ABSTRACT

Title of Document:	SUPPLEMENTATION STRATEGY AND ITS IMPACT ON HEMATOLOGICAL STATUS IN THE CONTROL OF ANEMIA OF PREGANCY IN SENEGAL
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Anemia of pregnancy remains highly prevalent in Senegal despite the national iron/folic acid (IFA) supplementation program, which consists of providing prescriptions to purchase IFA to women during prenatal visits. The purpose of this study was to provide a framework for recommendations to improve the effectiveness of the program. We determined the prevalence and risk factors of anemia in a cohort of 480 pregnant women at 6 prenatal health centers in Dakar; we compared compliance after 20 weeks of supplementation between women who received prescriptions at 3 control centers and those who received free IFA at 3 intervention centers; and we assessed the factors that influenced high and low compliance in both groups. Overall, 39% of women were anemic and 71% were iron deficient (ID). Twelve percent were infected with P. *falciparum;* 21% had intestinal helminthes, and 6.5% had Hb AS. Women consumed foods containing iron absorption inhibitors at high frequency. ID > quadrupled the risk of anemia; malaria and Hb AS also

significantly increased the risk for anemia. Compliance was 48% and 86% in the control and intervention groups, respectively (P<.001). Anemia prevalence was 62% among controls versus 31% among interventions (P<.01); ID prevalence was 84% and 57% in the control and intervention groups, respectively (P<.01). Women with high compliance were motivated by 1) the perception of improved health upon taking the tablets, 2) the insistence by midwives that they take them, and 3) the mention that the tablets would improve health. Women with low compliance indicated 1) experiencing side effects that they associated with the tablets, 2) misunderstanding that they needed to continue taking the tablets throughout pregnancy, and 3) forgetfulness. Our findings indicate that for effective control of anemia in Senegal, iron supplementation is needed in addition to educating women about better food choices. Antimalarial chemoprophylaxis and helminthes screening should be made available to all women. In addition, increasing access to IFA and educating women about the health benefits of the tablets can dramatically increase compliance and therefore improve iron status and decrease the incidence of anemia.

SUPPLEMENTATION STRATEGY AND ITS IMPACT ON HEMATOLOGICAL
STATUS IN THE CONTROL OF ANEMIA OF PREGANCY IN SENEGAL

By

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Dedication

To my father, Ousmane Seck and my mother, Ndeye Mengué Faye, who supported me

every step of the way.

To my brother, Serigne Seck, who put up with my doubts and my breakdowns and made

me believe that I could reach and grab the moon.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

It was in the 1970's that iron deficiency was identified as the leading single-nutrient deficiency worldwide. Today, at the dawn of the 21st century, iron deficiency still affects more than 2 billion people around the world despite the numerous and extensive strategies used to decrease its prevalence (1, 2).

The pathogenesis of iron deficiency can be divided in three stages. The earliest stage involves the loss of iron stores without a decrease in the iron supply to red blood cells (RBC). Continued deficiency leads to iron-deficient erythropoiesis (decline in the iron supply to RBCs), which nevertheless does not affect circulating hemoglobin (Hb) levels. A continued loss of iron or lack of replenishment of iron stores results in the third and final stage, which is termed iron deficiency anemia (3). Anemia can therefore be defined as a state in which the number of RBCs, the quantity of Hb, or the volume of packed red cells (hematocrit) in blood is reduced below normal levels (4).

The World Health Organization estimates that about 1.3 billion individuals worldwide suffer from anemia, which is 22% of the world's population and 3.5 billion people have iron deficiency (5). Most of these individuals reside in the developing countries of Sub-Saharan Africa and South East Asia and most of them are pregnant women and children (1, 2, 6). In Western Africa, it is estimated that at least 47% of pregnant women are anemic (6). Pregnant women are particularly susceptible to iron deficiency and eventually anemia partly because of the normal physiological changes that occur during pregnancy. These changes include a dramatic maternal red cell expansion

around 20 to 25 weeks of gestation and fetal erythropoiesis (which directs the placenta to accumulate iron) during the 3rd trimester of gestation such that the expectant mother needs up to more than 1000 mg of iron in order to cover the cost of pregnancy (7, 8). In the developing world, this increased need for iron can rarely be met through dietary adjustments alone because of low iron intake, low bioavailability of iron in foods consumed, and women entering pregnancy with low iron stores (6, 9).

Even though iron deficiency is the most common cause of nutritional anemia among pregnant women, it is not the only cause, especially in developing countries. Multiple causes of anemia often coexist among women in Sub-Saharan Africa. These causes include deficiencies in other hematopoeitic nutrients such as folate and vitamin B_{12} (10-13), deficiencies in vitamin A (14), genetic diseases causing hemolysis (sickle cell and thalassemias) (15), concurrent infections and inflammations (16-18), Malaria (19), and parasitic infections that cause gastrointestinal blood loss: *Acylostoma duodenale, Necator americanus, Trichuris trichiria, Ascaris lumbricoides,* and *Schistosomes* (20-22).

Anemia during pregnancy and specifically iron deficiency anemia during the first and second trimesters of gestation increases the risk of low birth weight (LBW) and preterm delivery, which are the strongest predictors of perinatal mortality (23-24).

Iron supplementation of pregnant women who visit prenatal care clinics is one of the most widely used strategies to combat anemia and iron deficiency anemia in Sub-Saharan Africa (25). However, although clinical trials have repeatedly shown that iron supplementation increases pregnant women's iron stores and Hb concentrations, community and population-based public health iron supplementation programs, including

the ones implemented in antenatal clinics, have failed to improve iron status and decrease the prevalence of anemia (26). This failure may be due to failures in the distribution of supplements to antenatal clinics, to a lack of resources or availability of supplements, to poor training of the health care staff in understanding the importance of the supplements and conveying it to patients, and to the fact that iron supplements do not treat the underlying causes of anemia other than iron deficiency. In addition, compliance of pregnant women with the regimen of supplements is relatively low (27) because of limited resources to purchase the supplements, the gastrointestinal side effects that may be associated with iron supplements, and because the health care staff does not convey the importance of the supplements to women; community attitudes may also influence supplements consumption (27-28).

As is typical of most countries in Sub-Saharan Africa, the prevalence of anemia among pregnant women in Senegal is high. Unfortunately, there is a paucity of data on the prevalence and major causes of anemia in that country. No national survey has been conducted to gather this data. In the year 2000, World Vision Senegal performed an end of project survey in four rural districts in which they found that 55% (n = 278) of pregnant women were anemic (29). Ndiaye et al (30) found that 45% (n = 79) of pregnant women in a hospital in Dakar, the capital city of Senegal, were anemic. An unpublished paper (31) reported that 64% (n = 309) of low-income women at an antenatal care clinic in Dakar were anemic.

The high fertility rate in Senegal accounts for 450,000 births yearly; the prevalence of low birth weight in 15%; maternal and infant mortality rates are high at 560/100,000 and 58/1,000 live births, respectively. In order to combat anemia, in the

1990's the Senegalese Ministry of Health instituted a program of universal prescription of iron/folic acid tablets for all pregnant women attending health centers for prenatal care around the country. No data are available to indicate whether this strategy has been effective; however, given what we know of the low compliance associated with community-based iron supplementation programs of this type elsewhere, we suspected that effectiveness may need improvement.

The main objective of this study was to assess the effectiveness of the Senegalese supplementation program and to provide a framework for recommendations for improvement, if necessary. We assessed the prevalence and major risk factors for anemia in a cohort of pregnant women attending health centers for prenatal care in order to evaluate the efficacy of iron/folic acid tablets alone in raising Hb concentration when causes of anemia other than iron deficiency may be prevalent in this population. We then determined compliance with supplementation and compared it with that of women who received iron/folic acid tablets free of charge during the prenatal visit. We hypothesized that giving women iron/folic acid tablets during the prenatal visit rather than merely prescribing the tablets for purchase would eliminate some of the known barriers to program success. The net effect would be an increase in compliance and therefore improved iron status, and decreased incidence and prevalence of anemia among pregnant women. Lastly, we determined the factors that influence compliance in this population because understanding these factors can be the basis of recommendations to improve patient care and counseling.

Chapter 2: Review of Selected Topics in the Literature

1.Causes of anemia

1.1 Iron deficiency

The major cause of anemia around the world, across groups and among pregnant women is iron deficiency. Indeed, the World Health Organization (WHO) estimates that around 3.5 billion individuals suffer from iron deficiency (5) and that nutritional deficiency due to the low bioavailability of dietary iron accounts for more than 50% of the cases (32-33).

The role of iron in the human body

Iron is an essential mineral in the metabolism of all living organisms. The most important iron-containing compounds in the body are the heme proteins hemoglobin (Hb), myoglobin (Mb), and cytochromes, which have an iron-protoporphyrin prosthetic group. Iron is bound at the center of the heme group (**Figure 1**), which is the site of oxygen uptake by Hb and Mb. In turn, Hb plays an important role in transferring oxygen from the lungs to tissues, while the primary function of myoglobin is to transport and store oxygen within muscles. Cytochromes are critical to respiration and energy metabolism through their role in mitochondrial electron transport. Thus, iron plays central roles in energy metabolism and electron transfer (34).

Total body iron averages 4.0 g for men and 2.5 g for women. Seventy five percent of this iron is in the functional form mostly as Hb in circulating RBCs and 15% as Mb. The remaining 10% of body iron is in storage form in the liver, the reticuloendothelial cells, and bone marrow; storage iron exists in the form of two proteins, ferritin and hemosiderin. (35).

Figure 1: The chemical structure of heme

Heme
(Fe-protoporphyrin IX)
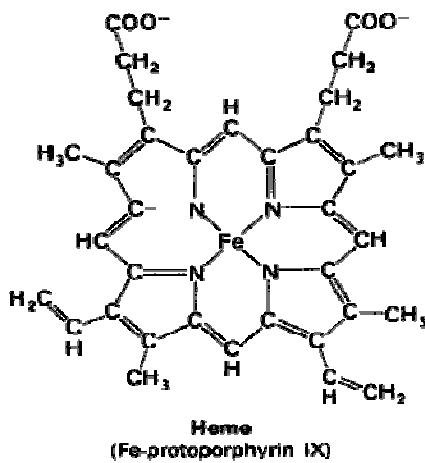
Iron digestion and absorption

Iron bioavailability from foods varies widely. It is greatest from mammalian meat, less from poultry or fish, and least from liver, eggs, milk, and foods of plant origin such as legumes (36). This difference in absorbability of iron is related to the difference between heme and non-heme iron, which form different iron pools in the body. Non-heme iron consists mostly of iron salts; it is found mainly in plants, dairy products, and iron-fortified foods. More than 85% of the human diet consists of this form of iron (37). Heme iron is mostly found in Hb in meat, poultry, and fish. Although heme iron is less

frequent in our diet than non-heme iron, the former is 2 to 3 times better absorbed than the latter (34).

In meats of mammalian origin, iron in the ferric form (Fe^{3+}) is complexed with the heme groups of Hb and Mb. Prior to absorption, the heme group is hydrolyzed from the globin portion of Hb and/or Mb by proteases in the stomach. The acidity in the stomach reduces Fe^{3+} to the ferrous form (Fe^{2+}), which dissociates from ligands more readily than Fe^{3+}. In the small intestine, further hydrolysis of the heme complex by proteases occurs and because of the alkaline environment, heme is readily absorbed in the duodenum and upper jejunum (38). Within the mucosal cell, heme oxygenase liberates iron from the protoporphyrin ring of heme. Iron is then transferred successively to the cytosolic proteins mobilferrin and paraferritin. It is then transported to the serosal surface of the cell and enters circulation via the basolateral transporter known as ferroportin. As it enters the blood, iron is oxidized by ceruloplasmin to Fe^{3+} and then binds to transferrin (38).

The digestion and absorption of non-heme iron differs from that of heme iron, which accounts for the difference in bioavailability. Stomach secretions and hydrochloric acid that release non-heme iron from food components are thought to delay or prevent its reduction to the ferrous state; this means that iron dissociates less readily from its ligands and is therefore less readily absorbed. Non-heme iron is taken up by one or more proteins of the luminal surface of the mucosal epithelium of the duodenum, including a transporter protein called divalent metal transporter 1 (DMT1) which facilitates transfer of iron across the intestinal epithelial cells (38). Once inside the cell, Fe^{3+} is reduced to Fe^{2+} and absorption continues as described above. Non-heme iron absorption can be

enhanced or hindered by food components ingested in the same meal. Some chelators that increase absorption are ascorbic acid and other undefined factors contained in meat, fish, and poultry. Non-heme iron absorption from a meal containing meat, fish, or poultry is about 4 times greater than that from a meal containing equivalent portions of milk, cheese, or eggs (34). Some chelators that decrease absorption are tannins (contained in tea), phosvitin (contained in egg yolk), calcium and phosphate salts, and phytates in cereals (39-41). Diets poor in heme iron and rich in iron absorption inhibitors have been documented all over sub-Saharan Africa (42-46). The staples in Senegal are white rice and millet, which are high in phytates. The Senegalese diet also includes a high consumption of green and black tea.

Iron transport and metabolism

Regardless of which form of iron is ingested, once absorbed, iron either remains in the mucosal cells for intestinal use or is bound by the glycoprotein transferrin for transport through the bloodstream to other body tissues (under normal conditions, transferrin binding sites are about 20 to 45% saturated with iron). Most of the absorbed iron is transported to the bone marrow where Hb is formed in RBCs and later released into circulating blood. RBCs have a life span of 120 days; to replace each 1/120 of erythrocytes, the daily iron turnover for an adult is about 20 mg. Most of the iron from hemolysis is recaptured for the synthesis of Hb (34). A smaller portion of iron enters iron stores as ferritin and hemosiderin in many organs, as myoglobin in the muscle cells, and as iron in cytochromes and cytochrome oxidase. An even smaller portion of iron remains bound to transferrin (34).

Daily external losses of iron are very limited in healthy individuals [gastrointestinal blood loss (Hb): 0.35 mg; gastrointestinal mucosal loss (ferritin): 0.10 mg; biliary loss: 0.20 mg; urinary loss: 0.08 mg; and skin loss: 0.20 mg]; thus, iron balance is maintained primarily through the regulation of iron absorption. Healthy individuals absorb about 5 to 10% of dietary iron whereas individuals who are iron deficient absorb 10 to 50% of dietary iron (34).

The pathogenesis of iron deficiency

When dietary iron intake is inadequate or when the diet is high in non-heme iron and iron absorption inhibitors over a significant period of time, the process of iron deficiency begins with depletion of iron stores and impaired iron supply to various tissues. During this phase of deficiency, the continuous supply of iron for erythropoiesis is adequate but no iron reserves exist to cover short-term needs. Generally, no clinical symptoms are noted at this point. If iron deficiency persists, the second stage of physiological iron deficiency ensues. This stage is characterized by an impairment of erythropoiesis with little if any effect on circulating red blood cells. Some clinical symptoms may be seen in this stage. The third and last stage of iron deficiency, when left untreated, is anemia where the impairment of erythropoiesis is so severe that the number of RBCs is reduced, Hb concentration, and hematocrit concentration fall below normal levels. The degree of anemia can range from mild to severe (Hb <70 g/L) to very severe (Hb <40 g/L). Mild anemia can have very few health consequences, especially in sedentary individuals, because compensatory mechanisms maintain oxygen supply to the tissues (47). Clinical symptoms associated with mild to severe anemia include fatigue,

decreased work capacity (48), behavior and cognitive impairment in children (49-50), an impaired capacity to maintain body temperature in a cold environment (34), impaired immunity and resistance to infections, and adverse perinatal outcomes in pregnancy (23).

Assessment of iron status

Iron status can be measured using hematological and biochemical indices. Each iron status index reflects changes in different body iron compartments and is affected at different levels of iron depletion. Serum ferritin (SF) is directly proportional to body iron stores in normal individuals (51). Quantitative phlebotomy studies indicate that 1 μg/L SF is equivalent to 8-10 mg of stored iron for an average-sized adult (34). SF is considered the most specific test for iron deficiency because very low levels of the protein are almost always indicative of low iron stores. Enzyme-linked immunosorbent assay (ELISA) with colorimetric fluorescent or chemoluminescent end points is widely used to measure SF (52). The SF cutoff commonly used to identify low iron stores is <15 μg/L for adults. There is however a caveat for the use of SF as an indicator: SF is an acute phase reactant, which means that in individuals with acute or chronic inflammations or diseases that cause tissue destruction (acute phase), SF is elevated. The SF measurement under these circumstances would yield false negatives for iron deficiency because levels can reach >20 μg/L even in the presence of advanced iron deficiency (53). The acute phase response is a short-term metabolic change characterized by increased plasma concentrations of certain proteins, such as C-reactive protein (CRP) [CRP >190 nmol/L is considered elevated] and haptoglobin, and decreased concentrations of other proteins, such as albumin and retinol binding protein. The

inflammatory response may last for several days or several weeks and months, in which case it is termed "chronic inflammation". Acute and chronic infections/inflammations are common in West Africa (e.g. malaria) and complicate the diagnosis of iron deficiency using SF. However, SF has been shown to be a useful indicator when the cutoff is raised to <30 µg/L for pregnant women in areas where infection is prevalent (10, 54). In a research setting, it is also advisable to measure SF and an acute phase protein (e.g. CRP) simultaneously in order to be able to control for the potential confounding effect of the acute phase response in the data analysis.

Another measure of iron status is Erythrocyte protoporphyrin (EP), which is the precursor of Hb: the heterocyclic ring system of heme is a porphyrin derivative known as protoporphyrin, which has a centrally bound iron atom. When there is insufficient iron available to combine with the ring to form heme during erythropoiesis or when too much lead is present and interferes with the process, there is an accumulation of protoporphyrin in RBCs (34). The EP test was originally used to detect lead poisoning in children, but was later found to respond to iron deficiency and to respond earlier than the lowering of Hb concentration. Thus EP can be used as a screening test for iron deficiency in both children and adults, including pregnant women (34, 55). Schifman et al and others have found that the diagnostic sensitivity and predictive value of EP for evaluating iron depletion and risk of anemia in children and in pregnant women compared favorably with ferritin and transferrin saturation measurements (56-57). The widely used technique to measure EP is to measure its natural fluorescence in a drop of capillary blood with a hematofluorometer. Several EP cutoffs have been proposed to define iron deficiency, the most commonly used in the literature is >70 µmol/mol heme, but a value of >125 has

been suggested in areas with high prevalence of severe forms of anemia and high incidence of infections (58). EP is highly correlated with iron deficiency; however, it can also be elevated in cases of chronic infection and lead poisoning. Thus, EP should not be used as a single indicator of iron deficiency.

Transferrin saturation (TS) is an indicator of the adequacy of iron transport to tissues. It is determined by dividing serum iron concentration by transferrin concentration, which is measured by total iron-binding capacity. When TS is <16%, it is often associated with iron deficiency. As with other indicators of iron status, TS is affected by infections/inflammations, which cause it to decrease (34).

Transferrin receptors (Tfr) are found on the surface of erythrocyte precursor cells. When the supply of iron is inadequate for the production of Hb, Tfrs increase, which make them good indicators of tissue iron depletion. However, any other condition that results in a high rate of RBC turnover or increased erythropoiesis, such as hemolytic anemia, can also lead to an elevation of Tfrs because the iron supply cannot keep up with the increased demand for heme synthesis. A widely used cutoff value for Tfr is >8.0 μg/L (34). Several studies have shown that Tfr is more stable than most indices of iron status in the face of infection/inflammation, which makes it an interesting index to use in West Africa. Beesly et al (59) found that changes in Tfr levels between the infected and non-infected state in the same individual with malaria were <10% whereas the change in ferritin was fivefold.

Because serum ferritin (SF) reflects the storage iron compartment and Tfr reflects the functional iron compartment, these two values have been combined into a ratio,

which is reciprocally regulated, the Transferrin receptor-ferritin index. Punnonen et al (60) found that the log of the ratio provides an outstanding index of iron depletion when compared to SF and Tfr indices alone.

There are several other red blood cell measurements that are useful in differentiating iron deficiency anemia from anemia due to other causes and that are easily measured by modern electronic blood counters. Erythrocyte indices are useful in defining anemia that results from iron deficiency and mild anemia resulting from thalassemic trait: Mean Corpuscular Volume (MCV) and Erythrocyte distribution width decrease as iron deficiency becomes more severe (34).

Hb concentration is the most widely used index of the last and most severe stage of iron deficiency, anemia. Hb is traditionally measured by spectrophotometry through a method that was first described by Drabkins back in 1942. Today, there are newer and less cumbersome methods such as the HemoCue system, which consists of a portable photometer and microcuvettes that contain sodium deoxycholate, sodium nitrite, sodium azide, and sodium fluorsceine. When a drop of capillary blood obtained by skin puncture is placed on the chemically treated microcuvette, sodium deoxycholate lyses the RBCs, which triggers the release of Hb. Sodium nitrite and sodium azide then convert Hb to methemoglobin and azide methemoglobin whose absorbance is then read by the photometer at a wavelength of 565 nm. The advantages of the HemoCue system include the following: 1) it is useful for surveys where high accuracy is important; 2) it provides accurate and objective measurements comparable to Drabkins' method; 3) the instrument is portable and blood specimens need no processing; 4) results are available in 45 to 60

13

seconds (61-62). The Centers for Disease Control and Prevention (CDC) has proposed anemia cutoff values for pregnant women that take into account the maternal plasma volume expansion and therefore hemodilution: <110 g/L for the first and third trimesters of gestation and <105 g/L for the second trimester (63). WHO considers that Hb levels below 90 g/L represent moderate to severe anemia while Hb values below 70 g/L are an indication of severe anemia (64).

Iron deficiency: the demands of pregnancy

Pregnant women are highly prone to iron deficiency and iron deficiency anemia because of their high need for the nutrient, needs that can rarely be met through diet alone. The total iron costs of pregnancy can be classified into five categories and are estimated at as much as 1190 mg (**Table 1**): 1) basal iron losses from the body, 2) fetal iron deposition (the rate of deposition varies with the stage of pregnancy so that most of the deposition occurs during the second and third trimesters of gestation), 3) iron deposition in the placenta, 4) the iron cost of maternal plasma volume expansion, which occurs during the second and third trimesters and is calculated as iron needed to achieve an average hemoglobin concentration of 130 g/L at the end of pregnancy, and 5) maternal blood loss at delivery (65).

Table 1: Iron requirements during pregnancy*

Expense	Amount (mg)
Basal obligatory iron losses	230
Fetal iron deposition	270
Placental deposition	90
Red blood cell mass expansion	450
Maternal blood loss at delivery	150
TOTAL COST	**1190**

*Adapted from reference 65

If only 5% to 10% of dietary iron is available for absorption, which is often the case for diets in many developing countries that are low in iron absorption enhancers and high in iron absorption inhibitors, then dietary iron intakes of 40-80 mg/d are needed to meet the iron needs of pregnancy. As mentioned above, the iron is not utilized at the same rate throughout pregnancy. Dietary iron needs in the third trimester could be 60-125mg/d in developing countries (65-66). The few dietary surveys conducted in sub-Saharan African countries such as Senegal confirm the low availability of bioavailable dietary iron. In a nutrition survey in the Lindi District of Tanzania, Tatala et al (32) found that the mainly cereal-based diet of their subjects (660 households; 2320 individuals of all ages) with additional legumes and green vegetables contained high amounts of total iron of low bioavailability. Estimation of the amount of iron absorbed and of the major cause of anemia in that region confirmed inadequate iron nutrition. Low intake of iron has also been documented in the Gambia, Somalia, Kenya, Tanzania, and Zambia (67). Sikosana et al (68) conducted an anemia prevalence survey among pregnant women in Zimbabwe from which they concluded that the 33% prevalence rate of iron deficiency anemia was linked to the diet of the sample subjects: the main staple food was maize meal porridge; beans and meats were only consumed on a weekly basis; however, bread and tea were consumed on a daily basis, both of which contain iron absorption inhibitors (i.e. phytates and tannins, respectively).

1.2 Folate deficiency

Deficiencies in hematopoeitic nutrients other than iron can also lead to anemia. Folate deficiency is the second most common cause of anemia after iron deficiency. It

causes a megaloblastic type of anemia because folate and its derivatives are necessary for the synthesis of deoxyribonucleic acid (DNA) and amino acids by being coenzymes in one-carbon transfer reactions. Thus a folate deficiency impairs cell division and protein synthesis, resulting in large, hypochromic, and immature RBCs (69).

Folate, also known as pteroylglutamate or pteroylmonoglutamate, is made of 3 distinct parts, which must all be present for vitamin activity: Pteridine, Para-amino benzoic acid (PABA), and multiple glutamic acid residues. Pteridine is conjugated to PABA to form Pteroic acid; the carboxy group of PABA is then peptide bound to the alpha amino group of glutamic acid to form folate. Although humans can synthesize all the component parts of folate, they lack the enzyme necessary for the coupling of the Pteridine molecule to PABA (70). Thus, folate is an essential nutrient that must be provided in the diet. Folate in foods exists mostly in the form of pteroylpolyglutamates (which contain up to nine glutamic acid residues), the most common in foods being tetrahydrofolate or THF. Good dietary sources of folate include green leafy vegetables, legumes, yeast, whole grains, and liver.

Once folate is ingested, the excess glutamic acid residues of the vitamin must be hydrolyzed from its side chain by enzyme conjugases in the intestinal lumen because the bioavailability of folate monoglutamate is far greater than that of folate polyglutamate in humans. Folate is then absorbed primarily from the proximal third of the small intestine. However, conjugase action may be specifically inhibited by food factors described in yeast and beans and may be non-specifically impaired at acid pH. The absorbed folate is then largely converted in the intestinal lumen and enterocytes to reduced forms and then methylated or formylated and transferred to the circulation (69-70). The transfer process

16

is poorly understood, but a carrier-mediated system has been suggested and identified. Normal total-body folate stores range from 5 to 10 mg of which about half is in the liver. Folate is excreted in urine and bile (about 100 μg of the biologically active vitamin is excreted in bile daily) (70).

Pregnancy is characterized by a marked acceleration in one-carbon transfer reactions, including those required for cell division (rapidly growing fetus and placenta, expansion of maternal red cell mass and reproductive organs), which is why the folate needs of pregnant women are dramatically higher than those of non-pregnant or lactating women (71). Folate bioavailability from foods varies widely from as high as 96% in cooked lima beans to as low as 25% in romaine lettuce. Therefore, the dietary reference intake (DRI) for the vitamin is expressed in terms of "dietary folate equivalents" or DFE. One DFE is equivalent to 1 μg food folate or to 0.06 μg folic acid consumed in fortified foods or as a supplement. It is recommended that pregnant women consume 600 μg of food folate per day in addition to 400 μg of folic acid in fortified foods or supplements, and 200 μg of folate from fruits and vegetables. The Food and Agriculture Organization (FAO) and WHO recommend a daily folic acid supplement of 400 μg from the beginning to the end of pregnancy (71).

Folate status is usually assessed by measuring serum/plasma or red blood cell folate. However, serum/plasma folate levels only reflect recent dietary intake. In contrast, RBC folate levels are more reflective of status because they represent vitamin status at the time the RBC was synthesized (70).

The sequence of events in developing a folate deficiency is somewhat similar to that of developing an iron deficiency. Early negative folate balance is characterized by a

drop in serum folate to below 3 ng/mL; however, neither folate stores nor RBC folate are affected. The second stage of deficiency is characterized by an even lower serum folate and a fall in RBC below 160 ng/mL. The third stage can be termed folate-deficient erythropoiesis, which is characterized by defective DNA synthesis. The fourth and final stage of deficiency is manifested by gross macroovalocytosis, elevated mean corpuscular volume, and anemia (72). The clinical symptoms that accompany a megaloblastic anemia include weakness and tiredness, sore tongue, constipation, headaches, and palpitations. The most severe consequence of folate deficiency during pregnancy is neural tube defect in the offspring. Studies have documented the contribution of folate deficiency to the prevalence of anemia in sub-Saharan Africa. In South Malawi, Van den Broek et al (10) found that 52% of pregnant women in their sample were iron deficient (making iron deficiency the main cause of anemia) while 34% were folate deficient, making folate deficiency the second most frequent cause of anemia in the sample. Ingram et al (73) reported that the second most common cause of megaloblastic anemia (32%) in a sample of pregnant South African women was folate deficiency. In the Congo, folate deficiency accounted for only a 5% prevalence of anemia; however, it was the second most prevalent nutrient deficiency in the etiology of anemia in a sample of pregnant women (74). Fleming (75) described the causes of severe anemia in a small sample of Zambian pregnant women and found that 62% of women were folate deficient, a deficiency that was mostly secondary to malarial hemolysis.

1.3 Malaria

The population in sub-Saharan Africa that was exposed to stable malaria in 1995 has been estimated at 447 million (76). An estimated 21% of severe anemia cases among

pregnant women is attributable to malaria according to Guyatt and Snow's review of 26 research studies on the levels of Hb in all parities and in a wide range of intensities of malaria transmission in 26 sub-Saharan African countries, including Mali, the Gambia, and Mauritania (all bordering Senegal) (19). Malaria is the leading cause of morbidity in Senegal.

Malaria is caused by the *Plasmodium* species, especially *P. falciparum*. WHO defines severe malarial anemia as Hb concentration < 50 g/L or a hematocrit of < 15% in the presence of a normocytic blood film and *P. falciparum* parasitemia > 10,000 parasites/μL of whole blood. In practice, severe malarial anemia is defined at the levels of Hb and hematocrit cited above, but in the presence of any *P. falciparum* parasitemia (77). The pathogenesis of malarial anemia is complex and results from both increased hemolysis and decreased erythropoiesis. Increased hemolysis is due to various factors including phagocytosis and splenic removal of both parasitized and unparasitized erythrocytes, autoimmune hemolysis of unparasitized erythrocytes, and hapten-induced intravascular hemolysis all resulting in decreased Hb concentration below normal levels and therefore anemia (77). Pregnant women in malaria endemic areas of Africa often do not receive adequate prenatal care to prevent and/or cure malaria. In areas of high or moderate (stable) malaria transmission such as Senegal, adult women become semi-immune to the disease so that many malarial infections are asymptomatic. However, these forms of infection can contribute to mild to severe maternal anemia depending on the level of acquired immunity to malaria prior to pregnancy and the efficacy of immune responses during pregnancy (Figure 2) (78); and this anemia attributable to malaria leads to negative perinatal outcomes (Table 2).

Of the other parasites that cause malaria in humans, *P. vivax is* not present in Senegal*, P. malariae and P. ovale* are low and their effects are less clear. *P. falciparum* was found to be the only parasite responsible for malaria in Dakar (Senegal) in a large survey involving 2,583 individuals (80). *P. falciparum* infections observed in all age groups (1 to 80 years) was greatest (39%) between October and December, the period that immediately follows the rainy season. The annual incidence of parasitemia in the sample was 5%. In the 1970's, WHO recommended that pregnant women in malaria-endemic areas systematically receive treatment upon their first visit to a prenatal center followed by weekly chemoprophylaxis (78). The most commonly used prophylactic drugs in prenatal clinics in Dakar are chloroquine and sulfadoxine pyrimethamine, which are prescribed to pregnant women in addition to the iron/folic acid tablets. However, the implementation of the WHO recommendation has been hampered by several factors in Africa. These factors include the spread of chloroquine resistance and poor compliance with a daily or weekly regimen of the drug throughout pregnancy (81).

Figure 2: Malaria during pregnancy*


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                    Malaria during pregnancy
                  in areas of high or moderate (stable)
                            transmission
─────────────────────────────────────────────────────────────

Acquired immunity – High
        │
        ▼
Asymptomatic infection
        │                    ╲
        ▼                     ╲
Anemia              Placental Sequestration
        ╲           Altered Placental Integrity
         ╲                   ╲
          ╲             Less Nutrient Transport
           ╲                  │
        │   ╲                 ▼
        ▼    ╲
Maternal Morbidity   Low Birth Weight ──────► Higher Infant Mortality
```
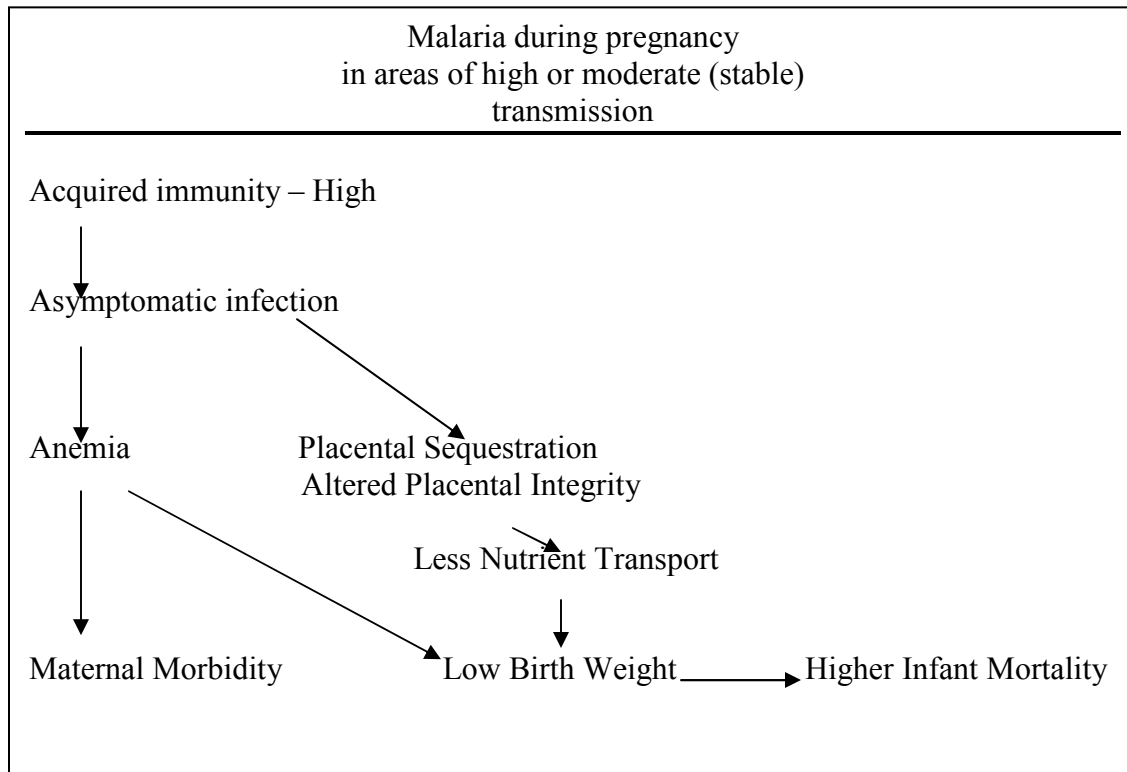
*Adapted from reference 78

Table 2: Malaria's contribution to adverse health outcomes in Sub-Saharan Africa*

Adverse health outcome	Percentage of the total caused by malarial infection
Maternal anemia	2-15
Low birth weight	8-14
Preterm - low birth weight	8-36
IUGR** – low birth weight	13-70
Infant death	3-8

*Adapted from reference 78; **Intrauterine growth retardation

1.4 Helminth infection

WHO estimates that about 1.2 billion and 1 billion individuals around the world are currently infected with the hookworms (*Necator Americanus and Ancylostoma*

21

duodenale) and *Trichuris trichuria*, respectively. Schistosomiasis and *Ascaris lumbricoides* infections are also prevalent. Most of these individuals reside in tropical developing countries. These parasites are common soil-transmitted helminths that propagate in areas of poverty, poor nutrition, poor sanitation, and where there are shortages of clean drinking water (21).

Hookworm infection is a major contributor to iron deficiency anemia in women of childbearing age, including pregnant women in most developing countries and is associated with high maternal mortality and morbidity (21). The hookworms are nematodes; transmission and infection by N. *americanus* depend on the penetration of human skin by third-stage larvae, which develop from eggs passed in human stools and into the environment (A. *duodenale* larvae can occasionally pass the placenta in the case of pregnant women and invade the developing fetus). After transmission, the larvae migrate through tissues and then return, via the respiratory system, to the digestive tract. The maturing larvae will then usually invade the jejunal mucosa where eventually they develop into adult worms and bite into the tissues and feed on blood. While in the small intestine, the female hookworms can produce thousands of eggs each day. It is estimated that a single N. *americanus* can be responsible for a blood loss of 0.05 ml per day; a single A. *duodenale*, which is the more virulent species that can be swallowed, can be responsible for 0.25 ml of blood lost per day; (82). Because of this fact, anemia from chronic infection follows 3 to 5 months after exposure. Heavily infected individuals (>2000 eggs per gram of feces) are most at risk of developing iron deficiency anemia (21). The effects of the parasites will also be mediated by the host's iron and general nutritional status, by the quantity and quality of iron sources in the diet, by the ability of

the gut to absorb iron, and by whether the host has infections that may adversely affect iron status. Pregnant women in sub-Saharan Africa are therefore especially susceptible to iron deficiency anemia caused by hookworms. In a sample of 2104 near-term pregnant Nigerian women, Egwunyenga et al (83) found that 48% were infected with intestinal nematodes of which 14% were specifically infected with hookworm; Hb concentrations among these women were lowest (86 g/L) among those with malaria coexisting with helminth infection. In Kenya, 75% of anemic pregnant women had hookworm infection compared to only 25% of non-anemic women (N = 279) (84).

WHO recommends that a single dose antihelminth treatment be provided to pregnant women after the first trimester of gestation in areas of endemic infection (21). Many studies have shown the effectiveness of treatment to decrease morbidity. In Sri Lanka, 115 pregnant women had marked improvements in Hb concentration when they were given an antihelminth drug in addition to iron/folic acid supplements (85). A longitudinal study in Sierra Leone involved 184 pregnant women who were given a single dose of 400 mg albendazole and iron/folic acid supplements. The women showed a significantly smaller decline in Hb and SF concentrations than those women who only received iron/folic acid (86)

Schistosomiasis caused by S. *haematobium,* S. *japonicum, and* S. *mansoni* affects more than 200 million people worldwide, 85% of whom live on the African continent. The infection is acquired from water containing worm larvae, which develop in snails. In 1989, 71.5% of more than 2,000 routine stool examinations in St Louis (Senegal River Valley) tested positive for S. *mansoni*. In sub-Saharan Africa where there have been few control programs and where the population has increased by close to 70% over the past

23

25 years, many individuals are infected or at risk of infection (87). Unfortunately, accurate data on country-specific prevalence of schistosomiasis are limited and therefore global estimates of the number of people infected must be based on extrapolations from the limited data generated from prevalence survey data in some countries. Senegal is considered to be an area of high endemicity. Two of the schistosome species are responsible for increased blood loss that can lead to iron deficiency anemia. S. *haematobium* causes chronic bleeding into the urinary tract while S. *mansoni* causes bleeding into the large intestine but both infections can contribute to the anemia of pregnancy (82).

Trichuris trichiura or the whipworm is transmitted feco-orally; once the infective egg is swallowed, it hatches; larvae invade the mucosa of the small intestine and mature in the large intestine. The mature worms then remain in the large intestine and feed on tissues therefore causing blood loss. Each worm can cause blood loss of about 0.005 ml per day; thus, a heavy T. *trichiura* infection can cause iron deficiency that can lead to anemia. A single female worm can produce up to 70 eggs per day (21-22).

Ascaris lumbricoides or the roundworm is transmitted in a similar fashion as the whipworm. A female nematode can produce >200,000 eggs per day in the small intestine. Adult worms then latch on to the intestinal tissues and cause blood loss. A. lumbricoides infection is believed to affect more than 10 million individuals in Africa alone (22).

Hookworm, T. *trichiuria,* A. *lumbricoides, and* S. *mansoni* infections are believed to be prevalent in Dakar, Senegal (93).

1.5 Hemoglobin variants

The normal Hb molecule is a tetramer of two pairs of unlike globin chains with each chain sharing a covalent bond with a heme group. Normal types of Hb have α chains combined with β(Hb A, $\alpha_2\beta_2$), δ chains (Hb A$_2$, $\alpha_2 \delta_2$), or γ chains (Hb F, $\alpha_2\gamma_2$). As with all polypeptides that perform specific functions in the human body, Hb has a specific sequence of amino acids. Inherited Hb variants or disorders can either result from structural mutations in the sequencing of the amino acids or mutations in the molecular structure of Hb (thalassemias). The structural Hb variants mostly result from single amino acid substitutions in the α or β chains. Over 700 structural Hb variants have been identified; however, only three of them, Hb S, Hb C, and Hb E, are prevalent around the world (15). In many cases, these substitutions are innocuous, but in others, substitutions may affect the stability and function of Hb, thus leading to a clinical disorder.

Such is the case with sickle cell Hb, or homozygous Hb SS, which was first identified in 1945 when Pauling discovered that the normal Hb A has an anionic charge two units more negative than that of Hb S. About ten years after Pauling's discovery, Ingram demonstrated that the charge difference between the two forms of Hb results from the replacement of the Glu residue in the 6[th] position of each of the Hb A β chains with a Val residue in the Hb S β chains (88). Hb variants are hereditary. For example, if two individuals both have Hb AS (heterozygous sicklers) and have children, half of their offspring will also have Hb AS, while one fourth will have normal Hb AA, and the remaining one fourth will be homozygous sicklers (Hb SS). For the latter, the amino acid mutation in Hb causes Hb S to aggregate into filaments when deoxygenated. These

filaments are of sufficient size and stiffness to give red blood cells an irregular crescent-like shape (or sickled shape) that causes vaso-occlusion. Individuals with Hb SC and Hb S [Hyphen] β-thalassemia are also homozygous sicklers. During a sickle cell crisis, the blood flow through tissues is decreased and may be completely blocked in some areas thereby creating severe pain and tissue damage. These crises include episodes of sequestration of blood into the lungs, liver, or spleen, infection, or the occlusion of cerebral vessels with resulting stroke. Furthermore, because of their mechanical fragility, the life span of erythrocytes is reduced to 60 days instead of the normal 120 days, leading to hemolytic anemia in individuals with homozygous Hb SS, thus the term sickle cell anemia (88). In sub-Saharan Africa, Hb S gene frequency reaches 0.15 in some populations in which about 30% of adults have sickle cell trait and 2% of newborns have homozygous sickle cell anemia. In Senegal, the Hb S gene frequency is greater than 0.02 (89-90). The heterozygous state for sickle cell, Hb AS and Hb SC, often results in a milder form of sickle cell anemia. It is associated with a high frequency of aseptic necrosis of the femoral or humoral heads, hematuria, proliferative retinopathy, and a thrombotic tendency, which in pregnancy may lead to massive pulmonary thrombo-embolic disease and death (89). Hemoglobin C is another mutation in the gene for the β chain. About 2-3% of people of West African descent are heterozygotes for hemoglobin C. Hemoglobin C disease (seen in homozygotes) is rare and relatively mild. It usually causes mild hemolytic anemia and a mild to moderate enlargement of the spleen.

Thalassemias are another form of Hb variants in which globin chain production is reduced. Thalassemias are classified as the α or β types depending on whether the α or β chains are reduced. The symptoms of α thalassemia are variable. The milder forms in

their homozygous state produce a mild hypochromic anemia. The more virulent forms can result in stillbirth and extremely difficult pregnancies. The carrier frequency of the milder forms of α and β thalassemia is as high as 10% to 20% in sub-Saharan Africa (88).

1.6 Chronic infection

In otherwise healthy populations, anemia is generally caused by nutritional deficiencies. However, in many developing countries anemia due to chronic infectious and/or inflammatory diseases, also known as the anemia of chronic disease (ACD) is also common.

The pathogenesis of ACD is thought to involve immune activation from contact with either a foreign infectious agent or a foreign neoplasm. This activation releases cytokines (i.e. tumor necrosis factor, interleukin-1, gamma-interferon, and beta-interferon), which lead to the inhibition of colony-forming units-erythroid (CFU-E) development (i.e. reduced erythropoiesis) and produce anemia (91). ACD is one of the most frequent forms of anemia encountered in sub-Saharan Africa and among pregnant women, especially with the growing numbers of HIV/AIDS infections. In a sample on 1064 HIV –infected pregnant Tanzanian women, iron deficiency and infectious diseases were found to be the predominant causes of anemia (92). Fortunately, Senegal has one of the lowest prevalence of the disease in sub-Saharan Africa with an estimated 1.7%. Nevertheless, other forms of chronic infection are prevalent during pregnancy, such as tetanus, yellow fever, respiratory infections, and tuberculosis (93).

2. Consequences of anemia during pregnancy: why it is important to treat the disease

Several studies have supported the correlation between anemia or iron deficiency anemia and increased risk of preterm delivery and low birth weight. Since birth weight is the strongest predictor of perinatal mortality, the eradication of anemia during pregnancy, especially in developing countries where health care is often limited, is a necessity.

Strong evidence indicates that there is an association between maternal hemoglobin concentration and birth weight and preterm birth (23, 94-96). Anemia, especially during the second and third trimesters of gestation, has also been linked to increased risk of small-for-gestational age (97). Scholl et al (98) found a strong association between anemia specifically caused by iron deficiency and preterm delivery. Furthermore, a recent study by Cogswell et al (24) has added promising new evidence that iron supplementation during pregnancy improves birth weight.

Although WHO has issued statistics on maternal mortality attributable to anemia, the relationship remains a source of controversy. Researchers do agree though that there is a proportion of maternal mortality in developing countries that is attributable to anemia, especially severe anemia (99). They also agree that iron deficiency is likely to be a major contributory cause.

3. Interventions to combat iron deficiency and iron deficiency anemia

Various intervention strategies have been employed to control iron deficiency anemia among pregnant women around the world. They include dietary diversification, iron fortification of widely accessible staple foods, iron supplementation, and parasite control.

The nature of the intervention that best controls the disease depends on the context (i.e. available physical, human, and financial resources and the target population).

3.1 Food-based interventions

If a fortifiable food consumed by many in the target population exists, in this case iron deficient/anemic pregnant women, fortification can be a cost-effective strategy to control the disease (100). There is no national policy for iron fortification programs in Senegal even though there are many staples, such as rice and millet, which are fortifiable. In West Africa, only Nigeria is exploring the possibility of dual fortification of salt with iodine and iron. Mali, Burkina Faso, and Cote d'Ivoire are exploring the possibilities of fortifying sugar, bouillon cubes, and wheat flour. Fortification of wheat flour is technically relatively easily done; it has worked in countries in South America and the Caribbean (101). The possibility of fortifying processed foods sold in the streets of Dakar has been suggested since large numbers of the population consume those foods (102).

General nutrition education messages have proven effective for controlling iron deficiency anemia, even when poverty limits dietary choices. Pregnant women should be educated to consume foods that are high in iron, even if bioavailability is low (103). Even though there are some nutrition education programs for pregnant women led mostly by non-governmental and charitable organizations in Senegal, there is no national program to specifically inform women on proper iron nutrition.

3.2 Helminth control

As previously mentioned, in areas where intestinal parasite infection is endemic and anemia is very prevalent, parasitic infection is likely to be an important cause of moderate to severe anemia. WHO recommends that in these areas, anthelminthic treatment (in combination with iron/folic acid supplements) be given prophylactically to pregnant women suffering from severe anemia. The treatment is safe and much less expensive than diagnosing parasitic infection. The combination of chemotherapy and iron/folic acid supplements enhances the Hb response to supplementation; mebendazole, albendazole, levamisole, and pyrantel may all be safely administered to pregnant women after the first trimester (21,103). There is no national policy for the control of helminthiasis among pregnant women in Senegal.

3.3 Malaria control

Where P. *falciparum* or other Plasmodium species are endemic, detecting and treating malaria is an essential part of the control of anemia among pregnant women. Several studies in Burkina Faso and other African countries have shown that the proper usage of insecticide-treated bed nets (ITNs) can decrease the prevalence of severe anemia in young children and decrease infant mortality by up to one third (100). The same could be true for pregnant women; however, the evidence is less clear. In Senegal, 33% of pregnant women use bed nets; however, only 10% of those use treated bed nets (106). Malaria prophylaxis combined with iron/folic acid supplements has proven more effective than supplements alone (78-81). The Senegalese national policy for malaria control among pregnant women consists of prescribing chloroquine or sulfadoxine

pyrimethamine prophylactically to all pregnant women who attend public prenatal clinics.

3.4 Reproductive intervention

Attempts to decrease the prevalence of iron deficiency anemia among pregnant women also include preventing early or adolescent pregnancies, which can lead to complications not just because of the gynecological immaturity of adolescents but also because of the competition for nutrients between the growing adolescent and her growing fetus (105). According to the 2005 Demographic and Health Survey (DHS) for Senegal, 19% and 38% of 17 and 19-year old adolescents, respectively, already have children. Because of the efforts of the Senegalese Ministry of Health and of non-governmental organizations to initiate education programs and promote birth control to reduce adolescent pregnancy, the median age at which women have their first child in Dakar is 22 years. Only 9% of girls aged 15-19 years experience pregnancies compared to 30% in rural areas (106).

In addition to reducing the number of adolescent pregnancies, reducing gravidity in women and increasing the interval between pregnancies will also contribute to the control of iron deficiency anemia in women. When this interval is two or more years, women are more likely to enter the subsequent pregnancy with adequate iron status if dietary iron deficiency is not severe (103).

3.5 Iron supplementation

Of the strategies that have commonly been used to combat iron deficiency and iron deficiency anemia among pregnant women, supplementation is by far the most

widespread in sub-Saharan Africa. The decision to supplement any population group should be and is usually based on the likelihood of iron deficiency in the target population and on the potential benefits of supplementation. Thus, pregnant women are excellent candidates for supplementation because they are more prone to iron deficiency than other groups and because clinical trials have shown that women who are supplemented with iron during pregnancy have improved iron and Hb status compared to women who are not supplemented.

3.6 Selecting the appropriate intervention

According to a International Nutritional Anemia Consultative Group (INACG) report (103), the appropriateness of an intervention for a specific region depends on three factors. First, it is important to define the epidemiology of anemia in that region; in other words, one needs to determine what populations are most at risk of anemia and very importantly the etiology of anemia. No published epidemiological information of this sort is yet available for Senegal; in this case, it is safe to assume that young children and pregnant women are at greatest risk for anemia because of their high physiological demands for iron. The second factor that determines an appropriate intervention is the available infrastructures that determine the cost and feasibility of the different approaches. As is the case in most developing countries, prenatal care clinics in Senegal are the strategic and chosen structures of intervention in the case of pregnant women. However, by definition, the same intervention does not reach those women who do not seek prenatal care or who do not seek it early enough during pregnancy for effective intervention. The third factor that determines the appropriateness and success of an intervention is the acceptability of the intervention by the health care workers and by the

community being served. It is important that this community develops a sense of active partnership with the health system based on their conviction that the intervention is designed to benefit the members of the community. This third factor is often absent from prenatal care programs in developing countries, including Senegal. **Figure 3** summarizes the elements of successful iron supplementation programs.

The monitoring and evaluation of iron supplementation programs are essential to their success. Monitoring is the process of continually collecting information about the different parts of the program while evaluation may be periodic and involves judgment about whether the program is effective (103). In the case of iron supplementation programs for pregnant women, the effectiveness of the program can be judged by whether it fosters a significant improvement in women's iron status and therefore decrease the incidence and prevalence of anemia during pregnancy. Further judgment can be based on whether supplementation improves pregnancy outcome, whether women make better dietary choices, whether they know about anemia, its causes, and its consequences.

Figure 3: Elements of successful iron supplementation programs (Adapted from reference 103)

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                        ┌─────────────────────┐
                        │   Policy-making     │
                        └─────────────────────┘

┌──────────────────┐   ┌──────────────────┐   ┌──────────────────┐
│     TABLET       │   │    DELIVERY      │   │     CONSUMER     │
│     SUPPLY       │   │     SYSTEM       │   │      DEMAND      │
│                  │   │                  │   │                  │
│ • Adequate       │   │ • Accessible to  │   │ • Good quality   │
│   budget         │   │   target group   │   │   supplements    │
│ • Data-based     │   │ • Motivated,     │   │ • Communications │
│   ordering       │   │   well-trained,  │   │   to educate     │
│   number in      │   │   approachable   │   │   public and     │
│   target group   │   │   staff          │   │   promote        │
│   + 35% surplus  │   │                  │   │   program        │
│ • Timely         │   │                  │   │                  │
│   ordering       │   │                  │   │                  │
│ • Distribution   │   │                  │   │                  │
│   system         │   │                  │   │                  │
└──────────────────┘   └──────────────────┘   └──────────────────┘
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COVERAGE	COMPLIANCE
Do intended recipients get supplements?	Do recipients take the supplements?

IMPACT
Reduction of iron deficiency anemia

Monitoring and Evaluation

4. The iron supplementation program for pregnant women in Senegal

The iron supplementation program for pregnant women in Senegal was implemented in the 1990's in all public health centers that deliver prenatal care in the country. Upon women's first visit, attending midwives write them a prescription to

purchase iron/folic acid tablets after a physical examination. The supplement tablets contain 65 mg of elemental iron (as 200 mg of ferrous sulphate) and 250 µg of folic acid. Women are directed to purchase as many tablets as necessary for daily supplementation until the end of pregnancy. The iron/folic acid tablets are supposed to be available for purchase at the health center pharmacies.

The Senegalese program is only partly monitored and not evaluated. The monitoring that is conducted by the Ministry of Health is actually a monitoring of prenatal care services as a whole: every six months, the Ministry of Health delegates a team to the health centers in Dakar and elsewhere to determine: 1) the number of women who have attended the center to receive prenatal care; 2) whether they all received the needed clinical examinations; 3) whether blood and urine samples were given for analyses; and 4) whether they all received a prescription for iron/folic acid tablets and malaria chemoprophylaxis. However, data is not collected on whether the women have actually purchased and taken the supplements. Thus, there is no data currently available on whether the Senegalese iron supplementation program fosters adequate compliance to reduce the prevalence of iron deficiency anemia among pregnant women. The absence of an evaluation of this program means that the potential problems of this program may never be identified so that the program can be improved.

5. Iron supplementation of pregnant women clinically improves their iron status: have supplementation programs around the world contributed to decreasing the prevalence of anemia? Why or why not?

5.1 A selective review of iron supplementation trials

Since the late 1960's, a large number of iron supplementation trials have demonstrated the efficacy of iron supplementation in improving the health of pregnant women. Sloan et al (107) have presented a comprehensive review of the impact of iron supplementation on maternal Hb in 23 randomized controlled trials, 15 of which were conducted in developing countries (including The Gambia, Nigeria, Tanzania, and South Africa) and all were published in peer-reviewed journals between 1966 and 1998. Almost all studies drew their samples from women attending prenatal clinics; 2 drew their samples from rural prenatal centers. The authors found that iron supplementation alone increased Hb concentration by 10 ± 0.13 g/L (P<0.001, n = 1118). Women receiving iron combined with folic acid had 13.7 ± 0.93 g/L (P<0.001) better change in Hb concentration than did women not receiving supplements. In studies reporting a change in SF, iron supplementation alone improved SF concentration by 9.48 ± 0.0174 μg/L (P<0.001, n = 578). Iron supplementation alone reduced the proportion of women with Hb concentrations <110 g/L (considered anemic by WHO standards) by 38%. The effect of iron supplementation was greatest in women from developing countries with the lowest baseline Hb concentrations. The dose of iron administered to women varied from one study to the next. The authors found a positive dose response relationship between iron dose and change: those women receiving no more than 60 mg of daily iron had the lowest change in Hb concentration compared to women who were receiving 61-90 mg, 91-120 mg, and >120 mg iron daily (Table 3).

Table 3: Meta-analysis of trials of iron supplementation during pregnancy[1]

Daily dose of elemental iron and mean initial Hb concentration and effect	Mean change compared with control subjects (g/L)
Daily dose (mg Fe/d)[2]	
≤60	2 (-17-11)
61-90	10 (6-15)
91-120	12 (6-22)
>120	16 (9-26)
Mean initial Hb (g/L)[3]	
≤100	10 (2-17)
<110	13 (-3 to 25)
<120	5 (-12 to 12)
≥120	__ (---)

[1]Adapted from reference 107
[2]Data from all countries
[3]Data from developing countries only

WHO makes the following recommendations for supplement dosage: where the prevalence of anemia is <40%, pregnant women should routinely receive a daily supplement containing 60 mg of elemental iron and 400 μg of folic acid for 6 months during pregnancy; where prevalence of anemia is ≥40%, the same treatment should be given for 6 months during pregnancy and continued until 3 months postpartum. WHO further advises that in either case, if the full 6 months of supplementation cannot be achieved, that the daily dosage should be increased to 120 mg of iron or that postpartum supplementation should continue for 6 months. If the recommended dosage of 400 μg of folic acid is not available, the widespread tablets of 250 μg should be used until higher doses are available (103).

According to the Sloan et al meta-analysis, in addition to the dose of iron, the duration of supplementation is also linked to the magnitude of improvement of Hb: countries

providing up to 10 weeks, 11 to 13 weeks, and 14 to 19 weeks of iron found benefits relative to no supplementation.

The bulk of iron supplementation trials of pregnant women were done in South East Asia where the prevalence of anemia among pregnant women is the highest in the world. Studies conducted specifically in sub-Saharan African countries, which have the second highest prevalence of anemia worldwide, are fewer but support the assertion that under certain conditions, iron supplementation, often in combination with at least one other nutrient, improves iron status and decreases the incidence of anemia among pregnant women. The following is a brief review of such studies.

Jackson and Latham (108) reported a 33% decline (78% at enrollment to 45% at term) in the prevalence of anemia among pregnant Liberian women (N = 621) supplemented with 60 mg of iron at different frequencies (once daily or thrice daily) and/or 500 μg of folic acid and/or anti-malarial drugs over 12 weeks of supplementation. This study showed the benefits of supplementation when there is a high prevalence of anemia and when supplementation is given late in the second trimester of gestation and early in the third when nutrient needs for both the developing human and the mother are greatest (88% of women in the Liberian sample were 24 weeks pregnant at enrollment). In a follow-up study involving a sub-sample of the Liberian women (n = 80), Jackson et al (109) found that there was a positive and significant correlation between maternal Hb concentration at 6 months of gestation and infant birth weight.

In a randomized, double-blind, controlled clinical trial, Semba et al (14) found that pregnant women in Malawi (n = 109) who were supplemented with a lower dose of iron than in the previous study (30 mg) and 400 μg of folic acid daily from 23 weeks

(enrollment) to 38 weeks of gestation had a mean Hb improvement of 7.3 ± 2.3 g/L (P = 0.003). The prevalence of anemia of 50% at enrollment was reduced to 35% at 38 weeks.

Another supplementation trial in rural Malawi (110) compared a weekly iron/folic acid supplement (120 mg iron, 500 µg folic acid) to a daily regimen (60 mg iron, 250 µg folic acid) in pregnant women attending a prenatal clinic. The women were stratified by grade of anemia at enrollment and randomly assigned to either group (n1 = 202; n2 = 211). Supplementation was continued for an average of 10 weeks. The mean final Hb concentrations of both groups significantly increased compared to initial Hb by the end of the trial, but did not significantly differ from each other. Compliance as indicated by self-reports and pill counts was significantly higher in the weekly group (76% compared with 60%, P<0.05); however, compliance was equal in both groups as assessed by a stool test for elemental iron. Even though weekly supplementation did significantly decrease the incidence of side effects in women, it had similar hematologic effects than daily supplementation when administered through an existing primary health care unit.

In Niamey (Niger), Preziosi et al (111) demonstrated the benefits of iron supplementation during pregnancy and during the postpartum period on both the mothers and their infants. The authors conducted a placebo-controlled trial where women in their third trimester of gestation (n = 197; stage of gestation: 28 weeks ±21 days) were randomly assigned to either receive 100 mg of elemental iron throughout the remainder of their pregnancies or to receive placebo. At delivery (after 3 month of supplementation) women in the treatment group had a significantly better iron status than women in the placebo group as measured by higher Hb, hematocrit, mean corpuscular volume, serum iron, and SF concentrations and lower EP concentration. These

hematologic differences persisted 3 months postpartum: the prevalence of anemia had decreased in both groups but was significantly higher in the placebo group. The prevalence of anemia decreased by 24% from enrollment to delivery in the iron group (66 to 42%) while it remained constant in the placebo group. The benefits of maternal iron supplementation on infants were noted in the infants' SF concentrations at 3 months postpartum: they were significantly higher than for infants in the placebo group. Furthermore, mean length and Apgar scores were also significantly higher in the iron group. These results show that iron supplementation even when started late in gestation have positive effects on the course and outcome of pregnancy.

In Abidjan (Cote d'Ivoire), Carré et al (112) provided iron supplements and malaria prophylactics to pregnant women (n = 631) during their first prenatal visit at 4 antenatal clinics. Most of the women were in their second trimester of gestation (69%) at enrollment and were given enough supplements to carry them until term. Hb concentration increased from 104 g/L at enrollment to 109 g/L at term; consequently, the prevalence of anemia decreased from 63 to 50% (P <0.01). This study suggests the importance of making iron supplements available to pregnant women to combat anemia.

In Western Uganda (113), the effect of daily iron (60 mg) and weekly folic acid (400 µg) supplementation on maternal Hb was investigated in a sample of pregnant women in a randomized, double-blind, placebo-controlled intervention trial. This treatment was compared to the effects of chloroquine prophylaxis alone or passive case management alone. The importance of combining iron/folic acid supplementation with malaria prophylaxis was evident; the authors found that it significantly increased maternal Hb concentrations during pregnancy as compared to case management (P =

0.01and P = 0.007, respectively) and the increase was positively correlated with the duration of the intervention.

The following lessons can be drawn from this selective review of iron supplementation trials in sub-Saharan Africa: 1) iron/folic acid supplementation is effective in decreasing the prevalence of anemia and improving the iron status of pregnant women both in urban and rural settings in the region; 2) combining the supplements with malaria prophylaxis increases their efficacy; 3) this combination remains efficacious even when given late in pregnancy; 4) an improved maternal hematologic profile, improves pregnancy outcome.

5.2 Success or failure of iron supplementation programs: has the prevalence of anemia among pregnant women decreased worldwide?

Despite the demonstrated efficacy of iron supplementation in the clinical setting, community-wide and population-based iron supplementation programs in the developing world have been much less successful. Since the 1972 WHO global policy recommendations to supplement all pregnant women with iron/folic acid in developing countries, more than half of those countries report having implemented national supplementation programs; however, the prevalence of the anemia of pregnancy has not decreased according to a United Nations report based on worldwide anemia surveillance data (114).   It has been suggested that until 80% of pregnant women are taking the recommended number of iron tablets, the worldwide prevalence of anemia will not decrease. The Demographic and Health Surveys from various countries do not indicate this level of coverage. In Tanzania (1999), Eritrea (1996-1997), and Yemen only 44%,

41

30%, and 21% of pregnant women receive iron supplements, respectively. Higher rates were reported for Ghana (25).

5.3 Factors that affect the effectiveness of supplementation programs

Several scientists, including Sloan et al (115), Yip (116-118), and Cook and Reddy (119) have reflected on the discrepancy between the results of clinical trials and population-based iron supplementation trials. The failure of programs is thought to be due to a combination of factors.

The factor that is most often cited is low compliance of women with the recommended dosage and frequency of intake of supplements. This low compliance is related to at least two factors, the occurrence of side effects and women's limited knowledge about anemia. Side effects that have been noted include heartburn, nausea, vomiting, constipation, and dark stools and are dose dependent. A recent study in Bangladesh (120) suggests that a dose of iron of 30-60 mg per day may have a low or acceptable level of gastrointestinal side effects but did not find that the occurrence of side effects was a significant determinant of compliance when daily was compared to weekly supplementation.   Furthermore, experiences from many countries including India, Indonesia, and Bangladesh have shown that systematic counseling on methods to prevent or ameliorate side effects enhances compliance. The counseling includes explaining to women that they should take iron with food, preferably in the evening, and explaining to them that most of the side effects are transient. Side effects may not play as big a role in compliance as originally thought though. In a study of women's perceptions of iron deficiency and anemia prevention and control in eight developing countries, Galloway et

al (121) found that only one tenth of women stopped taking iron tablets due to side effects.

Women's knowledge about anemia and the utility of iron tablets is thought to be even more important factors that influence compliance than side effects. In a qualitative study in urban and rural Nigeria among women attending prenatal clinics, Ejidokun (27) found that maternal anemia was not perceived as a priority health problem by pregnant women; their knowledge of the signs and symptoms of anemia was very limited. Furthermore, women attributed adverse health outcomes, such as severe blood loss at delivery, to the use of iron/folic acid supplements. Among pregnant Liberian women, Jackson et al (123) found that women's traditional attitudes toward pregnancy minimized the symptoms of anemia. Galloway et al (121) reported beliefs among pregnant women against consuming medications during pregnancy and fears that consuming too much iron may make one's baby "big", making delivery difficult. Studies have shown that when women are better informed about anemia and its treatment, compliance increases (124). This lack of knowledge about anemia and suspicion about iron tablets is also due to inadequate counseling from health care workers (midwives in Senegal). In most of the countries studied in the Galloway et al (121) study, women attending prenatal services recognized iron tablets and may take them as instructed, but were not told why they are prescribed or how to take them. In the Nigerian study (27), the author found that health care providers themselves often did not recognize maternal complications associated with anemia.

A second major barrier to the success of programs is the limited availability and access to the iron tablets. Prenatal clinics, where most supplementation programs are implemented, often run out of stock. This problem does exist in Senegalese clinics

whose pharmacies are supplied in tablets by the National Pharmacy, which obtains its stock for the most part from the United Nations.

A third barrier to program success is the use of iron to treat anemia when iron deficiency is not the primary cause of anemia. As was discussed in previous sections, anemia has multiple causes in sub-Saharan Africa and more studies investigating its etiology in specific countries are needed in order to "customize" anemia intervention programs.

Table 4 presents a summary of behavior goals that foster effective iron supplementation programs and challenges to those goals in the real world. Programs that have proven more successful than others with using iron/folic acid supplementation to prevent and control iron deficiency and anemia in pregnant women have focused on 1) increasing availability of supplements, 2) increasing acceptability of iron/folic acid tablets, 3) improving provider performance, 4) increasing compliance, and 5) encouraging iron supplementation earlier in life (124).

Table 4: Behavior goals and challenges of an effective iron supplementation program*

Agent	Behavior Goal	Challenges
Pregnant women	Obtain and use iron supplements at the right dose and frequency	-Women not asking for services or knowing where they are. -Lack of awareness of anemia and how to prevent it. -Lack of knowledge on how to manage side effects. -Fears, beliefs, and suspicions (e.g. iron pills will make the baby too big; iron pills will cause hemorrhage) -Forgetfulness
Health care providers/midwives	Distribute or prescribe iron supplements and counsel women properly about their use	-Lack of awareness and knowledge -Poor communication skills -Infrequent contacts with pregnant women -Providers may act disrespectfully towards women
Health planners and drug managers	Train and supervise staff, monitor supplies, and manage resources	-Lack of awareness of purpose of program
Agents in complementary services (e.g. family planning and pediatrics)	-Support and reinforce messages of iron supplementation program -Integrate anemia education into their activities	-Lack of awareness of anemia and iron supplementation activities -False sense of competition or threat between health care workers
Policy makers	Make and enforce necessary policies and allocate sufficient resources	Lack of awareness of cost of iron deficiency anemia to health and economy of society

*Adapted from reference 103

Chapter 3: Senegal

1. History and Geography

The Republic of Senegal is located on the west coast of Africa. It covers an area of 196,722 square kilometers, making it slightly smaller than South Dakota. It is bordered by the Islamic Republic of Mauritania, the Republic of Mali, Guinea and Guinea-Bissau, the Gambia, and in the west by the Atlantic Ocean (with a coastline of 531 kilometers) (124). The location of Senegal in relation to neighboring countries is shown on **Figure 4**. Most of the terrain in Senegal is composed of low, rolling plains rising to foothills in the southeast. In fact, the country's highest peak, Mount Assirik, stands only at 581 meters near Nepen Diakha in the south. The climate in Senegal is tropical. It is composed of a rainy season, which is accompanied by southeast winds and extends from May to November; and of a dry season, which extends from December to April (accompanied by dry harmattan winds) (124).

The region that later became Senegal was settled in the middle of the first millennium A.D. by Wolof and Serer peoples followed by the Tukolor (9[th] century A.D.) whose state of Tekur dominated the Senegal River Valley until the 14[th] century. The territory of Senegal was occupied successively by the Portuguese, the Dutch, and lastly the French who established it as a colony in 1895. Senegal remained part of French West Africa (AOF) until it was granted independence in 1960 (125).

Figure 4. Map of Africa
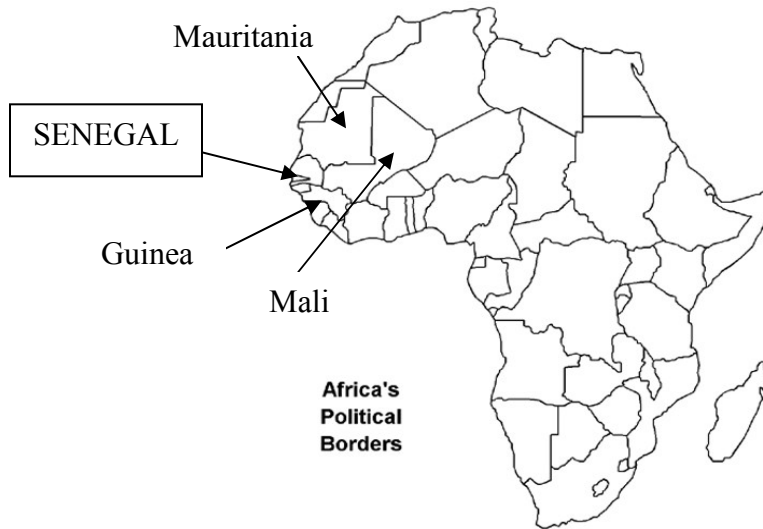
2. Population

According to the most recent census data, the population of Senegal is estimated at 10,284,929 million individuals who are unevenly distributed across the land. The capital city, Dakar, which constitutes only 0.3% of the territory, is home to 22% of the total population and 80% of the urban population.

The Senegalese population is composed of a number of ethnic groups (**Table 5**). The Wolofs constitute the largest group, representing 43% of the total population (106). Most people in Senegal are Muslim (94%); Christians, Animists, and individuals of other religions constitute the remaining 6%.

Table 5: Ethnic composition of the Senegalese population

Ethnic Group	Percent Total Population*
Wolof	43
Pular	24
Serer	15
Jola	4
Mandinka	3
Soninke	1
Europeans/Lebanese	1
Other	9

*Total population is estimated at 10,284,929

The Senegalese are a young people; 58% of the population is 20 years of age or younger and only 3% are 65 years of age and over (**Table 6**). The population rate of growth is estimated at 3%, which is fairly high in part due to the high fecundity rate. Senegalese women have on average 5.3 children over the course of their reproductive years (106).

Table 6: Age structure of the Senegalese population

Age Group (years)	Percent Total Population*
≤ 20	58
0- 14	45
15-64	52
≥ 65	3

* Total population is estimated at 10,284,929

The literacy rates in Senegal are relatively low. Only 33% of the total population is literate (i.e.15 years of age and can read and write), 43% of males and 23% of females (106).

3. Health Statistics

Table 7: Selected Senegalese health statistics

Statistic	Rate
Fecundity rate	6 children/woman (on average)
Birth rate	38 births/1,000
Infant mortality rate	58 deaths/1,000 live births
Maternal mortality rate	560 deaths/100,000 births
Rate of low birth weight	15%

As seen on **Table 7**, infant and maternal mortality rates are very high in Senegal even when these rates are compared to rates in other West African countries of similar income (92). Among the chief contributing factors to these high rates is the poor monitoring of pregnancies (including a high proportion of women delivering infants without professional assistance) resulting in poorly treated bouts of malaria, various causes of dystocia, and untreated nutritional deficiencies (especially iron). Indeed, only 12% of at-risk pregnancies are detected during prenatal visits. These problems are compounded by the fatigue and malnutrition due to short intervals during pregnancy (6% of births occur less than 18 months before the previous one and nearly 12% of births occur 18 months to two years after the previous birth), precocious pregnancies (18% of women between the ages of 15 and 19 years have given birth at least once), and advanced-age pregnancies (106).

Eighty three percent of women aged 20-34 years do seek antenatal care across Senegal. The overall rate of the first prenatal visit is only 50% though and the percentage of women who attend the follow-up visits decreases to 32% and 20% on the second and

third visits, respectively. In Dakar, antenatal care is provided in public hospitals, in health centers, or in private facilities for the few who can afford them. Unfortunately, the frequency of antenatal visits decreases as parity increases; 87% of primipares seek care while only 78% of women who have had six children or more seek help (106, 126). Only 23% of women obtain their first prenatal visit before 4 months of gestation (106).

Life expectancy is only 54 years for men and 57 years for women. However, Senegal is one of the few countries in Sub-Saharan Africa where the HIV/AIDS epidemic is under control. Prevalence of the disease among adults is estimated to be 1.7%. Malaria remains the most preoccupying epidemic in Senegal. One third of all visits to hospitals, clinics, and health centers are because of malaria (127).

On average, 7% to 8% of Senegal's annual budget is devoted to public health in addition to the contributions of multi-lateral and non-governmental agencies and the private sector (106).

4. Organization of the health care system (reference 127)

The Senegalese territory is divided into ten medical regions, which have at least one hospital each: Dakar, Djourbel, Fatick, Kaolack, Kolda, Louga, Saint-Louis, Tamba, Thies, and Ziguinchor. Each of these regions is subdivided into health districts, which may contain one or several health centers. Each health center oversees several health posts that are dependent on the larger center and dispense basic health care services.

The medical region of Dakar is the largest in Senegal. It has seven hospitals; it is subdivided into 8 health districts, 11 health centers, and 130 health posts (**Figure 5**). Each health district serves 200,000 to 300,000 inhabitants. Each health center is staffed

with a general practitioner medical doctor, a pediatric doctor, an obstetrician-gynecologist, two dentists (not in all cases), a variable number of midwives (depending on the size of the center), and a pharmacist. All centers are under the jurisdiction of the Ministry of Health and deliver the same package of health care services. These include maternity care, both prenatal and postnatal and at a low cost. The first prenatal visit, which includes a clinical examination and laboratory blood work, costs on average $2.00; subsequent visits cost about $1.00 each. Prenatal care is delivered primarily by midwives who are trained at government accredited Midwifery Schools. The health center doctor only intervenes if patients show signs of pathology in which case they are most often referred to the district hospital for treatment. Typically, there are 500-600 births per month at each center; however, up to 1,000 pregnant women attend each clinic. This disparity is due to the fact that women do not necessarily give birth where they receive prenatal care; in addition, many women give birth at home (20%) (106).

There is only one health center for every 180,000 inhabitants in Dakar. This is far from the WHO norm of one health center for every 50,000 inhabitants. The ratio of health care workers in these centers to patients has been steadily decreasing in recent years in part due to the rapid population growth of Dakar but also to a decrease in the number of health professionals. In Dakar, there is only one physician for 3,952 inhabitants and one midwife for 1,358 women of reproductive age (15-49 years) (127).

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                        ┌─────────────────────────────────────┐
                        │      MEDICAL REGION OF DAKAR         │
                        └─────────────────────────────────────┘
                                         │

              Health Districts
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South	North	Center	West		Pikine	Rufisque	Guediawaye	Mbao

Health Centers

| 1. IHS | 1.Gaspard Kamara | | Dominique | R. Baudoin |
| 2. CM**I** | 2.Hann sur Mer | | | |

| 1.Nabil Choucaire | | 1.Philippe Senghor | Y. Mbargane | K. Rassoul |
| 2.Parcelles Assainies | | 2.Ouakam | | |
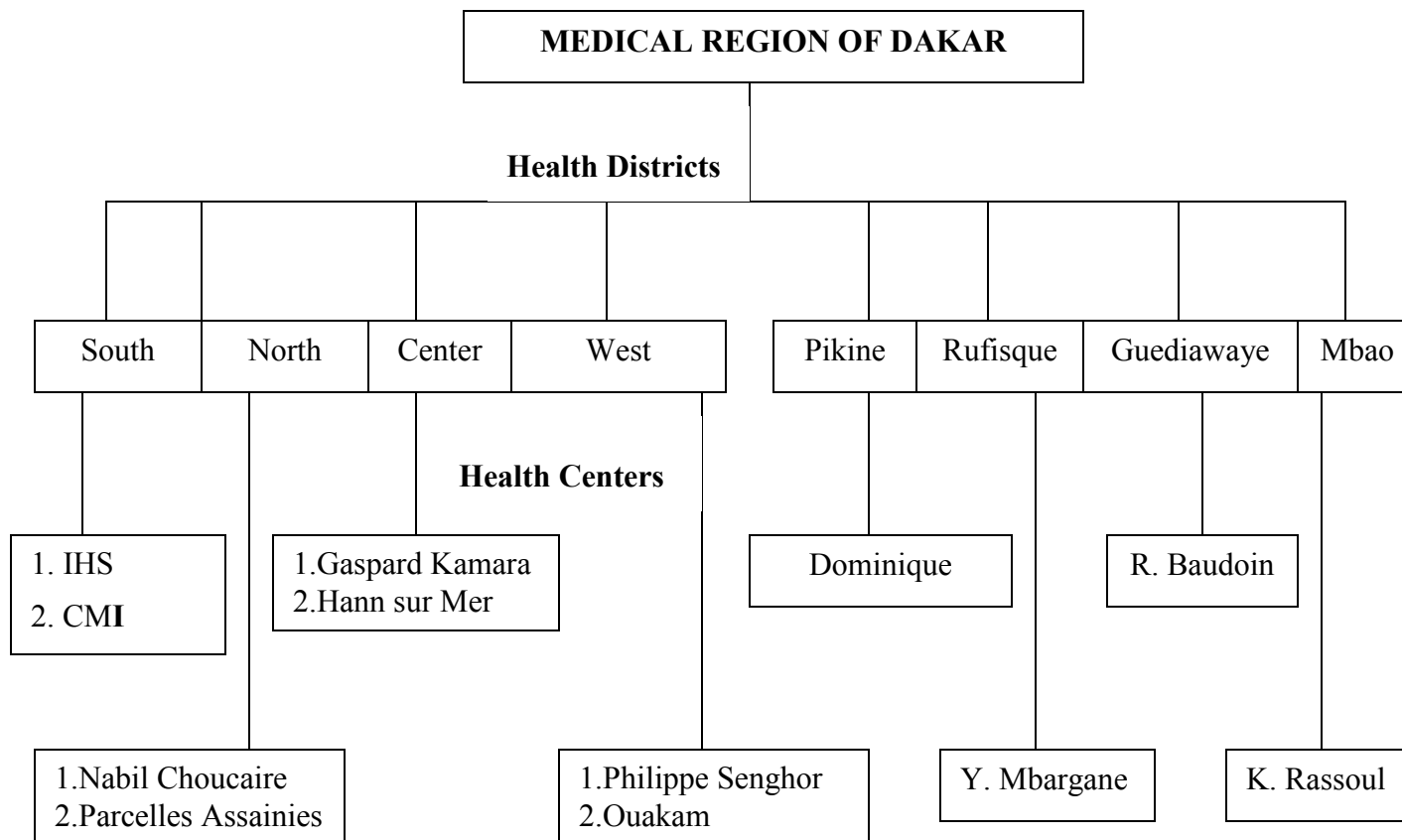
Figure 5. Organization of the health delivery system in Dakar, Senegal

5. The Economy

Senegal is considered a poor or developing country. The World Bank estimates Senegal's gross domestic product (GDP) at $22 billion and GDP per capita at $1,800. The composition of the GDP by sector is the following: agriculture = 19%, industry = 20% (agricultural and fish processing, phosphate mining, fertilizer production, petroleum refining), and services = 61% (128). Most of the Senegalese workforce (80%) labors in the agricultural sector. The major commodities produced are the groundnut (principal cash crop), millet, sorghum, rice, maize, and peas.

In January 1994, Senegal undertook a bold economic reform, which began with a 50% devaluation of the Senegalese currency, the CFA franc. Government price controls and subsidies were progressively eliminated and real growth in GDP rose from 2% in 1993 to an average of 5% annually from 1995 to date. Since the beginning of the implementation of the reform plan, annual inflation has been reduced to 2% and the fiscal deficit has also decreased to about 1.5% of GDP. Senegal has also achieved full Internet connectivity in 1996, an achievement that has led to the burgeoning of information technology-based services in the private sector (106, 128).

Despite these advances and improvements in the economy, Senegal remains a poor country with rising unemployment rates (40% among the urban youth), with 26.3% of the population living on less than one dollar a day and 68% living on less than two dollars a day. Only 32% of households have electricity and only 49% have running water (106, 128).

Chapter 4: Research Objectives

1. Summary of the problem

The prevalence of anemia among pregnant women in Senegal is at least as high as 50%. Anemia has multiple causes and increases the risk of adverse pregnancy outcomes. As the current strategy to combat anemia among pregnant Senegalese women stands, when women visit public health centers for prenatal care, they are examined by the center midwives (e.g. height, weight, height of fundus, and blood pressure are measured, stage of gestation is estimated, symptoms and complaints are recorded) who then give them a written prescription to purchase iron/folic acid tablets at the health center pharmacy. Each tablet contains 65 mg elemental iron (200 mg ferrous sulfate) and 250 μg folic acid and is to be taken once daily. Women are instructed to purchase enough tablets for daily supplementation until term. Studies show that these types of programs are generally less effective than desired mainly because of low compliance. Low compliance is strongly related to the following factors: 1) limited knowledge of women about anemia and the utility of tablets, 2) erroneous beliefs and fears about iron tablets, 3) health care workers' inadequate knowledge and counseling of pregnant women, 4) the occurrence of gastrointestinal side effects with high doses of iron, 5) limited resources for women to purchase supplements, 6) hindered access of women to supplements (e.g. health center pharmacies may often run out of stock); another important barrier to program

effectiveness is the fact that iron supplements alone cannot effectively treat anemia if other important underlying causes of the disease coexist with iron deficiency.

2. Objectives

The main objective of this study was to evaluate and, if necessary, provide a framework for recommendations for the improvement of the supplementation program for pregnant women in Senegal. First, we wanted to determine the prevalence and major risk factors for anemia in this population in order to ascertain whether iron/folic acid tablets alone constitute an effective treatment of anemia. Next, we hypothesized that if pregnant women attending health centers were given free iron/folic acid tablets during the prenatal visit instead of a prescription to purchase them, some potential barriers to program success would be eliminated, including the inadequacy of tablet supply at the designated health center pharmacy and the lack of financial resources for women to purchase the supplements. In addition, giving the supplements during the visit could encourage midwives to explain their utility and allay possible misgivings that patients have about the tablets (e.g. that iron supplements will make their infant « big » and therefore difficult to deliver). At the health center pharmacy, midwives have no control or final input into what is communicated to patients. The net effect of the elimination of these barriers could be an increase in compliance with supplementation. Lastly, we wanted to determine the factors that influence compliance because understanding these factors in this population can be the basis of recommendations to improve patient care and counseling.

Chapter 5: Materials and Methods

1. Subjects

The study was conducted in the capital city of Senegal, Dakar. It is subdivided into 8 health districts, that each represents a different geographical area, and counts 11 health centers. Subjects were recruited from the 6 "reference" health centers, which are larger than the others and offer more services, such as surgery and radiography. Two of the selected health centers are located in the South health district; the remaining 4 are each located in the Center, West, Pikine, and Guediawaye health districts. Pikine and Guediawaye are located in the suburban area of the city. The selected health centers each cover a population of about 180,000 (127).

All women who registered for routine prenatal care and who fulfilled the following criteria were invited to participate in the study after giving informed consent: 1) residing in Dakar or its suburbs, 2) not having used iron/folic acid supplements prior to enrollment, 3) being at the beginning of the second trimester of gestation as estimated by fundal height and date of last menstrual period, 4) and being apparently healthy. We chose to recruit women in their second trimester of gestation because we needed to maximize the duration of intervention, because iron needs are highest during the second trimester of gestation; and because most women do not seek prenatal care before this stage of gestation (106). This study was reviewed and approved by the Committee on Ethics of the Senegalese Ministry of Health and the Institutional Review Board of the University of Maryland.

2. Methods

2.1 Randomization

To avoid bias, we chose to randomize the health centers instead of the subjects themselves. Health centers were randomly assigned to either the control or intervention group by a computerized random digit generator. Assignment was as follows:

Control Group	**Intervention Group**
Institute of Social Hygiene	Center for Maternal and Infant Protection
Philippe Senghor	Gaspard Kamara
Roi Baudouin	Dominique

Women at the control centers received routine prenatal care such as devised by national policy. At the end of their visit, they received a prescription to purchase enough iron/ folic acid tablets [65 mg elemental iron as 200 mg dried ferrous sulfate + 250 μg folic acid)] at the health center pharmacy for daily supplementation until their next prenatal visit. At follow-up visit, the prescription was renewed until term.

Women at the intervention centers underwent the same prenatal treatment regime as women in the control group, except that at the end of their visit, the midwives gave them enough free iron/folic acid tablets for daily supplementation until term. The free tablets had the same appearance and composition as those prescribed for women in the control group. They were pre-packaged in plastic bags and women were asked not to discard empty tablet packages. The iron/folic acid tablets are provided to Senegal by the United Nations International Children's Fund (UNICEF) through the Poverty Reduction Funds.

2.2 Anthropometric measurements

Weight was measured to the nearest 0.1 kg on a calibrated beam balance scale. Height was measured to the nearest 0.1 cm with a stationary height board or measuring tape affixed to the wall.

2.3 Hematological measurements

Two venous blood samples were collected from each subject: one in an EDTA-treated vacutainer tube and another in an evacuated non-treated tube. The samples were packaged in dry ice for transport to the laboratory where analyses were performed.

Anemia status was assessed by measuring hemoglobin (Hb) concentration using the HemoCue photometer (HemoCue, Angelhölm, Sweden); each sample was analyzed in duplicate and calibration of the photometer was checked daily using the control cuvette provided by the manufacturer. Anemia was defined according to WHO guidelines as Hb < 110 g/L, moderate to severe anemia as Hb <90 g/L, and severe anemia as Hb< 70 g/L. Where appropriate, the CDC cut-off values were used to account for hemodilution: Hb < 105 g/L in the second trimester and a Hb < 110 g/L in the third trimester of gestation (63, 64).

Iron status was assessed by measuring erythrocyte protoporphyrin (EP), serum ferritin (SF), and serum transferrin receptors concentration (Tfr). EP was measured with a front-face hematofluorometer (Aviv Biomedical, Lakewood, NJ) in duplicate. Calibration of the device was checked daily using control solutions provided by the manufacturer. EP concentration was used as an indicator of iron-deficient erythropoiesis. Values indicative of iron deficiency were defined as EP >70 μmol/mol heme (129). Blood collected from

the evacuated tubes was used for SF analysis. The blood was centrifuged (481 x g, 8 min, 28ºC). The resulting serum was then frozen at -20 ºC. Later, it was thawed in a refrigerator (2-8ºC) for no longer than 12 hours before being analyzed by enzyme-linked immunosorbent assay with (ELISA) monoclonal antibodies using a kit and the Mini-Vidas (Biomerieux, France). An SF concentration <15 µg/L was indicative of depleted iron stores (130). Tfr was measured by ELISA in duplicate using a kit (Ramco Laboratories, Inc, Houston). A best-fit straight line was generated by regression using the standards provided by the manufacturer. A Tfr concentration >8.3 µg/L was indicative of iron-deficient erythropoiesis (131).

Hb, EP, SF, and Tfr concentrations were measured at enrollment and 20 weeks later.

2.4 Malaria parasitemia

Thick and thin blood films were collected, fixed, and stained with Giemsa buffer solution to detect all species of plasmodium parasites. Malaria parasites were counted as a ratio to leukocytes by an experienced parasitologist.   The calculation of parasite density was based on 8000 leukocytes per µL of blood. Densities >5,000 parasites/µL blood were considered evidence of clinical malaria (132).

2.5 Helminth infection

Study participants were asked to produce a stool specimen in containers provided by the researchers on the morning of their visit. When they were unable to do so, they were asked to try again that evening or the following morning and to return the sample to the health center. The samples were conserved in 5% formaldehyde solution until analyses

could be performed. They were stained using the Kato Katz method and read within one hour of staining by a trained microscopist for presence of intestinal parasites (133-134).

2.6 C-Reactive Protein

C-reactive protein (CRP) was measured from the serum at enrollment as an indicator of infection or inflammation, which can affect Hb, EP, and SF concentrations, by latex-enhanced immunonephelometry on a BN II Analyzer (Dade Behring, Newark, DE). A cutoff value of > 6 mg/L was used (135).

2.7 Hemoglobin S

Blood samples were screened for Hb S within a few hours of collection at enrollment using the sodium metabisulfite reduction test. All samples that were positive for Hb S were re-analyzed by hemoglobin electrophoresis of a red blood cell hemolysate on cellulose acetate plates to confirm the phenotype (136).

2.8 Dietary evaluation

A modified non-quantitative food frequency questionnaire (FFQ) was used to estimate dietary intake of iron (Fe) (see appendix A). The 45-item FFQ was constructed after reviewing the food list of the Worldfood Dietary Assessment System (WDAS), which was developed at the University of California at Berkeley in collaboration with the Food and Agriculture Organization; it is designed primarily for dietary research projects in developing countries. The database contains a list of 1800 foods reported from 6 countries, including Senegal. The nutrient composition of foods that were reported as frequently consumed in that country was reviewed using the International Minilist (IML)

food composition table, which is built into the WDAS. For each food on the IML, there are values for 52 constituents: the data are taken from published food composition tables or imputed where no analytic data are available (137). Foods were included in the FFQ if they contribute significant amounts of Fe in the Senegalese diet, or are non-heme Fe absorption enhancers (due to their high content of ascorbic acid and citric acid), or are non-heme Fe absorption inhibitors (due to their high content of phytate, calcium, polyphenols, and tannins). For intake of heme-Fe, only foods of animal origin were included. For non-heme Fe intake, only plant foods containing >0.35 mg Fe per serving were included. For example, foods were considered important contributors of vitamin C if they contain >24 mg of vitamin C per serving. Foods rich in phytate, tannins, and calcium were identified on the basis of the IML and published values.

Study participants were asked to indicate how often they consumed each item on the FFQ over the 12-month period preceding the interview.

2.9 Socio-demographic and health questionnaires

Women were interviewed at enrollment using structured questionnaires that were pre-tested on 10 non-study participants at each center for face validity and the necessary changes were made prior to data collection. Characteristics such as age, education level, employment status, and household possessions were collected. Data on parity, known diagnosis of disease, and general perception of health were also collected.

2.10 Assessment of compliance and of factors that affect compliance

Compliance was on average 20 weeks after the date of enrollment. Women were contacted by telephone and given appointments to return to the health center where they

had originally registered for prenatal care. This mean of communication was chosen because most women could not provide an exact street address, as is common in low-income areas of Dakar. They were asked to provide a phone number where they could be reached; it could be their home or cellular phone number, their husband's cellular phone number or neighbors' and relatives'. Ninety seven percent of women in the study sample provided a phone number where they could be reached. On average, three attempts were made to contact the participants; once they were reached, they were given an appointment date and time to return to the health center for follow-up. For women who did not come to their first appointment, two subsequent attempts were made to schedule another appointment if needed.

During the follow-up visit, women in the control group were interviewed to determine the number of iron/folic acid tablets they had purchased since their first prenatal visit and the number of tablets that remained in their supply. In addition, women were asked whether they had actually ingested the missing tablets to ensure that the tablets had not been given away to friends or relatives or lost. Compliance in this group was then calculated as: (Number of tablets ingested ÷ Number of days elapsed since enrollment x 100). It was assumed that the number of days that had elapsed between enrollment and follow-up corresponded to the number of tablets that the patient should have ingested.

Women in the intervention group were asked to bring the bag of supplements they were given at enrollment, including the empty tablet packages. The number of tablets left in the bag was determined and women were questioned about actual tablet ingestion. Compliance was calculated using the same formula as for the control group.

During the follow-up visit, each subject's compliance was computed and classified as either "low compliance", i.e. < 70% or "high compliance", i.e.≥ 70%. This classification was chosen on the basis of WHO's recommendation stating that in countries where the prevalence of anemia among pregnant women is ≥40% that supplementation be given for 6 months (24 weeks) of pregnancy, i.e. 168 tablets (103). For this study, participants were monitored over an average period of 20 weeks during which the maximum number of tablets they could have ingested as prescribed would have been 140; 70% of 140 is 98 tablets, which is 83% of WHO recommended number. The remaining 28 tablets that women would have consumed if monitoring was continued over the full 24 weeks would account for about 100% compliance.

An in-depth interview, which lasted 20 minutes on average, was then conducted with each subject. Women with low compliance were asked to identify the reason or reasons why they did not take the iron/folic acid tablets as directed most of the time. Women with high compliance were asked to identify the factor or factors that motivated them to take the tablets as directed most of the time. All interviews were conducted in the major local language of Senegal, Wolof. The questions were open-ended and were asked in a non-threatening or accusatory manner. Women's answers were recorded and then transcribed word for word by the interviewer. For example, women in the study sample often refer to anemia as "lacking blood"; if a woman answered "I took the tablets because I thought they would increase my blood", the interviewer wrote down the exact phrase. The translation and interpretation was done during data entry and analysis. Where women identified more than one factor that influenced their compliance, all factors were

recorded; however they were asked to identify what they thought to be the strongest factor.

3. Sample size and power analyses

The primary outcome for this study was the prevalence of anemia after 20 weeks of supplementation; i.e. the proportions of women in the control and intervention groups with below normal Hb concentration at follow-up. Thus, on the basis of two independent samples with a two-sided chi-square test with continuity correction, a significance level of .05, and a β-error specification of 0.20, 183 subjects were needed per group (control and intervention) in order to detect a 15% relative difference between the 2 groups in the prevalence at follow-up. A total of 480 subjects were recruited (30% more than needed) to account for potential loss of subjects.

4. Statistical analysis

All data were coded where appropriate and analyzed using SPSS (version 15.0). The statistical analyses that were performed are explained in detail in the next chapters. Briefly, the primary outcomes of this study were comparisons at follow-up, between the control and intervention groups, of: 1) compliance, 2) the prevalence of anemia and iron deficiency, and 3) mean Hb, EP, and SF. Other outcomes included the risk factors for anemia and the factors that influence compliance. Statistical significance for all tests was accepted at $P < .05$.

Chapter 6: Results

1. Iron deficiency is a major risk factor for anemia among pregnant women in Senegal

Abstract

Anemia during pregnancy is major public health problem in Senegal. For effective intervention, its causes must be known. We determined the prevalence of anemia and the relative contribution of various risk factors in a cohort of 480 women visiting health centers in Dakar. Hemoglobin (Hb), erythrocyte protoporphyrin (EP), serum ferritin (SF), serum transferrin receptors (Tfr) malaria parasitemia, helminthes infection, Hb phenotype, and dietary iron intake were assessed. Overall, 39% of women were anemic (Hb<110 g/L), 71% were iron deficient (EP>70µmol/mol heme, SF<15µg/L, or Tfr>8.3 µg/L), and 34% had iron deficiency anemia. Twelve percent were infected with P. *falciparum;* 21% had intestinal helminthes, most of which resided in the suburban areas of the city. About 7% had sickle cell trait (Hb AS). Women consumed heme-iron containing foods more frequently than non-heme iron containing foods; however, they also consumed iron absorption inhibitors at a high frequency. Iron deficiency >quadrupled the risk of anemia. Malaria parasitemia and Hb AS significantly increased the risk of anemia. Our findings indicate that for effective control of anemia during pregnancy in Senegal, iron supplementation is needed in addition to education of women about better food choices. Antimalarial chemoprophylaxis should be made available to all women and populations at risk of helminthes infections should be screened and treated.

Introduction

      Anemia of pregnancy is highly prevalent in Senegal (West Africa). Although no recent survey has been conducted, it is estimated that >50% of pregnant women in that country are anemic (1-2). Anemia, specifically iron deficiency anemia early in pregnancy, has adverse consequences on pregnancy outcome, including low birth weight, preterm birth, and small-for-gestational age, which are the strongest predictors of perinatal mortality; in severe cases, anemia increases the risk of maternal death at the time of delivery (3-7). In Senegal the prevalence of low birth weight is at least 15%; maternal and infant mortality rates are high at 560/100,000 and 58/1,000, respectively. The proportions of these rates attributable to anemia are unknown; however, studies suggest that they are significant (8).

      There are multiple causes of anemia in tropical developing countries such as Senegal. More than half of the cases are generally due to dietary iron deficiency often resulting from a diet poor in bioavailable iron but rich in iron-absorption inhibitors such as phytates in cereals and polyphenols in coffee and tea. In addition, nutritional deficiencies of folate, vitamin B12, and vitamin A can also cause anemia. Other coexisting causes include malaria parasitemia, intestinal parasite infections and to a lesser extent hemoglobinopathies such as sickle cell (Hb S) (9-10).

      As part of its new national health strategic plan the government of Senegal has taken steps to improve maternal health, including reducing anemia. Since >80% of women aged 20-34 years seek prenatal care and that the majority of those (83%) obtain it in the government-run health centers, improvement efforts have mainly been

concentrated on these centers (11). The government's program to reduce anemia can be summarized as follows: when women go to their first prenatal visit, they are given a prescription to purchase iron/folic acid tablets (65 mg elemental iron and 250 μg folic acid) and malaria chemoprophylaxis. The efficacy, effectiveness, and cost-effectiveness of this program, which has been in place for the past twenty years, are not known although maternal mortality rate has decreased from 1200 to 560/100,000 live births between 1990 and 2005 (11).

An effective program to combat anemia should address all the major causes of anemia. One of the first steps in designing such a program for a specific country is to investigate the contribution of each of the known causes of anemia in that country. Thus the purpose of this study was to determine the prevalence and major risk factors for anemia in a cohort of pregnant women in urban Senegal and more specifically the relative contributions of iron status, malaria parasitemia, helminthes infection, and Hb S to anemia in order to make recommendations that could improve the current policy of supplementation.

Subjects and Methods

The study was conducted in the capital city, Dakar, which is home to 80% of the urban population of Senegal. The country's fertility rate results in approximately 450,000 pregnancies annually (11).

Dakar is subdivided into 8 health districts, which have a total of 11 health centers. Each district represents a different geographical area. Subjects were recruited from the 6 "reference" health centers, which are larger than the others and offer more services, such as surgery and radiography. Two of the selected health centers were located in the South

health district; the remaining 4 were each located in the Center, West, Pikine, and Guediawaye health districts. Each health center covers a population of about 180,000 (12).

The subjects were participating in a randomized intervention trial of the effect of free iron/folic acid tablets versus a prescription to purchase the tablets on compliance. All women who registered for routine prenatal care and fulfilled the following inclusion criteria were invited to participate after giving informed consent: 1) healthy according to physical examination, 2) in their second trimester of gestation according to fundal height and date of the last menstrual period (this criterion was included, in part, because 69.5% of women in Dakar do not seek prenatal care before this stage of gestation (11)), 3) residing in Dakar or its suburbs, and 4) not having received iron/folic acid supplements prior to enrollment. For adequate statistical power in the randomized study, 480 subjects were enrolled, i.e. 80 women per center.

Socio-demographic, socio-economic and health survey

Women were interviewed at enrollment using structured questionnaires that were pre-tested on 10 non-study participants at each center for face validity and the necessary changes were made prior to data collection. Characteristics such as age, education level, employment status, and household possessions were collected. Data on parity, known diagnosis of disease, and general perception of health were also collected. Weight was measured to the nearest 0.1 kg on a calibrated beam balance scale. Height was measured to the nearest 0.1 cm with a stationary height board affixed to the wall.

Anemia, iron, and infection status

Two venous blood samples were collected from each subject: one in an EDTA-treated vacutainer tube and another in an evacuated non-treated tube. The samples were packaged in dry ice for transport to the laboratory where analyses were performed.

Anemia status was assessed by measuring hemoglobin (Hb) concentration using the HemoCue photometer (HemoCue, Angelhölm, Sweden); each sample was analyzed in duplicate and calibration of the photometer was checked daily using the control cuvette provided by the manufacturer. The Pearson correlation between the 2 Hb measurements was .998 (P<.001). Anemia was defined according to WHO guidelines as Hb < 110 g/L, moderate to severe anemia as Hb <90 g/L, and severe anemia as Hb< 80 g/L. This last cut-off was used instead of the traditional <70 g/L because only a few women (n =14) had Hb concentrations that low (13).

Iron status was assessed according to two indicators, erythrocyte protoporphyrin (EP) serum ferritin (SF), and serum transferrin receptors (Tfr). EP was measured in duplicate with a front-face hematofluorometer (Aviv Biomedical, Lakewood, NJ). Calibration of the hematofluorometer was checked daily using control solutions provided by the manufacturer. The Pearson correlation between the 2 EP measurements was .997 (P<.001). EP concentration was used as an indicator of Fe-deficient erythropoiesis. Values indicative of iron deficiency (ID) were defined as EP >70 μmol/mol heme (14). Blood collected from the evacuated tubes was used for SF analysis. The blood was centrifuged (481 x g, 8 min, 28ºC). The resulting serum was then frozen at -20 ºC. Later, it was thawed in a refrigerator (2-8ºC) for no longer than 12 hours before being analyzed

by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies using a kit and the Mini-Vidas (Biomerieux, France). An SF concentration <15 µg/L was indicative of depleted iron stores (14). Tfr was measured by ELISA (Ramco laboratories, Inc, Houston). A best-fit straight line was generated by regression using the standards provided by the manufacturer. A Tfr concentration >8.3 µg/L was indicative of iron-deficient erythropoiesis

Iron deficiency anemia (IDA) was defined as Hb<110 g/L and EP>70 µmol/mol heme or SF <15 µg/L or Tfr>8.3 µg/L.

C-reactive protein (CRP) was measured from the serum as an indicator of infection or inflammation, which can affect Hb, EP, and SF concentrations, by latex-enhanced immunonephelometry on a BN II Analyzer (Dade Behring, Newark, DE). A cutoff value of > 6 mg/L was used (15).

Malaria and intestinal parasitic infection

Thick and thin blood films were collected and fixed and stained with Giemsa to detect malaria parasitemia. Malaria parasites were counted as a ratio to leukocytes by an experienced parasitologist. The calculation of parasite density was based on 8000 leukocytes per µL of blood and all species were identified as P. *falciparum*. Densities >5,000 parasites/µL blood were considered evidence of clinical malaria (16).

To assess helminthes infection, women were asked to produce a stool specimen in containers provided by the researchers on the morning of their visit. When they were unable to do so, they were asked to try again that evening or the following morning and to return the sample to the health center. The samples were conserved in 5%

formaldehyde solution until analyses could be performed. They were stained using the Kato Katz method and read within one hour of staining by a trained microscopist for presence of intestinal helminthes. Parasite density was expressed in number of eggs per gram of stool (17).

Hemoglobin S

Blood samples were screened for Hb S within a few hours of collection using the sodium metabisulfite reduction test. All samples that were positive for Hb S were re-analyzed by hemoglobin electrophoresis of a red blood cell hemolysate on cellulose acetate plates to confirm the phenotype (18).

Dietary evaluation

A modified non-quantitative food frequency questionnaire (FFQ) was used to estimate dietary intake of iron (Fe). The 45-item FFQ was constructed after reviewing the food list of the Worldfood Dietary Assessment System (WDAS), which was developed at the University of California at Berkeley in collaboration with the Food and Agriculture Organization; it is designed primarily for dietary research projects in developing countries. The database contains a list of 1800 foods reported from 6 countries, including Senegal. The nutrient composition of foods that were reported as frequently consumed in that country was reviewed using the International Minilist (IML) food composition table, which is built into the WDAS. For each food on the IML, there are values for 52 constituents: the data are taken from published food composition tables or imputed where no analytic data are available (19). Foods were included in the FFQ if they contribute

significant amounts of Fe in the Senegalese diet, or are non-heme Fe absorption enhancers (due to their high content of ascorbic acid and citric acid), or are non-heme Fe absorption inhibitors (due to their high content of phytate, calcium, polyphenols, and tannins). For intake of heme-Fe, only foods of animal origin were included. For non-heme Fe intake, only plant foods containing >0.35 mg Fe per serving were included. Foods were considered important contributors of vitamin C if they contain >24 mg of vitamin C per serving. Foods rich in phytate, tannins, and calcium were identified on the basis of the IML and published values. Study participants were asked to indicate how often they consumed each item on the FFQ over the 12-month period preceding the interview.

Statistical analysis

Multinomial logistic regression models were performed to estimate the adjusted odds ratios (AOR) and 95% confidence intervals (CI) for anemia, iron-deficient erythropoiesis, and depleted iron stores. Since EP, SF, and Tfr were skewed, they were log-transformed for analysis. Adjusted mean differences in Hb, EP, SF, and Tfr were calculated with forward selection multiple linear regression. All regression models included variables for malaria parasitemia, intestinal parasite infection, CRP, Hb S, stage of gestation, time elapsed between the previous and current pregnancies (birth interval), socio-economic, demographic, and anthropometric data. Variables were retained in the models only if statistically significant. Since the intensities of malaria parasitemia and parasitic infection were relatively low in the population that was studied, the data were entered as present or absent.

Differences in the prevalence of anemia and/or iron deficiency were assessed using chi-square for dichotomous variables. Differences in Hb, EP, and SF concentrations were ascertained by Student t test and ANOVA for categorical variables. Where appropriate, Dunnett's C test was used for post-hoc comparisons for samples with unequal variance.

Foods in the FFQ were grouped into 4 categories: sources of heme-Fe, sources of non-heme Fe, sources of Fe absorption enhancers, and sources of Fe absorption inhibitors. Mean frequencies of consumption were computed and coded to reflect the following 3 categories: almost never/a few times a month, several times a week, and several times a day.

An index of socio-economic status (SES) was created from 4 binary indicators: paid employment, home ownership, refrigerator ownership, and literacy. Subjects were assigned a score of 0 to 4 depending on how they scored on each of the indicators. All data were analyzed using SPSS (version 15.0) and statistical significance was defined as a P value <0.05.

Results

Characteristics of the study sample (Table 1)

The mean age of the sample was 25.7±5.9 years with a range of 15-43 years. Most women were aged between 20 and 29 years (55%). Only 18% could read and write and 68% were unemployed. About 35% were primipares and very few had 5 or more children (6%). For 49% of the non-primipares, the time elapsed between the previous

and current pregnancy was of at least 24 months. Most of the women (81%) had never been diagnosed with a serious illness such as HIV/AIDS, diabetes, or cancer; the remainder reported diagnoses of chronic heartburn, respiratory disorders, and migraines. Most described their current state of health as either good or excellent (51%). Mean weight and height were 60.40±12.7 kg and 1.64±0.6 m, respectively. Mean SES score was 1.6±1.1 on a scale of 4.0 and on average, women spent <$1 per day on food (mean = $.99±.98). Mean stage of gestation at enrollment was 12.4±4.7 weeks.

Prevalence of anemia, iron deficiency, and iron deficiency anemia (Table 2 & 3)

Overall, 39% of the women were anemic, of which only 7% suffered from severe anemia (Hb <80 g/L). Around 70% were iron deficient; 40% had low SF, 63% had elevated EP, and 18.5% had elevated Tfr.   The prevalence of iron deficiency anemia was 34%.

Malaria

Malaria parasitemia was detected in only 12% of women (n = 433; 47 blood films were unreadable at the time of analysis). P. *falciparum* was the only species detected. Mean parasite density was low at 342.4±2091.9/µL blood; only 5 women had parasite densities indicative of clinical malaria (>5,000/µL blood). Mean Hb, EP, SF, and Tfr were not significantly different between women with and without malaria parasitemia.

Helminth infection

Parasitic infection was assessed for a sub-sample of 298 women; the remainder of the women either did not provide a stool sample or the sample that they provided was

deteriorated at the time of analysis. Overall, only 13% (n = 63) tested positive for intestinal helminthes. Most (82% n = 52) were infected with A. *lumbricoides* with a mean density of 866 (S.E 120.6) eggs/g; 13% (n = 8) were infected with T. *trichiura* with a mean density of 262.5 (76.6) eggs/g. About 5% (n = 3) were infected with A. *duodenale* with a mean density of 48.0 (13.8) eggs/g. Sixty two percent of infections were found among women who resided around and visited the two suburban health centers. Mean Hb, EP, SF, and Tfr were not significantly different between women with and without parasitic infections.

Hb S analysis

The sodium metabisulfite screening test was performed on 415 subjects (65 samples were missing from women who were enrolled in the study before the test was available). Only 6.5% tested positive (n = 27) and were all heterozygote for sickle cell trait according to cellulose acetate electrophoresis.

Dietary intake of heme and non-heme iron, iron absorption enhancers and inhibitors (Table 4)

Approximately 35% of women reported consuming heme-Fe containing foods several times a day; 25% consumed non-heme Fe containing foods at that frequency; 34% and 40% reported consuming foods containing Fe absorption enhancers and foods containing Fe absorption inhibitors, respectively, several times a day. Mean Hb concentration was significantly higher among women who consumed Fe inhibitors only several times a month as opposed to several times per day (P<.05). Mean Hb, EP, SF, and did not differ

significantly across frequencies of consumption of heme-Fe, non-heme Fe, Fe enhancers and inhibitors; however, there was a general trend of increasing mean Hb and SF with increased consumption of heme-Fe, non-heme Fe, and enhancers and decreased consumption of inhibitors. There was also a trend of decreasing EP concentration with increased consumption of heme-Fe, non-heme Fe, and enhancers and decreased consumption of inhibitors.

Relation of anemia to iron deficiency, malaria parasitemia, parasitic infection, and Hb AS

In the multiple logistic regression, the adjusted odds ratio (AOR) of anemia associated with the combination of elevated EP and low SF was 4.63 (95% CI 2.05 − 10.41). The AORs for Hb AS, malaria parasitemia, and parasitic infection were 4.01 (95% CI 1.20 − 13.72), 2.36 (95% CI 1.00 − 5.58), and .923 (95% CI .45 − 1.87). The association between anemia and helminthes infection is not significant since the confidence interval includes 1.

A stepwise multiple linear regression analysis was conducted with forward selection to predict Hb concentration from iron status indicators while controlling for various factors: Block 1 included: the intensity of parasitic infection, malaria parasitemia, Hb AS, and CRP. Block 2 included: stage of gestation, age, weight, height, and SES score. Block 3 included: log EP, log SF, and log Tfr. The three blocks were so ordered to determine the proportion of variation in Hb concentration accounted by the iron status indicators in block 3 above and beyond the factors in the remaining blocks. The first block of predictors accounted for a significant amount of Hb variability with an R^2 = .042 (P = 0.014). The second block, controlling for the first, also accounted for a significant

amount of Hb variability, R^2 change was .11 (P <.001). The third block, controlling for the first two, accounted for the largest Hb variability with R^2 change of .44 (P < 0.001).

Relation of anemia with other factors

The prevalence of anemia was not associated with age category, parity, birth interval, or SES score (Chi-square tests, P>.05). Neither iron-deficient erythropoiesis nor depleted iron stores or the combination of the indicators were associated with the three maternal characteristics mentioned above.

Discussion

Anemia among pregnant women is a major public health problem in Senegal. This is the first study in recent years to investigate the multiple risk factors for anemia of pregnancy among low-income urban women. This study can provide a framework for recommendations for effective prevention and treatment.

We found that almost 40% of women in a cohort of pregnant women in Dakar were anemic. This prevalence is somewhat lower than WHO estimates of anemia in West Africa (20). It is also lower than previous estimates in Senegal (1-2). The fact that all women in our sample were recruited in the beginning of the second trimester of gestation may explain these discrepancies; the stage of gestation at which women were sampled to generate the WHO and other data is unclear. If a large proportion of women in those samples were at more advanced stages of gestation, we would expect their hemoglobin concentration to be lower because of plasma volume expansion (21). We recruited women at the start of the second trimester for two reasons: 1) it is the stage at

which most women go to their first prenatal visit (11) and 2) it is when expansion of blood volume begins and when iron needs rise dramatically (21).

Iron deficiency was very prevalent at 71%; however, serum ferritin concentrations were not as low as expected, suggesting that most women did not enter pregnancy with depleted iron stores. Thirty four percent of cases of anemia were attributable to iron deficiency. Iron deficiency more than quadrupled the odds of anemia after controlling for other causes; serum ferritin, erythrocyte protoporphyrin, and serum transferrin receptor concentrations accounted for 44% of the variability in hemoglobin concentration. Analysis of the food frequency questionnaire showed that women consumed heme-iron foods more frequently that non-heme iron foods; however their consumption of absorption inhibitors was more frequent. These inhibitors included rice, millet, and maize, which contain phytates, which all women consumed at least once a day because they are staples in Senegal. These inhibitors also included a local black tea and coffee which contain phenolic compounds and were consumed several times a day by 40% of the women. Our findings indicate that dietary iron, although available to this population, is hindered by the consumption of absorption inhibitors. Furthermore, the 2005 Senegal Demographic and Health Survey (DHS) reported that only 7% of mothers of children <5 years of age are undernourished in Dakar whereas 40% are over-nourished as indicated by body mass index (11). This, again, suggests that inadequate iron intake in this population is more related to poor food choices than to the unavailability of food.
Our findings support the current policy of universal iron/folic acid supplementation for pregnant women who visit public health centers for prenatal care. DHS reports that 95% of women in Dakar receive a prescription to purchase supplements at their first prenatal

visit; however there is no data available to suggest that women actually purchase the tablets and comply with daily supplementation. Women also need to be educated about better food choices so that they consciously increase bioavailable iron intake and decrease their intake of absorption inhibitors. Unfortunately, the DHS survey also indicates that only 8% of women in Dakar reported having received nutritional counseling at their prenatal visit (11).

Seventeen percent of women who were tested for helminthes were infected with A. *lumbricoides*, which is a nematode that invades the gastrointestinal tract after ingestion of its eggs in contaminated foods or drinks. A high parasite load can cause iron deficiency anemia, especially when dietary iron quality and quantity are marginal, when the host's general nutritional status is poor, and when coexisting infections are present (22). However, we found that the parasite load in the sample was low. The same was true for hookworms. Mature hookworm infection causes intestinal bleeding leading to fecal bleeding and eventually to anemia depending on the worm burden (>2000 eggs/gram stool) (9). Only 1% of women were infected with A. *duodenale* and the worm burden was light. The third parasite that was detected was T. *trichiuria*, a whipworm, which invades the intestinal tract after consumption of contaminated foodstuffs and can cause iron deficiency depending on the parasite load (9). Again, worm burden was low. We expected to find a larger prevalence of helminthes infection in this population and much heavier worm burdens because an increasing number of people in Dakar consume street foods prepared in unsanitary make-shift restaurants. A survey of the risk of parasitic infection among consumers of these foods in Dakar found that 60% and 45% of food sellers and consumers in the sample were infected with protozoans (23).

Since worm burdens were low for all three parasites, we pooled them as one variable in the analysis of risk factors for anemia. Infection did not increase the odds of anemia. WHO recommends that anti-helminth drugs be given prophylactically to pregnant women in conjunction with iron/folic acid tablets only in areas where infection is endemic and anemia is very prevalent; the combination of therapy and supplementation enhances Hb response (24). Our data does not indicate that this is the case in Dakar although this may have been due to the fact that we only obtained stool specimens from 62% of the original sample. However, since the proportion of women who were infected was not negligible (21%, n = 298) and since most of those who were infected were recruited from the suburban health centers (Pikine and Guediawaye), pregnant women can benefit from a routine stool examination, especially in areas where infection rates are higher. The suburban neighborhoods of Dakar are home to many of the low-income migrants who come from rural areas of the country where parasitic infections are more prevalent. They often reside in cramped houses where sanitary conditions are poor and conducive to helminthes transmission.

Malaria parasitemia was detected in 12% of women and the only species found was P. *falciparum*. This is consistent with the findings of a survey involving 2,583 individuals in Dakar where P. *falciparum* was the only parasite observed in all age groups (1 to 80 years). Infection rates were greatest between October and December, the period that immediately follows the rainy season. The annual incidence of parasitemia in the sample was 5% (25). Women were enrolled in our study between February 2005 and March 2006, which covers the period of greatest infection rates. Although WHO defines severe malarial anemia as Hb concentration < 50 g/L in the presence of a normocytic

blood film and *P. falciparum* parasitemia > 10,000 parasites/μL of whole blood, in practice, severe malarial anemia is defined at the level of Hb concentration cited above, but in the presence of any *P. falciparum* parasitemia (25). We found that only 2 women in our sample had malarial anemia according to this definition. Malaria parasitemia more than doubled the risk of anemia after controlling for other causes. Currently, 91% of women who visit health centers receive a prescription to purchase antimalarial drugs; however, only 8% receive the very effective intermittent preventive treatment (Fansidar) during the prenatal visit (11). As a result of untreated mild forms of malaria in malaria endemic areas, adult women become semi-immune to the disease so that many malarial infections are asymptomatic. However, these forms of infection can contribute to mild to severe maternal anemia depending on the level of acquired immunity prior to pregnancy and the efficacy of immune responses during pregnancy; and this anemia attributable to malaria leads to negative perinatal outcomes (26). Much improvement is therefore needed in the delivery of antimalarial chemoprophylaxis in health centers.

We chose to investigate the Hb variant form that would be most common in the population that was studied, Hb S. In Senegal, the Hb S gene frequency is > 0.02 (27). For the homozygous form, Hb SS, the amino acid mutation in Hb causes Hb S to aggregate into filaments when deoxygenated. These filaments are of sufficient size and stiffness to give red blood cells an irregular crescent-like shape (or sickled shape) that causes vaso-occlusion and their destruction, hence the term sickle cell anemia. The heterozygous state for sickle cell, Hb AS, often results in a milder form of anemia (28). We found that 6.5% of the women in our sample had Hb AS, which more than quadrupled the risk of anemia. Careful screening of women should be done at the time of

the prenatal visit. A simple sodium metabisulfite test can be performed. The test is currently performed on only 79% of women who seek prenatal care (11).

Recommendations

Since iron deficiency was very prevalent in this sample and was the strongest risk factor for anemia among the factors studied, we recommend that public health efforts to control anemia in Senegal include two phases during the prenatal visit: 1) the education of pregnant women about making better food choices to limit the consumption of iron absorption inhibitors and 2) an effective iron supplementation program. The current program which consists of writing a prescription for women to purchase supplements has not been evaluated.

Since helminthes infection was not prevalent and was not a significant risk factor for anemia, systematic anthelminthic therapy may not be necessary for this population. However, we do recommend that women who reside in areas where the prevalence of infection is generally high to undergo stool analysis. The analysis is available at almost all health center laboratories. A single course of anthelminthic therapy coupled with iron/folic acid supplementation can dramatically improve hemoglobin and iron status (29). Malarial chemoprophylaxis should be made available to all pregnant women. Efforts should be made to significantly increase the proportion of women who receive the actual treatment instead of a prescription to purchase it.

TABLES

Table 1: Characteristics of the study sample of Senegalese pregnant women

Age, y	
<20	14.0
20-29	55.2
≥30	30.8
Parity	
0	34.9
1-2	40.3
3-4	18.5
≥5	6.3
Age of the last child (months)	
<24	13.7
24-48	34.1
≥49	14.8

Table 2: Anemia and iron status indicators in the sample of Senegalese pregnant women

Hematological status	%	Mean (SE)	Mean (SE)	Mean (SE)
Normal (Hb≥110)	61	121.1 (.5)	81.2 (2.9)[1]	37.5 (2.4)[4]
Anemic (Hb<110)	26	101.6 (.4)	110.7 (5.5)[2]	29.5 (3.3)[5]
Moderately to severely anemic (Hb<90)	6	85.6 (.5)	205.4 (22.6)	11.4 (2.3)
Severely anemic (Hb<80)	7	68.0 (1.9)	271.6 (26.5)[3]	15.9 (3.9)

[1]Significantly different from anemia, moderate to severe, & severe anemia, ANOVA, P<.05
[2]Significantly different from moderate to severe & severe anemia, ANOVA, P<.05
[3]Not significantly different from moderate to severe anemia, ANOVA
[4]Significantly different from moderate to severe & severe anemia, ANOVA, P<.05
[5]Significantly different from moderate to severe anemia, ANOVA, P<.05

Table 3: Prevalence of iron deficiency in the sample of Senegalese pregnant women

EP, µmol/mol heme (n)	%	Mean (SE)	SF, µg/L (n)	%	Mean (SE)
≤70 (167)	36.9	42.3 (1.3)	≥15 (277)	59.8	48.2 (2.6)
>70 (285)	63.1	149.1 (5.1)	<15 (186)	40.2	8.3 (.3)

Table 4: Dietary intake of heme and non-heme iron (Fe), iron absorption enhancers and inhibitors in the sample of Senegalese pregnant women

Frequency of consumption	Heme-Fe N = 480				Non-heme Fe N = 480				Fe absorption Enhancers N = 480				Fe absorption Inhibitors N = 480			
	%[1]	Hb[2]	EP[3]	SF[4]	%	Hb	EP	SF	%	Hb	EP	SF	%	Hb	EP	SF
Few times/ month	26	108.1 (1.6)	115.0 (7.8)	29.8 (3.2)	44.8	108.0 (1.2)	113.2 (6.4)	29.5 (2.3)	3.8	104.9 (5.4)	133.2 (2.9)	30.4 (8.4)	0	--	--	--
Several times/ month	38.8	109.5 (1.3)	107.9 (5.9)	42.5 (3.2)	29.8	110.8 (1.7)	109.7 (8.5)	35.5 (4.4)	61.8	110.6 (1.0)	108.1 (5.1)	32.0 (2.4)	60	111.6[5] (1.1)	106.1 (5.4)	43.5 (3.2)
Several times/ day	35.2	111.3 (1.5)	108.0 (7.8)	36.7 (2.9)	25.5	111.9 (1.5)	103.9 (6.3)	31.8 (2.8)	34.3	108.9 (1.5)	110.1 (7.3)	32.0 (2.9)	40	107.7 (1.3)	115.8 (6.3)	34.9 (2.1)

[1]Percent sample (N = 480)
[2]Mean hemoglobin (g/L) (standard error)
[3]Mean erythrocyte protoporphyrin (µmol/mol heme) (standard error)
[4]Mean serum ferritin (µg/L) (standard error)
[5]Significantly different, Student t test, $P<.05$

2. Providing free iron/folic acid tablets improves compliance in pregnant women in Senegal

Abstract

Iron (Fe) deficiency and anemia during pregnancy remains highly prevalent in Senegal because of low compliance with Fe supplementation. Improving women's access to supplements may increase compliance. Six prenatal centers in Dakar were randomly assigned to either a control group in which women received routine prenatal visits, including prescriptions to purchase Fe/folic acid tablets (IFA) according to the guidelines of the current Senegalese supplementation program (n = 112); or to an intervention group in which women received free IFA (n = 109) in addition to routine prenatal care. Compliance was assessed 20 weeks after enrollment by pill count and interviews. Hemoglobin, erythrocyte protoporphyrin, and serum ferritin were measured at baseline and follow-up. Compliance was 48% and 86% in the control and intervention groups, respectively (P<.001). After adjustment for confounding, prevalence of anemia was 62% among controls versus 31% among interventions (P<.01); prevalence of Fe deficiency was 84.5% and 56.9% in the control and intervention groups, respectively (P<.01). Improving access to IFA for pregnant women visiting health centers could dramatically increase compliance, improve Fe status and decrease the incidence of anemia.

Introduction

Anemia among pregnant women continues to be a major public health problem in West Africa. The World Health Organization (WHO) estimates that it affects 47% of women in that region (1). In Senegal, the prevalence of anemia is estimated at >50% (2-

4). Anemia during pregnancy and specifically iron deficiency anemia (IDA) during the first and second trimesters of gestation has been linked to increased risk of low birth weight and preterm delivery, which are the strongest predictors of perinatal mortality. Severe anemia during pregnancy also increases the risk of maternal mortality (5-6). The etiology of anemia is often multifold in West Africa; however, more than half of the cases are generally due to iron deficiency (ID) (7).

Iron (Fe) supplementation of pregnant women who visit prenatal care clinics is one of the most widely used strategies to combat anemia and IDA in West Africa, including in Senegal (8). In the early 1990's, the Senegalese Ministry of Health implemented a program of universal Fe/folic acid (IFA) supplementation by which pregnant women who visit any public health center for prenatal care are given a written prescription to purchase tablets containing 65 mg of elemental Fe (200 mg ferrous sulphate) and 250 µg of folic acid for the remainder of their pregnancy. The tablets are generally available at the health center pharmacies for a subsidized price of about 1 cent for 10 tablets. However, although clinical trials have repeatedly shown that Fe supplementation increases pregnant women's Fe stores and hemoglobin (Hb) concentrations (9), this type of community-based supplementation program have generally failed to show evidence of improved Fe status in large part due to low compliance. Studies have suggested that among other factors that affect compliance, inadequate access, supply, and distribution of supplements are major ones (10-12).

We hypothesized that if pregnant women attending health centers were given a free supply of IFA during the prenatal visit instead of receiving a prescription to purchase tablets, i.e. if their access to supplements was improved, compliance would also increase.

Thus, the objective of this study was to compare the effectiveness of free IFA versus a prescription at increasing compliance, therefore improving Fe status and decreasing the prevalence of anemia among pregnant women.

Subjects and methods

Study area and population

The study was conducted in Dakar, the capital city of Senegal, where 22% of the total population and 80% of the urban population resides. The population rate of growth is estimated at 3%, which is fairly high in part due to the high fecundity rate. Senegalese women have on average 6 children over the course of their reproductive years. Senegal has an estimated prevalence of low birth weight of 15%, an infant mortality rate of 58/1,000, and a maternal mortality rate of 560/100,000. Eighty seven percent of women aged 20-34 years seek antenatal care across Senegal, 98% of women in Dakar do. Most of this care is provided in government-run health centers by midwives. The centers cater mostly to low-income women at a lower cost than hospitals and at a much lower cost than private clinics (13).

For this study we chose the 6 reference centers (Gaspard Kamara, Philippe Senghor, Institute of Social Hygiene, Center for Maternal and Infant Protection, Dominique, and Roi Baudouin) of the 11 health centers in Dakar, which cover a population of about 180,000 individuals each. The centers are designated as "reference" because they are larger than the others, they have hospitalization beds and surgical units, and they train future health care workers. The first 4 centers are located in the inner city

and primarily cater to residents around them while the last 2 are located in suburban low-income areas and cater to residents of those areas.

Each health center was randomly assigned to either the control group or the intervention group using a computerized random number generator. In the control centers (Philippe Senghor, Roi Baudoin, Institute of Social Hygiene), women were offered the routine prenatal visit at the end of which a prescription to purchase IFA [200 mg ferrous sulphate (65 mg elemental Fe) and 250 µg folic acid] was given. As is routine, midwives instructed women to purchase as many tablets as they could after their first visit and to refill the prescription as necessary until their follow-up visit, at which time they were given another prescription; the time period between the first visit and the second varied depending on the stage of gestation. In the intervention centers (Gaspard Kamara, Dominique, Center for Maternal and Infant Protection), women received the routine prenatal visit at the end of which the midwife gave them a free supply of tablets with instructions to take one tablet per day until the end of their pregnancy. These tablets were provided by the researchers who purchased them from the National Pharmacy, which supplies the same tablets to the health center pharmacies where they are eventually purchased by patients. The tablets are produced in India and provided by the United Nations Children's Fund (UNICEF). They were pre-packaged in plastic bags; each patient was given the number of tablets sufficient until the date of follow-up and was asked not to discard empty tablet packages.

All women who registered for prenatal care and who fulfilled the following criteria were invited to participate in the study after giving informed consent: 1) residing in Dakar or in its suburbs, 2) not having used IFA supplements prior to enrollment, 3) being at the

beginning of the 2nd trimester of gestation (as estimated by fundal height and date of last menstrual period), 4) and being apparently healthy. We started recruitment at a control center where we remained until all subjects were enrolled and then moved on to a treatment center. We alternated between control and intervention centers until all subjects were enrolled; the average time spent at each center was 8 weeks.

This study was reviewed and approved by the Committee on Ethics of the Senegalese Ministry of Health and by the Institutional Review Board of the University of Maryland.

Compliance

Twenty weeks after enrollment, study participants were contacted by telephone for follow-up. We chose this mean of communication because most women could not provide an exact street address, as is common in low-income areas of Dakar. They were asked to provide a phone number where they could be reached, whether it was a home or cellular phone number, their husband's cellular phone number or neighbors' and relatives' phone numbers. An average of three attempts was made to contact the participants; once they were reached, they were given an appointment date and time to return to the health center for follow-up. For women who did not come to their first appointment, two attempts were subsequently made to schedule another appointment if needed.

Women in the intervention group were asked to bring the bag of supplements they were given at enrollment, including the empty tablet packages. The number of tablets left in the bag was counted. In addition, women were asked whether they actually ingested the missing tablets; this was to ensure that the tablets had not been given away to friends or

relatives or lost. According to their answers, the "number of tablets ingested" was calculated. Compliance was calculated as (Number of tablets ingested ÷ Number of days elapsed since enrollment) x 100.

At follow-up, women in the control group were asked how many tablets they had purchased since they were given the prescription at their first prenatal visit. They were asked how many tablets they had actually ingested and how many tablets remained in their supply. Compliance was calculated as (Number of tablets ingested ÷ Number of days elapsed since enrollment) x 100.

After follow-up, women in both groups were given IFA sufficient for daily supplementation until at least 3 months postpartum.

Hemoglobin (Hb), Erythrocyte protoporphyrin (EP), Serum ferritin (SF), Serum transferrin receptor (Tfr), and C-reactive protein (CRP) concentrations

Venous blood samples were collected in EDTA-treated vacutainer tubes at enrollment then at follow-up, 20 weeks later. Hb concentration was determined using the HemoCue photometer (HemoCue, Angelhölm, Sweden); each sample was analyzed in duplicates and calibration of the photometer was checked daily using the control cuvette provided by the manufacturer. Anemia was defined as Hb < 105 g/L in the second trimester and Hb < 110 g/L in the third trimester of gestation according to CDC cutoffs (14).

EP was measured, in duplicate, at enrollment and follow-up with a portable front-face hematofluorometer (Aviv Biomedical, Lakewood, NJ). Calibration of the hematofluorometer was checked daily using control solutions provided by the manufacturer. EP increases when Fe is unavailable for erythropoiesis. EP values

indicative of ID were defined as EP >70 μmol/mol heme, which is the cutoff that has been suggested by WHO (15).

Venous blood was also collected in evacuated non-treated tubes for SF analysis at enrollment and follow-up. After obtaining the blood, the samples were packaged in dry ice for transport from the field to a laboratory where they were centrifuged (481 x g, 8 min, 28ºC). The resulting serum was then frozen at -20 ºC. Later, it was thawed in a refrigerator (2-8ºC) for 12 hours before being analyzed for SF concentration by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies using a kit and the Mini-Vidas (Biomerieux, France). A SF concentration <15 μg/L was indicative of ID (15). Tfr was measured by ELISA in duplicate (Ramco Laboratories, Inc, Houston). A best-fit straight line was generated by regression using the standards provided by the manufacturer. A Tfr concentration >8.3 μg/L was indicative of iron-deficient erythropoiesis (16).

C-reactive protein (CRP) was measured at enrollment from the serum by latex-enhanced immunonephelometry on a BN II Analyzer (Dade Behring, Newark, DE). In research studies such as the one proposed here, CRP is widely used as the marker of infection; the cutoff value of > 6 mg/L was used to indicate the presence of infection or inflammation (17), which can affect Hb, EP, and SF concentrations.

Baseline characteristics

Structured questionnaires were administered to each subject to gather socio-demographic data (such as age, ethnic group, years of education, money spent on food), medical, and obstetric history. The questionnaires were pre-tested for face validity on 10 women in each health center and all necessary changes were made prior to actual data collection.

91

Statistical Analysis

We estimated that there would be at least a 15% relative difference in the prevalence of anemia between the control and intervention groups at follow-up. Therefore, on the basis of two independent samples with a two-sided chi-square test, a significance level of 0.05, and a β-error specification of 0.20, 183 subjects were needed per group in order to detect a 15% relative difference. Thirty percent was added in anticipation for loss to follow-up.

The demographic, health, and dietary questionnaires were coded where appropriate and analyzed using SPSS (version 15.0; SPSS Inc, Chicago). To correct data entry errors, we checked frequency analyses for all the variables as well as variable ranges. The primary outcomes of this study were the following: 1) comparison of the prevalence of anemia at follow-up in the control and intervention groups, 2) comparison of the prevalence of ID (EP>70 μmol/mol heme or SF <15μg/L or Tfr>8.3μg/L) at follow-up in the control and intervention groups, 3) comparisons of mean Hb, EP, Tfr, and SF in the control and intervention groups at follow-up, 4) comparison of compliance between the control and intervention regimen groups. The statistical methods included: chi-square test of homogeneity to compare the proportions of anemia and ID, and compliance; and the McNemar test was performed separately on the control and intervention groups to assess the significance of change in the proportions from baseline to follow-up; Student t test and analysis of variance (ANOVA) to compare means, Dunnett's C test for multiple comparisons (for samples of unequal variance), and paired sample t test to test the significance of mean differences between baseline and follow-up were also used. The Kruskal-Wallis for k independent-samples test was used to evaluate differences in socioeconomic status (i.e. percent literacy, home ownership, and employment) across the

6 health centers. Variables were tested for normality of distribution and where appropriate log transformations were used for parametric testing. Multivariate analysis of covariance (MANCOVA) was used to test the confounding effects of maternal age, stage of gestation at follow-up, parity, gravida, baseline Hb, EP, SF, Tfr, and CRP to generate the adjusted mean Hb, EP, SF, and Tfr mean differences between baseline and follow-up. Statistical significance was set at $P<0.05$ for all analyses.

Results

Participation

Of the 480 women enrolled at baseline, 221 (46%) completed the study; 112 (47%) in the control group and 109 (45%) in the intervention group (**Figure 1**). The only differences between women who remained in the study and those who were lost to follow-up were in maternal age, stage of gestation, and number of years of formal education. The sample size that was needed to detect a 15% relative difference in the prevalence of anemia between the two groups was 366 (183 per group); however, we actually found a >30% relative difference (results below), which would have required a much smaller sample size of 44 per group. Thus, our analyses were fully powered despite dropout. The main reasons for loss to follow-up in both groups were: not being able to be reached by telephone, delivering before the 20-week follow-up appointment, and refusing to come to follow-up. Women with complete data had a significantly higher number of years of education and were slightly, but significantly older than women with incomplete data. Women with complete data were at a slightly, but significantly less advanced stage of gestation (**Table 1**).

93

Socio-demographic characteristics and hematological status at baseline

Most of the women who had complete data were married (93.5%), 74% were illiterate (could not read or write), 56% were unemployed. Seventy six percent reported never having been diagnosed with a major illness, such as HIV/AIDS, cancer, or diabetes (the rest of the sample reported conditions such as asthma, chronic heartburn and migraines); 51% described their state of health at the time of enrollment as either "good" or "excellent". There was no significant difference in mean age, parity, gravida, stage of gestation, weight, or height between the control and intervention groups at baseline. However, mean Hb concentration in the intervention group was significantly higher (**Table 1**). The prevalence of anemia and ID (EP >70 µmol/mol heme, SF <15 µg/L, or Tfr>8.3µg/L) at baseline were not significantly different between the two groups.

Since the unit of randomization in this study was the health center instead of the individuals, descriptive characteristics at baseline were compared across the 6 health centers to test for large variations between health centers, which could have introduced a bias in subsequent analyses. Mean parity and gravida were significantly higher at one control center compared to an intervention center. Height was significantly higher in one intervention center compared to a control center. The proportions of women employed were significantly higher at two control centers compared to an intervention center (**Table 2**). From this analysis, we concluded that the clustering effect in this study is minimal.

Compliance and hematological outcome

Compliance was 67.1% (SE 2.3) in the overall sample; 48.5% (2.9) and 86.2% (2.4) in the control and intervention groups, respectively (P<0.0001).

Hematological status and change was analyzed both within and between the control and intervention groups at follow-up.

Within group analysis: paired sample t tests showed that mean Hb significantly decreased in the prescription group (control), but increased in the free IFA group (intervention) compared to baseline concentrations. Mean EP significantly increased in the control group while decreasing in the intervention group. Mean Tfr significantly increased in the control group; however it did not change significantly in the intervention group. Mean SF decreased in both groups; however the decrease was only significant in the control group. We compared the proportions of women who were anemic and those who were ID at baseline and follow-up within each group using the McNemar test of the significance of change. At follow-up, the proportion of women with anemia increased significantly only within the control group (37% versus 62%; P<0.001). The proportion of women with ID increased significantly from baseline to follow-up in the control group, going from 68.5% to 84.5% (P=0.000). In the intervention group, the proportion of iron deficient women decreased significantly from baseline to follow-up, from 69.8% to 56.9% (P=0.003) (**Table 3**).

Between group analysis: Independent Student t tests showed that mean Hb was significantly higher in the intervention group compared to the control group after 20 weeks of supplementation [115.9 (Std. error 2.2) versus 99.8 (2.2); P<0.0001] and mean EP was significantly lower in the intervention than in the control group [96.5 (8.5) versus

145.3 (9.4); P<0.0001]. Mean SF was higher in the intervention group; however, the difference was not significant. Mean Tfr was not significantly different between the two groups. The proportion of women who were anemic was significantly lower in the free IFA group (31% versus 62%; P<0.0001). The same trend was observed for the proportion of women who were ID deficient (84.5% versus 56.9%; P<0.0001) (**Table 3**). To control for confounding, a multivariate analysis of covariance (MANCOVA) was run where Hb, EP, Tfr, and SF concentrations at follow-up were entered as dependent variables and the following were entered as covariates: Hb, EP, SF, Tfr, and CRP concentrations at baseline, stage of gestation at baseline and follow-up, parity, and gradiva. We found that only Hb, EP, SF, Tfr, and CRP at baseline were significant covariates (P <0.05). After adjustment, the mean difference in Hb concentration at follow-up between the control and the treatment groups remained significant (P = 0.015) as well as the mean difference in EP concentration at follow-up (P = 0.037).

Discussion

To our knowledge, this is the first study that tested the assumption that pregnant women's poor access to iron supplements negatively affects compliance. We improved access to tablets by providing them for free during women's prenatal visits at health centers in Dakar, Senegal. We found that women who received free IFA had compliance of 86% compared to only 48% for women who received prescriptions to purchase tablets. The differential in compliance was corroborated by the hematological profiles in each group. The prevalence of ID in the prescription group increased from 68% at baseline to 84% at follow-up. In the free IFA group, the prevalence of ID significantly decreased

from 70% at baseline to 57% at follow-up. Furthermore, the prevalence of anemia in the prescription group increased from 37 to 62%, but did not significantly change in the free IFA group. As expected, we also found that Hb and SF concentrations were significantly higher at follow-up in the free IFA group and EP concentration was significantly lower. This analysis was tested for the potentially confounding effects of maternal age, parity, gravida, CRP, gestational age at follow-up (this was important since women entered the study and were seen for follow-up at different stages of gestation), and most importantly baseline Hb, EP, Tfr, and SF concentrations. After adjustment for significant covariates, mean differences remained significant for Hb and EP. This study did not address the issue of "efficacy" of supplementation, which is the "extent to which a specific intervention, procedure, regimen, or service produces a beneficial effect under ideal conditions" (19-20). Efficacy of IFA tablets has been demonstrated in many studies conducted in West Africa and elsewhere (9, 21). We wanted to address the issue of "effectiveness", which is defined as "the extent to which a specific intervention, procedure, regimen, or service, when deployed in the field, does what it is intended to do for a defined population" (20). Therefore, beyond giving the treatment group free IFA tablets, no attempt was made to influence behavior by supervising tablet intake. We wanted to determine whether giving a free supply of IFA instead of a prescription would increase compliance and translate into improved Fe status and decreased incidence of anemia.

The overall prevalence of anemia at baseline (30%) was relatively low compared to the >50% prevalence that has been reported elsewhere (3-4). It is unclear at which stage of gestation this prevalence was determined, whereas in our study women were just

entering the second trimester of gestation when iron needs are not as high as later in pregnancy. The follow-up measurements were performed 20 weeks after enrollment when women were only a few weeks from delivery because we wanted to see whether the strategy of giving free tablets was beneficial when risk of ID and anemia is highest during pregnancy (4).

The units of randomization to the two supplementation groups in this study were the health centers instead of the women because we wanted to minimize bias and confounding: we avoided the risk of women in the control and intervention groups communicating among themselves or sharing IFA tablets. Since only 6 centers were included in this study, any data analyses at the cluster level would not have had enough statistical power; therefore statistical analyses were conducted at the individual level. However, we did compare subject characteristics at baseline across health centers to ascertain whether there were large differences between the health centers. The direction of the few differences we found suggested that any clustering effect that may have introduced bias was minimal.

We had an unexpectedly high dropout rate >50% mainly due to the fact that the population we were studying was highly mobile, making the task of contacting women for follow-up visits very difficult. We had calculated the required sample size to detect a 15% relative difference in the prevalence of anemia between the groups and then added 30% to the sample to account for loss to follow-up. The actual difference in prevalence was far greater than 15%, thus the high dropout rate did not affect our primary outcome. We were conservative in our estimate of a 15% difference in prevalence because we were aware of no studies of this type that have previously been conducted in Senegal. The

high dropout rate did not introduce significant selection bias in this study. By comparing the baseline characteristics of women who remained in the study and those who were lost, we found only slight differences in age and stage of gestation. Hematological status (Hb, EP, and SF), socio-demographic characteristics, and pregnancy factors (parity and gravida) were not significantly different between the groups. One possible concern is that women who remained in the study had a significantly higher number of years of formal education than those who were lost to follow-up, i.e. 4 years versus 2 years; however, the fact that the number of years of education was relatively small in both groups minimizes bias. In addition, the two groups were similar in all other aspects of socioeconomic status we assessed.

Conclusions

Increasing access to IFA tablets by providing them for free to pregnant women in Dakar during prenatal visits increased compliance and iron/anemia status dramatically. The Senegalese supplementation program could be more effective if women received free IFA instead of prescriptions to purchase the tablets. The effectiveness of supplementation programs depends greatly on women's compliance. Understanding the factors that influence compliance is therefore paramount to designing and implementing successful programs. Data on the factors that influenced compliance of women in this study were collected through in-depth interviews with each participant and are presented below.

The Senegalese government's ability to provide free tablets to women will depend on cost. Even though IFA tablets are provided by UNICEF through the Poverty Reduction

Funds, the government does incur operating costs for receiving, warehousing, and distributing the tablets to the various health centers, which justifies why pregnant women have to pay for the tablets. It is therefore necessary to conduct cost-benefit analyses to determine whether a strategy of free tablets is more beneficial and whether the resources allotted to Senegal by UNICEF (i.e. IFA tablets) are being used efficiently. The cost of treating pregnant women for severe anemia (requires large doses of Fe) which occurs when IFA tablets are not taken regularly during the course of pregnancy, and the cost of managing negative pregnancy outcomes resulting from anemia of pregnancy, such as preterm birth, low birth weight, anemia in infancy is most likely much higher than the cost of providing free tablets at the prenatal visit. In a study of the cost-effectiveness of iron supplementation on disability adjusted life years (DALY) in 4 sub-regions of the world, Baltussen et al (21) estimated that iron supplementation would avert almost 2.5 million DALYs in African sub-regions with very high adult and child mortality rates. The estimation of cost-effectiveness included the costs of health care for pregnant women with provision of iron supplements at 4 prenatal visits. The cost-effectiveness of iron supplementation was $30 per DALY averted, which represents < 3 times the GDP per capita in the African sub-regions that were studied; this is consistent with the Commission on Macroeconomics and Health's recent definition of interventions that are cost-effective. The lessons learned in Senegal could be applied widely to other sub-Saharan African countries in the region, which have similar supplementation programs.

TABLES & FIGURE

Figure 1: Participation and loss to follow-up

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                    ┌─────────────────────────┐
                    │  6 Health centers randomly│
                    │        assigned          │
                    └─────────────────────────┘
                       ╱                    ╲
          ┌────────────────────┐   ┌────────────────────────┐
          │  3 Health centers   │   │   3 Health centers      │
          │ Prescription (control)│ │ Free tablets (Intervention)│
          │     (n = 240)       │   │      (n = 240)          │
          └────────────────────┘   └────────────────────────┘
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Lost to follow-up (n = 128)
- went out of town/country (n = 17)
- delivered before 20 wks (n = 18)
- spontaneous abortion (n = 6)
- could not be reached (n = 71)
- did not give blood samples (n = 3)
- did not want to come to follow-up (n = 12)
- was too ill to come to follow-up (n = 1)

Lost to follow-up (n = 131)
- went out of town/country (n = 12)
- delivered before 20 wks (n = 3)
- spontaneous abortion (n = 3)
- could not be reached (n = 73)
- did not give blood samples (n = 10)
- did not want to come to follow-up (n = 27)
- were too ill to come to follow-up (n = 3)

Final sample (n = 112)

Final sample (n = 109)

101

Table 1: Baseline characteristics of women with complete and incomplete data in the study of compliance with iron/folic acid supplementation among pregnant women

	Complete			Incomplete		
	All (n = 221) mean ±SEM	Prescription (n = 112)	Free IFA (n = 109)	All (n = 259)	Prescription (n = 147)	Free IFA (n = 150)
Age (yrs)	26.7 (.4)[1]	26.6 (.6)	27.3 (.6)	24.4 (.4)	24.3 (.6)	24.4 (.6)
Gravida (no.)	2.9 (.1)	3.2 (.2)	2.9 (.2)	2.8 (.2)	2.6 (.2)	2.9 (.2)
Parity (no.)	1.6 (.1)	1.8 (.2)	1.6 (.2)	1.5 (.1)	1.3 (.2)	1.6 (.2)
Gestation (wks)	11.8 (.3)[1]	11.8 (.4)	11.8 (.4)	13.3 (.4)	13.6 (.6)	13.0 (.6)
Weight	60.9 (.9)	60.4 (1.2)	61.4 (1.3)	58.9 (.9)	58.4 (1.3)	59.3 (1.3)
Height	1.6 (.003)	1.6 (.003)	1.6 (.004)	1.6 (.005)	1.6 (.007)	1.6 (.006)
[Hb][3] (g/L)	109.1 (1.2)	106.5 (1.9)[2]	111.8 (1.6)	108.3 (1.6)	109.5 (2.3)	107.0 (1.6)
[EP][4] (µmol/mol)	113.5 (6.4)	117.8 (10.5)	109.1 (7.2)	119.0 (7.9)	99.6 (9.4)	137.6 (12.3)
[SF][5] (µg/L)	32.9 (2.2)	31.7 (2.9)	34.1 (3.3)	28.1 (2.7)	28.1 (4.1)	28.1 (3.5)
Formal education (yrs)	4.4 (.3)[1]	4.2 (.4)	4.7	2.5 (.2)	2.4 (.4)	2.5 (.4)
% Employed	29.4	32.1	26.6	30.3	22.2	38.3
Money spent on food/day ($)	.96 (.08)	.95 (.08)	.99 (07)	.97 (.06)	.91 (.09)	1.0 (.13)

[1]Significantly different from incomplete (Student t test): P<0.05
[2]Significantly different from free IFA group (Student t test): P<0.05
[3]Hemoglobin concentration
[4]Erythrocyte protoporphyrin concentration
[5]Serum ferritin concentration

Table 2: Baseline characteristics of pregnant women across 6 control and intervention health centers in the study of compliance with iron/folic acid supplementation among pregnant women

	Control Centers (C)				Intervention Centers (I)		
	C1 (n = 32) mean ±SEM	C2 (n = 38)	C3 (n = 42)		I1 (n = 34)	I2 (n = 34)	I3 (n = 41)
Age (yrs)	25.6 (1.0)	25.8 (1.0)	27.8 (1.1)		27.6 (.9)	27.8 (.9)	25.6 (.8)
Gravida (no.)	2.7 (.3)	2.7 (.4)	3.9 (.4)[1]		3.6 (.3)[1]	2.6 (.3)	2.1 (.3)
Parity (no.)	1.5 (.3)	1.3 (.2)	2.2 (.3)[1]		2.2 (.3)[1]	1.3 (.2)	1 (.2)
Stage of Gestation (wks)	11.7 (.5)	11.9 (.8)	11.8 (.6)		11.6 (.9)	12.7 (.6)	11.4 (.5)
Weight (kg)	62.1 (2.5)	59.6 (1.7)	59.1 (1.9)		64.9 (2.6)	58.8 (1.6)	61.6 (2.2)
Height (m)	1.6 (.007)	1.6 (.008)	1.6 (.008)		1.6 (.009)	1.7 (.01)[2]	1.6 (.01)
Formal education (yrs)	5.0 (.6)	4.0 (.8)	3.7 (.6)		3.7 (.6)	3.8 (.7)	6.3 (.6)
% Employed	43.7[4]	36.8[5]	19.0[3]		41.2	8.8[3]	29.3
Money spent on food/day ($)	1.0 (.9)	1.0 (.1)	.8 (.1)		1.1 (.1)	.8 (.1)	1.1 (.1)

[1]Significantly different from center I3: Dunnett's C P<0.05
[2]Significantly different from center C2: Dunnett's C P<0.05
[3]Significantly different from centers I1 & I3: Kruskal-Wallis P<0.05
[4]Significantly different from centers I2 & C3: Kruskal-Wallis P<0.05
[5]Significantly different from center I2: Kruskal-Wallis P<0.05

Table 3: Hematological status at baseline and follow-up for women in the control and intervention groups in the study of compliance with iron/folic acid supplementation of pregnant women

	Prescription (n = 112)	Free IFA (n = 109)
Hemoglobin (g/L)		
Concentration at baseline	106.5 (1.9)[1]	111.8 (1.6)
% Anemic[2]	37	25.6
Concentration after 20 weeks	99.8 (2.2)	115.9 (2.2)[12]
% Anemic[3]	62[14]	31[15]
Change after 20 weeks	-6.4 (2.2)[4]	+4.1 (2.4)[5]
Erythrocyte protoporphyrin (µmol/mol heme)		
Concentration at baseline	117.8 (10.5)	109.1 (7.2)
Concentration after 20 weeks	145.3 (9.4)	96.5 (8.5)[13]
Change after 20 weeks	+26.8 (9.2)[6]	-13.7 (9.6)[7]
Serum ferritin (µg/L)		
Concentration at baseline	31.7 (2.9)	34.1 (3.3)
Concentration after 20 weeks	22.0 (2.3)	28.7 (2.9)
Change after 20 weeks	-8.9 (2.8)[8]	-6.1 (4.1)[9]
Serum transferrin receptor (µg/L)		
Concentration at baseline	5.58 (.39)	5.82 (.53)
Concentration after 20 weeks	6.65 (.47)	6.27 (.31)
Change after 20 weeks	+1.07[10]	+.45[11]
Iron deficiency[18]		
% Iron deficient at baseline	68.5	69.8
% Iron deficient after 20 weeks	84.5[16]	56.9[17]

[1]Mean (±SEM)

[2]Hemoglobin <105 g/L in 2nd trimester; [3]Hemoglobin <110 g/L

[4,5,6,7,8,9]Paired sample t test; [4]P=.004; [5]P=.135; [6]P=.004; [7]P=.176; [8]P=.002; [9]P=.144; [10]P= .006; [11]P=.299

[12,13]Significantly different from control group; [11]P<.0001; [12]P<.0001

[14,15,16,17]McNemar test; [14]P<.001; [15]P=.442; [16]P=.001; [17]P=.003

[18]Erythrocyte protoporphyrin>70µmol/mol heme or serum ferritin<15 µg/L or serum transferrin receptor>8.3 µg/L

3. Determinants of compliance with iron supplementation among pregnant women in Senegal

Abstract

Community-based iron supplementation programs for pregnant women have lacked effectiveness, in large part, because of low compliance. The objective of this study is to determine factors that influence compliance among pregnant women in Senegal. Pregnant women (N=221), recruited from 6 health centers in Dakar during their first prenatal visit, were randomly assigned to receive either a prescription to purchase iron/folic acid tablets (control, n = 112) to be taken daily, according to official policy or to receive free tablets (intervention, n = 109). Compliance was assessed 20 weeks after enrollment through interviews and pill count. Women with low compliance (<70%) and those with high compliance (≥70%) were asked to explain what influenced their adherence to supplementation. Overall compliance was 67%; It was significantly higher in the intervention than in the control group (86% versus 48%; P<0.0001). Women with high compliance (58%) were motivated by 1) the perception of improved health upon taking the tablets (Intervention = 24%; control = 10%), 2) the insistence by midwives that they take the tablets, and 3) the mention that the tablets would improve health. Women with low compliance (42%) indicated 1) the experience of side effects that they associated with the tablets (Intervention = 13%; control = 14%), 2) misunderstanding that they needed to continue taking the tablets throughout pregnancy (intervention = 0%; control = 18%), and 3) forgetfulness. Compliance with Fe/folic acid supplementation in Senegal can be increased by improving women's access to the tablets and by educating them on the health benefits of the tablets.

Introduction

The World Health Organization (WHO) estimates that anemia affects >47% of pregnant women in West Africa and that more than half of the cases are due to iron deficiency (ID) (1-2). In Senegal prevalence of anemia is even higher (>50%) (3-5). Anemia has adverse consequences including fatigue, decreased work capacity, and poor pregnancy outcomes such as preterm birth, low birth weight, and increased risk of maternal death both during delivery and during the postpartum period (6-7).

To combat ID and anemia, many West African countries, including Senegal, have implemented iron (Fe) supplementation programs for pregnant women (8). However, although clinical trials have repeatedly shown the efficacy of supplementation (9), most if not all community based programs have not been shown to be effective, i.e. they have not decreased the incidence and prevalence of anemia when deployed in the field. Studies conducted in South East Asia, Latin America, and in only a few African countries have shown that one of the main reasons why these programs have been less effective than anticipated is low compliance of women with taking daily Fe supplements. Low compliance has been associated with a number of factors, including: 1) gastrointestinal side effects that can occur with taking Fe, 2) inadequate supply of tablets (including limited resources to purchase tablets), 3) inadequate counseling of patients by health care providers concerning the utility of tablets and possible transient side effects, 4) poor utilization of prenatal health care services, 5) lack of knowledge and/or patient fears about the tablets, and 6) community beliefs, attitudes, and practices that affect women's perception regarding tablet use (10-13).

In Senegal, all women who seek prenatal care at government-run public health centers are given a written prescription by midwives to purchase Fe/folic acid tablets (IFA) that contain 65 mg of elemental Fe (200 mg ferrous sulphate) and 250 µg of folic acid. The tablets are sold at the health center pharmacies and cost about 1 US cent per tablet. The supplementation program in Senegal has been in place for nearly two decades; however, the overall prevalence of anemia among pregnant women who attend prenatal centers has not declined (4).

The present study is part of a larger study which sought to compare the effectiveness of the current Senegalese program to the effectiveness of a modified supplementation strategy whereby women would receive a free supply of IFA from the hands of the midwives during the prenatal visit instead of a prescription to purchase the tablets from the pharmacist. The hypothesis of the study was that the fact that women receive free tablets would eliminate several potential barriers to program success thereby increasing compliance. The results of the randomized controlled trial showed that women who received free tablets did indeed have a significantly higher compliance (86%) compared to women who received prescriptions (48%) (14).

The purpose of the present study is to determine and understand the specific factors that influence compliance with supplementation among pregnant women in Senegal; such understanding is crucial to the design and implementation of a successful and effective program to prevent and treat anemia.

Subjects and methods

Study area and population

The study was conducted in Dakar, the capital of Senegal, home to 22% and 80% of the total and urban populations, respectively. Senegalese women have a high fecundity rate of 6 children, which accounts in part for a population rate of growth of 3%. Low birth weight is prevalent (15%), infant and maternal mortality rates are high (58/1,000 and 560/100,000, respectively). 98% of women aged 20-34 years in Dakar seek antenatal care; the vast majority of them seek it in public health centers (15). This care is provided by licensed midwives who receive a three-year training course at government-accredited midwifery schools.

Six reference health centers of the 11 health centers of Dakar, which cover a population of about 180,000 individuals each (the vast majority of whom are residents of Dakar and its suburbs), were chosen to implement the study. "Reference" health centers are larger than other health centers, they are equipped to provide more sophisticated medical care, such as radiography and surgery, and they train future health care workers. The randomization procedures used in the larger study are described in detail elsewhere (14). Briefly, the health centers were randomly assigned to either dispense the routine prenatal visit services during which a prescription to purchase IFA (65 mg elemental Fe + 250 µg folic acid) was given to women (control group; n = 3 centers) with instructions to purchase as many tablets as they could afford after their first visit, to ingest one tablet per day, and to refill the prescription as necessary until their follow-up visit; or to dispense the routine prenatal visit during which the midwife gave women a free supply of tablets with instructions to take one tablet per day until the end of pregnancy (intervention group; n = 3 centers). The tablets were purchased from the National Pharmacy, which

108

supplies the health center pharmacies with tablets of the same appearance and composition for sale in pharmacies. The researchers pre-packaged the tablets in plastic bags which contained the number of tablets sufficient until the date of follow-up. Women in the intervention group were asked to keep empty tablet packages in the plastic bag that was provided.

All women who registered for prenatal care in the 6 study centers, who had not used Fe supplements prior to enrollment, who were at the beginning of the 2nd trimester of gestation (as determined by fundal height and date of last menstrual period), and who were apparently healthy, were invited to participate in the study after giving informed consent.

This study was reviewed and approved by the Committee on Ethics of the Senegalese Ministry of Health and by the Institutional Review Board of the University of Maryland.

Baseline characteristics

Demographic data (such as age, ethnic background, education) were gathered using structured questionnaires. Medical and obstetric histories were obtained through interviews and by consulting patient health center records when available. For face validity, all questionnaires were pre-tested on 10 non-study participants at each health center and all necessary changes were made prior to the start of actual data collection.

Compliance

Compliance was assessed 20 weeks after the date of enrollment. Women were contacted by telephone and given appointments to return to the health center where they had originally registered for prenatal care. This mean of communication was chosen because most women could not provide an exact street address, as is common in low-

income areas of Dakar. They were asked to provide a phone number where they could be reached; it could be their home or cellular phone number, their husband's cellular phone number or neighbors' and relatives'. On average, three attempts were made to contact the participants; once they were reached, they were given an appointment date and time to return to the health center for follow-up. For women who did not come to their first appointment, two subsequent attempts were made to schedule another appointment if needed.

During the follow-up visit, women in the control group were interviewed to determine the number of IFA they had purchased since their first prenatal visit and the number of tablets that remained in their supply. In addition, women were asked whether they had actually ingested the missing tablets to ensure that the tablets had not been given away to friends or relatives or lost. Compliance in this group was then calculated as: (Number of tablets ingested ÷ Number of days elapsed since enrollment x 100). It was assumed that the number of days that had elapsed between enrollment and follow-up corresponded to the number of tablets that the patient should have ingested.

Women in the intervention group were asked to bring the bag of supplements they were given at enrollment, including the empty tablet packages. The number of tablets left in the bag was determined and women were questioned about actual tablet ingestion. Compliance was calculated using the same formula as for the control group.

During the follow-up visit, each subject's compliance was computed and classified as either "low compliance", i.e. < 70% or "high compliance", i.e.≥ 70%. This classification was chosen on the basis of WHO's recommendation stating that in countries where the prevalence of anemia among pregnant women is ≥40% that supplementation be given for

6 months (24 weeks) of pregnancy, i.e. 168 tablets (16). For this study, participants were monitored over a period of 20 weeks during which the maximum number of tablets they would have ingested as directed would have been 140; 70% of 140 is 98 tablets, which is 83% of WHO recommended number. The remaining 28 tablets that women would have consumed if monitoring was continued over the full 24 weeks would account for about 100% compliance.

A semi-structured interview, which lasted 20 minutes on average, was then conducted with each subject. Women with low compliance were asked to identify the reason or reasons why they did not take the IFA as directed most of the time. Women with high compliance were asked to identify the factor or factors that motivated them to take the tablets as directed most of the time. All interviews were conducted in the major local language of Senegal, Wolof. The questions were open-ended and were asked in a non-threatening or accusatory manner. Women's answers were recorded and then transcribed word for word by the interviewer. For example, women in the study population often refer to anemia as "lacking blood"; if a woman answered "I took the tablets because I thought they would increase my blood", the interviewer wrote down the exact phrase. The translation and interpretation was done during data entry and analysis. Where women identified more than one factor that influenced their compliance, all factors were recorded; however they were asked to identify what they thought to be the strongest factor.

Hemoglobin (Hb), Erythrocyte protoporphyrin (EP), Serum ferritin (SF), Serum transferrin receptors (Tfr), and C-reactive protein (CRP) concentrations

Venous blood samples were collected at enrollment then at follow-up, 20 weeks later. Hb concentration was measured with the HemoCue photometer (HemoCue, Angelhölm, Sweden). Anemia was defined as Hb < 105 g/L and < 110 g/L in the second and third trimester of gestation, respectively (17). EP was measured at enrollment and follow-up with a portable front-face hematofluorometer (Aviv Biomedical, Lakewood, NJ). EP >70 μmol/mol heme, was considered abnormal (18). Venous blood was also collected for SF analysis at enrollment and follow-up. Serum samples were then obtained and analyzed for SF concentration by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies using a kit and the Mini-Vidas (Biomerieux, France). An SF concentration <15 μg/L was considered abnormal (19). Tfr was measured by ELISA in duplicate (Ramco Laboratories, Inc, Houston). A best-fit straight line was generated by regression using the standards provided by the manufacturer. A Tfr concentration >8.3 μg/L was indicative of iron-deficient erythropoiesis (20). ID was defined as EP >70 μmol/mol heme, SF <15 μg/L, or Tfr >8.3 μg/L.

Statistical Analysis

The questionnaires were coded and the data entered into SPSS (version 15.0; SPSS Inc, Chicago). Each compliance questionnaire was analyzed to determine the key reason why the subject had a low or high compliance and that reason was assigned an individual code; the frequency distribution of the different reasons were then calculated. To correct data entry errors, frequency analyses for all the variables were checked as well as variable ranges. Student t tests were used to compare means. Chi-square tests of

homogeneity were used to compare compliance between women who received prescriptions (control) and women who received free tablets (intervention) and to compare the proportions of anemia and ID between the two groups. To assess the significance of change in the proportion of women with anemia and women with ID from baseline to follow-up within the two groups, the McNemar test was performed. Statistical significance was set at $P<0.05$ for all analyses.

Results

Participation

Of the 480 women who participated in the study, 221 (46%) had complete information on compliance and were included in the analysis. The only differences between women who remained in the study and those who were lost to follow-up were in maternal age, stage of gestation, and number of years of formal education (see previous chapter). **Figure 1** shows the number of subjects recruited in each center.

Socio-demographic, health, and hematological characteristics

The mean age in the sample was 27±6 years. Most of the women were married (93.5%), 57% were illiterate. Seventy six percent reported never having been diagnosed with a major illness, such as HIV/AIDS, cancer, or diabetes (the rest of the sample reported illnesses such as asthma, chronic heartburn and migraines); 51% described their state of health at the time of enrollment as either "good" or "excellent". The 2 groups did not differ by age, parity, gravida, stage of gestation, weight, or height between the controls and the interventions at baseline. The women had achieved, on average, 4.4 ±4.2 years of formal education and 71% were not employed at the time of enrollment (**Table 1**). **Table 2** shows the sample's ethnic distribution.

Overall compliance and determinants

Overall, compliance in the control and intervention groups was 67.5% ±29 (n = 221); 42% of the women had compliance <70% (Mean = 42 ±21; n = 93) and 58% had compliance ≥70% (Mean = 88 ±13; n = 128). Among the compliant, the four main reasons for taking the tablets as directed most of the time were: 1) that they perceived a health benefit when they took the tablets and therefore continued taking them (i.e. alleviation of fatigue and dizziness and improved appetite) (32% of answers), 2) because the midwife directed them to take the tablets was a strong motivator to do so (19% of answers), 3) because the midwife specifically indicated that the tablets would improve their health (13% of answers), and 4) that they always take prescribed medications because they are fearful of illness (11% of answers). About 14% of the women stated that they knew that the tablets would "increase their blood" (remedy anemia) or prevent them from "losing blood" (prevent anemia), which is what motivated compliance (**Table 3**).

Among women who had low compliance (<70%), the five main reasons why they did not take the tablets as directed most of the time were: 1) that they experienced gastrointestinal side effects (i.e. one or a combination of nausea, vomiting, diarrhea, and constipation) (27% of answers), 2) that the midwife did not explain or they did not understand that they needed to purchase more tablets after their first prescription ran out (18% of answers) (this answer is only relevant for the control group), 3) that they forgot to take the tablets most days (17% of answers), 4) that they could not afford to purchase the tablets (13% of answers) (this answer is only relevant for the control group), 5) that

they were tired of having to take so many tablets (11% of answers). In 4% of the cases, the midwife should have but did not prescribe the tablets (**Table 4**).

Compliance and determinants by group assignment

Women who received free IFA (intervention) had a compliance of 86% compared to those who received a prescription (control) and had a compliance of 48%, a difference that was statistically significant (P <0.0001).

Control group

Of the 112 women in the control group, 42% had ≥70% compliance (Mean = 88 ±10.5; n = 54). The four main reasons why women took the tablets as directed most of the time were: 1) the fact that the midwife directed them to do so (24% of answers), 2) that they always take prescribed medications because they are fearful of illness (20% of answers), 3) that they perceived a health benefit when they took the tablets and therefore continued taking them (18% of answers), and 4) the fact that the midwife specifically indicated that the tablets would improve their health (17% of answers). About 10% of the women knew that the tablets would "increase their blood" (remedy anemia) or prevent them from "losing blood" (prevent anemia), which is what motivated compliance (**Table 3**).

Fifty eight percent of women in the control group had < 70% compliance (Mean = 36 ±21; n = 58). The four main reasons why they did not take the tablets as directed most of the time were: 1) that the midwife did not explain or they did not understand that they needed to purchase more tablets after their first prescription ran out (28% of answers), 2) that they experienced gastrointestinal side effects (22% of answers), 3) that they could not afford to purchase their prescription (21% of answers), and 4) that they forgot to take

the tablets most days (12% of answers). In 3% of the cases, the midwife should have but did not prescribe the tablets (**Table 4**).

Intervention group

Of the 109 women in the intervention group, 68% had ≥70% compliance (Mean = 88 ±15; n = 74). The three main reasons why women took the tablets as directed most of the time were: 1) that they perceived a health benefit when they took the tablets and therefore continued taking them (42% of answers), 2) the fact that the midwife directed them to do so (15% of answers), 3) the fact that the midwife specifically indicated that the tablets would improve their health (11% of answers). About 18% of the women knew that the tablets would "increase their blood" (remedy anemia) or prevent them from "losing blood" (prevent anemia), which is what motivated compliance; 8% of the women felt that the tablets had to be important since they were given to them during the prenatal visit (**Table 3**).

Thirty two percent of women in the intervention group had < 70% compliance (Mean = 51 ±19; n = 35). The three main reasons why they did not take the tablets as directed most of the time were: 1) that they experienced gastrointestinal side effects (34% of answers), 2) that they forgot to take the tablets most days (26% of answers), and 3) that they grew tired of taking the tablets (23% of answers). In 19% of cases either the midwife did not give the free tablets to the women or the women themselves lost them or gave them away to friends and relatives (**Table 4**).

Prevalence of anemia and ID in the control and intervention groups at follow-up

Twenty weeks after enrollment, the proportion of women with anemia significantly increased among women who received a prescription (control) (37% versus 62%;

P<0.001), but did not increase significantly among women who received free tablets (intervention) (26% versus 31%; P=0.442). The proportion of women who were Fe deficient significantly increased in the control group (68.5% versus 84.5%; P=0.000) but decreased in the intervention group (69.8% versus 56.9%; P=0.003) (**Table 5**). The prevalence of anemia and iron deficiency was significantly higher among women who received prescriptions compared to those who received free IFA. Mean differences in Hb and EP concentrations remained significant after control for baseline Hb, EP, SF, Tfr, and C-reactive protein, and baseline and follow-up stage of gestation in a multivariate analysis of covariance.

Discussion

This study is the first attempt to understand the factors that influence adherence to iron supplementation among pregnant women in Senegal. It is also one of the few studies that examine the specific factors that facilitate high compliance with supplementation in a community environment. The study sample was drawn from six different health centers that each cater to a large group of low-income women residing in different neighborhoods of Dakar. The ethnic distribution of the sample is similar to that of the general population of Senegal (15). The sample size was large compared to other studies of this kind.

Compliance with supplementation in the entire sample was 67%; however, women who received IFA during the prenatal visit had significantly higher compliance than women who received a prescription, i.e. 86% compared to 46%. The overall compliance of 67% is relatively high compared to compliance previously reported for pregnant women in developing countries (11-12, 21-24).

In the overall sample, women with the highest compliance (≥70%) were motivated by four major factors which accounted for 75% of the responses: 1) the perception of improved health upon taking the tablets, 2) the admonishment from midwives to take the tablets, 3) the specific mention by midwives that the tablets would improve their health, and 4) the fear of becoming ill.

The first factor in the overall sample was also the single most important motivator among women who received free tablets during the prenatal visit (42% of answers) but was only the third motivating factor among women who received prescriptions (18% of answers). This strongly suggests that dispensing the tablets during the visit increases the likelihood that women will start IFA treatment rapidly and will continue long enough to reap health benefits, which in turn may motivate them to continue the treatment. For women who are given written prescriptions and directed to purchase the tablets, some barriers may prevent or delay them from complying, one of which is financial: a significant proportion (21%) of women who had the lowest compliance in the control group indicated that they could not afford to purchase their prescriptions. Furthermore, a significant proportion of women in the same group indicated that they did not purchase and take more tablets after their first prescription ran out because they did not know that they were supposed to do so. This problem could not have occurred among women who received free tablets since they were given a supply for the remainder of their pregnancy; thus, the risk of an interruption of treatment in this group was decreased.

The second and third factors in the overall sample suggest that the quality of counseling during the prenatal visit is a very important determinant of compliance. The combination of midwives telling women to take the tablets and of midwives specifically explaining to

them that the tablets will improve health accounted for 32% of responses. Conversely, we found that among women who had the lowest compliance, 18% either misunderstood the directions given by the midwife or the midwife's directions were unclear. Inadequate counseling by health care providers has been documented by Galloway et al (11-12) as a major barrier to compliance in eight other developing countries and by Oluwatoyin in Nigeria (12). It was the single most important reason for low compliance with supplementation as directed (28% of answers) among women who only received prescriptions; whereas among women who received free tablets but had low compliance, inadequate counseling was not a factor. Researchers in Mali also found a strong link between the quality of counseling by health care providers and compliance. They reported that pregnant women complied with supplementation when they were provided with "minimum, consistent, and easily understandable information and counseling" (25). Results from the current study suggest that giving the tablets to women during the prenatal visit may encourage the midwives to clearly explain their purpose; this is further supported by the fact that 18% of women who had the highest compliance in the intervention group indicated that they took the tablets as directed most of the time because they knew their specific function, i.e. they knew that the tablets would either prevent them from becoming anemic or would treat their existent anemia. This knowledge was apparent in only 10% of women who received a prescription and had the highest compliance. Adequate verbal counseling is especially important for women with little or no formal education. On average, women in this sample had received only four years of formal education and most could not read or write. In this study, it was assumed that midwives at the intervention and control centers dispensed the same overall quality

of prenatal care since they all receive the same training at government accredited midwifery schools.

The fourth motivating factor in the overall sample, the fear of becoming ill, cannot be tied to specific knowledge about the function of IFA. Women who evoked it as a factor indicated that they generally take any medication they are prescribed. It accounted for 20% of answers among women with the highest compliance in the control group but was not a major determinant among women with the highest compliance in the intervention group. This may suggest that the specific knowledge of the role of the tablets is a stronger motivator than just the fear of illness.

In the overall sample, women with the lowest compliance were influenced by five factors, which accounted for 81% of responses. The single most important factor was the experience of gastrointestinal side effects (27% of responses). Side effects have traditionally been considered the major obstacle to compliance, leading many to advocate weekly instead of daily supplementation; however, more recently, studies have shown that side effects have a limited influence on compliance (especially when it is unclear how women distinguish the symptoms associated with taking iron tablets and those associated with pregnancy) (26). Findings in this study support this view: despite the fact that more women cited gastrointestinal side effects as a major reason for low compliance among women who received free tablets than among women who received prescriptions, women who received free tablets still had a significantly higher compliance. This may be a further suggestion that adequate counseling is a stronger determinant of whether or not women adhere to supplementation. Midwives should explain to women that Fe tablets should be taken with food and that side effects associated with taking the tablets are

generally transient. Although verbal exchanges between the patients and midwives during the prenatal visit were not studied, women who received free tablets may have received more thorough counseling.

The second important barrier to compliance, inadequate counseling, was discussed above. The third determinant of low compliance was forgetfulness among women with the lowest compliance in the overall sample (17%) and both in the control group (12%) and the intervention group (26%). This problem could be addressed through better counseling during the prenatal visit by suggesting to women strategies to remember to take their tablets; for example, placing the tablets in a spot that they see everyday (e.g. breakfast or night table, kitchen counter).

The fourth barrier was related to the financial inability of women to purchase the tablets; this only concerned women who received prescriptions rather than free tablets (21% of responses). In fact, the written prescription that women are given during the prenatal visit contains items other than IFA. The content of the prescription will vary according to individual patient afflictions; women who did not purchase the tablets for financial reasons generally did not purchase any other medication on the prescription, even though the IFAs are affordable (1 cent for 10 tablets). In addition, among women in the intervention group who had the highest compliance, no one indicated that the fact that the tablets were provided free of charge was a facilitator of compliance. The results from this study suggest that the benefit of IFA being given during the prenatal visit lies more in the fact that it encouraged midwives to explain the purpose of the tablets and less in the fact that the tablets were free.

The fifth barrier to compliance was expressed by women as being "tired of taking pills". This sentiment was even stronger in women in the intervention group than in women in the control group. Women who expressed this sentiment were further questioned about the reason for this "pill fatigue", but they did not give further details. This "fatigue" may also explain why more women in the intervention group gave away tablets to friends and relatives or lost the tablets. This suggests that daily supplementation is impractical for certain individuals who may benefit more from weekly or bi-weekly supplementation.

Tablet ingestion was not supervised in this study because our intention was to examine the determinants of compliance under usual field conditions. Calculations of compliance were mostly based on the number of tablets that women reported taking in the control group and on counting the number of tablets remaining in the bag of tablets that were given to women in the intervention group. However, the finding that women in the latter group had significantly higher compliance is supported by the fact that women in this group also had a significantly improved Fe status and a lower incidence of anemia compared to women in the other group.

Conclusions and recommendations

Compliance of pregnant women with IFA supplementation in Senegal can be greatly improved by increasing women's access to the supplements. In this study, access was facilitated by asking midwives to hand the tablets to women (free of charge) during the prenatal visit, an effort which led to another facilitator of compliance, providing women with specific and concise information on the health benefits that the tablets confer. This information should include a mention of possible transient side effects of IFA and strategies for women to remember to take the tablets on a daily basis. Although the fact

122

that the tablets were free facilitated compliance, it was not found to be as important a facilitator as proper counseling. Thus, modifying the Senegalese supplementation program to increase compliance may not involve large additional governmental costs. It may just entail a rerouting of the tablet delivery system whereby the tablets would be distributed by the very people who are directly involved with delivering care to pregnant women, the health center midwives, rather than the health center pharmacists. However, pharmacy workers should also be trained to give appropriate counseling to women regarding tablet use. Since adequate counseling is an important determinant of compliance, future inquiries about the effectiveness of IFA supplementation in Senegal and other countries should focus on the nature of the interaction between midwives and patients during the prenatal visit and on midwives' training and knowledge about anemia.

Figure 1 **Number of women in the control and intervention groups with complete hematological and compliance data**

6 Health Centers Randomly Assigned

3 Control (prescription) centers **3 Treatment (free tablets) centers**

Philippe Senghor (n = 32) Roi Baudoin (n = 42) Institute of Social Hygiene (n = 38)	Gaspard Kamara (n = 34) Dominique (n = 41) Center for Maternal & Infant Protection (n = 34)

N = 112 **N = 109**
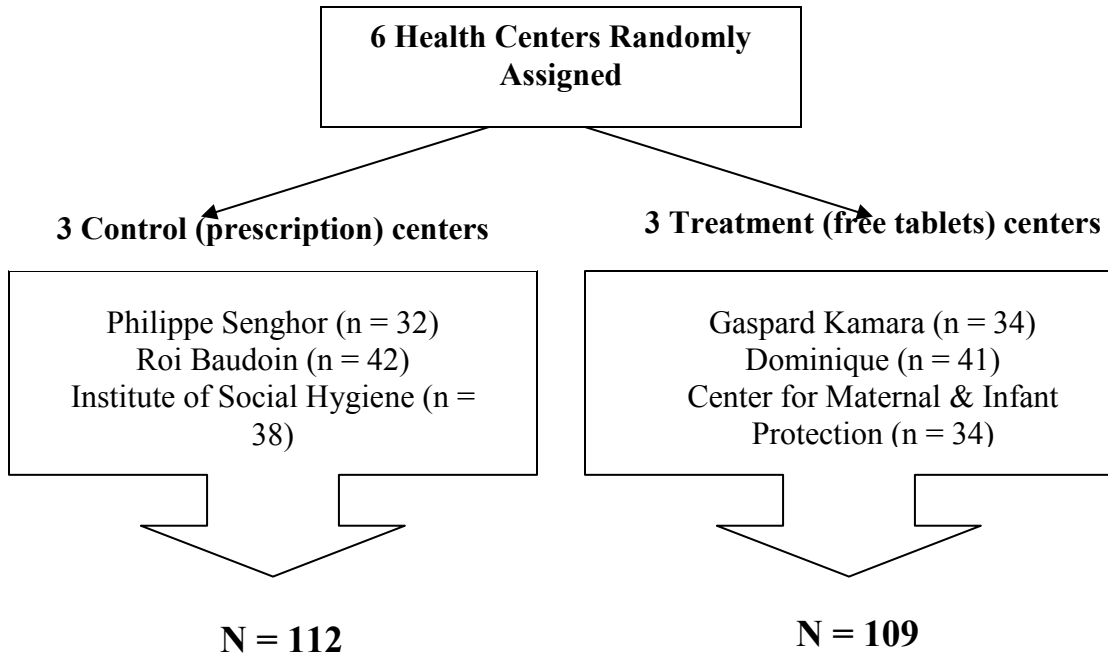
Table 1 Baseline characteristics of women in the intervention and control groups in the study of compliance with iron/folic acid supplementation among pregnant Senegalese women

Variable	All n = 221 Mean ±SEM	Control n = 112 Mean ±SEM	Intervention n = 109 Mean ±SEM	P value[1]
Age (yrs)	26.7 (.4)	26.6 (.6)	27.3 (.6)	.364
Number of yrs of formal education	4.4 (.29)	4.2 (.41)	4.7 (.39)	.350
% Employed	29.4	32.1	26.6	.366[2]
$ Spent on food daily	0.97 (.05)	0.95 (.82)	0.99 (.07)	.663
Gravida	2.96 (.14)	3.2 (.2)	2.9 (.2)	.305
Parity	1.57 (.11)	1.8 (.2)	1.6 (.2)	.458
Stage of gestation (wks)	11.8 (.27)	11.8 (.4)	11.8 (.4)	.930
Weight (kg)	60.96 (.86)	60.4 (1.2)	61.4 (1.3)	.560
Height (m)	1.64 (.003)	1.63 (.003)	1.65 (.004)	.158

[1] Student's t test P value of the mean difference between treatment and control groups
[2] Pearson chi-square test of significance

Table 2 Ethnic distribution of the sample pregnant Senegalese women

Ethnic Group	Frequency	Percent n = 221
Wolof/Lébou	89	40.3
Poular	56	25.3
Sérère	34	15.4
Dioula	11	5
Other	31	14

Table 3 **Factors that influenced compliance with supplementation with iron/folic acid among Senegalese pregnant women with high compliance (≥70%) in the overall sample and in the control and intervention groups**

Motivating Factor	Total Sample (n = 128)[1]		Control Group (n = 54)[2]		Intervention Group (n = 74)[3]	
	Frequency	%	Frequency	%	Frequency	%
Felt better when they ingested the tablets	41	**32.0**	10	**18.5**	31	**41.8**
Midwife instructed them to take the tablets	24	**18.8**	13	**24.0**	11	**14.8**
Midwife explained that the tablets would improve health	17	**13.3**	9	**16.6**	8	**10.8**
Always take whatever they are prescribed because of fear of illness	14	**10.9**	11	**20.4**	3	**4.0**
Knew that the tablets would "increase their blood"	8	**6.2**	2	**3.7**	6	**8.1**
Were afraid of "losing blood"	6	**4.7**	1	**1.9**	5	**6.8**
Since the tablets were given to them, they felt that it was important they take them	6	**4.7**	---	---	6	**8.1**
Husband/mother felt that the tablets were very important for their health and made sure they took them	5	**3.9**	3	**5.6**	2	**2.6**
Knew that they "lacked blood" and that the tablets would increase it	4	**3.2**	2	**3.7**	2	**2.6**
Thought that the tablets would make them feel better	3	**2.2**	3	**5.6**	---	---

[1]N = 221 (Total % may not equal 100 due to rounding)
[2]Total number of subjects in control group = 112
[3]Total number of subjects in intervention group = 109

Table 4 **Factors that influenced compliance with supplementation with iron/folic acid among pregnant Senegalese women with low compliance (<70%) in the overall sample and in the control and intervention groups**

	Total Sample (n = 93)[1]		Control Group (n = 58)[2]		Intervention Group (n = 35)[3]	
Motivating Factor	Frequency	%	Frequency	%	Frequency	%
Experienced gastrointestinal side effects	25	**26.8**	13	**22.5**	12	**34.2**
Took the tablets until prescription ran out; but did not purchase more tablets because the midwife did not say so	17	**18.3**	17	**29.3**	---	---
Forgot to take the tablets on most days	16	**17.2**	6	**10.3**	9	**25.7**
Could not afford to purchase the prescription	12	**12.9**	12	**20.6**	---	---
Grew tired of taking tablets	11	**11.8**	3	**5.1**	8	**22.8**
Midwife did not prescribe/give the tablets (Patient is correct)	4	**4.3**	2	**3.2**	2	**5.7**
Lost the prescription/tablets but were afraid to ask for another	3	**3.2**	2	**3.2**	2	**5.7**
Midwife did not prescribe the tablets (Patient is mistaken)	2	**2.1**	2	**3.2**	---	---
Was afraid that too many tablets would harm her and/or her baby	1	**1.1**	---	---	1	**2.8**
Never came to follow-up prenatal visits and therefore could not get another prescription to purchase tablets	1	**1.1**	1	**1.7**	---	---
Gave away many tablets	1	**1.1**	---	---	1	**2.8**

[1]N = 221 (Total % may not equal 100 due to rounding)
[2]Total number of subjects in control group = 112
[3]Total number of subjects in intervention group = 109

Table 5 Prevalence of anemia and iron deficiency at baseline and follow-up among pregnant Senegalese women who received a prescription (control) and women who received free iron tablets (intervention)

	Control n = 112	Intervention n = 109
% Anemic at baseline[1]	37	25.6
% Anemic after 20 weeks[2]	62[3]	31[4]
% Iron deficient at baseline[5]	68.5	69.8
% Iron deficient after 20 weeks	84.5[6]	56.9[7]

[1]Hb <105 g/L
[2]Hb <110 g/L
[3]McNemar test (P<0.001); significantly different from intervention group, Chi-square test (P<0.001)
[4]McNemar test (P = 0.442)
[5] EP >70µmol/mol heme or SF <15 µg/L or Tfr >8.3µg/L
[6]McNemar test (P = 0.001); significantly different from intervention group, Chi-square test (P<0.001)
[7]McNemar test (P = 0.003)

Chapter 7: Summary and Conclusions

Despite a national policy of universal iron/folic acid (IFA) supplementation, anemia of pregnancy remains a major public health problem in Senegal. The policy mandates that all women who attend public health centers for prenatal care be prescribed to purchase and take IFA throughout pregnancy. However, the effectiveness of this policy may be limited by two main factors, low compliance with supplementation and the fact that iron and folate deficiencies may not be the only important risk factors for anemia in this population such that IFA alone cannot effectively treat anemia. In this longitudinal study we examined a cohort of pregnant women visiting large public health centers in Dakar and 1) determined the prevalence of anemia and the relative contribution of several risk factors, 2) evaluated the effectiveness of the current national supplementation program by determining compliance and comparing it to that of women who received free IFA during the prenatal visit, and 3) determined the factors that influence compliance in this population. This is the first study in Senegal to evaluate supplement delivery to pregnant women and to provide a framework for recommendations to improve compliance and therefore improve iron and anemia status.

Six reference health centers that cater to large low-income population groups in Dakar were randomly assigned to either a control group or an intervention group. In the 3 control centers, women received routine prenatal care including a prescription to purchase IFA. In the 3 intervention centers, women received routine prenatal care with a free supply of IFA during the visit. At baseline, iron and anemia status were determined and the following assessments were made: 1) dietary intake of iron, 2) malaria

parasitemia, 3) intestinal parasite infection for a sub-sample of 298 women, and 3) hemoglobin phenotyping (Hb S). Twenty weeks after enrollment (follow-up) 1) compliance was assessed in both groups as the ratio of the number of IFA that were ingested to the number of days elapsed between enrollment and follow-up, 2) iron and anemia status, and 3) the factors that influenced compliance.

Objective #1:
Determining the prevalence of anemia and the relative contribution of various risk factors for anemia; this was done to ascertain whether a policy of IFA supplementation alone can eradicate anemia of pregnancy.

Findings
Eighty healthy women in their 2nd trimester of gestation were recruited from each center for a total of 480. We found that 39% were anemic, of which 34% had iron deficiency anemia. Twelve percent had P. *falciparum* malaria parasitemia of which only 1% had clinical malaria (>5,000/ μL blood). Thirteen percent were lightly infected with A. *lumbricoides*, A. *duodenale*, and T. *trichuria*. Most of the infected women resided in the suburban areas of Dakar. Six percent had Hb AS. In a multiple logistic regression, we found that iron deficiency was the most important contributor to anemia, more than quadrupling the risk of anemia. Malaria parasitemia and Hb AS also significantly increased the risk of anemia. We also found that women had a higher frequency of consumption of heme-iron containing foods (the most bioavailable form) than non-heme iron containing foods; however, this was accompanied by a high frequency of consumption of iron-absorption inhibitors, such as phytates and polyphenols.

Conclusions:

Since iron deficiency was prevalent and was the greatest risk factor for anemia among the factors that were studied, a policy of universal IFA supplementation for pregnant women is well justified in the context of Senegal. However, IFA should be accompanied by malaria prevention and treatment. Currently, prenatal care services do include providing prescriptions for IFA and antimalarial drugs; however, whether or not women purchase and take them as prescribed in unknown and is the focus of the second objective of this study. We recommend that in areas where helminthes infection is prevalent, routine stool screening be performed and treatment prescribed when necessary. A single affordable course of treatment after the first trimester of pregnancy cures infection and elevates hemoglobin concentration in cases of severe infection. We also recommend that midwives take the opportunity afforded by the prenatal visit to educate women on choices of foods available in the market to maximize their iron intake while minimizing absorption inhibitors intake.

Objective #2:

Comparing compliance among women who received prescriptions to purchase IFA (control) to that of women who were given free IFA during the prenatal visit (intervention); this was done in order to ascertain whether the latter mode of supplementation is more effective at improving iron and anemia status.

Findings

Two hundred and twenty one women had complete baseline and follow-up data. We found that compliance [(Number of tablets ingested/Time elapsed between enrollment and follow-up) x 100] was only 48% in the control group (n = 112) compared to 86% in

the intervention group (n = 109). After adjustment for confounding, the prevalence of anemia after 20 weeks of supplementation was significantly higher in the control than in the intervention group (62% versus 31%). The same was true for the prevalence of iron deficiency (84% versus 57%).

Conclusion:

Impaired access to IFA has been shown to be one of the major causes of low compliance with supplementation in developing countries such as Senegal. We have shown that by increasing access to IFA (i.e. modifying the delivery system of IFA and making them free of charge), compliance can be dramatically improved, which translates into much improved iron and anemia status. More than 95% of women who visit public prenatal centers do receive a prescription for IFA, but there is no monitoring of whether or not women actually purchase and use the supplements. Our data indicate that a mere prescription alone yields relatively low compliance. The current Senegalese supplementation program could be more effective if IFA were provided directly to women during the prenatal visit. Moreover, we need to gain a better understanding of what drives the motivation of women to take or not take IFA in order to make more precise recommendations for program improvement.

Objective #3:
Assessing the factors that determined high (≥70%) and low (<70%) compliance through in-depth interviews

Findings:

Women who had the highest compliance in the control group were motivated mainly by the fact they were told to take the IFA by the midwife; whereas among women who received free IFA the chief motivators were the perceived health benefits which they associated with taking the tablets and the knowledge of the utility of the tablets (i.e. IFA "increase blood"). Women who had the lowest compliance in the control group cited a misunderstanding of instructions given by the midwife about the frequency of intake of tablets as being the main reason for non-compliance. Not being able to afford the tablets was only the fourth reason for non-compliance. Women who had the lowest compliance in the intervention group cited the occurrence of gastrointestinal side effects that they associated with taking the tablets. None of them cited misunderstanding midwife instructions as a justification for non-compliance.

Conclusions:

Providing women with clear and concise information about the frequency of intake of supplements and their utility can dramatically improve compliance. Achieving this necessitates training of health care providers to disseminate this information effectively. In addition, dispensing the supplements directly to women during the prenatal visit may improve compliance by fostering better communication about the tablets between the patient and the midwife. In the context of the Senegalese national program of supplementation, this entails a rerouting of supplements from the pharmacists to the midwives; and possibly enhanced midwife training.

Implications, recommendations, and future research

This study showed that the prevalence of anemia and iron deficiency is high among pregnant women in Senegal. It also showed that the current policy of prescribing pregnant women with iron/folic acid supplements for purchase yields low compliance. An alternative distribution policy where women received the actual supplements during their prenatal visits improved compliance dramatically. This improvement was in part due to the fact that the supplements were offered free of charge and also to the fact that the new distribution strategy fostered better counseling of patients about the utility of the tablets. This was evidenced by the fact that more women in the intervention group knew that the tablets would prevent/cure anemia.

These findings suggest that improving the effectiveness of supplementation in Senegal will involve the following:

1. Changing the distribution flow of supplements: this change can be operated either by providing the tablets completely free of charge during the prenatal visit (such as was done in this study) or by factoring the price of the supplements in the price of the visit. Both possibilities require a cost analysis. In the first case this analysis would determine whether the government can bare the cost of the tablets. In the second case, the analysis would determine whether patients can bare the burden of an increased prenatal visit cost. In either case, the iron/folic acid tablets would be dispensed separately from the other drugs that women are prescribed routinely at their prenatal visit (e.g. ibuprofen or antimalarial drugs). In this study we found that women who stated that they could not comply with supplementation for financial reasons in fact could not afford to purchase their

134

entire prescriptions, not just the iron/folic acid tablets that are purposefully made very affordable (1 US cent per 10 tablets). Furthermore, making the tablets available during the prenatal visit allowed women to start treatment sooner than those who received prescriptions; the former were therefore able to notice the health benefits from the tablets, which encouraged them to continue the treatment. Low-income women typically cannot purchase their prescriptions on the same day they receive them. They generally inquire about the total cost of the prescription at the pharmacy and then work out a way to purchase it either on their own or aided by their husbands or relatives. This process may take weeks or months if they ever succeed to gather enough funds for their purchase. Thus, treatment is delayed or does not occur at all.

2. Training midwives and other prenatal care providers to give women concise and precise information about the purpose of iron/folic acid tablets (i.e. the health benefits that they provide), the mode and frequency of ingestion, and the occurrence of transient side effects should be a public health priority even if the supplementation policy is not modified. This training should involve uniform procedures that all midwives at all health centers must follow. This is to insure that all women who go to prenatal visits at any health center receive the same quality of counseling. Improving the quality of counseling may also involve decreasing the case load of midwives so that they have more time to devote to each patient. Currently, Senegal does not meet the World Health Organization's (WHO) standards for midwife/patient ratio, which results in long lines at health

centers, short patient/midwife interactions, and frustration both on the part of midwives and on the part of patients. Midwife frustration manifests itself as rudeness and lack of respect towards patients who in turn feel intimidated. Several women from this sample stated that they refrained from asking health-related questions to the midwives for fear of being humiliated. One woman in the control group stated that she did not purchase any of the iron/folic acid tablets because she had lost her prescription shortly after her first prenatal visit and was fearful to return and ask for another one. The current patient/midwife relation in health centers does not foster adequate counseling and in part explains why a large percentage of pregnant women do not return for follow-up prenatal visits at health centers. This increases the risk of negative pregnancy outcomes not only because iron deficiency/anemia can be left undiagnosed and untreated but other pregnancy complications may not be detected. Unfortunately, maternal and infant morbidity and mortality remain high in Senegal.

3. Modifying the composition of the tablets that are currently available: WHO recommends that women receive daily supplementation of 60 mg of iron and 400 μg folic acid. The tablets that are available in Senegal only contain 250 μg folic acid. Although folate status was not determined in this study, folate deficiency as a major cause of anemia has been documented in other countries in West Africa. Thus, supplementing women with the recommended dose of folic acid may contribute in decreasing the risk of anemia.

Future research

Causes of anemia other than iron deficiency, malaria parasitemia, helminthes infection, and Hb S were not investigated in this study. These potential causes include thalassemias, vitamins A, B_{12}, and folate deficiencies. A better understanding of the contribution of these factors to the risk of anemia can foster a more holistic approach to the treatment of anemia among pregnant women in Senegal.

A second important contribution to the prevention and treatment of anemia would be an ethnographic research to uncover the beliefs and attitudes of women towards the causes of anemia and its prevention and treatment. Cultural beliefs and traditional remedies have been largely dismissed by the public health community instead of being used to frame supplementation policies and programs. Such ethnographic research should include key informants such as doctors, midwives, pharmacists, traditional healers (most people in Senegal have great faith in them and often seek both traditional and modern medicine), and of course the women themselves. For example, a qualitative study in Nigeria showed that pregnant women did not perceive anemia and its symptoms as serious and therefore did not see the point of treating it. Another study in Tanzania showed that women believed that anemia was caused by evil spirits who sucked pregnant women's blood. Even though the Tanzanian women recognized the symptoms of anemia, they did not take the iron tablets they were given, but instead turned to a traditional healer who promised to ward off the spirits with amulets and incantations. Such examples, illustrate the great need to gain a deep understanding of where iron/folic acid tablets fit in the tapestry of traditional medicines in Senegal and to use that understanding to make them acceptable to women.

Finally, a rigorous survey of the dietary iron intake of pregnant women is also needed. In this study, we used a modified food frequency questionnaire which did show that women consumed iron absorption inhibitors frequently, but which did not allow us to relate dietary intake with hematological outcomes. Such relationship could have only been established by collecting multiple diet records. A better understanding of the dietary sources of iron in the Senegalese diet is essential for diet counseling.

Appendices

Appendix A: <u>Food Frequency Questionnaire</u>

Enrollment Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2; Roi
Baudoin = 3; Center for Maternal and Infant Protection = 4; Gaspard
Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

Address

Type of Food	Frequency								
Heme Fe-contributors	**Never or < 1/mo**	**1/mo**	**2-3/mo**	**1/wk**	**2/wk**	**3-4/wk**	**5-6/wk**	**1/d**	**2+/d**
Beef									
Mutton									
Goat									
Liver (beef/mutton)									
Kidney (beef/mutton)									
Heart (beef/mutton)									
Chicken									
Hen									
Turkey									
Fresh fish									
Dry fish									
Smoked fish									
Crustaceans									

Non-heme Fe contributors	Never or < 1/mo	1/mo	2-3/mo	1/wk	2/wk	3-4/wk	5-6/wk	1/d	2+/d
Green beans									
Parsley									
Lentils									
Bissap									
Baobab leaf									
Groundnut									
Groundnut oil									
Fe absorption enhancers	**Never or < 1/mo**	**1/mo**	**2-3/mo**	**1/wk**	**2/wk**	**3-4/wk**	**5-6/wk**	**1/d**	**2+/d**
Orange (fruit/juice)									
Lemon (fruit/juice)									
Tangerine (fruit/juice)									
Pineapple (fruit/juice)									
Mango (fruit/juice)									
Papaya									
Fresh tomato									
Tomato paste									
Dry tomato									
Fe absorption inhibitors	**Never or < 1/mo**	**1/mo**	**2-3/mo**	**1/wk**	**2/wk**	**3-4/wk**	**5-6/wk**	**1/d**	**2+/d**
Biscuit									
Bread (white/whole wheat)									
Maize (bran/flour/whole kernel)									
Millet (flour/meal/whole grain)									
Rice (white)									
Sorghum (bran/flour)									
Wheat (bran/flour)									

Fe absorption inhibitors	Never or < 1/mo	1/mo	2-3/mo	1/wk	2/wk	3-4/wk	5-6/wk	1/d	2+/d
Milk									
Milk curds									
Milk powder									
Yogurt									
Kew									
Eggs									
Coffee									
Tea									

Appendix B : <u>Socio-demographic questionnaire</u>

Enrollment Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2; Roi Baudoin = 3; Center for Maternal and Infant Protection = 4; Gaspard Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

Address

_____

Phone Number _____

1. Date of birth _____   2. Age (years) _____
   [bdate]   [age]

3. What is your ethnic group? [ethgrp]
____ Wolof/Lebou (1)   ____ Bambara (7)
____ Poular (2)   ____ Manjaak (8)
____ Serer (3)   ____ Mancagne (9)
____ Mandingue/Soce/Malinke (4)   ____ Balant (10)
____ Diola (5)   ____ Other (11)
____ Soninke/Sarakole (6)   _____

4. What is your religion? [faith]
____ Muslim (1)   ____ No religion
____ Christian (2)
____ Animist (3)
____ Other (4)

5. Years of formal education _____   6. Can you read and write: [read]
   [educ]   ____ Easily (1)
                                                 ____ With difficulty (2)

143

____ Not at all (3)

7. Are you employed? _____
[employ] (Y = 1; N = 2)

8. Occupation?
_____
[occtype]

9. Who is the head of your household?
_____

10. Occupation of the head of the household?

_____
[occtypeh]

11. Marital status? [marital]
____ Married (1)
____ Separated (2)
____ Divorced (3)
____ Widowed (4)
____ Single (5)

12. Are you the only wife to your husband?
[onlywife] (Y = 1; N = 2]
_____

13. Do you own your home? _____
[ownhome] (Y = 1; N =2)

14. If renting, how much rent do you pay per month? [morent]
_____

15. What source of water do you use for washing dishes, washing hands, and showering? [source1]
____ Tap in the home (1)
____ Public fountain (2)
____ Delivery (3)
____ Bottled (4)

16. Do you use the same source for drinking water? [source2]
_____
If not, which source do you use?
____ Tap in the home (1)
____ Public fountain (2)
____ Delivery (3)
____ Bottled (4)

17. Type of toilets in the household? [toilet]
____ Flush toilet (1)
____ Private flush toilet (2)
____ Cesspool/latrine (3)
____ Other (4)
____ No toilets (5)

18. Do you possess the following? (Y = 1; N = 2)
____ Radio [radio]
____ Television [tv]
____ Refrigerator/freezer [refer]
____ VCR [vcr]
____ Phone [phone]

19. Do you have electricity? _____
[electr] (Y = 1; N = 2)

20. How many rooms do you use for sleeping? [sleep] _____

21. What material covers your floor? [material]
____ Dirt/ Sand floor (1)
____ Wood floor (2)
____ Linoleum (3)
____ Tile floor (4)
____ Concrete floor (5)
____ Carpet (6)

22. Does someone in the household own the following? [hown]
____ Bicycle (1)
____ Motorcycle (2)
____ Car (3)
____ Horse-drawn cart (4)
____ Other mode of transport (5)

144

____ Other (7)

23. How is used water evacuated from your household? [waterdisp]
____ City mains (1)
____ Closed canal (2)
____ Open canal (3)
____ Septic tank (4)
____ Street (5)
____ Other (6)

24. Where do you keep the household wastes? [waste]
____ Trash bin with cover (1)
____ Trash bin without cover (2)
____ Plastic bag (3)
____ Used kitchen utensils (4)
____ Other (5)

25. Where do you place the household waste pending disposal? [befdisp]
____ Inside the household (1)
____ Outside the household (2)

26. How are the household wastes evacuated? [aftdisp]
____ Pick up (1)
____ Burial (2)
____ Authorized depot (3)
____ Unauthorized depot (4)
____ Incineration (5)

27. How much money do you spend on food each day? [foodexp] _____

Appendix C: <u>Pregnancy and health questionnaire</u>

Enrollment Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2; Roi
Baudoin = 3; Center for Maternal and Infant Protection = 4; Gaspard
Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

1. How many times have you been pregnant? [gravida] _____

2. How many children did you give birth to? [parity] _____

3. Did any children die? [childdie] (Y = 1; N = 2) _____

4. If yes, how many? [nokiddie] _____

5. Age of your last child? _____months
[lastch]

6. What medications are you currently taking? (list and specify why used) [meds]

_____

_____

7. Who gave you the medicines? [medsfrom]
____ Medical doctor (1)
____ Nurse (2)
____ Midwife (3)
____ Pharmacist (4)
____ Friend/relative (5)
____ Other (6)

8. Have you been told that you have: [diseases]
____ Diabetes (1)
____ High blood pressure (2)
____ Cancer (3)
____ Kidney disease (4)
____ Liver disease (5)
____ HIV/AIDS (6)
____ Other

9. How would you describe your current state of health? [health]
____ Excellent (1)
____ Good (2)
____ Fair (3)   ____ Poor (4)

10. Have you received or donated blood in the last 2 months?

_____
[blood] (Y =1; N =2)

Present pregnancy prenatal record

Enrollment Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2; Roi
Baudoin = 3; Center for Maternal and Infant Protection = 4; Gaspard
Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

1. 1st day of last period? _____
[lmp]

7. Stage of gestation
_____ wks

2. Weight? [ewt] _____ Kg

8. Height? [ht]
_____ m

3. Weight at follow-up? _____ Kg
[fwt]

4. Blood type? [bloodtyp] _____

9. RH factor? [rh]
_____

5. Blood pressure?
Systolic _____ mmHg
[syst]
Diastolic _____mmHg
[diast]

10. Serologic test for
syphilis _____
[syphill] (negative = 1;
positive = 2)

6. Albumin [album] _____ g/L

11. Blood glucose
[gluc] _____
mg/dL

Laboratory measurements at enrollment

Enrollment Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2; Roi
Baudoin = 3; Center for Maternal and Infant Protection = 4; Gaspard
Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

---

1. Hb _____ g/L
[ehb]

2. EP _____ μmol/mol
heme
[eep]

3. SF _____ μg/L
[esf]

4. Malaria _____
[malaria] (negative = 1; positive =
2)
Parasite count per μL blood

_____
[malcount]

5. CRP _____mg/L
[crp]

6. Intestinal Helminths_____
[parasite] (negative = 1; positive =
2)
Parasites detected

_____
[partype]
#Eggs per gram of stool

_____
[pdense]

7. Hb variant_____
[Hbvar]

Laboratory measurements at follow-up

Follow-up Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2; Roi Baudoin = 3; Center for Maternal and Infant Protection = 4; Gaspard Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

1.Hb _____ g/L
[hb2]

2. EP _____
μmol/mol heme
[ep2]

3. SF _____ μg/L
[sf2]

Follow-up interview

Follow-up Date _____ (mm/dd/yyyy)
[enrolld]

Name _____ (First/Last)

ID# _____
[idno]

Group Assignment _____
[grpass] control = 1; treatment = 2

Health Center _____
[hcid] Institute of Social Hygiene = 1; Philippe Senghor = 2;
Roi Baudoin = 3; Center for Maternal and Infant Protection = 4;
Gaspard Kamara = 5; Dominique = 6

Patient Number _____ [hcno]

- The midwife prescribed/gave me iron/folic acid tablets: ____Yes; ____No
- How many tablets have you taken/purchased since the last visit? _____
- How many tablets remain (count) _____

Compliance:

If compliance is ≥70%:
What influenced your decision to take the tablets as prescribed?

If compliance is <70%:
Why didn't you take the tablets as prescribed?

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