A STUDY TO ASSESS SERUM ELECTROLYTES AND HbA1c LEVELS IN NONDIABETIC IRON DEFICIENCY ANEMIA INDIVIDUVALS

BY

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LIST OF ABBREVIATIONS

ADA	:	American Diabetes Association
DCCT	:	Diabetes Control and Complications Trial
DM	:	Diabetes Mellitus
DMT	:	Divalent Metal Transporter
EPO	:	Erythropoietin
FBS	:	Fasting Blood Sugar
Fpn	:	Ferroportin
Hb	:	Hemoglobin
HbA1c	:	Glycosylated Hemoglobin
HGI	:	Hemoglobin Glycation Index
HPLC	:	High Performance Liquid Chromatography
IDA	:	Iron Deficiency Anaemia
IEC	:	International Expert Committee
IFCC	:	International Federation of Clinical Chemistry
NGSP	:	National Glycohemoglobin Standardization Program
OGTT	:	Oral Glucose Tolerance Test
PPBS	:	Postprandial Blood Sugar
RDW	:	Red cell Distribution Width
TfR	:	Transferrin Receptor
TIBC	:	Total Iron Binding Capacity
UIBC	:	Unsaturated/Latent Iron Binding Capacity
WHO	:	World Health Organization

LIST OF TABLES

SL.NO	TABLE	PAGE.NO
1	Iron compartments	9
2	Recommended Dietary Allowance (RDA) for Iron	12
3	Bone Marrow Iron stores and corresponding Serum ferritin levels	24
4	Differential Diagnosis of microcytic anaemia	28
5	Oral preparations of Iron	30
6	Parenteral iron preparations	31
7	American Diabetic Association (ADA) criteria for the diagnosis of diabetes	38
8	Age Distribution	48
9	Sex distribution	49
10	Mean HbA1c among cases	50
11	Symptoms and signs among cases	51
12	Diet distribution among cases	52
13	Comparison of Haemoglobin with HbA1c	53
14	Correlation between Haemoglobin and HbA1c	54
15	Comparison of Haemoglobin with serum sodium	55
16	Comparison of Haemoglobin with serum potassium	56
17	Mean value of other lab parameters	57

LIST OF GRAPHS

SL.NO	GRAPH	PAGE.NO
1.	Age distribution among Cases	48
2.	Sex distribution	49
3.	Mean HbA1c among cases	50
4.	Clinical features among cases	51
5.	Diet distribution among cases	52
6.	Comparison of Haemoglobin with HbA1c	53
7.	Correlation between Haemoglobin and HbA1c	54
8.	Comparison of Haemoglobin with serum sodium	55
9.	Comparison of Haemoglobin with serum potassium	56

LIST OF FIGURES

SL.NO	FIGURE	PAGE.NO
1	Absorption and distribution of Oral Iron	11
2	The relationship between oral iron dosage and amount of iron absorbed	13
3	Schematic diagram of iron uptake dcytb –duodenal cytochrome-b, HCP-1 – Heme carrier protein-1, HO-Heme Oxygenase, DMT – Divalent metal transporter	14
4	Iron recycling in and out of plasma	15
5	Hepcidin Negative regulator of Iron metabolism at the level of absorption	16
6	Development of iron deficiency anaemia	19
7	Peripheral smear in iron deficiency anaemia	23
8	Serum ferritin levels as a function of sex and age	25
9	Approach to iron refractory iron deficiency (IRIDA)	32

TABLE OF CONTENTS

SL.NO	CONTENT	PAGE.NO
1.	ABSTRACT	1-3
2.	INTRODUCTION	4-6
3.	AIMS OF THE STUDY	7
4.	REVIEW OF LITERATURE	8-43
5.	MATERIALS AND METHODS	44-47
6.	RESULTS	48-57
7.	DISCUSSION	58-62
8.	CONCLUSION	62-63
9.	SUMMARY	64
10.	BIBLIOGRAPHY	65-71
11.	ANNEXURES	72-78
I	ETHICAL CLEARANCE CERTIFICATE	72
II	CONSENT FORM	73-75
III	CASE TAKING PROFORMA	76-77
IV	MASTER CHART	78

ABSTRACT

TITLE: A STUDY TO ASSESS SERUM ELECTROLYTES AND HbA1C LEVELS IN NON-DIABETIC IRON DEFICIENCY ANEMIA INDIVIDUALS.

NEED FOR THE STUDY:

Diabetes Mellitus (DM) is defined as a group of metabolic diseases of multiple etiologies characterized by hyperglycemia with disturbances of carbohydrate, protein & fat metabolism resulting from defects in insulin secretion, insulin action, or both.

Iron deficiency anaemia is commonest form of nutritional anaemia worldwide. Glycated haemoglobin, or HbA1c, makes up the majority of HbA. It is the gold standard approach for evaluating a person's glycemic management during the last three months. In 2009 International expert committee recommended HbA1c level >6.5 as cutoff to diagnose Diabetes and HbA1c between 5.7-6.5 as Prediabetes. Limitations imposed by non-glycemic factors influence us to question the application of HbA1c in diagnosis of Pre-Diabetic and diabetic individuals in undernourished population like India where burden of IDA and Diabetes are high.

Electrolyte imbalance and iron deficiency anaemia are widespread issues in the Indian population. Few studies have conclusively linked anaemia to an imbalance in serum electrolyte levels. The results of various studies on relationship between HbA1c and iron deficiency anaemia, and its effect on serum electrolytes were conflicting. Very Fewer studies have been conducted in Indian population on this topic.

Objective: To know whether the Iron deficiency anaemia has an effect on Glycated haemoglobin (HbA1c) levels and Serum electrolytes in non-diabetic individuals.

Materials and method

Our study was a hospital-based cross-sectional study conducted on 65 patients admitted to the wards with a history and clinical findings for Iron deficiency anaemia in BLDEDU Shri B M Patil Medical College and Research Centre, Vijayapura, after getting approval from the institutional ethical committee.

RESULTS

The study results were conducted to evaluate the correlation between haemoglobin and HbA1C levels. Also, between haemoglobin and serum electrolytes (serum sodium and serum potassium) in a total 65 patients are as follows,

Correlation between HB and HBA1c:

In this study, we can observe that when haemoglobin levels decreased due to iron deficiency, HbA1C levels gradually increased, and there was a statistically significant negative correlation between haemoglobin and HbA1C levels in iron deficiency anaemia ($p = 0.0001$).

Comparison between Haemoglobin (Hb) and Serum electrolytes:

In our study, 16 patients had hyponatremia with a mean Hb of 4.8, and 1 patient had hypernatremia with a mean Hb of 7.0. Whereas, 48 patients had sodium levels in the normal range with a mean Hb of 4.9. Also, our study shows 2 patients had hypokalemia with a mean Hb of 6.2, and 1 patient had hyperkalemia with a mean Hb of 3.2. Whereas, 62 patients had potassium levels in the normal range with a mean Hb of 4.9.

The correlation between Haemoglobin and HbA1c levels had a P-value < 0.0001 which is statically significant and SPERSON'S CORRELATION COEFFICIENT was **r=0.433** which signifies the mild correlation.

Conclusion:

- The prevalence of iron deficiency anemia is more common in reproductory age group women. Haemoglobin and HbA1c showed statistically significant negative correlation in patients with iron deficiency anaemia. Before using the HbA1c to diagnose diabetes and pre-diabetes, iron deficiency anaemia must be taken into consideration as there is false positive elevation in HbA1C levels. Hyponatremia which is commonly seen in iron deficiency anaemia must be kept in mind before treatment.
- Treating physicians should always be aware of the effect of alcoholism, uremia, Aspirin intake, hemolytic anaemia, Vit B12 deficiency anaemia, iron deficiency on HbA1c before making a therapeutic decision based on HbA1c. And, should always closely monitor serum electrolytes in patients with iron deficiency anemia as the prevalence of hyponatremia and hyperkalemia is high and to avoid complications.

INTRODUCTION

Iron deficiency anaemia is the commonest form of nutritional anaemia worldwide. According to the WHO (World Health Organization), 2.1 billion people worldwide—or almost 30% of the world's population—have iron deficiency anaemia. Iron deficiency is a late sign of anaemia.[1]

Anaemia is projected to be present in 39.8% of children under the age of 5, 48% of children aged 5 to 14, 42% of women aged 15 to 59, 30% of males aged 15 to 59, and 45% of individuals aged 60 and older in developing nations. These data demonstrate the considerable negative effects of anaemia on the economies and health of middle- and low-income nations. Adults who are anaemic or iron-deficient have severe productivity loss. Higher maternal mortality, premature labour, low birth weight, and increased infant mortality are all linked to iron deficiency in pregnant women.

Children who are iron deficient have impaired cognitive and motor development and are more susceptible to illnesses.[1]

In India, anaemia is the publics main health issue. Anaemia affected 53.4% of children aged 6 to 59 months, 57% of girls aged 15 to 49, and 23% of boys aged 15 to 49 in 2019, according to the National Family Health Survey-5 (NFHS-5). According to NFHS-5 statistics, rural regions have a greater prevalence of anaemia. Anaemia affects 51% of urban and 53% of rural women, 25% of rural males, and 19% of urban men. However, there is a dearth of information about the epidemiology of anaemia in the rural population. Anaemia prevalence has barely changed in past 10years.At the national level, the findings of the NFHS-5 reveal that there has been an increase in the prevalence of anaemia among women and children compared to the previous NFHS-4 survey that was conducted in 2015-16, about 4 years ago.[2]

The most common form of haemoglobin A is called haemoglobin A1c (HbA1c), often known as glycated haemoglobin. It serves as the gold standard for evaluating glycemic control. It displays the person's glycemic status over the last three months. It is created by glycating the haemoglobin

chain's NH2-terminal valine. The goal HbA1c for all diabetes patients is below 7%, according American Diabetes Association recommendations, in order to prevent the emergence of secondary microvascular problems.[1,2]

High HbA1c levels are associated with an increased incidence of retinopathy, much like high plasma glucose levels. The use of HbA1c to identify diabetes has now been advised by the ADA and an international expert committee. The WHO also stated that, when used in conjunction with the proper precautions, such as a standardised assay, calibration against IFCC standards, and a low coefficient of variability, HbA1c may be used to diagnose diabetes.

In addition to the classic clinical signs of diabetes and plasma glucose levels of more than 200 mg/dl, an international expert committee proposed the HbA1c level of more than 6.5% as a cut-off point in 2009. The Committee also suggested that people with HbA1c levels between 6.0 and 6.5% should take preventative measures for diabetes because they are at a higher risk. In addition to blood glucose level, genetic factors, hematologic factors, multi-system illness and drug intake has variable effects on HbA1c levels.[2]

Based on structural changes and variations in the levels of HbA1c in both old and young red blood cells, it was hypothesised that HbA1c levels and iron deficiency anaemia were related in early research. There were not many research that discovered any alterations in the HbA1c levels of anaemic individuals. Another group of research reported that individuals with iron deficiency anaemia had higher HbA1c values, which considerably decreased following therapy. Only a small number of studies have also shown that patients with iron deficiency anaemia have lower HbA1c values than controls.

Electrolyte imbalance and iron deficiency anaemia are widespread issues in the Indian population. Few studies have conclusively linked anaemia to an imbalance in serum electrolyte levels as a result of changes in the sodium-potassium adenosine triphosphatase (Na+/K+ ATPase) pump activity that

controls intra and extracellular cation homeostasis and is related with red cell membranes. The serum's four electrolytes—sodium (Na+), potassium (K+), chloride (Cl), and bicarbonate—are crucial for maintaining red blood cells (RBCs') normal shape, as well as for the exchange of gases between RBCs and tissues. They also play a crucial role in nerve conduction, muscle contraction, and acid-base balance. Increased Na + /K + ATPase activity in anaemic patients counteracts the physiological effects of patient's tolerance to low oxygen levels in the cell, change in membrane-bound enzymes directly affects the Na + and K + in the serum.[3]

Recent studies have shown that people with iron deficiency have low sodium levels and high potassium levels. Lethargy, tiredness, and muscular cramps are examples of minor symptoms that can result from an alteration in blood electrolyte levels. More serious symptoms include an irregular heartbeat, disorientation, convulsions, and even death.[4] The postulated relationship between the two carries major clinical significance since anaemia and serum electrolyte abnormalities are the two public health problems with the greatest prevalence. Global statistics indicate a link between electrolyte imbalance and anaemia, especially IDA. The local statistics, however, are few.[5]

Studies on the association between HbA1c and iron deficiency anaemia and how it affects serum electrolytes have produced inconsistent results. On this subject, very few research have been done among the Indian population. In Indian population where incidence of both diabetes mellitus, nutritional iron deficiency and electrolyte imbalance is very high, and the conflicting results of previous similar studies have influenced us to take up the current study.

AIM OF THE STUDY

To know whether the Iron deficiency anaemia has an effect on Glycated haemoglobin (HbA1c) levels and Serum electrolytes in non-diabetic individuals.

REVIEW OF LITERATURE

IRON DEFICIENCY

Iron deficiency is the state in which the body's iron content is less than normal. Depletion of iron stores is the first sign of iron deficiency; during this stage, haemoglobin, serum iron, and transferrin saturation will all be within normal ranges, but the amount of storage iron will either be low or absent. Iron deficiency without anaemia is a more advanced stage marked by low serum iron and transferrin saturation as well as depleted iron reserves. Anaemia is not evident, though.[6]

The stage of iron deficiency that is by far the most advanced is anaemia. Iron stores are depleted, serum iron levels are low, transferrin saturation is poor, and haemoglobin levels are low. The most prevalent type of nutritional anaemia is iron deficiency anaemia. It is more common in women and children in areas with poor meat consumption, iron-deficient food, endemic areas of malaria, and a higher prevalence of intestinal helminthic diseases.[6,7]

IRON METABOLISM

One of the essential components of the basic metabolism is iron. Heme contains a significant amount of iron. When producing energy in mitochondria, cytochrome oxidase uses it as the active site for electron transport in cytochromes. O2 is carried from the lungs to the different tissues and stored by the heme moiety as haemoglobin and myoglobin. Heme is the peroxidases active site, which protects cells from oxidative damage by converting peroxides to water and inducing granulocytes to create microbicidal hypochlorite.[6]

Table 1: Iron compartments[9]

Compartment	Iron Content (mg)	Total Body Iron (%)
Hemoglobin iron	2000	67
Storage iron (ferritin, hemosiderin)	1000	27
Myoglobin iron	130	3.5
Labile pool	80	2.2
Other tissue iron	8	0.2
Transport iron	3	0.08

DISTRIBUTION OF IRON [1,2,3,4]

I. HEMOGLOBIN

About 1 mg of iron may be found in 1 ml of packed red blood cells. Given that red blood cells have a 120-day lifespan, macrophages recycle 1/120 of the iron in haemoglobin each day and release it back into the plasma. They are mostly transported from plasma to marrow erythroblasts where they are incorporated into freshly made haemoglobin.[6]

II. STORAGE COMPARTMENT: Hemosiderin or ferritin is the iron storage form. Hemosiderin is water insoluble, but ferritin is water soluble.

A. FERRITIN

The ferritin molecules H and L subunits are of different types. The rapid uptake or release of iron by ferritin is improved by the ferroxidase activity of H subunits. Except in cases of inflammation, the body's entire iron store is represented by plasma ferritin concentration. The average adult male's iron storage compartment is between 800 and 2000 mg, whereas an adult female's is between 300 and 500 mg. To be released from ferritin storage, iron must be reduced from the ferric state (Fe^{3+}) to the ferrous state (Fe^{2+}), which diffuses out of the apoferritin shell. It is reoxidized by hephaestin or

ceruloplasmin as it diffuses from the cytosol into the plasma. It also binds to transferrin and flows to the target organs, including the liver and bone marrow. Autophagy can also release iron from ferritin after lysosomal degradation has taken place.

B. HEMOSIDERIN

Hemosiderin is being abundantly distributed in macrophages. It is similar to the iron centre of ferritin chemically. It could have come from ferritins, whose outer protein shells were destroyed in lysosomes. When the body's supply of iron is reduced, the iron found in hemosiderin deposits in cells is not readily available.[6]

III. MYOGLOBIN

All skeletal and cardiac muscles contain trace levels of myoglobin. It acts as a storage space for oxygen. It shields cells from hypoxia damage. Nitric oxide and reactive oxygen species are also scavenged by it.[6,7]

IV. LABILE IRON POOL

It depicts iron that is still in the interstitial compartment before it is converted into heme or storage compounds. A portion of the iron returns to the plasma. The labile iron pool typically ranges from 80 to 90 mg.[7]

V. TISSUE-IRON COMPARTMENT

This amounts to approximately 6 to 8 milligrammes (exclusive of hemoglobin, ferritin, hemosiderin, myoglobin and the labile compartment). Tissue iron is made up of cytochromes and other iron-containing enzymes. It is a crucial component of the iron compartments.[7]

VI. TRANSPORT COMPARTMENT

It normally weighs 3 mg and is the iron compartment's smallest and most functional component. This portion of the iron travels almost entirely through transferrin. There are at least ten times a day where transferrin normally turns over. Iron is moved in this fashion between different compartments.[7]

TRANSFERRIN

A glycoprotein called transferrin has two globular domains and binding clefts for Fe3+. Transferrin, which can bind 250–480 mcg/dl of iron and carry 50–180 mcg/dl of iron, is present in human plasma at levels of 200–360 mg/dl. Hepatocytes and the cells of the monocyte-macrophage system produce apotrasferrin, which is devoid of iron, from which transferrin is formed.[8]

METABOLISM OF IRON

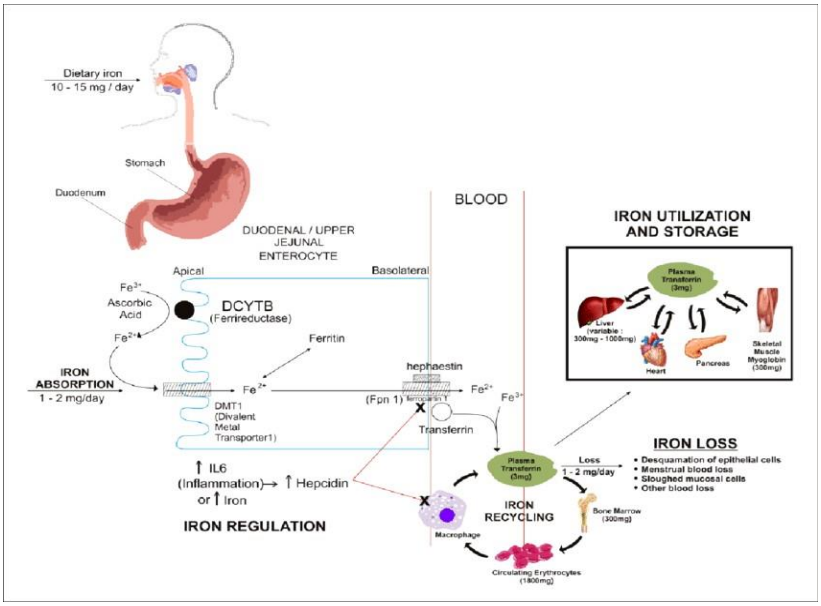


Figure 1: Absorption and distribution of Oral Iron[11]

DIETARY IRON:

Iron intake for average adult males and females is 9 to 10 mg and 12 to 14 mg daily, respectively. The adult males need for iron is to counteract the trace amounts of iron lost in stools due to duodenal epithelial cell desquamation. One milligram each day, roughly. The need for iron rises during periods of active growth or following blood loss. The need for iron in women increases during menstruation, pregnancy, and lactation.[8,9]

Table 2: Recommended Dietary Allowance (RDA) for Iron[10]

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27 mg*	0.27 mg*		
7–12 months	11 mg	11 mg		
1–3 years	7 mg	7 mg		
4–8 years	10 mg	10 mg		
9–13 years	8 mg	8 mg		
14–18 years	11 mg	15 mg	27 mg	10 mg
19–50 years	8 mg	18 mg	27 mg	9 mg
51+ years	8 mg	8 mg		

BIOAVAILABILITY OF IRON:

Dietary sources of iron are two types:

- Haem sources- meat is main source of haem iron
- Non-Haem sources- Vegetables, iron fortified cereals, lentils, jaggery

Heme from haemoglobin and myoglobin makes up about 15% of the dietary iron in meat-based foods. It absorbs more effectively than non-heme iron. Iron-binding dietary ingredients including phytates, oxalates, and phosphates have an impact on the absorption of non-heme iron. These chemicals bind to iron and prevent it from being absorbed.

Reducing chemicals like ascorbate, pyruvate, lactate, succinate, fructose, cysteine, and sorbitol improve the absorption of iron. Iron absorption is also regulated by gastric pH, mucus secretion, and how quickly food particles move through the intestine.[8,9]

IRON ABSORPTION[6]

The duodenum is where the majority of iron is absorbed. Iron absorption is influenced by the body's requirements. Iron absorption is -

- Increased in: active production of red cells, iron deficiency, increased physiological demand.
- Decreased in: iron overload states, systemic inflammation.

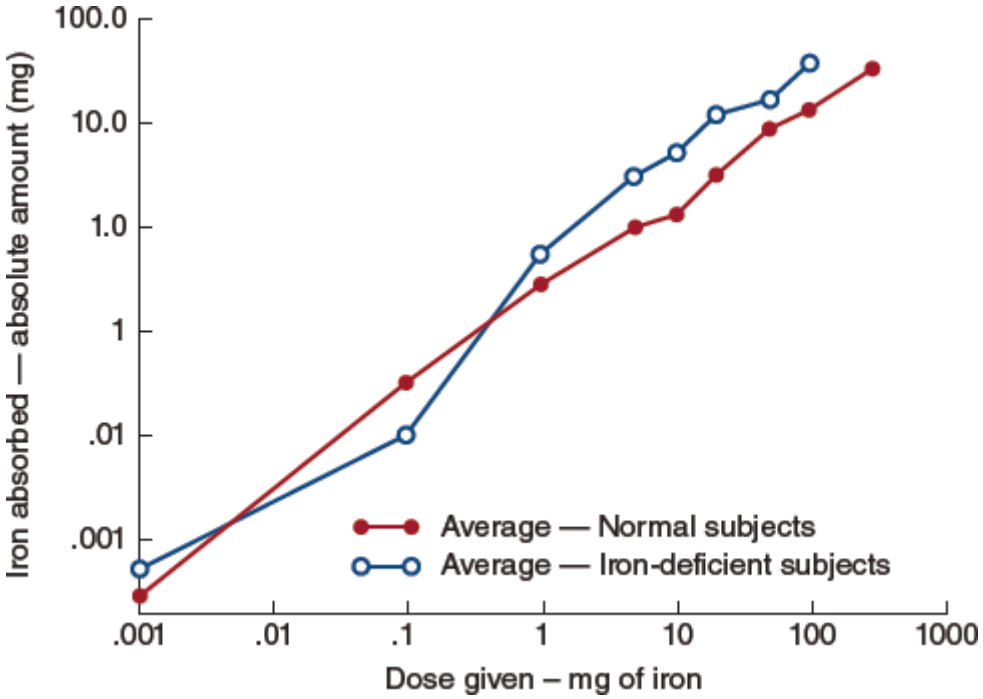


Figure 2: The relationship between oral iron dosage and amount of iron absorbed[6]

TRANSPORT ACROSS INTESTINAL MUCOSA

Duodenal cytochrome b reductase transforms ferric iron into ferrous iron. Then, it is moved by the Divalent Metal Transporter into the Intestinal Villous Cell (DMT). The basolateral membrane allows the ferric form of the iron to be exported once it has been oxidised back to ferrous by

ferroportin (Fpn) in association with hepaestin and ceruloplasmin. Subsequently, plasma apo transferrin transports ferric iron.

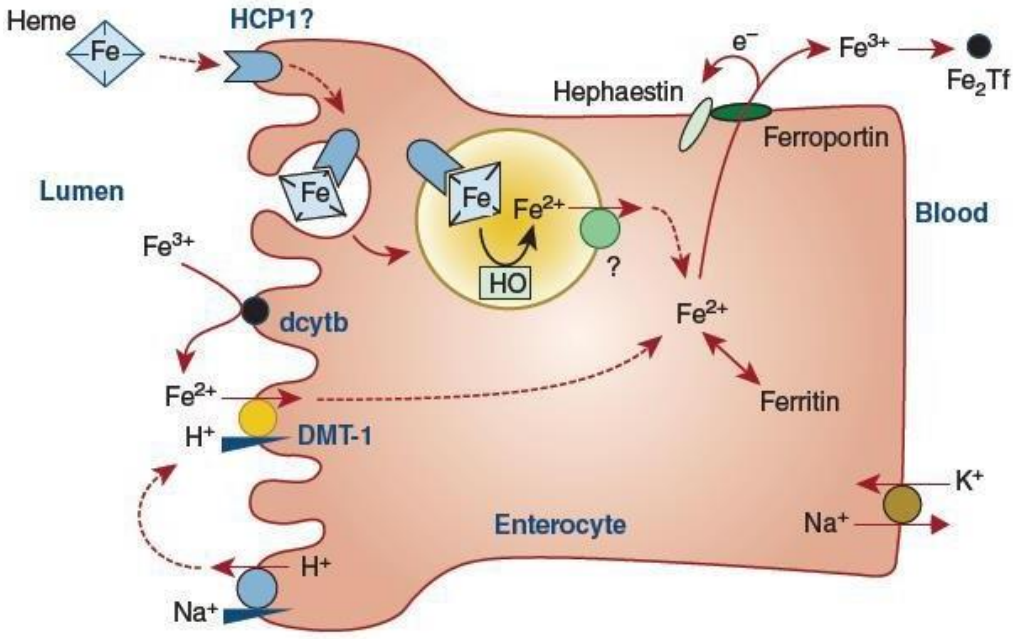


Figure 3: Schematic diagram of iron uptake [(2)]dcytb –duodenal cytochrome-b, HCP-1 – Heme carrier protein-1, HO-HemeOxygenase, DMT – Divalent metal transporter.[12]

IRON RECYCLING

The majority of the iron flux in and out of plasma is roughly 20 to 25 mg/day, compared to its absorption of 1 to 2 mg/day in adults, which is produced by the breakdown and formation of RBCs. The monocyte-macrophage system is where senescent aged RBCs are destroyed and haemoglobin is degraded. In order to produce haemoglobin in younger RBC, 80% of the iron that enters the plasma after being removed from senescent RBCs is reincorporated into the heme moiety.

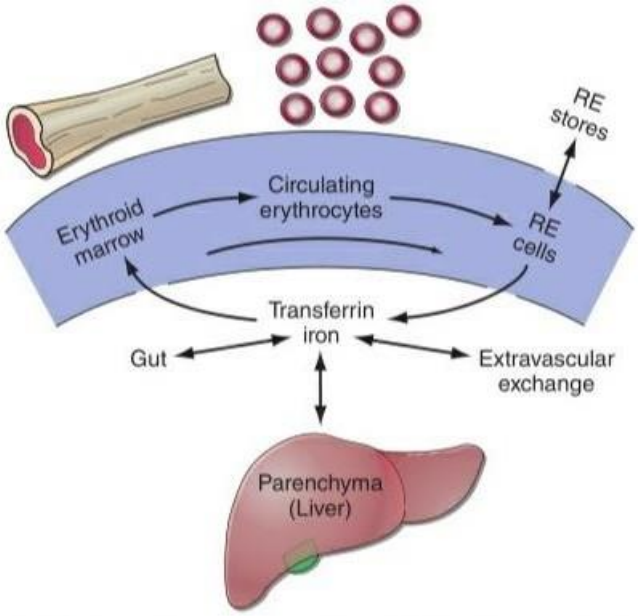


Figure 4: Iron recycling in and out of plasma.[6]

Iron that is still present is stored as ferritin or hemosiderin. If haemoglobin synthesis is required, the stored iron can be quickly activated. Iron stores don't need to be used as long as transferrin saturation is kept between 20 and 60% and erythropoiesis isn't enhanced. Up to 40mg/dl of iron can be mobilised from reserves for erythropoiesis in the event of blood loss, dietary iron shortage, or poor iron absorption. Anaemia results from the sluggish reutilization of iron caused by infections and other inflammatory processes.[6]

HEPCIDIN

Hepatocytes are the main producers of the peptide hormone hepcidin. It is essential for maintaining the balance of systemic iron. At the level of absorption, it is a negative regulator of iron metabolism. Hepcidin tightly controls how much iron is absorbed by intestinal epithelial cells and how much is released from iron storage, depending on the level of plasma iron concentration. Hepcidin levels are

abnormally high in inflammatory situations, although serum iron levels are normal or low as a result of iron storage in macrophages, the liver, and intestinal epithelial cells.[13]

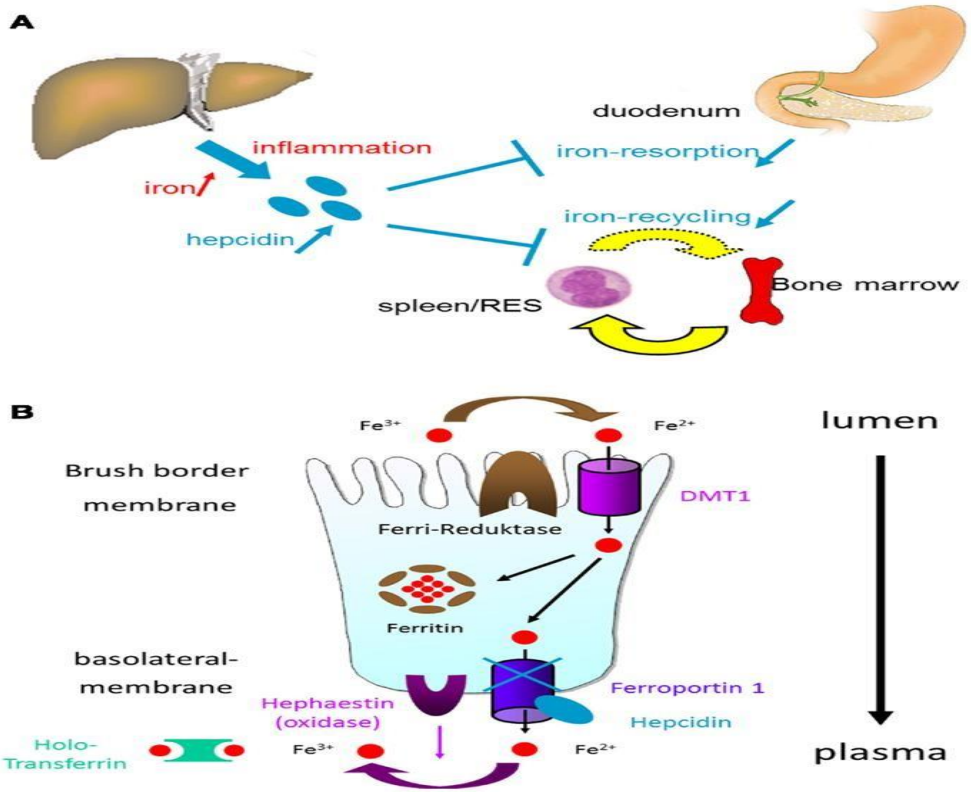


Figure 5: Hepcidin Negative regulator of Iron metabolism at the level of absorption[13]

IRON EXCRETION

Every day, around 1mg of iron is lost in faeces. Through sweating and skin exfoliation, minor losses also happen. Menstruation in women causes a low iron level. Males lose an average of 1 mg of total iron daily, compared to 2 mg for women who are menstruation. Daily loss during instances of iron overload can reach 4 mg.[8]

ETIOLOGIC FACTORS OF IRON DEFICIENCY ANEMIA[8,9]

Decreased iron intake and absorption-

- Inadequate diet
- Inadequate absorption:
    1. Achlorohydria
    2. Gastric Surgery
    3. Celiac disease
    4. Helicobacter pylori infection
    5. Duodenal Bypass
    6. Drugs that increase gastric PH
    7. Tannins, phytates, bran
    8. Competing divalent metals
    9. Inflammation causing increase in Hepcidin

Increased Iron Loss-

1. Blood donation
2. Iatrogenic- Diagnostic phlebotomy for testing
3. Gastrointestinal bleeding
4. Intestinal parasites
5. Anatomic lesions like hemorrhoids, gastritis, diverticulitis, varices, hiatal hernia, Meckel's diverticulum, arteriovenous malformation, peptic ulcer.
6. Neoplasm
7. Inflammatory bowel disease
8. Milk induced enteropathy in infants
9. Use of NSAIDS

10. Pulmonary Hemosiderosis
11. Tuberculosis
12. Bronchiectasis
13. Hereditary hemorrhagic telangiectasia
14. Anticoagulant, antiplatelet therapy
15. Chronic hemodialysis
16. Runner's anemia
17. Excessive menstrual flow
18. Gynecological neoplasm
19. Bladder neoplasm
20. Epistaxis
21. Hemoglobinuria

Increased Requirements-

1. Infancy
2. Pregnancy
3. Puberty
4. Lactation

PATHOGENESIS

IRON-CONTAINING PROTEINS

The body's storage of iron becomes depleted in the early stages of an iron deficiency. This causes dyserythropoiesis, which results in erythrocytes with low levels of haemoglobin. Other iron-containing proteins like cytochromes, myoglobin, and other mitochondrial ferroproteins concentrations are also impacted.[7]

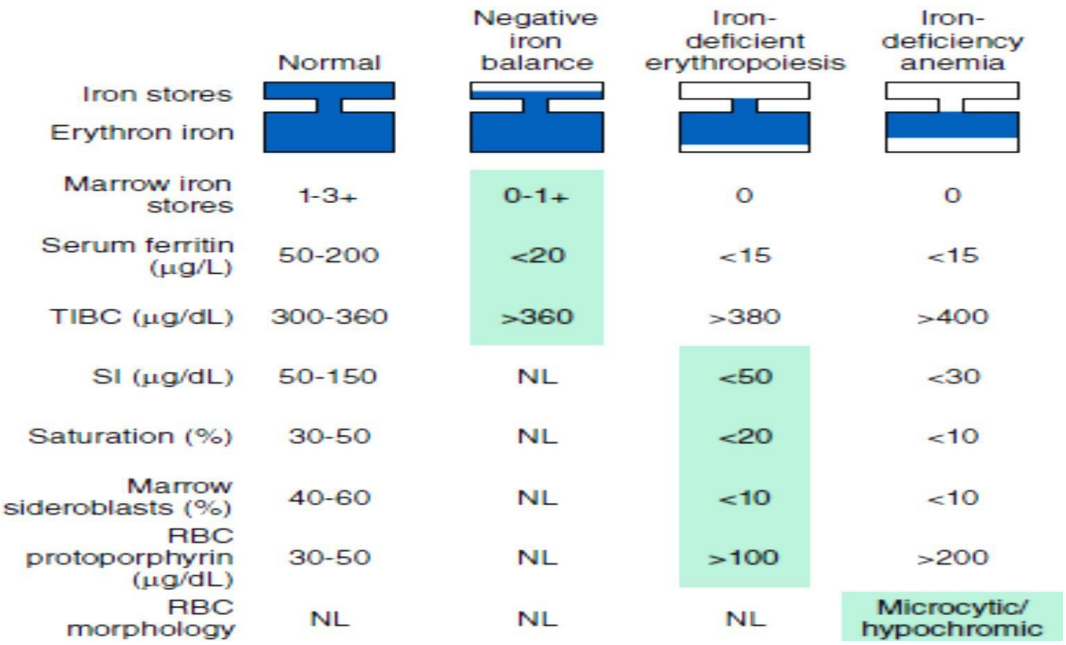


Figure 6: Development of iron deficiency anemia[6]

MUSCULAR FUNCTION AND EXERCISE TOLERANCE

Even in an iron deficient state without anaemia, patients have trouble engaging in high-intensity exercise, a problem that gets worse as anaemia levels rise. This exercise restriction is brought on by the blood's lower haemoglobin level and decreased oxygen delivery to tissues.

The levels of spontaneous activity, ventilator threshold, endurance, and muscle exhaustion will all decline. These effects are linked to the reduction of mitochondrial proteins involved in energy metabolism that contain iron. By taking an iron supplement, these effects can be reversed.[7]

NEUROLOGIC CHANGES

- Iron deficiency is associated with irritability, disruptive behaviour, developmental delay, breath holding episodes, short attention spans and lack of interest among school going children, PICA, stroke, cranial nerve palsy in children.
- In adults it is associated with cerebral venous sinus thrombosis and restless leg syndrome. Rarely iron deficiency also causes Nuralgias, Vasomotor disturbances, numbness and tingling, Ischemic stroke, Idiopathic intracranial hypertension, Papilledema, Psuedotumour cerebri[3,9]

HOST DEFENSE AND INFLAMMATION

A lack of iron impairs bacterial phagocytosis and results in decreased lymphocyte-mediated immunity. There is a 35% decrease in the quantity of T cells in circulation. Helper and T cells are both impacted. Phagocytosis is accompanied by a smaller oxidative burst, which results in diminished neutrophilic functions.

Hepcidin is lost as a result of iron shortage, and hepcidin is necessary for lipopolysaccharide to have its systemic effects amplified. Asthma-like allergic inflammation was also increased by IDA.

GROWTH AND METABOLISM

Children that are iron deficient experience growth retardation. When exposed to cold, there will be a decline in thermoregulation. This is linked to the contradictory effects of blood flow with decreasing oxygen content and the desire to reduce heat loss, as well as the effect on thyroid function. Chronic hemolytic anaemia and thalassemia both cause skeletal and bone abnormalities that are comparable to those seen in chronic iron deficiency anaemia. Chronic IDA results in expanded diploic gaps, thinning of the outer table of the skull, and vertical straits that resemble hair on end. Metacarpels and phalanges exhibit medulla enlargement and cortices thinned.

EPITHELIAL TISSUES

The rapidly growing cells of the tongue, mouth, hypopharynx, oesophagus, and nails are frequently impacted by iron deficiency in the upper section of the gastro intestinal system. The upper GI tract will experience mucosal atrophy. The lateral margin of the tongue's epithelium has less epithelial thickness, which is due to rapid epithelial cell exfoliation. The filliform papillae that cover the tongue's anterior two thirds are the first to atrophy and may eventually disappear entirely. The fungiform papillae may also be impacted in extreme cases, giving the tongue a smooth, waxy look. These modifications cause pain and a burning feeling on the tongue. Buccal mucosa has keratinization and thinning due to angular stomatitis. esophageal webs and dysphagia in the

hypopharynx. IDA causes achlorohydria and gastritis in the stomach. Fingernails may become brittle, fragile, longitudinally ridged, platonychia and koilonychia may also be present.[7-9]

CLINICAL FEATURES OF IRON DEFIICIENCY ANEMIA[6-9]

Symptoms of anaemia result from decreased oxygen supply to cells and the body's response to it.

- Tachycardia-perceived as palpitations
- Lightheadedness.
- Decreased work performance
- Pounding sensations in ears
- Headache

Neurological

In infants and children

- Poor attention
- Retarded behavioral and delayed developmental milestones.
- Iron deficiency also contributes to tourette syndrome
- Attention deficit hyperactivity disorder
- Breath-holding spells in children
- Restless leg syndrome.

Alimentary tract

- Burning sensation in tongue
- Post cricoids web develops as a result of dysphagia brought on by mucosal atrophy in the laryngopharynx, which makes swallowing difficult (Plummer Vinson syndrome).It is precursor lesion for Pharyngeal carcinoma. It is more prevalent in middle aged women. The condition should be identified and treated with Iron supplementation.

- **PICA-** A well-known manifestation of iron deficiency is pica. Pica is a heightened desire to consume uncommon or unhealthy foods, such as clay, paint, cardboard, laundry starch (8), and even hair. Iron therapy generally cures the condition.

PHYSICAL SIGNS:

- Pallor of lower palpebral conjunctiva, oral mucosa, nails, palms, Pallor of disc on fundus examination, pallor of anal and cervical mucosa.
- Loss of papillae over the surface of tongue- Bald tongue
- Flatening and spooning of nails.
- Fundus examination of the severely anemic patients may reveal hemorrhages/exudates in retina.
- Water hammer pulse, functional ejection systolic murmur secondary to high output cardiac state.
- Congestive Cardiac failure in very severe form of anemia.

LABORATORY FINDINGS[7,8]

ERYTHROCYTES

Anisocytosis is the first observable morphologic change of a red blood cell in iron deficient anaemia. Mild ovalocytosis might be present in addition to it.

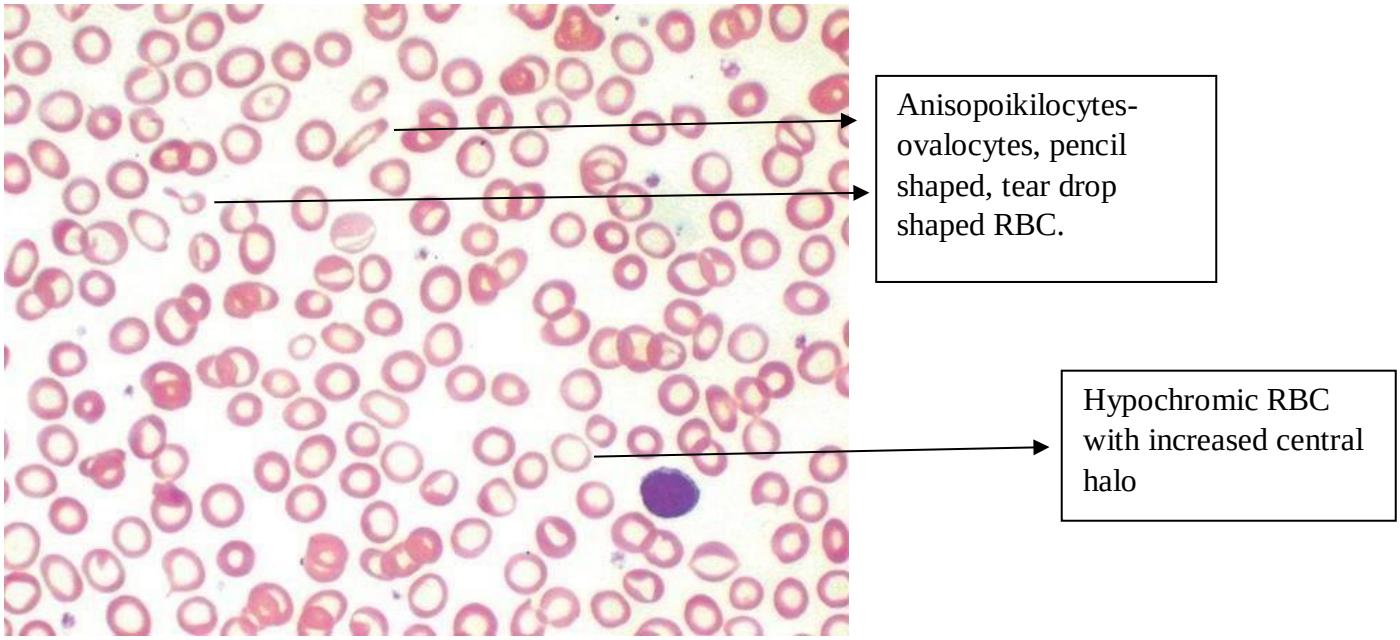


Figure 7: Peripheral smear in iron deficiency anaemia

- A modest normocytic normochromic anaemia first appears. Erythrocyte count, mean corpuscular volume, mean haemoglobin concentration, and mean corposcular haemoglobin concentration all decrease as the iron deficiency worsens. Red blood cells seem microcytic and hypochromic as these indices fluctuate. Anisopoikilocytes or target cells, can occasionally be detected. In iron deficiency anaemia, the red cell distribution width (RDW) is increased.
- Most people with iron deficiency anemia have normal white cell count.

  Although the exact process is unclear, thrombocytopenia and thrombocytosis can both be caused by iron deficient anaemia.

• The bone marrow frequently exhibits a little increase in reticulocyte count as a result of increased erythroid activity.

BONE MARROW

In iron deficiency anaemia, the iron stores are depleted before the red cell mass is compromised. Therefore, determining iron store is the most accurate and sensitive way to distinguish between iron deficiency anaemia and all other anemias.

Table 3: Bone Marrow Iron stores and corresponding Serum ferritin levels[6]

IRON STORES	MARROW IRON STAIN, 0–4+	SERUM FERRITIN, µg/L
0	0	<15
1–300 mg	Trace to 1+	15–30
300–800 mg	2+	30–60
800–1000 mg	3+	60–150
1–2 g	4+	>150
Iron overload	—	>500–1000

Hemosiderin levels in the bone marrow are typically low or absent in iron deficiency, which is determined using the Prussian blue method of staining. Measuring the iron level of marrow macrophages is the gold standard for determining whether an individual is iron deficient. Both prior transfusions and parenteral iron therapy change it.[6]

SERUM IRON CONCENTRATION

The serum iron concentration will be low in iron deficiency anaemia, though it very rarely maybe normal. Inflammatory and malignant diseases change the serum iron concentration. Chemotherapy may cause an increase in it because the cytotoxic medications impair erythropoiesis. If the patients used iron supplements before to the study, their serum iron concentration will be normal or even high.[6]

IRON BINDING CAPACITY AND TRANSFERRIN SATURATION

The amount of transferrin in the blood is represented by the total iron binding capacity. Using a spectrophotometric approach, the unsaturated or latent iron binding capacity (UIBC) can be measured quickly. Total iron binding capacity (TIBC) is calculated as the sum of UIBC and serum iron. Since the serum iron concentration is decreased and both UIBC and TIBC are elevated in iron deficiency anaemia, the transferrin saturation is decreased.[6,7]

SERUM FERRITIN

The total body iron stores are represented by the serum ferritin concentration. The serum ferritin level can be as low as 10mcg/L in an iron deficient state. In inflammatory conditions like rheumatoid arthritis and chronic renal disease, ferritin levels are increased. The value of normal serum ferritin varies with age and gender. Because of the negative iron balance brought on by menstruation and childbirth, serum ferritin levels are maintained in the female reproductive age group at the lower limit of normal and are typically constant.[6]

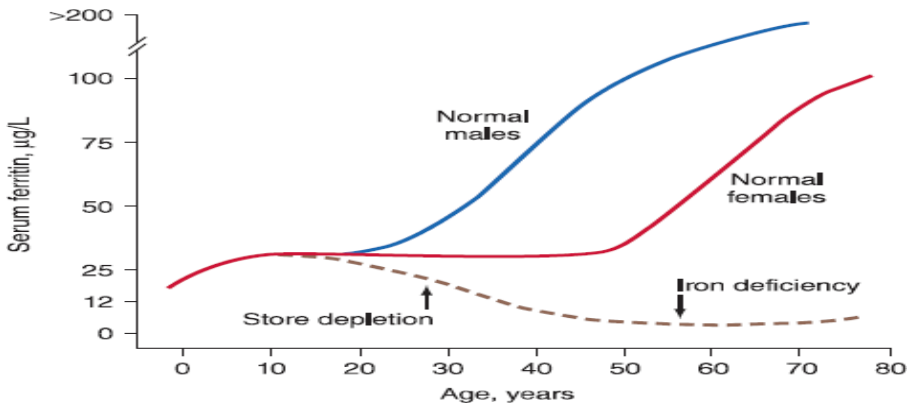


Figure 8: Serum ferritin levels as a function of sex and age[6]

RED CELL PROTOPORPHYRIN LEVELS

Heme production is impaired in iron deficiency anaemia, which allows protoporphyrin to build up inside red blood cells. Normally, it will not exceed 30mcg/dl. Red cell protoporphyrin levels are greater than 100mcg/dl in iron insufficiency.

TRANSFERRIN RECEPTOR PROTEIN LEVELS

In erythroid cells, there are several transferrin receptors (TfR), which are glycoproteins. Circulating transferrin enters cells through them. Iron response protein controls how the TfR molecule is made. The amount of erythroblasts expressing the receptor and tissue iron deficit are proportional to serum TfR levels, which are related with the number of cellular receptors.

TfR production is increased during iron deficiency, which increases the amount of circulating receptors. Cytokines decrease TfR production in anaemia brought on by persistent inflammation. Because there is a substantial inter-assay variation, it is not used frequently.[6]

NOVEL ERYTHROCYTE INDICES[7]

- **Ratio of sTfR/ Serum Ferritin-** In mild iron deficiency without anemia is usually missed by Serum ferritin alone. It can be picked up by the ratio of sTfR/ Serum Ferritin
- **Reticulocyte Hemoglobin Content (CHr) -** It is an indicator of iron restricted of hemoglobin synthesis. Reduction in CHr occurs in few days of Iron deficiency anemia as half-life of reticulocytes is 1-2 days. It has low specificity. False positive results occur in macrocytosis and thallasemia.
- **Percent hypochromic erythrocytes-** It extends the iron restriction that had been in place for the few months prior.

DIFFERENTIAL DIAGNOSIS:[6-8]

I. THALASSEMIA:

It is brought on by a hereditary defect in the synthesis of the globin chain. Serum iron levels can be used to distinguish it from iron deficiency. Thalassemias will have normal or elevated serum iron levels and transferrin saturation levels. In thalassemia, the RDW index will be normal. In cases of iron deficiency anaemia, it is increased.

II. ANEMIA OF CHRONIC INFLAMMATION:

It is caused by a lack of iron reaching to the erythroid marrow. Anaemia of inflammation is usually normocytic and normochromic. The level of ferritin may be low or high. In chronic disease-related anaemia, both the TIBC and the percent transferrin saturation are lowered.

III. MYELODYSPLASTIC SYNDROMES:

Sideroblastic anemia is a Myelodysplastic anemia with defective utilization of Iron. There is accumulation of iron in the mitochondria of devoloping RBC which leads to defective iron incorporation into heme and thereby causing Hypochromia. The iron store levels will be normal and excess.

Test	Iron Deficiency	Chronic Inflammation	Thalassaemia	Sideroblastic Anaemia
MCV	Decreased	Normal or decreased	Markedly decreased	Decreased
RDW	Increased	Increased or normal	Increased or normal	Increased or normal
Red cell morphology	Microcytic, hypochromic, pencil cells, anisocytosis	Normocytic, normochromic or microcytic, hypochromic	Microcytic, hypochromic, basophilic stippling, target cells, polychromasia	Dimorphic
Red cell count	Decreased	Decreased	Normal	Normal
Serum iron	Decreased	Decreased or normal	Normal or increased	Normal or increased
Total iron binding capacity	Increased	Decreased	Normal	Normal
Per cent saturation	Decreased	Decreased to normal	Normal or increased	Normal or increased
Ferritin	Decreased	Increased or normal	Normal	Normal
Serum transferrin receptor	Increased	Normal	Increased	Normal
Free erythrocyte protoporphyrin	Increased	Increased	Normal	Increased
Haemoglobin pattern on electrophoresis	Normal	Normal	Abnormal (Hb A_2 >3.5%)	Normal
Marrow Iron	Low or absent	Normal or increased	Normal	Ring sideroblasts seen >15%

Table 4: Differential Diagnosis of microcytic anemia[6]

TREATMENT

Depending on the severity and underlying reason, different therapy approaches are used for iron deficiency anaemia. Once the cause and diagnosis of iron deficiency are established, there are three main therapy modalities.

I.RED CELL TRANSFUSION[14]

Transfusion therapy is reserved for patients with

- Hemodynamic instability
- In patients requiring intervention, upcoming major surgery
- Continued/excessive blood loss
- Symptomatic anemia

In addition to correcting the anaemia transfusion provides iron for reutilization.

II. ORAL IRON THERAPY[14]

In patients with established iron deficiency anaemia who are asymptomatic, oral iron therapy is sufficient. There are many different iron preparations available, varying from simple salts to complex substances designed for prolonged release. Ascorbic acid is a constituent in several treatments that improves the iron absorption. Divided doses of up to 200 mg of elemental iron are administered each day for iron replenishment. Food determines how well iron is absorbed. As a result, iron supplements are taken on an empty stomach. 50 mg of the provided 200 mg of iron will be absorbed each day. The amount of iron absorbed depends on:

- The hemoglobin levels
- Severity of iron deficiency
- Marrow function and
- The degree of erythropoietin stimulus.

Iron therapy aims to treat anaemia and supply at least 0.5 to 1 grams of iron reserves. Following the correction of the anaemia, this goal requires ongoing treatment for six to twelve months. =The most frequent side effect of oral iron treatment is GI discomfort. Other side effects associated with oral iron preparations, such as nausea, vomiting, abdominal pain, or constipation, contribute to poor treatment compliance. Lower incidences of the gastrointestinal side effects would be seen with sustained release formulations or small doses of iron. The response to oral iron depends on:

i) Erythropoietin stimulus and
ii) Rate of absorption.

The increase in reticulocyte count within 4–7 days after the start of therapy is a sign of an adequate response. Poor absorption, an incorrect diagnosis, or noncompliance (commonest) might all indicate an inadequate or absent response.

Table 5: Oral preparations of Iron.[14]

Iron Salt	Dose	Elemental iron
Ferrous sulphate ($FeSO_4, 7H_2O$)	300mg	60mg
Ferrous sulphate dried	200mg	65mg
Ferrous fumarate	200mg	65mg
Ferrous gluconate	300mg	35mg
Ferrous glycine Sulphate	225mg	40mg
Ferrous Succinate	100mg	35mg
Ferrous calcium citrate complex	-	250mg Fe+ 85mg Ca^{2+}
Ferrous aminoate	-	60mg
Ferric amonium citrate	100mg	20mg
Iron polysaccharide complex (Iron polymaltose)	370mg	100mg
Sodium Feredetate	231mg	33mg
Carboryl iron		100%

III. PARENTERAL IRON THERAPY[15]

Parenteral iron is reserved for patients with intolerance to oral iron and patients with higher iron deficient.

Formula for iron requirement calculation [(1)]:

Body weight (kg)×2.3×(15 –patient's hemoglobin, gm/dl)+500

Table 6: Parenteral iron preparations[15]

Table 2 - Some characteristics of the different intravenous iron formulations

	Iron gluconate	Iron sucrose	Low-molecular-weight iron dextran	Ferric carboxymaltose	Iron isomaltoside 1000	High-molecular--weight iron dextran	Ferumoxytol
Brand name	Ferrlecit®	Venofer®	Cosmofer® INFeD®	Ferinject® Injectafer®	Monofer®	Dexferrum®	FeraHeme®
Carbohydrate shell	Gluconate (monosaccharide)	Sucrose (disaccharide)	Dextran (branched polysaccharide	Carboxymaltose (branched polysaccharide	Isomaltoside (linear oligosaccharide)	Dextran (branched polysaccharide)	Polyglucose sorbitol carboxymethyl-ether
Complex type	Type III Labile and weak	Type II Semi-robust and moderately strong	Type I Robust and strong	Type I Robust and strong	Type I Robust and strong	Type I Robust and strong	Type I Robust and strong
Molecular weight (kD)	289-440	30-60	165	150	150	265	750
Initial distribution volume (L)	6	3.4	3.5	3.5	3.4	3.5	3.16
Plasma half-life (h)	1	6	20	16	20	60	15
Labile iron release	+++	±	-	-	-	-	-
Direct iron donation to transferrin (% injected dose)	5-6	4-5	1-2	1-2	< 1	1-2	< 1
Test dose required	No	No	Yes	No	No	Yes	No
Iron content (mg/mL)	12.5	20	50	50	100	50	30
Maximal single dose (mg)	125	200-300	20 mg/kg	15 mg/kg (max 1000 mg in one infusion)	20 mg/kg	20 mg/kg	510
Premedication	No	No	No	No	No	TDI only	No
Life-threatening a dverse effects ($x10^6$ doses)	0.9	0.6	3.3	??	??	113	

TDI = total dose infusion

The main issue with intravenous iron dextran is anaphylaxis. Anaphylaxis has become far less common due to the availability of improved parenteral iron formulations. There may be other symptoms such arthralgias, a skin rash, and a low-grade fever. Usually, these symptoms are dose-related. In these patients, further parenteral iron therapy is not contraindicated.[15]

IRON REFRACTORY IRON DEFICIENCY ANEMIA[16]

Iron deficiency persists in certain people even with appropriate iron supplementation. The causes are:

i) Poor compliance (most common)

ii) Reconsider the diagnosis, supplementation of other hematinics along with iron supplementation.

iii) Iron malabsorption secondary to celiac disease, autoimmune gastritis, H.Pylori infection and Achlorohydria.

iv) Continuous ongoing loss- Menorrhagia metropathica, GI malignancies, Angiodysplasia, Hookworm infestation

v) Inherited defects in iron uptake, transfer and release [4] defect in DMT, low ceruloplasmin levels, increased hepcidin levels.

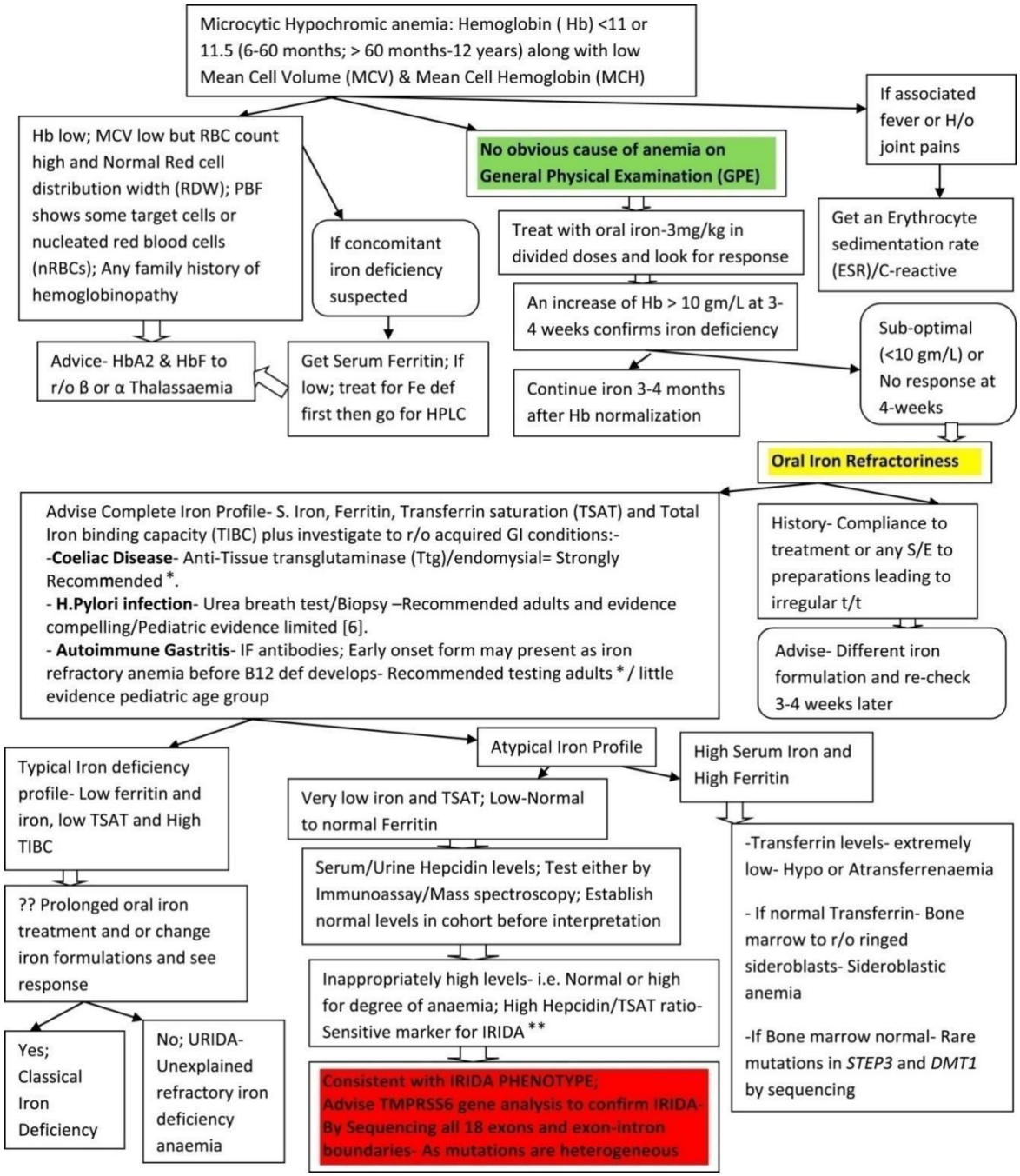


Figure 9: Approach to iron refractory iron deficiency (IRIDA)[16]

Serum electrolytes

Electrolytes are necessary for the fundamental processes of life, including the generation and conductivity of action potentials in the muscles and nerves, as well as the maintenance of electrical neutrality in cells. Significant electrolytes include magnesium, calcium, phosphate, sodium (Na), potassium (K), and chloride in addition to bicarbonates, calcium, and phosphate. Our food and liquids provide electrolytes. Elevated or depleted levels of these electrolytes can result from an imbalance. Electrolyte imbalances can have a serious impact on health and can even be fatal because they interfere with normal bodily processes.[17]

Serum Sodium

Sodium is one of the most crucial electrolytes in the extracellular fluid because it is an osmotically active cation. It controls the modulation of the membrane potential of cells as well as the volume of extracellular fluid. As part of active transport, sodium and potassium are exchanged across cell membranes. The kidneys regulate sodium levels. Most of the sodium reabsorption occurs in the proximal tubule. Sodium is reabsorbed in the distal convoluted tubule. Through sodium-chloride symporters, which is regulated by the hormone aldosterone, sodium is transported in the body.

The most common electrolyte imbalance is hyponatremia. When the serum sodium level is less than 135 mmol/L, a diagnosis is made. There are neurological effects of hyponatremia. Patients may exhibit delirium, headaches, confusion, and/or nausea. When serum sodium levels exceed 145 mmol/L, hypernatremia develops. Tachypnea, trouble falling asleep, and agitation are all signs of hypernatremia. Rapid sodium corrections have the potential to cause major side effects such cerebral edoema and osmotic demyelination syndrome.[17]

Serum Potassium

The most of potassium ions are intracellular. The fundamental mechanism for controlling the balance between sodium and potassium is the sodium-potassium adenosine triphosphatase pump, which pumps sodium out in exchange for potassium, which enters the cells. The glomerulus in the kidneys is where potassium is filtered. At the thick ascending loop of Henle and the proximal convoluted tubule, potassium is reabsorbed. At the distal convoluted tubule, potassium is secreted. K+ secretion is enhanced by aldosterone.[19] Potassium is also secreted through potassium channels and potassium-chloride cotransporters at the apical membrane.[18]

Cardiac arrhythmias are associated with potassium imbalances. Low serum potassium levels, or hypokalemia, are characterised by weakness, exhaustion, and twitching of the muscles. Arrhythmias may develop as a result of hyperkalemia, which happens when serum potassium levels are above 5.5 mmol/L. Myoglobinuria, rhabdomyolysis, muscle spasms, and weakness are some of the symptoms of hyperkalemia.[20]

Normally, the intracellular environment has more K + ions and the extracellular environment has larger quantities of Na + ions. On the cell membranes of RBC, there is a protein called Na +/K + ATPase that keeps this environment stable. It pumps three Na + ions out of the cell and two K + ions in, and is primarily sensitive to changes in pH, membrane integrity, and volume.[21] Studies found Na + /K ATPase to be a strong indicator for blood disorders, including anaemia. In vitro hydrogen peroxide incubation of iron-deficient RBC produced chemical malonyl dialdehyde, a byproduct of lipid peroxidation, according to the literature. The increased membrane stiffness brought on by this peroxidation in iron-deficient RBCs may help to shorten the lifespan of red cells. Since a change in membrane dynamics and permeability can have an impact on the Na + /K + pump, the peroxidation factor may therefore be to blame for abnormal serum electrolytes.[22]

In another study, polycarbonate filters were used to study the rheological properties of erythrocytes in patients with IDA. It concluded that the ratio between the cell surface and cell volume is

unfavourable in IDA; hence, RBC can be prematurely sequestered during its passage through the spleen. There might be yet another factor responsible for Na + /K + pump impairment, thus, leading to electrolyte imbalance in IDA.[23]

GLYCATED HEMOGLOBIN:

A kind of haemoglobin known as "glycated haemoglobin" (HbA1c) is one that has a persistent glucose adduct covalently attached to the N-terminal valine of the β-chain. Adult's normal haemoglobin is made up of 97%, 2.5%, and 0.5% of HbA (α2β2), HbA2 (α2δ2), and HbF (α2γ2), respectively. About 6% of the total HbA is categorised as HbA1. Among HbA1, there are HbA1a1, HbA1a2, HbA1b, and HbA1c. These fractions differ from one another in terms of electrophoretic and chromatographic characteristics.

Despite having similar amino acid sequences, HbA1 and HbA0's electrophoretic and chromatographic characteristics are slightly different from those of the main component HbA0[12,13]. The most common HbA fraction is HbA1c. In healthy individuals, it makes up about 5% of total HbA. HbA1c doesn't appear to have any physiological function.[24]

ESTIMATION OF HbA1c[24]

1. **Cation exchange chromatography**

   Based on the slight differences in their isoelectric points, HbA1c and HbA0 can be distinguished from one another. These days, high performance liquid chromatography (HPLC) methods are used which are not impacted by haemoglobin variations such as carbamylated or Schiff base haemoglobin.

2. **Affinity chromatography**

   M-amino phenyl boronic acid is used in this assay technique. This approach is based on the interaction of the glucose molecule on HbA1c with the immobilised boronic acid.

3. **Immunoassay**

   Antibodies against the N-terminal glycated tetrapeptide and or hexapeptide group are used in this assay. In this test, electrical charge has no impact. It is applicable to standard medical laboratories. However, they have the problem of necessitating frequent and multilayer calibration.

4. **Capillary electrophoresis**

This approach is based on free-solution liquid flow capillary electrophoresis. This method makes use of the variable electrophoretic mobility of charged molecules at a specific pH in an alkaline buffer. Hemoglobin fractions are separated in silica capillary tubes, and the migration process is carried out at high voltage under strict temperature control. An optical detector immediately detects the hemoglobins at the cathode end at a particular absorption wavelength of 414 nm.

MARKER FOR GLYCEMIA

The main purpose of glycated haemoglobin is to measure the average plasma glucose level over a period of 12 to 16 weeks. The proportion of glycated haemoglobin rises gradually as the average plasma glucose rises. Poor glycemic control is indicated by higher levels of glycated haemoglobin in people with diabetes mellitus.

Although haemoglobin glycation happens during the course of a red blood cell's life, the most recent glycemia has a significant impact on the HbA1c score. The first, second-, and third-month's average blood sugar levels each contribute roughly 50%, 40%, and 10%, respectively, to the final HbA1c score. HbA1c measures average plasma glucose but does not indicate how stable glycemic management is. So, a patient can still have the same HbA1c as someone with little variance even when their blood glucose levels vary substantially. The approximate mapping between eAG (estimated average glucose) measurements and HbA1c values is given by the equation:[24]

$$eAG\ (mg/dl) = 28.7 \times A1C - 46.7$$

$$eAG\ (mmol/l) = 1.59 \times A1C - 2.59$$

While American Diabetes Association recommends that HbA1c below 7.0% as the target. American College of Endocrinology and the International Diabetes Federation recommend HbA1c below 6.5% [16]. Target HbA1c level should be individualized. The American Diabetes Association recommends performing the HbA1c test quarterly in diabetics whose therapy has changed or who are not maintaining recommended glycemic levels, and twice a year in diabetics who have met their treatment goals.[24-26]

HBA1C AND DIAGNOSIS OF DM

Diabetes-specific complications are caused by chronic hyperglycemia, which is the characteristic of the disease. So, rather than relying solely on a single glucose measurement, the HbA1c, which indicates long-term glucose exposure, is a stronger predictor of diabetes-specific problems. Studies showed retinopathy and HbA1c levels correlated more consistently and significantly than fasting glucose levels with retinopathy.[27] As this HbA1c level is associated with an increased prevalence of diabetes-specific complications, especially retinopathy, assigning a HbA1c cut off point of more than 6.5% for diagnosing diabetes is strongly supported by large volumes of data from various populations and numerous controlled clinical trials. This threshold does not perfectly separate diabetes from normal glucose control. However, this level is sensitive and particular enough to identify patients who are at risk of developing retinopathy. The HbA1c level of greater than 6.5% has been suggested by an international expert committee for the diagnosis of diabetes. The American Diabetes Association has acknowledged this. However, the National Glycohemoglobin Standardization Program (NGSP) certification and traceability to the Diabetes Control and Complications Trial (DCCT) reference assay are required for this diagnostic HbA1c test.[27-30]

Table 7: American Diabetic Association (ADA) criteria for the diagnosis of diabetes[26,31,32]

Test[a]	Threshold	Qualifier
Hemoglobin A_{1c} or	≥ 6.5%	Lab NGSP-certified, standardized DCCT assay
Fasting glucose or	≥ 126 mg/dL (7.0 mmol/L)	No caloric intake for at least 8 hours
2-hour glucose or	≥ 200 mg/dL (11.1 mmol/L)	After 75 g of anhydrous glucose
Random glucose	≥ 200 mg/dL (11.1 mmol/L)	Plus classic hyperglycemia symptoms or crisis

NGSP, National Glycohemoglobin Standardization Program; DCCT, Diabetes Control and Complications Trial.

[a] Results must be confirmed by repeated testing.

ADVANTAGES OF HbA1c[19,27,28,29,31,32]

- The 2-hour OGTT and the two fasting glucose tests are less reliable in detecting persistent hyperglycemia than the HbA1c test.[19]
- HbA1c is better associated with chronic complications than FPG. Individuals with higher HbA1c are prone for retinopathy, nephropathy, peripheral neuropathy[27]
- Fasting is not needed for HbA1c assessment and acute changes due to sepsis, drugs, stress do not affect HbA1c.[27]
- HbA1c has a greater preanalytical stability than FPG. Glucose concentration reduces by 5-7% per hour delay in sample processing. In contrast HbA1c values are stable after sample collection variance from day to day or person to person is less than 2%, however FPG variation ranges from 12 to 15%.[28]
- Standardization of the HbA1c assay is equivalent to that of the glucose assay.[29]
- Individual glycation susceptibility may provide additional value for HbA1c measurement.[31]
- Same biomarker can be used for diagnosis and monitoring.[32]

LIMITATIONS OF HBA1c[19,20,27-32]

- Diabetes is defined by high blood glucose and not by glycation of proteins. Primary pathology i.e., High plasma glucose levels have to be given importance over secondary results
- HbA1c is a poor marker of pathophysiological abnormalities featuring diabetes. HbA1c is a weaker correlate of Plasma glucose peaking in postprandial period, impaired pancreatic β cell function[19]
- Factors that influence HbA1c and its measurement

  Increased HbA1c: Iron deficiency, Vitamin B12 deficiency, Chronic renal failure, splenectomy, Hyperbilirubinemia decreased erythropoiesis, alcoholism, Carbamylated Haemoglobin

  Decreased HbA1c: Administration hematincs, Aspirin consumption, Vitamin C and Vitamin E consumption, Reduced RBC life span like in haemolytic anaemia, Splenomegaly, Rheumatoid arthritis, Drugs like antiretrovirals and dapsone

  Variable HbA1c: Hemoglobinopathies, methemoglobin[20]
- Diabetes cannot be accurately diagnosed using HbA1c, which would alter the epidemiology. When compared to HbA1c, which only detects around 40% of previously undetected diabetes, epidemiological studies have shown that FBS detects about 50% and 2 hours of PPBS detects about 90% of previously undiagnosed diabetic patients.
- Fasting PG is a poor predictor of CVD events and death related to diabetes. However, the HbA1c and 2 hrs PG are more reliable predictors. Patients with reduced glucose tolerance had a higher death rate (about 40%). Neither FPG nor HbA1c testing can identify these persons.[27-28]
- Standardization of HbA1c assay is very poor and has not been achieved worldwide yet

- The physiologic fluctuation in plasma glucose from day to day may indicate a problem with glucose metabolism. The person's activities and nutritional habits also reflect it. This information will not be provided by HbA1c.[29-30]
- Assays for HbA1c are more costly. Additionally, hardly many nations have these available.[32]

EFFECTS OF IRON DEFICIENCY ANEMIA ON HbA1c AND SERUM ELECTROLYTES

The purpose of this study is to evaluate the HbA1c values in people with iron deficiency anaemia. Despite the fact that there have been several research looking at the association between HbA1c and iron deficiency anaemia in the past, the outcomes have been inconsistent.

Horton and Husiman conducted the earliest study on evaluating the influence of iron deficiency anaemia over HbA1c levels. Their study shows that “patients with iron deficiency anaemia the mean concentration of HbA1c was 4.9% compared to 5.3% in healthy individuals. Since 2000, there were studies evaluating the effects of iron deficiency anaemia on HbA1c. In contrast to the earlier studies HbA1c levels decreased as much as 17% after treatment of iron deficiency in many new studies. It was postulated that a fall in hemoglobin under stable glycemic conditions could cause an increase in the Glycated fraction” [43].

Ford et al conducted a study on evaluating the influence of iron deficiency anaemia over HbA1c levels. This study showed a “positive association between HbA1c and hemoglobin levels. The mean HbA1c value in participants with Hb below 10 g/dl was 5.28% and in participants with Hb above 17 g/dl was 5.72%. The participants with and without iron deficiency had the adjusted mean HbA1c concentration of 5.56% and 5.46% respectively with p value 0.095. They suggested that iron deficiency anaemia had little effect on HbA1c levels. The difference in HbA1c concentrations between extremes of hemoglobin concentration was 0.2%. They concluded that, people who were close to the diagnostic threshold with anaemia should be retested or undergo another diagnostic method” [44].

Nithin Sinha et al study was on 50 diagnosed iron deficiency anaemia cases. “They compared the HbA1c levels in iron deficiency anaemia patients before and after treatment. They showed that in anemic patients the mean baseline HbA1c level is lower and it increased after treating with iron” [45]. Contrasting to the above results Brooks et al carried out a study in 1980 on 25 iron deficiency anaemia. “They estimated HbA1c values before and after treating them with iron. And they noted that the mean concentration of HbA1c was elevated in iron deficiency anaemia patients (9.9±0.3) and it decreased after treatment (7.9±0.13).” [46]

Sluiter et al postulated that “the glycosylation of hemoglobin is an irreversible process and the concentration of HbA1c in a red blood cell increases with cell age. The levels of HbA1c should be normal in individuals with normal glycemic status and normal red blood cell life span. In the event of chronic iron deficiency anaemia, red blood cell production will decrease leading to anaemia and a longer span for the red blood cells present in the circulation. After treating with iron, HbA1c levels will decrease which is attributable to the production of newer RBC.” [47]

Coban et al carried out a study that compared “the HbA1c levels in patients with iron deficiency anaemia to normal control groups. They observed that the mean HbA1c level in patients with iron deficiency anaemia was higher i.e 7.4±0.8% than mean HbA1c of control group (5.9±0.5%). And after a 3-month course of iron therapy, the mean HbA1c level significantly decreased to 6.2±0.6%.” [48]

Kim et al stated that “iron deficiency anaemia increased the HbA1c, from the observations of iron deficiency and HbA1c in non-diabetic adults in the National Health and Nutrition Examination Survey (NHANES). This effect was seen at the lower spectrum of HbA1c levels i.e., between 5.5 – 6.0% and below 5.5%. Observations on iron deficiency anaemia and normal iron states, using cross sectional data on HbA1c levels from the NHANES study, stated that there was a significant positive correlation between HbA1c and hemoglobin among adults with and without iron deficiency.” [49]

In 2012 Hardikar et al conducted a study in Indians, which analysed the effect of glycemia and other non glycemic parameters over HbA1c levels. They postulated that if HbA1c is used to diagnose prediabetes and diabetes in iron deficiency anaemia patients, it will result in false high prevalence. [54]

Balasubramanian Shanthi et al studied "the effect of iron deficiency on glycation of haemoglobin in non-diabetics. They concluded that mean HbA1c was higher in iron deficiency patient group than control group. They opined that due consideration should be given to anaemia before making a therapeutic decision based on HbA1c." [55]

In 2015 Emma English, Iskandar Idris et al[56] published a systematic review on the effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis. "They concluded that HbA1c is likely to be affected by iron deficiency anaemia with spurious increase in HbA1c conversely non-iron deficiency anaemia led to decreased HbA1c."

Rafiq et al studied that variance in serum electrolyte levels exists among patients with IDA and those without anaemia. [57]

Antwi-Boasiako et al. studied that there was low Na + levels and high K + and Cl − in patients with sickle cell anaemia. [58]

Rhoda et al. proposed that in sickle cells, an abnormal activation of potassium chloride (K+ Cl−) cotransport system was involved in cell potassium (K+) loss and dehydration. [59]

THEORIES ON EFFECT OF IDA OVER HBA1C LEVELS

- It was postulated that there may be an alteration in the quarternary structure of the hemoglobin molecule in iron deficiency anemia. That alteration will result in increased level of glycosylation of the β-globin chain during iron deficient state.[60]
- Patients with iron deficiency anaemia may have greater HbA1c levels due to the possibility that anaemic conditions prolong the lifespan of the red blood cells already in circulation. Lower HbA1c levels occur from enhanced bone marrow red cell synthesis and the release of new, immature red cells following iron therapy.

MATERIALS AND METHODS

Study Title:

"A STUDY TO ASSESS SERUM ELECTROLYTES AND HbA1c LEVELS IN NON-DIABETIC IRON DEFICIENCY ANEMIA INDIVIDUALS"

Aims and objectives:

To know whether the Iron deficiency anaemia has an effect on Glycated haemoglobin (HbA1c) levels and Serum electrolytes in non-diabetic individuals.

Study centre:

Department Of General Medicine, BLDE DU Shri B M Patil medical college hospital and research centre.

Study design: Cross sectional study

Study Period: From January 2021 to June 2022

Inclusion criteria:

- Patients with moderate to severe Iron deficiency anemia attending medicine OPD and medical wards in Shri B M Patil medical college hospital and research centre.
- Age above 18 years.

Exclusion criteria:

- Known case of Diabetes mellitus
- Chronic renal failure
- Haemolytic anaemia
- Pregnancy
- Chronic alcoholism and known liver disease
- Known case of malignancy
- Recent history of blood loss
- Patient on treatment of IDA in last 3 months

Data collection:

A detailed history was recorded along with complete clinical examination as in the proforma. A provisional diagnosis was made and was subsequently revised after the completion of the routine investigations. Individuals who fulfilled the inclusion criteria proceeded to HbA1c analysis.

Laboratory investigations:

Samples were collected from all the participants to estimate

- Fasting blood sugar, Postprandial blood sugar levels
- Complete Blood Count
- Renal function tests
- Liver function tests
- Iron Profile- Serum Iron, Serum Ferritin, Total Iron Binding Capacity (TIBC)
- HbA1c level
- Peripheral smear
- USG Abdomen & Pelvis
- Electrocardiogram &
- Chest X-RAY (PA View)

The final data was entered into Microsoft excel sheet**.**

Study protocol:

Patients with moderate to severe iron deficiency anaemia based on WHO criteria cut-off point were assigned for study. History taking, clinical assessment and investigations including Iron profile, HbA1c and serum electrolytes were done.

SAMPLE SIZE:

- With Anticipated correlation coefficient between haemoglobin and HbA1c level in non-Diabetics -0.391 (ref), at 95%confidence level and 90 power in the study, the sample size worked out is **65**.

  Formula used is

  $N=[(\frac{Z_\alpha+Z_\beta}{C})]^2+3$

  The standard normal deviate for $\alpha = Z_\alpha = 1.9600$

  The standard normal deviate for $\beta = Z_\beta = 1.6449$

  $C=0.5*\ln[\frac{1+r}{1-r}]=0.4130$

STATISTICAL ANALYSIS:

The data obtained will be entered in a Microsoft Excel sheet, and statistical analysis will be performed using statistical package for the social sciences (Verson 20).

Results will be presented as Mean (Median)±SD, counts and percentages and diagrams.

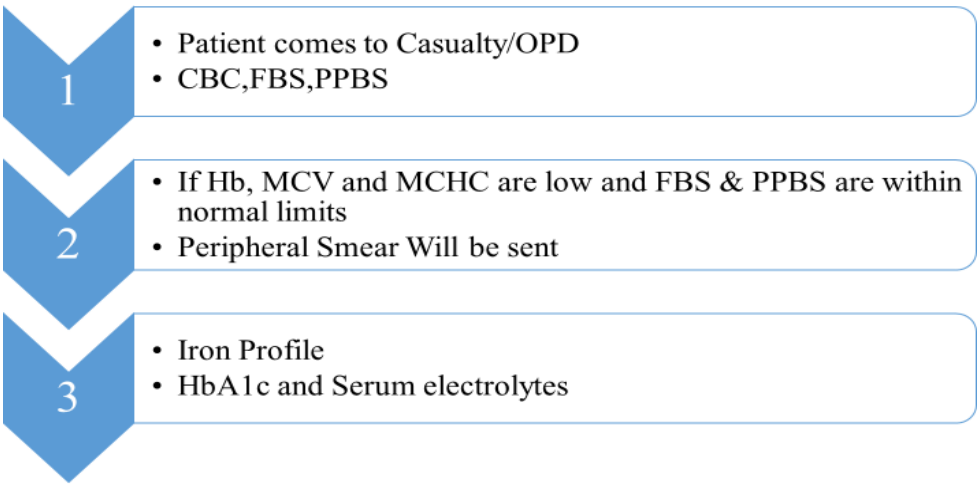
For normally distributed continuous variables between two groups will be compared using independent t-test for not normally distributed variables Mann Whitney U test will be used.

Categorical variables between the two groups will be compared using Chi-square test.

Correlation coefficient will be used to find the correlation between quantitative variables.

$p<0.05$ will be considered statistically significant. All statistical tests will be performed two-tailed.

STUDY PROTOCOL



RESULTS

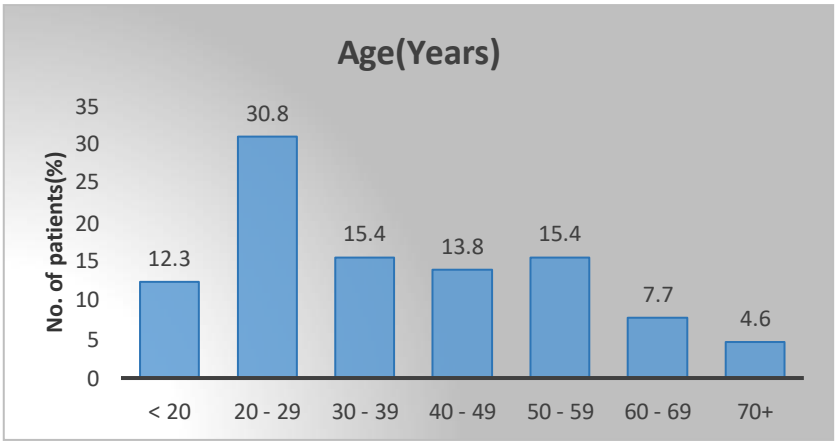
1. Age distribution:

65 patients were included in our study. The mean age of patients in our study group is 37years.

Minimum age of patient in our study group is 18 years and maximum age of patient is 76 years.

Table 8: Age distribution among cases

Age (Years)	No. of patients	Percentage
< 20	8	12.3
20 – 29	20	30.8
30 – 39	10	15.4
40 - 49	9	13.8
50 - 59	10	15.4
60 - 69	5	7.7
70+	3	4.6
Total	65	100.0



Graph 1: Age distribution among cases

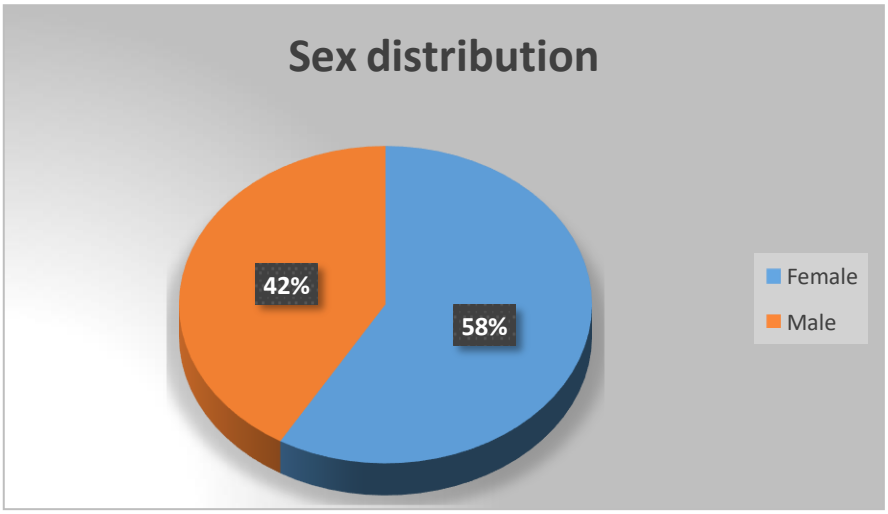
Our study group includes 12.3% (8) of patients of age group <20 years. 30.8% (20) of patients are from age group 20-29, 15.4% (10) of patients from age group 30-39years, 13.8% (9) of patients from age group 40-49years, 15.4%(10) of patients from age group 50-59years, 7.7%(5) of patients from age group 60-69years and 4.6%(3) of patients who are >70 years of age.

2. Sex distribution:

The study group includes 38 females and 27 males. The case distribution among the two sexes is consistent with higher prevalence of iron deficiency anaemia among females.

Table 9: Sex distribution among cases

SEX	No. of patients	Percentage
Female	38	58.5
Male	27	41.5
Total	65	100.0



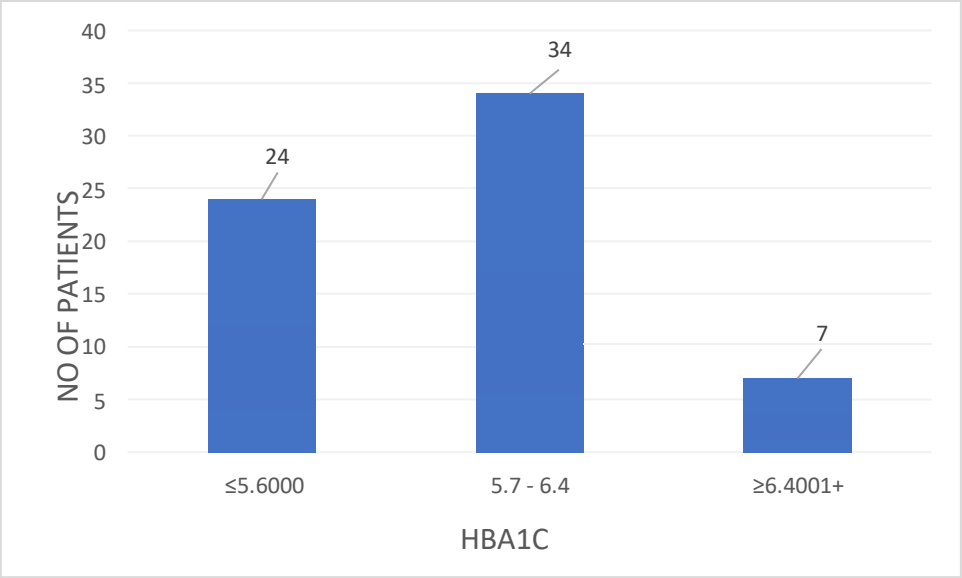
Graph 2: Sex distribution among cases

3. Comparison of HbA1c:

Table 10: Mean HbA1c among cases

HbA1c	N	Percent	Valid percent	Mean HbA1c
≤5.6000	24	36.9	36.9	5.67
5.7 - 6.4	34	52.3	52.3	
≥6.4001+	7	10.8	10.8	
Total	65	100.0	100.0	

The variable under study is HbA1c. Mean HbA1c among study group is 5.67. Minimum HbA1c among our study group is 4.8%. Maximum HbA1c among 6.7%. Standard deviation is 0.664 and standard error of mean being 0.06. HbA1c is 5% elevated among our study group.



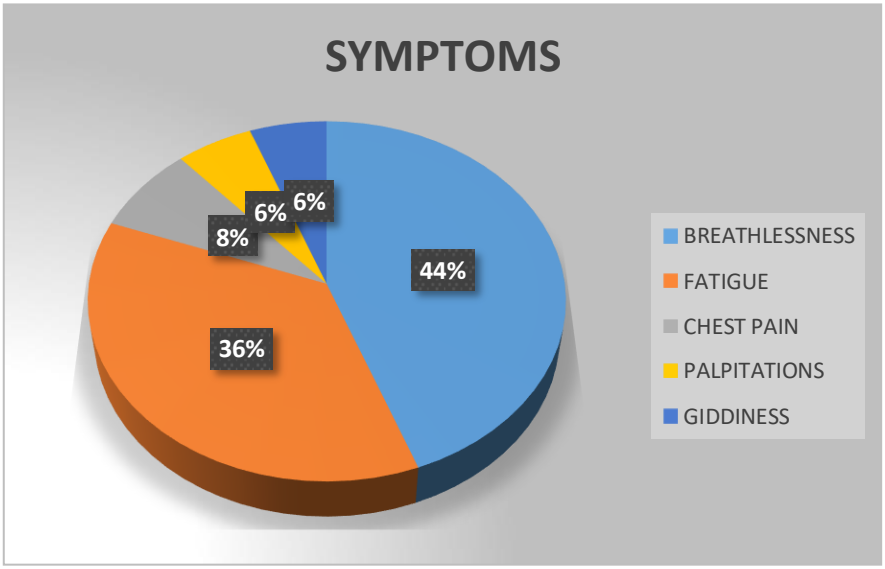
Graph 3: Mean HbA1c among cases

Among 65 iron deficiency anaemia patients, 36.9% (24 in number) had HbA1c within normal limit i.e., within 5.7%, 52%(34 in number) had HbA1c in pre-diabetic range, 10.8%(7 in number) had HbA1c in diabetic range.

4. Symptoms and Signs:

Table11: Clinical features and findings

Symptoms & Signs	N (No of patients)	Percent (%)
Breathlessness	62	95.4
Fatigue	51	78.5
Chest pain	11	16.9
Palpitations	8	12.3
Giddiness	8	12.3
Pallor	64	98.5



Graph 4: Clinical features among cases

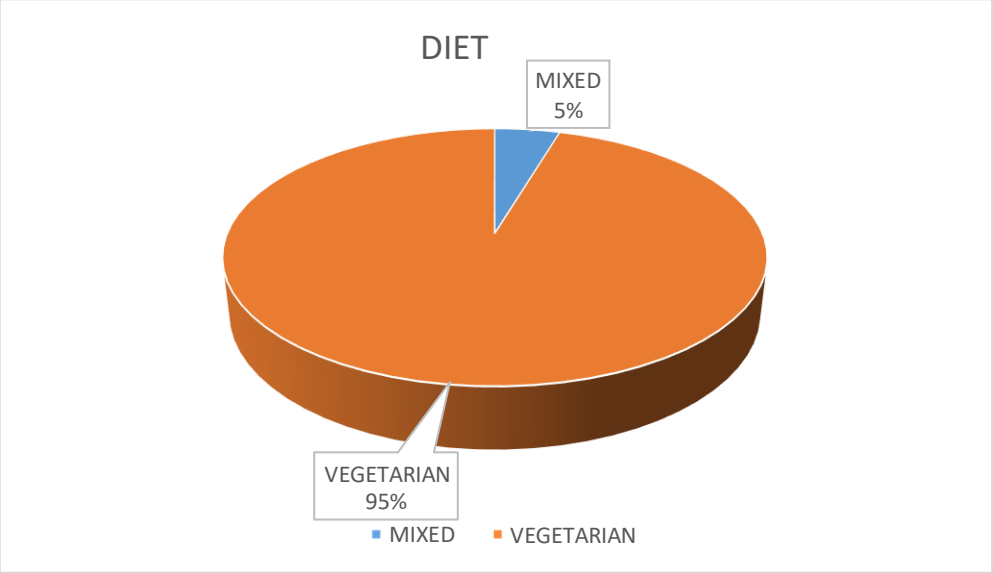
In our study group, 62(95.4%) of the patients had breathlessness, 51(78.5%) patients had easy fatiguability, 11(16.9%) patients had chest pain, 8(12.3%) patients had palpitations and giddiness. Whereas, 64(98.5%) patients in our study had pallor.

5. Diet distribution

Table 12: Diet distribution among cases

DIET	FREQUENCY	PERCENT
MIXED	3	4.6
VEGETARIAN	62	95.4

In our study which included 65 number of patients, 62(95.4%) patients were vegetarian and 3(4.6%) patients had mixed diet.



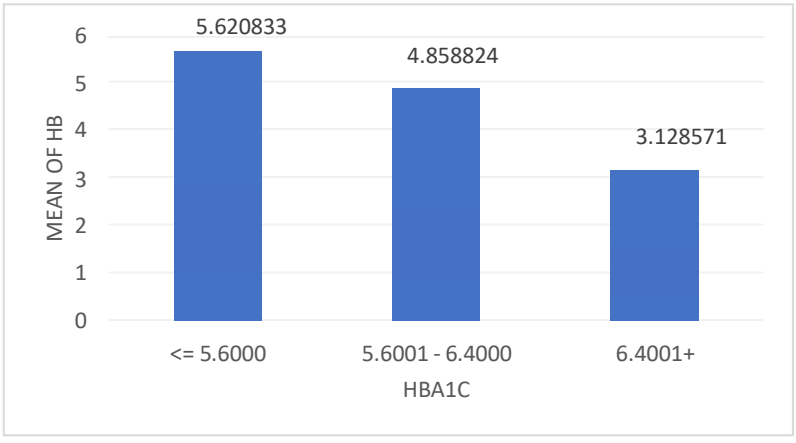
Graph 5: Diet distribution among cases

6. Comparison of Haemoglobin (Hb) with HBA1c

Table 13: Comparison of Haemoglobin (Hb) with HBA1c

HbA1c	No of patients	HB Mean	Standard Deviation	Kruskal-Wallis Test	p Value
≤ 5.6000	24	5.620833	1.0236249	17.416	0.0001*
5.7 - 6.4	34	4.858824	1.1542064		
>6.4001	7	3.128571	1.1220729		
Total	65	4.953846	1.3105434		
*-Statistically Significant					

In our study group which included 65 patients, 24 patients had HbA1c levels ≤5.6 (normal range) whose mean haemoglobin was 5.62 gm/dl, 34 patients had HbA1c levels were between 5.7 to 6.4 (pre-diabetic range) whose mean haemoglobin was 4.85 gm/dl and 7 patients had HbA1c levels were >6.4 (diabetic range) whose mean haemoglobin was 3.1 gm/dl.



Graph 6: Comparison of Haemoglobin (Hb) with HBA1c

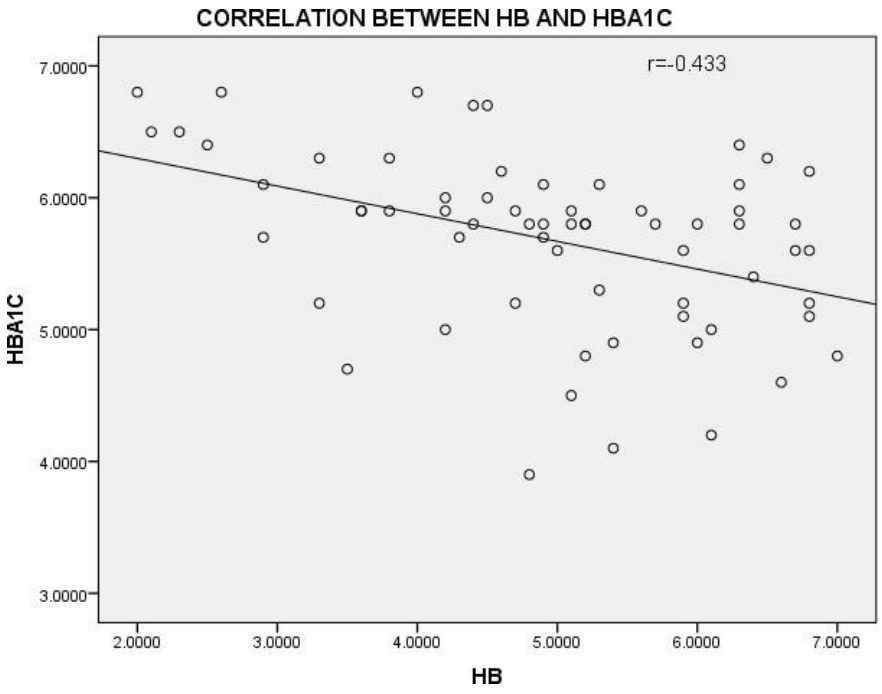
7. Correlation between HB and HBA1c:

Among 65 iron deficiency anaemia patients, 34 patients were in the pre-diabetic range whose mean Hb was 4.8 gm/dl, 7 patients were in the diabetic range whose mean Hb was 3.1gm/dl and 24 patients were in the normal range with mean Hb of 5.6gm/dl.

In our study, we can see that (graph 8), as the haemoglobin levels dropped secondary to iron deficiency the HbA1c levels gradually increased. As seen in Table 7, there is a mild negative correlation between haemoglobin and HbA1c levels in iron deficiency anaemia which is statistically significant (p = 0.0001).

Table 14: Correlation between HB and HBA1c

Correlation between	Correlation coefficient	Significant value	Remark
HB and HBA1c	r=-0.433	P=0.0001	Mild Negative correlation. Statistically significant



Graph 7: Correlation between HB and HBA1c

8. Comparison between haemoglobin (Hb) and serum electrolytes:

In our study, 16 patients had hyponatremia with a mean Hb of 4.8, and 1 patient had hypernatremia with a mean Hb of 7.0. Whereas, 48 patients had sodium levels in the normal range with a mean Hb of 4.9.

Table 15: Comparison between Hb and Serum Sodium

Serum Sodium	No of patients	Haemoglobin (Mean)	Standard Deviation (SD)
< 135	16	4.837500	1.0763364
135 - 145	48	4.950000	1.3694229
146+	1	7.000000	0.3225469

Graph 8: Comparison between Hb and Serum Sodium

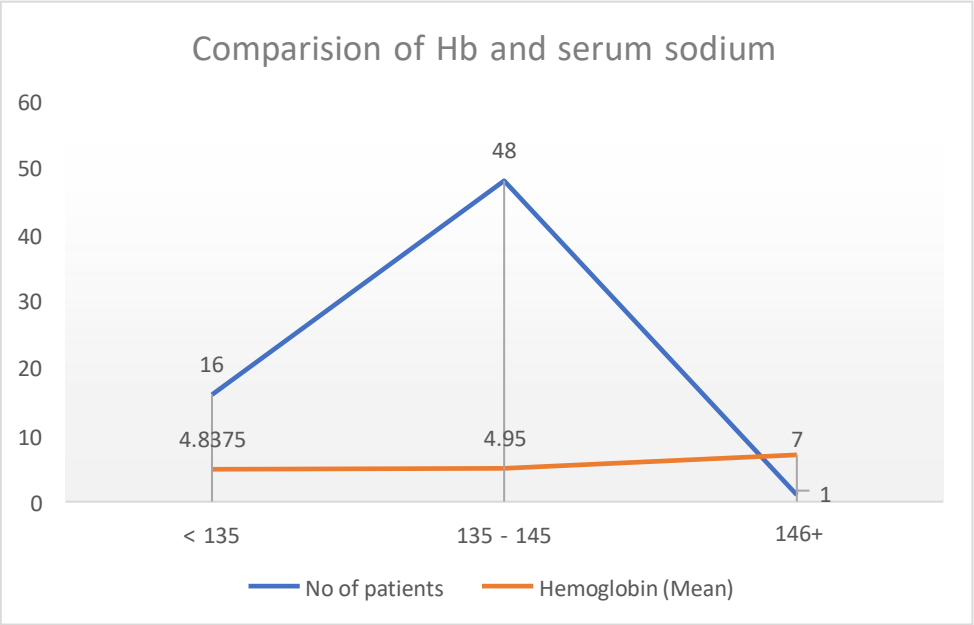
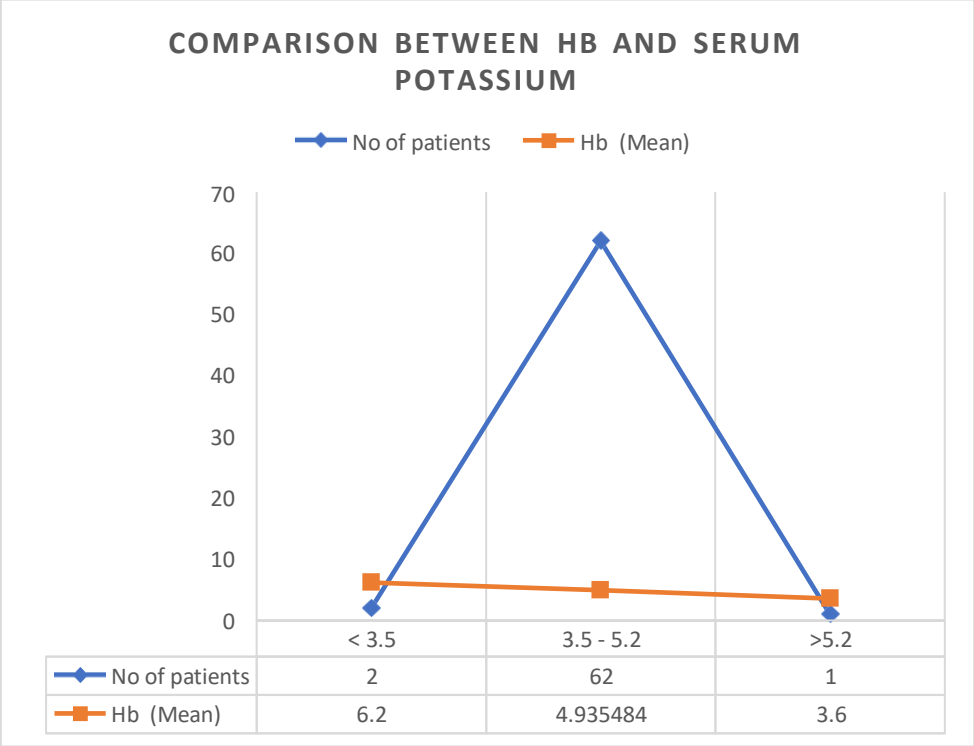


Table 16: Comparison between Hb and Serum Potassium

Serum Potassium	**No of patients**	**Hb (Mean)**	**Std Deviation**
< 3.5	2	6.200000	.7071068
3.5 - 5.2	62	4.935484	1.3086217
>5.2	1	3.600000	.0224536

In our study, 2 patients had hypokalemia with a mean Hb of 6.2, and 1 patient had hyperkalemia with a mean Hb of 3.2. Whereas, 62 patients had potassium levels in the normal range with a mean Hb of 4.9.



Graph 9: Comparison between Hb and Serum Potassium

9. Mean values of other lab parameters:

Our study which included 65 patients, had mean value of 16.2% PCV, 68.3fl MCV, 13.3% RDW, 19.1 ug/dl Serum iron, 24.2 ng/ml Serum ferritin, 340 ug/dl TIBC, 93mg/dl FBS, 124mg/dl PPBS, 31mg/dl Blood urea, 0.7mg/dl Serum creatinine, 0.8 mg/dl total bilirubin, 27.5 U/L SGPT, 35.5 U/L and 3.7 g/dl of Serum albumin.

Table 17: Mean values of other lab parameters

Lab Parameters	Mean value
PCV	16.2%
MCV	68.3 fl
RDW	21.9 ug/dl
Serum Iron	19.1 ug/ml
Serum Ferritin	24.28 ng/dl
TIBC	340 ug/dl
FBS	93 mg/dl
PPBS	124 mg/dl
Blood urea	31 mg/dl
Serum creatinine	0.7 mg/dl
Total bilirubin	0.8 mg/dl
SGPT	27.5 U/L
SGOT	35.5 U/L
Serum albumin	3.7 g/dl

DISCUSSION

Iron deficiency anaemia is the commonest cause of nutritional anaemia. It is a major public health problem in under nourished population like India. Children and women of reproductive age group are more vulnerable for development of Iron deficiency anaemia because of poor nutritional intake, menstrual loss, quick succession of pregnancy.

HbA is slowly and non-enzymatically glycosylated in a physiological manner. The rate of glycosylation depends on the concentration of glucose. The most prevalent type of glycated haemoglobin is HbA1c. The NH2 group in the terminal valine of the β-globin chains become permanently linked to glucose. Red blood cells undergo glycosylation during their 120-day lifespan. As a result, the measured levels of glycohemoglobin represent the glycemic state of the previous three months.

In addition to plasma glucose levels, other factors can also have an impact on HbA1c levels. Falsely lower or higher numbers can occur under a variety of circumstances. The HbA1c assays are affected by hemolytic anaemia, hemoglobinopathies, uremia, and prolonged blood loss. HbA1c has thus far shown to be an invaluable tool for tracking diabetics glycemic management. HbA1c has recently been endorsed by the American Diabetic Association and an international expert committee for the diagnosis of diabetes and pre-diabetes. HbA1c level of 6.5% has been proposed as a diagnostic cut off point for Diabetes, HbA1c value between 5.7-6.5% is considered as Prediabetic.

Few studies have strongly associated anaemia with an imbalance in the serum electrolyte levels due to alteration in red cell membrane bound sodium-potassium adenosine triphosphatase (Na+/K+ ATPase) pump activity that regulates intra and extracellular cation homeostasis. For basic life functions, such as generation and conduction of action potential in nerves and muscles, and maintenance of electrical neutrality of cells, electrolytes play a significant role.

Iron is important for oxygen supply and utilization in the human body by maintaining the shape of RBC. In this study, it was observed that serum levels of sodium levels were significantly lower in anemic patients.

There were multiple studies investigating the relationship between iron deficiency anaemia and HbA1c and its effect on serum sodium and potassium. But the results were inconsistent. Our study aims to measure the HbA1c and serum electrolyte levels in non-diabetic iron deficiency anaemia patients to estimate the error that can occur due to anaemia in HbA1c analysis.

Age and sex distribution of the study population:

The study included 65 non diabetic iron deficiency anaemia subjects. The mean age of patients in our study group is 37years. Minimum age of patient in our study group is 18 years and maximum age of patient is 76 years. Our study group includes 12.3% (8) of patients of age group <20 years, 30.8% (20) of patients are from age group 20-29, 15.4% (10) of patients from age group 30-39years, 13.8% (9) of patients from age group 40-49years, 15.4% (10) of patients from age group 50-59years, 7.7%(5) of patients from age group 60-69years and 4.6%(3) of patients who are >70 years of age.

The study group includes 38 females and 27 males. The case distribution among the two sexes is consistent with higher prevalence of iron deficiency anaemia among females. This implies that maximum iron deficiency anaemia cases are concentrated in reproductory age group that is from 18-45years with peak incidence in age group less than 30years. This could be secondary to increase in iron demand during puberty, menorrhagia, pregnancy, and quick succession of pregnancies leading to supply demand mismatch resulting in high prevalence of Iron deficiency anaemia these patients.

In age group of <30years iron deficiency anaemic patients were 30.8%, which is higher when compared to other age groups. This is consistent with the finding that iron deficiency anaemia in reproductive age group is more severe because of negative iron balance which is due to menstrual loss.

HbA1c level of the study population:

The variable under study is HbA1c. Mean HbA1c among study group is 5.67. Minimum HbA1c among our study group is 4.8%. Maximum HbA1c among 6.7%. Standard deviation is 0.664 and standard error of mean being 0.06. HbA1c is 5% elevated among our study group.

Symptoms and Signs of the study population:

In our study group, 62(95.4%) of the patients had breathlessness, 51(78.5%) patients had easy fatiguability, 11(16.9%) patients had chest pain, 8(12.3%) patients had palpitations and giddiness. Whereas, 64(98.5%) patients in our study had pallor.

Comparison between Haemoglobin (Hb) and Serum electrolytes:

In our study, 16 patients had hyponatremia with a mean Hb of 4.8, and 1 patient had hypernatremia with a mean Hb of 7.0. Whereas, 48 patients had sodium levels in the normal range with a mean Hb of 4.9. Also, our study shows 2 patients had hypokalemia with a mean Hb of 6.2, and 1 patient had hyperkalemia with a mean Hb of 3.2. Whereas, 62 patients had potassium levels in the normal range with a mean Hb of 4.9.

A study done by Mansoor et al. concluded that sodium levels and potassium levels are impacted in anaemic patients compared to patients without anaemia.

Rafiq et al. also found the variance in serum electrolyte levels exists among patients with IDA and those without anaemia.

Comparison of Haemoglobin (Hb) with HBA1c

In our study group which included 65 patients, 24 patients had HbA1c levels ≤5.6 (normal range) whose mean haemoglobin was 5.62 gm/dl, 34 patients had HbA1C levels were between 5.7 to 6.4 (pre-diabetic range) whose mean haemoglobin was 4.85 gm/dl and 7 patients had HbA1C levels were >6.4 (diabetic range) whose mean haemoglobin was 4.9 gm/dl.

Correlation between HB and HBA1c:

Among 65 iron deficiency anaemia patients, 34 patients were in the pre-diabetic range whose mean Hb was 4.8 gm/dl, 7 patients were in the diabetic range whose mean Hb was 3.1gm/dl and 24 patients were in the normal range with mean Hb of 5.6gm/dl.

Iron deficiency alters the quaternary structure of haemoglobin thereby alter its rate of glycation. Therefore, HbA1c among iron deficiency individuals is elevated independent of glycemia. Similar study was done by Alap L. Christy et al [(32)] concluded that "iron deficiency anaemia elevates HbA1c levels in diabetic individuals with controlled plasma glucose levels. They postulated that iron deficiency anaemia has a positive correlation with increased HbA1c levels."

Similar to this study, in 2014 a study was conducted by Vishal Kalasker et al[63] on the effect of iron deficiency anaemia on glycosylated haemoglobin levels in non-diabetic Indian adults. "They postulated that Hb concentrations are positively corrected with HbA1c concentration and that HbA1c concentration tended to be lower in the presence of iron deficiency anaemia."

A study done by Catherine Kim et al concluded that "iron deficiency shifted the HbA1c slightly upwards independent of fasting glucose level." El-Agouza et al[53] reported that "iron deficiency anaemia patients had higher HbA1c levels and it decreased after treatment. They believed that there was a balance between haemoglobin concentration and HbA1c level. That is if the plasma glucose was maintained, the lower haemoglobin concentration would lead to rise in HbA1c levels."

Study done by Van Heyningen et al[62] found out that "there was no significant influence of iron deficiency anaemia over HbA1c concentrations. They suggested that differences observed in previous studies could be due to the various laboratory methods used in estimating the HbA1c."
In our study, we can see that as the haemoglobin levels dropped secondary to iron deficiency the HbA1c levels gradually increased with negative correlation between haemoglobin and HbA1c levels in iron deficiency anaemia which is statistically significant ($p = 0.0001$).

CONCLUSION

- The prevalence of iron deficiency anaemia is more common in women of reproductive age group.
- Haemoglobin and HbA1c showed statistically significant negative correlation in patients with iron deficiency anaemia.
- Before utilising the HbA1c to identify diabetes and pre diabetes, iron deficiency anaemia must be taken into consideration as there is false positive elevation of HbA1c levels in iron deficiency anemia.
- Caution should be taken when therapeutic decision is needed in patients with co-existing diabetes and iron deficiency anaemia.
- Treating physicians should always be aware of the effect of alcoholism, uremia, aspirin intake, hemolytic anaemia, Vit B12 deficiency anaemia, iron deficiency on HbA1c before making a therapeutic decision based on HbA1c.
- Electrolyte disturbances like hyponatremia and hyperkalemia which are commonly seen in iron deficiency anaemia must be kept in mind before treatment.
- Physicians should always closely monitor serum electrolytes in patients with iron deficiency anaemia to avoid complications and life-threatening conditions.

LIMITATIONS

- The sample size of the study was small.
- The study should be done ideally to be a community-based study rather than a hospital based to know anaemia and its correlation with HbA1c and to prove its statistically correlation.
- Male population in study was low when compared to females. Variation of HbA1c based on sex could not be assessed.

FUTURE PROSPECTIVES

- Large scale trials over longer durations may give accurate information about the influence of iron deficiency anaemia over HbA1c levels and serum electrolytes.
- Prospective trial assessing HbA1c levels prior to correction of anaemia after correction of anaemia can be done. This would study HbA1c variation at individual level.
- Extensive study may be done to determine the value of alternative glycemic control indicators in individuals with iron deficient anaemia, such as glycated albumin and fructosamine.

SUMMARY

Iron deficiency anaemia is the most common type of nutritional anaemia. More than half of the world's anaemia burden is caused by it. To evaluate the glycemic control during the previous 120 days, HbA1c was used. Numerous non-glycemic factors, such as alcoholism, hemolytic anemia, vitamin B12 deficiency, uremia, and iron deficiency might have an impact on HbA1c levels. This study was conducted to study the influence of iron deficiency anaemia over HbA1c and serum electrolyte levels. Our study confirmed that iron deficiency anaemia was highly prevalent among female population in 18-45 years age group i.e., reproductive age group and the prevalence of hyponatremia in iron deficiency anaemia is common.

Study included 65 non diabetic iron deficiency anaemia patients. Mean HbA1c of patients with iron deficiency anaemia was 5.67%. Mean HbA1c increased by 5% with mild negative correlation between haemoglobin and HbA1c levels in iron deficiency anaemia which is statistically significant ($p = 0.0001$).

Among 65 anaemic individuals, 24 patients had HbA1c within normal limit i.e., <5.7%, 34 patients had HbA1c in pre-diabetic range 5.7-6.5%, 7 individuals had HbA1c in diabetic range. 16 patients had hyponatremia and 1 patient had hyperkalemia.

Caution should be taken when diagnosing diabetes and pre diabetes among people with anaemia and when HbA1c levels near to diagnostic value. Also, as hyponatremia is commonly seen in iron deficiency anaemia patients one must promptly treat electrolyte disturbances to prevent further complications.

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ANNEXURE I

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

Iec/110-09/2021

B.L.D.E. (DEEMED TO BE UNIVERSITY) Date-22-01-2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: A study to assess serum Electrolytes & HbA1c Levels in Non-Diabetic Iron deficiency Anemia indivuduals.

Name of PG student: Dr Shirish Patil, Department of Medicine

Name of Guide/Co-investigator: Dr S M Biradar, Associate Professor of Medicine

DR .S.V.PATIL
CHAIRMAN
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

21

ANNEXURE II

SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA-586 103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: " A STUDY TO ASSESS SERUM ELECTROLYTES AND HbA1c LEVELS IN NON-DIABETIC IRON DEFICIENCY ANEMIA INDIVIDUALS"

PG GUIDE : DR. S M BIRADAR

PG STUDENT : DR. SHIRISH PATIL

PURPOSE OF RESEARCH: I have been informed about this study. I have also been given a free choice of participation in this study.

BENEFITS:-

I understand that my participation in this study will help the investigator to diagnose the disease better and will help in the management of the disease.

PROCEDURE:-

I understand that relevant history will be taken and I will undergo detailed clinical examination after which necessary investigations will be done and accordingly treatment will be given.

RISK AND DISCOMFORTS:-

I understand there is no risk involved and I will experience no pain during the procedures performed.

CONFIDENTIALITY:-

I understand that medical information produced by this study will become a part of my hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purposes no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION: -

I understand that I may ask more questions about the study at any time Concerned. The researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION: -

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that the researcher may terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician, if this is appropriate.

INJURY STATEMENT: -

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient's / relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in patient's own language.

_____ _____

Investigator / P. G. Guide Date

I confirm that ……. (Name of the PG guide / chief researcher) has explained to me the research, the study procedures that I undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and I understand this consent form. Therefore, I agree to give my consent for my participation as a subject in this research project.

_____

_____

Participant / guardian Date

_____ _____

Witness to signature

Date:

ANNEXURE-III: SCHEME OF CASE TAKING PROFORMA

B.L.D.E (DEEMED TO BE UNIVERSITY)

SHRI B M PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

Informant:

Case No:

Name: IP No:

Age / Sex: DOA:

Occupation:

Address:

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS:

PAST HISTORY:

PERSONAL HISTORY:

1. Diet:
2. Appetite:
3. Sleep:
4. Bowel and bladder:
5. Habits:

FAMILY HISTORY:

MENSTRUAL HISTORY:

TREATMENT HISTORY:

GENERAL PHYSICAL EXAMINATION:

Vitals:

1. PR:
2. BP:
3. RR:
4. TEMPERATURE:
5. SpO2

Pallor:

Icterus:

Cyanosis:

Clubbing:

Lymphadenopathy:

Edema:

SYSTEMIC EXAMINATION:

1. CARDIO VASCULAR SYSTEM:
2. RESPIRATORY SYSTEM:
3. PER ABDOMEN:
4. CENTRAL NERVOUS SYSTEM:

INVESTIGATIONS:

1. Complete blood count:
2. Peripheral smear:
3. Iron profile:
   Serum ferritin
   Serum iron
   Tibc:
4. HbA1c:
5. Fbs & Ppbs:
6. Renal function test:
7. Liver function test:
8. USG Abdomen and pelvis:
9. Ecg:
10. Chest X-Ray:

FINAL DIAGNOSIS:

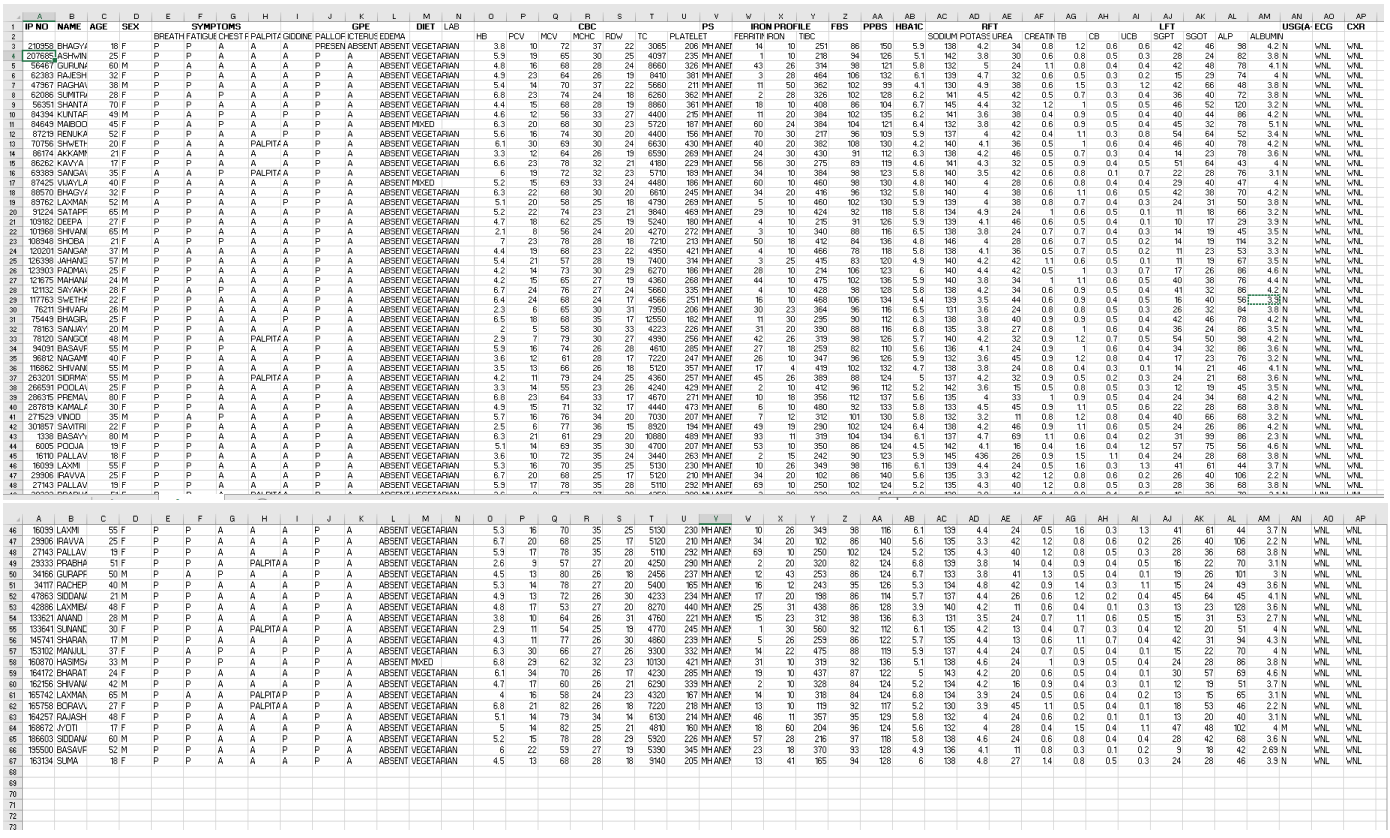
TREATMENT:

Date:

ANNEXURE IV

MASTER CHART

KEY TO MASTER CHART
A: ABSENT
P: PRESENT
HB: HAEMOGLOBIN
PCV: PACKED CELL VOLUME
MCV: MEAN CORPUSCULAR VOLUME
MCHC: MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION
RDW: RED CELL DISTRIBUTION WIDTH
TC: TOTAL COUNT
TIBC: TOTAL IRON BINDING CAPACITY
TB: TOTAL BILIRUBIN
CB: CONJUGATED BILIRUBIN
UCB: UNCONJUGATED BILIRUBIN
ALP: ALKALINE PHOSPHATASE



IP NO	NAME	AGE	SEX	SYMPTOMS						GPE			DIET	LAB	CBC					PS		IRON PROFILE			FBS	PPBS	HBA1C	RFT					LFT					USG(A	ECG	CXR
				BREATH	FATIGUE	CHEST P	PALPITA	GIDDINE	PALLOR	ICTERUS	EDEMA				HB	PCV	MCV	MCHC	RDW	TC	PLATELET		FERRITI	IRON	TIBC				SODIUM	POTASS	UREA	CREATIN	TB	CB	UCB	SGPT	SGOT	ALP	ALBUMIN			
210958	BHAGYA	18	F	P	P	A	A	A		PRESEN	ABSENT	ABSENT	VEGETARIAN		3.8	10	72	37	22	3065	206	MH ANEI	14	10	251	86	150	5.9	138	4.2	34	0.8	1.2	0.6	0.6	42	46	98	4.2 N		WNL	WNL
207685	ASHWIN	25	F	P	P	A	A	A	P	A	ABSENT	ABSENT	VEGETARIAN		5.9	19	65	30	25	4097	235	MH ANEI	1	10	218	94	126	5.1	142	3.8	30	0.6	0.8	0.5	0.3	28	24	82	3.8 N		WNL	WNL
5646	GURUNA	60	M	P	A	A	A	A	P	A	ABSENT	ABSENT	VEGETARIAN		4.8	16	68	28	24	8680	326	MH ANEI	43	26	314	98	121	5.6	132	5	24	1.1	0.8	0.4	0.4	42	48	78	4.1 N		WNL	WNL
62363	RAJESH	32	F	P	P	A	A	A	P	A	ABSENT	ABSENT	VEGETARIAN		4.9	23	64	26	19	8410	391	MH ANEI	3	28	464	106	132	6.1	139	4.7	32	0.6	0.5	0.3	0.2	15	29	74	4 N		WNL	WNL
47967	RAGHAV	38	M	P	P	A	A	A	P	A	ABSENT	ABSENT	VEGETARIAN		5.4	14	70	37	22	5660	211	MH ANEI	11	50	362	102	99	4.1	130	4.9	38	0.6	1.5	0.3	1.2	42	66	48	3.8 N		WNL	WNL
62086	SUMITR	28	F	P	A	P	A	P	P	A	ABSENT	ABSENT	VEGETARIAN		6.8	23	74	24	18	6260	362	MH ANEI	2	28	326	102	128	6.2	141	4.5	42	0.5	0.7	0.3	0.4	36	40	72	3.8 N		WNL	WNL
56351	SHANTA	70	F	P	P	A	A	A	P	A	ABSENT	ABSENT	VEGETARIAN		4.4	15	68	28	19	8980	361	MH ANEI	16	10	408	86	104	6.7	145	4.4	32	1.2	1	0.5	0.5	46	52	120	3.2 N		WNL	WNL
84394	KUNTAP	49	M	P	A	P	A	P	P	A	ABSENT	ABSENT	VEGETARIAN		4.6	12	56	33	27	4400	215	MH ANEI	11	20	384	102	135	6.2	141	3.6	38	0.4	0.9	0.5	0.4	40	44	86	4.2 N		WNL	WNL
84649	MAIBOO	45	F	P	A	P	A	A	P	A	ABSENT	MIXED			6.3	20	68	30	23	5720	187	MH ANEI	60	24	384	104	121	6.4	132	3.8	42	0.6	0.9	0.5	0.4	45	32	78	5.1 N		WNL	WNL
87219	RENUKA	52	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.6	16	74	30	20	4400	156	MH ANEI	70	30	217	96	109	5.9	137	4	42	0.4	1.1	0.3	0.8	54	64	52	3.4 N		WNL	WNL
70756	SHWETH	20	F	P	A	A	PALPITA	A	P	A	ABSENT	VEGETARIAN			6.1	30	69	30	24	6630	430	MH ANEI	40	20	382	108	130	4.2	140	4.1	36	0.5	1	0.6	0.4	46	40	78	4.2 N		WNL	WNL
86174	AKKAMM	21	F	P	A	A	A	P	P	A	ABSENT	VEGETARIAN			3.3	12	64	26	19	6590	269	MH ANEI	24	30	430	91	112	6.3	138	4.2	46	0.5	0.7	0.3	0.4	14	23	78	3.6 N		WNL	WNL
86262	KAVYA	17	F	P	A	A	A	A	P	A	ABSENT	VEGETARIAN			6.6	23	78	32	21	4180	229	MH ANEI	56	30	275	89	119	4.6	141	4.3	32	0.5	0.9	0.4	0.5	51	64	43	4 N		WNL	WNL
69389	SANGAM	35	F	A	A	P	PALPITA	A	P	A	ABSENT	VEGETARIAN			6	19	72	32	23	5710	189	MH ANEI	34	10	384	98	123	5.8	140	3.5	42	0.6	0.8	0.1	0.7	22	28	76	3.1 N		WNL	WNL
87425	VIJAYLA	40	F	P	A	A	A	P	P	A	ABSENT	MIXED			5.2	15	69	33	24	4480	186	MH ANEI	60	10	460	98	130	4.8	140	4	28	0.6	0.8	0.4	0.4	29	40	47	4 N		WNL	WNL
88570	BHAGYA	32	F	P	A	A	A	A	P	A	ABSENT	VEGETARIAN			6.3	22	68	30	20	6810	245	MH ANEI	34	20	416	96	132	5.8	140	4	38	0.6	1.1	0.6	0.5	42	36	70	4.2 N		WNL	WNL
89762	LAXMAN	52	M	A	P	P	A	P	P	A	ABSENT	VEGETARIAN			5.1	20	58	25	18	4780	269	MH ANEI	5	10	460	102	130	5.9	139	4	38	0.8	0.7	0.4	0.3	24	31	50	3.8 N		WNL	WNL
91224	SATAPP	65	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.2	22	74	23	21	9840	469	MH ANEI	29	10	424	92	118	5.8	134	4.9	24	1	0.6	0.5	0.1	11	18	66	3.2 N		WNL	WNL
109182	DEEPA	27	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.7	18	62	25	19	5240	180	MH ANEI	4	10	215	31	126	5.9	139	4.1	46	0.6	0.5	0.4	0.1	10	17	29	3.9 N		WNL	WNL
101968	SHIVANI	65	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			2.1	8	56	24	20	4270	272	MH ANEI	3	10	340	88	116	6.5	138	3.8	24	0.7	0.7	0.4	0.3	14	19	45	3.5 N		WNL	WNL
108948	SHOBA	21	F	A	P	A	A	A	P	A	ABSENT	VEGETARIAN			7	23	78	28	18	7210	213	MH ANEI	50	18	412	84	136	4.8	146	4	28	0.6	0.7	0.5	0.2	14	19	114	3.2 N		WNL	WNL
120201	SANGAM	37	M	P	A	A	A	P	A	A	ABSENT	VEGETARIAN			4.4	19	68	23	22	4950	421	MH ANEI	4	10	466	78	118	5.8	138	4.1	36	0.5	0.7	0.5	0.2	11	23	53	3.3 N		WNL	WNL
126398	JAHANE	57	M	P	A	A	A	P	P	A	ABSENT	VEGETARIAN			5.4	21	57	28	19	7400	394	MH ANEI	3	25	415	83	120	4.9	140	4.2	42	1.1	0.6	0.5	0.1	11	19	67	3.5 N		WNL	WNL
123903	PADMA	25	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.2	14	73	30	29	6270	186	MH ANEI	28	10	214	106	123	6	140	4.4	42	0.5	1	0.3	0.7	17	26	86	4.6 N		WNL	WNL
121675	MAHANA	24	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.2	15	65	27	19	4360	266	MH ANEI	44	10	475	102	136	5.9	140	3.8	34	1	1.1	0.6	0.5	40	38	76	4.4 N		WNL	WNL
121132	SAYAKK	28	F	P	A	A	A	A	P	A	ABSENT	VEGETARIAN			6.7	24	76	27	24	5960	335	MH ANEI	4	10	428	98	128	5.8	138	4.2	34	0.6	0.9	0.5	0.4	41	32	86	4.2 N		WNL	WNL
117763	SWETHA	22	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			6.4	24	68	24	17	4566	251	MH ANEI	16	10	468	106	134	5.4	139	3.5	44	0.6	0.9	0.4	0.5	16	40	56	3.8 N		WNL	WNL
76211	SHIVARA	26	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			2.3	6	65	30	31	7950	206	MH ANEI	30	23	364	96	116	6.5	131	3.6	24	0.8	0.8	0.5	0.3	26	32	84	3.8 N		WNL	WNL
75449	BHAGIR	25	F	P	P	A	A	P	P	A	ABSENT	VEGETARIAN			6.5	18	68	35	17	12550	182	MH ANEI	11	30	295	90	112	6.3	138	3.8	40	0.9	0.9	0.5	0.4	42	46	78	4.2 N		WNL	WNL
78163	SANJAY	20	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			2	5	58	30	33	4223	226	MH ANEI	31	20	390	88	116	6.8	135	3.8	27	0.8	1	0.6	0.4	36	24	86	3.5 N		WNL	WNL
78120	SANGOI	48	M	P	P	A	PALPITA	A	P	A	ABSENT	VEGETARIAN			2.9	7	79	30	27	4990	256	MH ANEI	42	26	319	98	126	5.7	140	4.2	32	0.9	1.2	0.7	0.5	54	50	98	4.2 N		WNL	WNL
94091	BASAVE	55	M	P	P	A	A	P	P	A	ABSENT	VEGETARIAN			5.9	16	74	26	26	4610	285	MH ANEI	27	16	259	82	110	5.6	136	4.1	24	0.9	1	0.6	0.4	34	32	86	3.6 N		WNL	WNL
96812	NAGAMM	40	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			3.6	12	61	28	17	7220	247	MH ANEI	26	10	347	96	126	5.9	132	3.6	45	0.9	1.2	0.8	0.4	17	23	76	3.2 N		WNL	WNL
116862	SHIVANI	55	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			3.5	13	66	26	18	5120	357	MH ANEI	17	4	419	102	132	4.7	138	3.8	24	0.8	0.4	0.3	0.1	14	21	46	4.1 N		WNL	WNL
263201	SIDRAM	55	M	P	P	A	PALPITA	A	P	A	ABSENT	VEGETARIAN			4.2	11	79	24	25	4360	257	MH ANEI	45	26	389	88	124	5	137	4.2	32	0.9	0.5	0.2	0.3	24	21	68	3.6 N		WNL	WNL
266591	POOLA	25	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			3.3	14	55	23	26	4240	429	MH ANEI	2	10	412	96	112	5.2	142	3.6	15	0.5	0.8	0.5	0.3	12	19	45	3.5 N		WNL	WNL
286315	PREMA	80	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			6.8	23	64	33	17	4670	271	MH ANEI	10	18	356	112	137	5.6	135	4	33	1	0.9	0.5	0.4	24	34	68	4.2 N		WNL	WNL
287818	KAMALA	30	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.9	15	71	32	17	4440	473	MH ANEI	6	10	480	92	133	5.8	133	4.5	45	0.9	1.1	0.5	0.6	22	28	68	3.8 N		WNL	WNL
275231	VINOD	35	M	P	A	P	A	A	P	A	ABSENT	VEGETARIAN			5.7	16	76	34	20	7030	207	MH ANEI	7	12	312	101	130	5.8	132	3.2	11	0.8	1.2	0.8	0.4	40	66	68	3.2 N		WNL	WNL
301857	SAVITRI	22	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			2.5	6	77	36	15	8920	194	MH ANEI	49	19	236	102	124	6.4	138	4.2	46	0.9	1.1	0.6	0.5	24	26	86	4.2 N		WNL	WNL
1338	BASAVA	80	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			6.3	21	61	29	20	10880	489	MH ANEI	93	11	319	104	134	6.1	137	4.7	69	1.1	0.6	0.4	0.2	31	99	86	2.3 N		WNL	WNL
6005	POOJA	19	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.1	14	69	35	30	4700	207	MH ANEI	53	10	350	86	124	4.5	142	4.1	16	0.4	1.6	0.4	1.2	57	75	56	4.6 N		WNL	WNL
16110	PALLAV	18	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			3.6	10	72	35	24	3440	263	MH ANEI	2	15	242	90	123	5.9	145	436	26	0.9	1.5	1.1	0.4	24	28	68	3.8 N		WNL	WNL
16039	LAXMI	55	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.3	16	70	35	25	5130	230	MH ANEI	10	26	349	98	116	6.1	139	4.4	24	0.5	1.6	0.3	1.3	41	61	44	3.7 N		WNL	WNL
23906	PAVVA	25	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			6.7	20	68	25	17	5120	210	MH ANEI	34	20	102	86	140	5.6	135	3.3	42	1.2	0.8	0.6	0.2	26	40	106	2.2 N		WNL	WNL
27143	PALLAV	19	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.9	17	78	35	28	5110	282	MH ANEI	69	10	250	102	124	5.2	135	4.3	40	1.2	0.8	0.5	0.3	28	36	68	3.8 N		WNL	WNL
16039	LAXMI	55	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.3	16	70	35	25	5130	230	MH ANEI	10	26	349	98	116	6.1	139	4.4	24	0.5	1.6	0.3	1.3	41	61	44	3.7 N		WNL	WNL
29333	PRABHA	51	F	P	P	A	PALPITA	A	P	A	ABSENT	VEGETARIAN			2.6	9	57	27	20	4250	290	MH ANEI	2	20	320	82	124	6.8	139	3.8	14	0.4	0.9	0.4	0.5	16	22	70	3.1 N		WNL	WNL
34166	GURAPP	50	M	P	A	P	A	A	P	A	ABSENT	VEGETARIAN			4.5	13	80	26	18	2456	237	MH ANEI	12	43	253	86	124	6.7	133	3.8	41	1.3	0.5	0.4	0.1	19	26	101	3 N		WNL	WNL
34117	RACHEP	40	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.3	14	78	27	20	5400	165	MH ANEI	16	12	243	95	126	5.3	134	4.8	42	0.9	1.4	0.3	1.1	15	24	49	3.6 N		WNL	WNL
47863	SIDDAM	21	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.9	13	72	26	30	4233	234	MH ANEI	17	20	398	86	114	5.7	137	4.4	26	0.6	1.2	0.7	0.4	45	64	45	4.1 N		WNL	WNL
42886	LAXMIB	48	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.8	17	53	27	20	8270	440	MH ANEI	25	31	438	86	128	3.9	140	4.2	11	0.6	0.4	0.1	0.3	13	23	128	3.6 N		WNL	WNL
133621	ANAND	28	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			3.8	10	64	26	31	4760	221	MH ANEI	15	23	312	98	136	6.3	131	3.5	24	0.7	1.1	0.6	0.5	15	31	53	2.7 N		WNL	WNL
133641	SUNANC	30	F	P	P	A	PALPITA	A	P	A	ABSENT	VEGETARIAN			2.9	11	54	25	19	4770	245	MH ANEI	1	30	560	92	112	6.1	135	4.2	13	0.4	0.7	0.3	0.4	12	20	51	4 N		WNL	WNL
145741	SHARAN	17	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.3	11	77	26	30	4860	239	MH ANEI	5	26	259	86	122	5.7	135	4.4	13	0.6	1.1	0.7	0.4	42	31	94	4.3 N		WNL	WNL
153182	MANJUL	37	F	P	A	P	A	P	P	A	ABSENT	VEGETARIAN			5.3	30	66	27	26	3300	332	MH ANEI	14	22	475	88	119	5.9	137	4.4	24	0.7	0.5	0.4	0.1	15	22	70	4 N		WNL	WNL
160870	HASIMS	33	M	P	P	A	A	A	P	A	ABSENT	MIXED			6.8	29	62	32	23	10130	421	MH ANEI	31	10	319	92	136	5.1	138	4.6	24	1	0.9	0.5	0.4	24	28	86	3.8 N		WNL	WNL
164172	BHARAT	24	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			6.1	34	70	26	17	4230	285	MH ANEI	19	10	437	87	122	5	143	4.2	20	0.6	0.5	0.4	0.1	30	57	69	4.6 N		WNL	WNL
162156	SHIVAN	42	M	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.7	17	60	26	21	6290	339	MH ANEI	2	10	328	84	124	5.2	134	4.2	16	0.9	0.4	0.3	0.1	12	19	51	3.7 N		WNL	WNL
165742	LAXMAN	65	M	P	A	A	PALPITA	P	P	A	ABSENT	VEGETARIAN			4	16	58	24	23	4320	167	MH ANEI	14	10	318	84	124	6.8	134	3.9	24	0.5	0.6	0.4	0.2	13	15	65	3.1 N		WNL	WNL
165758	BORAVV	27	F	P	P	A	PALPITA	A	P	A	ABSENT	VEGETARIAN			6.8	21	82	26	18	7220	218	MH ANEI	13	10	119	92	117	5.2	130	3.9	45	1.1	0.5	0.4	0.1	18	53	46	2.2 N		WNL	WNL
164257	RAJASH	48	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5.1	14	79	34	14	6130	214	MH ANEI	46	11	357	95	129	5.8	132	4	24	0.6	0.2	0.1	0.1	13	20	40	3.1 N		WNL	WNL
168672	JYOTI	17	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			5	14	82	25	21	4810	160	MH ANEI	18	60	204	96	124	5.6	132	4	28	0.4	1.5	0.4	1.1	47	48	102	4 M		WNL	WNL
186603	SIDDAM	60	M	P	P	A	A	P	P	A	ABSENT	VEGETARIAN			5.2	15	78	28	29	5920	226	MH ANEI	57	28	216	97	118	5.8	138	4.6	24	0.6	0.8	0.4	0.4	28	42	68	3.6 N		WNL	WNL
185500	BASAVE	52	M	P	P	A	A	P	P	A	ABSENT	VEGETARIAN			6	22	59	27	19	5390	345	MH ANEI	23	18	370	93	128	4.9	136	4.1	11	0.8	0.3	0.1	0.2	9	18	42	2.69 N		WNL	WNL
163134	SUMA	18	F	P	P	A	A	A	P	A	ABSENT	VEGETARIAN			4.5	13	68	28	18	3140	205	MH ANEI	13	41	165	94	128	6	138	4.8	27	1.4	0.8	0.5	0.3	24	28	46	3.9 N		WNL	WNL