Chapter

Potential Marker for Diagnosis and Screening of Iron Deficiency Anemia in Children

Yulia Nadar Indrasari, Siti Nurul Hapsari and Muhamad Robiul Fuadi

Abstract

Iron plays a role in multiple physiological functions, naming oxygen transport, gene regulation, DNA synthesis, DNA repair, and brain function. Iron deficiency anemia (IDA) may happen following iron deficiency, but iron deficiency alone may cause negative impacts on the health risk of pediatric patients. The degree of iron deficiency is described by total body iron (measured by ferritin), transport iron (measured by transferrin saturation), serum iron, and other hematologic and biochemical markers. Iron deficiency anemia is a result of insufficient iron supply causing the inability to maintain normal levels of hemoglobin. The most common causes of microcytic anemia in children are iron deficiency and thalassemia minor. There are various hematologic and biochemical parameters used for screening and diagnosis of iron deficiency anemia in children, but there is no single "best" test to diagnose iron deficiency with or without anemia. The "gold standard" for identifying iron deficiency is a direct test-bone marrow biopsy with Prussian blue staining. This article aims to explain iron metabolism in children and discuss the role of hematologic and biochemical parameters for screening and diagnosis of iron deficiency anemia in children.

Keywords: iron deficiency anemia, health risk, children, diagnosis, screening

1. Introduction

Iron deficiency is the most common nutritional deficiency across the world and an important public health problem, particularly in developing countries [1]. Anemia, defined as a low hemoglobin concentration, is a public health problem that affects low-, middle-, and high-income countries, having significant adverse health consequences, as well as adverse impacts not only to the health of citizens, but also to the socio-economic development [2]. The high prevalence of iron deficiency anemia in developing countries most often is attributed to nutritional deficiencies worsened by chronic blood loss due to parasitic infections and malaria. In the industrialized nations, the most common cause of iron deficiency with or without anemia is insufficient dietary iron [3].

Approximately 50% of cases of anemia are considered to be an iron deficiency, but the proportion probably varies among population groups and in different areas,

according to the local conditions [2]. Unfortified complementary foods particularly have a low iron content, iron deficiency, also iron deficiency anemia (IDA) are consequently major public health problems in infants and young children, especially in poor populations [4].

Anemia resulting from iron deficiency adversely affects cognitive and motor development, causes fatigue and low productivity [2]. Iron plays a role in various essential physiological functions, such as oxygen transport, gene regulation, DNA synthesis, DNA repair, and brain function [5]. Iron serves important functions in biochemical processes including the development of the central nervous system, and it is essential to neural myelination, neurotransmitter function, neuronal energy metabolism and neurite differentiation [6].

Many studies have shown an association between iron deficiency anemia and poor neurodevelopment in infants that lasts beyond the period of deficiency [6]. This article aims to explain iron metabolism in children, also discuss the role of hematologic and biochemical parameters for screening and diagnosis of iron deficiency anemia (IDA) in children.

2. Iron requirements, absorption, and metabolism in infants and children

Hemoglobin levels at birth are normally quite high and primarily consist of fetal hemoglobin (HbF or $\alpha_2\gamma_2$), which comprises 80–90% of the total hemoglobin synthesized, gradually decreasing to <1% by 10 months of age in normal infants. The switch from hemoglobin F to adult hemoglobin (HbA or $\alpha_2\beta_2$) begins around 12 weeks of gestation, although the production of hemoglobin A occurs in the bone marrow where it remains throughout the life [7].

Iron requirements in late infancy are higher than during any other period in life due to rapid growth. A unique feature of human iron metabolism is the absence of an excretory pathway and regulation of iron absorption is very important for homeostasis [4]. At birth, most of the body iron is found in the blood hemoglobin, but a term, healthy, normal birth weight infant also has some iron stores, appropriate to about 25% of the total body iron [8].

Knowledge of iron metabolism in infants and children has been enhanced due to recent discoveries of protein and peptides regulating iron absorption. Iron is absorbed in the small intestine by divalent metal transporter 1 (DMT1) and is stored by ferritin inside the mucosal cells or taken by ferroportin to the systemic circulation, while being oxidized by hephaestin to be integrated into transferrin. Hepcidin, a small peptide that is synthesized by the liver, can sense iron stores and regulates iron transport by ferroportin inhibition [7].

2.1 Iron requirements

The majority of iron required by the body is obtained from the reuse of iron released from erythrocyte catabolism. However, sufficient amounts of iron must be supplied by the diet to replace the iron that is lost from the body (through exfoliation of the skin and gastrointestinal cells; and blood loss) and the iron that is needed for growth [9]. At birth, most of the body iron is found in blood hemoglobin, but a term, healthy, normal birth weight infant also has some iron stores, corresponding to about 25% of the total body iron [8].

Healthy, full-term, normal birth weight infants are born with sufficient stores of iron to cover their needs during the first 4–6 months of life [9]. The healthy infant

at term is born with iron stores which can be partially mobilized and utilized for growth during early infancy. In addition to these stores, the high levels of hemoglobin at birth will decrease and the iron will be recycled and also used for growth and blood-volume expansion [7].

The huge demand for iron in the late fetal and early postnatal period is for hemoglobin (Hb) synthesis [10]. Some theories are estimating the iron requirements of infants. Total body iron varies with birthweight and has been estimated to be approximately 268 mg for an infant with a birthweight of 3.5 kg and approximately 183 mg for an infant with a birthweight of 2.5 kg [7].

Premature infants are at high risk of iron deficiency (ID) due to inadequate iron storage caused by the factors of preterm birth, early onset of postnatal erythropoiesis, and rapid growth after birth. There is a lack of a gold standard to describe iron status clinically for healthy preterm infants [10].

2.2 Iron absorption in children and mechanism regulating iron absorption

Iron bioavailability is commonly assumed to be 50% from breast milk and 10% from mixed foods. The stable isotope method can be applied to assess iron absorption in children [4]. Iron homeostasis is mainly controlled through tightly regulated changes in iron absorption in adults. Three "regulators" of iron hemostasis mechanisms have been identified which are referred to as the "erythropoietic regulator", the "stores regulator", and the "dietary regulator" [7].

Iron deficiency and overload are protected by the regulation of these compartments which are integrated to control iron absorption. The store's regulator has a predominant role in maintaining iron homeostasis in response to endogenous iron stores. The dietary regulator may functionally respond to acute changes in iron intake, primarily to prevent iron overload [7].

Iron is absorbed from the diet in primarily the duodenum and jejunum. Iron cannot pass through cellular membrane unassisted. The primary importer of iron across the apical membrane of the intestinal epithelial cell is divalent metal transporter 1 (DMT1, also known as Nramp2, and DCT1). To date, only 1 transmembrane transporter protein, solute carrier family 11, member 2 (Slc11a2, also known as DMT1, is known to have physiological importance in bringing iron into cells. DMT1 is essential for iron absorption, based on a murine study explained that lack the gene encoding DMT1 develop severe IDA. Slc11a2 acts as a proton-dependent iron importer of Fe^{2+}. It can also transport a variety of other divalent metal cations, including $Mn^{2+,}$ $Co^{2+,}$ $Cu^{2+,}$ and Zn^{2+} [11].

Iron homeostasis is regulated at the level of intestinal absorption. Several proteins must synchronize the transfer of iron across the enterocyte and into the systemic circulation. Iron acquired from the diet, is generally in the ferric ($Fe^{3+)}$ state and must be reduced to the ferrous form ($Fe^{2+)}$ before uptake into the enterocyte, presumably by an apical membrane-associated ferric reductase, possibly duodenal cytochrome *b* (Dcytb), aiding the uptake of ferrous iron across the apical membrane into the enterocyte via divalent metal transporter 1 (DMT1) [7]. In adults, the only known transport mechanism for iron from the intestinal lumen into the enterocyte, is DMT1. In the fetal and infant human small intestine, a lactoferrin receptor has been found, and may be important for iron uptake lactoferrin binds most iron in breast milk. The functional importance of the lactoferrin receptor in human infants, is still not yet determined [4].

Hepcidin has an important role in the regulation of iron absorption. The hepatic synthesis of this peptide is induced by high serum iron concentrations and circulating hepcidin leads to decreased expression of ferroportin on the basolateral membrane of enterocytes, thereby blocking the dietary iron transport into the blood. On

the contrary, hepcidin is downregulated in iron deficiency leading to an increase in intestinal iron absorption. It is not yet known whether hepcidin is involved in the significant developmental changes in iron metabolism that occur during the first year of life [4].

Iron homeostasis is primarily regulated at the level of intestinal absorption in adults; thus, the ontogeny of this homeostatic system has developmental consequences. The study from Lőnnerdal and Kelleher explained a hypothesis that the increase in iron absorption that occurs during infancy reflects the maturation of the small intestine iron absorption mechanism to facilitate iron transfer into the systemic circulation [7].

2.3 Iron metabolism

Despite the magnitude of the difference in bioavailability of iron from breast milk and infant formula varies among studies, most investigators agree that iron is absorbed better from breast milk. A major part of iron in breast milk is associated with lactoferrin. Human lactoferrin is absorbed across the apical membrane of the intestinal cell via a specific lactoferrin receptor and internalized with its bound iron. Thus, lactoferrin facilitates a unique mechanism for the absorption of iron from breast milk. The molecular reasons for the lack of homeostasis of iron metabolism in young infants are not yet known. Iron absorption is refractory to hepcidin during the neonatal period, despite intact hepcidin signaling during this period. The mechanism for iron absorption and its regulation is different during early life than in adults, so further research is needed in this area [6].

Transferring transports absorbed iron to the liver, where it is taken up into hepatocytes by transferrin receptors and stored sequestered in ferritin until needed. Iron is released from ferritin and mobilized into the hepatic circulation for further distribution to the tissue, during times of high demand. The regulation of this process is just beginning to be explained, and our concept has been aided by the identification of several genes expressed in the liver that when mutated cause hereditary hemochromatosis, resulting in iron overload. These genes contain those for hepcidin, hemochromatosis protein (HFE), transferrin receptor 2 (TfR2), and hemojuvelin [7].

3. Iron deficiency Anemia (IDA) in children

3.1 Etiology of iron deficiency anemia

Anemia may be caused by decreased RBC production, increased RBC destruction, or blood loss [12]. In developing countries, iron deficiency (ID) and iron deficiency anemia (IDA) typically result from insufficient dietary intake, loss of blood due to intestinal worm colonization, or both. In high-income countries, certain eating habits (e.g., vegetarian diet) and pathologic conditions (e.g., chronic blood loss or malabsorption) are the most common causes [13].

Inadequate intake together with rapid growth, low birth weight and gastrointestinal loss due to excessive consumption of cow's milk are the most common causes of IDA in children. Iron crossing through the placenta is the only source of iron during the intrauterine period. In the final period of pregnancy, the total amount of iron in the fetus is 75 mg/kg. If there is no significant cause of blood loss, physiological anemia begins in the postnatal period and iron stores are sufficient to provide erythropoiesis in the first 6 months of life. Stores are exhausted earlier in babies with perinatal blood loss and in low birth weight infants, since they are

smaller. Improvement of the iron status and reduction of the risk of iron deficiency can be done by delaying umbilical cord clamping [1].

The iron-fortified formula helps ensure adequate iron supplies for infants. However, toddlers often have diets that contain large amounts of cow milk and minimal amounts of iron-rich foods. The risk of iron deficiency may be increased by the early introduction of whole cow milk (before 1 year of age) and consumption of greater whole cow milk after the first year of life. Cow milk is not only low in iron, it also interferes with iron absorption. Cow milk may cause unknown gastrointestinal bleeding in some infants [14].

Adolescent females may become anemic due to menstrual losses. Some children develop anemia due to Meckel diverticulum, chronic epistaxis, or inflammatory bowel disease, which all cause blood loss. Iron is absorbed from the gastrointestinal tract and transported into the blood bound to transferrin. Excess iron is stored primarily in the liver, bone marrow, and spleen as ferritin [14].

3.2 Iron deficiency: clinical classification and clinical findings

Three main body iron compartments describe iron status inadequacy: iron stores, transport iron, and functional iron. Depletion of each component leads to a different iron deficiency stage. Short-term variations in physiologic iron needs are met by the release of iron stores, the majority of which are available as intracellular ferritin, predominantly in hepatocytes and specialized macrophages [15].

Iron deficiency (ID) can be divided into 4 major categories: 1) iron depletion (a state in which the low level of iron affects nonhematologic pathways (e.g., brain, muscle); where microcytic anemia that is classically seen in iron deficiency anemia (IDA) is not found, 2) iron-restricted erythropoiesis (a condition with some impairment of hematologic function without evidence of anemia or microcytosis), 3) IDA (a clinical picture with reduced hemoglobin levels, in which neurodevelopmental and musculoskeletal functions have been inhibited), 4) Functional iron deficiency (a state in which iron stores are adequate but unavailable for biological use). This typical laboratory findings of each category can be seen in **Table 1** [5].

Iron deficiency affects a variety of physiological functions [5]. Iron deficiency refers to the reduction of iron stores that precedes overt iron deficiency anemia or persists without progression. Iron deficiency anemia is a more severe condition in

Laboratory finding	Iron depletion	Iron-restricted erythropoiesis	Iron deficiency anemia	Functional iron deficiency
Hemoglobin	Normal	Normal	Reduced	Normal
MCV	Normal	Normal to reduced	Reduced	Reduced
Serum iron (SI)	Normal	Reduced	Reduced	Normal
Serum ferritin	Reduced	Reduced	Reduced	Normal to elevated
TIBC	Normal	Increased	Increased	Increased
sTfR	Normal	Increased	Increased	Increased
CHr or Ret-He	Normal	Decreased	Decreased	Decreased
Hepcidin	Reduced	Reduced	Reduced	Elevated

MCV: mean corpuscular volume; TIBC: total iron-binding capacity; sTfR: soluble transferrin receptor; CHr or Ret-He: reticulocyte hemoglobin content.

Table 1.
Classification of the iron states and associated laboratory findings.

which low levels of iron are associated with anemia and the presence of microcytic hypochromic red cells [13].

Serum ferritin represents a small fraction of the body's ferritin pool, but the concentration of ferritin reflects the amount of iron stores. Once iron stores are depleted, the first stage of iron deficiency (ID) is reached, namely iron depletion, but there are no erythropoietic consequences yet [15].

The iron supply provided by the transport iron compartment is mainly for red blood cell (RBC) production because the demand of iron for erythropoiesis is much larger than other tissues. The second stage of ID, namely iron-deficiency erythropoiesis, occurs without showing a notable decrease in hemoglobin concentration, when the supply can no longer be met. Laboratory parameters providing information about the adequacy of iron supply are transferrin saturation (TSAT) and the concentrations of erythrocyte protoporphyrin (EP), and soluble transferrin receptor (sTfR). The percentage of binding sites on all transferrin molecules occupied with iron molecules is represented by TSAT, and is calculated as the ratio of serum iron to transferrin or serum iron to total iron-binding capacity (TIBC) [15].

Impairment of the delivery of iron to erythroid is indicated by iron-restricted erythropoiesis, no matter how replete the stores. In cases of anemia of chronic inflammation, stores may be normal or even increased because of iron sequestration, which is observed in patients with autoimmune disorders, infections, and chronic kidney diseases [13]. Common indicator considerations require biological confounding caused by the inflammation. Inflammation is a highly complex biological process, confounding the interpretation of iron status indicators, especially serum ferritin concentration because it increases in response to inflammation as well as to increased iron stores [15].

In uncomplicated IDA (without inflammation response), there is a reduction in iron stores, transport iron, and functional iron. Transferrin production is upregulated to increase iron transport, as soon as the iron supply to erythropoiesis becomes insufficient. Upregulation of transferrin receptor production happens to facilitate iron delivery to cells increasing sTfR, and zinc protoporphyrin (ZPP) is produced instead of heme resulting in an increase of erythrocyte protoporphyrin (EP). Serum ferritin and Hb concentration are important indicators in uncomplicated IDA [15].

The functional iron deficiency is a state of iron-poor erythropoiesis in which there is an insufficient mobilization of iron from stores in the presence of increased demands, as is observed after treatment with erythropoiesis-stimulating agents [13].

3.3 Diagnosis and laboratory findings

A detailed history (anamnesis) of the patient and physical examination is crucial in the diagnosis of all diseases in medical science. A study has shown that a detailed history can diagnose anemia with a sensitivity of 71% and specificity of 79% [16]. Particularly, prenatal period, times of starting breastmilk and solid foods, bleeding history and nutrition should be considered in detail, also signs other systemic diseases and anemia that may accompany [1].

A hemoglobin (Hb) value 5 percentile below the normal hemoglobin value specified for that age or reduced erythrocyte count in healthy individuals is defined as anemia. Anemia should be defined by paying attention to the lower limit of the normal value for different age groups and gender [1]. Hemoglobin concentration is the key indicator for a functionally important iron deficit, specifically iron deficiency anemia (IDA). The hematocrit does not reveal any additional information other than hemoglobin [15]. Based on the size of RBC, hematologists categorize the anemia as macrocytic, normocytic, or microcytic [12].

Anemia in children has a broad differential diagnosis, but it narrows once the anemia is classified further as microcytic. The most common causes of this in children are iron deficiency and thalassemia minor. Microcytosis also results from lead poisoning, chronic diseases (e.g., inflammation, infection, etc.), sideroblastic anemia, and other rare conditions [14].

Reduction in MCV and MCH (mean corpuscular hemoglobin) in a CBC result is a manifestation of reduced hemoglobin in erythrocytes. The erythrocytes are paler and smaller than normal on the peripheral blood smear, (microcytic and hypochromic). MCV and MCH are parallel to each other; meaning erythrocytes may be microcytic and hypochromic at the same time. An MCH below 27 pg. is considered low. The normal value of MCV ranges between 80 and 99 fL, but in children, normal values differ according to age. Laboratory findings in iron deficiency are shown in **Table 2** below [1]. The data from our study (**Table 3**) shows significant differences in hematologic parameters between the β-thalassemia minor and IDA groups. The higher RBC increase in the IDA group compared to the β-thalassemia minor (BTMi) group was probably related to the administration of iron therapy in children with IDA [17].

Differential diagnosis of microcytic hypochromic anemia is very important to consider because the interpretation of its' peripheral blood can be found in iron deficiency anemia and β-thalassemia trait. Iron deficiency and β-thalassemia minor are best differentiated using serum ferritin level, serum iron level, total iron-binding capacity, transferrin saturation, and Hb A2 level, along with a complete blood count (CBC) and examination of peripheral blood film [18]. Carriers of β-thalassemia are usually clinically asymptomatic. However, they have characteristics of the CBCs with mean corpuscular volume (MCV) less than 80 fL and mean corpuscular hemoglobin (MCH) less than 27 p. [19].

Complete blood count:
RDW >14
RBC: low
Hb, Hct: low according to age and gender
MCV: low according to age and gender
When specifying the lower limit of MCV: 70 + age (for >10 years)
(if MCV is <72, generally abnormal)
Upper limit of MCV: 84 + age x 0.6 (for >6 months)
(if MCV is >98, always abnormal)
MCH <27 pg.
MCHC <30%
Thrombocytosis
Rarely: thrombocytopenia, leukopenia
Peripheral blood smear:
Hypochromic
Microcytosis
Anisochromic
Anisocytosis
Pencil cells
Rarely: basophilic stippling, target cells, hyper segmented neutrophils
Serum ferritin <12 ng/mL
*Serum iron <30 mcg/dL
*TIBC >480 mcg/dL
Transferrin saturation (SI/TIBC × 100%) <16%
Mentzer index (MCV/RBC) <13

**May change by age, gender, and other factors. Should be evaluated together.*

Table 2.
Iron deficiency laboratory findings.

Parameter	BTMi (n: 159)		IDA (n: 64)	
	Range	Mean ± SD	Range	Mean ± SD
Hb (g/dL)	4.49–14	8.53 ± 1.62*	5.07–16.3	10.96 ± 2.13*
Hct (%)	13.6–43.8*	27.57 ± 5.06	14.7–51.6*	34.15 ± 6.89
RBC ($\times 10^6/\mu L$)	1.9–6.77*	3.86 ± 0.81	2.06–6.05*	4.46 ± 8.86
MCV (fL)	55.0–99.3	71.95 ± 6.76*	63.4–90.7	76.48 ± 4.85*
MCH (pg)	16.6–31.7	22.34 ± 3.02*	20.0–28.7	24.59 ± 2.11*
MCHC	26.5-34.9	30.96 ± 1.86*	27.6–34.9	32.20 ± 1.45*
RDW-CV (%)	8.3–34*	20.15 ± 4.77	10.5–30.9*	14.6 ± 3.28

*Note: Hb: hemoglobin; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-CV: red cell distribution width-coefficient of variation. *Significant, $p < 0.001$.*

Table 3.
Hematological parameters of the group of β-thalassemia minor (BTMi) and iron deficiency anemia.

Anemia evaluation can be done by an array of tests, but there is no single "best" test to diagnose iron deficiency, with or without anemia. The "gold standard" for identifying iron deficiency is bone marrow biopsy with Prussian blue staining. Since, bone marrow aspiration is an invasive procedure, indirect assays are used for routine use. The laboratory tests that may be used to support and consider the diagnosis of iron deficiency are complete blood count (CBC), peripheral blood smear, reticulocyte, iron profile (SI, TIBC, and transferrin saturation index), sTfR level, and biochemical tests based on iron metabolism (e.g., zinc protoporphyrin-ZPP, serum ferritin concentration) [14]. In CBC, if anemia is present, it should be primarily checked if hemoglobin and hematocrit values are normal for age and gender. In infants younger than 6 months, lower values are observed because of physiological anemia, but hemoglobin levels are not expected to be lower than 9 g/dL in physiological anemia in term infants if there is no other accompanying factor [1].

4. Conclusion

Iron has a role in various essential physiological functions, such as oxygen transport, gene regulation, DNA synthesis, DNA repair, and brain function. Depletion of and inability to use iron disturbs these pathways and causes multiple morbidities. Iron deficiency anemia (IDA) is a well-known complication, but iron deficiency alone may cause negative impacts on the health risk of pediatric patients.

The high prevalence of iron deficiency anemia in developing countries most often is attributed to nutritional deficiencies worsened by chronic blood loss due to parasitic infections and malaria. The differential diagnosis for anemia in children is broad, but it narrows once the anemia is classified further as microcytic. Iron deficiency and thalassemia minor are the most common causes of microcytic anemia in children.

An array of tests can be used for evaluating anemia, but there is no single "best" test to diagnose iron deficiency, with or without anemia. The "gold standard" for identifying iron deficiency is bone marrow biopsy with Prussian blue staining. The laboratory tests that may be used to support and consider the diagnosis of iron deficiency are complete blood count (CBC), peripheral blood smear, reticulocyte, iron profile (SI, TIBC, and transferrin saturation index), sTfR level, and biochemical tests are based on iron metabolism.

Conflict of interest

The authors declare no conflict of interest.

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