REVIEW ARTICLE

Revisiting Iron Metabolism, Iron Homeostasis and Iron Deficiency Anemia

Muhammad Saboor 1, 2, Amtuz Zehra 3, Hassan A. Hamali 1, Abdullah A. Mobarki 1

1 Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia
2 Medical Research Centre (MRC), Jazan University, Jazan, Saudi Arabia
3 Basic Science Department, King Saud Bin Abdulaziz, University, Al Ahsa Branch, Saudi Arabia

SUMMARY

Background: Iron deficiency anemia (IDA) is one of the commonest clinical scenarios especially in children, women of childbearing age, and elders. The crux of this review was revisiting iron homeostasis, mechanism of iron absorption, causes, laboratory diagnosis, and management of IDA.

Methods: This narrative review is compiled after a relevant literature search from electronic databases including, but not limited to, Google, Google Scholar, PMC, PubMed, Science Direct, and Scopus. The key words used for searching relevant literature include iron, iron deficiency, iron deficiency anemia, iron metabolism, hepcidin, transferrin, causes of iron deficiency anemia, and laboratory diagnosis of iron deficiency anemia. Reference hematology books were also consulted.

Results: According to the published literature, about one mg of iron is required daily which equals its loss, although the iron requirement increases in pregnancy and lactating mothers. Dietary non heme iron (oxidized Fe3+) is reduced to the ferrous (Fe2+) form by ferrireductase present in the brush border of duodenal enterocytes. Ferrous iron is absorbed in the brush border of duodenal enterocytes through a carrier protein, divalent metal transporter 1 (DMT1). Heme iron is absorbed by the duodenal enterocytes through a mechanism that is not well understood or a receptor yet to be discovered. Transferrin receptor helps in the internalization of iron in the cells. Hepcidin acts as a gatekeeper and controls iron absorption by enterocytes and macrophages. IDA may be caused by decreased intake of iron, increased iron requirements or loss of iron from the body.

Conclusions: Iron deficiency anemia is the most common nutritional anemia that affects large numbers of people in developed as well as in developing countries. It is estimated that approximately 2 billion people around the world have IDA. Microcytosis with marked reduction in serum iron, decreased % saturation of transferrin, low ferritin, and reduced or even undetectable hepcidin are the laboratory features of IDA. In addition, total iron binding capacity and soluble transferrin receptors increase significantly in IDA. Management of IDA is incomplete if the underlying cause is not ruled out and left untreated.


Correspondence:
Muhammad Saboor
Department of Medical Laboratory Technology
Faculty of Applied Medical Science
Jazan University
Jazan
Saudi Arabia
Phone: +966 54 495 9029
Email: msaboor@jazanu.edu.sa

KEY WORDS
iron, iron deficiency anemia, hepcidin, transferrin

INTRODUCTION

1. Introduction
Iron is an essential micronutrient for all living organisms. It is pivotal for a number of biological functions such as oxygen transport, mitochondrial metabolism, and DNA synthesis [1,2]. It is also an important compo-
nent of various enzymes (cytochromes, catalases, oxidases) that are essential for normal cellular activities e.g., energy generation and drug metabolism [3]. Under aerobic conditions, iron has pro-oxidant activity because it can produce reactive oxygen species and free radicals [4]. Iron is essential for the production of erythrocytes; if its supply is inadequate, red cell production declines. This subsequent lack of iron leads to the development of microcytic and hypochromic anemia [5,6]. Iron deficiency anemia (IDA) is the most common type of anemia across the globe [5,7,8]. It is the only nutritional anemia that affects a large number of populations in developed as well as in developing countries [9]. There is currently no reliable available data regarding global prevalence of IDA. However, it is estimated that approximately 2 billion people around the world i.e., nearly 25% of world population has anemia [6,10-13]. It is estimated that approximately 50% of anemic individuals are afflicted with iron deficiency [6]. According to WHO Global Database on anemia (1993 - 2005), children and pregnant women are the most vulnerable group in which prevalence of anemia is significantly higher than postmenopausal women and adult men [14].

1.1. Distribution of iron in the body
Iron is present in the body in two forms, i.e., functional and storage. The functional form is about 80% of the total iron and includes hemoglobin, myoglobin, and enzymes (cytochromes and catalases). Of the remaining iron, 15 - 20% is in the storage form (ferritin and hemosiderin) [15]. The normal adult has approximately 3.5 g (3,500 mg) of iron in the body. Sixty-five percent (2,300 mg) of the total body iron is found in hemoglobin. Nearly 10% (350 mg) is present in muscle fibers (myoglobin) and other tissues (cytochromes and catalases). The remaining iron, i.e., 25 - 30%, is stored in liver (200 mg), macrophages (500 mg), and bone marrow (150 mg) [16-18].

1.2. Dietary sources of iron
A normal balanced diet contains about 15 - 20 mg of iron. About 1 mg of iron is required daily which equals its loss, although the iron requirement increases in pregnancy and lactating mothers. The two forms of iron present in the food are divided according to the bioavailability of iron to the body.

1.2.1. Heme iron
This form of iron is present in animal products such as meat (beef, mutton, and chicken) and liver. It is present either in the form of hemoglobin or myoglobin as heme-protein complex. Gastric acid is important to separate heme iron from its apoprotein. This type of iron readily absorbs in the intestine [19,20].

1.2.2. Non-heme iron
Non heme iron is present in fruit, vegetables, eggs, nuts, and grains. It is present in the form of ferric hydroxide or is attached to organic compounds such as phytates, oxalates, sugars, citrates, lactate or amino acids in these foods. This form of iron is not easily absorbed [20,21].

1.3. Iron absorption
Dietary non heme iron is in the oxidized (Fe³⁺) form. This oxidized form is not bioavailable and must be reduced to ferrous (Fe²⁺) form. Ferrireductase present in the brush border of duodenal enterocytes converts Fe³⁺ to Fe²⁺ [16-18]. During this conversion vitamin C acts as a coenzyme. Fe²⁺ iron is absorbed in the brush border of duodenal enterocytes through a carrier protein called divalent metal transporter 1 (DMT1) [22]. Absorption of non heme iron is influenced by various factors, i.e., phytates (high fibrous diet), calcium, phenolic compounds (coffee and tea), and co-administration of some drugs like tetracyclines, proton pump inhibitors, and antacids. In addition, Helicobacter infection also affects bioavailability of non heme iron [23,24]. Heme iron is absorbed by the duodenal enterocytes through a mechanism that is not well understood. It was proposed that heme iron is transported across the intestinal epithelium through heme carrier protein 1 (HPC1). Once heme iron enters the enterocytes, it is released as Fe²⁺ by the action of heme oxygenase. Eventually Fe²⁺ is exported across the basolateral membrane into the bloodstream via Fe²⁺ transporter called ferroportin 1 [22]. Before it enters into circulation, Fe²⁺ is re-oxidized to Fe³⁺ by hephaestin that physically interacts with ferroportin. Exported Fe³⁺ is captured by transferrin which delivers it to the tissues for different metabolic purposes [4,16,17].

There are certain factors that affect iron absorption. Some of the factors increase while others decrease its absorption. Factors increasing iron absorption include ferrous form of iron, vitamin C, hydrochloric acid (HCl), gastric juice or low gastric pH, sugars and amino acids present in the food, pregnancy and low serum hepcidin levels, e.g., in IDA. Factors that culminate in decreased iron absorption are the ferric form of iron, phytates, phosphates, calcium present in the food, increased serum hepcidin levels, and inflammatory conditions.

1.4. Iron metabolism
Transferrin bound iron is transported to the tissues for storage or utilization e.g., liver, myoglobin and most importantly to the developing erythroblasts present in the bone marrow. Two mechanisms have been described for the incorporation of iron into developing erythroblast. Rhpoeocytosis was previously thought to be the main mechanism for iron incorporation. This mechanism suggests that the developing erythroblasts surround a macrophage which acts as a nurse cell. This macrophage extends its cytoplasmic processes and delivers iron into the erythroblasts by the process of pinocytosis [25]. However, this mechanism has gone into oblivion. The transferrin mechanism is widely accepted for iron entrance into erythroblasts. Transferrin-iron complex binds with the transferrin receptor 1 (Tfr1) present on
erythroblasts. This transferrin-iron complex enters the cell by the process of endocytosis and is enclosed within the endosome in the cell. This endosome is acidic in nature and dissociates the iron and transferrin molecule. Transferrin is released from the cell into the circulation and is reused [9,26]. Iron after dissociation from transferrin is used for the formation of hemoglobin within the cell.

Iron circulates in the body in a closed system from plasma to the marrow for heme synthesis in the red blood cells and also for other metabolic activities. After 120 days, macrophages recycle iron after the engulfment and destruction of old or damaged red blood cells. Hemoglobin is digested, and iron is released into the plasma and reutilized in the iron cycle [15]. Within each cycle, a small proportion of iron is transferred to storage sites to be stored in the form of ferritin or hemosiderin. A small proportion of storage iron is released into the plasma to maintain a normal level of iron pool and a very small amount is lost in urine, sweat, and feces. Iron absorption through intestine is 1 - 2 mg per day which barely matches the normal daily iron loss [20,27,28].

There is no well-established regulatory mechanism of iron excretion from the body. Approximately 1 - 2 mg of iron is lost daily via intestinal sloughing of mucosal and skin cells, menstruation, and other blood losses. The body’s secretions like saliva, intestinal and biliary juices also contain minute amounts of iron. Similarly, a small amount of iron is lost through sweat and urine [4].

1.5. Iron homeostasis

Iron is regulated by two pathways in the body. Systemic iron regulation is the main mechanism that is controlled by hepcidin. Secondly, iron is also maintained at the cellular level in the duodenal enterocytes.

1.5.1. Hepcidin mechanism

Hepcidin is synthesized in the liver. It is regulated by the status of the body’s iron content, hypoxia, anemia, and inflammation. Hepcidin acts as a gatekeeper and controls iron absorption by enterocytes and macrophages. It binds with ferroportin and promotes its phosphorylation, internalization, and degradation [29]. High levels of hepcidin lead to internalization and degradation of ferroportin 1 (as in inflammation or iron overload). As a result, ferroportin loses its function and iron availability from intestinal crypt cells, liver, and macrophages is diminished. In IDA or hypoxia, hepcidin levels are decreased. Consequently, ferroportin mediated release of iron from enterocytes, hepatocytes, and macrophages is increased [16,17,30,31].

In inflammation or iron overload, hepatocytes secrete high amounts of hepcidin. Hepcidin binds with ferroportin (FPN1) and induces FPN1 internalization and degradation. Consequently, the export of iron is decreased from enterocytes and cells fill with iron. These iron-filled cells are shed in the intestinal lumen and are excreted. [1].

1.5.2. Intracellular iron regulation (IRP-IRE system)

Iron at the cellular level is regulated by IRP1 and IRP 2. IRPs are capable of binding with iron regulatory elements (IRE) which are stem-loop structures present on mRNA at either the 3’ or 5’ untranslated regions. The mRNA of DMT1 and transferrin receptors 1 (TFR1) has a 3’ IRE whereas ferritin and ferroportin have a 5’ IRE [8,19,23,29]. When intracellular iron level is high IRP1 contains an iron-sulfur cluster and acts as an enzyme, cystolic aconitase. In this form it converts citrate to isocitrate and has low binding affinity for the 3’ IRE and, thus, inhibits production of DMT1 and TFR1 [15]. It binds with the 5’ IRE and increases the production of ferritin and ferroportin. As a result, iron storage and export are increased. When intracellular iron level is low, IRP1 loses its aconitase activity due to the absence of the iron-sulfur cluster and binds with the 3’ IRE, thus increasing DMT1 and TFR1 production. This increases iron absorption and intracellular iron levels. IRP2 acts in the same way but does not contain the aconitase activity and is degraded when intracellular levels of iron are high [15,23,29].

In IDA, DMT1 and TFR1 levels are increased by binding of IRP with 3’ IRE but ferroportin which is present on the 5’ IRE is not increased by IRE/IRP mechanism. There is another mechanism by which ferroportin is increased. Ferroportin B (FPNB), another ferroportin transcript present in duodenal enterocytes, is not repressed by IRPs, that continuously express ferroportin in IDA [1].

1.6. Causes of IDA

Causes of iron deficiency anemia may be poor intake (diet), increased demand (physiological, or pathological), decreased absorption, and blood loss as shown in the Table 1. Some of the important causes of IDA are described below.

1.6.1. IDA in pregnancy

Anemia in pregnancy is the most common problem. The World Health Organization (WHO) has defined anemia in pregnancy as having hemoglobin levels below 11 g/dL [32]. Anemia in pregnancy is multifactorial, of which IDA is the most prevalent cause. Improper diet, increased demand of the fetus and increased blood volume are some of the causes of IDA in pregnancy. In developing countries, it is the major problem as inadequate diet does not fulfill the increased iron requirement [33,34].

1.6.2. Blood loss

Blood loss is most commonly accompanied with IDA. There are two types of blood loss, i.e., acute and chronic. Both conditions lead to the depletion of body’s iron stores. As a result, subsequent iron is insufficient for restoring iron homeostasis in the individual and leads to the development of IDA. Blood loss may be internal (ulcer or hemorrhage) or external (trauma, surgery)
Table 1. Causes of iron deficiency.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor diet (dietary deficiency)</td>
<td>iron deficient diet, vegetarian diet, poverty</td>
</tr>
<tr>
<td>Increased demand</td>
<td>pregnancy, lactation, adolescence</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>dietary factors (tannins, phytates, calcium), total gastrectomy, malabsorption (celiac disease, tropical sprue, Inflammatory Bowel Syndrome, Helicobacter pylori infection)</td>
</tr>
<tr>
<td>Blood loss</td>
<td>Menorrhagia, parturition (loss during childbirth), intestinal parasites (Necator americanus and Ancylostoma duodenale), recurrent epistaxis, hematuria, GIT bleeding (esophageal varices, esophagitis, gastric ulcer, duodenal ulcer, H. pylori gastritis, chronic gastritis, GIT carcinoma, gastric lymphoma, chronic/recurrent hemoglobinuria)</td>
</tr>
</tbody>
</table>

[35,36].
Acute or sudden blood loss may be associated with surgery, accidents, childbirth, esophageal varices, genitourinary bleeding, gastro-intestinal bleeding, stomach ulcers, and blood vessel rupture. Chronic or gradual blood loss may be observed in parasitic infestation, heavy menstrual bleeding, and gastric and colonic cancers.

1.6.3. Surgical procedures
Surgical procedures, such as post gastrectomy, pancreatic resection, terminal ileal resection, jejunoileal bypass, and intestinal resections cause malabsorption due to removal of a part of intestine which in turn causes malnutritional deficiencies including IDA.

1.6.4. Malabsorption
Malabsorption is characterized by the abnormal function of the gastrointestinal tract leading to the inhibition of nutrient absorption in intestine [37]. Malabsorptive disorders can lead to decreased absorption of iron that may develop IDA [37-40].

1.6.5. Hookworm infestation
Hookworm infestation is also associated with IDA. It afflicts two million people around the world. Nector americanus and Ancylostoma duodenale are the most common soil transmitted helminth that cause anemia. It is frequently found in Sub Saharan Africa and South Asia (Tropical and sub-Tropical areas). In these areas, hookworm infections are widespread due to improper sanitation and contaminated water. Larvae reach the host’s (human) duodenum orally or via skin [36]. Once it enters the gut of the host, it may remain there for several years and release eggs in the stool. Adult worms invade and attach to the mucosa and sub-mucosa of small intestine. This invasion causes damage to the capillaries and arterioles. Adult worms release anticoagulant and ingest extravasated blood. Some of the red cells are lysed and release hemoglobin which is recycled by the parasitic gut proteases. Each hookworm uses 0.03 to 0.15 mL of blood every day from gastrointestinal tract. In moderate to severe infection, patients cannot replace this loss, as a result their iron reserves are depleted and they become anemic [2,41,42].

1.6.6. Helicobacter pylori
Helicobacter pylori is among one of the most causative agents of IDA. It contributes in the development of IDA through gastric ulcers, achlorhydria that impairs iron absorption, and autoimmune gastritis [43].

1.6.7. Iron deficient diet
Iron deficiency anemia may occur due to low intake of iron or poor absorption of dietary iron. Dietary iron comprises heme and non-heme iron. About 25% of iron content is absorbed from food of animal origin whereas only 2 - 5% of iron is absorbed from food of plant origin [5]. In most of the developing countries especially in Asia and South Africa, intake of meat and meat products is very low due to low socio-economic status. In certain communities these foods are prohibited as taboos for religious or cultural reasons. Furthermore, in some regions of the world, there is widespread use of diet that contains strong inhibitors of iron absorption like tea, phytates, calcium supplements, and dairy products [5,33].

1.6.8. Poverty
Anemia, one of the major global nutrition concerns, is caused by deficiency of iron and other nutrients. In developing countries, iron deficiency is a major cause of anemia, apart from vitamin B12, folic acid, vitamin A, and zinc. The greatest number of affected people is from the South-East Asia region [13,36]. Despite various advancements and improvement in health and malnutrition, poverty remains one of the main public health challenges of the 21st century, predominantly in developing countries.

1.7. Clinical features of IDA
With mild to moderate IDA, patients mostly present with no sign and symptoms. Clinical features mostly appear when the disease is severe. The most common presentation is generalized weakness, pallor, dizziness,
koilonychias (spoon-shaped nails). With greater severity the disease may lead to cardiorespiratory failure and ultimately death. Cognitive performance and delay of mental and motor development in children with iron deficiency has been reported in literature [44]. IDA adversely affects both the mother and fetus. Preterm labor and low neonatal weight have been observed in cases of mother with severe IDA [45].

1.8. Laboratory diagnosis
For the diagnosis and differentiation of IDA, a set of laboratory tests may help, including complete blood count, serum iron profile, bone marrow aspiration/iron stain, serum soluble transferrin receptors, and serum hepcidin. Almost all symptomatic patients with IDA show decreased hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and increased red cell distribution width (RDW) [21,46]. Increased immature reticulocyte fraction (IRF) and slightly decreased reticulocyte hemoglobin equivalent (Ret-He) are the hallmark of IDA [47,48]. Peripheral blood smears show microcytic and hypochromic red cells and pencil cells. The iron profile includes serum iron, serum ferritin, total iron binding capacity (TIBC), and transferrin saturation. In non-complicated IDA, all parameters of serum iron profile decrease except TIBC which increases [8,19,45].

These tests have some limitations. Serum iron increases immediately after oral supplementation. Ferritin is an acute phase protein and often does not reflect the true iron stores. Soluble transferrin receptors can be used to differentiate IDA and anemia of chronic disease [49]. IDA leads to markedly increased levels of soluble transferrin receptors. Serum hepcidin has shown a promising role in the diagnosis and differentiation of IDA [50]. Hepcidin decreases in IDA. However, soluble transferrin receptors and hepcidin are not readily available in general clinical laboratories and are of academic interest only. Bone marrow cytology and iron stores were the gold standard for the diagnosis of IDA; however, due to its invasive procedure it has gone into oblivion.

1.9. Management of IDA
Management of IDA includes the diagnosis, identifying the definite cause, and the treatment. IDA’s management is incomplete if the underlying cause is not ruled out and left untreated. Treatment such as giving iron therapy either in the oral or intravenous form and blood transfusion are supportive measures which temporarily control the condition; hence, proper evaluation of the disease is important.

Following is the therapeutic strategy of IDA:
- Dietary intake of iron
- Oral iron therapy
- Parenteral iron administration

1.9.1. Dietary intake of iron
The first and foremost step in correcting IDA is to increase dietary intake of iron. It is important to note the amount of iron present in food and its bioavailability. Spinach, apples, and milk are rich in iron but phytates and phosphates present in these substances inhibit iron absorption, so less amount of iron is available to the body. On the other hand, red meat is a good source of iron as it is a heme iron and is readily absorbed. It is also superior to white meat as it contains high myoglobin content and micro vessels which is a rich source of hemoglobin. Counseling should be done to the patients to have such substances which increase the iron levels in the body. Even though dietary intake of iron could keep the iron homeostasis maintained, it cannot replace the depleted stores of iron especially of females in reproductive age.

1.9.2. Oral iron therapy
The first line of treatment in IDA is oral iron. It is the safest, cheapest, and most effective treatment for IDA. It is present in various forms, i.e., tablets, syrups, capsules, and drops. Oral iron preparations are present in the form of inorganic iron salts. Inorganic salts contain iron in the ferrous form as the ferric form of iron is less absorbed in the duodenum. Three commonly found ferrous salt preparations are ferrous sulphate, ferrous gluconate, and ferrous fumarate. Ferrous sulphate is the most commonly used preparation and the cheapest. 200 mg of ferrous sulphate gives 67 mg of iron whereas 300 mg of ferrous gluconate supplies 36 mg of iron. The required dose to treat IDA in adults is 120 mg of iron/day for three months. After 1 month if there is an increase of 1 g of hemoglobin the diagnosis is confirmed and no further evaluation is needed. If, however, there is no rise in hemoglobin, further investigations should be done. Oral iron should be continued even after correction of anemia in order to replenish the iron stores [51]. For infants and children liquid preparations are used and the recommended dose is 3 - 6 mg/kg/day. The patients should be advised to take oral iron on an empty stomach or 500 mg of vitamin C should be given along with it to increase its absorption.

Gastrointestinal (GI) intolerance that includes nausea, diarrhea, constipation and epigastric pain are the common side-effects of oral iron tablets. Therefore, patients are often non-compliant when being treated with oral iron. Also, it is noteworthy that if a patient is on proton pump inhibitors or had a gastrectomy then there is reduced absorption of oral iron [51].

1.9.3. Parenteral iron therapy
Although, oral iron therapy is the first line of treatment and preferred route of administration, there are certain conditions in which parenteral iron is given. These include non-compliance, gastrointestinal intolerance, patients going for surgeries, malabsorption, iron refractory IDA, ulcerating lesions in GI tract, chronic bleeding in GI tract, patients on renal dialysis, and patients taking

Clin. Lab. 3/2021 5
erythropoietin stimulating agent (ESA) therapy [8,52, 53]. The intravenous iron preparations are present in the form of iron sucrose, iron dextran, and sodium ferric gluconate complex. Iron sucrose is the most commonly used intravenous iron preparation. One 5 mL ampoule of iron sucrose (100 mg) is diluted in 100 mL of 0.9% saline and administered as infusion. The advantage that parenteral iron has over oral iron, is the assurance of iron correction to the desired amount.

The most common side effects are nausea, vomiting, arthralgia, and light-headedness. Iron dextran made up of dextran polymers causes dextran induced hypersensitivity and the rate of hypersensitivity reaction is more than that of iron sucrose and ferric gluconate complex; therefore, its use has lessened [54].

1.10. Treatment of IDA in pregnancy
According to UK guidelines for IDA in pregnancy, anemia is defined as hemoglobin level less than 11 g/dL in first trimester and less than 10.5 g/dL in second and third trimester. According to the international organization, it is recommended that, in areas where prevalence of IDA is high, oral iron supplements are to be started in the third month. The recommended dose is 60 mg of elemental iron/day. Oral iron is mostly given in mild anemia. In moderate to severe anemia intravenous iron in the form of iron sucrose is mostly recommended as non-compliance is also an issue in our part of the world. Research shows its safety while giving in pregnancy. So, giving intravenous iron is safe and also treats the patient with IDA in pregnancy. However, if the hemoglobin level is less than 6 g/dL, then blood transfusion is recommended [51].

Source of Support:
None.

Declaration of Interest:
The authors declare no conflict of interest.

References:


