THE EFFECT OF DAILY VERSUS WEEKLY IRON SUPPLEMENTATION IN THE CONTROL AND PREVENTION OF IRON DEFICIENCY ANAEMIA IN LACTATING MOTHERS: A CASE STUDY OF URBAN SLUMS IN ADDIS ABABA, ETHIOPIA.

BY

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ABSTRACT

This study was designed to take a sequential step, comprising a cross sectional study with a longitudinal observation, to demonstrate the biological effectiveness and social feasibility of weekly versus daily iron supplementation in the control and prevention of anaemia in women of reproductive age. It compares the haemoglobin, serum ferritin and compliance in Teklehaimanot *Wereda,* Ethiopia between March and May 2001. A total of 1017 lactating women were recruited, and screened clinically and biochemically for anaemia; out of which, only 207 of the women were found to be eligible and enrolled in this study. After an informed written consent was obtained from all the subjects, they were then assigned to the daily, weekly supplementation or control groups following randomisation.

The daily group (n=71) received 60 mg of elemental iron containing 300 mg ferrous sulphate with 400 µg folic acid from Monday to Friday, while the weekly group (n=68) received only one tablet of iron of the same content once a week, every Monday supervised by the field workers; and the control group (n=68) was advised to take no iron tablets or other drugs without the knowledge of the investigator until the completion of the study. Before the supplementation of iron, every subject was dewormed with 120 mg levamisole, and then baseline information on socio-demographic, nutritional status, and the appropriate laboratory tests taken from all groups.

To evaluate the outcome of the study, haemoglobin from all and serum ferritin from a sub-sample of the subjects were measured before and after the completion of the study; and compliance was observed every two weeks by the researcher together with the field workers. The mean age, household size, parity, frequency of meals, body mass index, education, employment status, baseline haemoglobin and serum ferritin levels of the groups was not significantly different between groups indicating that the groups enrolled were homogenous.

Ascariasis (17.9%), strongyloidiasis (2.4%), and hookworm (1.9%) were found to be the commonest infestation, and had a close association with low serum ferritin concentration (P=0.05) in the *Wereda*. To see the confounding effect of contraceptive, information was collected and analysed among the groups. The commonest (55.4%) type of contraceptives used was pills and injection and were nearly uniform in all the groups. No association was observed between contraceptive use and level of anaemia (P=0.5). Chronic energy deficiency in the *Wereda* was higher (27.0%) when compared with the figure for Addis Ababa Administrative region, which was (17.9%). Prevalence of anaemia, as determined by haemoglobin concentration in the *Wereda* was 22.3% and iron deficiency (determined by serum ferritin below 12 μg/litre) 22.6% and iron deficiency anaemia (determined by serum ferritin below 12 μg/litre and haemoglobin less than 12 gm/dL) 22.3%. Following the supplementation, significant increase in haemoglobin concentration and a three fold reduction in the prevalence of anaemia in both the daily and weekly supplemented groups were found. When the daily and weekly supplementations groups were compared, the therapeutic effectiveness was equally significant in both schedules. The reports of side effects observed in this study were related to the frequency of iron supplementation, with fewer side effects in the weekly supplemented than in the daily group. This would suggest that weekly supplementation schedule is better tolerated and confirms to achieve same results as daily schedule.

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DECLARATION

I, **JEMAL HAIDAR ALI,** hereby declare that this thesis is my original work and it has not been presented in any other universities.

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated above all to my honourable late mother, Amina Ebrahim, father, Haidar Ali, whom I lost while I was doing the course work. I will always remember their love and care during the good days, when they brought me up.

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ACRONYM

ACC = Administrative Committee On Coordination

ANP = Applied Nutrition Program

ATP = Adenosine Triphosphate

BF = Blood film

BMI = Body Mass Index

BPS = Board of Post Graduate Studies

CBISDO = Community Based Integrated Development Organization

CED = Chronic Energy Deficiency

CSA = Central Statistics Authority

EDTA = Ethylenediaminetetra-acetic acid

EHNRI = Ethiopian Health and Nutrition Research Institute

epg = Egg per count

FAO = Food Agriculture Organization

Hgb = Haemoglobin

HIV = Human Immune Deficiency Virus

I DA = Iron Deficiency Anaemia

IMCI = Integrated Management Of Child Illness

IMR = Infant Mortality Rate

INACG = International Nutritional Ac anaemia Consultative Group

IUCD = Intra Uterine Device

MCH = Maternal and Child Health

MOH = Ministry of Health

NGO = Non Governmental organization

O/P = Ova or/and Parasites

RBC = Red Blood Corpuscles

SCN = Sub-Committee For Nutrition

SDA = Supplemental Daily Allowance

SF = Serum Ferritin

SPSS = Statistical Package for Social Science

µg = Microgram

USD = United State Dollar

WHO = World Health Organization

OPERATIONAL DEFINITIONS

Anaemia... A deficiency in the size or number of red blood cells or the amount of haemoglobin they contain, which limits the exchange of oxygen and carbon dioxide between the blood and the tissue cells.

Assignment... Individual chosen for the study have equal chance of being assigned either to the study groups or control groups.

Body Mass Index (BMI)... It utilises both height and weight and provides a better measurement of thinness than weight alone; it is the ratio of weight (in kg) and height (in meter) expressed as kg/meter squared.

Compliance... In this study, it was defined as the proportion of distributed iron tablets that were consumed by the study subjects. Thus, during the three months course, the consumption of 12 tablets, in the case of weekly schedule or 6o iron tablets in the case of daily supplementation would represent 100% compliance.

Enhancers... Refers to formation of soluble complexes with iron preventing precipitation and polymerisation and thus, increase iron absorption.

Ferritin...The non-haeme compound in which iron is stored in blood serum, liver, bone marrow, and the spleen.

Folic acid ... Refers to all forms of heterocyclic compounds that are based on the pteroic acid skeleton conjugated with one or more glutamic acid molecules in gamma peptide linkage and that are nutritionally active in human.

Fortification... refers to the process whereby nutrients are added to foods to maintain or improve the quality of the diet of a group a community or population.

Haeme iron... The organic form in which iron occurs in meat, fish and poultry.

Haeme... The non-protein, iron protoporphyrin constituent of haemoglobin.

Haemoglobin...A conjugated protein containing four haeme groups and globin; the iron containing protein mainly responsible for transporting, storing and using oxygen throughout the body.

Household size... It is the total number of people living in a household during the study period. This doesn't include guests and residents in transit.

Household.... All the people who had lived together for more than three months and operate as a unit, including such members as unrelated servants, labourers, and relatives who share food from the same pot.

Hypochromic.... Characterised by deficient haemoglobin content of red blood cells.

Inhibitors...Refer to factors that inhibit the absorption of iron such as phytates from cereals, polyphones from tea, coffee, spinach, oregano, legumes and wine; calcium and phosphorous from milk and cheese.

Injera... A pancake like leavened bread, which is the commonest traditional fermented food made from cereal flour in Ethiopia.

Intrinsic factor (IF)... A glycoprotein, secreted by the gastric glands, that is necessary for the absorption of exogenous vitamin B_{12} by ileal cell surface receptors for IF-B_{12} complexes.

Iron Deficiency Anaemia... Is the result of depleted iron stores (serum ferritin < 12 µg/l), depleted transport and low haemoglobin (less than 12 gm/dl).

Iron overload... It is a positive iron balance. It is said to be present when the percent transferrin saturation is greater than 50 in women and 60 in men, and if the serum iron level exceeds 180 mg/dL or serum ferritin above 300 µg/l.

Kebele... The lowest administrative unit in the urban context by which, urban dwellers are being administered.

Malnourished mother... In this study, a malnourished mother of, a mother with BMI is less than 18.5% kg/meter squared.

Microcytic.... Characterised by smaller than normal erythrocytes and less circulating haemoglobin; characteristics of iron deficiency anaemia.

Non-haeme iron... Iron that is not a part of the haeme complex and that is present in foods, such as eggs, grains, vegetables and fruits; also present in small amounts in meat, fish and poultry.

Power... The ability of a test to detect a statistically significant difference or association in sample data if a true difference is present.

Slums... Areas in a city or town where badly built, old, deteriorated and overcrowded unplanned houses/buildings are found.

Supplementation... Refers to giving of iron in medicinal form. This is often the only feasible approach when there is a large iron requirement and a relatively short time span, as occurs during pregnancy.

Wereda... It is an administrative unit equivalent to a district.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background

Iron is one of the most important elements in nutrition and is of fundamental importance to life. As a constituent of haeme, it is present in haemoglobin, myoglobin and a variety of enzymes, and also more non-haeme iron enzymes (INACG, 1982; Beaton and Benoga, 1976). These various compounds sub serve many vital functions, which is always present when there is sufficient amount of iron in the body. Most tissues normally contain only small amounts of stored iron, but the hepatocyte and the elements of the reticuloendothelial system in the various organs contain large amounts and serve as the main repository of the body's iron reserve (INACG, 1982; Beaton and Benoga, 1976). The size of the body's iron is very variable, reflecting the adequacy of iron balance over an extended period of time. In iron replete men it reaches 1 gram while in women it is about 300 mg (INACG, 1982). The lower figure in women is an indication of the more precarious balance between the amount of iron, which can be absorbed and their increased physiologic requirements. These points emphasise the fact that when iron nutrition is less than optimal, it is the storage reserve that first becomes depleted. Although the amount of iron contained even in the most inadequate diet is considerably more than human nutritional requirements, iron deficiency remains the most commonly recognised of the nutritional deficiencies; while iron deficiency anaemia reaches its greatest prevalence and severity in developing countries, and is also frequently encountered in developed societies (INACG, 1982). The explanation for

this paradox lies in the poor bioavailability of much of the iron in present day diets. The prevalence of iron deficiency in any population is a function of the bioavailability of the iron in the average diet, but varying requirements for iron largely determine which members of the population are affected. In this regard, infants and children are at risk because of the increased requirements related to rapid growth. Likewise, women also need more iron than men because of the superimposed requirements related to menstruation, pregnancy and lactation. Therefore, both the prevalence of iron deficiency and its severity is considerably greater in women during their reproductive years than they are in men (INACG, 1977; INACG, 1982). When iron deficiency is widespread and severe, the prevalence of morbidity and the effects on the individual's resistance to infectious disease are significant (Joyson *et al, 1972*). Some of the effects of iron deficiency are decreased in white cell phagocytic function and cell-mediated immunity, which can induce and serve to perpetuate a cycle of worsening malnutrition and infection, and also decreased gastric secretion, and reduced activity of intestinal enzymes (Dallman , Sunshine and Leonard, 1967; Mohammed *et al* 1988). Although the magnitude of iron deficiency and iron deficiency anaemia in Ethiopia has not yet been well documented, it is believed to be emerging as a public health problem particularly in the low land areas. This could be partly attributed to intestinal and haemoparasites, particularly, to hookworm and malaria. The poor bioavailability compounded by intestinal parasites and malaria also heralds the high prevalence among the vulnerable group (Hofvander 1968).

The magnitude of iron deficiency throughout the world and its short and long term negative health effects make this study a priority. Hence, a community based iron

supplementation programme, which is affordable and sustainable, should be adopted as a short-term strategy.

1.2. Statement of the Problem

Iron deficiency anaemia was recognised to be a problem and highly prevalent in many developing countries as early as 1967 (WHO, 1968; WHO, 1972). It is the most common type of nutritional anaemia affecting young children and women of reproductive age (DeMaeyer, and Tegman, 1985; Herceberg, Galan and Dubin, 1987; Felming, 1989; Hathirat *et al* 1992; Marita *et al*, 1996; Djko and Muhilal, 1996; Muhilal, Iman and Kumari, 1996). The prevalence tends to be greater in populations subsisting on diets containing less meat and fish and large amounts of cereals and legumes. Even though the total iron content of such diets is usually adequate, inhibitors such as phytates and polyphenols in the foodstuffs limit the bioavailability of the iron (Chanoenlary, 1988; Zewdie, 1992). Many causes of iron deficiency anaemia have been identified. However, nutritional deficiency due to lack of bioavailable dietary iron accounts for over 50% of the cases (Beaton, and Benoga, 1976; Hovfander, 1968; Combs et al, 1996).

Ethiopia with its wide range of agro-climatic conditions grows a wide variety of cereals such as *Tef* (*Eragrostis Teff*), barley, maize, Sorghum and *Enset* (*Enset Ventricosum*). Some of the cereals consumed in the country are not fully exploited by the general population in the urban settings. Thus, there seems to be dependency on a single crop,

resulting in shortage of minerals and vitamins, which affects the nutritional status of the community.

Cereal grains such as *Tef* and barely are good sources of iron, whereas Sorghum and maize supply low amount of iron. The amount of iron content in *Enset* and milk staple diet is very low. Overall, the energy and protein supplied by some of the above cereal ranges from 334 to 366 kcal and 7.1 to 10.3 gm/100gm edible portion respectively (Ågren and Gibson, 1968). A nation-wide nutrition survey in 1980 showed that 95% of the dietary energy in Ethiopia comes from plants and only 5% from animal source (Zewdie, 1992). The main source of energy was from cereals. This implies that the bioavailability of much of the iron in the average Ethiopian diet is restricted.

Although, the nation-wide iron deficiency anaemia status has not been yet established in Ethiopia, a number of focal studies have attempted to determine the extent of the problem in specified areas (GebreMedhn, Killander, Vahlquist and Wuhib, 1976; Hofvander, 1968; Zewdie, 1992; 1999). These studies indicate that iron deficiency anaemia is not a problem of pubic health significance especially in parts of the country where the staple diet is *Tef* (*Eragrostis Teff*), which is rich in iron. In the low land areas, much of the problem was attributed to intestinal parasites, particularly, to hookworm and to malarial diseases.

Few in-depth studies based on the use of multiple parameters for assessing iron deficiency anaemia has been done, however, none of these studies have determined the iron deficiency anaemia status by type of the various staple food consumed in Ethiopia. Hofvander (1968) showed a high prevalence, i.e., 60.0% of anaemia, of

which, 17% was nutritional anaemia. This nutritional anaemia was attributed to the type of food consumed such as milk, bulky dietary gruel and high prevalence of intestinal parasites. A recent survey conducted on a limited scale in all the agro-ecological zones of the country from 1990 to 1993 indicated that about 18 in a hundred of the pregnant and lactating women surveyed appear to suffer from iron deficiency anaemia (Haidar, NekaTibeb and Urga, 1999). The study also showed that the extent of iron deficiency anaemia varied in each agro-ecological zone with dietary patterns of the community and also differed with and without parasitic disease prevalence. As an immediate measure where the prevalence of iron deficiency anaemia (IDA) is high, iron tablet is supplied to those women who have the access to proper antenatal check-ups, and the mode of supplementing the iron tablets were on three times daily basis. The ongoing intervention system in the country seems to reach the few women in the urban settings and ignores the majority of the rural women in the country. In addition, compliance is shown to be low and the continuing high prevalence of anaemia is an indication that they are not working well because of several major constraints such as inadequate supplies of iron supplements, and poor coverage of the rural population. Therefore, as an immediate measure where the problem is serious, the only hope of reaching the vulnerable group is through an appropriate iron supplementation intervention programme, which works satisfactorily, economically advantageous and has fewer complaints of side effects. The present study is the first step in this direction and will substantiate these findings in lactating women, with different dosage schedules (weekly versus daily) in urban slum communities of Ethiopia. The magnitude of iron deficiency throughout the world and its short and long term negative health effects make this study

a priority. Hence, a community based trial with oral iron supplementation, of different dosage levels and appropriate outcome measurement was proposed.

1.3. OBJECTIVES

1.3.1. General Objective

To demonstrate the biological effectiveness and social feasibility of weekly iron supplementation in preventing or treating iron deficiency anaemia in lactating women.

1.3.2. Specific Objectives

1. To determine the prevalence of anaemia before and after supplementation trial of iron on daily and weekly schedule.

2. To test the therapeutic effectiveness of daily vs. weekly doses of 60-mg elemental iron clinically and biochemically.

3. To assess the frequency of the side effects/ undesirable effects of iron in weekly supplementation compared with daily supplementation.

4. To determine the level of compliance for the daily and weekly iron administration schedule.

1.4. Hypothesis

1. A weekly dosage schedule of iron in lactating women will not bring a return towards haemoglobin normality as rapidly, or almost so, when they have moderate anaemia as daily dosage schedule.

2. There are no significant differences in compliance, between weekly doses and daily dosage schedule.

3. Weekly doses of iron given to lactating women will not raise serum ferritin levels perceptibly in iron-deficient anaemic lactating women, as does the daily dosage schedule.

1.5. Expected Benefits of the Project

If the results of these studies are positive, adoption of community or clinic-based weekly iron supplementation could be nationally recommended rather than daily supplementation as part of a control and prevention guideline for the nation. This would result in great savings and consequently allow much wider coverage of communities than at present.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. World View of Iron Deficiency Anaemia

Iron deficiency anaemia (IDA) is a widespread and important nutritional problem worldwide. It is estimated that 30% of the developing world's population suffer from anaemia (FAO, 1988; ACC/SCN, 1990; ACC/SCN, 1991). Pre-school children in Africa have some of the highest rates of nutritional anaemia in the world, nearly 56% (FAO, 1988). There are many possible causes of iron deficiency anaemia (Figure 1). The condition can arise from

(1) Nutritional deficiencies,

(2) Inadequate absorption,

(3) Inadequate utilisation,

(4) Increased excretion,

(5) Defective release of iron from iron stores into the plasma,

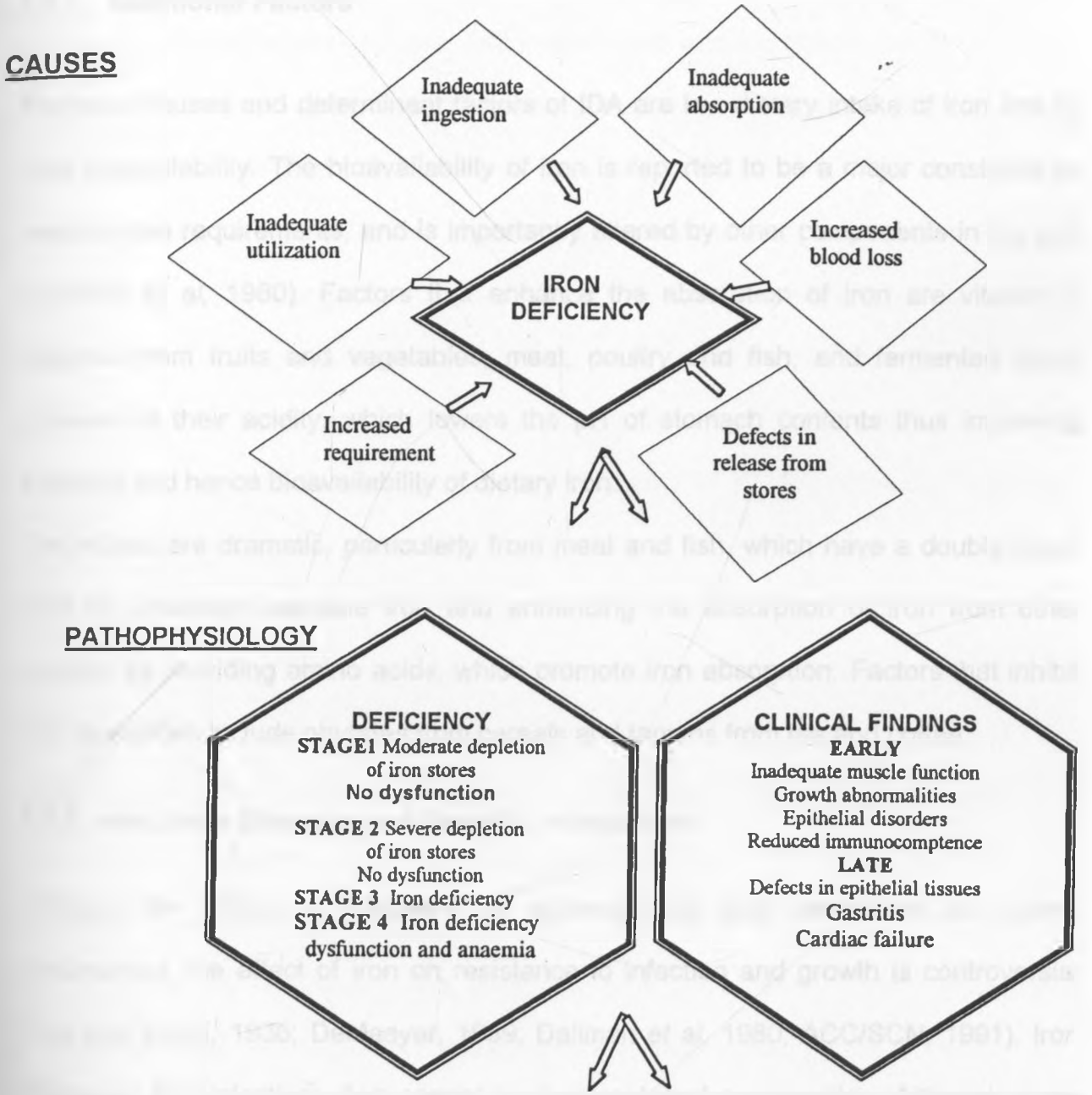
(6) Defective release of iron utilisation owing to a chronic inflammation,

(7) Physiological conditions,

(8) Environmental and,

(9) Socio-economic factors. The most important causes are discussed in the succeeding sections.

Figure 1. Pathophysiology Algorithm – Iron deficiency anaemia



Source: by john Anderson 2000

2.1.1. Nutritional Factors

The main causes and determinant factors of IDA are low dietary intake of iron and its poor bioavailability. The bioavailability of iron is reported to be a major constraint on meeting iron requirements, and is importantly altered by other components in the diet (Dallman *et al,* 1980). Factors that enhance the absorption of iron are vitamin C obtained from fruits and vegetables, meat, poultry and fish, and fermented foods because of their acidity, which lowers the pH of stomach contents thus improving solubility and hence bioavailability of dietary iron.

The effects are dramatic, particularly from meat and fish, which have a double effect both by providing available iron and enhancing the absorption of iron from other sources by providing amino acids, which promote iron absorption. Factors that inhibit iron absorption include phytates from cereals and tannins from tea and coffee.

2.1.2. Infectious Diseases and Parasitic Infestations

Although the effects of infections on erythropoiesis and haemolysis are widely documented, the effect of iron on resistance to infection and growth is controversial (Foy and Kondi, 1936; DeMaeyer, 1989; Dallman *et* al, 1980; ACC/SCN, 1991). Iron deficiency and infection often coexist in disadvantaged communities. Although some infestations like hookworm (Bever, 1951; Roche, 1964; Stephons, 1987), schistosomiasis (Chandra and Saraya, 1975) and malaria (FAO, 1988; Chandra and Saraya, 1975) are known to lead to iron deficiency, iron deficiency may also lead to infection due to reduced host defence cell mediated immunity (Joyson *et* al, 1976) and neutrophil function (Chandra and Saraya, 1975; Macdugali and Anderson, 1975;

Joyson *et* al, 1976) is affected by low iron, but the humeral component seems to be unaffected (Chandra and Saraya, 1975; Macdugali and Anderson, 1975). However, studies in human are less conclusive and contradictory (FAO, 1988; Mackay, 1928). Some community intervention studies in both developed (Aukett, 1986; Majia, 1988) and developing countries (Mackay, 1966; Oppenheimer, *et al*, 1986; Hershko, Peto and Weatherall, 1986) have provided evidence that supplementing iron can reduce diarrhoea and acute respiratory infection. However, other investigators have reported that supplemental iron reactivated pre-existing infections such as malaria, brucellosis and tuberculosis (Masawe and Munidi, 1974; Marray *et al,* 1975; Chwang, Soamantri and Politt, 1988; Barbin and Bernard, 1992).

In 1989, DeMaeyer reported that an increase in the rate of infection is not due to iron therapy per se, but the route of administration of the drug. Parenteral iron causes transferrin to be saturated making unbound iron available for growth and reproduction of microorganisms in the blood (ICN, 1978; Foy and Kondi, 1936). The degree of functional impairment or morbidity generally increases with the severity of iron deficiency. Some of the manifestations are due to anaemia itself, some due to the effect of iron deficiency on tissues, and some due to a combination of both (ICN, 1978; DeMaeyer, 1989). The following observations have been made:

- Iron deficiency in children reduces resistance to infection, growth faltering, and impaired intellectual development (ICN, 1978).
- In women of reproductive age, it is strongly associated with decreased work performance, increased risk of premature delivery and maternal and foetal mortality (INACG, 1977; INACG, 1982; ICN, 1978), while decreased gastric juice

secretion, together with gastric mucosal atrophy, shortened intestinal villous height and reduced activity of intestinal enzymes (ICN, 1978) has been documented in iron deficient individuals.

2.1.3. Physiological Factors

The requirement for iron increases with growth. Iron deficiency is most common during infancy and puberty when velocity of growth is rapid (Marray *et* al, Herberg and Galons, 1989). Iron deficiency reduces appetite and affects intestinal nutrient absorption (Judisch *et* al, 1966), thereby impairing growth. Preventing and correcting iron deficiency anaemia are urgent among those vulnerable group because of their negative consequences, some of which may be long lasting if not permanent (Dallman, Sushine and Leonard, 1967; Joyson, *et al*; Mohammed, *et al*, 1988). For this reason, treating of anaemia in the vulnerable group through supplementation and fortification has been proposed (Dallman, Sushine and Leonard, 1967; Mohammed, *et al*, 1988) and is carried out in many countries especially for pregnant women

2.1.3. Socio-economic and Cultural Factors

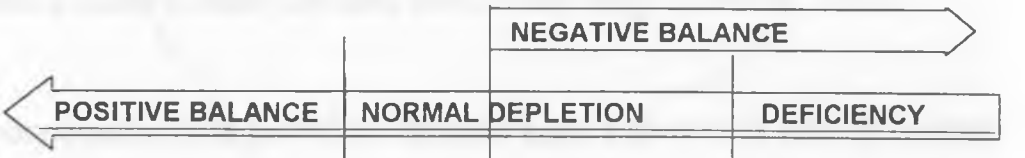
High poverty levels, inequitable availability, accessibility, and low coverage of health services, and inadequate knowledge and skill to reduce risk of developing anaemia in the general population are fundamental issues in the anaemia dialogue. In this regard, the policy of including iron supplementation for the vulnerable group mainly for the pregnant women has been going as an MCH package for many years with controversial results in order to reach a high number of these mothers in the low socio-economic status (INACG, 1997).

2.2. Stages of Deficiency

As shown in figure 2, one's iron status can range from iron overload to iron deficiency anaemia. Deviations from normal iron status have been summarised by Herbert (1992) as follows:

- Stages I and II negative iron balance- In these stages, iron stores are low but there is no dysfunction. In stage I negative iron balance, reduced iron absorption produces moderately depleted iron stores. Stage II negative iron balance is characterised by severely depleted iron stores. When persons treated in this category, they never develop dysfunctions.
- Stages III and IV negative iron balance- Iron deficiency is characterised by inadequate body iron-causing dysfunction. In stage III negative iron balance, dysfunction is not accompanied by anaemia; however anaemia does occur in stage IV negative iron balance.
- Stages I and II positive iron balance - Stage I positive iron balance usually lasts for several years with accompanying dysfunction. A supplement of iron and/or vitamin C promotes progression to dysfunction, whereas removal prevents progression to diseases. Iron overload disease develops in individuals with stage II positive balance after years of iron overload have caused progressive damage to tissues and organs. Removal of iron can stop disease progression

Figure 2. Sequential stages of Iron Status



	Stag II Iron overload	Stage I		Stage I Early	Stage II	Stage III Damaged metabolism	Stage IV Clinical damage
Iron stores	Excess	+ve iron balance	Normal	-ve Iron balance	Iron depleted	IDE	IDA
RE marrow iron	4+	3+	2-3+	1+	0-1+		0
Transferrin IBC(ug/100 ml)	<300	<300	330±30	300-360	360	0	410
Plasma ferritin(ug/L)	>300	>150	100±60	<25	20	390	<10
Iron absorption(%)	>15	10-15	5-10	10-15	10-15	10	10-20
Plasma iron(ug/100ml)	>175	>150	115±50	<120	115	10-20	<40
TS(%)	>60	>45	35±15	30	30	<60	>60
Sideroblasts (%)	40-60	40-60	40-60	40-60	40-60	<15	<10
RBC protoporphyrin	30	30	30	30	30	<10	200
Erythrocytes	normal	normal	normal	normal	normal	<100 normal	MH

Source: Herbert et al 1995. IDE= Iron deficient erythropoiesis, IDA= iron deficiency anaemia, IBC= iron b inding capacity, TS=transferrin saturation, RBC= red blood cells, MH= microcytic hypochromic

2.3. Iron Therapy and its Benefit

The chief treatment for iron deficiency involves oral administration of inorganic iron in the ferrous form. Daily administration of iron has been a standard and mode of treating iron deficiency anaemia. As a preventive measure, however, it has encountered serious problems of compliance because of adverse side effects that produces a gastrointestinal epithelium permanently loaded with iron (Brown, 1958). This condition is un-physiologically and possibly responsible for the side effects, which are directly proportional to the iron dose administered (Mohammed, *et al*, 1988). A follow up of growth charts of 156 children under 3 years of age with nutritional anaemia showed preponderance of lower weight, which shifted towards normal following the daily schedule iron therapy (Briend, Hoque and Aziz, 1990). Bangladesh children, living in a

community getting more than 1 mg of iron daily in their water, were taller and heavier compared to children in communities with less iron in their water (Tonkins, 1970).

A double blind randomised clinical trial of 91 children aged between 17 and 19 months in Birmingham, indicated faster weight gain among the iron supplemented children than placebo treated controls (Majia, 1988). A similar study in rural Indonesian school children supported this result (Marray, 1975), but a study in New Zealand showed only a gain in weight, but not height or head circumference (Jacques, 1992). A cross sectional study in Togo failed to show any difference in weight and height between iron deficient (n=241) and iron sufficient children (n=56) (Scrimshaw, 1989).

A daily iron supplementation therapy due to its side effects and continuous administration of the drug may be a major factor affecting treatment outcome (DeMaeyer, 1989). Hence, alternative strategies to control iron deficiency anaemia are required. Recent field trial studies conducted among pre-school children in China indicated that intermittent therapeutic dosage to be as effective as daily dosage in correcting mild to moderate anaemia and iron deficiency in same age group (Scrimshaw, 1989; Liu, Kang, Zha and Viteri, 1995; Bothwell, Bradlow and Jacobs, 1964). However, further studies are needed to substantiate this observation and to determine the effects in different age groups. Particularly in lactating women and children, and in other countries where the prevalence of iron deficiency anaemia is higher.

2.4. Metabolism of Iron

Haeme iron from animal products is present in its reduced form while non-haeme iron from vegetable products is in ferric state (Passmore and Eastwood, 1986). Iron is consumed in the ferric state and must be converted to the ferrous state prior to intestinal absorption. Co-administration of ascorbic acid facilitates this conversion and consequently facilitates binding of iron to ferritin and transferrin by a change of the valence in iron (WHO, 1972). Iron is absorbed in the ferrous state (reduced) and the amount of iron absorbed by the body depends on several factors, including the total amount in the diet, its absorbability, and the regulation of its absorption by the body. The absorption of iron in the food may be influenced by the simultaneous administration of other foods. Meat increases the absorption of iron from a number of sources, including maize, black beans, and inorganic iron salts. Cysteine has been shown to be one of the substances responsible for this enhancing effect (Bothwell, Bradlow and Jacobs, 1964).

Administration of ascorbic acid at mealtime improves absorption of non-haeme food iron, probably because of being a reducing agent, converting ferric to ferrous iron. However, phytates and eggs cause a marked reduction in absorption. Geophagia depresses iron absorption and may be a more common cause of iron deficiency in some countries than has been previously been realised (Bothwell, Bradlow and Jacobs, 1964; WHO, 1972).

Before iron can be absorbed in the upper part of the small intestine, it is first separated from organic material such as proteins, into a solubilised form. Once iron is absorbed, it is transported through blood bound to the protein transferrin. The iron ions transported

by transferrin can be released to cells for the manufacture of iron containing enzymes and other proteins, or released to the bone marrow for use in haemoglobin synthesis, or deposited within the ferritin and haemosedrin in the iron storage sites of the body (Guthrie and Piciano, 1995). When a cell demands iron, it produces more transferring receptors and is thus able to collect more iron from the circulating supply. If the amount of iron absorbed from the diet and obtained from the break down of RBC is insufficient to meet the body's needs, iron is removed from the storage sites and transported to the sites of demand bound to transferring molecules. Iron is stored in the body in the form of ferritin, in the bone marrow, liver, spleen, kidney, and symptoms of iron deficiency appear only when the body's stores of iron are depleted (Guthrie and Piciano, 1995).

2.5. Potential Toxicity and Side Effects of Iron

Iron can be extremely toxic if acutely ingested in large amounts. Accidental or unintentional consumption of large quantities of iron-containing nutritional supplements has been reported among the pre-school children. Iron toxicity can also arises because of the chronic absorption or accumulation of iron in amounts that exceed normal transport and storage mechanisms (Beard and Dawson, 1997).

Present evidence indicates that supplementing iron at recommended doses to women of reproductive age groups causes minor side effects such as constipation, abdominal pain, nausea, vomiting and faecal discoloration in less than 5% of the subjects (ICN, 1978). Overload in the body may however, occur depending on the daily administration schedule. For example, with the daily administration of iron, gastrointestinal complaints are reported to be common.

2.6.0. Biochemical and Physiological Functions

Iron is an integral part of heame and is needed in, or it is part of the following functions:

2.6.1. Transport and storage of oxygen: iron in its ferrous state within the metalloproteins haemoglobin and myoglobin can bind to oxygen molecules and transport them through the blood or store them within the muscles.

2.6.2. Cofactors of Enzymes and other proteins: the iron containing haeme group is a part of several proteins involved in the release of energy during the oxidation of nutrients and trapping of that energy within ATP. Iron is a cofactor of cytochromes, catalase and peroxidase. Iron on its own is a factor bound to several non-haeme enzymes required for the proper functioning of cells. Some of the other process that depend on the activities of iron containing enzymes are conversion of betacarotene to the active form of vitamin A; synthesis of carnitine synthesis of collagen, detoxification of drugs, and other toxic compounds in the liver and intestine; and synthesis of neurotransmitters (Guthrie and Piciano, 1995).

2.6.3. Electron Transport: the iron-sulphur proteins act as electron carries through the action of iron bound to either two or four sulphur and cysteine side chains. The 40 different proteins that constitute the respiratory chain contain six different haeme proteins, six iron-sulphur centres (Beard and Dawson, 1997).

2.6.4. Formation of Red Blood Cells (RBC): Since the iron containing protein haemoglobin is a major component of RBC, iron is required for the formation of these cells, which are formed in the bone marrow (Guthrie and Piciano, 1995).

2.7. Assessment of Iron Deficiency Anaemia

The initial detection of iron deficiency anaemia in an individual is usually made in the laboratory through the recognition of anaemia. Once the presence of anaemia has been established, tests are then done to establish that it is infact, the result of iron deficiency. However, it must be stressed that haemoglobin concentration varies considerably in normal subjects, such that lesser degrees of iron deficiency will go undetected if it is the only measurement used (INACG, 1982; Beaton, 1976). While it is customary to set arbitrary lower limits of normality for haemoglobin concentration at different ages in the two sex groups, it is appreciated that such figures can only be used as a rough guide. The most useful tests for the evaluation of iron status are serum ferritin concentration, the percentage saturation of the plasma transferrin, the red cell protoporphyrin concentration and haemeatocrit. For the mildest degree of iron deficiency, amounting to a reduction in the body iron reserve only, the plasma ferritin concentration provides the only convenient yardstick. An overt iron deficit is present when the haemoglobin concentration is less than 12 gm/dl and serum ferritin is below 12 μg/litre (INACG, 1982).

Table 1. WHO Guidelines on cut-off for Anaemia and Public Health Significance

Grades of anaemia	Cut-of point of Hgb concentration, in gm/dl	Prevalence of anaemia, percents	
		Mild to moderate	Severe
Mild	10.1 – 11.9	1 – 9	< 1
Moderate	7 – 10	9.1 – 39.9	1 – 9
Severe	4 - 6.9	≥ 40	≥ 9
Very severe	< 4	-	-

SOURCE: GUIDELINES ON CUT-OFF FOR ANAEMIA AND PUBLIC HEALTH SIGNIFICANCE BY STLZFUS, 1998.

2.6. WHO Guidelines on cut-off for Anaemia

Haemoglobin value below 11 gm/dl and 12 gm/dl in the pregnant and non-pregnant women respectively as being indicative of anaemia in these groups.

In order to facilitate the interpretation of observed anaemia from a public health view point, the above table recommended by WHO was used (Stlzfus and Dreyfus, 1998).

Some of the intermittent iron supplementation studies conducted earlier have only attempted to look into the effect of the supplementation in pre-school children and school children. None of these studies has addressed the effect of supplementation, in terms of the therapeutic effectiveness in lactating women. The present study is therefore, aimed at documenting, the effect or consequences of different iron supplementation schedules.

2.7. Justification

The problem of iron deficiency anaemia in Ethiopia appears to gain momentum and hence the needs to seek for appropriate and affordable control and prevention

strategies. Compliance has been shown to be poor in supplementation programmes. Further more it is important to monitor it and establish reasons for compliance. The continually high prevalence despite the presence of daily supplementation programme, suggests that in addition to poor compliance there could be other factors leading to this situation.

Weekly dosage schedule is not only a convenient unit of time, but also leads to less fewer incidence of overdose. Administering a supplementary dose every week once, cells loaded with iron from a previous dose will have been shed. As a consequence iron absorption is increased while avoiding a constant luminal and mucosal iron overload likely to provoke adverse effects.

CHAPTER THREE

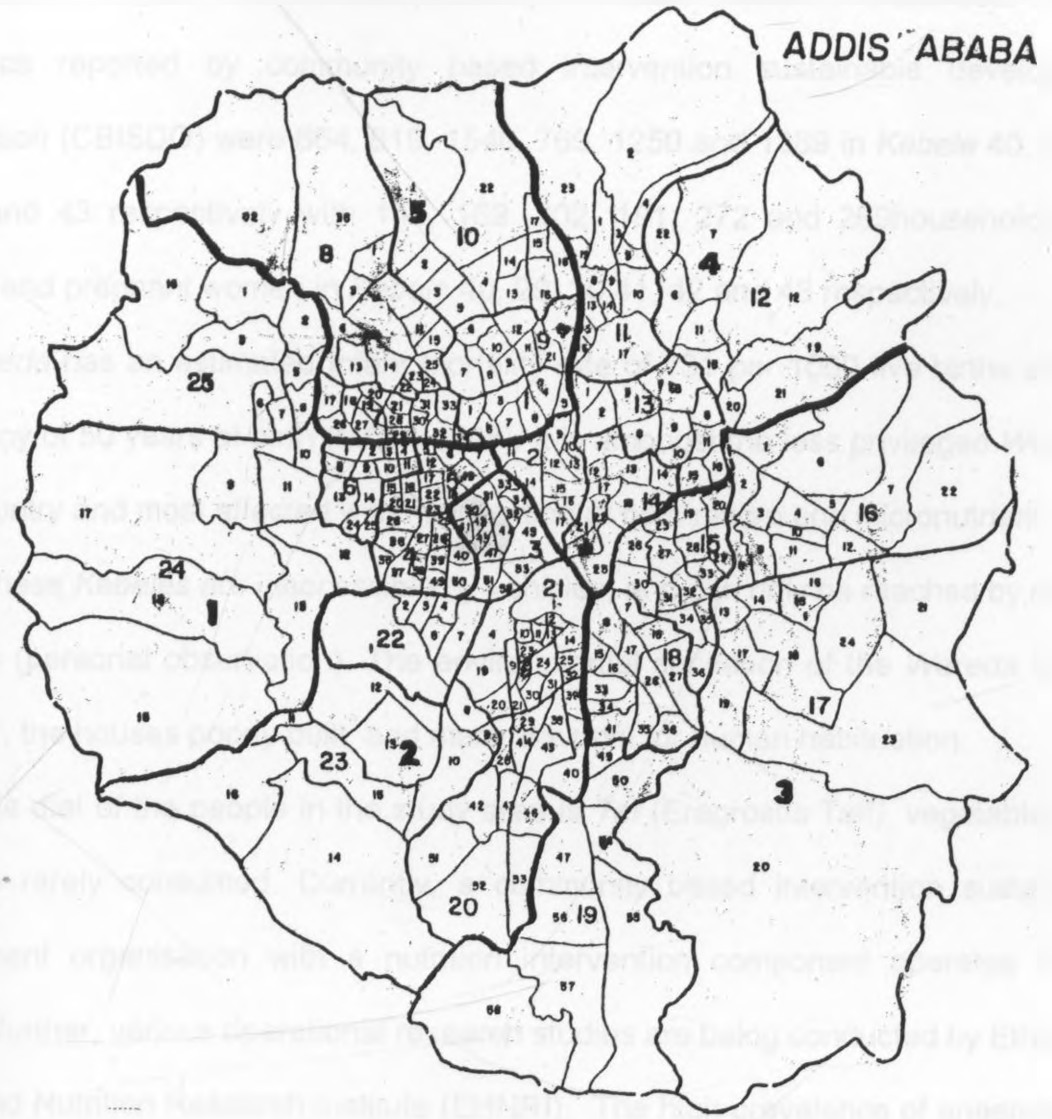
3. STUDY SETTINGS AND METHODOLOGY

3.1. Background information on the study area

Addis Ababa is the capital city, and one of the fourteen regions of Ethiopia with an estimated population of over two million, of which, according to the 1994 census, 48.4% are males and 51.6% females. Of the total number of females in the city, 31.06% of them are lactating women aged, 15 to 49 years. The capital city is administratively divided into six zones, which in-turn is divided into 28 *Weredas or* districts (Figure 1). The *Weredas* are subdivided into 305 administrative units known as *Kebeles.* Each *Kebele* has an average population of 5000 people or 1000 households. Around 79% of Addis Ababa population live in low grade and congested areas (Harpham, Lusty and Vaughan, 1988). These areas fit into the category of city slum. The principal health problems in the city are communicable disease and nutritional deficiencies (CSO, 1992).

Available data indicated that Protein energy malnutrition (PEM), deficiency of iodine, vitamin A deficiency and nutritional anaemia are reported to be the commonest nutritional problem in the city as well as in the entire country. In 1978, World Bank identified seven *Kebeles*, as typical slum in the capital city, which were extremely congested and the poorest amongst all the *Kebeles* where the bank was considering supporting upgrading activities in two *Weredas* namely *Teklehaimant* and *Cherkos Weredas* (Jember, 1985). Of the total seven slum *Kebeles,* six of them were found in *Teklehaimant Wereda* and the remaining was in *Cherkos Wereda.* Thus, the *Wereda* with more slum *Kebeles* was chosen purposively.

Figure 3. Map of Addis Ababa showing the Study area



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Scale:1;75,000

Source: Central Statistics Authority 1997

Study sites: *Kebeles* 28, 29, 30, 40, 41, 42 and 43

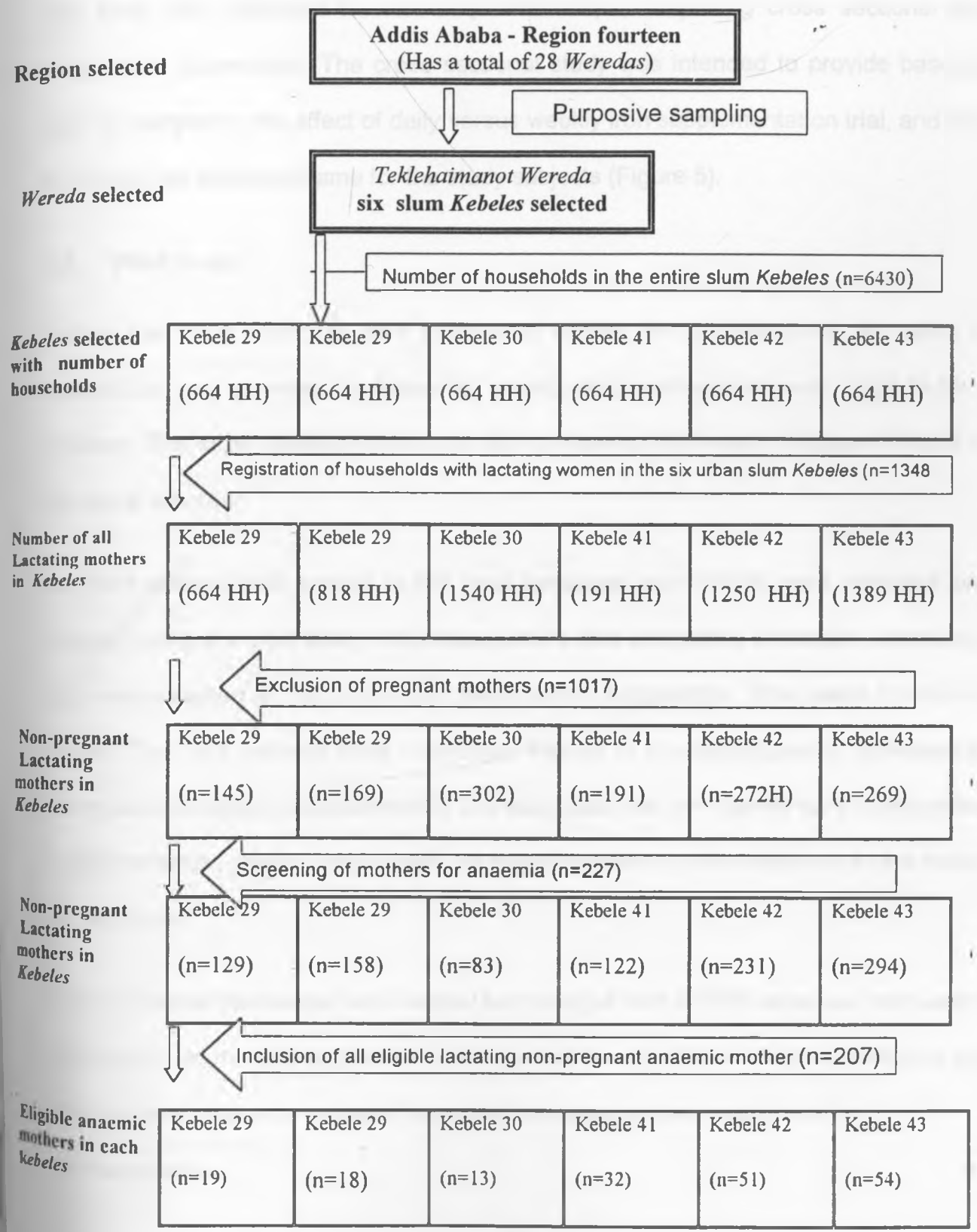
The six *Kebeles of Teklehaimant Wereda* of Addis Ababa Region where the study took place, shown in Figure 4 are in close proximity. The total number of households in each *Kebele* as reported by community based intervention sustainable development organisation (CBISDO) were 664, 819, 1540, 769, 1250 and 1389 in *Kebele* 40, 29, 30 41, 42 and 43 respectively with 145, 169, 302, 191, 272 and 269households with lactating and pregnant women in *Kebele* 40, 29, 30 41, 42 and 43 respectively.

The *Wereda* has an estimated Infant mortality rate of 131 per 1000 live births and life expectancy of 50 years at birth (MOH, 1998). It is amongst the less privileged *Weredas* in the country and most affected with malnutrition of both macro and micronutrient. Most parts of these *Kebeles* are inaccessible by vehicles, and can only be reached by narrow footpaths (personal observation). The environmental sanitation of the *Wereda* is also very poor, the houses poorly built, and many are unfit for human habituation.

The staple diet of the people in the study area is *Tef* (Eragrostis Teff*),* vegetables and meat are rarely consumed. Currently, a community based intervention sustainable development organisation with a nutrition intervention component operates in the *Wereda,* further, various operational research studies are being conducted by Ethiopian Health and Nutrition Research Institute (EHNRI). The high prevalence of anaemia and malnutrition, the availability of equipped clinics made the site favourable. In addition, the usual collaboration of the health workers and the presence of local NGO known as CBISDO operating in all the selected *Kebeles* of the *Wereda* were also favouring the site as priority; and one of the criteria for selecting the site. The previous experience of CBISDO, EHNRI in nutrition intervention and public health management and previous

exposure of the researcher in the *Wereda* was an advantage in implementing this community trial.

Figure 4. Flow chart showing the sampling and screening procedures of the eligible study subjects



3.2. Survey Design

The study was designed to take sequential steps comprising cross sectional and longitudinal observation. The cross sectional study was intended to provide baseline data for comparing the effect of daily versus weekly iron supplementation trial, and also to provide the sampling frame for the study subjects (Figure 5).

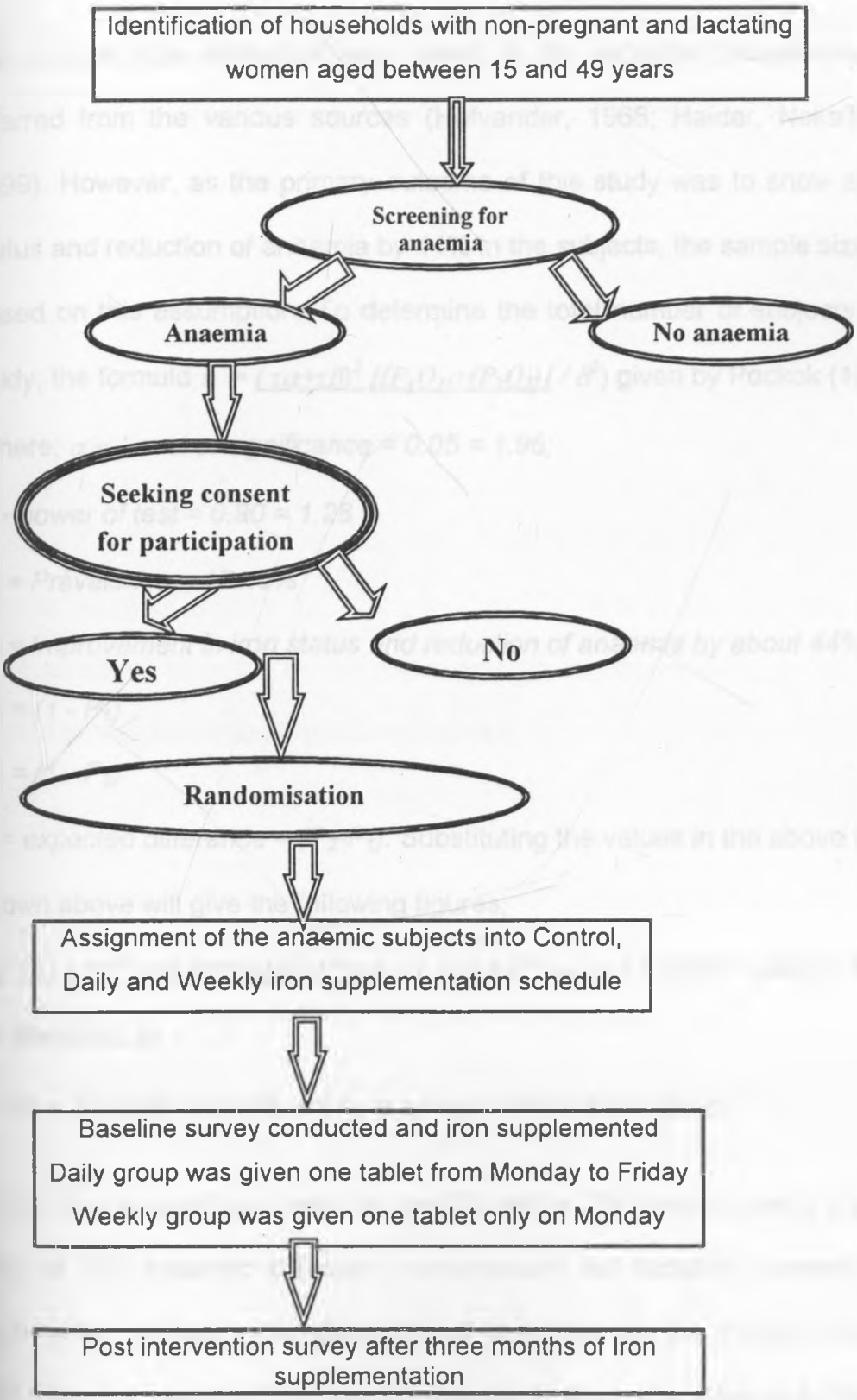
3.3. Pilot Study

Before the main study, a pilot study was carried out to determine the level of malnutrition and anaemia in *Kebele* 42 during which malnutrition was found to be a problem. The study questionnaire was also pre-tested the nearby adjacent *Kebele* of the same *Wereda*.

Six field workers well versed in the local language and culture were recruited and trained during the pilot study. The enumerators had completed secondary schoolings and were enrolled in the community development programme. They were intensively trained The field workers were intensively trained in the techniques of interviewing, taking anthropometric measurements and administer the iron tablets for the respective supplementation groups, and record the negative effects of the treatment in the follow-up tally sheet.

A senior clinical biochemist and medical technologist from EHNRI were also recruited to assist and train the field technician to take care of the specimen collection, handling and analyse stool for ova or parasite, haemoglobin measurement, and check for haemeoparasite.

Figure 5. Flow chart of the study design



3.4. Sample Size Estimation

The sample size estimation was based on the expected prevalence of anaemia as inferred from the various sources (Hofvander, 1968; Haidar, NekaTibeb and Urga, 1999). However, as the primary outcome of this study was to show an improved iron status and reduction of anaemia by 44% in the subjects, the sample size was calculated based on this assumption. To determine the total number of subjects needed for this study, the formula $\eta = \underline{(z\alpha+z\beta)^2 [(P_1Q_1)+(P_2Q_2)]} / \delta^2$) given by Pockok (1986) was used;

Where; α = *Level of significance = 0.05 = 1.96;*

β = *power of test = 0.90 = 1.28*

P_1 = *Prévalence = 18.40%*

P_2 = *Improvement in iron status and reduction of anaemia by about 44% (assumed)*

$Q_1 = (1 - P_1)$

$Q_2 = (1 - P_2)$

δ = *expected difference* = (P_2-P_1). Substituting the values in the above formula as shown above will give the following figures;

$\underline{(1.96+1.28)^2 [(18.4*81.6)+(44*56)]} / (44-18.4)^2 \Rightarrow 10.5 * [1501 + 2464] / 655.36$

$\Rightarrow$ ***3965/655.36***

$\Rightarrow$ ***64 + 7%*** drop out rate, will give a total of 69 in each group.

To achieve a significant result at the 5% with a 7% dropout rate at a power of 90%, a total of 207 anaemic subjects (non-pregnant but lactating women) were required. Fortunately, the figure calculated almost coincided with the number of lactating mothers and therefore, nearly all the lactating women in the entire *Wereda* fulfilling the inclusion

criteria were included in the study. For those procedures, which require serum ferritin analysis, a sub-sample of one in four subjects (25%) was selected systematically.

3.5.1. Subject Selection and Allocation of Groups

To select the study subjects, a census of all households with lactating women, aged 15 to 45 years within one to two kilometre radius of the working centre was made. Households, which had a lactating mother that was pregnant, were excluded altogether; and only lactating women were retained to maintain the homogeneity of the study subjects, and avoid the confounding effects.

The total number of households, with lactating non-pregnant women, recruited in the census was1017 (Figure 4), of whom, only 207 were eligible enrolled in this study and were assigned in either of the different supplementation schedule (daily or weekly) or control groups following simple randomisation.

To eliminate a major source of variation, subjects participating in the study were dewormed with 120 mg levamisole tablets before the intervention, and advised to avoid taking any iron or vitamin preparation apart from what was given to them throughout the study period.

For screening, the only convenient yardstick to be used was a haemoglobin concentration of less than 12 gm/dl (INACG, 1982), although clinical screening for pallor in the lower eye lids, palms, nail beds and other parts of the body was also checked side by side to screen for anaemia.

The daily supplemented group received one or 60 mg of elemental iron tablet (*300 mg ferrous sulphate containing 400µg folic acid manufactured by Cyprus, Greek; each*

tablet costing 16 Ethiopian cents or an equivalent of 0.02 USD) from Monday to Friday, while the weekly supplemented group received 60 mg of elemental iron tablet with the same content once a week every Monday for three consecutive months supervised by the field workers. Two weeks supply of iron tablets was issued to each field worker in a plastic bag labelled with the date and the group. All the field workers administered the tablets in the morning between 8 and 9, to make sure that the subjects swallowed the tablets accordingly. The field workers maintained the daily or weekly record sheets for administering the tablet and recorded any complaints throughout the study period (Appendix 8).

Each study subject received either the daily or weekly dose of iron supervised, and was checked for compliance prior to administering the second doses of iron and record complaints in the follow-up tally sheet.

3.5.2. Possible Confounding Factors

Study subjects were randomised across groups in order to, equally distribute potential confounders such as physiological status, age, nutritional status, illnesses, diurnal variation, drug intake including family planning use, exercise, sampling and the like between the two supplementing and control groups. Important potential confounders that are different between groups, despite proper randomisation, were controlled during the analysis.

5.3. Inclusion and Exclusion Criteria

3.5.3.1. All non-pregnant lactating women of reproductive group,,ranged from 15 to 49 years with anaemia, who were permanent residents of the study area or lived in the study areas for at least 12 months were eligible for the study.

3.5.3.2. Women with either chronic illnesses or acutely sick or physical disability less than 1%were excluded from the study for ethical reasons; in addition to this, all subjects who had started iron treatment or supplementation two weeks prior to the study were also excluded to guard the compounding effects.

3.5.4. Screening of Subjects and Screening Procedures

A central meeting place, where CBISDO is operating was found within the *Wereda*, as both a screening location and working place for the Laboratory technician. *Kebele* leaders and community elders were contacted to inform the households, two day in advance, that anthropometric and haemoglobin screening to be conducted in all lactating non pregnant women of the respective *Kebeles*. The clinical officer together with the researcher was present to identify cases with anaemia and other conditions.

3.5.5. Ethical Considerations

3.5.5.1. Ethical clearance was obtained from the research and ethical clearance committees of Ethiopian Health and Nutrition Research Institute, Ethiopia.

3.5.5.2. Subjects fully informed about the objectives of the study, the advantages and potential side effects of the treatment and asked for their consent to sign consent form (Appendix 6).

3.5.5.3. All participants had the benefit of getting treatment for anaemia during and after the study.

3.5.5.4. Subjects who did not want to continue with the study were also allowed to dropout at any stage of the study and were offered treatment or referred, when they were found anaemic or positive for any intestinal parasites and other illnesses.

3.5.5.5. All used expendable materials were disposed into pit latrines, including glassware, and the laboratory technicians who were handling the specimens wore gloves.

3.6.0. Data collection

The intervention and data collection took place from March to May 2001, which was followed by one-month follow-up of the subjects to obtain important missing data. All the collected data, which were shown in Figure 5, and discussed in the following sections, were coded, entered into a computer, and cleaned during the follow-up.

3.6.1. Standardisation

The interviewers trained in the proper use of the weight scales and recording, and intra- and inter-observer errors checked before data collection. The scales adjusted before each measuring session and re-calibrated every day.

3.6.2. Collection of Baseline Data

3.6.2.1. Socio-Demographic characteristics

Socio-demographic variables were collected in all the study subjects; these variables fell into individual (study subject) and household characteristics. The main individual variables were age, ethnic group, educational status, marital status, occupation, religion, parity, family planning use, nutritional status, qualitative food consumption and morbidity. The household variables were family size, latrine facility, garbage disposal and source of drinking water.

3.6.2.2. Anthropometric Measurements

To assess the nutritional status, weight and height measurements taken in all the study subjects, at baseline and recorded in the questionnaire by the enumerators. Weight was measured on all study subjects, and the measurement was taken with minimum clothing, in duplicate to the nearest 100 grams using a digital bath scale balance (Gibson, 1990). Height was measured using a wooden height-board, which was positioned straight, and then duplicate measurements were taken from each subject to the nearest 0.1 cm. All footwear was removed and hair was loose and flat (Gibson, 1990).

To detect inter-and intra–observer variability; duplicate measurements were obtained by the same measurer and recorder in 5% (n=11) of the sample and by the Principal investigator in 10% (n=27) of the sample; the variability was 0.2 and 0.3 cm for height and 0.1kg for weight.

3.6.2.3. Clinical assessment

All the study subjects were interviewed for chronic and acute illnesses, and checked for pallor in the eye, fingernail, palm, tongue, and undergo physical examination for organomegally and murmur by the principal investigator together with the physician from CBISDO clinic.

3.6.2.4. Dietary intake assessment

Qualitative information on, dietary practices and consumption from all the study subjects, using specific food, rich in vitamin and minerals, frequency checklist, was collected and recorded by the field workers under close supervision.

3.6.2.5. Biochemical parameters

For haemoglobin and blood film; capillary blood obtained from a finger prick was used. Prior to the finger prick, the finger was warmed to promote blood flow, then sterilised and punctured using a sterile disposable lancet. As the first drop was most likely contaminated with a tissue fluid, it was wiped away with dry gauze, and the next drop formed over the puncture was then collected on a glass slide for blood film analysis; for haemoglobin, two hundredth of millilitres of blood were collected using a micropipette for haemoglobin determination in duplicate, and recorded the average (DeMaeyer, 1989; 1974; Cheesbrough, 1981).

For serum ferritin assessment, venous blood was used using vaccutainer system. Venous blood was collected from the upper arm by applying a tourniquet only on the initial and final stages of the study on non-acutely ill subjects. The cubital area of the

arm was properly sterilised with alcohol and 5 ml of blood was collected from antecubital vein with a vaccutainer. The blood specimens were stored and transported in an icebox at 4° to EHNRI. Serum ferritin and haemoglobin, were measured at baseline, and repeated after three months to assess and compare the effect of the different supplementation and control groups. Blood film was also taken for those who were suspected to have any kind of fever.

3.6.2.5.1. Serum Ferritin Determination:

Serum ferritin values fall in iron deficiency anaemia before changes in serum iron; total iron binding capacity or haemoglobin levels are manifested. In most individuals the concentration of serum ferritin parallels the total amount of storage iron, and serum ferritin is the only iron status index that can reflect a deficiency, excess and normal iron status (Baker et al, 1974; Cheesbrough, 1981; Gibson, 1990). In conditions of frank iron deficiency anaemia, when classical microcytic hypochromic anaemia occurs, serum ferritin levels are very low or zero, reflecting the exhaustion of storage iron. A low concentration of serum ferritin (<12 µg/l) is characteristic only of iron deficiency (Baker et al, 1974; Cheesbrough, 1981).

Two aliquots were collected for ferritin status determination in a sub-sample of the subjects (n=53). One aliquot was analysed to find the initial status, while the second was for the final assessment, which was 3 months later. The sera collected from the study subjects over the study period were analysed in patch (Baker et al, 1974). Analysis was handled, throughout the study period, by the senior clinical biochemist of the institute (EHNRI).

Reagents:

All reagents used were supplied by Roche, Boerrhinger Manheim, German.

- Ferritin standards 250 ng/ml
- Normal saline
- Sample
- Pool serum for assessing the precision of the determination
- Serum ferritin reference material

Equipments:

- ELIZA (based on sandwich enzyme-linked immunosorbent assay (ELISA) technique using fully automated ES 300 analyser (Roche, Boerrhinger Manheim).
- Centrifuge (1500 x g).
- Pipettes
- 50μ/L for standards and samples
- 200μ/L for tracer reagents
- 3.0 ml/L repeating dispenser
- Graph papers-four-cycle semi-log
- Polypropylene tubes (12x75)
- Vortex mixer and suction apparatus for removing supernatant

Procedures:

Sterile disposable syringe used for drawing venous blood aseptically from the antecubital area of the arm in every fourth subjects as follows;

1. Label tubes pool, reference and each test subjects,
2. Add 50 μL of each standard, pool, reference and test serums to the respective tube, commencing with the lowest dilution set and mix,
3. Shake the rack of tubes to mix the contents,
4. Add 3.0 ml saline to all tubes,
5. Centrifuge all the tubes for 10 minutes at 1500 X g at 4 degree Celsius, to pick the solids into the bottom of the tube. Proceed promptly to the next steps,
6. Remove the supernatant in each tubes using suction apparatus,
7. Insert the tubes into the ELIZA reader, which is fully automated. The system dispenses reagents, calibrates and controls, evaluates all processes in an ELISA procedure, takes absorbency reading, establishes calibration curves and evaluates results automatically.
8. Count the tubes with the reader.

3.6.2.5.2. Haemoglobin Concentration Determination:

The cyanmethaemoglobin technique recommended by Valley *et al* (1988) was used to measure the haemoglobin concentration in all the subjects. This method was preferred for its accuracy and its ease for field situations (DeMaeyer, 1989; Cheesbrough, 1981), simpler, involves a visual colour comparison; and was used to measure whole blood concentration. The circulating haemoglobin in blood was converted to cyanmethaemoglobin and measured photo metrically. Blood from a finger prick drawn was mixed with 250 times its volume of Drab kin's solution (200 mg of ferric potassium cyanide, 50-mg potassium cyanide, and 140-mg anhydrous dihydrogen phosphate and

0.5 to 1.0 ml non-ionic detergent per litre of per litre of water). The diluted sample was measured after 3 to 5 minutes at around 540-nano meter wavelength with a calibrated spectrophotometer, and results were obtained from a direct read out. Content and spectral purity was checked regularly (DeMaeyer, 1989; Cheesbrough, 1981). To avoid diurnal variation in haemoglobin concentration, which is 1 gm/dl (Gibson, 1980), morning blood sample was taken in the entire group.

The haemoglobin concentration of each test sample was then calculated from the following formula:

$$\text{Hgb conc. of test sample (gm/dl)} = \frac{\text{Absorption of the test sample}}{\text{Absorbance of standard}} \times \text{Dilution factor} \times \frac{\text{Conc. Of standard of t}}{1000}$$

A dilution factor of 201 was used according to the 4.0 ml of Drabkins reagent used.

Reagents:

- Modified Drabkin's reagents,
- Aqueous certified standard solution of cyanmethaemoglobin,
- Samples, alcohol (70%) and swaps,
- Pool blood sampling for assessing the precision of the determination.

Equipments:

- Test tubes, (vaccutainers) and glass wares,
- Pipettes of 0.02 ml (Sahli), 5 ml volumetric and 10 ml graduated,
- Spectrophotometer (Beckman model 25 with 540 nm filter),
- Cuvettes,
- Automatic dispenser (5ml),

- Sterile disposable blood lancet,
- Disposable gloves and sterile gauze,
- Standing racks.

Procedures:

1. Sahli pipette used for taking the whole blood specimen,
2. Draw mixed blood in pipette,
3. Wipe off the tip,
4. Dispense the blood specimen into the tube with 5ml of Drabkin solution,
5. Wash out the inside of the pipette repeatedly and mix,
6. Wait for five minutes before the solution is taken for absorbency,
7. Set the 0% transmittance (without cuvette) with the help of the left control knob,
8. Insert the cuvette with the blank solution and set the 100% transmittance with the control knob on the right,
9. Finally insert the standard solution (S) and test solution (T1, T2, -----T_n) and read their respective absorbance on the scale.

3.6.2.5.3. Blood Slide for Malaria Parasite

Malaria blood films were prepared directly from capillary blood; this is best preparation (Monica, 1998; King, 1983). Capillary blood method (thin and thick films on the same slide), which is more convenient method, was used. One drop (0.05 ml) of blood from a finger prick was placed near one end of a 25 x 75 mm glass microscope slide. A second slide having a polished end was held at a 45 degree angle into the drop of blood, and

then rapidly and smoothly pushed to the opposite end of the slide, and such a prepared slide was stained with Wright's stain and checked for malarial parasite if there was any in the prepared slides.

All the blood film specimens described above were examined on the same day in the field by a hired laboratory technician, and crosschecked by a senior laboratory technician of EHNRI.

Reagents:

- Alcohol (70%) and alcohol swaps,
- EDTA-anticoagulant,
- Wright stain Field stain A (reagent no.25),
- Field stain B (reagent no.26),
- Buffered water PH 7.1___ 7.2 (reagent no.14)

Equipments:

- Pipette to measure the stain and buffered water
- Two containers of clean water (need not be buffered)
- Microscope
- Sterile disposable blood lancets and syringes,
- Disposable gloves and sterile gauze,
- Clean glass slides,
- Staining rack,

- Manual cell counter designed for differential counts, immersion oil, lens paper, set of reference slides.

Procedures:

1. Cleanse the lobe of the finger using with 70% alcohol. Allow the area to dry.
2. Using sterile lancet, prick the finger. Squeeze gently to obtain large drop of blood.
3. Add small drop of blood to the centre of the slide and larger drop about 15 mm to the right.
4. Immediately spread the small drop and large drop of blood to make thin film and thick film respectively using a smooth edged slide spreader.
5. Cover evenly in area about 15x15 mm.
6. Label the slide with date and the participant's name and number with a black lead pencil.
7. Allow the blood films to dry, with the slide in the horizontal position and placed in a safe place in the air.

Thin Film was prepared as follows;

1. Place the slide on staining rack and cover the methanol fixed thin film with approximately 0.5 ml of diluted field stain B.
2. Add immediately an equal volume of field's stain B. leave to stain for one minute.
3. Wash off the stain with clean water. Wipe the back of the slide, clean and place it in a draining rack for the film to air dry.

Thick Film was prepared as follows;

1. Holding the slide with dried films facing downwards, dip the slide into the field's Stain A for five seconds.
2. Drain off the excess stain by touching a corner of the slide against the side of the container
3. Wash gently for about 5 seconds in clean water. Drain the excess water.
4. Dip the slide into the field's stain B for three seconds. Drain off the excess stain.
5. Wash gently in clean water. Wipe the back of the slide clean and place it upright in drain rack for the film to air dry.

The thick blood film was examined microscopically, using 40x and 100x objective

1. When the thick film is completely dry, apply a drop of immersion oil to an area of the film, which appears mauve coloured.
2. Spread the oil to cover an area about 10 mm in diameter
3. Select an area well stained and not too thick using the lower objective. Change 100x object.
4. Examine for malaria parasites and malaria pigments.

The thin blood film also examined following similar procedures as above with more emphasis on to checking the stain, morphology and distribution of the cells and to detect malaria schizonts, gametocytes, and trophozoytes. Use colour plates 5.43, 5.46 to help identify the different plasmodium species.

Counting Parasite Numbers (Parasite Density)

Parasite numbers counted on the blood slides of the participants that tested positive and done by estimating parasite numbers/µl of blood by counting parasites against white blood cells as follows:

1. Select part of the thick film where the white blood cells are evenly distributed and the parasites well stained.
2. Using the oil immersion objective systematically count 100 white blood cells estimating at the same time the numbers of parasites (asexual) in each Field covered. The counting was done using two hand tally counters. This was repeated into two other areas of the film and an average of the three counts was taken.
3. Calculate the number of parasite per µl of blood as follows:

$$\frac{\text{WBC count x parasite counted against 100WBC}}{100}$$

3.6.2.6. Stool Microscopy

Faecal samples taken from all the subjects and a smear were prepared from each sample using the Kato-Katz techniques to determine the magnitude of the intestinal parasites. This technique was preferred mainly for its quantitative in nature, simplicity and rapidness. The commonly encountered parasitic infestation intensity is presented in Table 2.

Reagents:

- 10 % formal saline

- Glycerine and tincture of malachite green

Equipments:

- Small glass rods.
- Pestle and mortar
- Cellophane paper
- Wooden applicator
- Screw capped universal bottle with rubber liner
- Clean slides
- Pasteur pipette with rubber teat
- Stool containers
- Olympus model K microscope

Procedures:

1. Measure about 10 ml of 10% formal saline in the graduated glass centrifuge tube. Pour into the clean mortar.
2. Smear 50 μg of fresh stool specimen by cleaning the direct faecal and smearing pressed out thinly under glycerine impregnated cellophane.
3. A sample of fresh stool is pressed through a fine wire gauze sieve (#25) to remove fibrous tissue.
4. A template hole of 50 μg capillary is placed on a microscope slide.
5. A portion of sieved stool s transferred to a microscope slide using a wooden applicator stick to fill the template hole resting on the slide.

6. The template is removed, and a strip of cellophane impregnated with glycerine and tincture of malachite green is pressed over the faeces on the slide until it is evenly spread,
7. Label the slide with the participant's laboratory number. Place the slide on the microscope stage.
8. Swing the x10 objective into position and focus; examine the preparation systematically for cysts larvae and ova. Examine structures in more detail using the x40 objective,
9. Examine systematically for ova and parasites using x10 objective. Examine cysts in more detail using the x40 objective after 30 minutes. Record findings using digital telecounter, for parasitic intensity and record on the questionnaire (Monica, 1998),

Table 2. Definitions of Parasitic Intensity Used in the Study

Type of parasites	Intensity of infection	Egg count
A. Lumbricoides	Light	< 7000
	Moderate	7000-35,000
	Heavy	>35,000
A. Duodenale (Hook worm)	Light	< 5000
	Moderate	50,000-20,000
	Heavy	>20,000
E. Vermicularis	Light	< 100
	Moderate	101-400
	Heavy	>400
S. mansoni	Light	< 100
	Moderate	101-400
	Heavy	>400

Source: The impacts of helminthes infection on human nutrition by Stephenson and Hlland, 1996.

3.6.2.7. Study Instruments

3.6.2.7.1. Questionnaires

A structured questionnaire, which was pre-tested in a similar community but not in the study households, was developed and used. The questionnaire used comprised of six sections as follows:

1. The first part contained questions on selected socio-demographic characteristics of the study subjects including age, marital status, education, average monthly income, occupation, etc,
2. Health seeking behaviour and morbidity information, including water sanitation and hygiene, as well as possession of latrines, and how to handle the refuse disposal among other things.

3. Dietary intake assessments consisting of a list recall of fruit, vegetable, meat, egg and fish consumption of commonly and frequently eaten ones.

4. Anthropometric measurements, which included height and weight.

5. Baseline clinical and biochemical assessment, and parasitological examination.

6. Follow-up, which consisted of compliance, clinical and biochemical assessment.

Equipments:

- A locally made wooden height measuring board with a precision of 0.1 cm, was used for measuring the height of the subjects

- A digital scale balance with a precision of ±100grams was used for measuring the weight of the subjects.

3.6.3. Post Intervention or supplementation Assessment and Measures Taken

On the day after the last dose of iron administered, (after 90 days or 12 weeks), another sample of haemoglobin from every supplemented, and control group, and serum ferritin from a sub-sample (n=53) of previously tested mothers was collected and analysed as in baseline. The control groups were treated and given the three months course on weekly administration schedule.

3.6.3. Monitoring Adverse Experience on Supplementation

Present evidence indicates supplementing iron at recommended dose to women causes minor effects such as constipation, abdominal pain or nausea in less than 5%

(DeMaeyer, 1989). However, in this study the subjects were followed and checked for adverse effects of iron throughout the study period by the field workers and the researcher. All symptoms and complaints encountered during the study were recorded in the follow-up daily or weekly tally sheets.

3.6.5. Data Validity and Reliability

To improve validity of the information collected the interviewers were closely supervised during the pre-testing. Probing questions were asked to reduce errors arising from respondents' memory lapses. Each completed questionnaire was checked immediately after return from interview to ascertain all the question had been answered correctly and consistently, and also rechecked the information collected in a randomly selected sub-sample (10%) of the study subjects.

To control the quality of specimen, duplicate analysis were performed for haemoglobin in all the samples, and a sub-sample(5%)of the serum ferritin, and the variability was 0.2 1gm/dl and 0.52µg/l respectively (Baker, 1974). A 5-kilogram iron bar was used to regularly check scale accuracy and make sure measurements are correct.

3.6.6. Data Analysis

Data were entered and cleaned at the study site using the statistical package for social science (SPSS), version nine, and analysed for changes in haemoglobin, serum ferritin, compliance, and morbidity for the different groups.

Differences in means and variances, between two variables of same group (within group) were compared with paired t-tests; whereas differences in means and variances,

between groups were compared with independent t-test or analysis of variance (ANOVA). Potential confounders among groups were tested by either student t-test for continuous outcome, or chi-square for dichotomous outcome. The chi-squared test was also used to test association between dichotomous variables.

Confidence intervals (95%) were determined. The results were adjusted for level of anaemia at the beginning of the study and other confounders found to be different at baseline.

Altitude adjustments for haemoglobin was based on an increase of 0.3 gm/dl per 1000 meters at sea level to determine the prevalence of anaemia (INACG, 1982). Graphs on some descriptive information were done using Microsoft excel version nine. Differences were considered statistically significant when was $p<0.05$.

3.6.7. Limitation of the Study

The study was restricted to lactating women of reproductive age group residing in *Kebeles* of *Teklehaimant Wereda*, for at least one year.

It should be remembered that data obtained through a longitudinal study has sometimes limitations such as defaulting due to the undesirable effect of the iron supplementation, change of addresses and others. Furthermore, some doubtful responses may occur, as some respondents do not report the truth.

3.6.8. Constraints

Few mothers recruited refused to continue/dropped out from the study due to the undesirable effect of iron tablets; an anticipated extra cost for incentives arose.

CHAPTER FOUR

4. RESULTS

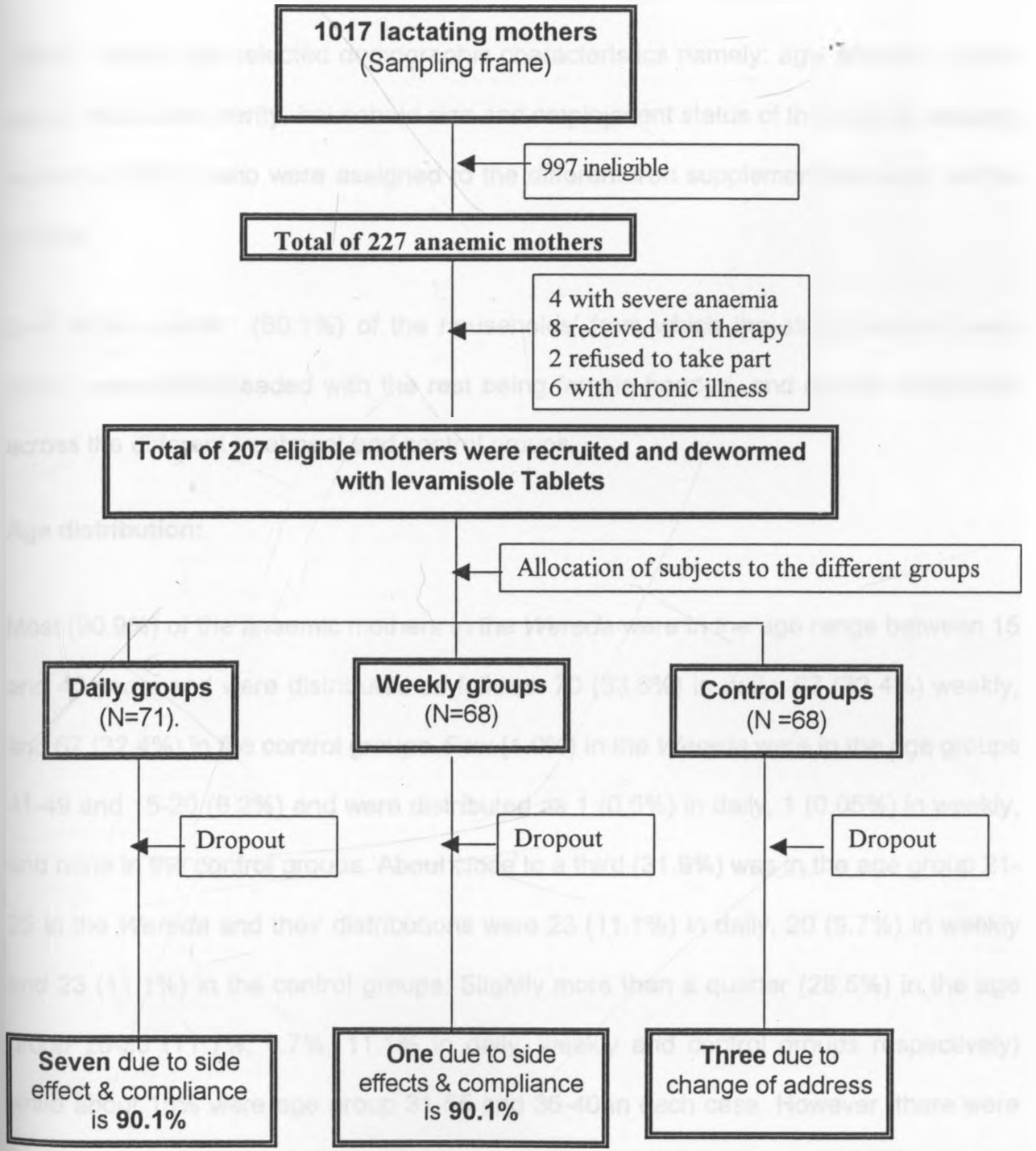
4.1. Introduction

This chapter mainly divided into two major parts. The first part deals with the descriptive results of the baseline or crossectional data on demographic, nutritional, morbidity status, clinical and biochemical findings of the control, weekly and daily iron supplemented groups. The second part deals with the outcome of the iron therapy following the different schedules of iron treatment.

4.1.1. BASELNE CHARACTERSTICS OF THE SUBJECTS

The total number of households, with lactating women, aged 15 to 49 years old, recruited in the census was 1017. A total of 227 of the mothers were identified as being anaemic. However, only 207 were considered for the stud because twenty were excluded. Of the twenty mothers, six had chronic illnesses, four had received iron supplements two weeks before the study, and four had severe anaemia while two had effused to take part in the study. The 207 eligible mothers were assigned to the different iron supplementation groups or controls. Of the 68 women assigned to the control group, 68 to weekly supplemented groups and 71 to daily supplemented groups, complete results over the 3 months period were obtained from 64, 65 and 67 women respectively (Figure 6). Although the groups should ideally have an equal number of subjects, this was not possible. For the daily and weekly supplemented groups, subjects dropped because of the negative side effects of iron while the control groups dropped out because of change of addresses. Thus, complete data on the post intervention survey are available only for 196 lactating women.

Figure 6. Trial profile



4.1.1.2. Socio-Demographic Status:

Table 3 shows the selected demographic characteristics namely: age, religion, marital status, education, parity, household size and employment status of the eligible lactating mothers (N=207) who were assigned to the different iron supplementation and control groups.

Over three quarter (80.1%) of the households' from which the study subjects were drawn, were male headed with the rest being female headed, and equally distributed across the different treatment and control groups.

Age distribution:

Most (90.9%) of the anaemic mothers in the *Wereda* were in the age range between 15 and 40 years and were distributed as follows: 70 (33.8%) in daily, 67 (32.4%) weekly, and 67 (32.4%) in the control groups. Few (1.0%) in the *Wereda* were in the age groups 41-49 and 15-20 (8.2%) and were distributed as 1 (0.5%) in daily, 1 (0.05%) in weekly, and none in the control groups. About close to a third (31.9%) was in the age group 21-25 in the *Wereda* and their distributions were 23 (11.1%) in daily, 20 (9.7%) in weekly and 23 (11.1%) in the control groups. Slightly more than a quarter (28.5%) in the age group 26-30 (11.1%, 9.7%, 11.1% in daily, weekly and control groups respectively) while about 15% were age group 31-55 and 36-40 in each case. However, there were no significant differences among the groups (X^2 value=7.1, Df=10, P=0.47). The mean age by groups ranged between 28.6±5.7 and 29.0±7.05 years.

Table 3. Selected Demographic Characteristics by Groups

Type of variables	Type of study subjects			Total (N=207)
	Daily (N=71)	Weekly (N=68)	Control (N=68)	
Age reakdown*(yrs)				
15 – 20	4 (1.9)	7 (3.4)	6 (2.9)	17 (8.2)
21 – 25	23 (1.1)	20 (9.7)	23 (11.1)	66 (31.9)
26 – 30	23 (11.1)	4 (6.8)	22 (10.6)	59 (28.5)
31 – 35	13 (6.3)	11 (5.3)	8 (3.9)	32 (15.5)
36 – 40	7 (3.4)	15 (7.2)	9 (4.3)	31 (15.0)
41 – 49	1 (0.5)	1 (0.5)	-	2 (1.0)
Mean±SD	28.62±5.73	29.0±7.05	27.75±5.48	28.46±6.12
Religion*				
Orthodox	50 (24.2)	49 (23.7)	51 (24.6)	150 (72.5)
Moslems	13 (6.3)	14 (6.8)	8 (3.9)	35 (16.9)
Catholic	-	1 (0.5)	3 (1.4)	4 (1.9)
Protestant	7 (3.4)	3 (1.4)	5 (2.4)	15 (7.2)
Other Christians	1 (0.5)	1 (0.5)	1 (0.5)	3 (1.4)
Marital status*				
Single	11 (5.3)	15 (7.2)	11 (5.3)	37 (17.9)
Married	49 (23.7)	45 (21.7)	48 (23.2)	142 (68.6)
Divorced	2 (1.0)	4 (1.9)	6 (2.9)	12 (5.8)
Separated	6 (2.9)	2 (1.0)	3 (1.4)	11 (5.3)
Widow	3 (1.4)	2 (1.0)	-	5 (2.4)
Education status*				
None	12 (5.8)	19 (9.2)	13 (6.30)	44 (21.3)
1-4	8 (3.9)	6 (2.9)	5 (1.9)	19 (9.2)
5-8	31 (15.0)	23 (11.1)	29 (14.0)	83 (40.1)
Secondary	18 (8.7)	17 (8.2)	21 (10.1)	56 (27.1)
Post secondary	2 (1.0)	3 (1.4)	-	5 (2.4)
Parity*				
Para one	11 (5.3)	26 (12.6)	22 (10.6)	59 (28.5)
Para two	23 (11.4)	10 (4.8)	15 (7.2)	48 (23.2)
Para 3-5	24 (11.6)	21 (10.1)	27 (13.0)	72 (34.8)
Multipara (≥6)	13 (6.3)	11 (5.3)	4 (1.9)	28 (13.5)
Mean ±SD	3.55±0.9	3.25±1.14	3.19±0.9	3.33±1.03
Household size*				197 (95.1)
< 10 family members	67 (32.4)	6 4 (30.9)	46 (31.9)	10 (4.9)
≥ 10 family members	4 (1.9)	4 (1.9)	2 (1.0)	6.34±2.7
Mean±SD	6.14±2.8	6.6±2.6	6.29±2.6	
Employment status*				
Self	2 (1.0)	9 (4.3)	4 (1.9)	15 (7.2)
Government	2 (1.0)	2 (1.0)	2 (1.0)	112 (54.1)
Daily labourer	7 (3.4)	7 (3.4)	6 (2.9)	20 (9.7)

*** P>0.05, Figures in parentheses are percentages.**

Religion:

Majorities of the study subjects were orthodox Christians (72.5%) followed by Moslems 35 (16.9%), and small number of Catholic 4 (1.9%), Protestants 15(7.2%) and other Christians 3 (1.4%); and the number of Orthodox Christians in the daily, weekly and control groups were 50 (24.2%), 49 (23.7%) and 51 (24.6%) respectively whereas the Moslems were 13 (6.3%), 14 (6.8%) and 8 (3.9%) in the daily, weekly and control groups respectively indicating that the groups were almost homogenous.

Marital Status:

Abut two-thirds of the study subjects were married (68.6%). The proportion of married subjects in daily, weekly and control groups (23.7%, 21.7% and 23.2% respectively) were not significantly different.($p>0.05$) The proportion of lactating mothers who were single (17.9% for the entire groups) 5.3%, 7.2% and 5.3% in the daily, weekly and control groups respectively, were not significantly different among groups (X^2 value=8.0,Df=8, P=0.4). The proportion of divorced, separated and widowed subjects was negligible.

Education Status:

The proportion of the study subjects who had formal education in the *Wereda* was slightly more than three-quarters (78.8%) distributed as follows: 59 (14.6%) in the daily, 33 (23.6%) in weekly and 55 (26.0%) in the control group. While the proportion of mothers in the *Wereda* who had no education at all was less than a quarter (21.3%) and were 12 (5.8%) in daily, 19 (9.2%) in weekly and 13 (6.3%) in the control groups.

However, the differences noted were not statistically significant in the different groups (X^2 value=7.1, Df=8, P=0.51).

Parity Size:

The mean parity among the daily and weekly iron supplemented, and control groups ranged between 3.19±0.9 and 3.55±0.9. The proportion of mothers in the *Wereda* with one child (parity) was 59 (28.5%) distributed as 11 (5.3%) in the daily, 26 (12.6%) in the weekly and 22 (10.6%) in the control groups. The proportion of mothers with 2 to 5 children was 120 (58.0%) in the *Wereda* distributed as 47 (23.0%) in the daily, 31 (14.9%) in the weekly and 42 (120.2%) in the control groups. However, the difference noted among the groups was not significant (X^2 value=7.1, Df=8, P=0.08). The proportion of mothers with more than 6 children (multipara) in the *Wereda* was 28(13.5%) and were 13 (6.3%), 11 (5.3%) and 4 (1.9%) in the daily, weekly and control groups respectively.

Household Size:

The mean household size in the different groups ranged from 6.1±2.8 to 6.6±2.6. Overall, only 10 (4.9%) of the households in the *Wereda* had a family member more than ten. The proportion of households with family member more than ten were 4 (1.9%), 4 (1.9%) and 2 (1.0%) in the daily, weekly and control groups respectively. However, no significant differences were observed among the groups (X^2 value=7.1, Df=2, P=0.5). The proportions of mothers with less than 10 members of household size were197 (95.1%) in the *Wereda* distributed as follows:, 67 (32.4%), 64 (30.9%) and 46 (31.9%) in the daily, weekly and control groups respectively.

Employment Status:

About a half of the subjects (54.1%) in the *Wereda,* were housewives and were evenly distributed among the different supplementation and control groups. Those who worked outside their home were engaged in either salaried 112 (54.1%) or self-employment 15 (7.2%) and the rest in casual or daily labour 20 (9.7%). The proportion in employment as daily labourers or government employees was not significantly different among the groups (X^2 value=9.1, Df=8, P=0.3). The proportion of mothers who had no job at the time of the study was nearly similar and were 16 (7.7%), 17 (8.2%) and 18 (8.7%) in the daily, weekly and control groups respectively.

4.1.1.3. Dietary Pattern/ Intake Habit

The food frequency pattern, cigarette smoking habits and coffee drinking after a meal by the study groups are shown in Table 4, while consumption of selected foods is presented in Table 5.

Meal frequency:

The mean consumption frequency of meal per day was practically the same for the entire group (i.e. About 3). This is was the practice for the majority (87.0%) of the mothers in the *Wereda* who had three meals a day. The proportion of mothers who had a meal two times a day was very small as that of mothers who had four meal a day or one meal a day. In all these cases there were no significant differences among the groups.

Smoking and tea or coffee drinking habits:

None of the mothers had the habit of smoking at all; but most (95.2%) were found to drink either coffee or tea after a meal as shown in Table 4. The proportion of mothers who had the habit of drinking coffee after meal were virtually similar for the entire groups (ie. 32.4%, 30.9% and 31.9% in the daily, weekly supplemented and control groups respectively).

Table 4. Frequency of Meal, Drinking of Tea or Coffee after Meal and Smoking Habits of Groups.

Type of variables	Type of groups			Total (N=207)
	Daily (N=71)	Weekly (N=68)	Control (N=68)	
Meals frequency				
Once a day	4 (1.9)	4 (1.9)	3 (1.4)	11 (5.3)
Twice a day	3 (1.0)	6 (2.9)	3 (1.4)	12 (5.8)
Three times a day	62 (30.0)	57 (27.5)	61 (29.5)	180 (87.0)
Four times a day	2 (1.0)	1(0.5)	1 (0.5)	4 (1.9)
Mean±SD	3.0±0.2	2.9±0.39	2.97±0.245	2.96±0.3
Smoking habits				
Yes	-	-	-	-
No	71 (34.3)	68 (32.9)	68 (32.9)	207 (100.0)
Drinking coffee				
Yes	67 (32.4)	64 (30.9)	66 (31.9)	197 (95.2)
No	4 (1.9)	4 (1.9)	2 (1.00)	10 (4.8)

Figures in parentheses are percentages

Injera made of *Tef* (**Eragrostis Teff**) a type of cereal together with locally prepared sauce from chickpea; little oil and chilli were the commonly consumed staple diet in the *Wereda*. The frequency consumption of iron rich foods from animal source was uniformly low in the *wereda* as shown in Table 5. The most commonly and frequently consumed foods were foods of plant source.

Table 5. Frequency and Consumption of Selected Foods by Group.

Selected foods rich in vitamin or minerals	Frequency consumed	Type of groups				X^2	P
		Daily (N=71)	Weekly (N=68)	Control (N=68)	Total (N=207)		
Plant source							
Kale	Daily	63 (30.4)	57 (24.5)	58 (28.0)	178 (86.0)	0.7	0.6
	Weekly	8 (3.9)	11 (5.3)	10 (4.8)	29 (14.0)		
Lettuce	Daily	41 (19.8)	30 (14.5)	31 (15.0)	102 (49.3)	3.1	0.2
	Weekly	30 (14.5)	38 (18.4)	37 (17.9)	105 (50.7)		
Spinach	Daily	62 (30.0)	58 (28.0)	51 (24.6)	171 (82.6)	4.1	0.1
	Weekly	9 (4.1)	10 (4.8)	17 (8.2)	36 (17.4)		
Fruits	Daily	52 (25.1)	45 (21.7)	40 (19.3)	137 (66.2)	3.2	0.1
	Weekly	19 (9.2)	23 (11.1)	28 (13.5)	70 (33.8)		
Animal source							
Eggs	Daily	53 (25.6)	53 (25.6)	54 (26.1)	160 (77.3)	0.47	0.7
	Weekly	18 (8.7)	15 (7.2)	14 (6.8)	47 (22.7)		
Fish	Daily	12 (5.8)	5 (2.4)	6 (2.9)	23 (11.1)	3.7	0.1
	Weekly	59 (28.5)	63 (30.4)	62 (30.4)	184 (88.9)		
Meat	Daily	29 (14.0)	26 (21..6)	22 (10.6)	77 (37.2)	1.1	0.5
	Weekly	42 (20.3)	42 (20.3)	46 (22.2)	130 (62.8)		
Milk	Daily	27 (13.0)	23 (11.1)	18 (8.7)	68 (32.9)	1.2	0.5
	Weekly	44 (21.3)	45 (21.7)	50 (24.2)	139 (67.1)		

Figures in parentheses are percentages.

Kale:

Kale was consumed daily by the majority ie. 176 (86.0%) of the mothers in the *Wereda,* distributed as follows: 63 (30.4%), 57 (28.5%) and 58 (28.05%) in the daily, weekly and control groups respectively while the proportion of mothers who consumed once weekly was 8 (3.9%) in daily, 11 (5.35%) in weekly and 10 (4.8%)in the control groups. However, no significant difference was observed among the groups (X^2 value=0.7, Df=2, P=0.6).

Spinach:

Over three quarter (82.6) of the subjects consumed Spinach daily in the *Wereda* distributed as follows: 62 (30.0%), in daily, 58 (28.0%) in weekly and 51 (24.65%) in the control groups. While the proportion of mothers who consumed once weekly was 9 (4.1%), 10 (4.8%) and 17 (8.2%) in the daily, weekly and control groups respectively. However, the difference noted in among the groups was not significant (X^2 value=3.1, Df=2, P=0.2).

Meat:

Meat was consumed daily by 68 (32.9%) of the mothers in the *Wereda* distributed as follows: 27 (13.0%), 23 (11.1%) in weekly and 18 (8.7%) in the control groups while the proportion of mothers who consumed once weekly was 44 (21.3%) in daily, 45 (21.7%) in weekly and 50 (24.2%)in the control groups. However, the difference observed between the groups was not statistically significant (X^2 value=0.4, Df=2, P=0.7).

Fish:

Fish was consumed weekly by 184 (88.9%) of the mothers in the *Wereda* distributed as follows: 59 (28.5%) in daily, 63 (30.4%) in weekly and 62 (30.4%) in the control groups. While the proportion of mothers who consumed daily was relatively low in all the groups (X^2 value=3.7, Df=2, P=0.1).

The sources of the foods consumed in the *Wereda* were, either produced (2.4%), purchased (92.8%), produced and purchased (1.0%), relief donations (0.5%) or combinations of all (3.5%). The proportion of mothers who purchased their food for consumption were close to a third in the entire group (33.3%, 28.5% and 30.9% in daily, weekly and control groups respectively). However, the differences were not significant (X^2 value=2.4, Df=2, P=0.08). This shows that almost all the subjects got their food from the market. Donations were very little.

4.1.1.3. Health Status and Morbidity

Table 6 shows the distribution of various health problems such as reported illnesses, anaemia, iron deficiency anaemia status and distribution of intestinal parasite by the different supplementation and control groups.

Reported illnesses or morbidity status

The number of mothers who reported illnesses in the last seven days prior to the study was about a third (33.8%) in the *Wereda* which practically equally distributed, ie. 23 (11.1%) in the daily, 25 (12.1%) in the weekly and 22 (10.6%) in control groups. The

types of reported illnesses in the *Wereda* were weakness or easy fatigue-ability 37 (17.9%), headaches 24 (11.6%) and flu 10 (4.8%). The proportion of mothers who had weakness was distributed as follows: 9 (4.3%) in the daily, 15 (7.2 %) in the weekly and 13 (6.2%) in control groups.

The proportion of mothers who had headache was distributed as follows:: 11(5.3%) in the daily, 8 (3.9%) in the weekly and 5 (2.4%) in control groups. While flu distribution was 4 (1.8%), 3 (1.4%) and 3 (1.4%) in the daily, weekly and control groups respectively. There was no significant difference observed among the groups (X^2 value=5.7, Df=8, P=0.6). Neither of the groups had murmur, organomegally, nor nail changes (koilonychias) nor oedema.

Table 6. Distribution of Various Health Problem by Groups

Type of variables	Type of groups			Total (N=207)
	Daily (N=71)	Weekly (N=68)	Control (N=68)	
Health problem	23 (11.1)	25 (12.1)	22 (0.6)	70 (33.8)
Type of reported illnesses				
Headache	11 (5.3)	8 (3.9)	5 (2.4)	24 (11.6)
Flu/fever	4 (1.8)	3 (1.4)	3 (1.4)	10 (4.8)
Weakness	9 (4.3)	15 (7.2)	13 (6.3)	37 (17.9)
Pallor				
Palmar	10 (4.8)	9 (4.3)	11 (5.3)	30 (14.5)
Nail	8 (3.9)	7 (3.4)	10 (4.8)	25 (12.1)
Anaemia (Hgb < 12 gm/dl)				
Mild type (hg 1011.9gm/dl)	71 (34.3)	68 (32.9)	67 (32.4)	206 (99.5)
Moderate type (7-10gm/dl)	-	-	1 (0.5)	1 (0.5)
Mean±SD	11.5± 0.38	11.4±0.35	11.41±0.45	11.45±0.4
Serum ferritin (IDA status)	(n=19)	(n=19)	(n=15)	(n=53)
<12 µg/l(deficient)	5 (9.4)	5 (9.4)	2 (3.8)	12 (22.6)
12 – 40µ g/l(marginal)	7 (13.2)	8 (15.0)	7 (13.2)	22 (41.5)
>40- 150µg /l(normal)	7 (13.2)	6 (11.3)	6 (11.3)	19 (35.8)
>150 (positive iron balance)	-	-	-	-
Mean±SD	55.15±48.17	43.03±30.7	60.9±62	52.4±47.2
Intestinal parasites				
Ascaris ova	12 (5.8)	12 (5.8)	13 (6.3)	37 (17.9
Hook worm ova	-	1 (0.5)	3 (1.4)	4 (1.9))
Strongyloid larva	3 (1.4)	1 (0.5)	1 (0.5)	5 (2.4)
Others	6 (2.8)	2 (1)	1 (0.5)	9 (4.3)

Figures in parentheses are percentages

Pallor of the Palms and Nail Bed

Over a quarter (26.6%) of the mothers were found to have palmar and fingernail pallor in the entire group. Palmar pallor was 30 (14.5%) in the *Wereda* and was distributed as follows: 10 (4.8%) in daily, 9 (4.3%) in weekly and 11 (5.3%) in the control groups, while

nail pallor was 25 (12.1%) in the *Wereda* distributed as 8 (3.9%) in daily, 7 (3.4%) in weekly and 10 (4.8%) in the control groups.

Haemoglobin Concentration and Anaemia Status

The degree and types of anaemia according to the different supplementation and control groups is presented in Table 6. While the study subjects enrolled were lactating mothers from the lower socio-economic strata, the overall prevalence rate of anaemia for the entire groups of lactating women was 22.3% according to the age specific cut-offs for initial haemoglobin values (Hgb.<12 gm/dl). The prevalence rate is slightly higher when compared with the prevalence of anaemia in rural lactating women of Ethiopia. The proportion of subjects who had haemoglobin below 12 gm/dl was almost equally distributed as follows: 71 (34.3%) in the daily, 68 (32.9%) in weekly, and 68 (32.9%) in the control group. Majority (99.5%) of the subjects had a mild type of anaemia, while few (0.5%) had the moderate type. The mean haemoglobin concentration for the entire group ranged between 11.4 and 11.5 gm/dl. No significant differences were observed across the different treatment and control groups (F value=2.4, Df=2, P=0.09).

Serum Ferritin (IDA) Status

Overall, 12 out of 53 of the subjects had a serum ferritin concentration (SFC) below 12 µg/L, and the remaining subjects had SFC above 12 ug/L, which shows that the prevalence of iron deficiency and iron deficiency anaemia in the entire group is 22.6% and 22.3 % respectively. The proportion of mothers with SFC below 12 ug/L was distributed as follows: 5(9.4%) in the daily, 5(9.4%) in the weekly and 2(3.8%) in the

control groups. The proportion of mothers with marginal iron deficiency (12-40 µg/L) anaemia and normal SFC (100±60 µg/L) are nearly uniform and was distributed as follows: 7 (13.2%) in the daily, 6 (11.3%) in the weekly and 7 (13.2%) in the control groups. The mean serum ferritin concentration (SFC) ranged between 43.01 and 60.97 µg/litre and no significant difference was observed among the groups (F value=0.6, Df=2, P=0.5).

Intestinal Parasites and Treatment of Reported Illnesses

The commonest type of intestinal parasite found in the *Wereda* is Ascariasis as shown by its prevalence of 17.9% distributed as follows: 12 (5.8%) in daily, 12 (5.8%) in weekly and 13 (6.3%) in the control groups. Strongyloidiasis was the second commonest parasitic infestation in the *Wereda* with a prevalence rate of 2.4% distributed as 3 (1.4%) in daily and 1 (0.5%) in the remaining groups. T he prevalence of hookworm was 1.9% in the *Wereda* and was distributed as follows: 1 (0.5%) in weekly, 3 (1.4%) in control and none in the daily groups. However, no significant difference was observed among the groups (X^2-value=12.0, Df=9, P=0.6).

The prevalence of other infestation such as Giardiasis, Hymenolepis nana and Teania saginata were uniformly low 9 (4.3%) in the *Wereda* and were distributed as follows:: 6 (2.8%) in daily, 2 (1.0%) in weekly and 1 (0.5%) in the control groups.

The proportion of mothers who received treatment for their health problem in the *Wereda* was 11 (5.3%) and distributed as follows: 4 (1.9%) in daily) 2 (1.0%) in weekly and 5 (2.4%) in the control groups. The type of treatments received was unidentified capsules 6 (54.5%) and injection 5 (45.5%). The proportion of subjects who received

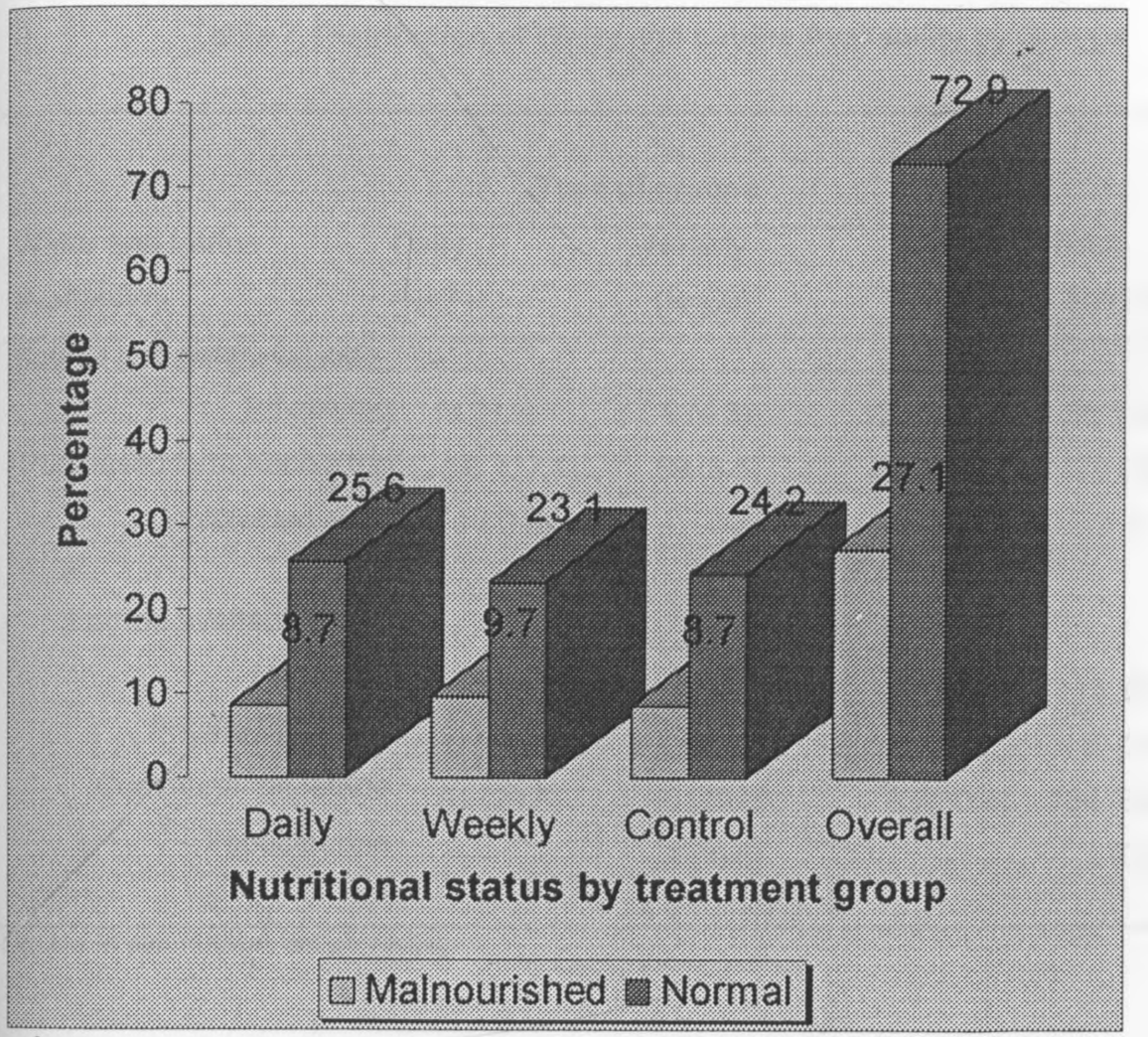
capsules was distributed as follows: 3 (27.3%) in daily, 1 (9.1%) in weekly and 2 (18.2%) in the control groups while those subjects who received injection were distributed uniformly (9.1%) in each group.

4.1.1.5. Nutritional Status of the Mothers

The prevalence of malnutrition as determined by body mass index (BMI) is presented in Figure 5. The overall prevalence of chronic energy malnutrition (BMI <18.5 kg/m^2) was 27.1% with a mean BMI ranged between 20.4 and 21.09 kg/m^2. These were distributed follows: 18 (8.7%,) 20 (9.7%), and 18 (8.7%) in the daily, weekly and control groups respectively.

While the proportion of mothers with desirable weight (BMI ≥ 18.5 kg/m^2) in the *Wereda* was 151 (72.9%) distributed as follows: 53 (25.6%) in daily, 48 (23.1%) in weekly and 50 (24.2%) in the control groups. However, no significant difference is observed among groups (X^2-value=0.30, Df=2, P=0.8).

Figure 7. Nutritional Status of the Mothers in the Study Areas by Groups



(X^2-value=0.30, Df=2, P=0.8)

4.1.1.6. Environmental Health Status in the *Wereda*

Water and Latrine Availability

The distribution of mothers by type of water availability in all the groups is shown in Table 7. The major water source for consumption in the community was tap water as reported by 205 (99.0%) of households. The rest of households 2 (1.0%) used well water.

Table 7. Distribution of Water and Latrine Availability by Groups

Water and latrine availability	Type of groups			Total
	Daily (N=71)	Weekly (N=68)	Control (N=68)	N=(207)
Source and water availability				
Private tap	14 (6.8)	9 (4.3)	12 (5.8)	35 (16.9)
Purchased form *Kebele* tap	56 (27.1)	59 (8.5)	55 (26.6)	170 (82.1)
Unprotected wells	1 (0.5)	-	1 (0.5)	2 (1.0)
Latrine availability				
No latrine	5 (1.9)	4 (2.4)	4 (1.9)	13 (6.3)
Private pit latrine	2 (1.0)	2 (1.0)	1 (0.5)	5 (2.4)
Communal pit latrine	61 (29.5)	60 (29.0)	63 (30.4)	184 (88.9)
Septic latrine	-	1 (0.5)	-	1 (0.5)

Figures in parentheses are percentages

Over a three quarter (82.1%) of the mothers purchased water from communal tap of the Kebele for consumption. While the rest 35 (16.9%) had water from a tap in their compound and distributed as follows: 14 (6.8%) in daily, 9 (4.3%) and 12 (5.8%) in the control groups. No significant difference was observed among the groups in respect of getting water from own private tap (X2 value=2.1, Df=4, P=0.7).

Communal pit latrines were available in majority (88.9%) of thee households in the *Wereda* distributed as follows: 61 (29.5%) in daily, 60 (29.0%) in weekly and 63 (30.4%) in the control groups, while private pit latrine was available in a few (2.4%) households

distributed as follows: 2 (1.0%) in daily, 2 (1.0%) in weekly and 1 (0.5%) in the control groups. However, the differences noted among the groups were not statistically significant.

None of the households in the entire group had garbage disposal at household level. The environmental sanitation was generally was poor; dirty stagnant water was seen around most (95.1%) of the households compound. The distribution of household sanitation condition was practically the same for all households; 95.1% each in the entire groups. In-addition, there was no drainage at all to depose for dirty water from their houses.

4.1.1.7. Knowledge of Mothers on Causes of Anaemia and Family Planning Use

Table 8 shows the proportion and type of family planning (FP) services used by the different study groups in the *Wereda*. Close to two thirds (59.9%) of the mothers in the *Wereda* reported to have access to family planning services distributed as follows: 43 (20.8%) in daily, 45 (21.7%) in weekly and 26 (17.4%) in the control groups. The remaining 83 (41.5%) were not using the services for religious reason and husband opposition. The distribution was 28 (13.5%) in daily, 21 (10.1%) in weekly and 32 (15.5%) in the control groups.

Injection was the commonest type of family planning method used in about a third of the mothers (33.3%) distributed as follows: 22 (10.6%) in daily, 28 (13.5%) in weekly and 19 (9.2%) in the control groups. Pills were used by slightly more than a quarter (25.1%) in the

Wereda distributed as follows: 20 (9.7%) in daily, 16 (7.7%) in weekly and 16 (7.7%) in the control groups.

Table 8. Knowledge of Mothers and type of family planning used by Groups

Maternal characteristics	Type of groups			Total (N=207)
	Daily (N=71)	Weekly (N=68)	Control (N=68)	
Causes of anaemia				
Low intake of vitamins/ minerals	8 (3.9)	4 (1.9)	8 (3.9)	20 (9.7)
Lack of food/poverty	53 (25.6)	47 (22.7)	52 (25.1)	152 (73.4)
Illnesses	4 (1.9)	14 (6.8)	3 (1.4)	21 (10.1)
Bleeding related with labour	-	-	1 (0.5)	1 (0.5)
Do not know	-	1(0.5)	-	1 (0.5)
Type of Family planning used				
Pills	20 (9.7)	16 (7.7)	16 (7.7)	52 (25.1)
Injection	22 (10.6)	28 (13.5)	19 (9.2)	69 (33.3)
Others	1 (0.5)	1 (0.5)	1 (0.5)	3 (1.50)

Figures in parentheses are percentages

Other methods of contraception used such as intra uterine contraceptive device (IUCD) were practiced by a few (1.50%) mothers in the *Wereda* with the same proportion, in each group. However, no significant difference was observed among between the groups (X^2 value=6.8, Df=8, P=0.5).

Most (95%) of the mothers distributed practically equally among the groups reported that anaemia was one of the commonest health problems in the *Wereda*. About three-

quarter (73.4%) in the Wereda distributed as 53 (25.6%) in daily, 47 (22.7%) in weekly and 52 (25.1%) in the control groups attributed anaemia to lack of food/poverty.

The proportion of mothers, who attributed anaemia to low intake of minerals or vitamins in the *Wereda* was 20 (9.7%) distributed as 8 (3.9%) in daily, 4 (1.9%) in weekly and 8 (3.9%) in the control groups. The proportion of mothers also about ten percent in the *Wereda* attributed anaemia to illnesses. Theses were distributed as follows: 4 (1.9%) in daily, 14 (6.8%) in weekly 3 (1.4%) in the control groups.

Only one mother (0.5%) in the control group attributed anaemia to bleeding during delivery, and one mother (0.5%) in the weekly group said that she did not know the cause of anaemia. The difference was statistically significant among the groups (X^2 value=18.6, Df=10, P=0.04).

4.2. Post Intervention Findings

This section deals with the outcome of iron supplementation effects such as side effects, dropout, compliance, and reduction of anaemia following the different schedules of iron treatment in the supplemented and control groups:

4.2.1. Side Effects of Iron Supplementation

The commonly reported side effects during the first two weeks and four weeks of the therapy in daily and weekly supplemented groups are presented in Table 9.

Eventhough side effects were more common in the daily than the weekly supplemented groups during the first two weeks of the therapy; the difference noted between groups was not statistically significant. The proportion of mothers with nausea, vomiting,

abdominal pain, constipation skin itching and changes in stool colour were 8 (5.7%), 14 (10.1%), 16 (11.6%), 8 (5.7%), 2 (1.4%), and 2 (1.4%) in the daily supplemented group. On the other hand, the proportion of mothers with nausea, vomiting, abdominal pain, constipation, skin itching and changes in stool colour was 6 (4.3%), 2 (1.4%), 4 (2.8%), 2 (1.4%), 1 (0.7%), and 1 (0.7%) respectively in the weekly supplemented groups.

Table 9. Distribution of side Effect after iron supplementation by groups

Type of Side effects	**Daily groups**		**Weekly groups**	
	Week2* (N=71)	week4 ** (N=67)	Week2* (N=71)	week4** (N=67)
Nausea	8 (5.7)	6 (4.3)	6 (4.5)	4 (3.0)
Vomiting	14 (10.1)	2 (1.4)	10 (7.5)	-
Abdominal pain	16 (11.6)	4 (2.8)	12(9.2)	6 (4.5)
Constipation	8 (5.7)	2 (1.4)	3 (2.1)	-
Skin itching	2 (1.4)	1 (0.7)	1 (0.7)	-
Change in stool colour	2 (1.4)	-	-	-
Total	50 (35.9)	16 (11.3)	16 (22.8)	10 (7.5)

Figures in parentheses are percentages, *X^2 value=28.54, Df=5, P=0.00 , **X^2 value=19.8.54, Df=5, P=0.00

The symptoms lowered during the fourth weeks in both the supplemented groups. These were distributed as follows: nausea 6 (4.5%), vomiting 10 (7.5%), abdominal pain 12 (9.2%), constipation 3 (2.1%) and skin itching 1 (0.7%) in the daily supplemented groups. Only 4 (3.0%) of the mothers with nausea and 6 (4.5%) with abdominal pain were reported in the weekly supplemented group.

The above symptoms became milder in the sixth and eight weeks in both groups and gradually subsided except in 8 women who were forced to discontinue 7 in daily and 1 in weekly supplemented group.

4.2.2. Dropout and Compliance

From the 207 eligible mothers who were randomised into the different treatment and control groups, eight of them stopped participating in the study, due to the undesirable effect of the drugs administered, and three due to change of residence. Overall, the dropout rate was 5.3% (11/207) for the entire group; the dropout rate was slightly higher in the daily (3.4%) than the weekly (0.48%) and control (1.4%) groups. There were eight (3.9%) non-compliant due to the negative side effects of iron therapy. Of whom 7(9.8%) of the cases were in daily and 1 (1.5%) in weekly supplemented groups. Another 3 (4.4%) mothers who were enrolled as control dropped out due to change of residence.

Overall compliance was 94.2% for both groups supplemented. The proportion was significantly higher in the weekly supplemented groups (98.5%) than the daily (90.1%) supplemented groups (x^2 value=4.5, Df=1, P=0.04).

4.2.3. Anaemia Status after Three Months of Intervention

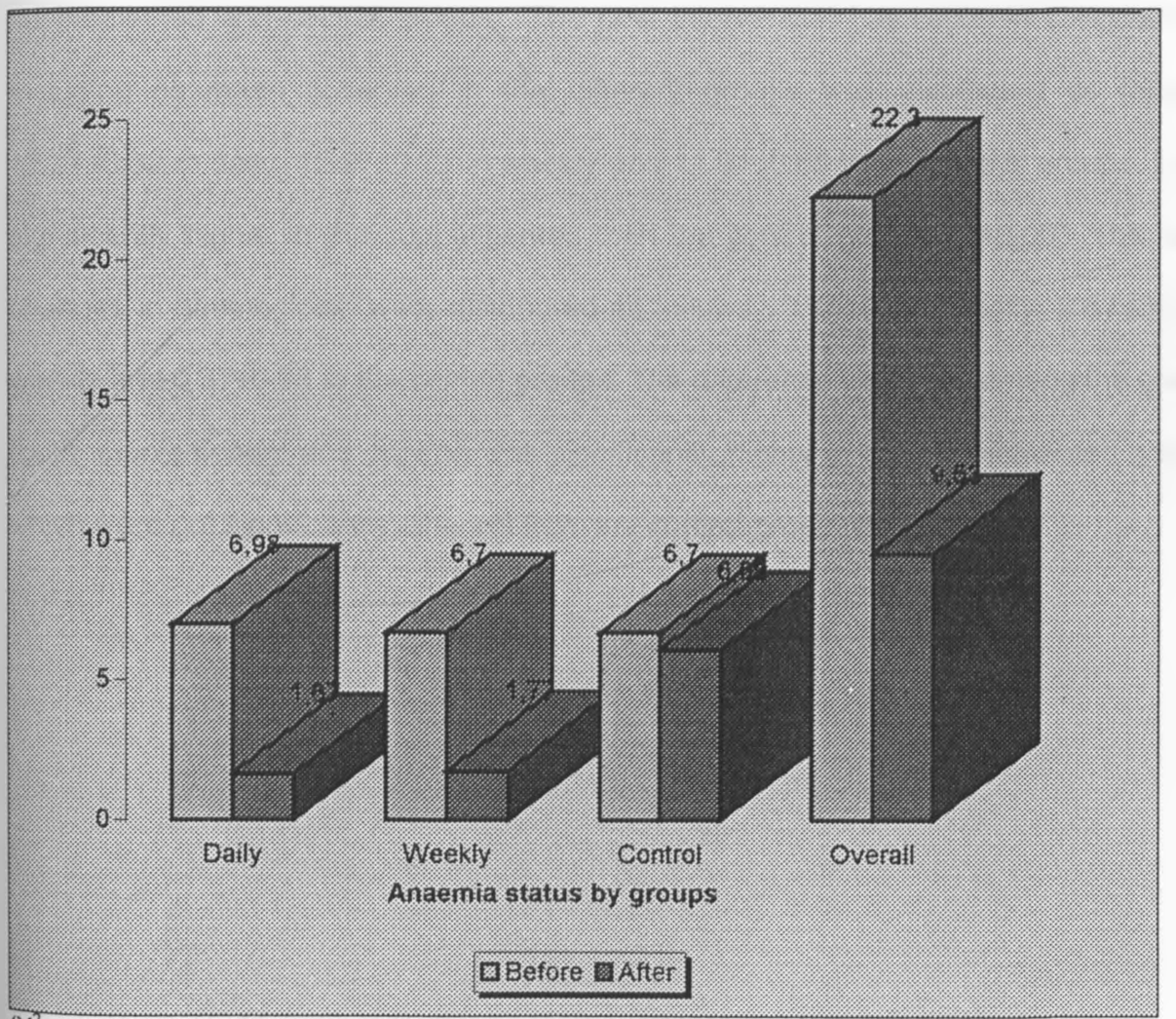
4.2.3.1. Anaemia status as determined by haemoglobin concentration:

Figure 8 shows the status of anaemia (Hgb <12 gm/dl) in the iron supplement and control groups. Overall, anaemia was reduced from **22.3%** to **9.5%** in the entire group distributed as follows: **6.9%** to **1.6 %** in the daily, **6.7%** to **1.7%** in the weekly and **6.7%** to

6.1% in the control groups indicating that the reduction was 5.31%, 5.0% and 0.6% in the daily, weekly and control groups respectively.

When the daily and weekly supplemented groups are compared, the proportion of mothers with normal haemoglobin was slightly higher in the daily than the weekly group. The difference however, was not statistically significant (X^2-value = 0.09, DF=1, P=0.76).

Figure 8. Anaemia Status Before and After Three Months of Intervention



(X^2-value = 85.43, DF=2, P=0.000 for iron supplemented vs. control group).

On the other hand, when both supplemented groups were compared with the control

groups, it was evident that the improvement in haemoglobin concentration was significantly higher in the supplemented groups than the control groups especially when the baseline haemoglobin values are considered (X^2-value = 85.43, DF=2, P=0.000).

4.2.3.2. Haemoglobin concentration before and after three months of intervention

Table 10 shows the 95 % confidence interval, mean±SD and changes in haemoglobin concentrations of the groups before and after three months of treatment in iron supplemented and control groups. The post intervention haemoglobin concentration including its mean observed in the different groups was distributed as follows:: 12.27±0.32 in daily, 12.27±0.32 in weekly and 11.01±0.65 in the control groups. It was significantly higher in the supplemented than the control groups (P=0.00). The mean changes in haemoglobin were distributed as follows:: 0.74±0.49 in daily, 0.83±0.51 in weekly and 0.37±0.76 in the control groups. The difference observed among the groups (both supplemented and control) was significantly higher in the supplemented groups, which were not significantly different from the control group (P=0.00).

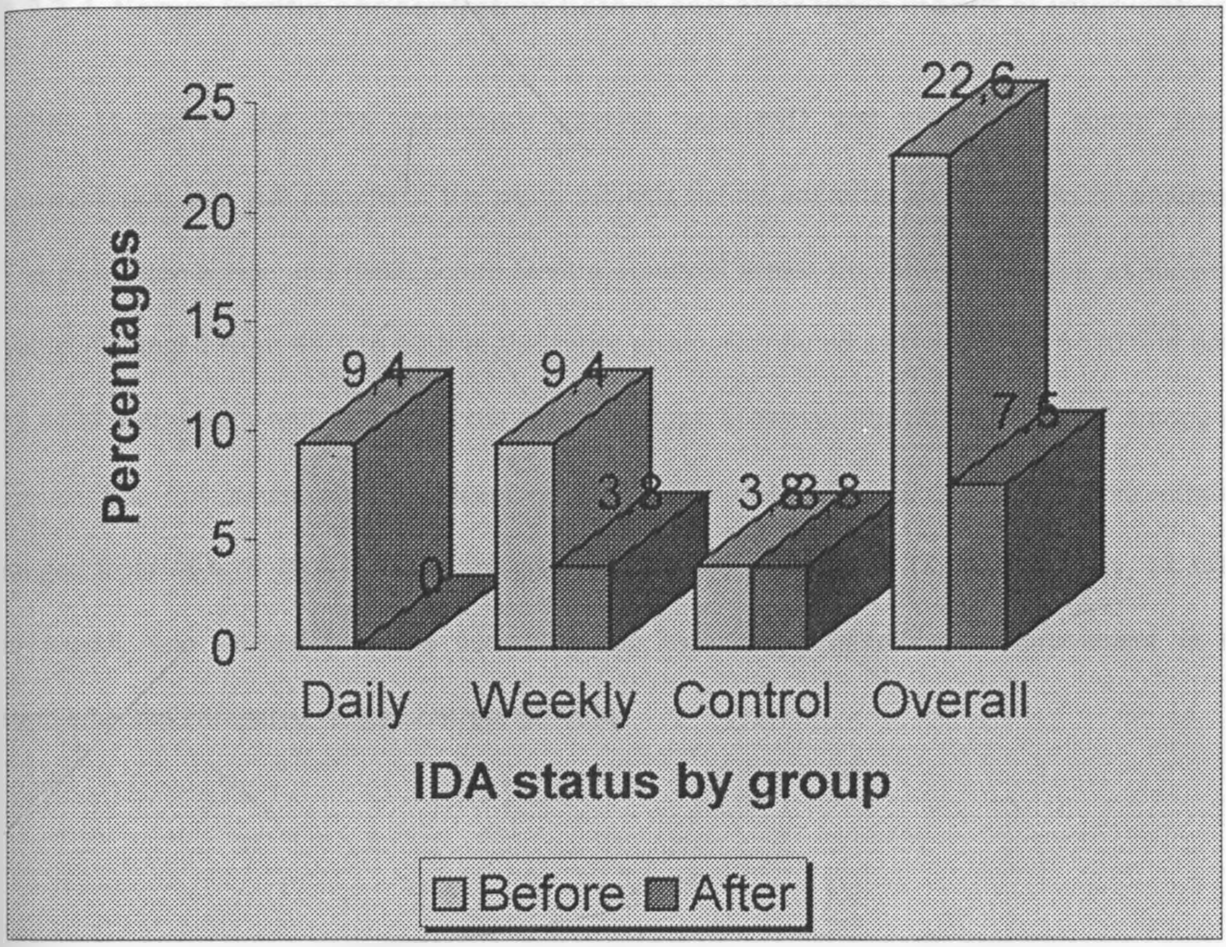
Table 10. **Haemoglobin concentration of mothers before and after three months of intervention**

	Haemoglobin Concentration (in gm/dL)				
Treatment groups	**Before (N=207)** Mean±SD [95% CI]	**After (N=196)** mean±SD [95% CI]	**Change((N=196)** mean±SD [95% CI]	t-value	p-value
Daily	11.5±0.39 [11.4 - 11.6]	12.27±0.32 [12.1 – 12.4]	0.74±0.49 [0.62 – 0.86]	-1.22	0.00
Weekly	11.4±0.36 [11.3 – 11.5]	12.2±0.39 [12.1 – 12.3]	0.83±0.51 [0.70 – 0.95]	-12.9	0.00
Control	11.4±0.44 [11.3 – 11.5]	11.01± 0.65[10.8 – 11.2]	-0.37±0.76 [(-0.56) – (-0.18)]	3.93	0.00
F-value	**2.4**	**144.7**	**80.3**		
p-value	**0.09**	**0.00**	**0.00**		

4.2.3.3. Serum ferritin status after three months of intervention

Figure 9 shows the prevalence of iron deficiency anaemia as determined by (SF below 12 µgm/L and Hgb below 12 gm/dl) before and after iron treatment in supplemented and control groups. Overall, iron deficiency anaemia status was reduced from **22.6 %** to **7.5%** in the entire groups. This was distributed as follows: 7.5**%** to 1.8**%** in the daily, **9.4%** to **3.7%** in the weekly and none in the control groups indicating that the reduction was 13.2% n the *Wereda* distributed as follow: 5.7% in daily, 7.6% in weekly and 0% in the control groups. However, the difference noted between the daily and weekly was not significant (P=0.5), and also among the groups (P=0.31).

Figure 9. Prevalence of Iron deficiency anaemia status before and after intervention by group



(X^2-valu=2.9, Df=2, P=0.31 for the 3 groups, X^2-valu=2.31, Df=1, P=0.05 for the 2 supplemented groups)

The proportion of mothers with normal serum ferritn concentration in the entire group was close to a half (49.1%) distributed as follows:: 11(20.8%) in daily, 9 (17.0%) in weekly and 6 (11.3%) in the control groups indicating that the improvements were 7.2%, 5.7% and 0 % in the daily, weekly and control groups respectively. However, there was no significant difference observed among the groups (P=0.31) neither between the iron-supplemented groups (P=0.5). Interestingly, the maximum level of serum ferritin concentration (SFC) attained were between 83 and 126 µgm/L in the entire group

suggesting that none of the supplemented groups in this study were observed to have a feature of iron load (SFC > 300 µgm/L).

4.2.3.4. Serum ferritin concentration before and after three month of intervention

Table 11 shows the 95% confidence interval, mean±SD and changes in serum ferritin concentration in a sub sample of the study subjects before and after three months of treatment. The post intervention and mean serum ferritin observed across the different groups, as shown in Table 11 ranged from 48.1 to 62.6 in the entire group distributed as follows: 62.6±30.6 in daily, 52.5±29.4 in weekly and 48.1±38.5 the control groups. However, the difference noted among the groups was not statistically significant (P=0.41). The changes in serum ferritin concentration ranged from –8.6 to 18.4 in the entire group distributed as follows:: 18.4±8.7 in daily, 18.3±8.7 in weekly and –8.6±0.7 in the control group indicating a significant improvement in the supplemented compared to the controls (P=0.00).

Table 11. Mean serum ferritin levels in a sub sample of mothers before and after supplementation.

	Serum Ferritin Levels (in µgm/L)				
Treatment groups	**Before (n=53)[a]** mean±SD [95% CI]	**After (n=53)[b]** mean±SD [95% CI]	**Change(n=53)[c]** mean±SD [95% CI]	t-value	p-value
Daily	44.3±28.36 [30.6 - 57.9]	62.6±30.6 [47.8 – 77.4]	18.4±8.7 [14.1-22.6]	-9.14	0.00
Weekly	42.4±28.7 [28.6 – 56.3]	52.5±29.4 [38.3 – 66.7]	18.3±8.7 [14.1 – 22.6]	-7.27	0.00
Control	48.2±38.5 [26.9 – 69.5]	48.1± 38.5[26.7 – 69.4]	-8.6±0.7 [(-0.48) – (0.31)]	0.46	0.64
F-value	**0.14**	**0.91**	**34.9**		
p-value	**0.87**	**0.41**	**0.00**		

CHAPTER FIVE

5. DISCUSSION

The key findings in chapter four are synthesised into baseline and post intervention results, and the likely interpretations relevant to the objectives of the study are presented.

5.1. BASELINE RESULTS

5.1.1. Demographic Characteristics

The study groups enrolled in the intervention trial have been found to have large household and parity size, which are manifestations of typical slum communities. There was no significant difference in the mean age, education, marital status, employment, and household size among the groups indicating that the study groups were homogenous in many ways.

The distribution of these characteristics of the entire group of the *Wereda* concurs with previous study results obtained from the community (Abate, Kogi and Muroki, 2000).

The mean age of mothers, which was 28.4± 6.12 years in the entire group, indicates the problem of anaemia to be more among the younger motherhood group in the community.

The education level of the mothers in the community was low, as expected in slum communities (Harpham *et al,* 1988) and was noted to have a positive association in determining the size of the household (P=0.01) and parity (P=0.000) (Appendix 5).

Dietary Intake Habit

Injera made of *Tef* together with locally prepared sauce from chickpea were the most commonly consumed staple diet in the *Wereda* indicating that the source of nutrients by and large is from plant source. It has been suggested that the absorption of dietary iron takes place from two independent pools namely: a haeme and non-haeme iron pool. In the present study, there is evidence that the mothers get their iron source from none haeme pool suggesting that the quality and inadequacy of food restricts the bioavailabilty of iron. The frequency of consumption of iron-rich foods from animal source was low and the consumption was almost uniform across the group. The lower frequency consumption of fruits, vegetables and meat in the household suggests that the community depends on diets that are lacking in some important vitamin and minerals. Tesemma and Hailu (1997) also reported similar low intake of animal food, where slightly over a quarter of the subjects did not have any of the animal or vegetable sources of diet respectively. This indicates low quality of the diet that might predispose the community to a number of micronutrient deficiencies,

Consumption of the selected foods was further analysed to see if it has any association with anaemia in the community. Egg consumption was noted to have a positive significant association with serum ferritin concentration ($r = 0.24$, $p = 0.05$), whereas, with vegetables, a positive significant association ($r = 0.02$, $p = 0.001$) was observed uniformly among the entire group (Appendix 5). Therefore consumption of vegetables should be encouraged.

5.1.2.1. Smoking habit, tea or coffee and consumption

Various studies documented that cigarette smoking to be associated with higher concentration of haemoglobin level in adults from 0.3 to 0.5gm/dl (Gibson, 1990). On the other hand, consumption of tea or coffee is documented to have a negative effect on the haemoglobin concentration. To avoid the confounding effects of these and avoid biases, information on habits of smoking and consumption of tea/coffee was obtained from all the subjects. There was no significant difference in the consumption of coffee or tea among the groups. In this study, there was no case with habit of smoking. The habit of drinking however, coffee was common. This was found to have a negative and significant association with iron deficiency anaemia ($r = -0.02$, $p = 0.003$) (Appendix 5). Therefore it is justifiable to restrict or lower consumption of coffee because of its negative effects on iron absorption.

5.1.3. Maternal Morbidity, Parasitic Infestation and Family planning use

The proportion of reported illnesses in the last seven days before the study in the entire group was similar. A large proportion of subjects with palmar and fingernail pallor was seen in the entire group, which was significantly and positively correlated with iron deficiency anaemia ($r = 0.36$, $P = 0.01$) suggesting that pallor can be useful for screening anaemia. Even though the proportion of subjects who received treatment for their health problem was more in the control and daily than the weekly groups, there was no significant difference observed among the groups ($P > 0.050$).

It is an established fact that poor hygienic and poor environmental conditions are usually associated with high prevalence of infection and hence is associated with nutritional anaemia (INACG, 1977; INACG, 1982). In the present study, the association between water source for consumption, which was purchased from communal tap of the *Kebele*, and prevalence of illnesses was significantly correlated ($r = -0.18$, $p = 0.008$), while the correlation of latrine availability with frequency of illness was also significant ($r = -0.2$, $p=0.01$) in the entire groups. These can contribute to incidence of diseases, which can ultimately cause maternal malnutrition and death (UNICEF, 1992).

In almost all of the households, there was no access to refuse disposal facility. This figure when compared with slum area of Nairobi where 36% of slum population lack access to facility is higher (UNICEF, 1992). The poor sanitation observed in the community is not different from previously reported studies (Jember, 1985; Harpham, Lusty and Vaughan, 1988; Abate, Kogi and Muroki, 2000).

Although the commonest types of intestinal parasite infestation were Ascariasis (17.9%), strongyloidiasis (2.4%), and hookworm (1.9%) in the *Wereda,* which were almost uniformly distributed among the groups, the correlation of parasites with low serum ferritin concentration (IDA) was positive and it is just about significant ($r = 0.24$, $p = 0.05$) suggesting that that the treatment of intestinal parasites should be considered together with iron supplement to control and prevent iron deficiency anaemia in the community. The observed pattern of Iron deficiency anaemia was consistent with exposure to parasites in all the groups.

To see the confounding effect of contraceptive uses, information was collected and analysed. Pills and injection were used by 55.4% in the entire groups. No significant association was seen with the iron deficiency anaemia in the entire groups ($r = -0.043$, $p = 0.7$) indicating that the confounding effect of family planning among the groups was negligible. However, family planning use was significantly correlated with maternal education ($r = -0.13$, $p = 0.04$). This shows that educated mothers are using the services more than mothers with no education.

5.1.4. Prevalence of Malnutrition

To avoid biases in the measurement of women's nutritional status, pregnant women and women who had given birth in the two months preceding the study were excluded. About 27.1% of the mothers in the present study were found malnourished with BMI of less than 18.5 kg/m^2. The prevalence of malnutrition in the *Wereda* was higher (27.1%) when compared with the figure obtained for Addis Ababa Administrative region, which was (17.9%) indicating that the communities of *Teklehaimant Wereda* are one of the most affected in the Addis Ababa area (CSA, 2001) indicating that nutrition intervention programme deemed to be necessary in the community. Because adequate diet for women has to be seen as a central issue and a major factor in relation to progress in the health and nutritional well being of women in developing as producer and consumer (UNICEF, 1983).

About eleven percent of the entire groups who had less than three meals a day were positively and significantly associated with the prevalence of malnutrition ($r = 0.37$, $p =$

0.001). This appeared to have contributed to the high level of malnutrition in addition to the low intake of nutrients as reported by most of the groups.

Malnutrition and anaemia was reported to be a common health problem in the community. Most (73.0%) of the mothers attributed the problem to be due to lack of food/poverty followed by illnesses (10.1%) and low intake which, is probably due to poverty (9.7%) in the entire group further substantiates the need for an intervention programme in the community.

5.1.5. Side Effects of Iron Therapy

The commonly reported side effects such as nausea, vomiting, abdominal pain, constipation skin itching and changes in stool colour which were reported by the supplemented groups were not uncommon to see them during the first 2 weeks of the therapy. In the present study, side effects were more common in a daily-supplemented group than the weekly supplemented groups. However, the difference observed between the groups was not statistically significant (P=0.4).

5.1.6. Prevalence of Anaemia

The prevalence of iron deficiency anaemia remains high in many parts of the world despite the considerable efforts made to alleviate the situation. The social, health, and economic costs of iron deficiency anaemia are important and should always be considered along with the public health implications.

The significance of iron deficiency anaemia across different age groups and population has been widely reported (ICN, 1978; INACG, 1977; INACG, 1982). It suffices to note that, the effects are significant enough to cause long term retardation of national development through individual's failure to realise full genetic potential and to optimally exploit opportunities. As a consequence of its deficiency in all age group, work capacity is reduced due to physical and mental lethargy and equally important increased susceptibility to infections arising from immunological impairment (WHO, 1972; INACG, 1977; INACG, 1982; DeMaeyer, 1989). Recently developed algorithms for calculating economic loss due to iron deficiency in countries with high burdens of anaemia estimate losses in the order of billions of US dollar every year (Ross and Horton, 1998). The objective of determining the prevalence of anaemia in the study group is achieved and it is of moderate public health problem. Cognisant of these facts, control and prevention of iron deficiency anaemia is a priority in the micronutrient intervention program.

The present study shows a high prevalence of anaemia in the *Wereda*, as determined by haemoglobin concentration, for the entire groups, which is 22.3% as well as iron deficiency anaemia of 22.3% as determined by serum ferritin below 12 μg/litre and haemoglobin less than 12 gm/dL. This figure is slightly higher than the figures (18.4%) obtained among the pregnant and lactating mothers in rural Ethiopia (Haidar, NekaTibeb, Urge, 1999) suggesting that the slum communities/*Kebeles* are at higher risk, and therefore underlines the need for iron supplementation for the women as well as the other at-risk groups in the community.

5.2.0. Post intervention results

After the three months of iron supplementation with various dosage schedules of iron tablets, a significant increase in haemoglobin concentration and a fall in the prevalence of anaemia in both the daily and weekly supplemented groups were found. When the groups receiving daily and weekly dosage schedules were compared, the therapeutic effectiveness was equally significant whether 60 mg of iron is supplemented once weekly for 12 weeks or 60 mg of iron administered daily for three months.

The findings in this study, lead to the rejection of the null hypothesis, which states that a weekly dosage schedule of iron in lactating women will not bring a return towards haemoglobin normality as rapidly, or almost so, when they have moderate anaemia as daily dosage schedule. Thus the alternative hypothesis stating that weekly dosage schedule equally improves the haemoglobin concentration as daily schedule does.

The effects of weekly supplementation to control the problem of iron deficiency anaemia appears to work satisfactorily in this study, which is comparable to the daily supplementation. In a sub-sample, daily administration of iron produced a slightly greater increase in serum ferritin than the weekly supplementation schedule. However, the rise in serum ferritin with daily administration of iron is of no practical significance as long as the preventive supplementation program is conducted and attains the referred haemoglobin increases. In-addition, the slower response of serum ferritin in the weekly schedule seems an important advantage in lessening the chances of iron overload.

The reports of side effects observed in this study were related to the frequency of iron supplementation. The fewer side effects observed in the weekly supplemented group

than the daily group shows that weekly supplementation is better and should be adopted especially where the problem of iron deficiency anaemia is highly prevalent in the country.

A study from China and Indonesia also investigated the potential effect of intermittent/weekly supplementation compared with daily iron supplements (Liu, Kang, Zhao, Viteri, 1995; Ridwan, Shultink, Dillan and Gross, 1996). The study showed the haemoglobin responses to be similar in daily and weekly groups, although the group given the supplement daily had a substantially larger rise in serum ferritin than did the weekly supplemented groups. Side effects were also reported to be more among the daily supplemented than the weekly group (John, 1998).

A recent study regarding the effectiveness of intermittent iron supplementation in young children, aged 2 to 5 years, which included a placebo intervention group but did not have a group that received the supplement daily for 9 weeks provided some perspective on the interpretation of the previous studies in china mentioned earlier (Palupi, Shultink, Dillan and Gros, 1997). The children in the placebo group showed no significant rise in haemoglobin concentration over the intervention period, while the prevalence of anaemia in both the intervention groups fell dramatically and significantly.

The observation that daily supplementation schedule generated more complaints of side effects and compliance was relatively lower when compared with the weekly/intermittent supplementation schedule also lead to the rejection of the null hypothesis stating that there are no significant differences in compliance, between when weekly doses are

compared with daily dosage schedule. Consequently, the alternative stating that hypothesis there is difference in compliance should be accepted.

The objective to determine the therapeutic effectiveness of the different supplementation schedules appeared to be achieved and showed the weekly supplementation to be a simple intervention to increase haemoglobin concentration status in women of reproductive age. Furthermore is economically advantageous; the unit cost of weekly supplementation per tablet was 0.16 Ethiopian Birr, equivalent to 0.02 $USD as compared to the daily cost of 0.80 Ethiopian Birr, equivalent to 0.10 $USD which is five times higher than the weekly schedule. This is an affordable cost by the majority of the women in the community as well as the country. The socio-economic costs of this micronutrient deficiency and its implication to the quality of life for the affected segment of population is enormous with an estimated GDP loss of 1.5%.

The present study is supporting a number of previous studies done in intermittent supplementation. In the clinical intervention studies reviewed in intermittent iron supplementation debate by John (1998), nearly 1000 subjects participated. The groups receiving intermittent iron supplementation achieved significant benefits in all trials regardless of age, culture, or research group. The prevalence of anaemia reduced in the weekly supplemented groups, which experienced a benefit that was equal to that of the groups supplemented daily. In some of these studies, side effects decreased and compliance improved in the intermittently or weekly supplemented groups (John, 1998).

Besides the significant increase in the haemoglobin concentration found in the iron-supplemented groups, a small improvement was also seen in the control groups, which

was suggested to be due to the positive deworming effect. Thus deworming of the subjects or the mothers in the community should be considered together with the weekly iron supplementation intervention to achieve a better programme of controlling and preventing the problem of anaemia in the *Wereda*. Similar effect of deworming also was reported previously in a study of pregnant women on a Sri Lankan plantation (Liu, Kang, Zhao, Viteri, 1995).

5.2.1. Compliance and Dropout

Iron supplementation is suggested when fortification programme or other food-based strategies fail to reach the vulnerable segment of the population. A debate has developed in the past five years regarding the desirability of intermittent iron supplementation compared with daily iron supplements. The primary proponents of the intermittent approach have a historical view based on their, and others, frustrations with the lack of effectiveness of daily iron intervention in developing countries (Schultink, 1986; Viteri, 1987). That is, compliance is low with many daily supplementation programs and there is a strong needs to improve the coverage of at-risk populations when human and financial resources are limited (Schultink, 1986).

Schultink et al (1993) studied compliance with a daily iron supplement program in the early 1990s and showed that as few as 36% of pregnant women were compliant even when they knew this issue was being studied. The authors concluded that coverage of the at-risk population was also quite low and the need existed for a new approach.

In the present study, compliance was high, ranged from 92 to 97 percents in both the supplementation schedules. It is evident that the dropout rate due to side effects was slightly higher among the daily than the weekly supplemented groups suggesting that the weekly supplementation schedule programme is more favoured than the daily schedule in our setting. This finding is in conformity with the study from Sri Lanka (Liu, Kang, Zhao, Viteri, 1995).

CHAPTER SIX

6.0.CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

1. The finding of this study has shown that the six slum *Kebeles* in the *wereda* were homogenous in socio-demographic indicators, including nutritional status and hygienic practices.

2. It has also shown that malnutrition to be a major health problem in the *Wereda*. In addition to this, anaemia is fund to be a public health problem.

3. Weekly iron supplementation to control the problem of iron deficiency anaemia is comparable to the daily iron supplementation. Besides, weekly supplementation is an economically advantageous, simple intervention to increase haemoglobin concentration with fewer complaints of side effects and good compliance.

4. Although daily supplementations shows a rise in serum ferritin, slightly higher than the weekly supplementation, this is of no practical significance as long as the preventive effect of weekly is also equally effective as shown by the increased haemoglobin.

5. Weekly iron supplementation can effectively reduce the prevalence of anaemia, and iron deficiency.

6.2. RECOMMENDATIONS

1. A weekly supplementation programme, as a short-term strategy to control problem of anaemia throughout the country should be initiated especially where the problem of iron deficiency anaemia is highly prevalent in the country since the weekly supplementation schedule programme is affordable in terms of cost and tolerable or less side effects.

2. In the improvement of maternal health through safe motherhood programme, an appropriate health education program addressing the household hygiene and sanitation practices together with deworming activities is recommended in order to make the supplementation programme more effective.

3. The status of iron deficiency anaemia in the at-risk groups such as the lactating women and pre-school children in the country should be studied in order to help plan and implement a weekly iron supplementation programme.

4. Increasing the availability and consumption of iron rich foods through promotion of horticulture and nutrition education strategies should be implemented as feasible long-term solution in-additions to a weekly supplementation programme.

5. For mothers without job, income-generating programmes should be planned to improve their living status to their nutritional status.

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Appendix...2 Baseline Health and related factors among the Groups

Type of characteristics	DF	F	P-value
Family planning	2	0.457	0.63
Illness in the last 2 weeks	2	0.194	0.82
Type of illnesses	2	0.564	0.57
Treatment of illness	2	0.663	0.51
Type of treatment received	2	0.272	0.76
Latrine availability	2	0.192	0.82
Water source	2	0.031	0.97
Garbage disposal	2	0.300	0.74

Appendix...3 Baseline consumption of foods among the Groups

Type of characteristics	DF	F	P-value
Dietary			
Meal frequency	2	0.404	0.66
Drink coffe/te3a after meal	2	0.391	0.67
Kale consumption	2	0.829	0.43
Spinach	2	1.51	0.22
Meat	2	1.16	0.31
Fish	2	0.224	0.80
Milk	2	1.981	0.14
Eggs	2	1.83	0.16
Source of food	2	0.65	0.52
	2	0.329	0.72

Appendix...4 Baseline anthropomtric and biochemical measurements among the Groups

Type of characteristics	DF	F	P-value
Anthropometry			
Weight	2	0.408	0.66
Height	2	0.546	0.58
Biochemical			
Hgb	2	2.78	0.06
Serum ferritin	2	0.40	0.66

Appendix...5 Association of various characteristic at baseline

Type of characteristics		R	P-value
Latrine availability with frequency of illnesses		-0.2	0.01
Water source with frequency of treatment for illnesses		0.13	0.04
Water source with frequency of type of illnesses		-0.18	0.008
Meal frequency with BMI		0.37	0.001
IDA with;	BMI	-0.1	0.3
	Nail pallor	0.36	0.004
	Palmar pallor	-0.2	0.03
	Coffee drinking	-0.2	0.2
	Vegetables	0.316	0.003
	Eggs	-0.02	0.01
	helminthiasis	0.24	0.05
Education with;		-0.19	0.01
	Household size	-0.13	0.04
	Family planning use	-0.19	0.001
	Treatment of illnesses	-0.13	0.03
	Source of water	-0.3	0.001
	parity		

Appendix...6 Iron and ascorbic acid content of commonly consumed foods in the *Wereda*

Type of foods	In milligram per 100 gram edible portion	
	Iron	Ascorbic acid
❖ *Tef (Injera)*	11.5	0.00
❖ Chilli	5.9	9.78
❖ Fish	1.2	4.2
❖ Kale	0.58	2.68
❖ Spinach	2	24
❖ Fruit(guava)	0.6	7.6
❖ Lettuce	1-17	2.1
❖ Meat(beef)	3-5	0.0
❖ Milk(cow)	2.2	1.32
❖ Egg (hen)	3.38	0.0

Source: Ägren and Gibson. Food composition tables for use in Ethiopia. 1968

Appendix...7 Questionnaire

Questionnaire for Determining the Effect of Daily Versus Weekly Iron Supplementation in Lactating Women; *Teklehaimanot Wereda*, Region Fourteen, Ethiopia (*Baseline information*).

I. Demographic Characteristics

Date of interview____/___/___/____ **Name of the *Kebele***_____

1. **Household number**_____
2. **Name of household head**_____
3. **Sex of household head**_____
4. **Name of study subject**_____
5. **Ethnicity of the study subjects**_____
5. **Type of the study subject**

   Daily=1 Weekly=2 Control=3

6. **Household size**_____
7. **Visit number**_____

_____ 8. **Age of study subject** (in years)

_____9. **Marital status**

1. Single
2. Married
3. Divorced
4. Separated
5. Widow

_____ 10. **Education status**

1. None
2. Studied 1-4
3. Studied 5-8
4. Secondary
5. Post-secondary

_____11. **Employment status**

1. None
2. Government employed
3. Self
4. Housewife
5. Other

_____12. **Religion**

1. Ortho.christ.
2. Catholic >>
3. Protestant>>
4. Other >>
5. Moslem

_____13. **Parity**

1. Para 0
2. Para 1
3. Para 2
4. Para 3-5
5. Para > 6

_____14. **Physiological status**

1. Lactating (>2months)
2. Non-lactating
3. Pregnant
4. Non-pregnant
5. 2 and 4

_____15. **Have you received any Iron supplement before (2 weeks) you were enrolled in this study?**

1. Yes 2. No

_____16. **Do you use any family planning method?**

1. Yes 2. No

_____17. **If Q.16 is yes, state type of family planning used.**

1. Pills
2. IUCD
3. Others
4. N/A

II. Morbidity and Hygiene

_____18.1. **Do you have a known chronic illness that you are told by a Doctor?**

1. Yes 2. No

_____18.2. **Have you had any health problem/illness in the last 2 weeks?**

1. Yes 2. No

19 . **If Q.18 is yes, sate type of illnesses.**

_____

_____20. **If Question 19 is yes; did you receive any treatment for your illness?**

1. Yes 2. No

21. **If Q.19 is yes, state type of treatment given.**_____

_____22. **Do you have latrine?**

1. Yes 2. No

_____23. **If yes state type of latrine,**

1. Pithiatiques
2. Communal pithiatiques
3. VIP latrines
4. Septic latrine
5. Others, specify
6. Not applicable

_____24. **What is the source of water used in the household?**

1. Piped water inside the house/private
2. Piped water outside the house/communal
3. River
4. Borehole
5. Protected wells
6. Other, Specify

_____25. **Do you have garbage disposal?**

1. Yes 2. No

III. Clinical Findings

26. **Observe the following and record** (1=Yes 2=N

_____1. Pallor in tongue and the palm

_____2. Nail changes

_____3. Organomegaly

_____4. Oedema

_____5. Others

IV. Dietary

27. **What is your common staple diet?**

List_____

_____28. **How frequent do you consume the above staple diet per day?**

1=once a day 2=twice a day

3=thrice a day 4= four times a day

_____29. **Do you often drink coffee/tea after meal?**

1=yes 2=no

_____29.1. **Do you often smoke cigarettes?**

1=yes 2=no

30. **How often do you consume the following iron and vitamin rich foods?**

(1=yes 2= no)

_____31. Kale

_____32. Spinach

_____33. Lettuce

_____34. Fruits

_____35. Meat

_____36. Fish

_____37. Milk

_____38. Eggs

_____39. **What is the source of this food consumed?**

1. Produced 4. Relief
2. Purchased 5. All
3. Both

V. Anthropometry

Type of variables	Reading 1	Reading 2	Average
40. Weight (kg.)			
41. Height (cm.)			

VI. Laboratory

_____42. BF for malaria 1= positive 2=negative

43. Stool for ovum or parasites;

_____1. Hook-worm	1= positive	2=negative
_____2.Schistosomiasis	1= positive	2=negative
_____3. Ascariasis	1= positive	2=negative
_____4. Amoebiasis	1= positive	2=negative
_____5.Others, state	1= positive	2=negative

44. Haematology:

_____Haemoglobin (in g/dl)

45. Serology:

_____Serum ferritin (in ug/L)

Date and time of specimen collection:_____

Signature of Laboratory technician: _____

VII. Follow-Up Of The Study Subjects

Date of interview __/___/___

1. **Name of the village**_____ **2.HNO**_____

3. **Name of study subject**_____

4. **Type of the study subject**: Daily=1 Weekly=2 Control=3

5. **Visit number**_____

VII.I. Compliance and side effects

6. Have you had any symptoms or illness after the iron supplementation?

1. Yes 2. No

7. **If yes, which of the following symptoms do you experience? Please, fill the symptoms in the follow-up sheet.**

_____7.1. Nausea 1=Yes 2=No

_____7.2. Vomiting 1=Yes 2=No

_____7.3. Epigastric pain 1=Yes 2=No

_____7.4. Constipation 1=Yes 2=No

_____7.5. Itching 1=Yes 2=No

_____7.6. Others 1=Yes 2=No

_____**8. What measures did you take after the symptoms developed?**

1. Nothing
2. Stopped the drug
3. Not applicable

VII.II. Laboratory

1. HGB (in gm/dl)_____

2. SF (ug/L)_____

Appendix...8 Iron supplementation follow-up or tally sheets

5.1. Weekly compliance follow-up (every Mondays for twelve weeks)

No	Name of subjects	M1	M2	M3	M4	m5	M6	M7	M8	M9	M10	M11	M12

5.2. Daily compliance follow-up sheets (Monday to Friday for three months)

No	Name of subjects	M	T	W	T	F	M	T	W	T	F	M	T

Appendix...9 Ethical Consent Form

Consent Form for Participation in Iron Deficiency Anaemia Supplementation Study

I the under signed study subject hereby have agreed fully to participate throughout the study period in the project entitled *the effect of daily versus weekly iron supplementation trial study in the control and prevention of iron deficiency anaemia.* The purpose of the study as well as its benefit was clearly explained to me in my local language by the researcher, and signed without hesitation.

Name and signature of the subject: _____

Date:_____

Name and signature of the researcher: _____

Date:_____