not necessarily indicate that the level of stored iron was normal and did not rule out iron deficiency as the cause for anaemia.

In such circumstances, the role of iron deficiency as a cause of anaemia can be assessed by measuring the change in haemoglobin concentration following iron supplementation. This approach is considered a more reliable method for diagnosing iron deficiency anaemia in communities exposed to malarial transmission than biochemical measurements (Cardoso *et al.*, 1994).

Although different levels of response are used as a criterion for responsiveness in different studies, a haemoglobin response of ≥ 1.0 g/dL to a therapeutic trial of iron has been considered an adequate response which can serve as an indirect measure the presence of iron deficiency (Dallman and Yip, 1989). The substantial proportion of iron supplemented women and children who achieved that level of haematological response in the present study indicates that iron deficiency was responsible, at least in part, for their anaemia.

The results of the present study support those of previous studies (Hofvander, 1968; Zewdie, 1993) that show the importance of parasitic diseases to the problem of anaemia in Ethiopia. However, the evidence from this study has shown clearly that a deficiency in dietary iron intake is also important and that the value of *teff* containing foods in preventing iron deficiency may have been overstated.

4.3. Response to oral iron supplementation

Major practical problems face the health authorities of most developing countries who wish to control anaemia. Because of its relative simplicity, iron supplementation is the approach of choice that is being most widely adopted. Although results of almost all iron supplementation trials and intervention studies show the beneficiary effect of iron, there is still lack of clear cut policy guidelines on this approach to the control of anaemia. Thus, further work is needed to improve our understanding and build up our experience in this area.

Unlike iron supplementation studies in the past, which have focused largely on pregnant women or infants, pre-school or school children, the present trial was carried out among anaemic women of reproductive age and among their children. Anaemia was highly prevalent in women and children in the study area. Despite the meager health resources in Ethiopia, maternal and child health programmes are provided in most health facilities and these include supply of iron supplements when drugs are available. However, it is uncertain how much impact this exercise has on the prevalence of anaemia in the community. No previous study has been done to evaluate the outcome of iron supplementation and to document any health effects of such a practice.

This study is probably the first randomized controlled placebo trial in the country to report on the effect of supplementation and to quantify the response of anaemic women and children to oral iron.

Nearly all women and children in this iron supplementation trial who received iron for twelve weeks showed an increase in their haemoglobin concentration compared with values at baseline or with those found in women and children receiving placebo.

Women and children who received iron achieved an average rise in haemoglobin of 1.43 and 1.28 g/dL compared with a rise of only 0.28 and 0.22 g/dL in the placebo group. The maximum rise in haemoglobin was seen among those with severe anaemia compared with those who had either moderate or mild anaemia at baseline; this effect was observed in both women and children, but it was more marked in women. The mean haemoglobin rise among the severely anaemic was 1.83 times higher in women and 1.57 times higher in children compared to the increase seen in those who were only mildly anaemic. The likely explanation for the inverse relationship between severity of the anaemia and the higher level of response was due to mucosal absorptive mechanism which is known to be stimulated by lower iron balance to increase absorption of the ingested iron (Finch and Huebers, 1982).

Although not all supplemented women or children achieved normal haemoglobin levels (10.0% for women and 18.2% for children), there was a substantial shift towards mild and moderate as opposed to severe anaemia. It is particularly gratifying that the effect was most marked in those who were severely anaemic, to whom the change is expected to have made a real difference to their well being.

The overall response to oral iron shown in the present study was very encouraging with an implication of public health significance. However, the observed positive response could not be attributed to the iron supplementation alone. There was also a small but positive response observed in the placebo group though the reasons for this were unclear. It is likely that this study contributed to the general health care improvement such as free medical treatment. To a certain extent this improvement may have helped study subjects to improve their anaemia. Women and children in both supplementation group received antimalarial treatment during the trial when this was needed and this may have improved their anaemia (van-Hensbroek *et al.*, 1995) and partly explain the positive haematological response seen among those in the placebo group. The study was carried out in the rainy season when most people face a considerable food shortage because of price increases. As an alternative, people grow green vegetables in their back yards and tend to consume such food items more frequently than is the case during the dry season. The increased consumption of green vegetables which are good sources of ascorbic acid, may have enhanced the absorption of *non-haem* dietary iron contributing to the haematological recovery and

this could be another reason for the small improvement in haemoglobin seen among women and children in the placebo group.

The results of the present study are in agreement with those of previous trials which have almost all documented the positive effect of iron supplementation, although the magnitude of the response has varied considerably between different studies. It is still uncertain what level of response can be regarded satisfactory and what would be the maximum attainable effect of iron supplementation. It is difficult to estimate the net health effect per unit rise in haemoglobin, but Dallman and Yip (1989) have suggested that any rise in haemoglobin concentration following iron repletion with a physiological relevance could be considered adequate and they have rather arbitrarily set an increase in haemoglobin of ≥ 1.0 g/dL as a response of sufficient magnitude to warrant iron supplementation. In the present study, a large majority of both women and children supplemented with iron achieved a rise in haemoglobin level of ≥ 1.0 g/dL and in only a small number of women and children (21.9% and 9.2% respectively) was the haematological response <0.5 g/dL.

Important practical questions in relation to iron supplementation that remain to be answered include the following. Why was the level of effect in some trials much more substantial than that found in others? Why do large scale intervention programmes often not show the beneficial effect of iron seen in supplementation trials? What other factors need to be considered to achieve the maximum attainable haematological response in larger intervention programmes?

To answer these questions, there is a need to address other issues which potentially determine the success and/or failure of intervention programmes so that due attention will be given to the planning and execution of supplementation programmes at the community level. Even though clinical and epidemiological studies regarding iron supplementation may all seem similar there is substantial heterogeneity between them which ranges from the study set up, design characteristics to the procedures for data collection, all of which may account in part for differences in the outcome of each study. It is difficult but informative to examine the characteristics of major studies and to see what lessons can be learnt from these that could guide the planning of future intervention programmes. Most iron supplementation trials reported in the literature were based in hospitals or on out-patient services where the environment is relatively conducive to manage the progress of the study. In field situations many external factors, some of which are beyond the control of investigators, play a major role even in well designed investigations. In hospital based studies, subjects come to seek medical assistance and are readily accessible for the supplements and evaluation

while in field trials study subjects are dispersed throughout the community and may be lost to follow up for very many reasons. This situation is even more difficult in intervention programmes conducted on a larger scale. In addition, iron supplementation studies have often been carried out in a relatively small study population. About 40% of the studies on iron supplementation trials published in the past two decades were on a sample size of less than 30 subjects and nearly 80% of them studied ≤ 60 subjects (Sloan, 1992). The problem with such small studies is that the measured effect can be biased, either failing to detect the effect or imprecisely estimating its outcome. In designing the present study appropriate statistical procedures were applied to ensure that the sample size of both women and children was large enough to give adequate power to detect an effect.

Other variables in iron supplementation trials which are often considered to play a major role in the outcome of the study include those related to the formulation, dosage, frequency and duration of iron supplementation. Although the recommended dosage of oral iron supplementation shown in most medical text books is 60-120 mg of elemental iron for women and 3 mg/kg for children given daily for up to a duration of six months, previous studies have rarely continued for more than three months. The present study lasted for three months on the basis that about two-thirds of any haemoglobin deficit may be corrected in one month (Dallman, *et al.*, 1981) and that it may be completely overcome by two months (DeMayer, 1989).

Although prolonged supplementation will provide adequate iron to correct the deficiency, replete storage iron and maintain the iron balance (DeMaeyer, 1989) it is difficult to achieve in field situations and this seems to be one of the reasons why many trials have used supplementation schedules of shorter duration. Studies conducted for as long as the recommended duration are rare.

In an earlier study undertaken in Mauritius, the effect of 8 months of supplementation given to school children showed an average rise in haemoglobin of 1.61 g/dL (Stott, 1960), while such a rise has been achieved in several trials, including the present study, with three months or less of supplementation (Agrawal, et al., 1991; Cardoso *et al.*, 1994). Driggers (1981) has even found a mean haemoglobin response of as much as 2.3 g/dL among anaemic 1-year-old infants supplemented for three months. The daily administration of oral iron for three months in the present study may not have been optimal, but it was a realistic choice. Supplementation as a single dose per day minimized the inconveniency for subjects; more frequent doses might have adversely affected compliance and consequently the end results.

Iron supplementation schedules, particularly dosage and duration, are currently being reviewed and evidence in favor of low dose and short duration of supplementation is emerging. Animal studies have shown that iron in low dose given infrequently have similar effect to that which can be achieved by high dose given daily.

This is because of the short term suppressive effect of routine daily oral iron dosing on subsequent absorption, (Wright and Southon, 1990; Viteri, *et al.*, 1995), though views to the contrary also exist (Cook and Reddy, 1995). The effect of infrequent dosage of iron has similarly been observed in humans whose haematological responses were comparable to those resulting from daily iron supply. Thus, Indonesian pre-school children supplemented twice weekly achieved a similar response to those supplemented daily (Schultink *et al.*, 1995) and pregnant women who were supplemented weekly showed an effect similar to that seen in those who received iron daily under conditions resembling a normal antenatal care programme (Ridwan *et al.*, 1996). Moreover, prolongation of supplementation over one month brought about only minor additional haematological changes (Fogelholm *et al.*, 1994). If these new developments regarding supplementation schedules could be affirmed, the implications will be of paramount importance in public health (Stephenson, 1995) An improved supplementation schedules will not only improve the feasibility of interventions using iron supplementation, but also lead to standardization of procedures for evaluation of supplementation programmes. Supply of iron supplements as a strategy for controlling and preventing iron deficiency and anaemia is not without complications. Subjects to whom iron is administered, particularly if issued for an extended period of time, may experience undesired consequences which could potentially jeopardize the intervention itself.

Among the notable negative effects of iron supplementation are clinical side effects which are directly related to dose and duration of intake of iron (DeMaeyer, 1989). Side effects of iron medication has been the single factor identified most frequently as the reason for unsatisfactory supplementation trials because it leads to loss to follow up and poor compliance of subjects (Charoenlarp, *et al.*, 1988; Kuizon, *et al.*, 1983; DeMaeyer, 1989).

Failure of compliance does not necessarily imply failure of study subjects alone, but can also arise from factors such as the inadequacy of the service provider or investigators (Morrow, 1990; Galloway, 1994). To overcome this potential problem it is important to understand the behavioral and cultural characteristics of the community, to educate the study subjects and to maintain communications with them.

The study team for the present study, recruited locally, contributed to establishing a mutual understanding and relationship between the study subjects and the investigators. A survey conducted at the end of the trial showed that most study women and parents of children expressed their general satisfaction with the study and a large majority were convinced that they have benefited from it. Such a positive attitude and trust of the study team coupled with the continuous effort to encourage subjects may have helped to maintain the relatively high rate of compliance observed in this study.

The use of free medical treatment as an incentive for women and children, to most of whom medical services were unaffordable, was also an important factor used to build the close contact and sense of sharing between the investigators and study subjects. Such incentives may have encouraged them to remain in the study and helped to minimize the problem of compliance.

It would have been ideal if measurement of compliance could have been validated biochemically, but in field studies such as the present one, this is often not feasible. In this study, compliance was assessed on the basis of witnesses by field workers and on reports from subjects themselves. The problem of using this method of assessing compliance is that it cannot confirm that the supplements were taken in accordance to instructions for the entire duration of supplementation. In public health programmes, a similar effort to achieve compliance, particularly one using incentives, may not be feasible and such a high level of compliance may not be attainable.

4.4. Effects of oral iron supplementation on morbidity and malaria

One of the primary objectives of this study was to test the hypothesis that oral iron supplementation would increase the risk of malarial illness and other morbid conditions in women and children. Overall oral iron administration was found to increase morbidity and to increase risk of malarial illness among women and children in the study area.

Data obtained from the biweekly morbidity survey generally showed that women and children in the iron group reported more illness episodes than those in the placebo groups. The significant increase in episodes of illness, particularly febrile episodes, observed in the iron group during the supplementation period was likely to have been due to the iron supplementation. The mean duration of febrile episodes was also significantly longer in the iron compared with the placebo group. A problem with the evidence obtained from the morbidity survey is that the definition of illness episodes was based on questionnaires which included a list of symptoms and signs and the response to them, as perceived by study women and mothers/guardians of children. The questionnaire was designed to contain signs and symptoms which could best suggest specific diseases particularly malaria.

Nevertheless, the information obtained by this method may not be reliable enough to identify individual diseases accurately. Febrile episodes, for instance, are suggestive but not definitive indicators of malaria for there are many other conditions which can cause fever. The occurrence of fever and its duration during the two-weeks period was recorded as described by the respondents and validation by measuring the temperature was possible only for those episodes reported on the day of a home visit. Even if it is assumed that all febrile episodes were reported, it does not necessarily imply that these were all due to malaria. For example, in the Gambia it has been suggested that only 40% of fever episodes among children are attributable to malaria (Greenwood *et al.*, 1987b; Schellenberg, *et al.*, 1994). The frequency of visits can affect the estimation of the incidence of illness episodes, the longer the interval between visits the higher the chance of missing episodes occurring during this period. In a study in the Gambia (Snow *et al.*, 1989), it was shown that increasing the frequency of active surveillance by home visiting increased the sensitivity of detection. Monthly surveillance detected only 25% of the number of episodes of fever detected by weekly surveillance and weekly surveillance detected 74% of the number of episodes detected by daily surveillance. Similar findings apply to detection of episodes of diarrhoea- twice weekly visits are more reliable than once weekly. In the present study subjects were visited every other week and it was likely that illnesses occurring between visits were missed or under reported. For all of these reasons there were limitations on the reliability of the morbidity data which would potentially lead to misclassification or information bias.

However, such bias should have been randomly distributed among the groups to be compared and thus unlikely to distort the effect of the intervention.

Clinical re-evaluation of the health status of all women and children after supplementation revealed a higher disease burden among the groups supplemented with iron compared with the groups in the placebo. Women in the iron group had a 28.0% higher prevalence of an overall morbidity compared with that experienced by women in the placebo group. The general health status of iron supplemented women also deteriorated significantly from their own status at baseline whilst women in the placebo group showed a slight, but insignificant, improvement in the overall disease prevalence compared with that observed at baseline. A similar picture was also observed among children supplemented with iron who were found to have a higher overall morbidity than children in the placebo group suggesting the adverse health effect of oral iron supplementation. When distributions of individually diagnosed disease entities were compared, no clear pattern emerged between supplementation groups.

Iron supplementation did not show any statistically significant specific effect on common maternal conditions such as non-specific genito-urinary tract infections, menstrual disorder, skin and respiratory infections although each of these entities was diagnosed more frequently in the iron than in the placebo group.

The re-distribution of subjects into individual disease groups led to a depletion in the number of women with any one disease in each supplementation group and weakened the ability of the study to show statistical significance between groups. The morbidity situation in children at the end of supplementation was somewhat similar to that of women. As found in women, the difference in the overall prevalence of illnesses (all diagnosed diseases put together) was significantly higher among children in the iron supplementation than in the placebo group.

Further analysis of individual disease entities showed that children in the iron group were not particularly disadvantaged in respect to specific diseases except clinical malaria and splenomegaly which are discussed later in this section. The prevalence of acute respiratory infections (ARI) and intestinal parasitic infections were generally low among all children but were marginally higher in those in the iron group than in those in the placebo group while diarrhoea was observed slightly more frequently in the placebo children. Several previous studies have suggested the existence of an impairment of cell mediated immunity and decreased bactericidal activity in association with iron deficiency (Joyson *et al.*, 1972; Bhaskaram, 1975; Chandra, 1973; Strauss, 1978) and anaemia with a haemoglobin <10 g/dL (Srikantia *et al.*, 1976), which implies that iron deficient and anaemic children could be more susceptible to respiratory tract and diarrhoea infections. Children in the present study were all anaemic before supplementation but the prevalences of both respiratory and gastrointestinal infections did not seem to reflect their vulnerability.

The prevalences of both ARI and diarrhoea in the present study were not very different from findings among children in other parts of the country (Muhe, 1994), further evidence against the view that anaemic children were more prone to an increased risk of these infections. Furthermore, correction of the anaemia and deficiency with iron supplementation has been shown to lead to the reversal of these abnormalities and reinstatement of normal phagocytosis and bactericidal capacity (Chandra, 1973; Strauss, 1978; Walter *et al.*, 1986) and consequently, children in the iron supplementation group might have been expected to show a reduction in the rate of infection compared with those in the placebo had they initially been at increased risk.

One possible reason for the lack of an effect of iron supplementation on the prevalence of these conditions may be the adverse health effects of poor environmental conditions in all children. Poor environmental sanitation has been suggested as the reason why iron supplementation of school children in two villages in India failed to show significant differences in the rate of clinical attacks and duration of respiratory conditions and diarrhoea compared with control children (Damodaran *et al.*, 1979).

In contrast, a relatively recent study in Papua New Guinea demonstrated the adverse effects of iron supplementation on overall infections among two-month-old infants (Oppenheimer *et al.*, 1986a). Following an intramuscular iron-dextran injection,

infants had more respiratory infections than control infants leading to frequent hospital admissions and a prolongation of stay in hospital by an average of 4 more days or 60%. The findings from the present study seem in agreement with the experience of PNG infants in showing the association of iron not only with the overall disease burden but also with malarial illness and splenomegaly. Unlike ARI and diarrhoea, prevalences of malarial illness and splenomegaly were significantly higher among the groups supplemented with iron than among placebo groups in this study. The number of children with malaria and splenomegaly was larger than the number of children with ARI or diarrhoea and this may have contributed to the ability of the study to show an effect.

In the present study, iron was given orally to relatively older children with known anaemia, while the study in Madang, PNG, involved new born infants given intramuscular iron dextran irrespective of their haemoglobin status at birth. Therefore it is difficult to make comparisons between the studies because of age factors, differences in the iron preparation itself or a combination of both.

In an earlier study conducted on Somali nomads in Eastern Ethiopia, (Murray *et al*, 1978), the increased frequency of disease episodes among those who were given iron was believed to have been due to activation of pre-existing infections of malaria, brucellosis or tuberculosis. In supplementation trials of shorter duration, as was the case in the study on the nomads during which supplementation was given for only 30

days, re-activation rather than new infections may be the rule while in the present study it is possible that re-activation and new infections may have occurred since the duration of iron supplementation was longer than the incubation period of most infectious diseases.

The present study has shown women and children in the iron group were more at risk for malaria than in those in the placebo group. The prevalence of clinical malaria was increased by 32.0% in women and by 49.0% in children compared with the respective placebo group. Moreover, children in the iron group showed a 43.0% increase in the rate of splenomegaly compared with those given placebo. The findings of higher spleen rate among children in the iron supplementation group is in agreement with findings of previous studies among Gambian children (Smith *et al*, 1989) and infants in Papua New Guinea (Oppenheimer *et al.*, 1986b).

In the Gambian study, the proportion of children with an enlarged spleen was higher in the iron supplemented group compared with those in the placebo (21.7% against 15.1%), with a peak at the age of 2 years (41.7%) in contrast to the peak at the age of 4 years onwards observed in the present study. In Papua New Guinea (Oppenheimer *et al.*, 1986b) the rate ratios of splenomegaly in infants six and twelve months after iron treatment were similar to those found in the present study (1.51-1.56 for PNG infants and 1.43 for children in the present study).

Microscopic examination demonstrated that in the present study, more blood films from women and children in the iron group tested positive for malarial parasites than did those in the placebo group. The geometric mean parasite count followed the same pattern, being higher among women and children in the iron group than in the placebo, but with the difference between groups being significant only in children and not in women. In addition, parasite count of $\geq 5,000$ / μ l was observed significantly more frequently in women and children in the iron compared with the placebo groups, further consolidating the evidence of a deleterious effect of iron supplementation on malaria. This was in accordance with an observation in The Gambia (Smith *et al.*, 1989) which showed an association between iron supplementation and fever episodes with parasitaemia, particularly in those with parasitaemia of ≥ 50 parasites per 100 microscopic fields, a parasite density approximately equivalent to 5,000 per μ l. A trend towards a higher parasite was also observed in another study done in the Gambia in a group of 5-14 year old children even though there was no significant difference in the overall malarial incidence related to the administration of combined supplements containing iron (Bates *et al.*, 1987). In this study, children were given iron combined with other vitamins, which interact with each other, so it is not straight forward to assess the net effect of iron on malaria.

The intricacy of the association between iron supplementation and increased malarial risk is highlighted by the fact that there are clinical and epidemiological studies which found no increase in risk when iron was given. Harvey (1989) found no adverse consequences of iron supplementation among Papua New Guinean school children; there were no significant difference in parasite rate, parasite density or spleen rates between children in the iron and placebo groups. Similarly, the prevalence and severity of peripheral or placental malarial infection among multigravid pregnant women in the Gambia did not show any difference between the groups given iron or placebo (Menendez *et al.*, 1994). There is no clear cut explanation for the causes of what seems irreconcilable discrepancy between these results. Although most of these studies were similar in design, procedural variations between studies and differences in the malaria situation between study areas could at least partially form the basis for the differences in study outcomes.

The malaria pattern in different geographical regions and even within the same area varies greatly with different seasons influencing the immune status of the study population. The effect of iron on populations with a high level of immunity may not be as marked as would be expected among those with low or no immunity at all.

Although immunological assessment was not included in the present study, the level of antimalarial immunity of the study population was possibly low since the malaria in the area is unstable and very much seasonal. This may be one of the reasons for the demonstration of the adverse effect of iron on malaria not found in some other studies.

The far ranging literature on iron and health and diseases of humans demonstrates the extent of research in this topic. In spite of such a wealth of knowledge the present understanding about the role of iron in the pathogenesis of certain diseases of public health importance, malaria in particular, appears incomplete. Epidemiological and clinical studies, including the present one, have provided evidence for the link between iron supplementation and increased risk of malarial illness, but they are still unable to unravel the pathways of interaction. It is still unclear whether the increased malarial morbidity was due to the effect of the ingested iron on host factors or due to its effect on the parasite behaviour or a combination of both.

The role of iron in living cells has been investigated at length, iron is an essential metal for metabolism and replication of all living cells including the plasmodium and other pathogens. The mammalian host has established defence mechanisms by which the invading organism is denied the iron it needs. Under normal situations, where the balance of body iron is maintained, the host possesses an array of mechanisms to withhold iron from an invading agent (Weinberg, 1984).

The deployment of iron binding proteins such as unsaturated transferrin and lactoferrin withholds iron at the absorption site and transports it to the reticuloendothelial cells where it is stored in a complex with the protein ferritin. The host also launches an anti-inflammatory and a hypoferraemic response to an invasion by pathogenic organisms by sequestering iron in tissue forms and moving into temporary storage sites (Hersheko *et al.*, 1988). In both situations, the concentration of free circulating iron salts is close to none (Hersheko, 1987) denying readily available iron to the invading agents. This sequence of events led to the idea that iron deficiency plays a protective effect against infection (Weinberg, 1984) and because of its similarity to the mechanism of acquired immunity, the term 'nutritional immunity' was coined (Kochan, 1973). The nutritional immunity theory was further strengthened by the increased risk of developing bacterial infections observed in patients with iron overload. An example of the association of iron overload and infection was the finding of an increased incidence of hepatic amoebiasis among young Zulu males whose daily iron intake was considered excessive as the result of a regular consumption of home made beer contaminated with iron (Diamond *et al.*, 1978). Similarly, the Masai nomads in Kenya whose milk diet was normally iron deficient, showed a dramatic increase in the prevalence of amoebiasis following supplementation of iron sulphate for one year, from < 9.0% at baseline up to 83.0% after supplementation (Murray *et al.*, 1980). The amount of iron supply from an oral iron supplementation programmes, however, would normally be much less than the magnitude of iron overload observed among the Zulu men and the Massai nomads.

Iron supplementation is unlikely to lead to an overload of this magnitude or to lead to an excess free circulating unbound iron available to the pathogenic organism. It is therefore, highly unlikely that oral iron in the dose and duration of supplementation, as was in the present study, would meet the need of the plasmodium and seem improbable to be an explanation for the excess malarial morbidity.

Many pathogenic micro-organisms obtain iron by competing with human iron transport systems and manage to capture iron successfully from the iron restricted environment of the host. The pathogen scavenges for iron by producing specific iron carrier molecules, known as *siderophores*, which interact directly with receptors on bacterial cell surface (Gutteridge, 1994). Siderophores are iron-chelating compounds capable of extracting iron from the surrounding environment in order to deliver it to the invading pathogen, which otherwise would be unavailable. The ability of the invading pathogens to overcome the host iron-withholding mechanisms and to acquire the iron essential for their growth and replication is an important component of their virulence (Weinberg, 1984; Gutteridge, 1994). Other non-bacterial pathogens, such as the plasmodium, are probably faced with a similar host defence response and an iron restricted environment. However, it is still uncertain how the plasmodium obtains the growth-essential iron from the host, and it may be appropriate to review pertinent information generated from other fields of research.

Because iron derived from the host plays an essential role in the successful replication of many pathogenic organisms, including the plasmodium, ways of depriving the parasites of iron has increasingly attracted biomedical researchers. Several natural and synthetic chelating compounds with high affinity for iron have already been identified which might inhibit the growth of pathogenic organisms including the malarial parasite. If the mode of action of these compounds could be established this might clarify the pathways through which the parasite obtains the iron it needs and demonstrate how iron supplementation can increase susceptibility to infectious diseases. The inhibitory effect of iron chelating agents on the growth of *P. falciparum* has been demonstrated in several *in vitro* studies (Raventoz *et al.*, 1982; Peto and Thompson, 1986; Iheanacho *et al.*, 1990) and also *in vivo* studies (Yinnon, 1989). It has also been documented that iron chelation reduce *P. falciparum* parasitaemia in adults with asymptomatic malaria (Gordeuk *et al.*, 1992a) and when given in combination with the standard antimalarial therapy, enhances the parasite clearance and hastens the recovery from deep coma in children with cerebral malaria (Gordeuk, *et al.*, 1992b). In an earlier experiment to study the inhibitory effect of an iron chelating agent (desferrioxamide) on the growth of *P. falciparum*, the iron uptake by the parasite was postulated to be, at least partially, from transferrin (Pollack and Fleming, 1984).

In contrast, the antimalarial effect of the iron chelating agent is independent of the iron status of the host, implying that the parasite does not take up iron from serum transferrin (Peto and Thompson, 1986), an observation supported by others who suggested that the effect of the chelating agent was by penetrating the infected erythrocyte and interacting with a labile iron pool as the action neither resulted in the reduction of transferrin saturation nor interfered with erythropoiesis (Hersheko and Peto, 1988; Yinnon *et al.*, 1989). Others have suggested that iron chelators interfere directly or indirectly with iron dependent functions of the parasite but not necessarily with iron acquisition per se (Lytton, *et al.*, 1994).

In spite of the studies that have been done the actual mode of action of the various chelating agents has not yet been clearly defined as shown by the lack of unanimity regarding the proposed mechanisms. The way in which oral iron supplementation can lead to an increased malarial risk among the human populations remains unanswered.

Chapter Five

5. Conclusion and Recommendations

5.1. The study and its implications

5.1.1. The study area and population

Rural populations in Ethiopia and other developing countries are faced with complex health problems. A poor economic situation, a rapidly growing population, natural and man made disasters, excessive morbidity and mortality compounded with acute shortage of health resources are common features of these countries. The present study area was a typical example of a rural underdeveloped area where these human calamities coexist. The study area located in the far north-western Ethiopia, was, until very recently, the site of a protracted civil conflict which has left several thousands people dead or displaced. Health facilities which were scarce in the first place, were either destroyed or severely depleted to meet the needs of the population who had already been victims of recurrent famine and drought.

Under such circumstances, vector borne diseases and nutritional deficiencies are often highly prevalent. The area was, thus, appropriate for this study, as the study objectives required an area where malaria was endemic and a population for whom iron supplementation would be justified.

The major demographic characteristics of the study area were typical of developing countries with a large majority of the population aged less than 15 years and with a small proportion of older subjects (>55 years). The male population aged 10-30 years was smaller than that of females in the same age group, probably because of the long standing civil war which recruited young males forcefully or otherwise. There was no apparent reason for the smaller number of elderly subjects (> 50 years) except the short life expectancy which one would expect in communities of this type. In general, selection of the study area and its population was appropriate to meet the study objectives.

5.1.2. The overall strengths and limitations of the study

To assess the haematological responses to oral iron supplementation and to investigate whether or not the supplement was associated with an increased risk of malaria, a randomized placebo-controlled design was most appropriate as it allowed for the control of the effect of potential confounding factors. This design ensured the similarity of the two treatment groups being compared; the subjects allocated to iron and placebo groups were similar in all characteristics at baseline.

Anaemia and malaria are both known to affect people of all ages. Studying the effect of iron supplementation on all population groups would, therefore, have been ideal. However, the practical implications of studying the whole population, particularly in terms of resources, are substantial and this was not possible. Limiting the study to anaemic women and children was necessary not only because it was logistically manageable but also because the prevalence of both malaria and anaemia is higher in women and children than in other age groups in the total population.

In spite of the apparent strength in the design and the effort made to minimise measurement errors, executing this study was not without limitations. The main problems were definition of the outcome measures, particularly malaria and anaemia.

Although the criteria used to diagnose clinical malaria may depend on the purpose of the definition, epidemiologic studies usually employ a combination of malariometric indices generated from clinical, parasitological, and serological data. The malariometric indices used in the present study were generated from both objective and partially subjective data which included clinical assessment, parasitaemia and morbidity surveys.

Because of methodological problems the definition of malariometric indices of this study may not have been sensitive and/or specific enough to discriminate the disease very well. The uncertainty over the sensitivity and specificity of each of these measures could have affected the estimation of the magnitude of the effect and may have introduced a misclassification bias which could potentially reduce the validity of the end results. However, the misclassification bias that may have occurred in this study will be non-differential since, by design, it should be similar in the supplementation and control groups under comparison. The non-differential effect was therefore unlikely to have affected the relative effect between the comparison groups which implies that the observed association between iron supplementation and malaria was valid although it is possible that it was under-estimated.

The other limitation of this study was in relation to the assessment of iron status and anaemia among women and children. Two major problems surfaced at the start of the study. The first problem was related to the unpopular, often hostile, attitude of

women and parents of children towards drawing venous blood on which estimation of most parameters were first planned. This problem resulted in the modification of procedures to accommodate collection of blood using the capillary method which was more familiar and more acceptable. As the result, serum iron was not measured and ferritin levels could be determined only in a small number of women and a fewer number of children.

The second problem was the irregularity and unavailability of electric power which made it difficult to use some of the laboratory equipment and which resulted in the need to measure haemoglobin by the *Sahli* method. The *Sahli* method, which is simple to operate and which does not require electric power, is still used widely in Ethiopia and in other developing countries. However, the *Sahli* method is now being used less frequently to estimate haemoglobin levels in community surveys because of the measurement error related to the manual procedures involved in the technique. In spite of this limitations, however, the *Sahli* technique is still a valid method in circumstances such as in the present study, and it has been shown that it can perform well, particularly in monitoring haemoglobin changes and in screening for anaemia (Lerberghe, 1983).

5.1.3. Implications of results of the study

The results of the present study have implications for two issues which are very much related but with distinctive characteristics of importance for public health. Firstly, evidence from this study strongly suggested that dietary iron intake in the community was inadequate to meet the increased iron demands of women and children leading to a high prevalence of anaemia. This finding strongly contests the long-standing view that dietary iron insufficiency is not the cause of anaemia in Ethiopia. The realisation that iron deficiency is one of the main causes of anaemia will have a significant impact on existing health policies for the control and prevention of anaemia. The observed beneficial effect will bolster the existing use of iron supplementation in maternal and child health activities and will contribute to revitalise this exercise which at present is fragmented.

Secondly, findings of this study indicated that women and children experienced a significant malarial risk in association with oral iron supplementation. This observation potentially jeopardises the public health effort of controlling anaemia using this strategy. This finding is in agreement with previous research results which showed potential drawbacks of iron supplementation in relation to infections, including malaria. The debate on the role of iron in the pathogenesis of some diseases seems likely to continue.

5.2. Conclusion and recommendations

5.2.1 Conclusions

dietary iron deficiency and anaemia

In the present study area, anaemia among women and children was highly prevalent. The food composition and eating habits of the population strongly suggested inadequacy of dietary iron intake. In addition to the low content of iron in the diet, the chemical form of dietary iron was largely non-haem, which is less readily bioavailable. The insufficiency of dietary iron was exacerbated both by inadequate intake of food items which enhance iron absorption and high intake of nutrients which inhibit the absorption of the ingested iron. The overall evidence clearly showed that iron deficiency is an important contributor to the anaemia in the study population.

response to oral iron supplementation

Anaemic women and children in the study showed an excellent response to twelve weeks of oral iron supplementation. The effect of oral iron supplementation in improving the anaemia was substantially higher among those with severe anaemia than those with either moderate or mild anaemia.

In the wake of anaemia resulting from either nutritional deficiency or infection, oral iron supplementation remains an effective strategy to the control of anaemia in the study area.

oral iron supplementation and risk of malaria

Oral iron supplementation to women and children during the malaria season was associated with a considerably increased risk of uncomplicated malaria. Similarly, study subjects suffered increased morbidity and poor general health status in association with oral iron supplementation.

5.2.2. Recommendations

- In view of the enormity of the problem of anaemia among women and children in the present study area, there is an urgent need to give this topic a higher profile and to undertake measures to prevent it.
- The policy for the control and prevention of anaemia in Ethiopia needs to be reviewed so that appropriate strategic approaches can be adopted. The contribution of iron deficiency in causing anaemia, which appears to have been underestimated should be given more consideration in the policy making process.

- In the study area there was a huge agricultural potential which could be focused towards improving the nutritional and eating habits of the community. Although changing deeply rooted cultural and religious practices may be difficult, there were factors which could be exploited for improvement of dietary habits. The high level of knowledge and awareness of good sources of haem iron was a promising target for nutritional education.
- In situations where the problem of anaemia exists and strategies for its control such as food fortification with iron are unrealistic, oral iron supplementation is a relatively feasible and effective choice. However, the necessary precautions should be taken when implementing iron supplementation programmes in malaria endemic areas due to the fact that it is associated with increased malarial risk.

- The contradictory role of iron supplementation on the health and disease of populations demands an extra effort from health planners and programme managers to skilfully weigh the health advantages against the drawbacks of iron supplementation. In the present study area, the problem alleviated by iron supplementation clearly outweighs the problem of malaria attributed to iron. Iron supplementation to anaemic women and children should thus be promoted in parallel with an increased malaria control effort in the area.
- The research effort need to resolve the controversy surrounding the role of iron on malaria and other infections should continue. The investigation of the iron-infection interaction requires a comprehensive and multidisciplinary approach involving scientists in various disciplines of the health sciences. Studying the effect of iron supplementation on the severity of malaria in populations in areas of varying endemicity will be particularly important.

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APPENDICES

Appendix I. General census and household questionnaire

IRON SUPPLEMENTATION & MALARIA STUDY AMONG WOMEN AND CHILDREN IN METEMA (SHEHDI), NORTH GONDAR

General Census and Household Questionnaire (Form GCHQ/94)

100 - IDENTIFYING INFORMATION

101. village: 102. HH No:

HEAD OF THE HOUSEHOLD

103. Name: _____

104. Age (yrs)

105. Sex: [1=Male 2=Female]

106. Education: [1=None 2=Read/Write 3=Above 2]

107. Occupation:

[1=Farming 2=Trade 3=Labourer 4=Govt./NGO
5=Other _____]

specify

instructions for completing the following table:

1. List all household members, in age order, living in the house, including those who temporarily are absent.
2. Ask their age as accurate as possible. Help them remember birth dates by cross questioning and associating events and public holidays.
3. Write down name of wife or husband of the head next and continue with the eldest child until all children are registered.

MEMBERS OF THE HOUSEHOLD: Use the following codes to complete the table.

AGE: in years or months. Indicate (Y) for years, and (M) for months next to age.

SEX: 1=Male 2=Female

RH: Relation to the head of the HH. 1=wife/husband 2=son/daughter
3=grandchild 4=other

ED: Educational status aged >5yr. 1=none 2=read & write 3 =above 3

No	NAME OF MEMBER	ID NUMBER	A G E	S E X	R H	E D	REMARK
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

200. VITAL INFORMATION REGISTRATION

201. Any birth in past 12 months? [1=Yes, 2=No]

(If Yes to 201, complete 202 & 203 below)

202. Infant's age in months

203. Infant's Sex: [1=Male, 2=Female]

204. Any death in past 12 months? [1=Yes,2=No]

(If yes to 204, complete 205, 206 & 207 below)

205. Age at time of death: yrs mths

206. Sex of the deceased: [1=Male,2=Female]

207. Actual/probable cause of death: _____

DIETARY INFORMATION

300. crops :Are the following crops grown locally? [1=Yes 2=No]

301. Barley: 302. Wheat: 303. Corn: 304. Teff:

305. Lentil: 306. Millet:

307. Oilseeds: 308. Sorghum: 309. beans,peas:

310. Others (specify): _____

400. vegetables & fruits: Are the following vegetables grown locally? [1=Yes 2=No]

401. Leafy & green vegetables (cabbage, onion..):

402. Rooty vegetables (carrot, radish, potato..):

403. Fruits (orange, lemon, banana...):

404. Others (specify): _____

500. dairy products: Are the following items locally available? [1=Yes 2=No]

501. Milk: 502. Butter: 503. Cheese: 504.

Chicken: 505. Eggs: 506. Meat:

507. Others (specify); _____

600. special dietary provisions:

Are there special dietary provisions for the following groups? [1=Yes

2=No; If yes, specify in the space provided]

601. Newborn infants: _____

602. Children: _____

603. Pregnant women: _____

604. Other women: _____

700. special dietary prohibitions:

Are there special dietary prohibitions for the following?

[1=Yes 2=No; if yes, specify in the space provided]

701. Newborn infants: _____

702. Children: _____

703. Pregnant women: _____

704. Other women: _____

800. contents of meals on ordinary days

What were the main composition of your meals yesterday?

801. Fasting: 1. _____ 2. _____ 3. _____ 4. _____

802. Non-fasting: 1. _____ 2. _____ 3. _____ 4. _____

What did/will you eat yesterday for :

803. Breakfast _____

804. Lunch _____

805. Dinner _____

How frequent do you eat the following?

- 806. Meat/chicken/eggs in: 1/wk 2/wk ≥3/wk
- 807. Milk/cheese/curd in: 1/wk 2/wk ≥3/wk
- 808. Butter/oil/telba in: 1/wk 2/wk ≥3/wk
- 809. Vegetables in: 1/wk 2/wk ≥3/wk
- 810. Beans/peas in: 1/wk 2/wk ≥3/wk
- 811. Lemon/orange in: 1/wk 2/wk ≥3/wk

900. socio-economy, water & sanitation

- 901. Housing ownership: [1=Owned 2=Rented]
- 902. Number of rooms:
- 903. Roofing: [1=Thatched 2=Corrugated iron]
- 904. Source of drinking water:
[1=Spring 2=River 3=Pond/Lake 4=Well 5=Other]
- 905. Do you have latrine? [1=Yes 2=No]
- 906. Do you have land of your own? [1-Yes 2=No]

How many of the following do you have?

- 909. Oxen for ploughing: 910. Cattle (cows):
- 911. Sheep/Goat: 912. Horse/Mule/Donkey:
- 913. Other means of income: (specify) _____

NAME OF INTERVIEWER: _____

DATE OF INTERVIEW: ____/____/____/

Appendix II Clinical Assessment questionnaire

IRON SUPPLEMENTATION & MALARIA STUDY

Clinical Assessment Record **FORM-clinical-92/D-1**

DATE: (d|m|y):|_|_|||_|_|||_|_|

SESSION NUMBER:|_|_|

100. IDENTIFICATION

101. Village Id:|_|_| 102. HH Id:|_|_|||_|_|

103. Name:_____ 104. Category (W=women;C=children) |_|

105. Code No.:|_|_|_|_| 105. Age:|_|_|yr |_|_|mo 105. Sex |_|

200. Anthropometry: [weight in kgs & length in cms]

201. Weight: |_|_|_|. |_| 202. Height: : |_|_|_|.

203. Child mid-arm circumference(cf): |_|_|_|.

204 Woman's hip cf: |_|_|_|_|. |_| 205.

Woman's waist cf: |_|_|_|_|. |_|

Any present complaint: _____

300. On Examination:

301. Temperature in degree celsius: |_|_|_|.

*Mark **Y** for the presence and **N** for the absence of the following signs and symptoms.*

302. pale conjunctiva: |_|

303. sunken eye balls: |_|

304. ear discharge: |_|

305. flaring of alae nasa: |_|

306. wheezing: |_|

307. chest indrawing: |_|

308. abnormal heart sound and rhythm: |_|

309. abdominal distention (except pregnancy): |_|

310. enlarged liver (hepatomegaly): |_|

311. enlarged spleen (splenomegaly): |_|

312. pitting oedema of legs : |_|

- 313. skin rash (if any):
- 314. any abnormal signs of CNS:
- 315. any other clinically significant signs:

400. For pregnant women only:

- 401. height of fundus:
- 402. foetal position: _____
- 403. any vaginal discharge:
- 404. any vaginal bleeding:
- 405. any other pregnancy related abnormal sign:

500. DOCTOR'S DIAGNOSTIC REMARK: (Y/N)

- 501. Anaemia:
 - 502. if yes, 1=Mild 2=Moderate 3=Severe
- 503. Malaria:
 - 504. if yes, is it severe (coma, jaundice..etc.):
- 505. Splenomegaly:
- 506. Acute Respiratory Infection (ARI):
 - 507. if yes, is it severe (rpm 50+, chest indrawing):
- 508. Diarrhoea:
 - 509. if yes, is dehydration present:
 - 510. is dehydration 1=mild 2=moderate 3=severe
- 509. Pregnancy related illness:
 - 510. if yes, specify _____
- 510. Intestinal helminthic infections:
 - 511. if yes, specify: _____
- 512. Other disease(s):
 - 513. if yes, specify: _____

514. any clinical condition that jeopardise supplementation (Y/N)

515. if yes specify, _____

516. Any laboratory investigation to be made:

517. if yes, specify _____

518. Any treatment prescribed:

519. if yes, specify _____

DOCTOR'S NAME & SIGNATURE: _____

Appendix III. Biweekly morbidity survey Questionnaire.

IRON SUPPLEMENTATION & MALARIA STUDY

Biweekly Morbidity Survey Questionnaire, FORM BWKLY/94 (CHILD)

1. IDENTIFICATION INFORMATION

1.1 Village:|_|_|_|

1.2 House. no.: |_|_|_|_|

1.3. Name: _____ 1.4 Code. no. |_|_|_|_|

2. MEDICAL HISTORY

2.1 Any visit to health facility in last 2 weeks [1=yes 2=no]

2.2 If yes, reason for visit _____

2.3 Any medication given [1=yes 2=no]

2.4 If yes, specify medication _____

3. Symptoms & signs experienced by child in the past 2 weeks.

[1=yes 2=no], If yes, indicate duration in days.

3.1 Fever (being hot than usual)

3.2 Child don't play as usual

3.3 Seizure &/or rigors

3.4 Body pain (joint, back)

3.5 Running nose & sneezing

3.6 Cough

3.7 Wheezing while breathing

3.8 Fast & difficult breathing

3.9 Pain of the ear (hands on ears)

3.10 Discharge from ears

3.11 Loss of feeding interest

3.12 Nausea &/or vomiting

- | | | |
|------------------------------------|--------------------------|--------------------------|
| 3.13 Abdominal pain | <input type="checkbox"/> | <input type="checkbox"/> |
| 3.14 Diarrhoea, 3 or more per day | <input type="checkbox"/> | <input type="checkbox"/> |
| 3.15 Is diarrhoea bloody | <input type="checkbox"/> | <input type="checkbox"/> |
| 3.16 Swelling of lower legs | <input type="checkbox"/> | <input type="checkbox"/> |
| 3.17 Intestinal worms passed | <input type="checkbox"/> | <input type="checkbox"/> |
| 3.18 Any other complaint (specify) | _____ | |
-
-

4. INTERVIEWER'S ACTION:

- | | |
|--------------------------------|--|
| 4.1. Temperature (if recorded) | <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> |
| 4.2. Supplementation inspected | [1=yes 2=no] <input type="checkbox"/> |
| 4.3. Any advice or remark | _____ |

NAME: _____ Date: __/__/__/__ Visit No.

Appendix IV. Biweekly Morbidity Survey Questionnaire for Women

IRON SUPPLEMENTATION & MALARIA (ISMAL) STUDY

Biweekly Morbidity Survey Questionnaire- WOMAN

1. IDENTIFICATION INFORMATION

1.1 Village Id. | 1.2 House.no.

1.3 Name _____ 1.4 Code number

2. MEDICAL HISTORY (in the past 2 weeks)

2.1. Any visit to health facility? [1=Yes 2=No]

1.2. If yes, reason for visit _____

1.3. Any medication given [1=yes 2=no]

1.4. If yes, specify medication _____

1.5. Your last menstruation period in weeks

1.6. If lactating [1=Continued 2=Discontinued]

1.7. If discontinued, when? ____/____/____/

3. ILLNESSES EXPERIENCED BY THE WOMAN IN THE PAST 2 WEEKS

[1=yes 2=no], If yes, indicate duration in days.

3.1. Fever

3.2. Headache

3.3. Chills &/or rigors

3.4. Joint &/or back pain

3.5. Blurring of vision

3.6. Generalised body weakness

3.7. Breathlessness on exertion

- 3.8. Cough
- 1=No 2=Dry 3=Productive 4=Bloody sputum
- 3.9. Epigastric pain (burning)
- 3.10 Loss of appetite
- 3.11 Nausea &/or vomiting
- 3.12 Lower abdominal pain
- 3.13 Urgency & painful urination
- 3.14 Vaginal discharge
- 3.15 Vaginal bleeding
- 3.16 Swelling of lower legs
- 3.17 Any other complaint
- 3.18. (specify) _____

4. INTERVIEWER'S ACTION:

- 4.1. Temperature .
- 4.2. Supplementation inspection
- [1=Continued 2=Discontinued]
- 4.3. If discontinued, when? ___/___/___/
- 4.4. Any advice or remark _____

NAME: _____ DATE: ___/___/___/ VISIT NO. |___|

