Iron- Refractory Iron Deficiency Anemia: Review Article

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Abstract

Background: IRIDA is an inherited recessive anaemia caused by a defect in the TMPRSS6 gene, which codes for the enzyme matriptase-2. This transmembrane serine protease suppresses hepcidin production, an iron regulator (suppressing intestinal absorption of iron). The biochemical results include normal to elevated hepcidin levels, inadequate transferrin saturation, and microcytic hypochromic anaemia. Although it can be identified at a later time, anaemia often manifests after birth. Despite the fact that effectiveness of oral iron therapy can depend on the degree of TMPRSS6 gene mutation. There are other therapies that either partially or totally correct anaemia such as intravenous iron infusions, liposomal oral iron therapy, anti hepcidin antibody and other information discussed later in these article.

Objectives: to examine IRIDA's genesis, diagnosis, and therapy.

Conclusion: When diagnosing microcytic anaemia, it is important to consider the separate disease entity known as iron refractory iron deficiency anaemia.

Keywords: Iron Refractory Iron Deficiency Anaemia(IRIDA); IDA; Iron deficiency; Hepcidin.

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Introduction

Studies indicate that anaemia brought on by a deficiency in iron is the kind that is most frequently observed worldwide, making it one of the most common types of the ailment (Camaschella, 2019).

This specific type of anaemia is caused by both a generalised shortage of iron in the body and the inability to provide the enormous amounts of iron that the bone marrow needs to produce enough red blood cells to maintain tissue oxygenation. A deficiency in iron can have effects other than anaemia. A long list of signs include pallor, shortness of breath, palpitations, and weakness. You can identify the source of your headache, nausea, numbness in your hands and feet, and brittle nails. Particularly throughout the earlier years of childhood (Deloughery, 2017).

The presence of hypochromia and microcytosis will most likely lead to a diagnosis of iron deficiency anaemia (IDA). The inquiry for further differential diagnoses, such as the beta-thalassemia trait, IRIDA, and chronic disease anaemia, should be sparked by a lack of response to iron. [Reference required] [Reference required] The most frequent cause of true iron deficiency anaemia that does not improve with oral iron therapy is celiac disease. Iron-refractory iron deficiency anaemia, or IRIDA, is a kind of iron deficiency anaemia that often does not get better when oral iron treatment is given. Red blood cells in children with IRIDA are unusually small and pale because of the absence of iron in their blood. Microcytic anaemia is the medical term for this illness (hypochromic) (Sanchez, 2013).

Iron absorption and balance

Iron intake, storage, recycling, and release are closely regulated by the systems that govern iron levels in plasma and cells. In order for the body to absorb iron, an enzyme located in the duodenum and upper jejunum must convert the insoluble ferric iron (Fe3+) present in grains and vegetables into the ferrous form (Fe2+). The divalent metal transporter-1 then permits the passage of ferrous iron across the enterocyte membrane. Heme iron, which is plentiful in red meat and more accessible than inorganic iron, appears to be absorbed by a unique chemical route than inorganic iron (Koury, 2019).

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Enterocytes, hepatocytes, and macrophages release extra iron to meet the body's needs when plasma iron levels are inadequate. In contrast, these cells will store iron even when plasma iron levels are high. Iron leaves the body when gastrointestinal cells are destroyed or blood is lost for any reason, such as during hemodialysis. In contrast to the regulation of plasma sodium levels, our systems lack a mechanism for iron removal from the kidney. Thus, the main mechanism for controlling iron levels in plasma is the controlled release of iron from intracellular storage sites (Nemeth, 2006).

Hepcidin

Hepcidin, a hormone composed of 25 amino acids (aa), is essential for regulating the amount of iron eaten and how it is distributed across the body's tissues. Hepcidin is largely produced by hepatocytes, but due to its modest expression in macrophages, adipocytes, and the brain, it is also essential for the autocrine and paracrine control of iron flux at the local level in these cells and tissues (Ganz et al., 2008).

The Workings of the Hepcidin Mechanism

The ferroportin protein performs this function by regulating the export of iron from cells into plasma and extracellular fluid. Ferroportin is the sole known receptor and iron exporter in vertebrates for the hormone hepcidin. Ferroportin is a protein generated exclusively by cells that effectively manage iron. During pregnancy, these cells consist of iron-storing hepatocytes, liver and spleen macrophages that recycle old erythrocytes, and iron-transporting placental trophoblasts. [Donovan et al., 2005]

Also included in this group are the duodenal enterocytes responsible for iron absorption. Ferroportin is also expressed by erythroid progenitor cells. Theoretically, ferroportin improves the sensitivity of erythroid precursor cells to the iron levels in the systemic environment and determines whether precursors will
develop or differentiate. [Zhang, et al., 2009].

In the absence of ferroportin expression, embryonic trophoblasts are incapable of transporting iron from the mother to the embryo, resulting in embryonic death in zebrafish and mouse models. Ferroportin-specific deletion mice with placental ferroportin presented with severe iron deficient anaemia. This was the result of insufficient iron absorption from diet, improper iron release from hepatic storage, and iron-recycling macrophages. [Donovan et al., 2005]

Hepcidin, the ferroportin ligand, modulates ferroportin production, which in turn regulates ferroportin levels post-translationally. When hepcidin binds to ferroportin, the receptor-ligand complex is carried into the cell and removed, (Nemeth et al., 2004).

It is assumed that disulfide exchange occurs between one of the disulfide links of hepcidin and Cys326 of the exofacial ferroportin, resulting in the contact. Individuals with C326S mutations are more susceptible to iron overload, and the mutant ferroportin cannot bind hepcidin in vitro. Lysosomes degrade the hepcidin ferroportin complex after digesting it, preventing the cell from exporting iron.[Fernandes, et al., 2009].

Control of hepcidin levels

(Fig.1) controls the production and release of hepcidin by the liver because hepcidin is an acute phase reactant. Hepcidin and iron homeostasis regulation. (Wallace 2016) Macrophages use a total of eight different proteins to communicate with hepatocytes in order to regulate the release of hepcidin into the circulation. These proteins are as follows: hemojuvelin, hereditary hemochromatosis protein, transferrin receptor 2, bone morphogenic protein 6 (BMP6), matriptase-2, neogenin, BMP receptors, and transferrin.

![Fig.1 Hepcidin control. (Zhao, et al., 2013)](image)

Diagram illustrating the substances and processes involved in regulating hepcidin (HAMP) gene expression in hepatocytes and ferroportin (FPN) surface expression in other cell types. Iron, inflammation, and erythropoiesis are regulated by the substances and mechanisms shown at the top of the diagram. The interaction between hepatocyte-derived hepcidin and FPN regulates iron absorption in duodenal enterocytes and iron recycling in macrophages. Iron circles are red. HJV, hemojuvelin; MT-2, matriptase-2; HFE, hemochromatosis protein; TF, transferrin; TFR1, TF receptor 1

Even with inflammation, severe anaemia is accompanied with low hepcidin levels. Abuga and co. During stress erythropoiesis, erythroferrone inhibits hepcidin and supplies additional iron for the synthesis of haemoglobin. 2019's Koury, Kautz, et al In cell cultures and when given to humans in high concentrations, vitamin D decreases hepcidin. Vitamin D supplementation might enhance hepcidin function. (Bacchetta, 2014)
Iron-refractory iron deficiency anemia

Definition of refractoriness to oral iron therapy - less than 1 g/dl Hb increase after 4 weeks of oral iron therapy, provided patient compliance is adequate and acquired forms of GI disorders have been ruled out (Hershko et al., 2014).

In IRIDA patients, intestinal iron absorption is inadequate (IDA). IRIDA maintains serum concentrations of hepcidin at or above normal levels. Her IRIDA and chronic anaemia resemble high hepcidin levels, demanding cautious diagnosis.

Microcytic hypochromic anaemia, low serum iron, poor transferrin saturation, and normal/high ferritin levels characterise IRIDA. Low haemoglobin, mean cell volume, and mean corpuscular haemoglobin concentration are also present. Hepcidin indicates iron insufficiency and explains a lack of or delayed treatment response. Frequently, children's anaemia is not severe (De Falco et al., 2013).

Acquired IRIDA

Chronic gastritis, which is characterised by stomach hypochlorhydria and H. pylori infection, reduces the conversion of ferric iron to ferrous iron absorption. (Kang, 2012)

Coeliac disease associated with impaired duodenal mucosal iron absorption resulting in a decreased absorptive surface area or superimposed illnesses, such as ulceration, neoplastic diseases, or continuous blood loss. (Yilmaz et al., 2015)

Autoimmune gastritis: the loss of parietal cells decreases stomach acid production, hence decreasing iron absorption (Kulnigg, 2016).

Combined anaemia and inflammation There are a number of factors for iron deficiency and delayed blood loss.

Relation between IRIDA and Corona pandemic

In December 2019, an outbreak of pneumonia of unknown origin was reported in Wuhan, Hubei Province. The worldwide spread of severe acute respiratory syndrome of coronavirus (SARS-CoV-2) and the thousands of deaths caused by it led the World Health Organization to declare a pandemic on 12 March 2020 (Sweta et al., 2021)

Severe COVID-19 appears to be characterized by increase hepcidin level leading to suppression of iron absorption and marked functional iron deficiency, as a response to hypoxia and lymphocyte function. (Girelli et al., 2021)

IRIDA genetics

Iron-refractory iron deficiency anaemia can be induced by a type II transmembrane serine protease produced by the liver and encoded by the TMPRSS6 gene. IRIDA is caused by mutations in the gene encoding matriptase-2, TMPRSS6 (MIM #609862). (MT-2) (Finberg et al., 2008; Folgueras et al., 2008; Du et al., 2008). There are multiple studies confirm that in (Table 1)

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Participants</th>
<th>HAMP gene of hepcidine</th>
<th>Results</th>
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<tbody>
<tr>
<td>Pandey, et al., 2018</td>
<td>Cross section</td>
<td>550 age- and sex-matched healthy controls and 500 IDA patients</td>
<td>Leukocytes from peripheral blood were extracted from total genomic DNA using a kit from Bioserve Frenchtown, NJ, USA.</td>
<td>Hepcidin may have a significant role in iron homeostasis under a variety of pathological circumstances, according to HAMP gene expression changes.</td>
</tr>
<tr>
<td>Finberg, et al., 2008</td>
<td>Cohort study</td>
<td>five multiplex kindreds of families (Turkish, coding areas and intron-exon borders by sequencing, haplotype analysis utilising</td>
<td>Mutations in TMPRSS6 cause IRIDA.</td>
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### An approach to IRIDA diagnosis

Microcytic hypochromic indications include low to normal serum ferritin levels, low transferrin saturation and serum iron levels (TSAT), and abnormally high blood hepcidin levels in relation to the severity of anaemia. Using known metrics to quantify the efficacy of oral iron, oral iron refractoriness mutations in the TMPRSS6 gene that are both heterozygous and homozygous in combination are associated with impaired oral iron function (Heeney et al., 2014).

Due to the similarity between the phenotypes of IRIDA and iron deficiency anaemia, it is likely that the illness will be misdiagnosed or ignored if testing is insufficient and clinical suspicion is absent. Nonetheless, the following minor clinical clues in the patient's history and early evaluation might trigger a differential diagnosis of IRIDA during the initial workup: The presence of anaemia in other siblings or in an older sibling who has been receiving iron deficiency therapy for an extended period without improvement.

a) The emergence of mild-to-moderate anaemia during infancy or early childhood.

b) Extreme hypochromia and microcytosis proportional to the degree of anaemia (Very low MCV and MCH)

d) The lack of organomegaly and the illusive manifestation of classic iron deficiency symptoms, including hair loss, dry skin, koilonychia, and angular cheilitis.

e) Low reticulocyte and high red blood cell count (Thalassemia trait may also have a high RBC count, but this disorder also has a high reticulocyte count, but with iron deficiency, even if the reticulocyte count is low, the RBC count would also be low proportionate to the severity of anaemia) (De Falco., et al 2013)

### Different diagnoses

One of the diagnoses for microcytic anaemia is **Thalassemia trait**: is asymptomatic or clinically manifests as mild anemia as a result of heterozygous mutation of β-globin gene (β thalassemia) or α globin gene (α thalassemia) leading to microcytic hypochromic anaemia refractory to oral iron therapy that can be distinguished by 1) haemoglobin electrophoresis, which demonstrates elevated Hgb A2 and hgb F may be elevated(2-6%) a low globin chain ratio in contrast to a normal result in IRIDA. Beta-Thalassemia is the most

<table>
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<tr>
<td>Luigia et al., 2014</td>
<td>Cross section</td>
<td>21 patients from 16 families</td>
<td>FCT cycle sequencing was used to evaluate the TMPRSS6 gene's exons, exon-intron boundaries, and varied amounts of the 5’ and 3’ flanking region. Patients with IRIDA may have problems with any domain of MT-2, which suggests that the TMPRSS6 gene has to be thoroughly examined for IRIDA-causing mutations.</td>
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<tr>
<td>Erika et al., 2014</td>
<td>Cohort study</td>
<td>113 participants, mean age 36 + 13 years, 12 men, 101 females (range 4–74 years)</td>
<td>PCR and direct sequencing were used to analyse the sequence variation of the TMPRSS6 gene in peripheral lymphomonocyte-isolated genomic DNA. TMPRSS6 polymorphisms may be risk factors in those who are already prone to iron shortage, such as celiac patients and fertile women.</td>
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common genetically inherited hemoglobin disorder in Egypt (El-Beshlawy et al., 2009)

2) RDW values are normal to slightly raised, although in IRIDA and sideroblastic anaemia are high. (Needs et al., 2022)

**Sideroblastic anemias** are characterised by an aberrant use of iron during the erythropoiesis process. Blood films from the periphery reveal the existence of ring sideroblasts, which are erythroid progenitors with non-heme iron deposits in mitochondria that create a ring-like distribution around the nucleus. At least one-third of the nucleus’ rim is covered by the iron ring. Iron levels in sideroblastic anemia patients may range from normal to excessive. (Ashorobi et al., 2022)

**Lead poisoning:** A blood film test may detect red blood cells with basophilic stippling if you have a history of lead poisoning exposure (dots visible on red blood cells under a microscope). Unlike other sideroblastic anemias, a bone marrow biopsy does not reveal ring sideroblasts. (Lewis, 2016)

Blood samples can also be examined for erythrocyte protoporphyrin (EP) to indicate lead exposure. It is known that when blood lead levels are elevated, the EP component of red blood cells increased a few weeks later. (Kosnett, 2005)

**IRIDA therapy**

**In the event of acquired IRIDA: Special care**

H pylori infection: The triple treatment includes of a proton pump inhibitor, clarithromycin, and amoxycillin for 10 to 14 days, or a bismuth-based regimen.

To ensure total eradication, the urease breath test must be administered one month after triple therapy has been completed (Kodama et al., 2008).

No special therapy exists for autoimmune gastritis. Cobalamin supplementation should be evaluated based on serum cobalamin levels. In individuals with autoimmune gastritis and active H pylori infection, it may be helpful to halt disease progression and improve oral iron response (Kang and others, 2012).

After starting a gluten-free diet, people with celiac disease should be monitored often to establish their reaction and compliance (Rubio-Tapia and others, 2013)

**Oral iron treatment** with 6 mg per kilogramme per day with 30 mg of ascorbic acid per day is used to treat partial anaemia for 6–8 weeks (the response to oral iron therapy is depending on TMPRESS6 gene mutation, with a strong mutation resulting in total refractoriness) (Cau 2012)

**Iron infusion treatment**

The Ganzoni formula, which runs as follows: total iron deficiency in mg = [body weight in kg x (target Hb - actual Hb in g/dL) x 0.24] + 500, was once used to calculate the quantity of parenteral iron required (Ganzoni, 1970)

**Repeating the dose based on the patient’s reaction.**

At the time of diagnosis, the mean haemoglobin and ferritin levels of the eleven children with IRIDA were 7.7 g/dL and 4.8 ng/mL, respectively. Six weeks after the initial prescription was administered, haemoglobin and ferritin levels increased to 9.5 g/dL and 24 ng/mL, respectively. Six months after the first treatment and six weeks after the second, ferritin levels continued to climb to 30 ng/mL and 47 ng/mL, respectively, but haemoglobin levels remained unchanged (Akin et al., 2014).

**Future medications** will seek to lower plasma levels of hepcidin. Anti-hepcidin antibodies were found to be beneficial in treating anaemia induced by chronic illness in a mouse model utilising heat-killed Brucella abortus injections. Anticalins® (Pieris AG, Germany), genetically modified lipocalins, having a high affinity for virtually every desired chemical (Sasu et al., 2010). (Hohlbaum et al., 2011)

In addition, the usefulness of siRNA technology (Alnyam, USA) in lowering HAMP mRNA has been proven in preclinical studies (Akinc et al 2011)

**Lysosomal iron therapy**: Liposomal iron, a new technique of oral iron preparation, technologically engineered through micronisation (increasing absorptive surface area) and microencapsulation, helps to overcome the limitation associated with the conventional iron preparations. (Manish et al., 2020)

If siblings develop anaemia, a complete haemogram and targeted mutation sequencing for genetic identification should be performed (Prateek et al., 2017)
Conclusions

Microcytic hypochromic anaemia, poor transferrin saturation, and normal to elevated hepcidin levels are biochemical indicators of IRIDA. Significant correlations were found between TMPRSS6 and serum iron, transferrin saturation, and haemoglobin levels. Resistance to oral iron and a sluggish and variable response to intravenous iron infusions characterise this disease. Parental iron treatment research is beneficial.

Conflicts of interest

Regarding this topic, no possible conflicts of interest were disclosed.

References


Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization.


- **Needs T, Gonzalez-Mosquera LF, Lynch DT. 2022** Beta Thalassemia. [Updated May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-

- **Prateek Bhatia, Richa Jain, Aditya Singh. (2017)** A structured approach to iron refractory iron deficiency anemia (IRIDA) diagnosis (SAID): The more is “SAID” about iron, the less it is, Pediatric Hematology Oncology Journal, Volume 2, Issue 2.


