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# IRON DEFICIENCY ANEMIA: A REVIEW

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## ABSTRACT

Anaemia is a condition in which the number of red cells necessary to meet the body's physiological requirements is insufficient .Iron deficiency anaemia is the one of the common cause of anaemia worldwide. This review will primarily focus on magnitude of the problem, causes and solutions and role of Iron studies in the diagnosis of Iron deficiency anaemia.

## INTRODUCTION

Iron comprises 5% of the earth's crust. Its redox states make iron useful for evolving biological processes. Growing lists of biomolecules that bind or incorporate iron are being catalogued according to their structural similarities. Four general categories of proteins contain iron:

(1) mononuclear iron proteins (e.g., superoxide dismutase),
(2) diiron-carboxylate proteins (e.g., ribonucleotide reductase, ferritin),
(3) iron-sulfur proteins (e.g., aconitase), and
(4) heme proteins (e.g., hemoglobin).

Among these four categories, the first three protein groups are detected at lower levels, but they are functionally important. Hemoglobin is the most abundant iron-containing protein in humans. More than one-half of total-body iron is contained within hemoglobin. Based on the location of hemoglobin in erythrocytes, anemia is a characteristic trait of iron deficiency. Despite iron's plentifulness on earth, iron deficiency is extremely common in humans, and is the most prevalent cause of anemia worldwide. To more fully understand iron deficiency anemia, consideration must be directed toward concepts of iron supply and demand for the production of erythrocytes. Erythropoiesis related demands for iron are created by three variables: tissue oxygenation, erythrocyte turnover, and erythrocyte loss from hemorrhage. Tissue oxygenation requirements and erythrocyte production generally remain stable during adulthood in the absence of hemorrhage, disease, or altered physical activity. As such, iron homeostasis (Fig. 1) also remains stable.

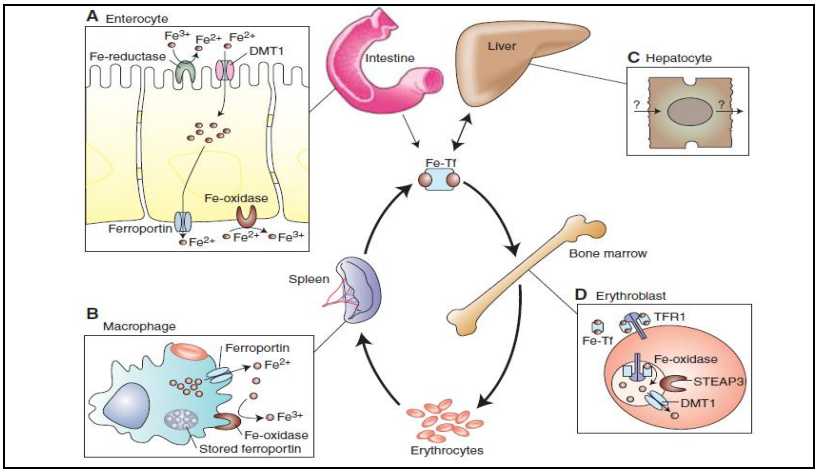
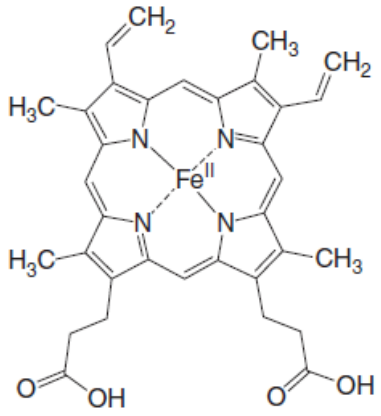



**Figure 1: Iron homeostasis in humans. Each day, 20 mg of iron is recycled between circulating transferrin (Fe-Tf) and erythrocytes. This recycling pathway is supported by (A) intestinal iron absorption, (B) erythrophagocytosis, (C) hepatic iron stores, and (D) iron incorporation into hemoglobin.**




Approximately 20 mL of senescent erythrocytes are cleared daily, and the 20 mg of iron in those cells is recycled for the production of new erythrocytes. Owing to a shorter half-life of circulating erythrocytes in iron deficiency anemia, iron is recovered sooner in those patients, but the amount of iron in each microcytic erythrocyte is reduced **(Macdougall *et al*. 1970).** In the event of hemorrhage, additional iron must be absorbed from the diet to meet the steady-state demands of the host.

Erythrocytes and their precursors require large amounts of iron for the production of heme (Fig. 2) and hemoglobin. Iron is central to hemoglobin structure and function **(Perutz 1982).** The most immediate source of iron for erythroblasts is mono- or diferric transferrin, found in high concentrations in the plasma. Iron deficiency anemia is typically associated with low iron saturation of available transferrin.

Iron is loaded onto diferric transferrin from three sources: the gut (diet), macrophages (recycled iron), and the liver (stored ferritin iron).

In general, iron stores are reduced or lost before the host develops anemia. Therefore, dietary and erythrocyte-recycled iron must meet the demands for erythrocyte production. If iron losses continue, the newly produced erythrocytes will have decreased hemoglobin, causing the amount of iron provided by the same number of senescent erythrocytes to be reduced. Unlike thalassemia trait, increased numbers of erythrocytes are not produced in the iron-deficient state to compensate for the reduction in intracellular hemoglobin content. For this reason, reticulocytosis is usually not present. In the absence of major hemorrhage, iron deficiency anemia generally develops slowly over the course of months or years. Resolution of iron deficiency anemia may be equally slow depending on the availability of iron in the diet as well as the adequacy of gastrointestinal function.

For decades, it has been possible to diagnose and fully reverse the anemia of iron deficiency at a relatively low cost. Unfortunately, iron deficiency has maintained itself as the most common anemia and nutritional disorder world-wide. This seemingly inexplicable paradox of high prevalence despite effective treatment represents a major challenge to public health efforts. Multiple obstacles involving economics, cultural barriers, and infectious diseases converge and make eradication of this disease more difficult. The additional challenges that are encountered by certain human subpopulations in select geographies need to be overcome to achieve therapeutic success in the global community.

**The Magnitude of The Problem**

30% – 50% of anemia in children and other groups is caused by iron deficiency (World Health Organization 2007). Because 1.6 billion people are anemic (**McLean *et al*. 2009**), several hundred million manifest iron deficiency anemia. As such, iron deficiency is the most common cause of anemia worldwide. Iron deficiency anemia afflicts a subset of the two billion people worldwide who are nutritionally iron deficient (**Viteri 1998).** Therefore, the health burden of iron deficiency may be extrapolated from the global prevalence of anemia. Anemia is not distributed evenly throughout the world, as there is a fivefold increase in underdeveloped geographies. In some global regions, the prevalence of anemia among young children is 50% (Table 1) and even approaches 100% in some locales **(Lutter 2008).** In the same regions, 40% – 50% of the population remains anemic at all ages with the exception of nonelderly men **(McLean *et al.* 2009).** By comparison, the disease burden is far less in developed nations. In the United States, the prevalence of anemia as a result of iron deficiency is reduced among all age and gender groups **(Clark 2008).** However, approximately 10 million people are iron deficient in the United States, including 5 million who have iron deficiency anemia.

Table 1. Prevalence of anemia in infants and young children (birth to 5 years of age)

| Global region | Prevalence (%) |
|---|---|
| Africa | 64.6 |
| Asia | 47.7 |
| Europe | 16.7 |
| Latin America | 39.5 |
| North America | 3.4 |
| Oceania | 28.0 |

**Recognizing Iron Deficiency Anemia**

The clinical constellation of signs and symptoms for this disease depends largely on the magnitude of the anemia. Hemoglobin values used for the definition of anemia vary according to age, sex, race, and whether the blood was sampled from the capillary (finger stick) or venous (phlebotomy) source **(Newman 2006; Ca-ble *et al.* 2011a)**. In the absence of automated testing, portable devices or visual matching of hemoglobin color have been proven to be 95% accurate for identifying the hemoglobin level within 1 – 2 g/dL of reference values **(Ingram and Lewis 2000; Lewis and Emmanuel 2001).**Patients may complain of poor mental performance or cold intolerance **(Rosenzweig and Volpe 1999).** Fatigue and exercise-associated dyspnea are regularly reported. Although rare, glossitis or dysphagia may be identified at presentation **(Cook 2005; Novacek 2006).** Recognition of these features may trigger appropriate laboratory tests and therapy.

Iron deficiency anemia is associated with some rather striking neurological sequelae. Some subjects possess the compulsion to move their lower extremities while at rest. The restless leg syndrome is now recognized as a reversible symptom of reduced brain iron levels that is particularly prevalent during pregnancy **(Vivarelli *et al.* 1976; Goodman *et al.* 1988).** Pica is another associated neurological comorbidity. Pica is defined as dietary compulsions for materials that may not usually be consumed in the diet of humans without iron deficiency. Pica, specifically geophagia, has been reported in a majority of pregnant African women living in regions where iron deficiency anemia is extremely common **(Njiru *et al.* 2011).** The molecular basis for this unusual behavior is unknown. Iron deficiency is also known to cause cognitive dysfunction. Neurological damage is particularly relevant during infancy brain development. Long-lasting cognitive challenges occur despite therapy later in life **(Lozoff *et al.*1991).** Therefore, iron deficiency anemia during infancy should be aggressively treated to avoid the potential for cognitive problems. Laboratory evaluation reveals characteristic changes in blood parameters for iron regulation storage, transport, and utilization. Hepcidin is the main regulator of iron in humans. Unfortunately, inter laboratory variation and lack of adequate standards have muted the advance of hepcidin assays in clinical care **(Kroot *et al.*2009).** Research studies suggest that low or absent hepcidin levels may be diagnostic of simple iron deficiency **(Tanno *et al.* 2010).**Currently, the central parameter for determination of significant iron deficiency as well as therapeutic response is ferritin **(Mei *et al.* 2005;O'Meara *et al.* 2011).** The ferritin protein complex structure acts as a cage to contain up to 4500 iron molecules (Fig. 3) **(Harrison and Arosio 1996)**. A serum ferritin level of 15mg/L or less is diagnostic of iron deficiency, and correlates specifically with the absence of stainable bone marrow iron (Baker 2000). Even at higher ferritin levels,40 mg/L, erythropoiesis may be affected. Iron deficiency also causes increased release of soluble transferrin from erythroblasts.

Therefore, ratios of soluble transferrin receptor and ferritin are used to detect iron-deficient erythropoiesis **(Cable et al. 2011b).** When significant inflammation is present, serum ferritin levels may not reflect accurate iron stores. In patients with chronic renal disease, ferritin levels of 400 mg/L are regularly detected in the absence of significant marrow iron stores **(Rocha *et al*. 2009).**

As expected, serum iron levels are reduced in iron deficiency anemia. Transferrin protein expression increases in iron-deficient states, so the iron saturation on transferrin is reduced to values of, 15%. Notably, apoferric and monoferric transferrin are the dominant species, and relatively minor amounts of diferric iron are present in the plasma **(Finch and Huebers 1982).** The reduction of diferric iron is pertinent for erythropoiesis owing to the relative levels and saturation kinetics of transferrin receptors on erythroblasts **(Cazzola *et al.* 1987).** In addition to iron parameters, a lack of iron has measurable effects on erythropoiesis and erythroid cells. Low reticulocyte hemoglobin content is derived from reduced hemoglobin production, and may be useful for screening infants and children for iron deficiency **(Brugnara *et al*. 1999; Ullrich *et al.* 2005).** However, thalassemia mutations may confound the interpretation of reticulocyte hemoglobin quantitation (Mast et al. 2002). Iron deficiency accounts for increased production of zinc protoporphyrin in association with decreased heme production during erythroblast maturation. Ratios of these two parameters may be quantified using washed erythrocytes. Although values 40 moles of zinc protoporphyrin per mole heme denote an iron-deficient

state **(Hastka *et al.*1992),** the presence of a recent illness may reduce the sensitivity of this test (**Crowell *et al.* 2006**).

The manifestation of anemia or microcytosis is usually somewhat delayed relative to the loss of body iron stores. About 1% of erythrocytes are replaced daily, and the recycling of iron from the senescent cells continues to support the production of new cells. Eventually, the complete blood count (CBC) will reflect the effects on erythropoiesis. A combination of increased red cell distribution width (RDW), decreased red blood cell (RBC) count, decreased RBC hemoglobin, and decreased mean cell volume may be manifested. Unless the iron deficiency is reversed, the hemoglobin and hematocrit levels decrease to sufficiently low levels to be classified as anemia.

**Major Causes of Iron Deficiency Anemia**

**Blood Loss**

Each milliliter of packed RBCs (2.5 mL of whole blood) contains 1.0 mg of iron. Each day, 1.0 mg of iron is absorbed from the diet and 20 mg of iron from senescent erythrocytes are available to support erythropoiesis. Once iron stores are depleted, dietary and recycled erythrocyte iron are not usually sufficient to compensate for acute blood loss. In all cases of iron deficiency anemia, blood loss should be considered. Hemorrhage itself is by far the most common mechanism for acute iron loss and anemia. Hemorrhage decreases the host's red cell mass, decreases the supply of iron for erythropoiesis, and increases the iron demand for erythropoiesis. Chronic blood loss from menstruation or hookworm infection (see below) has the greatest impact worldwide.

Less than 2 mL of blood is lost daily in the stool of healthy adults **(Ahlquist *et al.* 1985).** Detection of occult blood losses of up to 60 mL/dmay be difficult without specialized stool tests **(Rockey 1999).** Bleeding may occur from multiple sites along the intestinal tract, with an increased incidence of bleeding from the colon **(Lanas *et al.* 2009).** Sometimes overlooked causes of blood loss include blood donation and nosebleeds. Intravascular hemolysis with hemoglobinuria such as occurs in malaria results in iron loss in the absence of hemorrhage. After chronic physical exertion, significant iron is lost in sweat and may contribute to the deficient state (**Reinke *et al.* 2010).** A full history should be queried in all new cases of iron deficiency anemia.

**The Maternal – Fetal Bridge of Iron Deficiency**

Requirements for iron are greatest around the time of birth. Iron demand is high in menstruating as well as pregnant females. During pregnancy, it is estimated that 1200 mg of iron are required from conception through delivery **(Lee and Okam 2011).** Iron intake and stores in the mother must satisfy fetal development, and blood loss at delivery. Additionally, the maternal erythrocyte mass should increase from 350 to 450 mL. By comparison, pregnant women without iron supplements only increase their red cell mass by 180 – 250 mL **(Pedersen and Milman 2003).** One interpretation of this difference is that fetal iron demands are prioritized over the red cell mass of the mother. Postpartum, iron is lost as lactoferrin in breast milk. Those losses are balanced by the absence of menstruation in the lactating female. Maternal iron deficiency anemia during pregnancy and the perinatal period have devastating effects on both the mother and child. In addition to the direct effects of anemia, reduced fetal brain maturation, pediatric cognitive defects, and maternal depression are associated with iron deficiency anemia **(Black *et al.* 2011).**

The reversibility of cognitive defects caused at an early age by iron deficiency is unclear. Importantly, untreated iron deficiency in pregnant females will be passed to the infant. If left untreated during infancy, childhood, and adolescence, anemia and iron-associated cognitive defects may conceivably be passed between generations much like genetic traits. Unless the iron deficiency is treated at some stage of life, the cycle of iron deficiency from mother to child may remain unbroken for several generations.

In the fetus and during infancy, iron is required for the growth and development of all tissues. The human growth rate is almost logarithmic during this period **(Anderson and Holford 2008).** The hemoglobin and myoglobin requirements must be met before the accumulation of storage iron. Although serum ferritin levels are elevated in the fetus compared with the mother, the fetal iron stores correlate well with the iron stores of the mother **(Milman *et al.* 1987).** In anemic mothers with iron deficiency, the fetal ferritin levels remain 10 times higher than the maternal ferritin at the time of **delivery (Erdem *et al.* 2002**). At birth, the fetal red cell mass is 50 mL/kg (**Phillips *et al.* 1986**), compared with 25 – 30 mL/kg in adults **(Fairbanks *et al.* 1996).** During the first year of postnatal life, total-body iron increases by 240 mg **(Oski 1993).** Around 80% of that iron is used for expanded hemoglobin production (50%) and iron stores (30%). Beyond the first year, iron intake or stores must remain sufficient to support the ongoing growth and increased red cell mass **(Moser *et al.* 2011).** In children and young adults, iron deficiency remains one of the top three contributors to the overall disease burden in those populations **(Gore *et al.* 2011).**

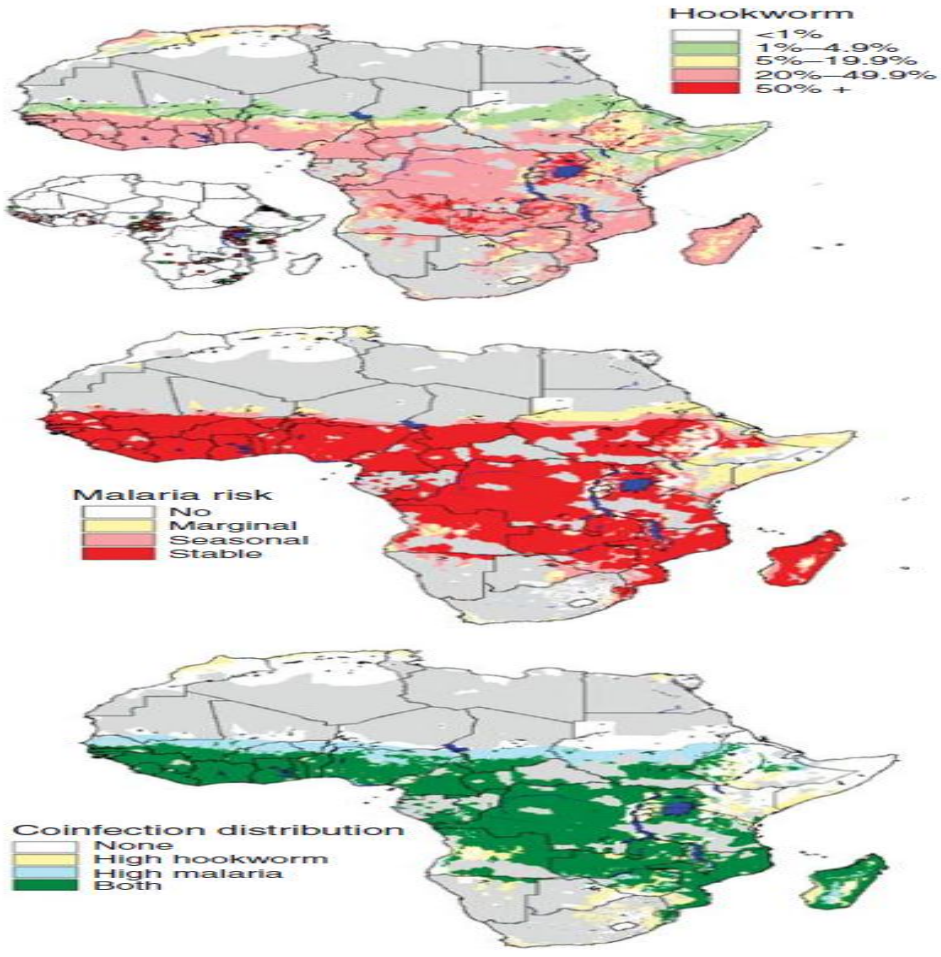
**Malaria**

Iron deficiency anemia and malaria coexist in most tropical regions of the world. Malaria contributes to iron deficiency anemia by causing intravascular hemolysis with subsequent loss of hemoglobin iron in the urine. This clinical feature was described in 1898 as black water fever **(Connolly 1898).** Malaria also causes an immune response that suppresses erythropoietin **(Burgmann *et al.* 1996)** as well as direct effects on erythropoiesis **(Skorokhod *et al.* 2010**). The host may also increase hepcidin expression for protection from

liver-stage malaria **(Portugal *et al.* 2011)**. Of course, increased hepcidin restricts iron and might delay erythroid recovery. It is essential to understand the complex interplay between iron, hepcidin, and malaria when considering efforts to eradicate iron deficiency in malaria-endemic regions. If iron redistribution by hepcidin is beneficial for malaria, restricted iron could benefit the infected host. This hypothesis may help explain the recent report of potential harm caused by iron supplementation among preschool children in malaria-endemic areas **(Sazawal *et al.* 2006).** In that study, iron and folic acid supplements were stopped owing to an increased risk for hospitalization or death from malaria in Zanzibar, Tanzania. Increased caution for iron supplementation in malaria-endemic regions was proposed **(World Health Organization 2007).** However, a recent Cochrane paper recommended, "iron supplementation should not be withheld from children living in malaria-endemic countries" **(Okebe *et al.* 2011).** Treatment of iron deficiency anemia is less clear in areas where access to proper malarial prevention and treatment are suboptimal. Further studies and resolution of this critical, but complex issue are awaited.

**Hookworm**

Like iron deficiency anemia, hookworm infection affects several hundred million humans worldwide **(Bungiro and Cappello 2011).** Amazingly, a recent study reported that there is a considerable overlap between malaria and hookworm in sub-Saharan Africa **(Fig. 4)** (**Brooker *et al.* 2006).** Worldwide, there are two hookworm species that infect humans. Both are found in tropical regions based on the requirement of moist soil for survival. The worm is introduced to the soil by fecal matter in regions where sanitation is not present. From the soil, the parasite accesses the duodenum of a new human host directly by mouth, or indirectly via the skin. Once in the gut, the worm may be retained for several years as it releases eggs in the stool. A hookworm infection should be suspected in cases of travel or habitation in the tropics, iron deficiency anemia, and mild eosinophilia. Owing to their location in the small bowel, capsule endoscopy is helpful for diagnosis if eggs are not present in the stool **(Li *et al.* 2007).** Hookworms live on 0.3 – 0.5 mL of blood extravasated daily from the intestinal mucosa. Heavily infected patients are simply unable to maintain adequate iron stores and become **anemic (Smith and Brooker 2010)**. Even without additional iron supplements, antihelminthic drugs can cause reversal of iron deficiency anemia **(Radhika *et al.*2011).** It is currently unknown whether hook-worm infection causes increased production of hepcidin in the host.



**Map of hookworm (top), Plasmodium falciparum (middle), and overlapping (bottom) prevalence.**

**Diet and Malabsorption of Iron**

Local economics generally dictate the level of nutrition worldwide. The diet, by itself, infrequently causes iron deficiency anemia in the absence of severe malnourishment or a comorbidity. A vegan diet is usually sufficient to prevent anemia even though the iron stores of the host may be low **(Craig 1994).** The diet becomes far more relevant when the iron stores are lost, or anemia has already developed, and the host requires additional iron absorption from the gut for recovery. This occurs in the multiple settings described earlier in this work: blood loss, rapid growth during infancy, malaria, and hookworm.

In these settings, the diet and iron supplements become critical for maintaining iron availability.

Supplemental dietary iron may be needed, because the average Western diet is not sufficient to meet the needs of pregnancy **(Lee and Okam 2011).** In addition to the iron content itself, the bioavailability of iron for absorption depends largely on the dietary components **(Sharp 2010).** Iron in the form of heme is especially bioavailable, and meat-containing diets are also beneficial **(Lopez and Martos 2004)**. Vitamin C improves dietary availability of iron as well as avoidance of tea or other iron-chelating substances **(Thankachan *et al.* 2008).** Numerous approaches are being tried to improve iron availability in the diet with a goal of low-cost and culturally acceptable implementation among the underprivileged **(Hurrell *et al.* 2004).** In addition to the dietary components, the gut must be able to absorb iron to prevent or treat iron deficiency anemia. Inorganic iron absorption requires multiple mechanisms for entry and exit from duodenal and jejunal epithelial cells (Fig. 1). In cases of suspected malabsorption, a simple oral iron absorption test may prevent or direct more invasive studies **(Alleyne *et al.* 2008).** If the host's hepcidin expression is increased, inorganic iron from the diet will not be optimally absorbed into the blood from the intestine. Instead, that iron will be trapped in the intestinal epithelial cells, and then sloughed into the lumen and stool **(Anderson *et al.* 2009).** Therefore, infections, inflammation, or other hepcidin up-regulating mechanisms are likely to inhibit nonheme iron absorption despite a high bioavailability in the diet. Other diseases, including helicobacter infections **(Vitale *et al.* 2011),** bariatric surgery **(Shankar *et al.* 2010),** or decreased gastrin **(Kovac *et al.* 2011)** can inhibit iron absorption. Finally, celiac disease is a common cause of anemia owing to low iron absorption from the gut **(Presutti *et al.* 2007).** There is a high incidence (15%) of celiac sprue among the subjects who undergo endoscopy for evaluation of iron deficiency anemia **(Oxentenko *et al.* 2002).**

**Solving the Global Problem**

**Iron Fortification**

Iron fortification remains the mainstay of efforts aimed toward the treatment or prevention of iron deficiency anemia. As a general rule, menstruating and pregnant women along with their children clearly represent the largest at-risk population for this disease. In the underdeveloped world, iron may be provided with other micronutrients to reduce anemia in schoolchildren **(Ahmed *et al.* 2010; Best *et al.* 2011; Lemaire *et al.* 2011).** In-home food preparations with iron supplements present an alternate to industrial scale supplementation of grains or commercial food products **(Lynch 2011).** Numerousoral preparations and dosing regimens are available for menstruating and pregnant females **(Fernandez-Gaxiola and De-Regil 2011).** Recently, intravenous iron preparations with improved toxicity profiles have been used for cases where rapid therapy was useful in reducing the need for transfusion **(Gozzard 2011).** Based on the large amount of iron that is provided with intravenous therapy, special care should be given toward dosing to prevent iatrogenic iron overload. As a general rule, therapy should be continued only until the anemia is resolved and iron stores are replenished. These goals are met with a normal hematocrit and serum ferritin level of 50 – 100 mg/L (corresponding to 400 – 800 mg iron in stores for otherwise healthy adults) **(Walters *et al.* 1973; Magnussen *et al.*2008)**

**Delayed Clamping of the Umbilical Cord**

Special attention must be given toward preventing iron deficiency in the newborn. In addition to maternal iron supplements, the simple maneuver of delayed umbilical cord clamping might help achieve this goal. In 1954, delayed umbilical cord clamping was found to be a relatively benign procedure (Colozzi 1954). The potential benefit of placental transfusion as an iron source was not initially considered. By the 1960s it was shown that placental transfusion causes a significant increase in the red cell volume when clamping is delayed for several minutes. A delay of 5 min results in 166 mL of added blood volume for a 3.5 kg infant **(Usher *et al.*1963).** Placental transfusion is also achieved by placing the newborn on the mother's abdomen after vaginal delivery, and clamping the cord once it stops pulsating **(Nelle *et al.* 1995).** In cases of cesarean section or other clinical setting that may preclude delayed clamping, clamping near the placenta should increase the volume of blood delivered to the newborn **(Daniel and Weerakkody 1996).** In the 1990s, the benefit of delayed clamping for the purpose of preventing iron deficiency during the first 6 months of life was more clearly appreciated **(Pisacane 1996).**

Widespread adaptation placental transfusion may have been prevented to date by unsupported concerns for polycythemia, hyperbilirubinemia, and increased blood viscosity **(van Rheenen and Brabin 2004)**. Importantly, none of those concerns were realized by a 3 min delay in

umbilical cord clamping at the time of delivery among 400 low-risk pregnancies **(Andersson *et al.* 2011).**

Instead, neonatal anemia and iron parameters by 4 months of age were improved. None of the infants who had undergone delayed (3min) clamping had ferritin levels below 20mg/L, versus decreased ferritin levels in 7.4% of subjects who had undergone clamping almost immediately after delivery. Overall, these studies suggest that some level of placental transfusion may be beneficial in most deliveries. Large, prospective multinational studies of this simple maneuver are needed to confirm these results.If those studies show a generic benefit toward preventing iron deficiency or anemia, then appropriate efforts should be made to widely institute placental transfusion via delayed umbilical cord clamping. This procedure may be particularly useful in underdeveloped communities, as it incurs little, if any, additional expense.

**Understand Fundamental Iron Biology**

It is predicted that advances in global therapy for iron deficiency anemia will be greatly assisted by basic research efforts. Perhaps the most significant advance in this regard is the discovery and development of hepcidin biology over the last decade **(Ganz 2011).** Hepcidin biology will undoubtedly evolve into applications for iron deficiency anemia among all world populations. For instance, the recognition that hepcidin expression is highly variable and influenced by a circadian rhythm should be advantageous in improving dosing regimens **(Kemna *et al.* 2007**). The kinetics of hepcidin expression in response to iron supplementation for iron deficiency remain largely unexplored as another research avenue aimed toward the optimization of therapy. Clinical comparisons of oral versus intravenous therapies will help determine if a rapid pulse of therapy can improve the chances of success for certain individuals or groups of patients certain populations do not benefit from universal iron supplementation **(Ghio 2011).**

With inherited hemochromatosis, the absorption of dietary iron increases. Some genetic variants are quite common, especially in northern Europeans **(van Bokhoven *et al.* 2011).** Although the clinical penetrance is quite variable in the most common forms of hereditary hemochromatosis, communal iron fortification for this group is generally not recommended. Another genetic disorder named thalassemia (**Fucharoen and Weatherall 2012; Gibbons 2012;Musallam *et al.* 2012; Nienhuis and Nathan2012; Cao and Kan 2013; Higgs 2013; Thein 2013; Vichinsky 2013)** has a more direct relationship with iron deficiency anemia, because both diseases are concentrated within malaria and hookworm-endemic regions of the world.

Thalassemia is caused by mutations in the globin genes that lead to decreased production of hemoglobin. Interestingly, iron deficiency itself may affect the production of hemoglobin in a -thalassemia **(O'Brien 1973).** Thalassemia trait patients present with microcytosis similar to iron deficiency. Homozygous thalassemia mutations lead to iron overload in the host by mechanisms that are not fully understood. As such, it is essential to recognize thalassemia and avoid iron supplements in that population.

Recent basic discoveries of several important genes or mutations that modify iron metabolism should also be mentioned. Iron is regulated, in part, by a recently discovered gene named TMPRSS6 **(Du *et al.* 2008).** Mutations in that gene cause iron deficiency that is refractory to iron supplements **(Cau *et al.* 2010).**

If patients with malaria or hookworm benefit from iron deficiency, then some populations may have evolved by incorporating mutations into this gene. Population-based studies will help determine if mutations in the TMPRSS6 gene could be confounding efforts to reverse iron deficiency in some global populations including those in sub-Saharan Africa.

As evidenced by the importance of iron for malarial pathogenesis, further research into the complex relationships between deprivation of iron for this pathogen and iron deficiency anemia are needed to determine the best course of therapy. Determination of hookworm effects on hepcidin expression should also be pursued. In populations afflicted with one or both of these parasites, efforts to supplement iron can be confounded by the host's inflammatory response.

Relationships between hookworm and intestinal iron absorption should be studied further, understood, and incorporated into eradication efforts. Ideally, strategies will be tested that in-corporate vaccination, sanitation, malarial treatment, deworming, and iron supplements into the same research plan. Although such strategies seem ambitious in a world of limited resources, it is crucial to remember that hundreds of millions stand to benefit worldwide.

**Iron Studies**

Iron studies are a panel of tests used to assess the amount of circulating iron and storage iron. These tests should be interpreted together. Below is a summary of the routine iron studies performed in most laboratories.

**Ferritin**

As the main iron storage protein in the body, the majority of ferritin is intracellular. However, a soluble form is found in the blood and can be assayed. **(Cohen LA *et al*;2010).** Ferritin concentrations vary by age and gender. From adolescence, males have higher values than females, a trend that persists into late adulthood. In females, ferritin concentrations remain relatively low until menopause and then rise. (**Gibson R. *et al;*2005).**In both sexes, ferritin increases from around 70 years of age.**(Loria A *et al*;1979).**

A ferritin concentration <15g/L in adults (WHO/NMH/NHD/MNM/11.1) is diagnostic of iron deficiency. An elevated ferritin may reflect iron overload; however, ferritin is an acute phase protein, so may also be increased in liver disease, malignancy, infection and inflammation. **(Gabay C and Kushner I 1999).**Therefore, a normal ferritin concentration alone does not)) necessarily exclude iron deficiency.

**Serum iron**

Serum iron is a measure of the amount of iron bound to transferrin in the plasma. Only a small proportion of the body's iron is bound to transferrin at any one time. **(Takami T and Sakaida I 2011).**

There is a rapid turnover of transferrin-bound iron and circulating iron concentration can be affected by dietary intake; as a result, there is significant variation in iron concentration within each day and between days. **(Dale JC: 2002)**

For this reason, assessment of serum iron alone provides little helpful clinical information. Total iron-binding capacity/transferring Total iron-binding capacity (TIBC) is an assay which determines the amount of iron that can be bound to unsaturated transferrin, i.e. the total number of transferrin binding sites per unit volume of plasma or serum. Historically, it was assessed by adding an excess of iron to plasma and measuring the amount of iron retained. **(Ramsay WN;1973).**

Therefore, TIBC is a proxy measure of transferrin. Unlike serum iron, TIBC does not have rapidly changing concentrations in the plasma. However, it is not a useful marker of early iron deficiency as values do not change until stores are depleted. **(World Health Organization, Assessing the iron status of populations, 2016).**

Transferrin is the transporter protein for iron and its concentration can be determined by immunological methods. **(World Health Organization, Assessing the iron status of populations, 2016).**

Both TIBC and transferrin rise in iron deplete states and fall in inflammatory and iron overload disorders.

**Transferrin saturation**

This is derived by dividing serum iron by TIBC. As the name suggests, it is the percentage of transferrin bound to iron. In iron deplete states, the amount of iron is reduced and therefore the transferrin saturation will be reduced (and vice versa). A transferrin saturation of <15% in association with an elevated TIBC is indicative of iron deficiency anaemia. A transferrin saturation of >45% is suggestive of iron overload and will usually require further investigation. **(Van Bokhoven MA *et al*;2011).**

As previously mentioned, the variation in plasma concentration of iron is considerable, and therefore, there will be daily variation in the transferrin saturation; as a result, transferrin saturation must be interpreted alongside other iron studies.

**Iron deficiency anaemia**

Iron deficiency anaemia is due to the lack of sufficient iron to form normal red blood cells; it is the most common cause of anaemia worldwide. **(Weiss G and Goodnough LT 2005).** Iron deficiency may be the result of blood loss, inadequate dietary intake or malabsorption. The gold standard for diagnosing iron deficiency is the absence of stainable iron on bone marrow biopsy; however, this is impractical, and iron deficiency is usually assessed by laboratory parameters on a peripheral blood sample.

**Laboratory diagnosis of iron deficiency anaemia**

**Full blood count and blood film**

By WHO criteria, anaemia is defined as a haemoglobin concentration (Hb) of<120 g/L in a female or<130 g/L in a male (WHO/NMH/NHD/MNM/11.1). In the early stages of iron deficiency, haematopoiesis is not affected; as stores diminish further, the red cells become microcytic first and then hypochromic before the Hb falls. As well as microcytos is and hypochromia,the blood film may feature poikilocytosis (variation inshape, including pencil cells) and anisocytosis (variation in size) **(Bain B 2006).** Microcytosis is reflected in the fullblood count (FBC) as a reduction in the mean cell volume (MCV) and hypochromia as a reduction in the mean cell haemoglobin concentration.

**Iron studies**

Hepcidin feedback is regulated by concentrations of iron; in iron deplete states, circulating concentrations of this hormone fall **(Guyatt GH *et al*; 1992)** .As hepcidin falls, ferroportin expression increases, leading to increased absorption of iron from enterocytes and increased iron export from storage cells. The IRP/IRE system also works to reduce the conversion of cytosolic iron into ferritin. Lastly, in order to optimize delivery of exported iron to areas of high demand, the production of transferrin is upregulated in the liver. Iron studies can reflect this physiological response .Circulating transferrin and TIBC are elevated. Serum iron falls; the relative decrease in supply compared with demand reduces the circulating pool. Transferrin saturation is reduced (typically<15%) due to increased TIBC and reduced serum iron. The increased export of iron from stores and decreased ferritin production lead to a fall in circulating ferritin; a concentration of<15 g/L is diagnostic of iron deficiency. **(WHO/NMH /NHD/MNM/11.1).** Although a low serum ferritin is both a highly specific and sensitive marker of iron deficiency; a normal ferritin can be falsely reassuring. As previously discussed, ferritin may rise with advancing age and inflammation, and therefore, diagnosing iron deficiency in these states can be challenging; however, a ferritin concentration above 100 g/L is unlikely to be associated with iron deficiency **(Goddard AF *et***

***al*;2011).**The British Society of Gastroenterology suggests that the threshold for diagnosing iron deficiency should be raised to a serum ferritin concentration of 50 g/L in people who have comorbidities.**(Cook JD *et al*;1993)**Table 1 summarizes these changes.

| Parameters | Iron deficiency anaemia |
|---|---|
| Serum Iron | Decreased |
| TIBC, Transferrin | Increased |
| Transferrin Saturation | Decreased |
| Serum Ferritin | Decreased |

## CONCLUSION

IDA is a major public health problem. Coordinated efforts should be made to control anaemia. Recognised risk factors should be considered in prevention and control strategies of IDA. Although predisposing factors for anaemia were documented, large scale studies should be done to identify specific aetiologies and root causes of anaemia among the groups by assessing micronutrients (serum iron, folate, and vit-b12 levels).

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