"EFFICACY OF ALTERNATE DAY VERSUS DAILY ORAL IRON THERAPY IN CHILDREN WITH IRON DEFICIENCY ANEMIA –A RANDOMIZED CONTROL TRIAL"

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LIST OF ABBREVIATIONS

CHr	-	Reticulocyte Hemoglobin content
CMPA	-	Cow milk protein allergy
DMT1	-	Divalent Metal Transporter 1
FA	-	Folic Acid
Fe	-	Iron
FEP	-	Free Erythrocyte Protoporphyrin
GIT	-	Gastrointestinal tract
Hb	-	Hemoglobin
IDA	-	Iron deficiency anemia
IL-6	-	Interleukin 6
MCH	-	Mean corpuscular hemoglobin
MCHC	-	Mean corpuscular hemoglobin concentration
MCV	-	Mean corpuscular volume
NFHS	-	National Family Health Survey
RBC	-	Red blood cell
RCT	-	Randomized control trial
Ret HE	-	Reticulocyte Hemoglobin equivalent
STfR	-	Soluble Transferrin receptor
TIBC	-	Total iron binding capacity
WHO	-	World health organization

ABSTRACT

"EFFICACY OF ALTERNATE DAY VERSUS DAILY ORAL IRON THERAPY IN CHILDREN WITH IRON DEFICIENCY ANEMIA- A RANDOMIZED CONTROL TRIAL"

Background:

Anemia means reduction in the Hb level, RBCs and hematocrit level below the expected normal range, for a given age as well as gender. It is the most important health problem, particularly in the developing countries, affecting approximately 33% of the paediatric population in the world. One of the commonest cause of nutritional anemia in India is IDA. Despite proper oral iron supplementation, optimal outcomes may not always be reached due to poor absorption and low adherence to the treatment. High doses of iron can also cause gut inflammation, which may result from irritation of the gut lining by elevated free iron concentration, leading to treatment failure. Hepcidin is an important regulatory molecule in Fe metabolism. Hepcidin binds to ferroportin, Fe transporter, which is mainly expressed on enterocytes, hepatocytes and macrophages, leading to degradation of ferroportin. High serum hepcidin reduces dietary Fe absorption and recycling of Fe from senescent erythrocytes. Hence the aim for our study is to assess, the effectiveness of alternate day Fe supplementation compared to daily supplementation in children with IDA and to see its effect on serum hepcidin levels and to compare the GI side- effects in the two groups which may affect compliance.

Methodology:

An open labelled RCT was carried out on 40 children, between the age group of 6 months to 10 years with IDA. Patients were randomly assigned into two groups. The first group of patients were subjected to oral Ferrous fumarate preparation of

Iron, (same iron preparation for all the patients) given at 4 mg/kg in 2 divided dosages on daily basis. The second group was subjected to oral Iron therapy at the same dose (4mg/kg) on alternate day as a single administration. Baseline CBC, RBC indices, Fe studies and hepcidin levels were done. Serum hepcidin level was repeated at 48 hours after starting Fe therapy. Change in hemoglobin level was noted after 1 month of intervention. Patients were followed up weekly (telephonically) to assess the side effects due to toxicity or any compliance issues.

Results:

The mean baseline Hb in daily group was 7.38 ± 1.49 g/dl whereas mean in the alternate day oral iron therapy group was 8.11 ± 1.46 g/dl. On follow up after 30 days, there was a statistically significant rise in the Hb level in alternate day group, with a mean rise of 2.3 ± 0.76 (P=0.002). 15 out of 20 children (75%) in the alternate group had decrease in serum hepcidin level after 48 hours, from 5.28 ± 2.87 to 4.1 ± 2.75 ng/ml, which was statistically significant (P value = 0.01). Patients were followed up weekly to assess the side effects and 12 out of 20 patients (60%) in daily group and 4 out of 20 in alternate group (20%) had gastrointestinal issues, such as constipation, abdominal pain and vomiting. The difference was statistically significant (P=0.01).

Conclusion:

Serum hepcidin concentrations were decreased in children undergoing alternate day iron therapy, leading to enhanced iron absorption and adherence with notable improvements in Hb levels. Therefore, for children suffering from IDA, administering alternate day iron supplementation, maximizes iron absorption with fewer gastrointestinal symptoms, and improved compliance, and could be more advantageous schedule.

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INTRODUCTION

Anemia means reduction in the Hb level, RBCs and hematocrit level below the expected normal range, for a given age as well as gender. It is the most important health problem, particularly in the developing countries, affecting approximately 33% of the paediatric population in the world. [(1)] One of the commonest cause of nutritional anemia in India is IDA. [(1)] The 2019-21 National Family Health Survey (NFHS) stated that, 67% of children in India are affected by some form of anemia, with value of Hb being less than 11 g/dl. [(2)] Gujarat has the highest anemia rate for children aged between 6-59 months at 80%, followed by Madhya Pradesh at 73%, and Punjab at 71%. In Karnataka, the projected rate is 65%. [(2)]

Iron deficiency is a global issue, particularly in the realm of public health. [(3)] IDA is microcytic- hypochromic anemia, that occurs because of inadequate amount of iron, available for the production of RBCs. [(4)] This shortage disrupts hemoglobin production in developing red blood cells, resulting in lower levels of circulating hemoglobin, smaller RBCs (microcytic), decrease in the number of RBCs, leading to anemia. This leads to impaired oxygen supply to the tissues, including the brain and muscles. IDA significantly impacts cognitive and physical functioning, hence it is a key factor in the global disease burden. [(4)] The primary causes in children include inadequate nutrition, absorption problems, early introduction of cow's milk, parasitic infections, obesity, maternal iron deficiency, and premature birth. [(5)]

Iron is necessary for various bodily processes, like generating energy, transport of oxygen, supporting immune function, and producing biomolecules. Fe content of the body, is the amount of Fe which is absorbed and utilized from diet. [(6)] This nutrient's homeostasis is established by the equilibrium between its intake and

release from the cells where it is regenerated and stored. A chronic lack of iron, particularly during times of rapid growth and development, can result in significant health problems. [6] IDA can cause haematological as well as non-haematological consequences in children and adolescents. [2]

Gastrointestinal complications often begin with anorexia as a common early symptom. Atrophic glossitis may develop, marked by flattened, diminished lingual papillae, resulting in a smooth, shiny tongue. Difficulty swallowing, or dysphagia, can also occur, sometimes accompanied by esophageal webs, as seen in Kelly Paterson syndrome. [7]

In the central nervous system, symptoms like irritability, tiredness, decreased energy, and impaired cognitive function are frequently observed. Scores on mental and motor development tests may be lower, and attention span may also be reduced. Breath-holding spells and papilledema can also be observed. Iron deficiency anemia also can lead to febrile seizures. [7]

The cardiovascular system may exhibit symptoms such as a higher heart rate during exercise and recovery, along with cardiac hypertrophy. The immune system may be weakened, leading to slower recovery from illness and a higher incidence of respiratory infections. [7]

At the cellular level, red blood cells may experience ineffective erythropoiesis and decreased survival. Cellular growth, as well as DNA, RNA and protein synthesis is also affected. [7]

The management of IDA involves providing the appropriate dosage, type and duration of treatment to normalize hemoglobin levels and replenish the body's iron reserves.

Treatment should also find and address any secondary causes of iron deficiency. Dietary changes may be necessary, and regular follow-up is needed to check how well the treatment is working. (8)

Oral iron therapy is usually the preferred treatment. It is safe, effective, affordable, and helps raise hemoglobin levels quickly when given in the right dose and followed up properly. Parenteral (injected) iron therapy is rarely needed in children.

Ferrous and ferric are the 2 forms of oral iron. Ferrous form (Fe^{+2}) is more easily absorbed by the body. The 3 common Fe^{+2} forms are- Fe^{+2} Sulphate (FS), Fe^{+2} Fumarate (FF), and Fe^{+2} Fluconate (FG) (8). Amount of elemental iron provided by the 3 supplements are 20% , 33%, 12% respectively. The form of Fe, easily absorbed by the human body, is the elemental form. (8)

Oral Ferrous sulfate (FeSO4) supplementation is recommended in the treatment of IDA. (9)

Despite proper oral iron supplementation, optimal outcomes may not always be reached due to poor absorption and low adherence to the treatment. High doses of iron can also cause gut inflammation, which may result from irritation of the gut lining by elevated free iron concentration, negative alterations in the gut microbiota, or both, ultimately leading to treatment failure. (10)

A recent multicentric Randomized controlled trial (RCT) in adults, has reported that the alternate day supplementation of Fe therapy is more efficacious in rising the Hb levels, compared to the daily therapy and this can be attributed to increased hepcidin level even after twice daily doses of iron. (10)

Hepcidin, a hormone made of 25 amino acids, which is synthesized by the liver and enters bloodstream mainly by hepatocytes near the portal vein. [4] It controls the body's iron balance. It attaches to ferroportin which is responsible for transporting iron out of cells, signalling for its degradation. Ferroportin is mainly found on the cell surface in intestines, spleen macrophages, and liver cells. When there is a rise in the hepcidin levels, it reduces iron absorption from food, reduces the recycling of iron from old RBCs, and restricts iron release from liver stores. Chronic high levels of hepcidin can lower iron available for tissues like the bone marrow, impairing RBC production and potentially leading to IDA. Conversely, low hepcidin levels, promote availability of iron for bone marrow to produce Hb and RBCs. Therefore, high hepcidin level, hinder the dietary absorption of iron, and recycling of Fe from aging RBCs. [4]

A recent study found that serum hepcidin levels significantly increase within 24 hours, in children with IDA, following oral Fe therapy, resulting in reduced Fe absorption. [11]

Few studies have been done in adults regarding the same, however there is paucity of clinical trial in children in this regard.

Hence the aim for our study is to assess, the effectiveness of alternate day Fe supplementation compared to daily supplementation in children with IDA and to see its effect on serum hepcidin levels and to compare the GI side- effects in the two groups which may affect compliance.

AIMS AND OBJECTIVES

PRIMARY OBJECTIVE-

- To assess the efficacy of alternate day versus twice daily oral Fe therapy in children with IDA.

SECONDARY OBJECTIVE-

1. Effect of alternate versus twice daily oral Fe therapy on serum hepcidin levels.
2. To compare the GI side effects in the two groups which may affect compliance

REVIEW OF LITERATURE

EPIDEMIOLOGY OF IRON DEFICIENCY ANEMIA-

Anemia impacts around 500 million women, in the age group between 15 and 49 years, and around 269 million children, aged between 6 and 59 months globally. [12] Groups at maximum risk for anemia are- children below 5 years, which includes infants and children less than 2 years of age, adolescent girls, women who are menstruating, as well as pregnant and postpartum women. [12] The most impacted WHO areas are, Africa and South east Asia. In Africa, around 106 million women and 103 million children are impacted, while in South-East Asia, approximately 244 million women and 83 million children are affected. [12]

In 2019, the worldwide incidence of anemia was documented at 39.8%, impacting 269 million children, with estimates ranging from 36.0% to 43.6%. Africa exhibited the highest number of anemia cases among children below 5 years, with rates reaching to 60.2%, fluctuating between 56.6% and 63.7%. The NFHS-5 in 2019-21, showed that the anemia prevalence among men aged between 15-49 years is 25%, whereas for women in the same age group, it is reported at 57%. Regarding adolescents, 31.1% of boys aged 15-19 years and 59.1% of girls have been reported to experience anemia. The prevalence among pregnant women aged between 15-49 years is 52%, while for children aged 6-59 months, it is recorded at 67.1%. [2]

When examining regional disparities, Gujarat has the highest anemia rate for children aged between 6 and 59 months at 80%, followed by Madhya Pradesh at 73%, and Punjab at 71%. In Karnataka, the projected rate is 65%. [2]

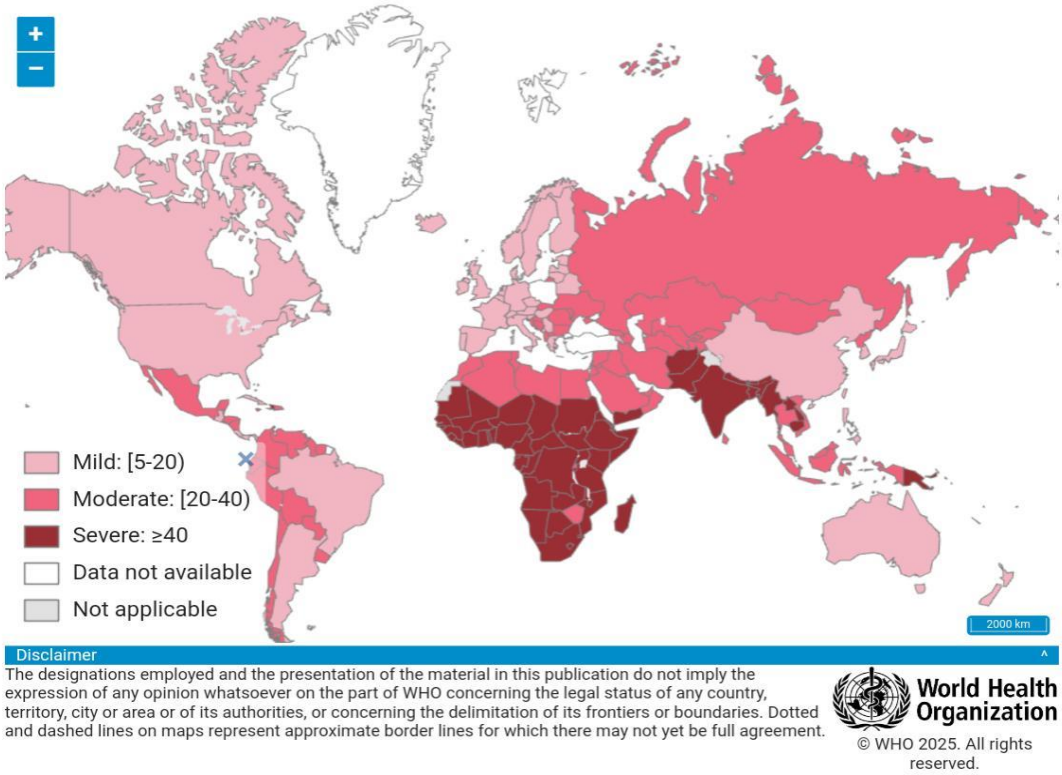
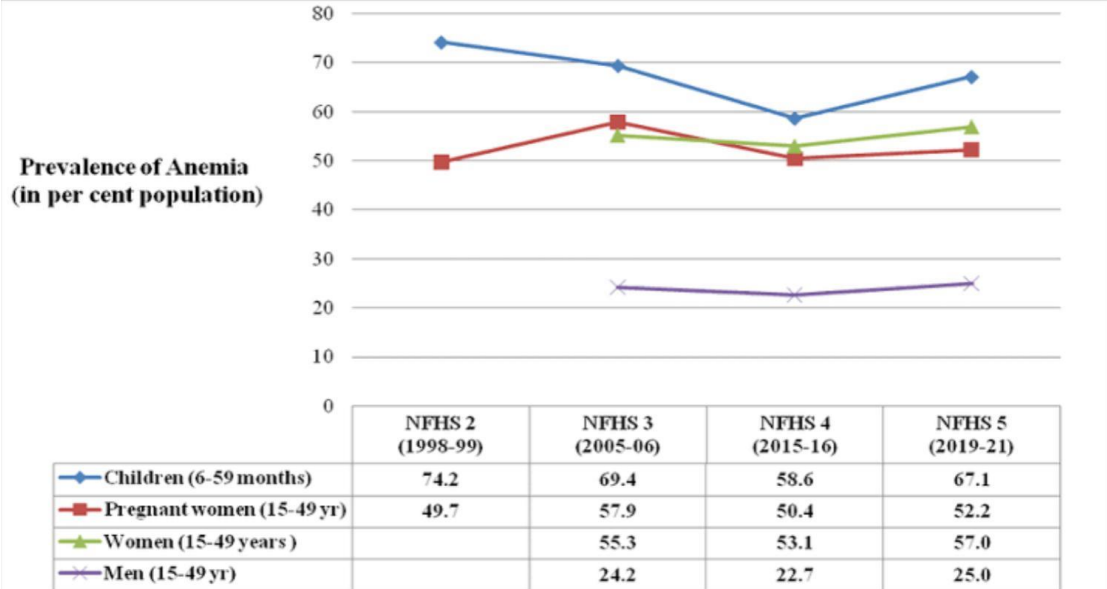


Figure 1- The prevalence of anemia in children, who are aged between 6-59 months in the world according to WHO [(1)]



	NFHS 2 (1998-99)	NFHS 3 (2005-06)	NFHS 4 (2015-16)	NFHS 5 (2019-21)
Children (6-59 months)	74.2	69.4	58.6	67.1
Pregnant women (15-49 yr)	49.7	57.9	50.4	52.2
Women (15-49 years)		55.3	53.1	57.0
Men (15-49 yr)		24.2	22.7	25.0

Figure 2- Prevalence of anemia, according to the NFHS [(2)]

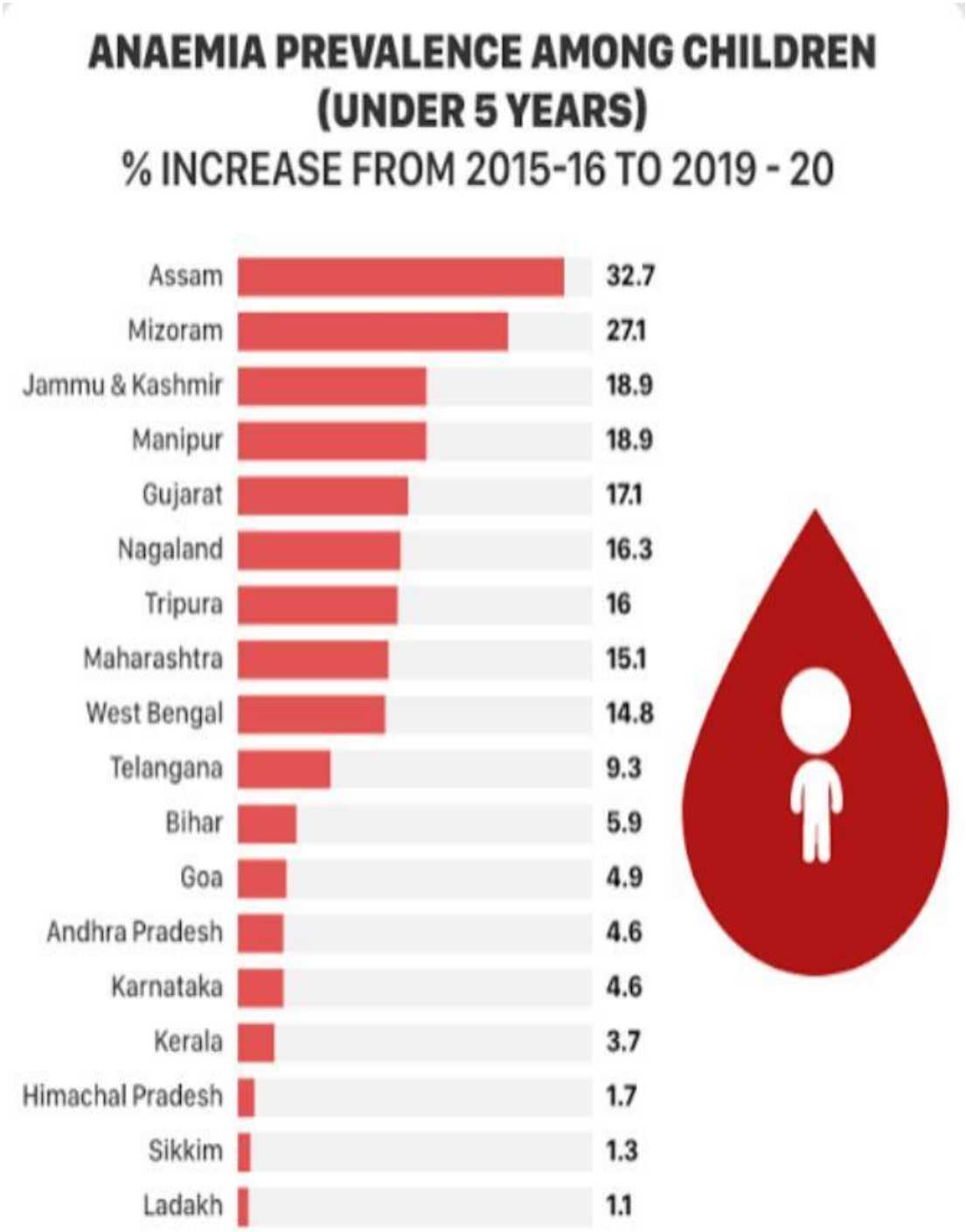


Figure 3- The prevalence in children below 5 years in India

IRON FUNCTIONS-

Iron is vital for enabling the blood to carry oxygen. It is indispensable for a variety of metabolic processes, such as oxygen delivery, cellular growth, and oxidative metabolism. [13]

In humans, iron primarily exists in complex forms that are protein bound, such as Hb (found in RBCs), myoglobin (present in muscles), and enzymes involved in energy production. Approximately 2/3rd of the body's Fe, is stored in Hb. 25% is kept in reserve for later use, and the remaining 15% is found in myoglobin and enzymes that assist with cellular functions. [13-14]

Deficiency of iron happens due to not enough iron intake, poor absorption of iron, or due to infections or blood loss raising the body's iron needs beyond what can be provided by diet alone. Deficiency of iron can be defined as- reduction in the number of the iron stores. If these iron stores are not replenished, it can lead to IDA. [15]

Iron Requirements in Different Age Groups

• Pregnant females	30 mg/day
• Females 11–30 years	15 mg/day
• Adult males	10 mg/day
– Up to 10 years	10 mg/day
• Full-term infants	1 mg/kg/day from 4 months of age
• Low birth weight babies	2 mg/kg/day from 2 months of age
• Babies < 1000 g	4 mg/kg/day from 2 months of age
– 1000–1500 g	3 mg/kg/day from 2 months of age

Figure 4- Iron requirements in different age groups [5]

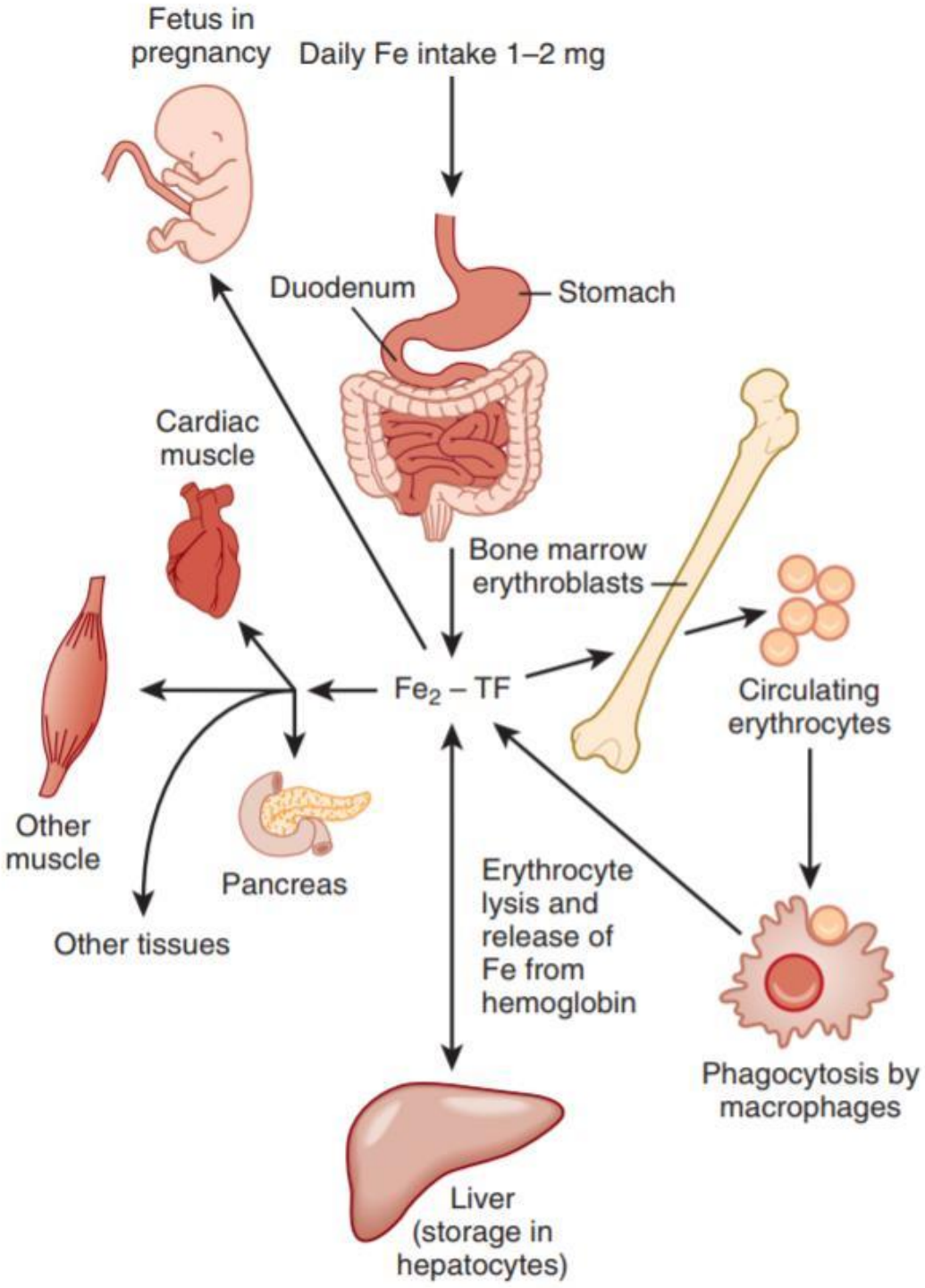


Figure 5- Fe homeostasis [16]

ABSORPTION OF Fe-

Fe absorption, takes place from the 1st part our small intestine (SI), that is the duodenum. Fe absorption depends on the form it enters the duodenum. At normal body pH, iron changes from a form that is easily absorbed (ferrous) to one that is less soluble (ferric). The stomach acid helps lower the pH in the duodenum, making iron easier to absorb. [14, 16] Heme iron, present in meat, is much better absorbed compared to the nonheme iron (present in plant sources). It doesn't depend on the pH of the duodenum. Meat is a great source of iron because of this. [16]

Certain foods can either enhance or impair iron absorption. Vitamin C (ascorbate) and citrate increase iron absorption by helping to dissolve iron in the duodenum. On the other hand, foods like bran, tannins, and phytates found in plants can block iron absorption by binding to it and preventing it from being absorbed. [17,18]

Iron enters the duodenum, in the ferric iron form (Fe3+). This ferric form is changed into a more absorbable form by the enzyme, Duodenal Cytochrome- B, present on the cells surface in the duodenum. This is mostly found in the upper part of the duodenum. Its level increases when there is decrease Fe content in the body. [19] Once Fe is in the Fe^{+2} form, it enters into the enterocytes with the help of a transporter called DMT1. This transporter works by moving protons (positively charged particles) along with the iron into the cells at the same time, creating an electrical gradient. Although DMT1 is found in many parts of the body, its levels in the duodenum increase a lot when the body is low in iron. [20]

DMT1 can also transport other metal ions besides iron, such as lead, manganese, cobalt, copper, and zinc. Studies show that these metals can use the same pathway as iron for absorption in the intestine. When iron absorption increases due to iron

deficiency, the absorption of these other metals also increases. This is particularly concerning for children, as iron deficiency often occurs along with lead poisoning, leading to serious health problems. However, copper is absorbed through a different process. [16] After Fe is absorbed into the enterocyte via DMT1, it may either remain within the cell and be discarded when the cell dies and is exfoliated, or it can be moved out of the cell into the bloodstream. Iron that stays in the cell is used for the cell's needs or stored in ferritin. The iron that leaves the cell is transported out through a special iron transporter called ferroportin. This process also requires the oxidation of iron, which is likely done by a protein called hephaestin, similar to ceruloplasmin found in the blood. [16] Usually only 10% of dietary nonheme Fe is absorbed in the small intestine, but this is increased in IDA. Excess iron reduces absorption, but doesn't stop it entirely, showing that the body regulates iron absorption based on its iron stores. This regulation has been called the "stores regulator." Conditions like iron deficiency anemia and anemia from ineffective RBC production also lead to increase in iron absorption, a phenomenon called the "erythroid regulator." Additionally, low oxygen levels (hypoxia) can increase iron absorption, even if there is no anemia. [14]

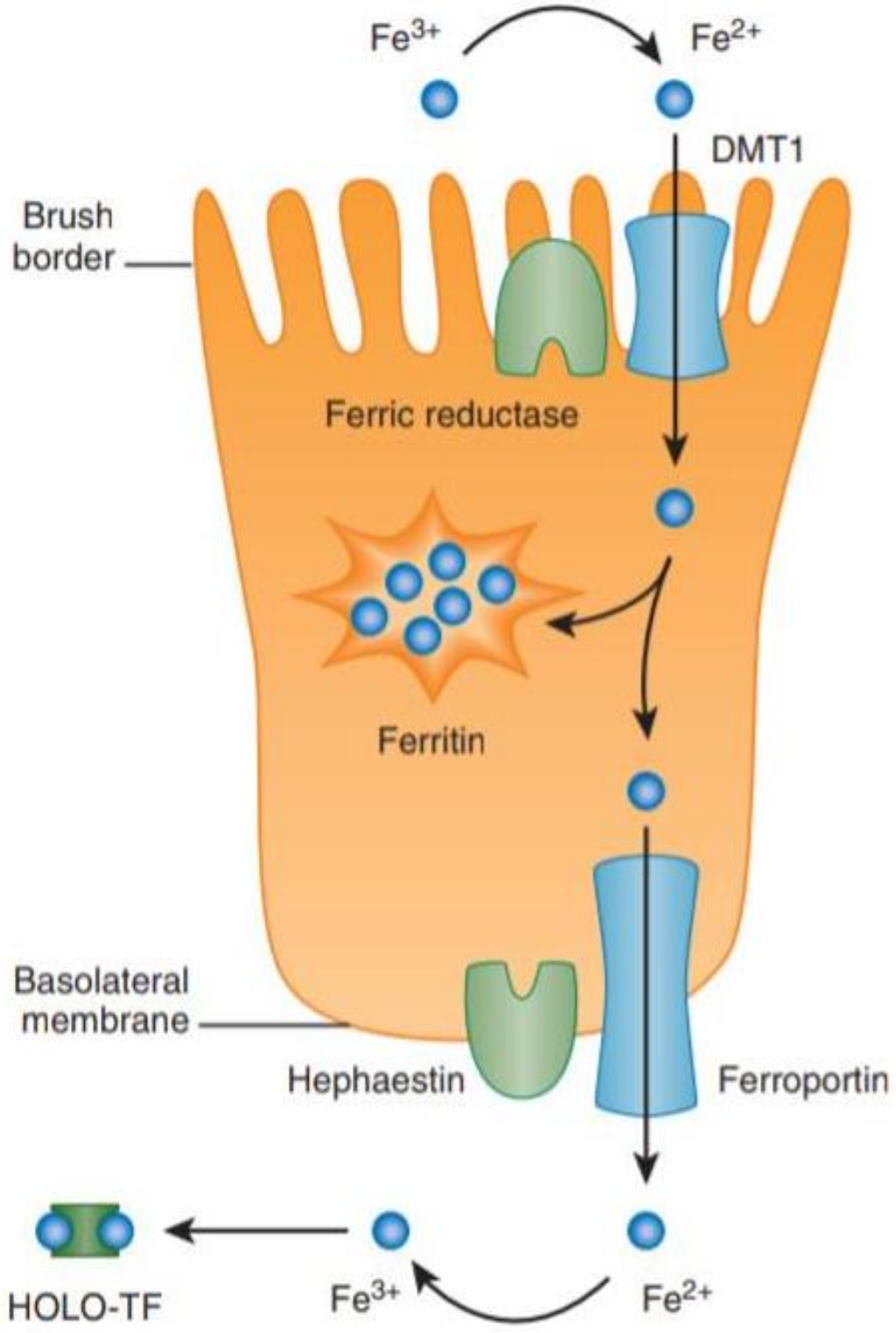


Figure 6- Duodenal Iron Transfer [16]

INTERCELLULAR TRANSPORT OF IRON

A small part of the body's Fe, is either absorbed or lost daily. As a result, the movement of iron between cells is more crucial than the absorption of Fe from the intestines. The majority of Fe is stored in RBCs, accounting for about 60% to 80% of the complete iron, in a healthy body. The body, recycles a significant amount of iron from old RBCs, which supports production of new Hb. [16]

About 0.1% (4 mg) of the total Fe is present in blood, where it is attached to a protein called Transferrin. Transferrin has three key roles: [16]

(1) It keeps iron in a soluble form in the body

(2) It protects cells from iron-related damage

(3) It helps transport iron inside the cell.

Transferrin, the primary transporter of iron to red blood cells, supplies Fe to various tissues throughout the body. It is a glycoprotein, with two sites that can bind iron. While the liver being the main organ is responsible for producing and releasing transferrin, other tissues such as Sertoli cells in the testes, oligodendrocytes in the brain, lymphocytes, muscle cells, and mammary cells are also capable of producing it [16]

Activated T lymphocytes also produce transferrin. When at rest, mature T cells don't make transferrin or have transferrin receptors on their surface, but after being stimulated, they start producing both. Only CD4+ helper T cells make transferrin, and the production of transferrin and its receptor happens before cell division, possibly as part of a self-regulating loop. [16]

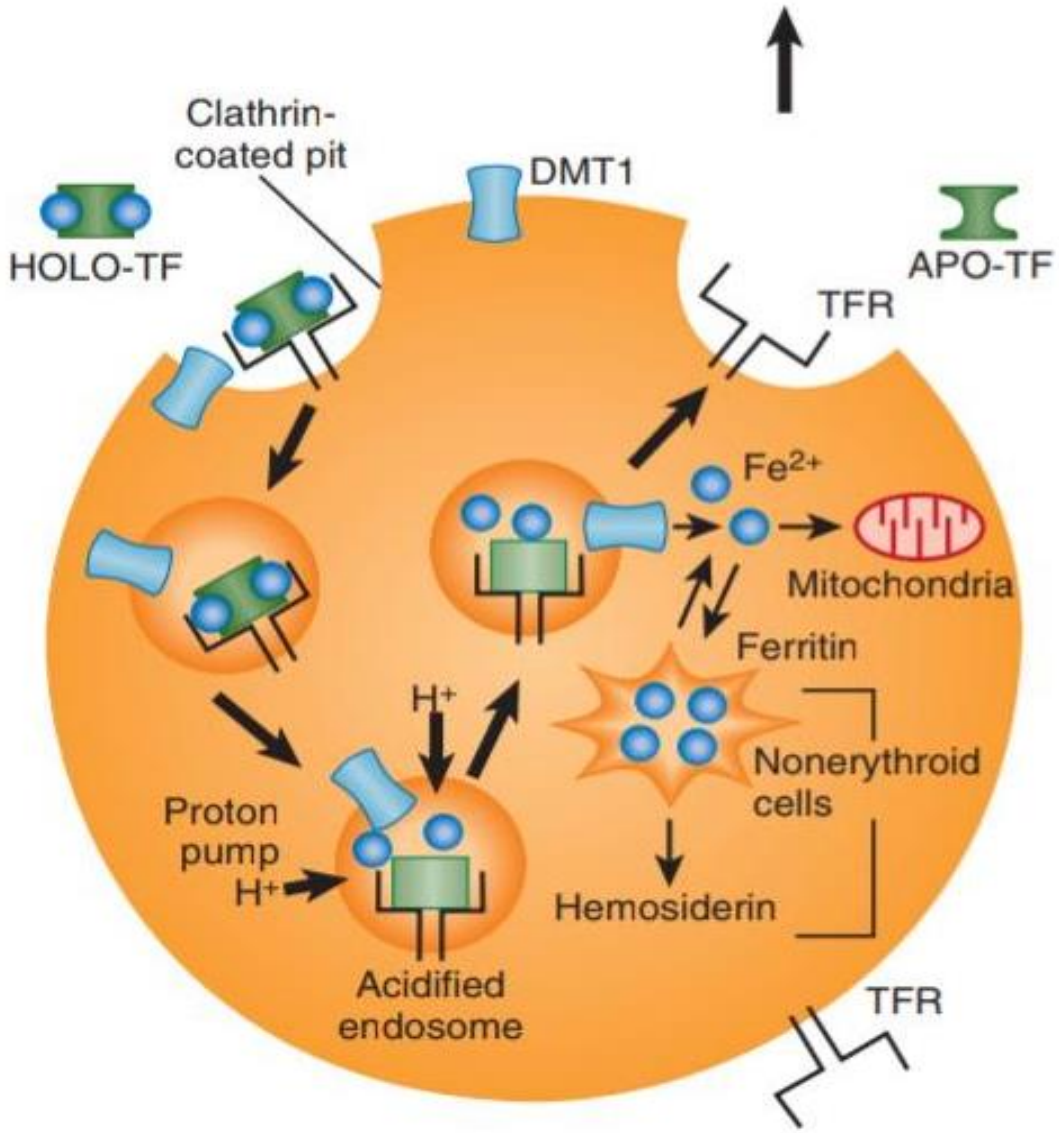


Figure 7-The endocytotic transferrin cycle [(16)]

HEPCIDIN-

Hepcidin controls the body's Fe balance. This 25 amino acid peptide hormone, prevents Fe from entering the bloodstream. Hepcidin is primarily produced in the hepatocytes, because of their presence near the Portal vein, their function in storage of Fe, and their closeness to the Kupffer cells, which detects pathogens and recycle RBCs. [4]

Hepcidin regulates iron from three key sources: absorption in the duodenum, stored Fe in hepatocytes, and recycled Fe present in the macrophages. The production of hepcidin is controlled by various signals related to iron levels, red blood cell production, and immune activity, which influence iron balance. [21-24]

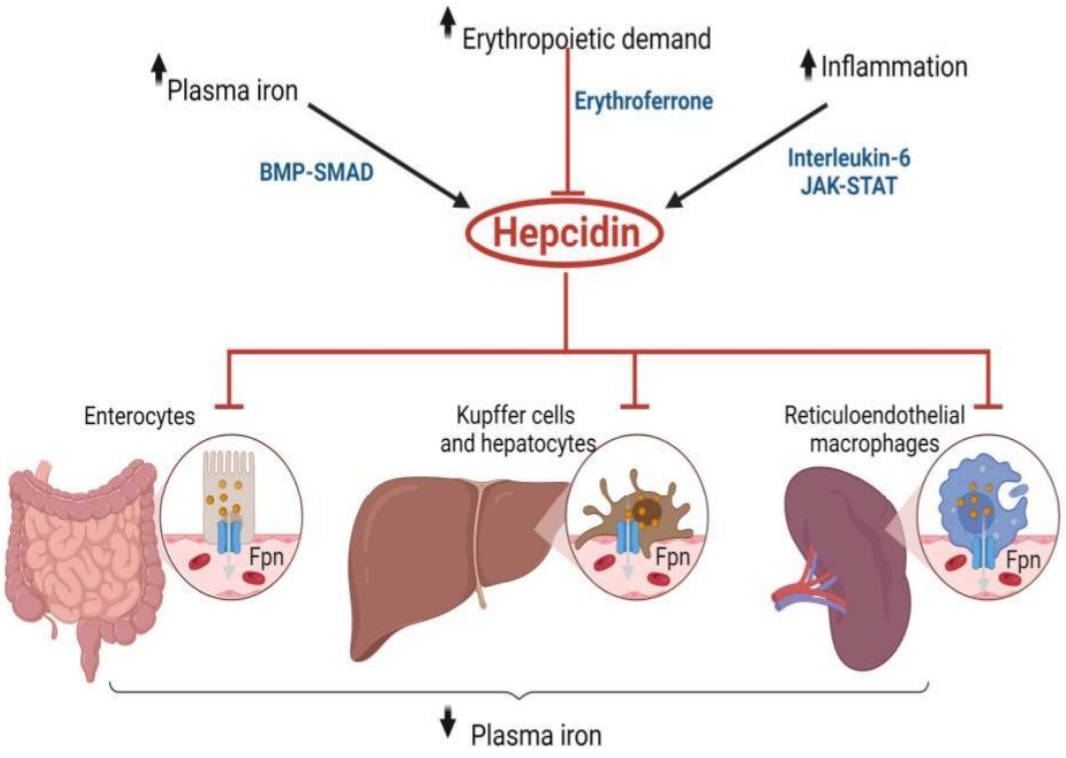


Figure 8- Systemic homeostasis of iron by Hepcidin [4]

In bloodstream, hepcidin attaches to ferroportin, responsible for transport of iron, outside the cells. Ferroportin is found in enterocytes of the intestine, splenic macrophages and liver cells. When hepcidin attaches to ferroportin, it signals the cell to destroy it, which reduces the release of iron.

Ferroportin, present on the baso-lateral membrane of the enterocytes, in duodenum, while absorption of iron occurs through the apical surface. [21-24]

Fe absorption is also regulated by the presence of a transporter called DMT-1, which adjusts according to body's iron status. To synchronize iron absorption with its export into the bloodstream, communication between the hepcidin's effect on basolateral Ferroportin, with apical surface must occur. There are three potential mechanisms that could be involved in this process.

1. When hepcidin attaches to ferroportin, it leads to iron accumulation within the cells, which in turn deactivates iron regulatory proteins (IRP-1, IRP-2). As a result, these proteins are unable to stabilize the mRNA of DMT1, reducing the production of DMT1.

2. Increased cellular iron activates prolyl hydroxylases, which degrade hypoxia-inducible factor HIF-2α, reducing DMT1 gene expression.

3. Hepcidin binding to ferroportin may activate ubiquitin ligases, which move through the cytoplasm, tag DMT1 for degradation, and possibly affect other iron transporters.

Hence, hepcidin decreases the absorption of Fe from food, limits the recycling of Fe from old RBCs and restricts release of iron stored in the liver.

If hepcidin levels remain high, it can limit iron supply to important areas like the bone marrow, reducing iron for making RBCs and leading to IDA.

Hepcidin was first discovered in 1998 as a peptide with antimicrobial properties, and its role in iron regulation was found shortly after.

- The main pathway for hepcidin gene transcription is influenced by iron levels and the BMP–SMAD signaling pathway.

When more iron enters the blood, liver cells detect iron through transferrin-bound iron and a complex of proteins, including the transferrin receptor (TfR), hemojuvelin (HJV), and HFE.

At the same time, liver cells release BMPs in response to iron, which bind to receptors on hepatocytes. This binding helps activate a complex that triggers the phosphorylation of the SMAD proteins.

These proteins move towards the nucleus and promote the expression of the hepcidin gene (HAMP). Since humans cannot easily excrete excess iron, the BMP–SMAD signaling pathway reduces Fe absorption from food and stores it in tissues to prevent too much iron in the bloodstream. Disruptions in this pathway, such as HFE gene mutations, can result in a reduced hepcidin response and iron overload, as seen in hereditary hemochromatosis.

Hepcidin is also inhibited when iron requirement increases, like during RBC production. This inhibition is thought to be driven by erythroferrone, a hormone released by developing red blood cells, which may block the BMP–SMAD pathway that normally activates hepcidin. [25-31]

A second pathway for hepcidin transcription is triggered by systemic infection through the JAK–STAT pathway. This pathway highlights hepcidin's role as an antimicrobial peptide.

During infection, hepcidin reduces circulating iron to limit iron availability for pathogens. When there is infection or tissue damage, immune cells, including macrophages, release IL-6. It activates the acute phase response and binds to the interleukin receptor on liver cells, triggering the JAK–STAT pathway to increase hepcidin production.

IL-6 is the main trigger for hepcidin during infection, but other inflammatory pathways can also increase hepcidin levels.

In conditions like anemia of inflammation or chronic disease, long-term inflammation can override the usual suppression of hepcidin, leading to iron-restricted erythropoiesis. IL-6-induced hepcidin also plays a role in iron declines seen with physical activity and in people with obesity.

The hepcidin response to inflammation, even without infection, might help remove iron from circulation to prevent infection or could be an unintended result of the immune response. [32, 33]

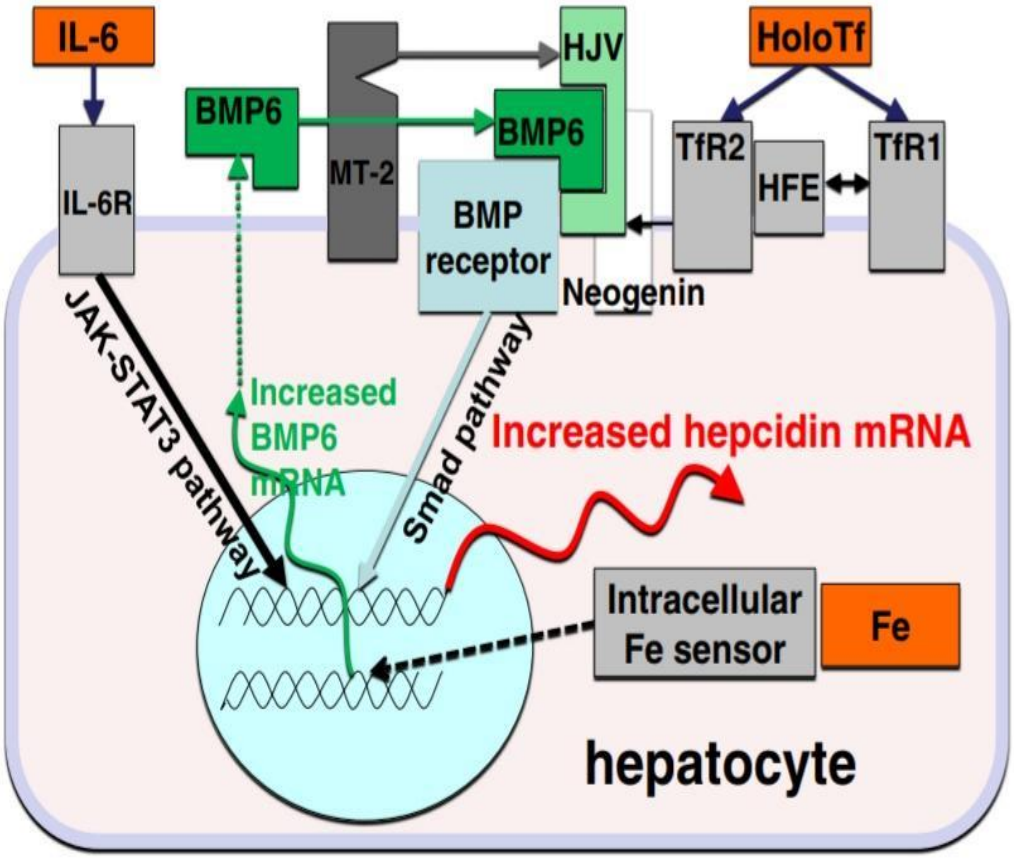


Figure 9- Molecular mechanism of hepcidin regulation.

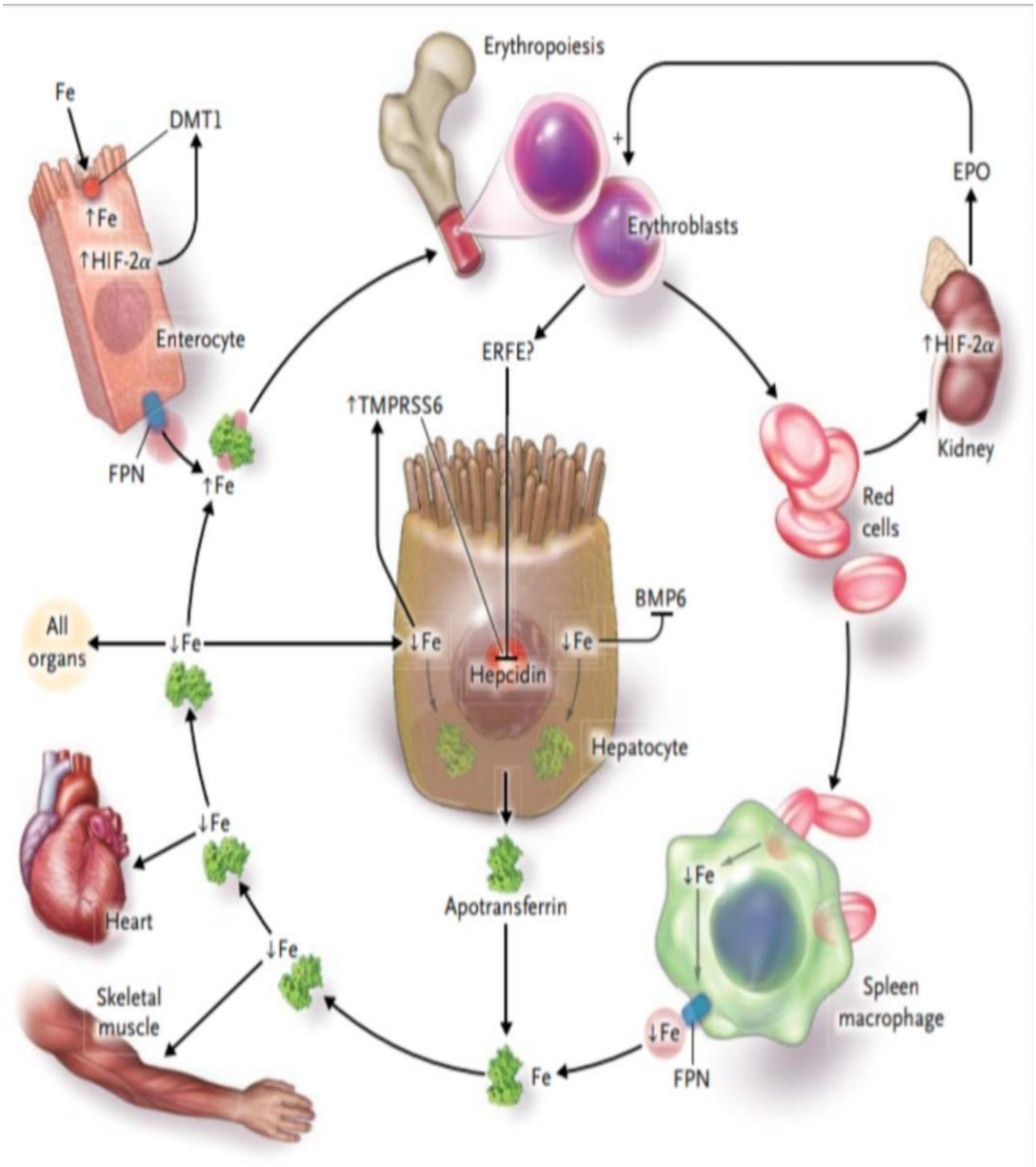


Figure 10- Mechanism of adaptation to IDA [(13)]

IRON DEFICIENCY ANEMIA-

IDA presents in 3 stages:

1. **Pre latent stage**: This phase is characterized by low iron stores in tissues, yet no noticeable changes in blood parameters such as hematocrit or the serum Fe levels. It can be identified by low serum ferritin levels.

2. **Latent Fe deficiency**: During this stage, the iron stored in macrophages is depleted. Blood tests reveal low serum Fe levels and high TIBC. RBC production (erythropoiesis) is restricted due to less iron, and markers such as sTfR and CHr may show alterations. This stage may not be easily detected through routine blood tests, as most red blood cells appear normal.

3. **IDA**: This is the most advanced and severe stage. RBCs become smaller (microcytic), and paler (hypochromic) due to prolonged iron deficiency, affecting the majority of RBCs being produced.

ETIOLOGY- IDA occurs due to insufficient intake of iron, increased iron demand, or due to loss of blood.

- **Decreased intake of iron**:

1. From dietary Sources: The heme Fe, found in animal food, is the easiest type of Fe for the body to absorb. It is absorbed no matter the stomach's pH level. In areas where people eat little or no meat and rely on plant foods like rice, which have less Fe, IDA is common. [16]

   In developing nations, IDA is worsened due to loss of blood from parasitic infections or infection like malaria. In developed nations, IDA typically occurs

due to improper consumption of iron-rich foods to satisfy the body's needs. Cow's milk can also lead to IDA. Milk of both cow and human contain less Fe, but the Fe present in human's milk is more easily absorbed by the body. Toddlers who switch to cow's milk from human milk, may have an increased risk of developing IDA. Cow's milk can supplant Fe rich foods in the diet, and specific components of cow's milk can hinder the absorption of Fe. [34]

Additionally, cow's milk may irritate the stomach, which can lead to minor blood loss resulting in Fe deficiency. Infants require a significant amount of iron for growth, making it less suitable. Infants who are on exclusive breastfeeding with cow's milk protein allergy (CMPA), symptoms are generally mild, and most infants do not exhibit anemia or failure to thrive. [34] Such infants should be exclusively breastfed for at least 6 months, and the mother should eliminate all dairy products, including milk, yoghurt, cheese, ghee, butter, and foods containing milk, from her diet. [34] It can take up to 72 hours for the antigens to exit the breast milk and for symptoms to improve following the removal of milk and dairy products. [34] Elimination diet of mother should last three to six days for infants with allergies which are IgE mediated; for non-IgE mediated ones, the duration should be two weeks for infants without atopy, four weeks for those with atopic dermatitis or allergic colitis. [34] If symptoms persist beyond this timeframe, alternative potential causes should be explored. [34] If symptoms improve or resolve, cow's milk protein can be reintroduced into the mother's diet to assess tolerance. [34] If the symptoms reappear, the mother should refrain from consuming cow's milk protein while breastfeeding. Calcium supplements of 1000 mg/day in divided doses, is essential for the mother during this period. [34] Restrictive diets that

exclude milk, fish, wheat, soy, and gluten can result in nutritional imbalances and should be avoided. [34]

In infants on mixed feeds, all CMP and unmodified animal milk proteins (from goat, sheep, buffalo, or camel) should be removed from the infant's diet. However, breastfeeding should continue without changes to the mother's diet. For infants under 6 months old with mild to moderate reactions, an extensively hydrolyzed formula (eHF) is recommended. Soy formula is not recommended for infants under 6 months due to safety concerns and the risk of cross-allergy in 10-15% of infants with CMPA. However, soy is cheaper and more palatable than eHF. Children with IgE-mediated CMPA usually tolerate soy protein better than non-IgE mediated CMPA. For infants over 6 months with mild to moderate reactions, soy protein formula can be used instead of eHF if there are financial concerns. If there is no improvement after 2 weeks of eHF, an amino acid formula (AAF) should be tried before CMPA is ruled out. In sick or severely symptomatic infants, AAF should be used first instead of eHF. [34]

In exclusively formula fed infants, if breastfeeding was recently stopped, it should be resumed. [34]

2. Poor bioavailability –

Iron is abundant in the environment, but it is not easily absorbed by the body. Most of the iron in the environment exists as insoluble salts. Gastric acid helps convert iron into a form that the body can absorb, but this process is not very efficient. Many plant foods contain iron, but the body often has trouble absorbing it because it is poorly soluble. Additionally, natural substances in plants, like phytates (found in wheat), can bind to iron and make it harder for

the body to absorb. High stomach acidity is needed for iron to dissolve properly. Conditions like stomach surgeries or the use of certain medications (such as H2 blockers or proton pump inhibitors) can lower stomach acid and make it harder to absorb iron.Environmental factors, like certain metals (e.g., cobalt) and lead, can interfere with iron absorption. Iron deficiency can increase the body's ability to absorb both iron and lead from the gut. Iron deficiency and lead poisoning often happen together.

3. Malabsorption occurs when certain disorders damage the intestines, resulting in decreased absorption.

   Crohn's disease (CD), which is a type of Inflammatory Bowel disease (IBD), can damage parts of the intestine, mainly the jejunum and the duodenum. This damage disrupts the tissue structure, decreasing absorption of iron and other nutrients. Occult (hidden) gastrointestinal bleeding can make the problem worse, leading to IDA and the Anemia of chronic inflammation. CD often affects the terminal ileum, which can also lead to a cobalamin (vitamin B12) deficiency. Treatment focuses on reducing inflammation. [16]

   Tropical sprue and Celiac disease can also disrupt absorption of iron. Both conditions cause damage to the intestinal lining and chronic inflammation, leading to poor absorption of nutrients. Some people with these conditions may not show visible or microscopic damage to the bowel, but still experience iron deficiency anemia and general nutritional deficiencies. For people with celiac disease, consumption of a gluten-free diet can improve both the anemia and overall health. [16]

Surgery that removes parts of the bowel, especially the duodenum, can also affect iron absorption. Conditions like severe inflammatory bowel disease, abdominal injuries, or structural problems such as intestinal volvulus or intussusception may require surgical removal of part of the intestine. Iron deficiency may develop slowly and may not be noticeable for several years after surgery. (16)

Iron malabsorption is rare unless there are significant structural issues in the intestines or the anemia of chronic inflammation. However, some people inherit a genetic condition called iron-refractory iron deficiency anemia (IRIDA). These individuals have severe iron deficiency anemia from an early age, and their bodies don't respond well to iron supplements or iron injections. Iron-deficiency anemia is defined as "refractory" when there is an absence of hematologic response (an increase of <1 g of hemoglobin) after 4 to 6 weeks of treatment with oral iron. This condition is caused by mutations in the TMPRSS6 gene, which encodes a transmembrane serine protease (also known as matriptase-2) expressed by the liver. which leads to too much hepcidin (a protein that regulates iron absorption), preventing iron from being absorbed properly. (16)

Mutations in DMT1, another protein involved in iron absorption, can also cause a similar condition, but these individuals typically have iron deficiency anemia with a buildup of iron in the body.

- Systemic Fe Loss

Gastrointestinal blood loss is one of the main reason for IDA worldwide. The gastrointestinal tract is where iron is absorbed, but it is also the commononest

site of blood loss, especially when the bleeding is not visible. If iron deficiency cannot be explained by other causes, blood loss from other areas should also be considered. [16]

Several gastrointestinal tract defects can cause blood loss and lead to iron deficiency:

Meckel's diverticulum is a common congenital defect caused by a persistent connection between the intestine and the umbilical cord. It can cause abdominal pain and, sometimes, intestinal blockage in young children. Adolescents with this condition may experience hidden blood loss, leading to iron deficiency.

Peptic ulcer disease is rare in children but should be considered if the clinical situation suggests it. The most common cause of gastrointestinal blood loss worldwide is parasitic infestations:

Hookworm infections, caused by Necator americanus or Ancylostoma duodenale, are common in tropical and subtropical areas. These infections are often without symptoms, but small blood loss from the intestines can lead to significant iron deficiency, especially in children. The larvae enter the body through the skin, usually from soil.

Whipworm infections, caused by Trichuris trichiura, affect 600 to 700 million people, especially children aged 2 to 10. While only 10% to 15% of these individuals show symptoms, heavy infestations can cause growth retardation and iron deficiency. [16]

CLINICAL FEATURES -

IDA is not just a blood condition, but it affects the entire body. The symptoms depend on several factors, including

Common symptoms include:

- Pallor (paleness)
- Tiredness and weakness
- Easy fatigue
- Loss of appetite (anorexia)
- Lack of concentration
- Shortness of breath
- Irritability
- Swelling in the feet (edema)

In mild anemia, pallor might not be present. In some cases, other symptoms like jaundice (yellowing of the skin), bluish color, or dark pigmentation can hide the pallor.

As anemia worsens, the body adjusts in other ways:

- Hyperdynamic circulation, which can cause palpitations, shortness of breath, and heart failure.
- Epithelial changes: These include brittle spoon shaped nails (koilonychia), flat nails (platynychia), tongue changes (atrophic glossitis), and cracks present at the corner of the mouth (angular cheilosis).

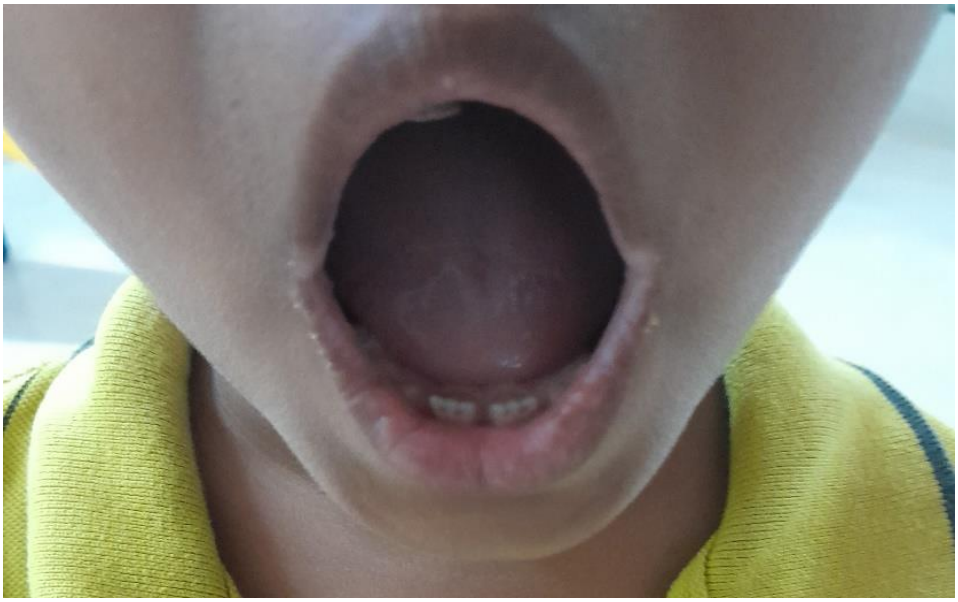


Figure 11- Angular Cheilosis

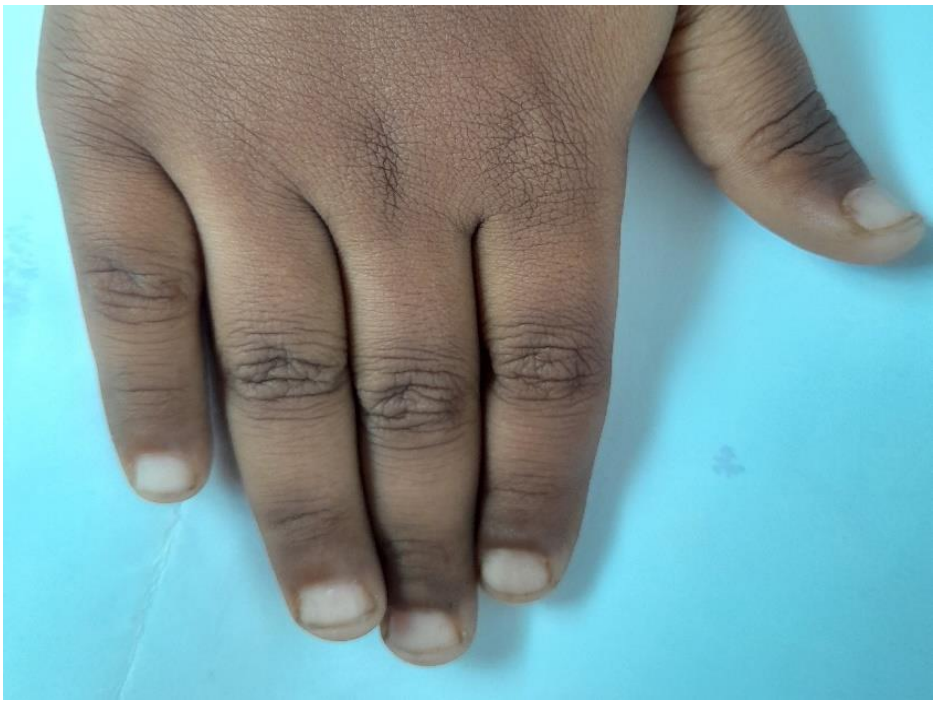


Figure 12- Platynychia

- Plummer-Vinson syndrome: This is a rare condition present in some children with IDA. It includes dysphagia (difficulty swallowing), koilonychias, and an enlarged spleen (splenomegaly).
- Mild liver and spleen enlargement (hepatosplenomegaly) is also common in children with IDA.
- Pica is another common symptom of IDA. It involves eating non-food items like-

    - Geophagia (eating dirt or clay), which can reduce iron absorption and worsen IDA
    - Amylophagia (eating starch or raw rice.
    - Pagophagia (eating ice), which is often seen in pregnant women

    Pica can also be caused by lead poisoning or psychological issues. In some cases, pica can both result from and contribute to iron deficiency anemia.

- In cases of mild anemia, there may be no noticeable symptoms, but after treatment, children may feel better and have improved exercise tolerance. Symptoms like fatigue, breathlessness, irritability, and loss of appetite may still be present. The spleen may also be slightly enlarged, but it remains soft and not hard.

❖ IDA can cause haematological as well as non-haematological consequences in children and adolescents. [(2)]

❖ Gastrointestinal tract issues often include anorexia, which is a common and early sign. Atrophic glossitis may develop, where the tongue's papillae become flattened, resulting in a smooth and shiny appearance. Dysphagia, or difficulty

swallowing, may also occur, along with the formation of esophageal webs, as seen in Kelly-Paterson syndrome. Presence of exudative enteropathy, or leaky gut syndrome, can lead to widespread malabsorption. [7]

- In central nervous system, individuals may experience irritability, fatigue, reduced activity levels, and a decline in cognitive performance. Mental and motor developmental test scores could be lower, and attention span may be shortened. Breath-holding spells and papilledema might also be present, and iron deficiency anemia can lead to febrile seizures.
- Cardiovascular signs might include an increased heart rate during exercise and recovery, as well as cardiac hypertrophy. The immune system may be compromised, leading to slower recovery from illnesses and a higher incidence of respiratory infections.
- At the cellular level, red blood cells may experience ineffective erythropoiesis and shortened lifespans. Cellular growth, along with the synthesis of DNA, RNA, and proteins, may also be disrupted. [7]

DIAGNOSIS OF IDA-

- In majority of instances, initial diagnosis of IDA can be established by evaluating the child's dietary history, clinical signs, and blood test results that indicate microcytic hypochromic anemia with anisopoikilocytosis.

  • The typical pattern of-

  i) low MCV

  ii) low MCH

  iii) low MCHC, relative to the child's age strengthens the case for IDA. The diagnosis is commonly confirmed by observing the child's response to iron supplementation. [8]

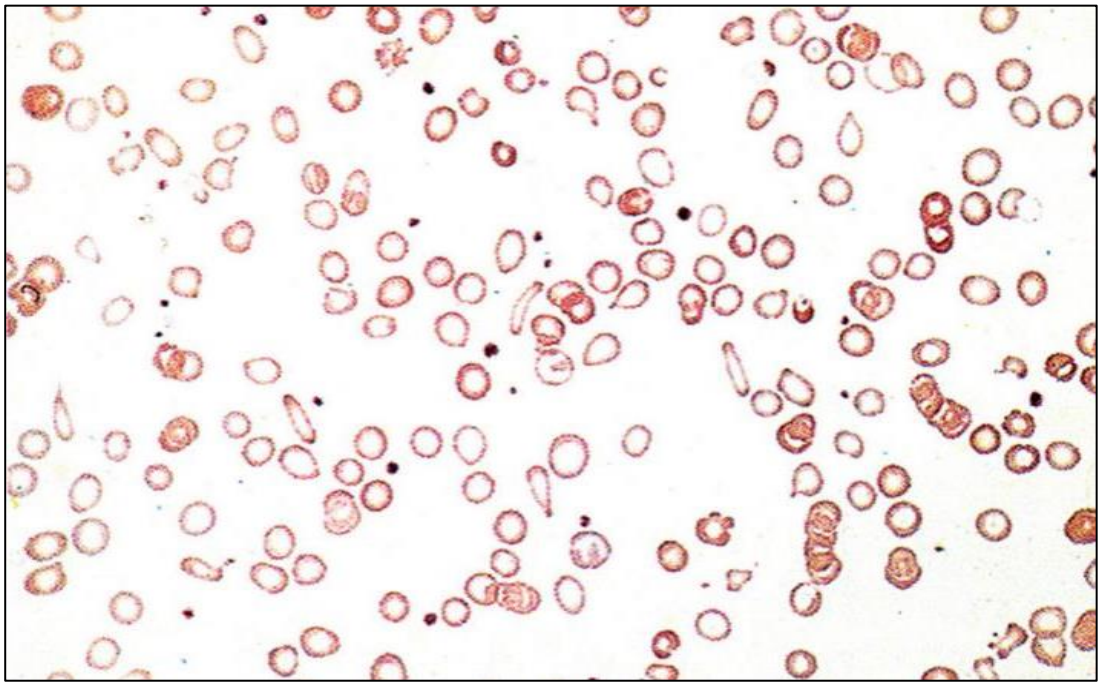


Figure 13- Peripheral smear examination showing Microcytic Hypochromic anemia [5]

- When an alternative diagnosis cannot be ruled out or when a child is not responding to iron therapy, additional investigations may be needed. These tests include:

- **Serum Ferritin**: It serves as an accurate indicator of Fe reserves in the body. This test is typically the first one performed to assess iron deficiency. A serum ferritin value below 12 µg/L in less than five years old and under 15 µg/L in older children indicates IDA, particularly in the absence of inflammation. When infection/inflammation is present, a level lower than 30 µg/L indicates IDA. [8]

- **Serum Fe, TIBC, Transferrin saturation (TS):** These tests are done, when the ferritin results are unclear. Serum Fe level will be decreased in IDA, but they can vary daily depending on recent food intake. Therefore, serum Fe alone is not enough for diagnosis. TIBC and TS are used to assess iron deficiency, with a TS level below 16% often indicating IDA. [8]

- **New Red Cell Indices**: These include measuring 'CHr' which stands for reticulocyte Hb content, and 'Ret-HE' which stands for reticulocyte Hb equivalent. These tests are useful for detecting early iron deficiency and assessing how the body is responding to iron treatment. Low levels of Ret-HE or CHr indicate early iron deficiency before anemia appears. Ret-HE values below 29 pg are often used to diagnose IDA in children.

- **Hypochromic Red Cells percentage (hypo%):** This measure indicates the iron status over the past 3 months. A Hypo% greater than 5% is used to diagnose IDA. It is also useful for identifying functional iron deficiency,

where iron is not being used properly in the blood cells despite adequate stores.

- **Soluble Transferrin Receptor (sTfR):** This test reflects active red blood cell production. A level greater than 27.3 nmol/L can indicate IDA. The sTfR/ferritin ratio helps distinguish anemia due to chronic disease from IDA. However, it is not typically recommended for early stages of iron deficiency.

- **Free Erythrocyte Protoporphyrin (FEP):** FEP levels are increased in IDA because of an accumulation of protoporphyrin due to limited heme synthesis. FEP levels quickly drop in response to iron treatment. A level above 80 µg/dL in children over five years old, or above 70 µg/dL in younger children, suggests IDA. However, high FEP levels can also occur in conditions like hyperbilirubinemia, thalassemia trait, and hemoglobin E disease, so it is not routinely recommended for diagnosing IDA.

- **Bone Marrow Studies**: Bone marrow aspiration is rarely needed in diagnosing IDA. It is an invasive test and can usually be avoided, as non/ less invasive tests are available to confirm the diagnosis. It is indicated only when Sideroblastic anemia is the differential diagnosis.

- Stool for occult blood when Anemia secondary to chronic blood loss is suspected. [8,35-44]

TREATMENT OF IDA-

The goal of IDA treatment is- to provide the right amount of iron, in the correct dose and for the right duration, to bring Hb level to normal and also to replenish the Fe stores. Treatment should also involve identification and management of any underlying causes of iron deficiency. Additionally, dietary changes are necessary, and regular follow-up is required to assess how well the treatment is working.

Iron therapy administration

Treatment of choice for IDA is generally oral iron therapy. It can be initiated based on the child's medical history, Hb levels, MCV levels, peripheral blood smear, without the need for any additional biochemical tests. Oral form of Fe is safe, very cost-efficient, and leads to a swift increase in Hb, when administered at the proper dosage with adequate follow-up. It is easier for the caregivers and also better tolerated by most children. Parenteral route of Fe is seldom needed for pediatric patients.

1. **Oral Iron:**

Iron in the oral form is primarily available in the Fe^{+2} (more easily absorbed) and Fe^{+3} forms.

The three most common Fe^{+2} iron formulations are Fe $^{+2}$ sulfate , Fe $^{+2}$ fumarate, Fe $^{+2}$ gluconate. [8]

The dosage should be determined based on the amount of elemental Fe in each formulation. All of these types are equally effective. Other available options include ferrous ascorbate and iron polymaltose complex (IPC).

Enteric-coated/delayed release iron, was developed to enhance tolerance by minimizing gastrointestinal side effects. However, these formulations are not absorbed as efficiently as regular ones and tend to be more costly. [8]

- Dose, schedule of oral Fe-

The dose is 3-6mg/kg/day for children. However, dose of 2-3 mg/kg/day is also effective and may reduce side effects like abdominal pain and constipation. [8]

Iron absorption is better tolerated post meal. It should never be taken along with milk and milk products like curd, calcium supplements, tea/ coffee as these can interfere with absorption. Medications like PPIs and antacids should also be avoided while taking iron. [8]

- Duration of therapy-

Iron therapy needs to be continued for 2-3 months following the normalization of hemoglobin levels. This time frame facilitates the replenishment of iron reserves and tackles erythropoiesis. It is essential to notify the family or caregivers regarding the significance of persisting with iron supplementation even after the anemia has been resolved. Prior to stopping iron therapy, the child's eating habits should be evaluated, and any underlying reasons for IDA should be dealt with.

Iron supplementation ought to persist into adolescence as a component of standard iron prophylaxis, according to national guidelines. Folic acid is commonly incorporated in all oral iron supplements and is regularly administered to children diagnosed with IDA. Oral form of vitamin B12 should be introduced when the response to iron is insufficient , or in instances of dimorphic anemia. Combined deficiencies are found in 30% of children experiencing nutritional type of anemia.

If approximately normal MCV is noted alongside dimorphic RBCs on the peripheral smear, or indications of Megaloblastic Anemia like hypersegmented neutrophils and macro-ovalocytes are observed, vitamin B12 levels and folate levels should be checked. If deficiencies are established, treatment with therapeutic levels of FA along with vitamin B12 is necessary. In contrast to previous assumptions, a RCT involving adults has demonstrated that there is no extra advantage to incorporating vitamin C into Fe therapy. Consequently, vitamin C in medicinal forms is not advised. Nevertheless, eating citrus fruits just prior to taking iron supplements may enhance iron absorption. [8, 45-51]

2. Parenteral Fe therapy

Majority cases of IDA can be treated with oral Fe supplementation. However, sometimes parenteral Fe therapy is needed. New intravenous iron formulations have few major side effects, so they are preferred for parenteral Fe therapy. The intramuscular route is usually not preferred because it is painful and can lead to skin staining.

Indications for parenteral iron therapy include-

- Poor adherence/ intolerance to oral iron – Gastrointestinal side effects often discourage patients from continuing oral iron supplements. This can lead to intolerance to the treatment regimen.
- Necessity for quick restoration of hemoglobin levels and iron reserves. – In situations where quick correction is necessary, such as before surgery, IV iron allows for faster replenishment compared to the gradual build up with oral supplements.

- Ongoing blood loss exceeding oral iron capacity – Chronic or heavy bleeding (e.g., uterine bleeding) can overwhelm the ability to absorb and utilize oral iron, making IV iron a more effective solution.
- Iron malabsorption due to pre-existing conditions – Some conditions like short bowel syndrome reduce the iron absorption from the digestive system, making oral iron ineffective.
- Coexisting inflammatory conditions – Inflammatory states, such as those seen in chronic diseases or infections, can disrupt iron metabolism and homeostasis, reducing the effectiveness of oral Fe.
- CKD patients may require IV Fe due to reduced erythropoiesis and difficulty in iron utilization, making oral iron less effective.
- Genetic conditions refractory to oral iron – Certain genetic disorders like IRIDA which stands for Iron Refractory IDA make it difficult to treat IDA with oral supplements, necessitating alternative treatments like IV iron.

Preparations of Intravenous Iron-

Fe Sucrose: It is the most, frequently utilized IV Fe for pediatric patients. Adverse reactions, including anaphylaxis, are rare, thus a test dose or standard premedication's are unnecessary. The recommended dosage is 1 to 4 mg/kg of elemental form of iron, delivered as an infusion over a one hour period once weekly. Adolescents can be given up to 200 mg per infusion, whereas children are limited to a maximum of 100 mg. The majority of patients need multiple infusions to fully replenish their iron levels. [(8)]

Ferric Gluconate: This is sanctioned for children identified with CKD who are undergoing dialysis and receiving erythropoietin-stimulating agents, starting from age

of 6 years and older. There is no need for a test dose or regular premedications. The highest allowable dosage is 125 mg for each infusion. Rarely any adverse reactions are mentioned. [8]

Fe Dextran: Usually given as one dose, reaching to a maximum 1000 mg in adults. [8]

Ferric carboxy-maltose (FCM): FCM is frequently utilized in adults who are not able to tolerate oral iron. Given as a complete replacement dose in one infusion. It is also employed in paediatric instances, although data is scarce. Hypophosphatemia (decreased phosphate levels) is a frequent adverse effect. The dosage is 15 mg/kg, administered as a quick IV infusion in 15 minutes and without giving any test dose. [8]

The U.S. Food and Drug Administration (FDA), has approved the administration of Fe dextran, Fe sucrose, Fe $^{+3}$ gluconate, FCM in paediatric patients (8). However, IV Fe dextran is currently not advised. In India, Fe sucrose, Fe $^{+3}$ derisomaltose known as isomaltoside and FCM have been sanctioned for addressing iron deficiency. [8]

Formula for calculation of dose for parenteral iron [8]

Total Fe deficit (in mg) = Body weight (in kilograms) x (target Hb – actual Hb) x 2.145 + storage Fe (mg).

Volume needed (in ml) = Body weight (kilograms) x 14 Hb x 2.145 ÷ elemental form of Fe (mg/mL)

Precautions-

Generally, premedication is not required. However, individuals with bronchial asthma, drug sensitivities, or inflammatory arthritis should be administered 1-2 mg/kg of IV methylpred prior to IV iron delivery.

Adverse effects from IV iron are usually mild and can consist of nausea, itching, vomiting, headache and flushing. Myalgia which is muscle discomfort, arthralgia which is joint discomfort, along with back or chest pain typically subside within 2 days, after the entire dose has been administered.

Hypersensitivity side effects occur infrequently, and severe or life-threatening responses are uncommon. The precise reason for these reactions is not completely understood but may be associated with the release of free iron, which is not a concern with more recent formulations.

Risk factors for reactions include rapid infusion and any history of drug allergies. To minimize risks, it's recommended to administer iron slowly, closely monitor the patient, and ensure the presence of trained medical staff and resuscitation equipment. A test dose may not always be reliable, and premedication with antihistamines is no longer recommended, as it can cause low blood pressure and rapid heart rate. [8]

Response to Parenteral Therapy

In uncomplicated cases, patients usually feel better within a few days after receiving IV iron. Reticulocyte levels peak around 10 days. Symptom improvement typically happens within 1-2 weeks, along with a rise in hematocrit levels. Full recovery may take about 4 to 6 weeks. A follow up evaluation should be done 6-8 weeks after treatment because IV iron can affect most iron tests. [8, 52-60]

The Anemia Mukt Bharat (AMB) initiative seeks to decrease anemia across six target groups:

i. Children in age group of 6 months - 59 months
ii. Children in age group of 5years to 9 years
iii. Adolescents (10 to19 years)
iv. Pregnant women
v. Lactating women
vi. Women in the reproductive age that is between 15 and 49 years.

This approach adopts a life cycle perspective and incorporates six primary interventions, all underpinned by a robust institutional framework. [61]

The 6 interventions under this strategy are [61]

Prevention using iron (Fe) and folic acid (FA) supplements-

- **Children in the age group of 6 months – 59 months**: Twice a week, oral Fe and FA- 1ml (20 mg elemental Fe with 100 mcg FA
- **Children in the age group of 5–10 years**: Once a week, 1 Fe and FA tablet (45 mg elemental Fe with 400 mcg FA).
- **Adolescents, 10–19 years**: Once a week, 1 Fe and FA tablet (60 mg elemental Fe with 500 mcg FA).
- **Non-pregnant and non-lactating women, aged between 20-49 years)**: Once a week, 1 Fe and FA tablet (60 mg elemental Fe with 500 mcg FA).

- **Pregnant women and lactating mothers (in first 6 months)**: Daily supplements, 1 Fe and FA tablet, beginning from 4th month antenatally and continuing for 6 months postpartum (60 mg elemental Fe with 500 mcg FA).

- **Pre-conception, first trimester**: 400 mcg of FA daily.

Regular deworming

- Twice a year mass deworming, for all children and even adolescents (1–19 years) on National Deworming Day (10th February, and 10th August).

- Pregnant women receive deworming treatment during the second trimester through antenatal care services.

Campaign for behaviour change and communication

- Encourage adherence to Fe and FA supplementation along with deworming practices.

- Promote proper IYCF which stands for 'Infant and Young Child Feeding', and ensure proper weaning for children 6 months and older.

- Advocate for increased consumption of foods rich in protein, Fe and Vitamin C, while supporting diversification and fortification of diet.

- Delayed clamping of cord.

Diagnosis and treatment of Anemia

- Utilize digital tools (Digital Invasive Hemoglobinometer) for anemia testing in health and wellness centres.
- Employ semi-automated analyzers at higher-level health facilities (PHC and above).
- Follow Anemia Management protocols as outlined in the guidelines for Anemia Mukt Bharat.

Providing Fe and FA fortified foods

- Ensure mandatory inclusion in health programs funded by the government.

Awareness, identification and management of other causes of anemia

- Address issues like malaria, inherited blood disorders like hemoglobinopathies and fluorosis, particularly in areas where these conditions are endemic

MATERIALS AND METHODS

Source of Data: Patients aged between 6 months to 10 years with IDA who come to the Paediatric OPD, Paediatric ward and Paediatric Hematology OPD at KLE's Dr. Prabhakar Kore Hospital.

Study Design: Randomized Control Trial (RCT)

Study Period: One Year

Sample Size:

The sample size, was determined based on the anticipated mean and the standard deviation of serum hepcidin in twice daily oral iron therapy as μ_1,σ_1(1.38,0.32) and in the alternate day oral iron therapy as μ_0, σ_0(1.09,0.32), as per the previous study by Stoffel nu et al (10).

Other parameters which were taken into consideration for sample size interpretation were, 80% power of study and 5% two-sided alpha error.

The required size of the study group was determined by using the following formula, which was proposed by Kirkwood BR et al.

Formula used for sample size calculation:

$$N = \frac{(u + v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

N	Number of participants
μ_1 , μ_0	Difference between the means (μ_1=1.38, μ_0=1.09)
σ_1, σ_0	Standard deviation (σ_1=0.32 , σ_0 =0.32)
U	Two-sided % point of the normal distribution corresponding to 100 % - the power of 80%, u =0.84
V	% point of the normal distribution, corresponding to the (two sided) significance level for significance level = 5%, *v* = 1.960

The necessary sample size according to the calculations stated above was 19 in every group. To address a nonparticipation rate/loss to follow-up rate of approximately 5%, an additional 1 subject was included in each of the group. Therefore, the ultimate required sample was 20 participants in each group.

Sampling technique: Open labelled Randomized Control Trial

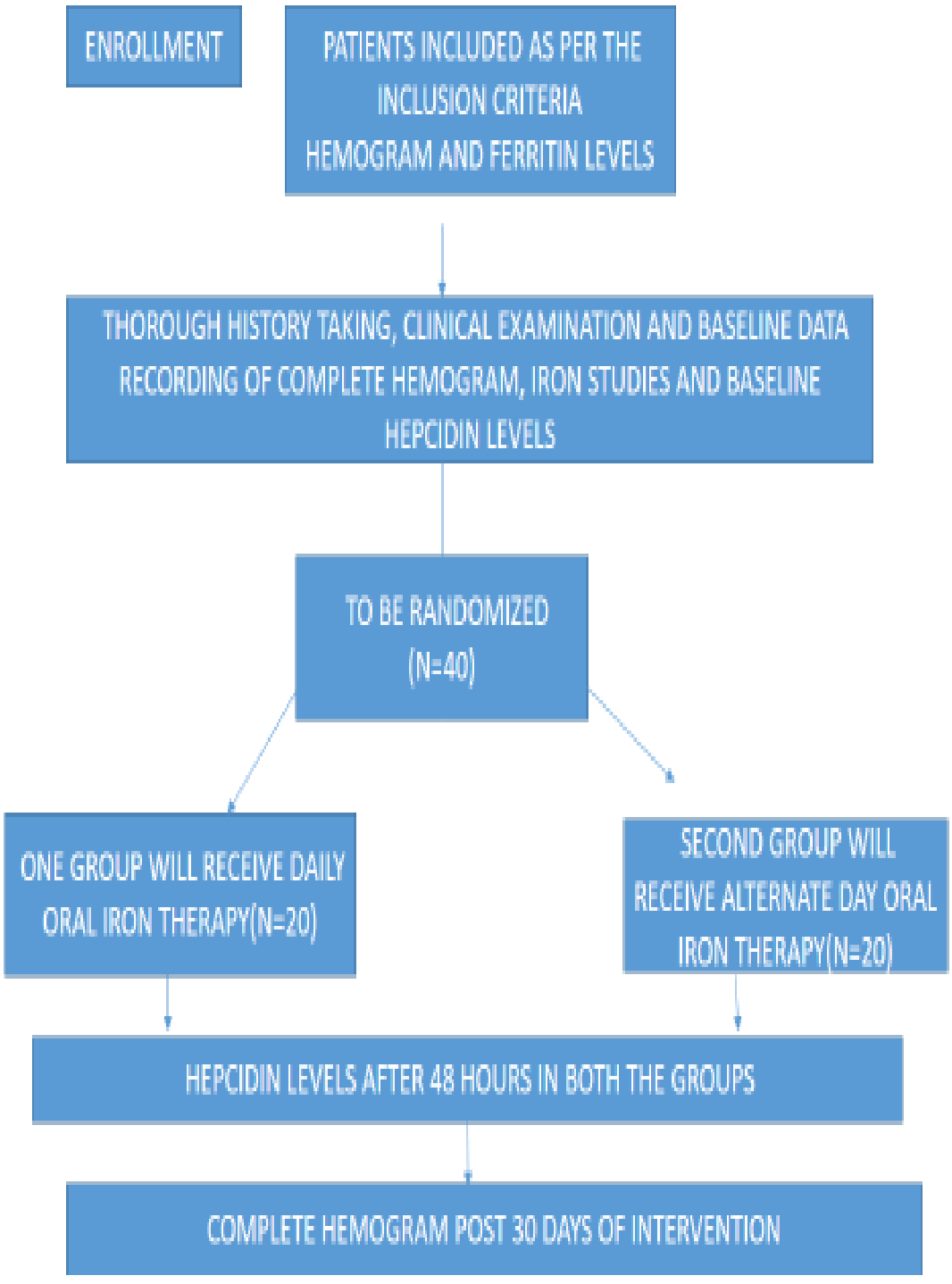
Inclusion Criteria:

Patients between 6 months to 10 years of age diagnosed with Iron Deficiency Anemia.

Exclusion Criteria:

- Patients who have received any blood transfusion in past 3 months.
- Patients who have received oral or injectable Fe supplements in past 3 months.
- Patients with hemoglobinopathies like Thalassemia Major or any other Hemolytic Anemia.
- Premature infants born before 32 weeks of gestation.
- Patients with chronic liver diseases.
- Any patient with history of acute infection or a known case of chronic inflammatory disorder.
- Patients not consenting for the study.

Study protocol:



Method of randomization- Open labelled Randomized Controlled Trial with participant allocation by Computer generated Random number sequence.

- Group 1: Twice daily oral iron therapy
- Group 2: Alternate day oral iron therapy

Data collection procedure:

An open labelled RCT was carried out on 40 children, between the age group of 6 months to 10 years with IDA. Following approval of the institutional ethical committee, and obtaining informed consent from the patients' parents in their native language, the patients were involved and randomly assigned in two groups.

The patients with pallor/ anemia were screened for IDA by doing complete hemogram and serum ferritin level. Patients with anemia (as per WHO reference range for hemoglobin, ferritin less than 12 below 5 years and less than 15, between 5-10 years) were enrolled into our study.

Thorough history taking, clinical examination and baseline data recording was done to look for features of IDA. After randomization, all participants were given age appropriate single dose of Albendazole.

The first group of patients were subjected to oral Ferrous fumarate preparation of Iron, (same iron preparation for all the patients) given at 4 mg/kg in 2 divided dosages on daily basis. The second group was subjected to oral Iron therapy at the same dose (4mg/kg) on alternate day as a single administration.

Baseline CBC, RBC indices, Fe studies and hepcidin levels were done. Serum hepcidin level was repeated at 48 hours after starting Fe therapy. Change in hemoglobin level was noted after 1 month of intervention. Patients were followed up weekly (telephonically) to assess the side effects due to toxicity or any compliance issues.

Complete blood count including RBC indices was processed by the Sysmex XN 1000 – 5part analyser using the Coulter Counter method. Iron studies were processed by the Ferrozine method using the Cobas Pro analyser. The Human Hepcidin ELISA kits were used as an analytical tool for the quantitative determination of serum Hepcidin levels.

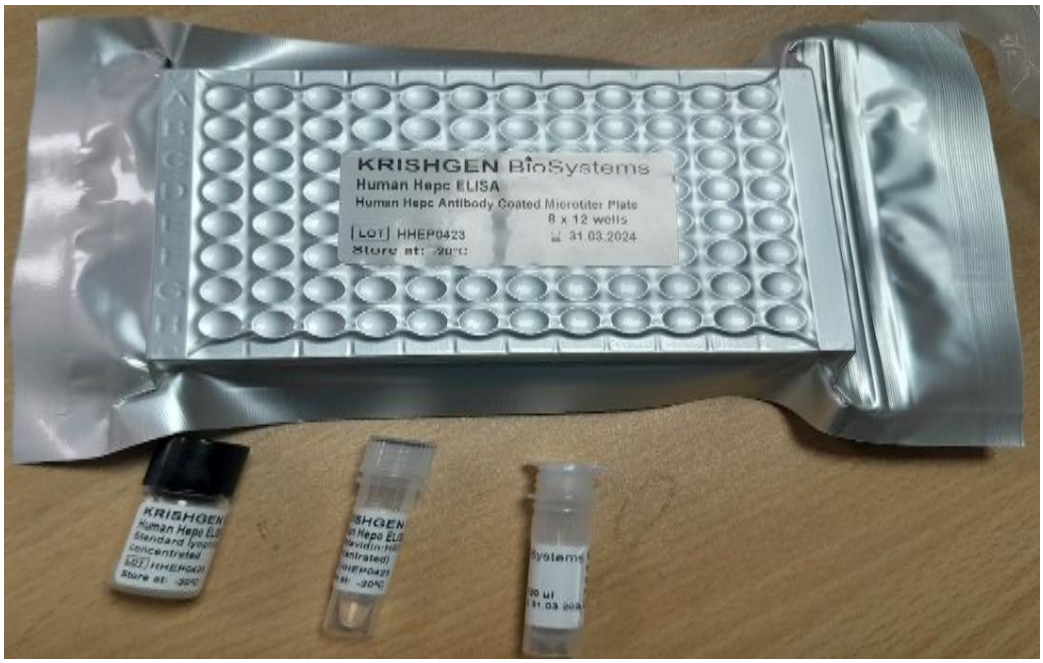
SAMPLES TAKEN-

- 1 EDTA VACUTAINER- Complete blood counts including RBC indices with peripheral smear on day 1 and day 30.
- 1 PLAIN VACUTAINER- Iron studies
- 1 PLAIN VACUTAINER- Serum hepcidin levels on day 1 and after 48 hours.

SYSMEX XN 1000 for RBC Indices

COBAS PRO for Serum iron studies and Iron profile

BioTek microplate washer for ELISA Assay



Human Hepcidin ELISA Kit

Data processing and analysis/statistical analysis:

The complete dataset was verified by identifying and rectifying any irregular values and typographical mistakes. All the quantitative variables were assessed for adherence to normal distribution, within each research group, by employing visual examination of histograms and normality Q-Q plots. Skewness and Kurtosis Z-Values, along with Shapiro-Wilk test P-values, were also utilized for this objective.

Data was examined using Intention to treat (ITT) analysis. First, all baseline variables were compared between the groups, to evaluate any notable differences in these variables between the two study groups. Following that, the main primary and secondary outcome variables were compared between the groups, to record the effectiveness and safety of the intervention.

The average and standard deviation of the normally distributed quantitative variables were analyzed between the groups through an independent sample t-test. The median and IQR of the non-normally distributed quantitative variables was analyzed between the groups using the Mann-Whitney U test. The qualitative variables were compared between the 2 techniques using the Chi-square test/Fisher's exact test.

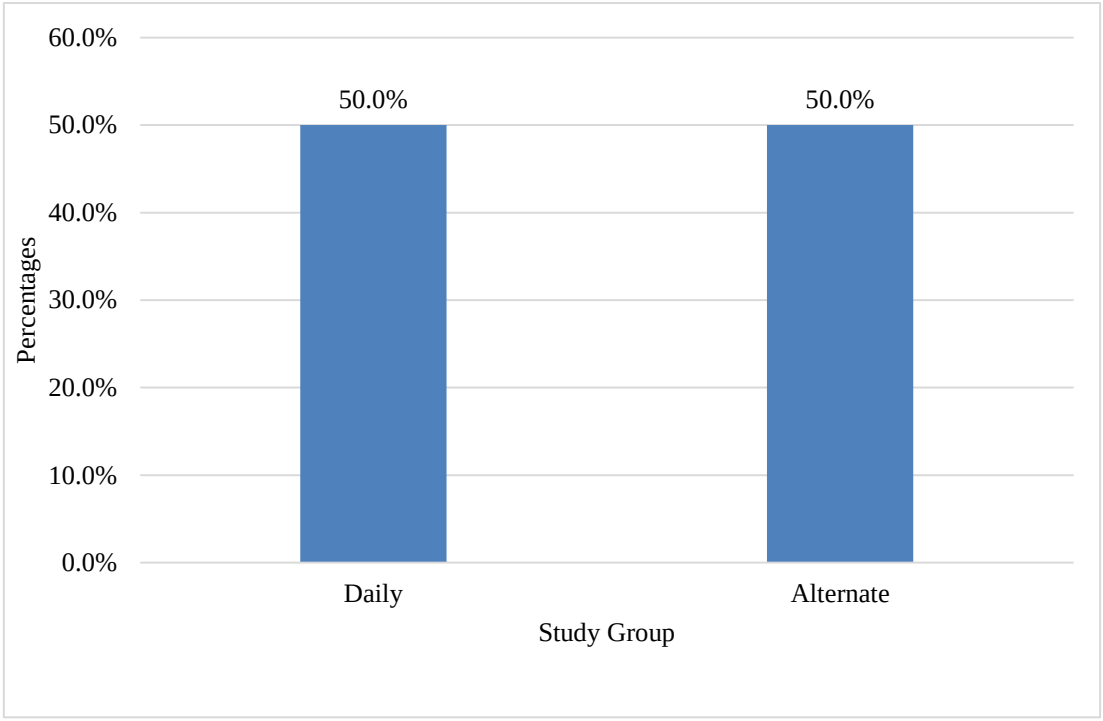
RESULTS

In this study, 40 participants with Iron deficiency anemia were enrolled. They were randomized into 2 groups of 20 each. Baseline descriptive analysis of lab parameters were compared in both the groups.

Table 1: Descriptive analysis of the study group in the study population (N=40)

	Frequency	**Percentages**
Daily	20	50.00%
Alternate	20	50.00%

Graph 1: Bar chart showing the study group (N=40)



The mean of all the ages of the participants was 3.16 ± 2.72, with a male is to female ratio of 0.9 as shown in the tables below.

Table 2: Descriptive statistics of age (years) (N=40)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Age in years	3.16 ± 2.72	2.00	0.58	10.00	2.29	4.03

Table 3: Descriptive statistics of gender (N=40)

Gender	Frequency	Percentages
Male	19	47.50%
Female	21	52.50%

Graph 2: Pie chart showing gender distribution in the study population (N=40)

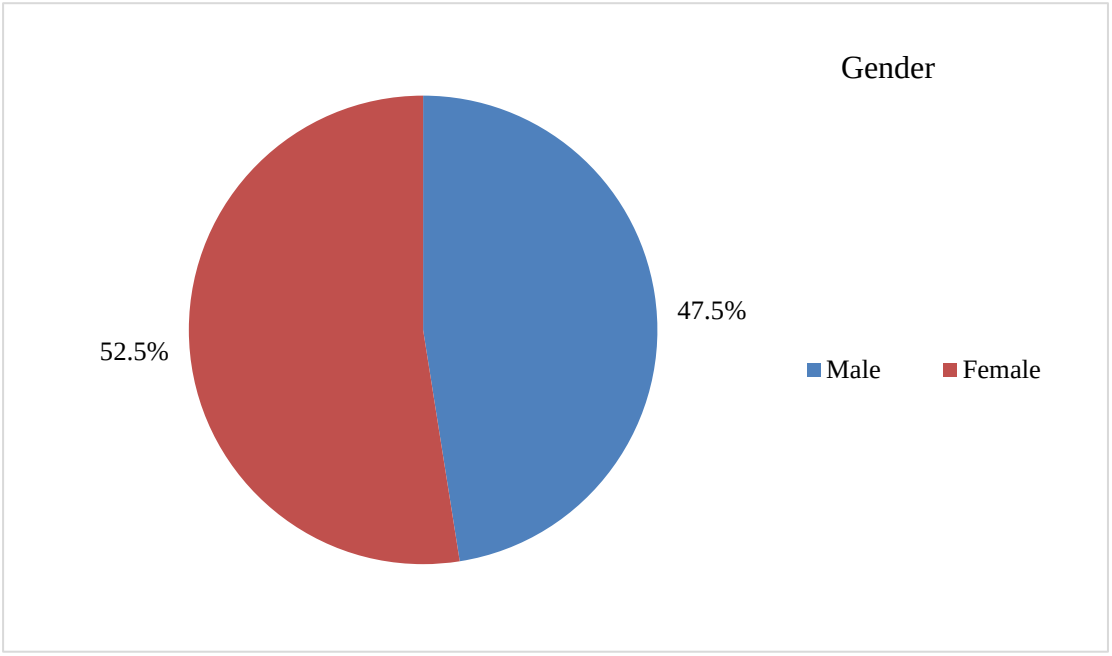


Table 4: Descriptive statistics of lab parameters (N=40)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Haemoglobin On Day 1(g/dl)	7.74 ± 1.51	7.7	5.4	10.5	7.3	8.2
Haemoglobin At 30 Days(g/dl)	9.65 ± 1.54	9.9	6.7	12.8	9.2	10.1
Platelet Count (Lakhs/microlitre)	4.21 ± 1.49	4.1	1.5	7.2	3.7	4.7
WBC (Thousands/Microlitre)	10.97 ± 3.87	10.3	4.2	17.6	9.7	12.2
MCV (fl)	61.87 ± 6.14	60.8	52.2	73.0	59.9	63.8
MCH (pg)	17.37 ± 3.25	16.8	12.0	27.0	16.3	18.4
MCHC (g/dl)	27.09 ± 2.73	27.5	20.8	33.8	26.2	28.0
RDW (%)	22.94 ± 6.36	21.3	14.3	50.9	20.9	25.0
Reticulocyte Count (%)	1.7 ± 0.94	1.4	0.3	4.0	1.4	2.0
Ferritin (ng/ml)	7.91 ± 4.65	7.6	1.6	30.0	6.4	9.4
S. Iron (mcg/dl)	11.4 ± 3.95	11.5	4.0	19.0	10.1	12.7
TIBC (mcg/dl)	380.4 ± 85.47	381.5	210.0	574.0	353.1	407.7
Transferrin Saturation (%)	3 ± 1.24	3.0	2.0	7.0	2.6	3.4
S. Hepcidin Baseline (ng/ml)	5.86 ± 3.4	4.5	0.6	13.7	4.8	7.0
S. Hepcidin At 48 Hours (ng/ml)	5.99 ± 4.73	3.9	1.1	18.2	4.5	7.5

The mean baseline hemoglobin in daily group was 7.38 ± 1.49 g/dl whereas mean in the alternate day oral iron therapy group was 8.11 ± 1.46 g/dl. On follow up after 30 days, there was a statistically significant rise in the Hb level in alternate day oral Fe therapy group, with a mean rise of 2.3 ± 0.76 (P=0.002) whereas rise of 1.51 ± 0.75 in the daily oral iron therapy group.

Table 5: Comparison of mean of day 1 and day 30 Hb between 2 study groups (N=40)

Parameter	Study group (Mean ± SD)		Independent t test (P value)
	Daily (N=20)	Alternate (N=20)	
Day 1 Hb	7.38 ± 1.49	8.11 ± 1.46	0.124
Day 30 Hb	8.89 ± 1.38	10.41 ± 1.32	<0.001

Graph 3: Cluster bar chart of comparison of mean of day 1 and day 30 Hb between study group (N=40)

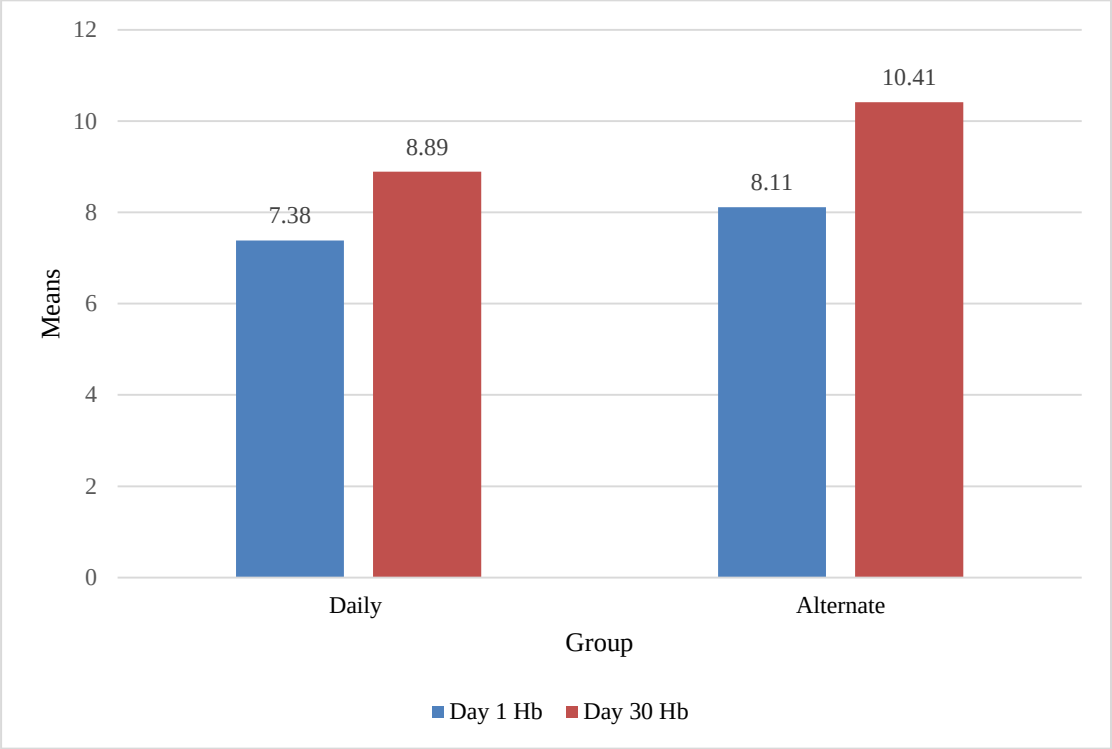
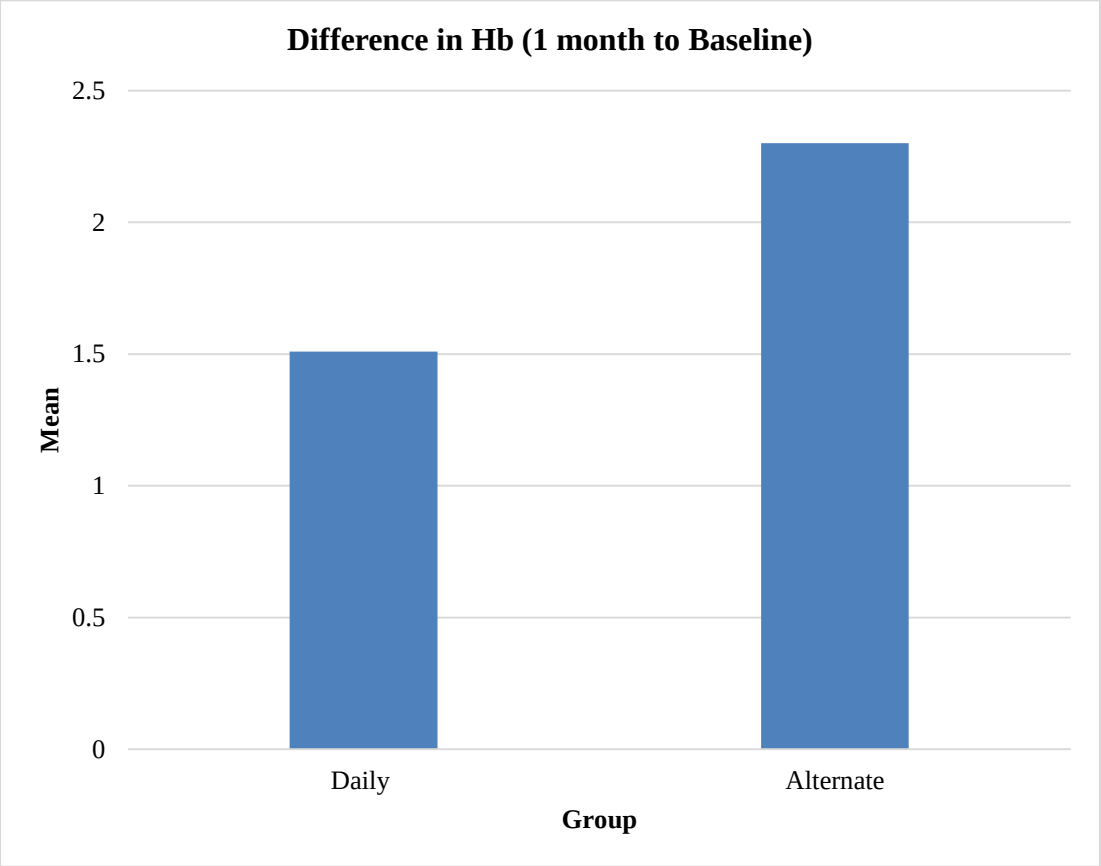


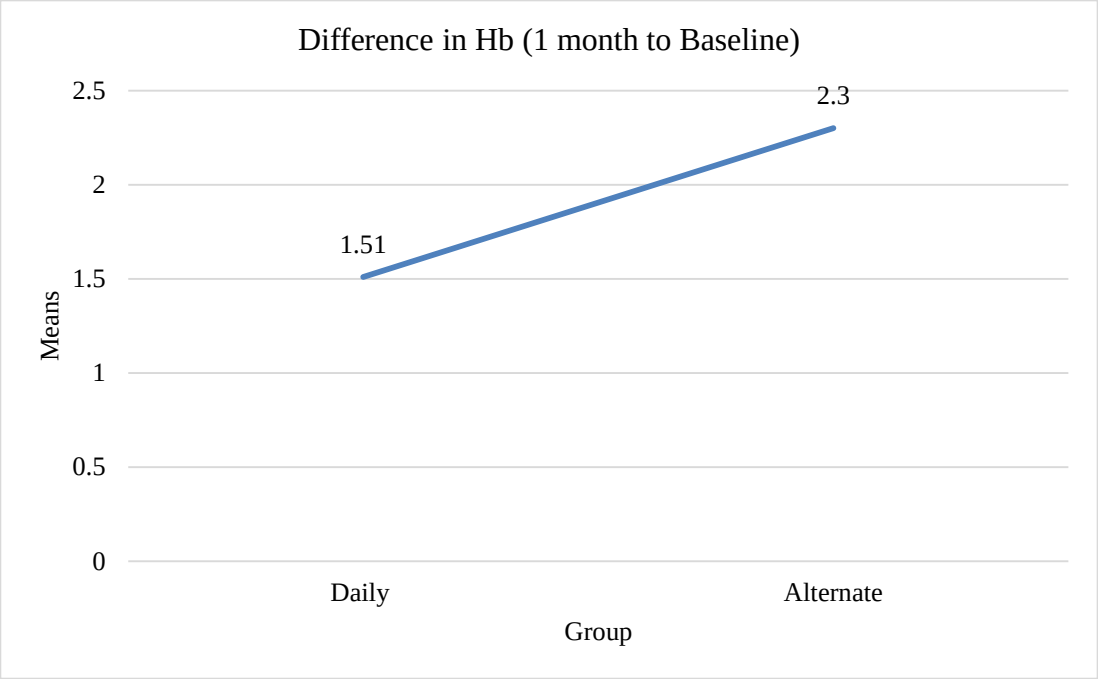
Table 6: Comparison of mean of Difference in Hb (Baseline to 1 month) between 2 study groups (N=40)

Parameter	Study Group (Mean ± SD)		Independent t test (P value)
	Daily (N=20)	Alternate (N=20)	
Difference in Hb (1 month to Baseline)	1.51 ± 0.75	2.3 ± 0.76	0.002

Graph 4: Bar chart of comparison of mean Difference in Hb (1 month to Baseline) between study group (N=40)



Graph 5: Line chart of comparison of mean Difference in Hb (1 month to Baseline) between study group (N=40)



The mean baseline serum hepcidin level in the daily group was 6.45 ± 3.84 ng/ml and 5.28 ± 2.87 ng/ml in the alternate day group. 15 out of 20 children (75%) in the alternate group had decrease in serum hepcidin levels after 48 hours. There was a statistically significant decrease in the serum hepcidin level to 4.1 ± 2.75 ng/ml in the alternate day supplementation group (P value = 0.01).

Table 7: Comparison of median of day 1 and day 30 serum hepcidin between 2 study groups (N=40)

Parameter	Study Group (Mean± SD)		Independent t test (P value)
	Daily (N=20)	Alternate (N=20)	
Baseline serum hepcidin	6.45 ± 3.84	5.28 ± 2.87	0.281
48 hours serum hepcidin	7.87 ± 5.56	4.1 ± 2.75	0.010

Graph 6: Cluster bar chart of comparison of mean of baseline and 48 hours serum hepcidin between the 2 study groups (N=40)

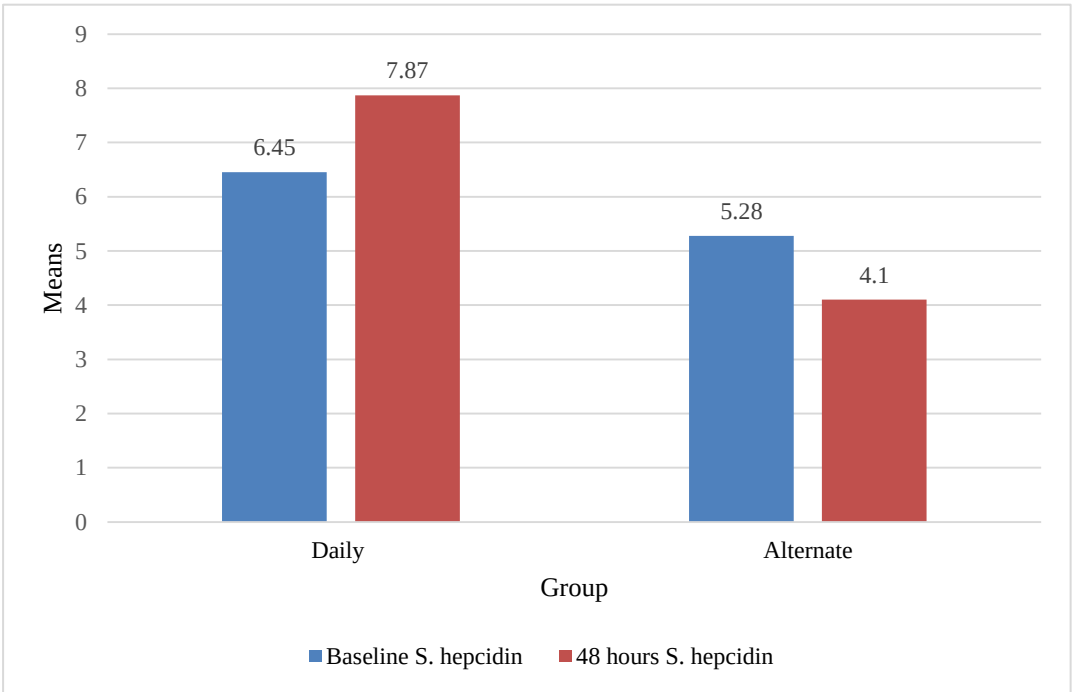
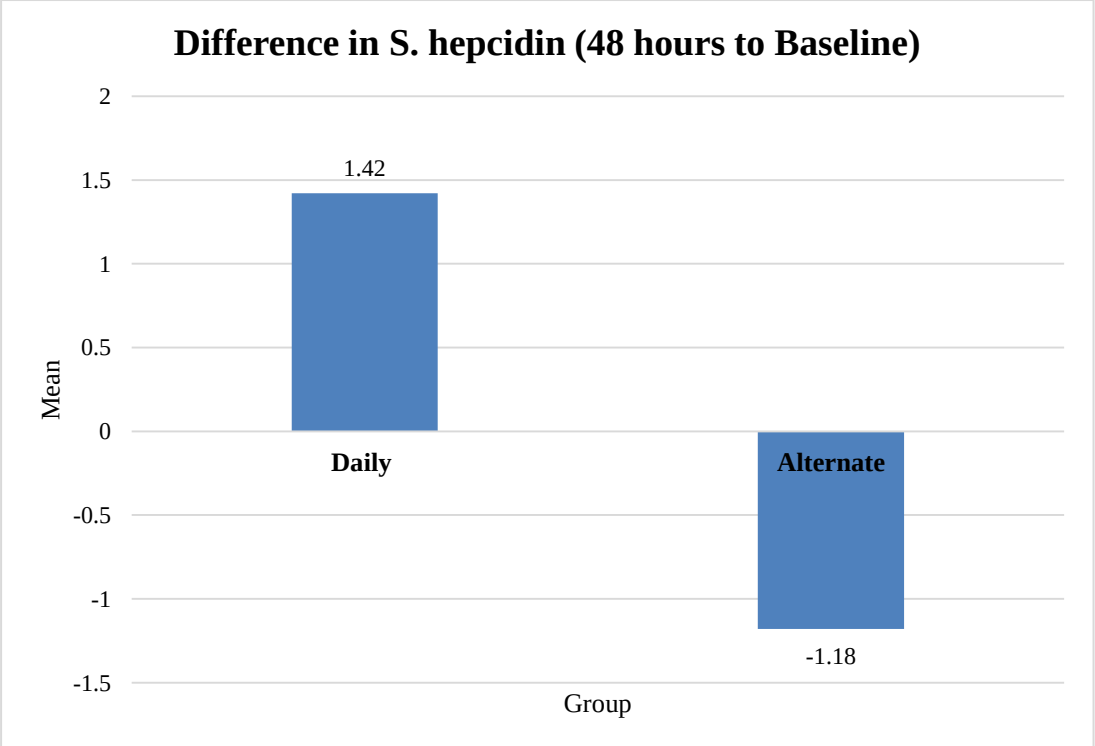


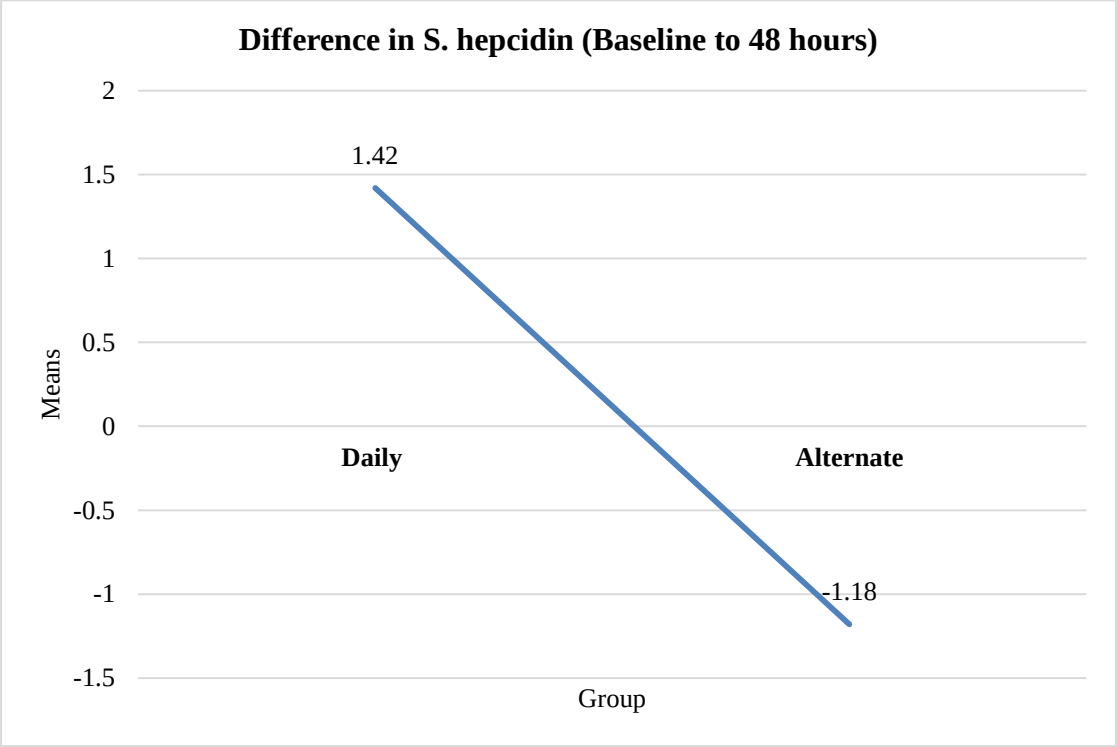
Table 8: Comparison of mean of Difference in serum hepcidin levels (Baseline to 48 hours) between study group (N=40)

Parameter	Study Group (Mean± SD)		Independent t test (P value)
	Daily (N=20)	Alternate (N=20)	
Difference in S. hepcidin (Baseline To 48 hours)	1.42 ± 3.86	-1.18 ± 2.47	0.015

Graph 7: Bar chart of comparison of Difference in serum hepcidin levels (48 hours to Baseline) between study group (N=40)



Graph 8: Line chart of comparison of Difference in serum hepcidin levels (48 hours to Baseline) between study group (N=40)

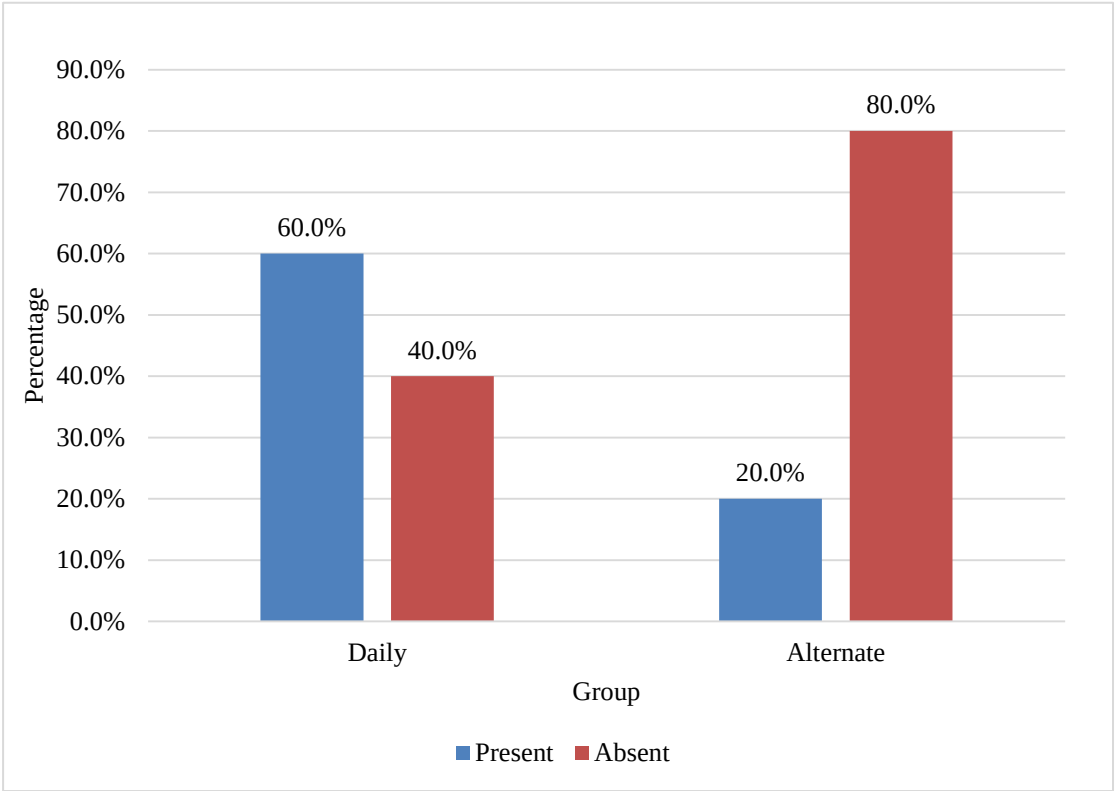


Patients were followed up weekly to assess the side effects and 12 out of 20 patients (60%) in daily group and 4 out of 20 in alternate group (20%) had gastrointestinal issues, such as constipation, abdominal pain and vomiting. The difference was statistically significant (P=0.01).

Table 9: Comparison of gastrointestinal side effects between group (N=40)

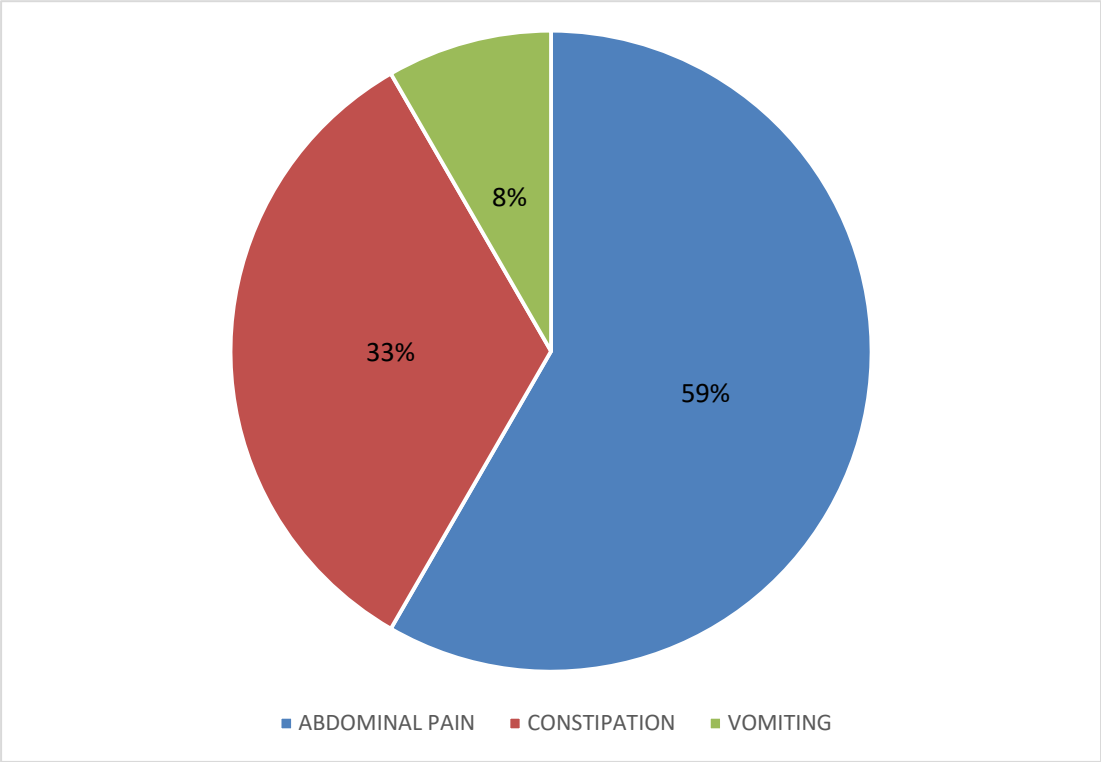
Gastrointestinal Side Effects	Group		Chi square	P value
	Daily (N=20)	Alternate (N=20)		
Present	12 (60%)	4 (20%)	6.667	0.010
Absent	8 (40%)	16 (80%)		

Graph 9: Cluster bar chart of comparison of gastrointestinal side effects between group (N=40)



Out of the 12 children having gastrointestinal side effects in the daily oral iron therapy group, 7 had complaints of abdominal pain (59%), whereas 4 children had constipation (33%) and 1 had vomiting (8%).

Graph 10: Pie chart of gastrointestinal side effects in daily oral iron therapy group (N=12)



DISCUSSION

IDA is a significant contributor to nutritional anemia. Sometimes, optimal results are not attained even after administering sufficient oral iron, due to inadequate absorption and decreased compliance (10). In children and adolescents, IDA can lead to both hematological and non-hematological effects such as stunted growth, compromised cognitive development, and diminished mental and motor performance. [(2)]

In this RCT involving 40 participants with IDA, we found that children receiving alternate day oral iron, exhibited low serum hepcidin levels at 48 hours, alongside a significant increase in haemoglobin levels by the end of one month.

These children also experienced fewer gastrointestinal side effects and demonstrated better adherence compared to the other group. Other analogous studies have been conducted in adults and pregnant women, but very few have focused on children.

Initial observations indicating possible benefits of intermittent over consecutive Fe supplementation stem from a Cochrane systematic review of RCTs, comparing the 2 methods in pregnant women. [(62)]

Research, which includes investigations involving menstruating women, has indicated that intermittent oral Fe supplementation could be more effective, than taking doses daily. In another study conducted by Moretti et al., it was noted that Fe supplementation elevates hepcidin levels, which subsequently impedes Fe absorption. [(9)]

Their research, which included 54 non-anemic, Fe-deficient women, found that lower Fe doses (40 mg and 80 mg of elemental Fe) improved the fractional absorption of Fe. [(9)] Consequently, intermittent iron supplementation may provide advantages.

However, the study had a brief duration (only 2 days), and the subjects were not anaemic. [9]

In a different study, Stoffel et al. analyzed the Fe absorption from oral Fe taken on consecutive days versus that taken on alternate days. [10] The research involved Fe deficient women, who received 60 mg of elemental Fe either continuously for 14 days or on an alternate day basis for 28 days. [10] The findings indicated higher serum hepcidin levels in the group taking Fe on consecutive day when compared to those taking it on alternate days. [10] Nevertheless, this study did not include participants who were anemic. Moreover, the small sample size and short duration of supplementation constrained the relevance of the findings to real-world contexts. [10] Another limitation was the lack of clinically significant outcomes, such as alterations in haemoglobin levels. [10]

A crossover study by Stoffel NU et al. was performed with women diagnosed with IDA. The primary objective was to assess whether alternate day supplementation of 100 mg and 200 mg of Fe results in superior Fe absorption compared to daily iron supplementation. [63] The secondary objective in this study, was to examine the correlation between Fe absorption, serum hepcidin levels, and the Fe status. [63] In women who have iron depletion but no anemia, oral Fe supplements induce an increase in serum hepcidin that persists for 24 hours, which consequently diminishes Fe absorption from subsequent doses taken on the same or the following day. [63] Consequently, Fe absorption is most effective when Fe is given on alternate days. The research revealed no noteworthy difference in the incidence of gastrointestinal side effects between the two dosing methods. [63] For women with anemia, alternate-day iron supplementation may be more advantageous as it significantly improves fractional Fe absorption. [63] Even when hepcidin release is markedly diminished due

to iron deficiency and the body's heightened requirement for erythropoiesis, ingesting oral iron still results in a temporary increase in hepcidin lasting up to 24 hours.

Alternate-day oral iron supplementation in anemic women might be favoured as it improves iron absorption. [63] A separate study conducted on anemic infants in Kenya reflected similar results to those of Stoffel observed in adults.

Mehta et al. reported a significant rise in Hb levels after 21 days in individuals who took iron every other day compared to those who received daily Fe supplementation. [64]

The primary result of their RCT was, the response of hepcidin. [64]

Although in patients in the alternate day group had no significant rise in serum hepcidin levels from baseline, it was notably higher in the daily group. [64]

The study concluded that a regimen of oral Fe administered every other day (60 mg of elemental Fe) was more effective and better tolerated, producing fewer gastrointestinal side effects, in comparison to daily supplementation for individuals with IDA. [64]

A limitation of the research was the small sample size, comprising only 20 participants in each group. [64]

A study conducted by David B. Bregman and colleagues investigated the use of serum hepcidin levels to predict non-responsiveness to oral Fe therapy in patients with IDA. [65] In the study, IDA patients were subjected to a 14-day oral Fe treatment trial. [65] Those who did not respond (defined as Hb rise of less than 1 gm/dl) were then randomly assigned to receive either 14 days of IV FCM or an additional 14 days of oral Fe therapy. [65] It was found that non-responders had higher baseline hepcidin

levels compared to responders after the oral iron treatment. [65] The sensitivity, the specificity and the positive predictive value (PPV) of hepcidin levels greater than 20 ng/mL in predicting non-responsiveness to oral Fe therapy were 41.3%, 84.4%, and 81.6%, respectively. [65] Additionally, ferritin levels above 30 ng/mL had a PPV of 59.2%. The study concluded that serum hepcidin is a better predictor of non-responsiveness to oral Fe therapy in patients with IDA than ferritin and transferrin saturation levels. [65]

Another study by Tarcio Aragao Matos et al. compared the impact of once a week versus twice a week Fe supplementation on hemoglobin (Hb) levels. [66] This study also determined the prevalence of anemia in infants, aged between 6-18 months, over a 16-week period. [66] The infants were randomly divided to receive 25 mg of elemental Fe either once a week (Group-A), or receive twice a week (Group-B). [66] The findings indicated that both groups experienced a rise in Hb levels, but the group with twice a week supplementation (Group-B) resulted in significant improvement in Hb (p value= 0.002), and a greater reduction in anemia prevalence (from 57.9% to 36.8%). [66] In contrast, the once-weekly supplementation (Group-A) showed only a modest and statistically insignificant increase in Hb ($p = 0.12$) and anemia prevalence ($p = 0.18$). [66] Overall, twice-weekly iron supplementation was more effective in boosting Hb levels and decreasing anemia. [66]

A study by Ahmad M. Faqih et al. involving Jordanian children, belonging to the age of 2-6 years with IDA, demonstrated that administering 5 mg of elemental Fe (Fe+2 sulfate) per kilogram of body weight once or twice a week for 3 months was as good as daily supplementation in correcting the anemia. [67] This finding suggests that less frequent Fe supplementation may be a viable approach for both preventing and

treating IDA, particularly in populations with an increased risk or prevalence of the condition. [67]

The limitations of our study were, that it is a unicentric study, with small sample size. We could not measure the fecal calprotectin, which is a marker for gut inflammation and we could not measure the fractional and total iron absorption using radioisotopes of iron.

OTHER STUDIES	**CONCLUSION OF STUDY**	**LIMITATIONS**
1. MORETTI ET ALL	Lower dosages of iron improved the fractional absorption of iron, hence alternate day iron supplementation is advantageous.	Shorter duration of iron supplementation, participants did not have anemia.
2. STOFFEL ET ALL	Consecutive day group had higher hepcidin levels compared to alternate day group.	Short duration of supplementation, lack of clinically relevant outcome such as change in Hb
3. MEHTA ET ALL	Alternate day with single dosing was more effective and well tolerated. Hepcidin levels raised in daily therapy group.	Small sample size.
4. DAVID B. BREGMAN ET ALL	Serum hepcidin predicts the non-responsiveness to oral Fe therapy in IDA patients.	Presence of confounding factors.
5. TARCIO ARAGAO MATOS ET ALL	Twice weekly iron supplementation improved hemoglobin levels better.	Small sample size. Short study duration. Presence of confounding factors.

CONCLUSION

Serum hepcidin concentrations were decreased in children undergoing alternate day iron therapy, leading to enhanced iron absorption and adherence with notable improvements in hemoglobin levels.

Therefore, for children suffering from IDA, administering alternate day iron supplementation, maximizes iron absorption with fewer gastrointestinal symptoms, and improved compliance, and could be more advantageous schedule.

SUMMARY

The study was carried out over 1 year from 2023 to 2024 at KLES Dr. Prabhakar Kore Hospital. Children in the age group, 6 months - 10 years with IDA, visiting the Paediatric OPD, Paediatric ward, and Paediatric Hematology OPD were included following informed consent and were randomly assigned into 2 groups. The first group of children received oral Ferrous fumarate iron preparation at a dosage of 4 mg/kg in 2 split doses daily. The second group received oral Iron therapy at the same dosage (4mg/kg) every alternate day as a single dose. Initial CBC, RBC indices, serum Fe levels, and hepcidin levels were measured. The hepcidin level was evaluated again 48 hours of beginning iron therapy. Hemoglobin levels were reassessed after 30 days of treatment. Patients were also contacted weekly via phone to track side effects and toxicity.

The relationship between the explanatory variables to the categorical outcomes, was evaluated by doing cross tabulation with percentage comparison. The Chi-square test, was employed to examine statistical significance. The P value of < 0.05, was statistically significant, and IBM SPSS Version 22 was utilized for the analysis of statistics.

- The mean age of the participants was 3.16 ± 2.72.
- There were 19 males (47.5 %) and 21 females (52.5%), with a male is to female (M: F) ratio of 0.9.
- The mean baseline hemoglobin in the daily group was 7.38 ± 1.49 g/dl whereas, in alternate day oral iron therapy group was 8.11 ± 1.46 g/dl.

- There was a statistically significant rise in the Hb level, in alternate day oral Fe therapy group after 30 days, with a mean rise of 2.3 ± 0.76 (P value = 0.002).
- The mean baseline serum hepcidin level in the daily group was 6.45 ± 3.84 ng/ml and 5.28 ± 2.87 ng/ml in the alternate day group.
- 15 out of 20 children (75%) in alternate group, had decrease in the serum hepcidin levels after 48 hours.
- Only 5 out of 20 children (25%) in the daily group, had decrease in the serum hepcidin levels after 48 hours.
- There was a statistically significant decrease in the serum hepcidin level to 4.1 ± 2.75 ng/ml in the alternate day group (P value = 0.01).
- 12 out of the 20 children (60%) in daily group, and 4 out of 20 in alternate group (20%), had gastrointestinal side effects.
- Out of the 12 children having gastrointestinal side effects in the daily oral iron therapy group, 7 had complaints of abdominal pain (59%), 4 had constipation (33%) and 1 had vomiting (8%).
- Serum hepcidin values were low in children in the alternate day group.
- A significant improvement, in Hb level was noted in children in alternate day group.
- Gastrointestinal side effects following oral iron supplementation, was significantly less in alternate day group.
- Hence in children with IDA, alternate day iron supplementation, optimises iron absorption with less gastrointestinal symptoms and better compliance and might be a preferable dosing regimen.
- Since this is a uni-center study, additional studies which are multi-centre, with larger sample sizes are needed to strengthen and validate the findings.

BIBLIOGRAPHY

1. World Health Organization. Anaemia. World Health Organization. Retrieved July 21, 2024, from www.who.int/news-room/fact-sheets/detail/anaemia
2. Ministry of Health and Family Welfare. National Family Health Survey (NFHS 5). 2019-2020. Compendium of Fact Sheets: Key indicators India and 14 states/UTs (phase II). Accessed July 7, 2024. Available from: https://main.mohfw. gov.in/sites/default/files/NFHS-5_Phase-II_0.pdfphase1
3. Ozdemir N. Iron deficiency anemia from diagnosis to treatment in children. Turk Arch Pediatr. 2015;50:11–9.
4. Barney, D. E. (2022). The Effects of a Prolonged Bout of Running on Hepcidin and Iron Homeostasis. Retrieved from https://purl.lib.fsu.edu/diginole/Barney_fsu_0071E_17493
5. Lokeshwar MR, Shah N. Nutritional Anemia in infancy, Childhood and Adolescents. In: Lokeshwar MR, Shah N, Agarwal B, Manglani M, Sachdeva A.(Ed). Textbook of Pediatric Hematology and Hemato-Oncology. Jaypee;2016,p.100-125
6. Chaber R, Helwich E, Lauterbach R, Mastalerz-Migas A, Matysiak M, Peregud-Pogorzelski J et all. Diagnosis and Treatment of Iron Deficiency and Iron Deficiency Anemia in Children and Adolescents: Recommendations of the Polish Pediatric Society, the Polish Society of Pediatric Oncology and Hematology, the Polish Society of Neonatology, and the Polish Society of Family Medicine. Nutrients. 2024 Oct 25;16(21):3623.

7. Powers J. Nutritional anemias. In: Fish J, Lipton J, Lanszkowsky P (Ed). Lanzkowsky's Manual of Pediatric Hematology Oncology. 7th edition. Elsevier; 2021, p.61-71.
8. Chandra J, Dewan P, Kumar P, Mahajan A, Singh P, Dhingra B et all. Diagnosis, Treatment and Prevention of Nutritional Anemia in Children: Recommendations of the Joint Committee of Pediatric Hematology-Oncology Chapter and Pediatric and Adolescent Nutrition Society of the Indian Academy of Pediatrics. Indian Pediatr. 2022 Oct 15;59(10):782-801.
9. Moretti D, Goede JS, Zeder C, Jiskra M, Chatzinakou V, Tjalsma H, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. Blood. 2015;126(17):1981–1989
10. Stoffel NU, Cercamondi CI, Brittenham G, Zeder C, Geurts-Moespot AJ, Swinkels DW, et al. Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: Two open-label, randomised controlled trials. Lancet Haematol. 2017;4(11):e524–e533.
11. Tanya Singh, Shilpa Khanna Arora,1 Parul Goyal. Hepcidin Levels in Response to Oral Iron Therapy in Children with Anemia. Indian Pediatrics, 2024
12. World Health Organization. Anaemia in women and children. World Health Organization. Retrieved July 21, 2024, from https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children

13. Camaschella C: Iron-deficiency anemia. N Engl J Med. 2015, 372:1832-43.
14. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014 Feb;19(2):164-74.
15. Ortolano, S. (2012). The interaction of iron deficiency and psychosocial care on child behavior and cognitive outcomes: A longitudinal study of Senegalese children. Tufts University, Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy.
16. Orkin S.H., Nathan D.G., Ginsburg D., Look A.T., Fisher D.E., Lux S. Nathan and Oski's Hematology of Infancy and Childhood (7th ed.). Saunders Elsevier; 2008,p.522-542.
17. Muir A, Hopfer U. Regional specificity of iron uptake by small intestinal brush-boarder membranes from normal and iron deficient mice. Am J Physiol 1985;248:G376-9. 17.
18. Frazer DM, Anderson GJ. Iron imports. I. Intestinal iron absorption and its regulation. Am J Physiol Gastrointest Liver Physiol 2005;289:G631-5. 18.
19. Nadadur SS, Srirama K, Mudipalli A. Iron transport and homeostasis mechanisms: Their role in health and disease. Indian J Med Res 2008;128:533-44
20. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. Biochem J 2011;434:365-81.
21. Kulaksiz H. Pro-hepcidin: expression and cell specific localisation in the liver and its regulation in hereditary haemochromatosis, chronic renal insufficiency, and renal anaemia. Gut 2004;53:735–43.
22. Nemeth E. Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization. Science 2004;306:2090–3.

23. Aschemeyer S, Qiao B, Stefanova D, Valore EV, Sek AC, Ruwe TA, et al. Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. Blood 2018;131:899–910.
24. Billesbølle CB, Azumaya CM, Kretsch RC, Powers AS, Gonen S, Schneider S et all. A. Structure of hepcidin-bound ferroportin reveals iron homeostatic mechanisms. Nature 2020;586:807–11.
25. Dulkadir, R., Turna Saltoğlu, G. & Güneş, A. Erythroferrone and hepcidin levels in children with iron deficiency anemia. BMC Pediatr **24**, 240 (2024).
26. Abioye AI, Aboud S, Premji Z, Etheredge AJ, Gunaratna NS, Sudfeld CR, et all. Hemoglobin and hepcidin have good validity and utility for diagnosing iron deficiency anemia among pregnant women. Eur J Clin Nutr. 2020 May;74(5):708-719.
27. Ćulafić J, Kolarović J, Pezo L, Čabarkapa V, Nikolić S, Stojadinović A et all. Serum Concentration of Hepcidin as an Indicator of Iron Reserves in Children. J Med Biochem. 2018 Dec 1;37(4):456-464.
28. Azab SF, Esh AM. Serum hepcidin levels in Helicobacter pylori-infected children with iron-deficiency anemia: a case-control study. Ann Hematol. 2013 Nov;92(11):1477-83.
29. Cherian S, Forbes DA, Cook AG, Sanfilippo FM, Kemna EH, Swinkels DW et all . An insight into the relationships between hepcidin, anemia, infections and inflammatory cytokines in pediatric refugees: a cross-sectional study. PLoS One. 2008;3(12):e4030.

30. Mupfudze TG, Stoltzfus RJ, Rukobo S, Moulton LH, Humphrey JH, Prendergast AJ et all. Plasma Concentrations of Hepcidin in Anemic Zimbabwean Infants. PLoS One. 2015 Aug 7;10(8):e0135227.
31. Vermeulen E, Vermeersch P. Hepcidin as a biomarker for the diagnosis of iron metabolism disorders: a review. Acta Clin Belg. 2012 May-Jun;67(3):190-7.
32. Pagani A, Nai A, Silvestri L and Camaschella C (2019) Hepcidin and Anemia: A Tight Relationship. Front. Physiol. 10:1294.
33. Sangkhae V, Nemeth E. Regulation of the Iron Homeostatic Hormone Hepcidin. Adv Nutr. 2017 Jan 17;8(1):126-136.
34. Matthai J., Sathiasekharan M., Poddar U., Sibal A., Srivastava A., Waikar Y. et all (2020). Guidelines on diagnosis and management of cow's milk protein allergy. Indian Pediatrics, 57(8), 723-729.
35. Baker RD, Greer FR; Committee on Nutrition, American Academy of Pediatrics. Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in Infants and Young Children (0-3 years of age). Pediatrics. 2010;126:1040-50.
36. Peyrin-Biroulet L, Williet N, Cacoub P. Guidelines on diagnosis and treatment of iron deficiency across indications: a systemic review. Am J Clin Nutr 2015;102:1585-94.
37. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO. Accessed May 23, 2024. Available from:https://www.who.int/publications/i/item/9789240000124

38. Fletcher A, Forbes A, Svenson N, Wayne Thomas D; A British Society for Haematology Good Practice Paper. Guideline for the laboratory diagnosis of iron deficiency in adults (excluding pregnancy) and children. Br J Haematol. 2022;196:523-29.
39. Yip R, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr. 1984;39:427-36.
40. Canals C, Remacha AF, Sarda MP, et al. Clinical utility of the new Sysmex XE 2100 parameter-reticulocyte hemoglobin equivalent-in the diagnosis of anemia. Haematologica. 2005; 90:1133-41.
41. Chinudomwong P, Binyasing A, Trongsakul R, Paisooksantivatana K. Diagnostic performance of reticulocyte hemoglobin equivalent in assessing the iron status. J Clin Lab Anal. 2020; 34:e23225.
42. Thomas DW, Hinchliffe RF, Briggs C, et al. Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol. 2013;161:639-48.
43. Jain S, Narayan S, Chandra J, et al. Evaluation of serum transerum ferritin errin receptor and sTfR ferritin indices in diagnosing and differentiating iron deficiency anemia from anemia of chronic disease. Indian J Pediatr. 2010;77:179-83.
44. Choi JW. Sensitivity, specificity and predictive value of serum soluble transerum ferritin errin receptors at different stages of iron deficiency. Ann Clin Lab Sci. 2005;35:435-39.

45. Snook J, Bhala N, Beales ILP, et al. British Society of Gastroenterology Guidelines for management of iron deficiency anemia in adults. Gut. 2021;70:2030-51.
46. Patil P, Geeverhese P, Khaire P, et al. Comparison of therapeutic efficacy of ferrous ascorbate and iron polymaltose complex in iron deficiency anemia in children: A randomized controlled trial. Indian J Pediatr. 2019;86:1112-17.
47. Bopche AV, Dwivedi R, Mishra R, Patel GS. Ferrous sulfate versus iron polymaltose complex for treatment of iron deficiency anemia in children. Indian Pediatr. 2009;46:883-85.
48. Name JJ, Vasconcelos AR, Maluf MCVR. Iron bisglycinate chelate and iron polymaltose complex for the treatment of iron deficiency anemia: A pilot randomized trial. Curr Pediatr Rev. 2018;14:261-68.
49. Yewale VN, Dewan B. Treatment of iron deficiency anemia in children: A comparative study of ferrous ascorbate and colloidal iron. Indian J Pediatr. 2013;80:385–90.
50. World Health Organization. Nutritional Anemias: Tools for Effective Prevention and Control. Geneva: World Health Organization, 2017. Accessed July 5, 2024.
51. Lee NH**.** Iron Deficiency Anemia**.** Clin Pediatr Hematol Oncol 2020;27:101-112.
52. Crary SE, Hall K, Buchanan GR. Intravenous iron sucrose for children with iron deficiency failing to respond to oral iron therapy. Pediatr Blood Cancer. 2011;56:615-19.
53. Laass MW, Straub S, Chainey S, et al. Effectiveness and safety of ferric carboxymaltose treatment in children and adolescents with inflammatory bowel disease and other gastrointestinal diseases. BMC Gastroenterol. 2014;14::184.

54. Cançado RD, Muñoz M. Intravenous iron therapy: How far have we come? Rev Bras Hematol Hemoter. 2011;33:461-9.
55. Koch TA, Myers J, Goodnough LT. Intravenous iron therapy in patients with iron deficiency anemia: Dosing considerations. Anemia. 2015; 2015:763576.
56. Raveendran AV, Shiji PV, Rajini P, Al Qassabi FS. Iron Deficiency Anemia: An Update. BMH Med. J. 2019;6:116-130.
57. Kendrick JG, Carr RR, Ensom MHH. Pharmacokinetics and drug dosing in obese children. J Pediatr Pharmacol Ther. 2010;15:94-109..
58. Kitsati N, Liakos D, Ermeidi E, et al. Rapid elevation of transerum ferritin errin saturation and serum hepcidin concentration in hemodialysis patients after intravenous iron infusion. Haematologica. 2015;100:e80.
59. Taneja S, Bhandari N, Strand TA, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr. 2007;86:1302-309.
60. Surico G, Muggeo P, Muggeo V, Lucarelli A, Martucci T, Daniele M, et all. Parenteral iron supplementation for the treatment of iron deficiency anemia in children. Ann Hematol. 2002 Mar;81(3):154-7.
61. National Health Mission. Anaemia Mukt Bharat. Retrieved July 5, 2024, from https://nhm.gov.in/index1.php?lang=1&level=3&sublinkid=1448&lid=797
62. Peña-Rosas, J. P., De-Regil, L. M., Gomez Malave, H., Flores-Urrutia, M. C. & Dowswell, T. Intermittent oral iron supplementation during pregnancy. Cochrane Database Syst Rev. 10, CD009997 (2015).
63. Stoffel NU, Zeder C, Brittenham GM, Moretti D, Zimmermann MB. Iron absorption from supplements is greater with alternate day than with consecutive day dosing in iron-deficient anemic women. Haematologica. 2020;105(5):1232–1239.

64. Mehta S, Sharma BS, Gulati S, Sharma N, Goyal LK, Mehta S. A prospective, randomized, interventional study of oral iron supplementation comparing daily dose with alternate day regimen using hepcidin as a biomarker in iron deficiency anemia. J. Assoc. Phys. India. 2020;68(5):39–41
65. Bregman DB, Morris D, Koch TA, He A, Goodnough LT. Hepcidin levels predict non-responsiveness to oral iron therapy in patients with iron deficiency anemia. Am J Hematol 2013; 88: 97-101.
66. Matos TA, Arcanjo FP, Santos PR, Arcanjo CC. Prevention and Treatment of Anemia in Infants through Supplementation, Assessing the Effectiveness of Using Iron Once or Twice Weekly. J Trop Pediatr. 2016 Apr;62(2):123-130
67. Faqih AM, Kakish SB, Izzat M. Effectiveness of intermittent iron treatment of two- to six-year-old Jordanian children with iron-deficiency anemia. Food Nutr Bull. 2006 Sep;27(3):220-227

ANNEXURES

ANNEXURE – I - INFORMED CONSENT FORM

EFFICACY OF ALTERNATE DAY VERSUS DAILY ORAL IRON THERAPY IN CHILDREN WITH IRON DEFICIENCY ANEMIA – A RANDOMIZED CONTROL TRIAL.

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Co Guide:

PRIMARY OBJECTIVE- To assess the efficacy of alternate day versus twice daily oral Fe therapy in children with IDA.

SECONDARY OBJECTIVE-

1. Effect of alternate versus twice daily oral Fe therapy on serum hepcidin levels.
2. To compare the GI side effects in the two groups which may affect compliance

Introduction: You are being invited to participate in this study to find out the benefits of alternate day versus twice daily oral iron supplementation in iron deficient children.

Explanation of procedure: The procedure is very simple, first baseline investigations like complete hemogram, serum ferritin levels and iron studies will be done to identify Iron deficiency anemia in children. Then patients will be randomized into two groups, one group of patients will be given twice daily oral iron supplementation (ferrous fumarate) and the other group will be given alternate day oral iron supplementation at the same dose and of the same composition. After 48

hours of intervention, the serum hepcidin levels will be measured and after one month of supplementation complete hemogram would be done to see the improvement in hemoglobin levels.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: By participating in this study, we can get to know the efficacy of alternate versus daily twice oral iron therapy. This can be utilised to improve the compliance and tolerability of oral iron supplements which is conventional approach to treatment of Iron deficiency anemia. The data gathered will help population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the **principal investigator.**

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups.

However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

"Name of student/PI, mobile number, email ID". If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "EFFICACY OF ALTERNATE DAY VERSUS DAILY ORAL IRON THERAPY IN CHILDREN WITH IRON DEFICIENCY ANEMIA –A RANDOMIZED CONTROL TRIAL". My signature below indicates that I have decided to participate and I have read the Information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE – II -PROFORMA

Randomization group-

Name: Weight:

Age:

Sex:

Address:

Contact:

History- 1) CHIEF COMPLAINTS-

	PRESENT	ABSENT
1) Lethargy		
2) Refusal to feeds		
3) Poor scholastic performance		
4) Failure to thrive		
5) Delayed attainment of milsestone/regression		
6) Oral ulcers		
7) h/o chronic diarrhoea		
8) h/o recurent infections		
9) h/o passing worms in stool		
10) Pica		
11) h/o blood transfusions		
12) h/o intake of oral hematinics		
13) h/o alternative medications		
14) Yellowish discolouration of skin		
15) h/o seizures		
16) h/o stroke		
17) Cognitive delay		

2) FAMILY HISTORY-

	PRESENT	ABSENT
1) Consanguinity		
2) h/o blood transfusion		
3) h/o intake of oral hematinics		
4) Celiac disease		
5) Malabsorption		

3) BIRTH HISTORY-

PERIOD OF GESTATION:

BIRTH WEIGHT:

ANY NICU ADMISSION:

4) ANTHROPOMETRY-

LENGTH	WEIGHT	HEAD CIRCUMFERENCE

Examination-

1) GENERAL PHYSICAL EXAMINATION-

	PRESENT	ABSENT
1) Pallor		
2) Icterus		
3) Angular cheilitis		
4) Platynychia		
5) Koilonychia		

2) SYSTEMIC EXAMINATION-

a)PER ABDOMEN	PRESENT	ABSENT
1) Hepatomegaly		
2) Splenomegaly		
3) Surgical scar		

b)CARDIOVASCULAR	PRESENT	ABSENT
1) Sinus tachycardia		
2) Decreased blood pressure		
3) Increased pulse pressure		
4) Hemic murmur		
5) Venous hum		

c) RESPIRATORY SYSTEM- positive findings if any

d) CENTRAL NERVOUS SYSTEM- positive findings if any

e) Any other positive findings-

Investigations-

1) COMPLETE HEMOGRAM-

	BASELINE	30 DAYS AFTER INTERVENTION
1) Hemoglobin		
2) RBC levels		
3) MCV		
4) MCH		
5) MCHC		
6) RDW		
7) Retic. Count		
8) Peripheral Smear		
9) Total count		
10) Differential count		
11) Platelet count		

2) IRON STUDIES-

	BASELINE
1) Serum Ferritin	
2) Serum Iron	
3) TIBC	
4) Transferrin saturation	

3) SERUM HEPCIDIN LEVELS

	BASELINE	AFTER 48 HOURS
1) Hepcidin		

4) LIVER FUNCTION/ RENAL FUNCTION TEST-

5) STOOL FOR OCCULT BLOOD-

6) ANY OTHER FOR SPECIAL FINDING-

a) Endoscopy

b) Serum Lead levels

c) USG

PATIENTS DIARY FOR GASTROINTESTINAL SYMPTOMS MONITORING-

	VOMITING	CONSTIPATION	ABDOMINAL PAIN
DAY 1			
DAY 2			
DAY 3			
DAY 4			
DAY 5			
DAY 6			
DAY 7			
DAY8			
DAY 9			
DAY 10			
DAY 11			
DAY 12			
DAY 13			
DAY 14			
DAY 15			
DAY 16			
DAY 17			
DAY 18			
DAY 19			
DAY 20			
DAY21			
DAY 22			
DAY 23			

DAY 24			
DAY 25			
DAY 26			
DAY 27			
DAY 28			
DAY 29			
DAY 30			
DAY 31			

IF PRESENT THEN TICK.

IF ABSENT THEN CROSS.

ANNEXURE III – MASTER CHART

S No.	Name	Age	Sex	Hemoglobin on day 1(g/dl)	Hemoglobin at 30 days(g/dl)	Platelet count(/microlitre)	WBC(/microlitre)	MCV(fi)	MCH(pg)	MCHC(g/dl)	RDW(%)	Reticulocyte count(%)	Peripheral Smear	Ferritin(ng/ml)	S. iron(mcg/dl)	TIBC(mcg/dl)	Transferrin saturation(%)	S. hepcidin baseline(ng/ml)	S. hepcidin at 48 hours(ng/ml)	Gastrointestinal Side effects	Alternate/Daily group
1	Shravya	2yrs	Female	8.3	9.4	2.8L	15k	56.7	16.8	29.7	27.6	1.3	Microcytic Hypochromic Anemia	8.42	8	322	2%	5.542	6.11	Present	Daily
2	Bhoomika	2yrs	Female	5.5	7.1	3.13L	12.3k	58.2	14.2	24.3	20.9	2.7	Microcytic Hypochromic Anemia	1.96	8	412	2%	1.536	3.429	Present	Daily
3	Zarina	3yrs	female	7.5	8.4	4.2L	6.8k	53.5	15.3	28.6	19.2	4	Microcytic Hypochromic Anemia	5.6	7	241	3%	6.331	8.318	Present	Daily
4	Amir	9 yrs	Male	5.4	9.5	4.8L	9k	56.3	13.4	23.8	50.9	1.9	Microcytic Hypochromic Anemia	1.6	19	385	2%	8.76	7.151	Absent	Daily
5	Mauli	1yr1m	Female	9.6	10.8	3.56L	7.2k	69	20.9	29.7	21	2.2	Microcytic Hypochromic Anemia	9.1	13	382	4%	9.012	4.343	Present	Alternate
6	Samarth	11months	Male	5.6	7	6.97L	16k	60.5	14.5	24	38	1.5	Microcytic Hypochromic Anemia	3.2	12	574	2%	11.031	2.703	Absent	Daily
7	B/o Reshma	11 months	female	5.6	6.7	3.81L	9.2k	58.7	13.7	23.3	24.5	2.2	Microcytic Hypochromic Anemia	7.12	10	298	3%	13.744	15.132	Present	Daily
8	Samarth	3yrs10m	Male	9.9	10.3	4.07L	12.6K	68.9	18.9	29	24	0.8	Microcytic Hypochromic Anemia	4.8	11	450	2%	4.722	3.397	Absent	Daily
9	Aradhya	2yrs	female	6.5	8	3.24L	9k	62.1	15	24.2	25.4	1.1	Microcytic Hypochromic Anemia	8.8	14	312	2%	13.271	17.813	Present	Daily
10	Shrinivas	4yrs	Male	7	9.9	2.4L	7.6K	55.2	14	25	28	1.5	Microcytic Hypochromic Anemia	10	16	484	2%	3.87	3.712	Absent	Alternate
11	Vedant	8m	Male	7.2	9	5.2L	13.1k	57.7	16	27.8	21.3	1.1	Microcytic Hypochromic Anemia	12	18	460%	2%	2.104	2.293	Absent	Daily
12	Izhaan	2yrs	male	10.5	12	2.71L	6.6K	67.2	20.2	30	17	0.8	Microcytic Hypochromic Anemia	11	14	343	4%	4.028	1.094	Absent	Alternate
13	Aishwarya	9months	female	10	12.8	4.2L	16k	62.7	20	22.8	14.3	1.3	Microcytic Hypochromic Anemia	30	18	414	5%	9.201	12.64	Present	Alternate
14	Adiba	1yr2m	Female	7.1	10.7	6.74L	17.6k	52.2	14.1	27.1	21.5	2.2	Microcytic Hypochromic Anemia	8	5	442	3%	3.744	2.703	Absent	Alternate
15	Shreenidhi	1y3m	Female	8.5	10	7.22L	13.4K	62.3	18.1	29	21.3	3.4	Microcytic Hypochromic Anemia	9	17	402	3%	2.829	2.23	Absent	Alternate
16	Praneeth	1.5yrs	male	6.4	10.1	4.07L	17.2K	52.6	13.6	25.8	22.2	3.6	Microcytic Hypochromic Anemia	7.1	8	448	2%	3.649	1.883	Absent	Alternate
17	Sandeep	1yr4m	Male	5.4	7.6	3.56L	7.2k	57.9	12	20.8	28.6	0.9	Microcytic Hypochromic Anemia	6.2	7	510	2%	3.113	3.776	Present	Alternate
18	Aditya	1y11m	male	10	12.5	3.38L	12.8k	69	19.7	28.6	21.3	1.1	Microcytic Hypochromic Anemia	9	10	418	3%	10.716	9.58	Absent	Alternate

19	Madhu	10yrs	female	8.7	10.1	2.12L	5.9k	73	21	28.4	18.1	1.2	Microcytic Hypochromic Anemia	11.1	10	318	7%	7.593	14.217	Present	Daily
20	Bhuvan	2yrs	Male	6	8.9	4.0L	9.1k	66	23	28	20	1.8	Microcytic Hypochromic Anemia	10	7	450	2%	9.769	5.668	Absent	Alternate
21	Ranvijay	1y 10m	male	7.8	8.9	1.54L	4.8k	61.1	16.3	26.7	16.8	0.9	Microcytic Hypochromic Anemia	14	17	407	4%	4.911	8.444	Present	Daily
22	Riza	10m	Female	9	10.1	3.88L	15.5k	69	21	25.9	23	2.1	Microcytic Hypochromic Anemia	8	13	340	5%	4.312	3.776	Absent	Daily
23	Shreenidhi	6yrs	Female	6.2	7.8	5.12L	14k	64	18	23	26	2.5	Microcytic Hypochromic Anemia	5	12	400	2%	4.091	4.659	Present	Daily
24	Pramod	4yrs	male	10.2	11.3	5.34L	8.1k	72	22.5	29	18.6	0.3	Microcytic Hypochromic Anemia	7	4	320	3%	7.687	3.776	Absent	Daily
25	B/O Jannifer	1y9m	Male	8.7	9.8	2.99L	9.7k	58.2	17.2	29.6	25.9	0.6	Microcytic Hypochromic Anemia	10	15	300	3%	3.776	3.8	Present	Alternate
26	Nikhil	2y 9m	male	8	9.6	5.14L	6.78K	53.5	13.5	25.2	21.2	0.6	Microcytic Hypochromic Anemia	2.99	16	530	3%	11.977	18.223	Present	Daily
27	Laxmi	8yrs	Female	8.5	10.5	4.86L	12.5k	70.9	20	30.2	16.9	2.1	Microcytic Hypochromic Anemia	7.1	15	381	2%	4.911	4.217	Absent	Alternate
28	Sanayna	9y	Female	5.7	7.2	2.0L	4.2K	55	14	25.5	28	1.3	Microcytic Hypochromic Anemia	3.78	9	488	2%	0.621	2.451	Absent	Daily
29	Sanvi	1y7m	Female	7.7	11.2	5.11L	15k	66.3	16.7	25.2	24.4	2.6	Microcytic Hypochromic Anemia	6.8	4	381	3%	5.069	3.902	Absent	Alternate
30	Pushpa	3y	Female	8.5	10	3.45L	8k	69	16	24.9	21	1.7	Microcytic Hypochromic Anemia	8	11	290	3%	2.356	3.618	Absent	Daily
31	Shriyan	2yrs	male	6.3	7.5	7.0L	8.6k	57.4	16.3	28.4	20.8	0.9	Microcytic Hypochromic Anemia	13.7	7	249	3%	6.552	15.858	Present	Daily
32	Amar	2yrs	male	7.6	9.4	6.36L	16k	57.1	14.6	25.5	19.4	0.9	Microcytic Hypochromic Anemia	6.27	12	508	2%	11	3.555	Absent	Alternate
33	Shreyas	6yrs	Male	7.7	10.2	4.29L	8.5k	57.8	17.6	30	18.9	1.2	Microcytic Hypochromic Anemia	4.42	9	445	2%	2.608	2.766	Absent	Alternate
34	Pavan	10yrs	male	9.3	11.5	3.17L	10.9k	71.7	27	33.8	20	1.1	Microcytic Hypochromic Anemia	3.24	12	342	3%	2.356	5.605	Absent	Alternate
35	Trisha	4y10m	Female	8.6	10.9	4.6L	15.7k	67	22	32	16.5	1.5	Microcytic Hypochromic Anemia	6	11	287	4%	4.343	4.028	Absent	Alternate
36	Umaiza	5yrs	female	8.4	11	4.15L	6.9k	56.7	16.5	28.7	18	0.9	Microcytic Hypochromic Anemia	9	13	285	7%	8.097	12.072	Present	Daily
37	Shivanya	1yr2m	Female	7.2	8.8	6.22L	13k	59	16.8	28	24	1.1	Microcytic Hypochromic Anemia	4	12	320	3%	3.744	4.028	Present	Daily
38	Keerthana	11m	Female	7	8.9	2.06k	16k	61	19	27	26	2.1	Microcytic Hypochromic Anemia	8	9	372	3%	3.807	1.504	Absent	Alternate
39	Preetam	4y	Male	9.8	11.6	2.7L	6.9k	70	19	28	19	4	Microcytic Hypochromic Anemia	6	15	210	3%	4.123	3.397	Absent	Alternate
40	B/O Laxmi	7m	Female	6.8	8.9	6.11L	14k	58.2	16.2	27	28	2.8	Microcytic Hypochromic Anemia	9	8	296	3%	3.618	1.536	Absent	Alternate