Dissertation On

A STUDY ON INFLUENCE OF IRON DEFICIENCY ANAEMIA OVER HBA1C LEVELS

Submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

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In partial fulfilment of regulations
For the award of the degree

M.D (GENERAL MEDICINE)

BRANCH – I

ESIC - MEDICAL COLLEGE& POSTGRADUATE INSTITUTE

OF MEDICAL SCIENCE AND RESEARCH,

K.K.NAGAR, CHENNAI – 78.

APRIL 2017

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DEAN

Dr. SRIKUMARI DAMODARAM,

M.S.,M.Ch.(SGE), M.A.M.S., F.A.C.S.,

F.I.C.S.,F.M.M.C

ESIC MEDICAL COLLEGE & PGIMSR

K.K.NAGAR, CHENNAI-78.

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PROF. DR. A.R.MALATHY. MD.,

Professor and Head,

Department of General medicine,

ESIC Medical College & PGIMSR,

Chennai -78

Date:

Place: Chennai

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PROF. DR. A.R.MALATHY. MD.,

Professor and Head,

Department of General medicine,

ESIC Medical College & PGIMSR,

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Place: Chennai

DECLARATION

I solemnly declare that this dissertation entitled "**A STUDY ON INFLUENCE OF IRON DEFICIENCY ANAEMIA OVER HBA1C LEVELS**" has been conducted by me at ESIC Medical College & PGIMSR, Chennai, under the guidance and supervision of **Prof.Dr.A.R.Malathy, M.D**., Professor and Head, Department of General Medicine, ESIC Medical College & PGIMSR, Chennai-78. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the University regulations for the award of the degree of M.D. Branch 1 (General Medicine).

SIGNATURE OF THE CANDIDATE
Dr. VIJAYA DURAIRAJ.K
M.D. Post Graduate
Dept. Of General Medicine
ESIC Medical College & PGIMSR
KK Nagar, Chennai-78

Date:

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ACKNOWLEDGEMENT

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Finally, I wholeheartedly thank the patients, who were the subjects of the study, without whom this would not have become a reality.

DR. K.VIJAYA DURAIRAJ

CERTIFICATE OF APPROVAL

To

Dr. K. Vijaya Durairaj,
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KK Nagar, Chennai-78.

Dear Dr. K. Vijaya Durairaj,

The Institutional Ethical Committee of ESIC Medical College & PGIMSR reviewed and discussed your application for approval of the proposal entitled "**A study on influence of iron deficiency anaemia over HBAIC levels**" at ESIC Medical College & PGIMSR, K K Nagar, Chennai 600 078", **No. 01/27/10/2014**..

The following members of the Ethical Committee were present in the meeting held on 27.10.2014 conducted at ESIC Medical College & PGIMSR, KK Nagar, Chennai-78.

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The proposal is approved to be conducted in its presented form.

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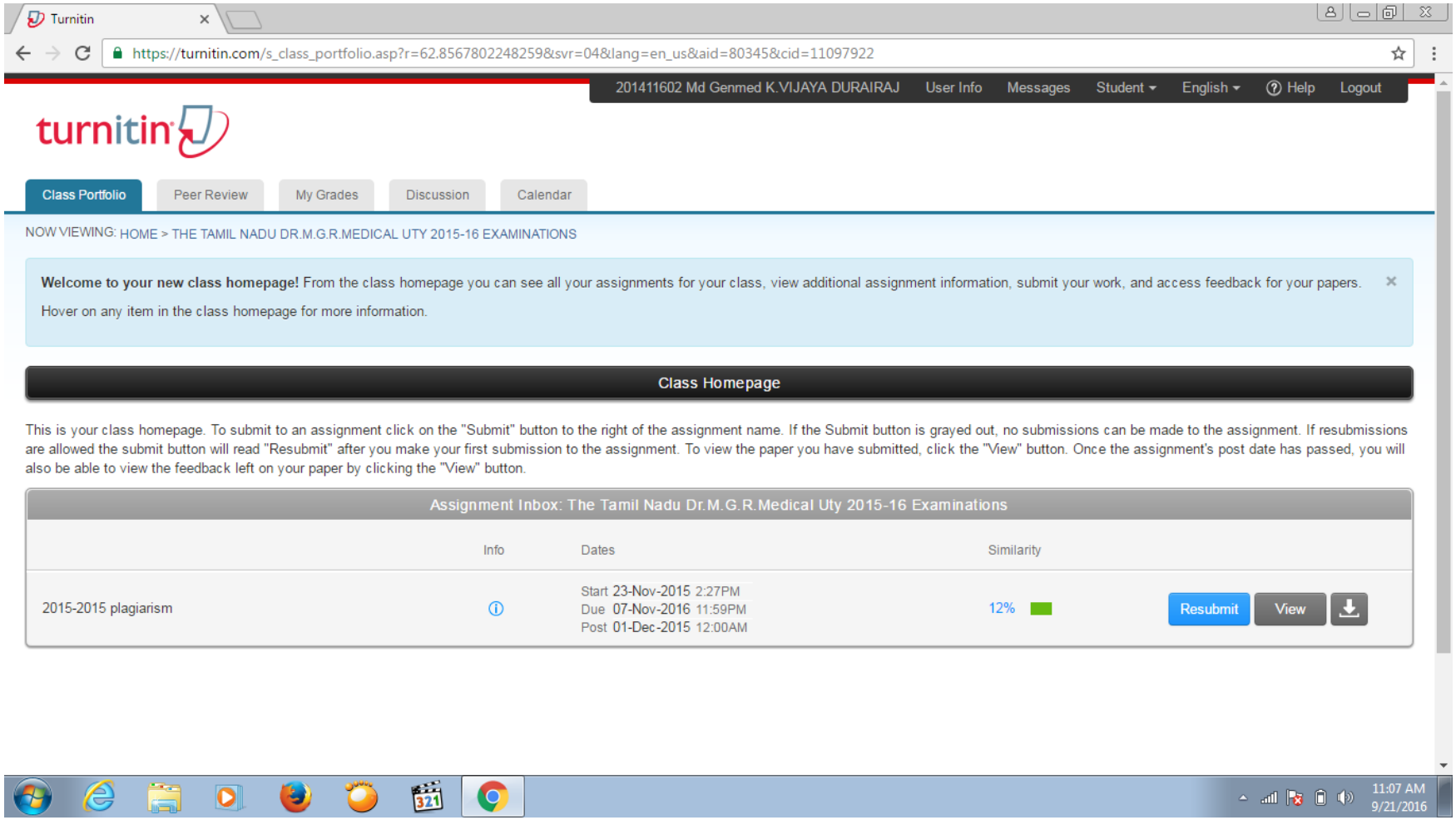
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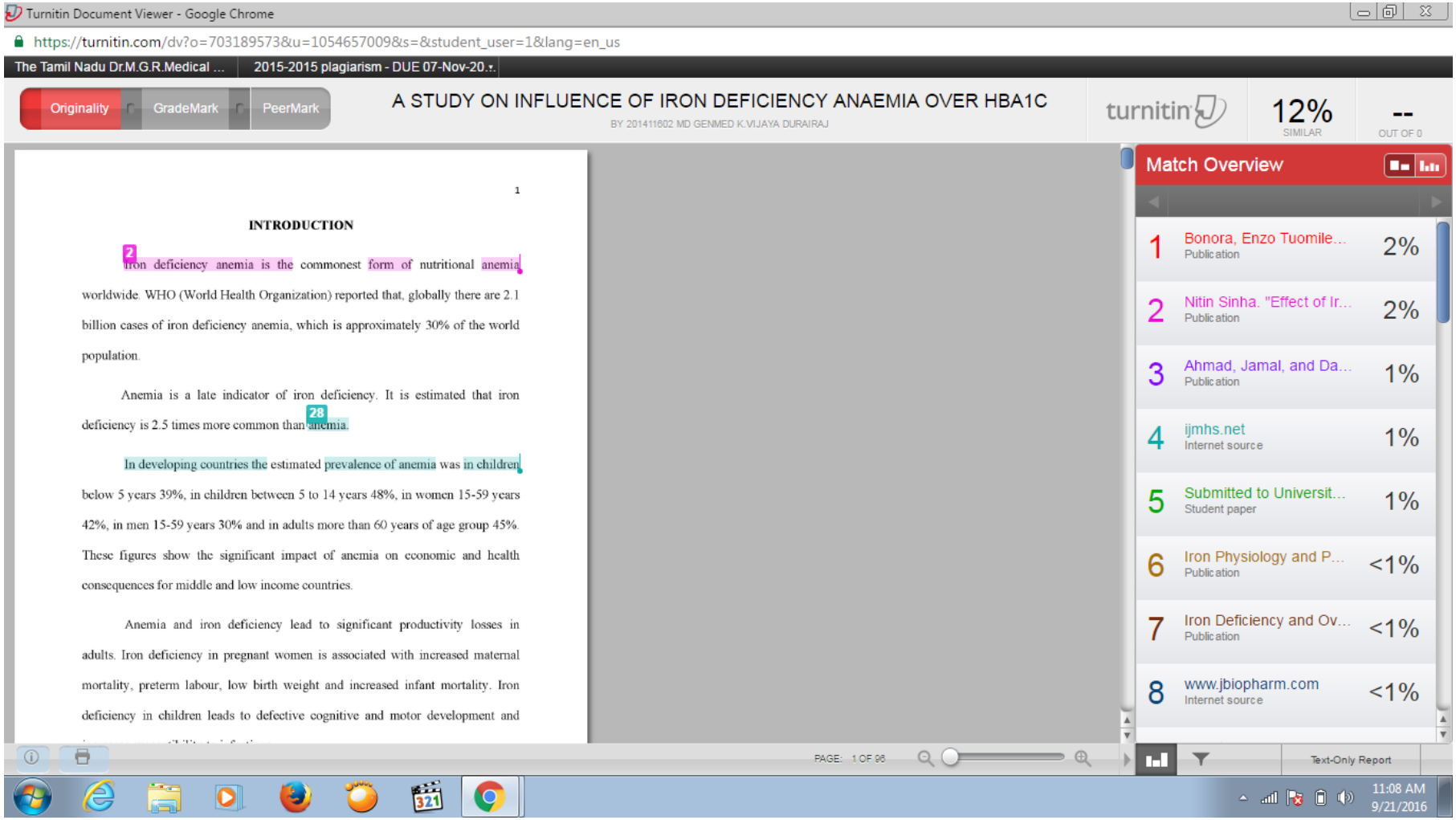
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INTRODUCTION
Iron deficiency anemia is the commonest form of nutritional anemia worldwide. WHO (World Health Organization) reported that, globally there are 2.1 billion cases of iron deficiency anemia, which is approximately 30% of the world population.
Anemia is a late indicator of iron deficiency. It is estimated that iron deficiency is 2.5 times more common than anemia.
In developing countries the estimated prevalence of anemia was in children below 5 years 39%, in children between 5 to 14 years 48%, in women 15-59 years 42%, in men 15-59 years 30% and in adults more than 60 years of age group 45%. These figures show the significant impact of anemia on economic and health consequences for middle and low income countries.
Anemia and iron deficiency lead to significant productivity losses in adults. Iron deficiency in pregnant women is associated with increased maternal mortality, preterm labour, low birth weight and increased infant mortality. Iron deficiency in children leads to defective cognitive and motor development and
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LIST OF ABBREVIATIONS

1.	**ADA**	**–**	**American Diabetes Association**
2.	**CVD**	**–**	**Cardiovascular Disease**
3.	**DCCT**	**–**	**Diabetes Control and Complications Trial**
4.	**DM**	**–**	**Diabetes Mellitus**
5.	**DMT**	**–**	**Divalent Metal Transporter**
6.	**eAG**	**–**	**estimated Average Glucose**
7.	**EPO**	**–**	**Erythropoietin**
8.	**ESA**	**–**	**Erythropoiesis stimulating agents**
9.	**FPG**	**–**	**Fasting Plasma Glucose**
10.	**Fpn**	**–**	**Ferroportin**
11.	**GDM**	**–**	**Gestational Diabetes Mellitus**
12.	**Hb**	**–**	**Hemoglobin**
13.	**HbA1c**	**–**	**Glycosylated Hemoglobin**
14.	**HGI**	**–**	**Hemoglobin Glycation Index**
15.	**HPLC**	**–**	**High Performance Liquid Chromatography**
16.	**IDA**	**–**	**Iron Deficiency Anemia**
17.	**IEC**	**–**	**International Expert Committee**
18.	**IFCC**	**–**	**International Federation of Clinical Chemistry**

19.	**NGSP**	**–**	**National Glycohemoglobin Standardization Program**
20.	**OGTT**	**–**	**Oral Glucose Tolerance Test**
21.	**PG**	**–**	**Postprandial Glucose**
22.	**RDW**	**–**	**Red cell Distribution Width**
23.	**TfR**	**–**	**Transferrin Receptor**
24.	**TIBC**	**–**	**Total Iron Binding Capacity**
25.	**UIBC**	**–**	**Unsaturated/Latent Iron Binding Capacity**

CONTENTS

ABSTRACT

Title: A STUDY ON INFLUENCE OF IRON DEFICIENCY ANAEMIA OVER HBA1C LEVELS

Background

Iron deficiency anemia is the commonest nutritional anemia worldwide. HbA1c, which is a valuable tool in monitoring the glycemic control, has been recently recommended for diagnosing diabetes. HbA1c can be affected by other non glycemic parameters like hemoglobin variants, anemia, uremia, pregnancy and acute blood loss. Reports on the effects of iron deficiency anemia on HbA1c levels were inconsistent.

Aim

This study aims to study the levels of HbA1c in iron deficiency anemia patients and to study the changes in HbA1c levels after correction of anemia.

Methods

120 patients confirmed to have iron deficiency were enrolled in this study. Complete blood count, anemia profile including serum ferritin and

HbA1c levels were measured at baseline and after treatment of anemia. These values were compared with those in the control population.

Results

The mean HbA1c level in iron deficiency anemia patients (4.619 ± 0.308%) was significantly lower than control group (5.446 ± 0.281%). A significant increase (5.816 ± 0.323%) was observed in the mean HbA1c of anemia group after treatment.

Conclusions

Our study showed that HbA1c levels were affected by iron deficiency anemia. The HbA1c levels are lower in iron deficiency anemia patients and it increases after treatment with iron supplements. So iron deficiency anemia has to be kept in mind before using the HbA1c to diagnose diabetes.

Keywords: Iron deficiency anemia, HbA1c, ferritin.

INTRODUCTION

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Iron deficiency anemia is the commonest form of nutritional anemia worldwide. WHO (World Health Organization) reported that, globally there are 2.1 billion cases of iron deficiency anemia, which is approximately 30% of the world population.

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Anemia and iron deficiency lead to significant productivity losses in adults. Iron deficiency in pregnant women is associated with increased maternal mortality, preterm labour, low birth weight and increased infant mortality. Iron deficiency in children leads to defective cognitive and motor development and increases susceptibility to infections.

Anemia is the major public health problem in India. According to National Family Health Survey (NFHS), 70% of children aged 6-59

months, 55% of females aged 15-49 years and 24% of males aged 15-49 years were suffering from anemia. NFHS-3 data showed that the prevalence of anemia was higher in rural areas. But there is a paucity of data about the epidemiology of anemia in rural population.

Hemoglobin A1c (HbA1c) or glycated hemoglobin is the predominant fraction of hemoglobin A. It is used as the gold standard method for assessing the glycemic control. It reflects the glycemic status of the individual over the past 3 months. It is formed by glycation of NH2-terminal valine of the hemoglobin β chain.

According to the guidelines of American Diabetic Association, the target HbA1c in all diabetic patients is below 7%, to prevent the development of secondary microvascular complications. Similar to plasma glucose, HbA1c level is related to the prevalent retinopathy.

The ADA and an International Expert Committee have now recommended the use of HbA1c to diagnose diabetes. The WHO also agreed that HbA1c may be used to diagnose diabetes, with appropriate measures i.e. standardized assay, calibration against IFCC standards and low coefficient of variability.

In 2009, an International Expert Committee recommended the HbA1c level of more than 6.5% as a cut-off point to diagnose diabetes. The test should be repeated to confirm the diagnosis. Repeat testing is not

required if there are classical clinical symptoms and the plasma glucose levels more than 200 mg/dl. The Committee also recommended, considering the diabetes preventive measures in individuals with HbA1c level between 6.0 to 6.5%, as they are at a higher risk.

In addition to blood glucose level, HbA1c is affected by multiple factors like genetic, hematologic and illness related factors.

Initial studies suggested a relationship between HbA1c levels and iron deficiency anemia. They tried to explain that on the basis of structural modifications and alterations in HbA1c levels in old and new red blood cells. Few studies reported no differences in the HbA1c levels of anemic patients compared to healthy controls.

Few studies stated that higher HbA1c levels were seen in iron deficiency anemia patients and it decreased significantly after treatment. The results of various studies on relationship between HbA1c and iron deficiency anemia were conflicting. Only fewer studies have been conducted in Indian population on this topic.

Our aim is to study the levels of HbA1c in iron deficiency anemia patients and the changes in HbA1c level after the correction of iron deficiency anemia.

AIMS
AND
OBJECTIVES

AIMS AND OBJECTIVES

Primary Objective

To study the levels of HbA1c in iron deficiency anemia patients.

Secondary Objective

To study the changes in HbA1c level with the correction of iron deficiency anemia.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

IRON DEFICIENCY

Iron deficiency is the state in which the iron content of the body is less than normal. The earliest stage of iron deficiency is depletion of iron stores, in which the serum iron, transferrin saturation and hemoglobin levels will be normal but the storage iron is decreased or absent. Further advanced stage is iron deficiency without anemia, characterized by depleted iron stores, low serum iron and transferrin saturation but without anemia.[2]

Iron deficiency anemia is the far most advanced stage of iron deficiency. It is characterized by absent iron stores, low serum iron levels, low transferrin saturation with low hemoglobin levels.

Iron deficiency anemia is most prevalent in women and children in regions where meat intake is low, food is not fortified with iron, malaria, intestinal infections and parasitic worms are common.

IRON METABOLISM

Iron is one of the key elements in the basal metabolism. Iron is an important component of heme. It acts as the active site for electron

transport in cytochromes and cytochrome oxidase involved in energy generation in mitochondria. The heme moiety in hemoglobin and myoglobin binds with O2 thereby transfers O2 from the lungs to the various tissues and to store it. Heme is the active site in peroxidases, the enzymes involved in protection of cells from oxidative injury by reducing the peroxides to water and generate microbicidal hypochlorite in granulocytes.

Table 3.1 Iron compartments

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

I. HEMOGLOBIN

Hemoglobin contains approximately2 gm of body iron in men and 1.5 gm in women. One ml of packed red cells contains approximately

1mg of iron. As the life span of red cells is 120 days, everyday 1/120 of the iron in hemoglobin is recycled by macrophages and they are returned to the plasma. From plasma they are delivered mostly to marrow erythroblasts for incorporation into newly synthesized hemoglobin.

II. STORAGE COMPARTMENT

The storage form of iron is ferritin or hemosiderin. Ferritin is water soluble and hemosiderin is water insoluble.

FERRITIN

The ferritin molecules have H (heavy), L (light) type subunits. H subunits have ferroxidase activity, which favors iron uptake or release by ferritin quite rapidly.

Total iron store of the body is represented by plasma ferritin concentration, except during inflammatory conditions. The iron storage compartment in normal adult male is ~ 800 to 2000 mg, in adult female it is ~300-500 milligrams.

To mobilize iron from ferritin storage it has to be reduced from Fe3+ to Fe2+ which diffuses out of the apoferritin shell. It gets reoxidized by hephaestin or ceruloplasmin as it diffuses in to plasma from cytosol.

Then it binds to transferrin. Iron can also be released from ferritin by autophagy following lysosomal degradation.

HEMOSIDERIN

Hemosiderin is present abundantly in macrophages. It is similar to the iron core of ferritin chemically. It may be derived from ferritins, whose protein shells have been degraded in lysosomes.

III. MYOGLOBIN

Myoglobin is present in all skeletal and cardiac muscles in small amounts. It serves as an oxygen reservoir. It protects the cells from hypoxic injury. It also scavenges nitric oxide and reactive oxygen species.

IV. LABILE IRON POOL

It represents iron in the interstitial compartment before getting incorporated into heme or storage compounds. Some of the iron reenters the plasma. Normally, the labile iron pool is 80 - 90 mg.

V. TISSUE-IRON COMPARTMENT

This amounts to 6 - 8 mg approximately (exclusive of hemoglobin, ferritin, hemosiderin, myoglobin and the labile compartment).

Cytochromes and other enzymes containing iron constitute tissue iron. It is one of the critical parts of the iron compartments.

VI. TRANSPORT COMPARTMENT

It is the smallest, normally about 3 mg, but very active part of the iron compartments. This part of iron is almost entirely carried by transferrin. Transferrin turns over minimum 10 times per day normally. This transports iron between various compartments.

TRANSFERRIN

Transferrin is a glycoprotein with two globular domains with binding clefts for Fe3+. Human plasma contains 200 to 360 mg/dl of transferrin, capable of binding 250 to 480 mcg/dl of iron, but carrying only 50 to 180 mcg/dl of iron. Transferrin is derived from apotrasferrin, which is devoid of iron, in hepatocytes and the cells of monocyte-macrophage system.

DIETARY IRON

IRON CONTENT

Average adult men and women ingest 9 to 10 mg and 12 to 14 mg of iron per day, respectively. The iron requirement of an adult male is to

balance the small amount that is excreted via stool, ~ 1mg per day. Iron requirement is increased during active growth periods or after blood loss. In women, due to menstruation or diversion of iron to the fetus during pregnancy or lactation raises the iron requirement.

Table 3.2 Recommended Dietary Allowances (RDAs) - for Iron

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

In non vegetarians, heme from hemoglobin and myoglobin comprises approximately 15 % of dietary iron, which is more efficiently absorbed than non-heme iron. The absorption of non heme iron is affected by iron binding components in food like Phytates, Oxalates, and Phosphates. These substances bind with iron and decrease its absorption. Iron absorption is increased by reducing substances (ascorbate, pyruvate, lactate, succinate, fructose, hydroquinone, cysteine and sorbitol).

Iron fortified cereals act as major sources of iron in countries where fortification is practiced. Cooking in iron pots also provide important exogenous iron. Gastric pH, mucus secretion and the transit time for food particles in the intestine also affect iron absorption.

IRON ABSORPTION

Majority of iron is absorbed in the duodenum. The absorption of iron depends on the body needs.

Iron absorption,

Increased in : active red cell production and/or iron deficiency.

Decreased in : iron overload states and systemic inflammation.

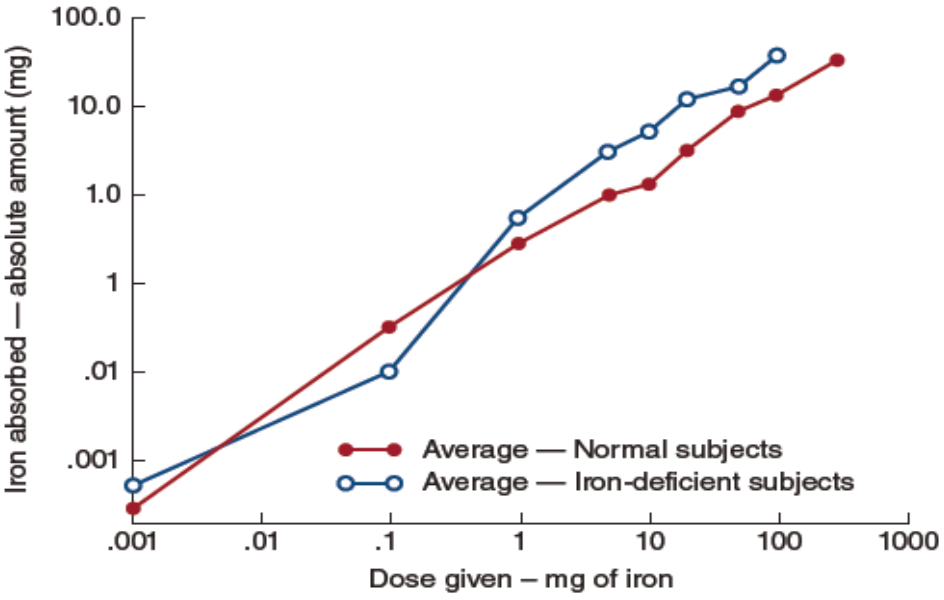


Figure 3.1The relationship between oral iron dosage and amount of iron absorbed

TRANSPORT ACROSS INTESTINAL MUCOSA

Ferric iron is reduced by duodenal cytochrome b reductase to ferrous iron. It is then transported into the intestinal villous cell by the Divalent metal transporter (DMT). Ferroportin(Fpn) in association with hepaestin and ceruloplasmin oxidizes the iron back to the ferric form, which is exported across the basolateral membrane. Then ferric iron is transported by plasma apotransferrin.

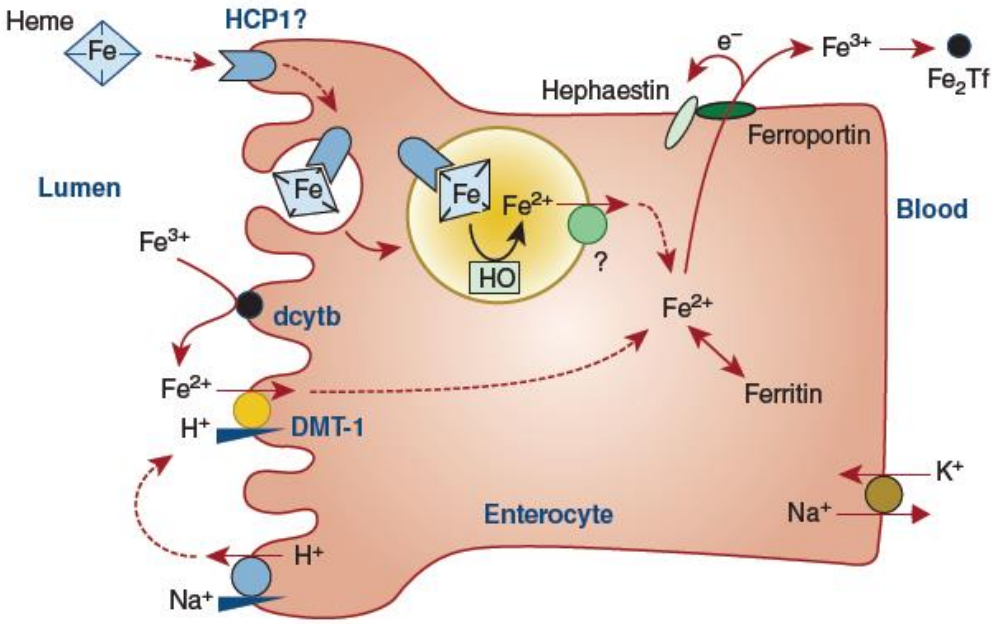


Figure 3.2 Schematic diagram of iron uptake[5]

dcytb –duodenal cytochrome-b, HCP-1 – Heme carrier protein-1, HO-Heme Oxygenase, DMT – Divalent metal transporter.

IRON RECYCLING

The destruction and production of RBCs generates most of the iron flux in and out of plasma, which approximates 20 to 25 mg/day recycled

in adults compared to its 1 to 2 mg/day of absorption. Destruction of the senescent erythrocytes and the degradation of hemoglobin occur within the monocyte-macrophage system. This occurs at a rate required to release ~20 % of the hemoglobin iron within a few hours. Among that, 80% of the iron is reincorporated into the hemoglobin. The remaining iron is stored as ferritin or hemosiderin.

The stored iron can be mobilized rapidly if there is need for Hb synthesis. Infections and other inflammatory processes will slow this reusage of iron leading to anemia.

HEPCIDIN

Hepcidin, a peptide hormone, is produced predominantly by hepatocytes. It plays a major role in systemic iron homeostasis. Depending on the level of plasma iron concentration, Hepcidin tightly regulates the absorption of iron by the intestinal epithelial cells and its release from iron storage.

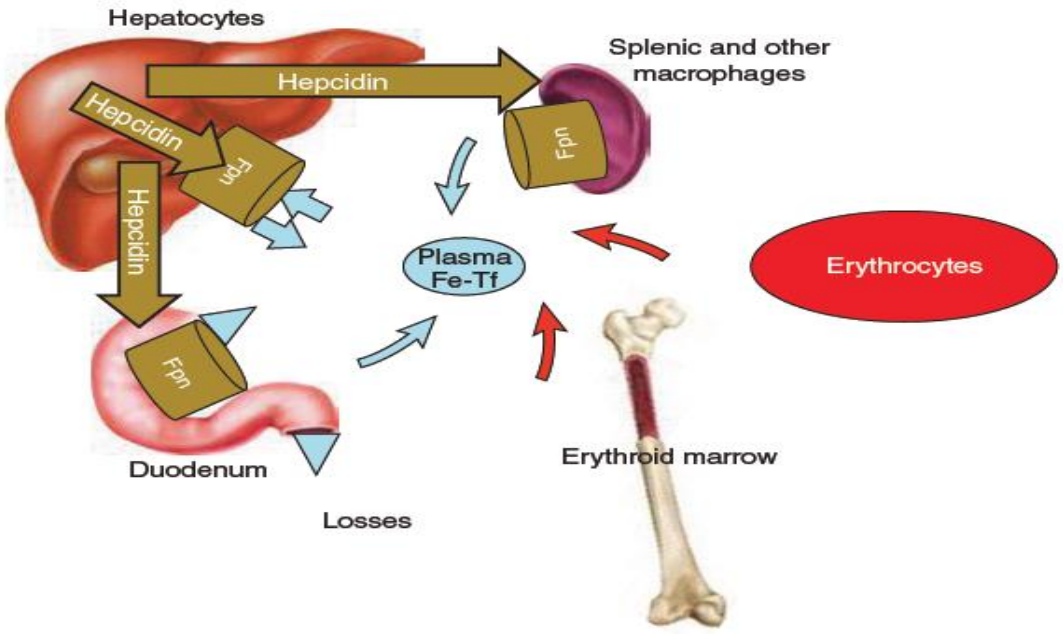


Figure 3.3 Regulation of plasma iron concentration by hepcidin[(2)] **Fpn- ferroportin; Tf- transferrin.**

IRON EXCRETION

Our body conserves iron effectively. About 1mg of iron is lost via feces everyday secondary to desquamation of intestinal epithelial cells. Other smaller losses occur through skin exfoliation and dermal appendages and sweating. In women menstruation leads to negative iron balance. Average total iron loss per day in male is 1mg, whereas in menstruating female it is 2mg. During iron overload states daily loss can be as much as 4mg.

ETIOLOGY AND PATHOGENESIS OF IDA

ETIOLOGY

I. Sources of blood loss:

A.Alimentary tract:	1.Esophagus-	• Varices, • Erosions
	2.Stomach and duodenum-	• Ulcer, • Gastritis, • Carcinoma, • Angiodysplasia, • Hemangioma, • Antralvascular ectasia, • Hypergastrinemia, • Watermelon stomach.
	3.Small intestine	• Vascular ectasia, • Tumors, • Ulceration, • Meckel's diverticulum
	4.Colon and anorectal	• Hemorrhoids, • Carcinoma, • Polyp, • Diverticuum, • Ulcerative colitis, • Angiodysplasia, • Hemangioma, • Telangiectasia, • Amoebiasis
B.Biliarytract	• Intrahepatic bleeding, • Carcinoma, • Cholelithiasis, • Trauma,	• Ruptured aneurysm, • Aberrant pancreas
C.Genitourinary tract	• Menorrhagia, • Uterine fibroids • Endometriosis,	• Carcinoma, • Vascular abnormalities
D.Respiratory tract	• Epistaxis, • Carcinoma • Infections	• Telangiectases, • Idiopathic pulmonary hemosiderosis

II. Increased demand:

A. Rapid growth during infancy or adolescence	
B. Pregnancy and parturition	In addition to hemodilution in pregnancy, true iron deficiency results in more severe anemia.

III. Dietary iron deficiency

IV. Malabsorption of iron

A. Due to Disease	1. Sprue, 2. Crohn's diease
B. Due to Surgery	1. Gastrectomy and 2. Forms of bariatric surgery

V. Genetic factors

VI. Acute or chronic inflammation

PATHOGENESIS

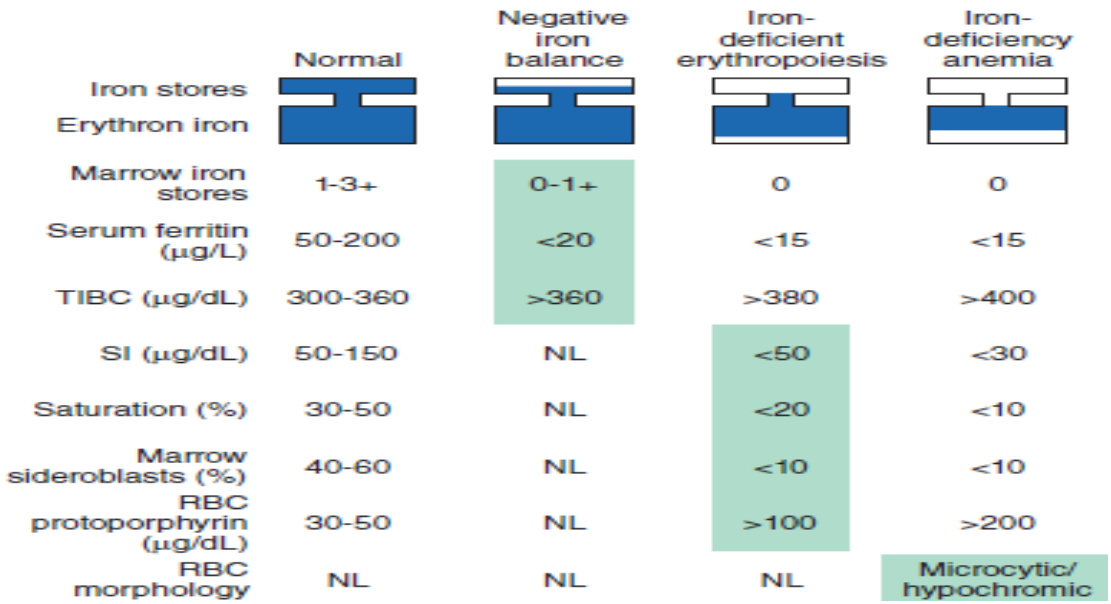


Figure 3.4 Stages in the development of iron deficiency anemia[6]

IRON CONTAINING PROTEINS

In initial stage of iron deficiency, Storage iron in the body becomes depleted. That leads to dyserythropoiesis resulting in hemoglobin deficient erythrocytes. The concentration of other iron containing proteins like myoglobin, cytochromes and other mitochondrial ferroproteins are affected in an organ specific manner.

MUSCULAR FUNCTION AND EXERCISE TOLERANCE

Patients experience difficulty in performing high intensity exercise even during non anemic iron deficiency state, which worsens with increasing anemia. This exercise limitation results from reduced hemoglobin content of blood and decreased oxygen delivery to tissues. There will be decreased spontaneous activity, decreased ventilatory threshold, decreased endurance and increased muscle fatigue. These effects are attributed to the depletion of iron containing mitochondrial proteins involved in energy metabolism. These effects are reversible with iron supplementation.

NEUROLOGIC CHANGES

Iron deficiency is associated with developmental abnormalities in children. In adults it is associated with restless leg syndrome.

HOST DEFENSE AND INFLAMMATION

Iron deficiency acts as a pro inflammatory state. It affects various immune functions through hepcidin. Iron deficiency potentiated the systemic effect of lipopolysaccharide in a hepcidin dependant manner. And it also promoted allergic inflammation like asthma.

GROWTH AND METABOLISM

Reports state that iron deficiency in children leads to growth retardation. There will be decreased thermoregulation in response to exposure to cold. This is attributed to the conflicting effects of blood flow with decreased oxygen content and need to minimize heat loss and also the effect on thyroid function.

HISTOLOGIC FINDINGS

Iron deficiency, depending on the severity leads to histological changes in various body organs. It commonly affects the rapidly proliferating cells in the upper part of gastro intestinal tract. There will be mucosal atrophy in the upper GI tract. The epithelial thickness of the lateral margin of the tongue is decreased despite increase in progenitor compartment, reflecting accelerated exfoliation of epithelial cells. There is thinning and keratinization of buccal mucosa with increased mitotic

activity. Widening of diploic spaces of bones like skull and hands will occur in chronic iron deficiency beginning in infancy.

CLINICAL FEATURES

Symptoms of anemia result from decreased oxygen supply to cells and the body's response to it. Resulting tachycardia can be perceived as palpitations and pounding sensations in ears, headache, light headedness. Rarely angina can occur if the anemia is very severe.

Neurological

Decreased work performance will be seen. In infants and children, iron deficiency may lead to poor attention, retarded behavioral and developmental milestones. Iron deficiency also contributes to tourette syndrome, attention deficit hyperactivity disorder and restless leg syndrome.

Breath holding spells in children, headaches and paresthesias have been attributed to iron deficiency. Association has been found between iron deficiency anemia and thrombocytosis that possibly triggers stroke in children and adults.

Alimentary tract

Iron deficiency accounts for burning sensation in tongue which diminishes with treatment. It could be a result of coexisting pyridoxine deficiency.

Mucosal atrophy in the laryngo pharynx leads to formation of post cricoids web, which results in difficulty in swallowing (Plummer Vinson syndrome). If it persists for longer duration, this condition may lead to pharyngeal carcinoma.

PICA

Pica is a well documented manifestation of iron deficiency. Pica is increased desire to eat unusual (unhealthy) substances like clay, paint, laundry starch, cardboard and even hair. It is promptly cured by iron therapy.

PHYSICAL SIGNS

The physical signs seen are pallor, smooth, red tongue, stomatitis / angular cheilitis and spooning of nails. Fundus examination of the severely anemic patients may reveal hemorrhages/exudates in retina.

LABORATORY FINDINGS

BLOOD CELLS

ERYTHROCYTES

In iron deficiency anemia the earliest recognizable morphologic change of red blood cell is anisocytosis. It may be accompanied by mild ovalocytosis.

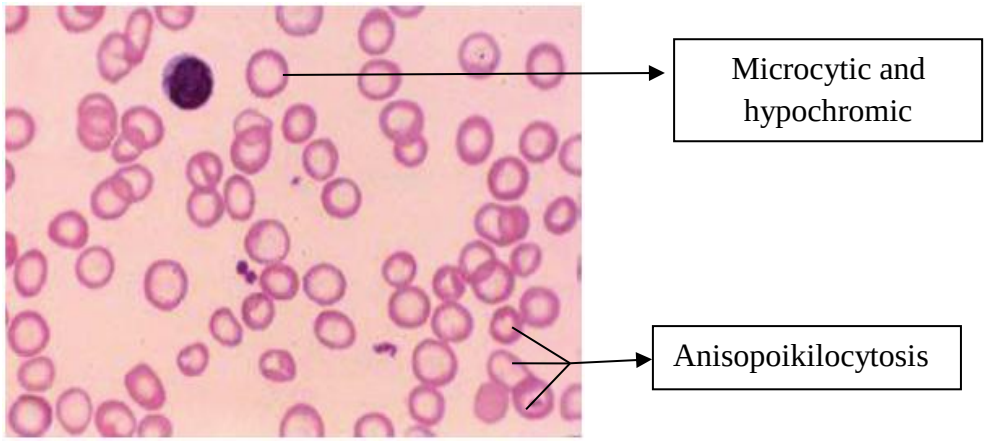


Figure 3.5 Peripheral smear in iron deficiency anemia

Initially mild normocytic normochromic anemia develops. As the iron deficiency progresses, erythrocyte count, mean corpuscular volume, mean hemoglobin concentration, mean erythrocyte hemoglobin content all decline together. As these indices change, red cells appear microcytic and hypochromic. Sometimes target cells, pencil cells may be seen.

The distribution of red cell volume (i.e. red cell distribution width-RDW) is increased in iron deficiency anemia.

LEUKOCYTES

Some patients may have leucopenia, but most people with iron deficiency anemia have normal white cell count.

PLATELETS

Iron deficiency anemia can be associated with both thrombocytopenia and thrombocytosis, though the mechanism is unknown.

RETICULOCYTES

Due to increased erythroid activity in the bone marrow reticulocyte count is often mildly increased.

MARROW

The iron stores are depleted earlier than the compromise in red cell mass in iron deficiency anemia. Thus evaluation of iron store is the most sensitive and reliable means of differentiating iron deficiency anemia from all other anemias. The characteristic finding in the marrow of iron deficiency is decreased or absent hemosiderin, which is evaluated by staining with Prussian blue method. The gold standard for diagnosing

iron deficiency is estimating the marrow macrophage iron content. It is altered by previous transfusion and treatment with parenteral iron.

SERUM IRON CONCENTRATION

In iron deficiency anemia, the serum iron concentration will be low, but rarely may be normal. The serum iron concentration has diurnal rhythm. It is maximum in morning between 7 to 10 am and decreases in late afternoon and evening. This diurnal rhythm may rarely influence the diagnosis. The serum iron concentration is altered in inflammatory conditions and malignancy. Conversely, it may be elevated during chemotherapy as the cytotoxic drugs inhibit erythropoiesis and related iron uptake by erythroblasts. Serum iron concentration will normal or even high if the patients have received iron medication before the investigation.

IRON BINDING CAPACITY AND TRANSFERRIN SATURATION

The total iron binding capacity represents the amount of transferrin in the blood. The unsaturated or latent iron binding capacity (UIBC) can be measured easily by spectrophotometric techniques. The sum of UIBC and the serum iron is total iron biding capacity (TIBC). In iron deficiency

anemia, both UIBC and TIBC are increased and serum iron concentration is decreased so the transferrin saturation is reduced.

SERUM FERRITIN

Serum ferritin concentration represents the total body iron stores. In iron deficiency state the serum ferritin level will be as low as 10mcg/L. Ferritin concentration is elevated in inflammatory disorders like rheumatoid arthritis, chronic kidney disease and malignancies. The normal serum ferritin value differs according to the age and gender.[7]

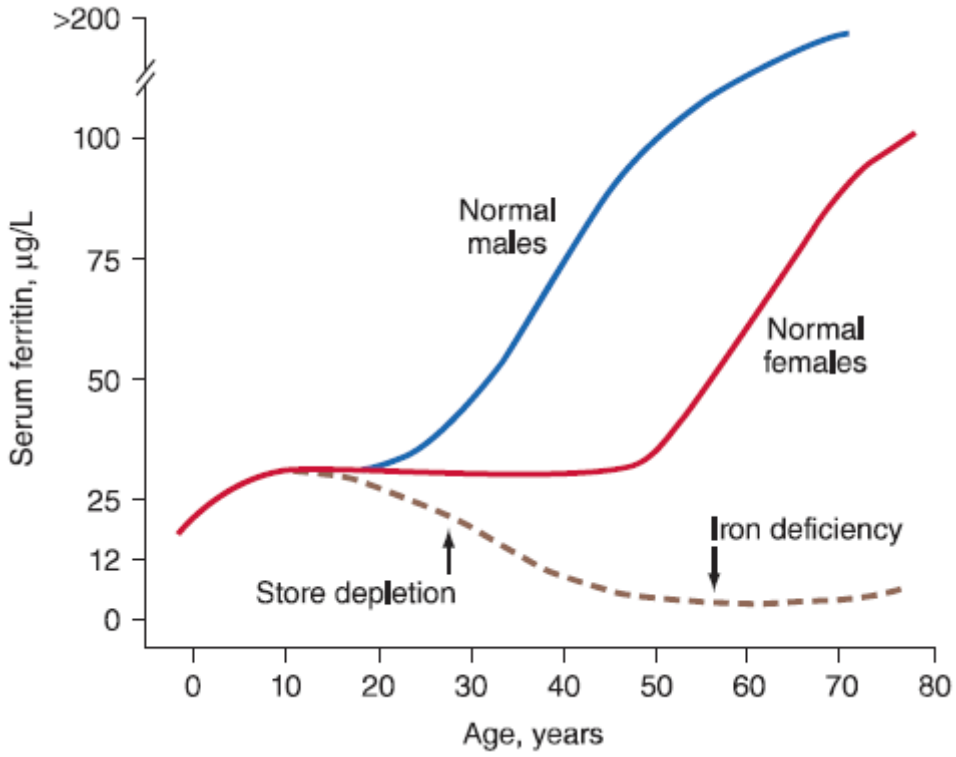


Figure 3.6 Serum ferritin levels as a function of sex and age

RED CELL PROTOPORPHYRIN LEVELS

In iron deficiency anemia, heme synthesis is impaired leading to accumulation of protoporphyrin within the red cells. Normally it will be less than 30mcg/dl. In iron deficiency, the red cell protoporphyrin levels are more than 100mcg/dl.

TRANSFERRIN RECEPTOR PROTEIN LEVELS

Tranferrin receptors (TfR) are abundant in erythroid cells. The levels of circulating TfR correlate with the amount of cellular receptors, which is proportional to the number of erythroblasts expressing the receptor. When cells lack iron, TfR synthesis is enhanced which results in increased circulating receptor levels. In anemia of inflammation the TfR synthesis is suppressed by cytokines.

NOVEL ERYTHROCYTE INDICES

Reticulocyte Hemoglobin Content can be measured by automated instruments. It is an indicator of iron restriction of hemoglobin synthesis during 3 to 4 days prior to the test.

Percent hypochromic erythrocytes give a longer term assessment of iron restriction during the preceding few months.

DIFFERENTIAL DIAGNOSIS

I. THALASSEMIA

It results from an inherited defect in globin chain synthesis. It can be differentiated from iron deficiency by serum iron levels. The serum iron levels and the transferrin saturation levels will be normal or increased in thalassemias. The RDW index will be normal in thalassemia. It is elevated in iron deficiency anemia.

II. ANEMIA OF CHRONIC INFLAMMATION

It results from inadequate supply of iron to the erythroid marrow. Anemia of inflammation is usually normocytic and normochromic. The ferritin level may be normal or elevated. The percent transferrin saturation and TIBC are decreased in anemia of chronic disease.

III. MYELODYSPLASTIC SYNDROMES:

Myelodysplastic patients will have impaired hemoglobin synthesis with mitochondrial dysfunction, which leads to defective iron incorporation into heme. The iron store levels will be normal and excess..

Table 3.3 Diagnosis of microcytic anemia

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%

TREATMENT

The treatment approach to iron deficiency anemia varies according to the severity and cause. There are three major therapeutic approaches once the diagnosis and cause of iron deficiency is made.

I.RED CELL TRANSFUSION

Transfusion therapy is reserved for patients with

- Symptomatic anemia
- Hemodynamic instability
- Continued/excessive blood loss
- In patients requiring intervention

In these patients management is related to the consequences of the severe anemia than the iron deficiency. In addition to correcting the anemia transfusion provides iron for reutilization.

II. ORAL IRON THERAPY

Treatment with oral iron is adequate in asymptomatic patients with established iron deficiency anemia. There are multiple iron preparations available like simple salts to complex compounds designed for sustained release. Even though they contain various amount of iron, they are well absorbed and equally effective in treatment. Some preparations contain ascorbic acid to enhance the absorption of iron.

For iron replacement up to 200 mg of elemental iron is provided per day in divided doses. Food interferes with iron absorption. So the iron tablets are taken in empty stomach. From the 200 mg of provided iron 50 mg will be absorbed per day.

The amount of iron absorbed depends on

- the hemoglobin level,
- marrow function and
- the degree of erythropoietin stimulus.

The goal of iron therapy is to correct the anemia and to provide at least 0.5 to 1 gm of iron stores. This goal requires continuous treatment for 6 to 12 months after correcting the anemia.

GI discomfort is the most common adverse effect with oral iron therapy. Nausea, vomiting, abdominal pain or constipation are the other adverse effects seen with oral iron preparations. These may lead to non compliance. Sustained release preparations or small doses of iron will have lower incidence of the gastrointestinal side effects.

The response to oral iron depends on i) the erythropoietin stimulus and ii) the rate of absorption. Adequate response is indicated by the rise in reticulocyte count within 4-7 days after the initiation of therapy. Inadequate or absence of response may be seen in non compliance (commonest), poor absorption or a faulty diagnosis.

Table 3.4 Oral preparations:

Generic Name	Tablet (Iron Content), mg	Elixir (Iron Content), mg in 5 mL
Ferrous sulfate	325 (65)	300 (60)
	195 (39)	90 (18)
Extended release	525 (105)	
Ferrous fumarate	325 (107)	
	195 (64)	100 (33)
Ferrous gluconate	325 (39)	300 (35)
Polysaccharide iron	150 (150)	100 (100)
	50 (50)	

III. PARENTERAL IRON THERAPY

Parenteral iron is reserved for patients with:

- intolerance to oral iron
- acute need
- Ongoing iron requirement, secondary to continuous GI blood loss.

Parenteral iron can be given in two ways

i) Requirement of iron to correct the Hb deficit and to replace minimum 500 mg of iron stores is calculated and administered.

ii) Regular small doses of parenteral iron are given as in patients on dialysis to improve the response to recombinant EPO.

Formula for iron requirement calculation[1]:

Body weight (kg) × 2.3 × (15 –patient's hemoglobin, gm/dl) + 500 or 1000 mg (for stores)

Anaphylaxis is the major concern with intravenous iron dextran. But with the availability of newer parenteral iron preparations

anaphylaxis has become much rarer. Other symptoms like arthralgias, skin rash and low grade fever may be seen. These symptoms are usually dose related. Further use of parenteral iron is not precluded in these patients.

Recommended test dose is 25 mg. Instead of separate test dose a slow infusion will provide early warning. Infusion of iron should be stopped immediately if the patient develops chest pain, breathing difficulty, hypotension or other allergic/anaphylactic symptoms.

Currently available parenteral iron preparations include iron sucrose, low molecular weight iron dextran, ferric gluconate, ferric carboxymaltose, ferumoxytol and iron isomaltose.

Table 3.5 Parenteral iron preparations

Preparation	Comments
Iron dextran	50 mg/mL, intramuscular and intravenous use Test dose required Total dose infusion possible Potentially severe side-effects
Iron sorbitol citrate	Only intramuscular use, 50 to 100 mg/day Test dose required
Iron sucrose	100 mg in 5 mL Only intravenous use. Maximum single dose 100 mg can be given as slow injection over 5 min or as infusion in 100 ml saline over 30 mins. Test dose not required, reactions very rare.
Ferric gluconate complex in sucrose (Sodium ferric gluconate)	12.5 mg in 10 mL. Slow injection over 10 mins or infusion in 100 mL saline over 1 hour Reactions very rare

IRON REFRACTORY IRON DEFICIENCY

In some patients, even with adequate iron supplementation iron deficiency persists. The causes are i) poor compliance (most common), ii) wrong diagnosis, iii) continuing losses, iv) iron malabsorption secondary to celiac disease, autoimmune gastritis and H.Pylori infection, v)inherited defects in iron uptake, transfer and release.[4]

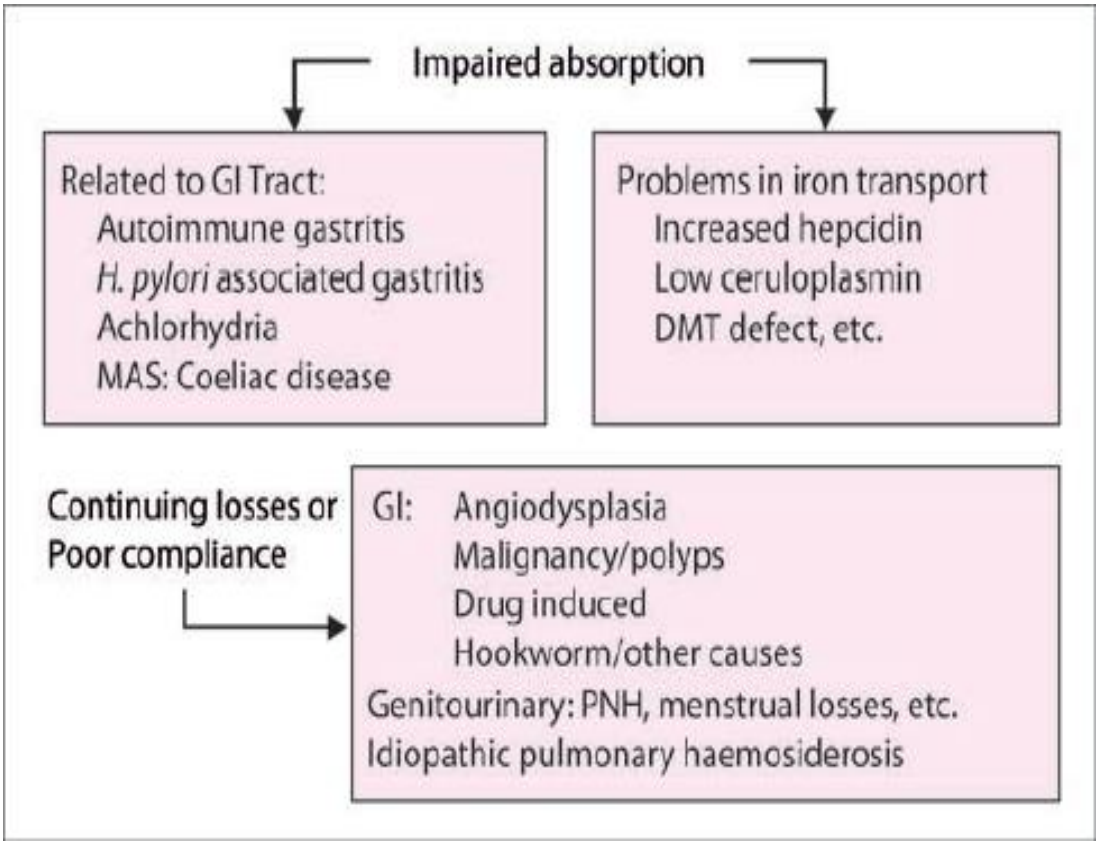


Figure 3.7 Approach to iron refractory iron deficiency

GLYCATED HEMOGLOBIN

Glycated hemoglobin (HbA1c) is a form of hemoglobin, modified with a stable adduct of glucose linked covalently to the N-terminal valine of the β-chain. In adults normal hemoglobin consists of HbA (α2β2), HbA2 (α2δ2) and HbF (α2γ2) in 97%, 2.5% and 0.5% respectively. Among the total HbA, about 6% is termed as HbA1. HbA1 consists of HbA1a1, HbA1a2, HbA1b and HbA1c. These fractions are characterized by their individual electrophoretic and chromatographic properties. Despite the identical amino acid sequences of HbA1 and HbA0, these fractions differ slightly in their electrophoretic and chromatographic properties from those of the major component HbA0.[9]

HbA1c is the predominant HbA1 fraction. In healthy people it constitutes approximately 5% of the total HbA fraction. There is no known physiological role for HbA1c.

ESTIMATION OF HbA1c[9]

1. Cation exchange chromatography

HbA1c and HbA0 can be separated on the basis of the subtle difference in their isoelectric points. Nowadays High performance liquid chromatography (HPLC) systems were used, which are not affected from

interference by the Schiff base or carbamylated hemoglobin, but by the hemoglobin variants.

2. Affinity chromatography

This assay method utilizes m-amino phenyl boronic acid. This method is based on the interaction between the glucose molecule on HbA1c and the boronic acid, which is immobilsed.

3. Immunoassay

In this assay the antibodies are directed against the β N-terminal glycated tetrapeptide or hexapeptide group. Electrical charge does not affect this assay. It can be used in the routine medical laboratory. But they have drawback of requiring multilevel calibration and frequent recalibration.

4. Capillary electrophoresis

This method is based on liquid flow capillary electrophoresis in free solution. This technique utilizes the principle of different electrophoretic mobility of charged molecules in an alkaline buffer at a particular pH. The separation of the fractions of hemoglobin occurs in silica capillary tubes and the migration is performed at the high voltage

under tight temperature control. The hemoglobins are detected directly at the cathode end at a specific absorption wavelength of 414nm by an optical detector.

MARKER FOR GLYCEMIA

Primarily, glycated hemoglobin represents the average plasma glucose concentration over a period of 12 to 16 weeks. The fraction of glycated hemoglobin increases in a predictable way as the average plasma glucose increases. Higher amount of glycated hemoglobin in diabetes mellitus indicates poor glycemic control.

Although hemoglobin glycation occurs throughout the life span of the red blood cell, major influence over the HbA1c value is by the recent glycemia. Mean blood glucose of the previous 1^{st}, 2^{nd} and 3^{rd} month contributes about 50%, 40% and 10% respectively to the final HbA1c value. The t1/2 of HbA1c is approximately 35.2 days by mathematical remodeling. So, the previous 35.2 days have contributed to about half of the glycation. HbA1c gives us the assessment of average plasma glucose but not about the stability of glycemic control. So even with widely fluctuating glucose levels a patient can have the same HbA1c as one with little variation. The approximate mapping between eAG (estimated

average glucose) measurements and HbA1c values is given by the equation:

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

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

While American Diabetes Association recommends the HbA1c below 7.0% as the target. American College of Endocrinology and the International Diabetes Federation recommend HbA1c below 6.5%. Target HbA1c level should be individualized. Patients at risk for developing diabetes associated complications have been proved to gain further benefits from reducing the HbA1c level below 7%. The American Diabetes Association advices to do the HbA1c test twice a year in diabetic patients who have achieved treatment goals and quarterly in those whose therapy has changed or not meeting the glycemic levels diagnosis of DM.

HBA1C AND DIAGNOSIS OF DM

The hallmark of diabetes is chronic hyperglycemia resulting in diabetes specific complications. So the HbA1c which represents the longterm glucose exposure predicts the diabetes specific complications better than single glucose measurement. Studies proved a consistent and

significant correlation between retinopathy and HbA1c levels than with the fasting glucose levels[16]. Multiple controlled clinical trials and large volume of data from different populations have provided a strong evidence for assigning an HbA1c cut off point of more than 6.5% for diagnosing diabetes as this HbA1c level is associated with an increased prevalence of diabetes specific complications especially retinopathy[15,19,25,29,62]. This cut off point is not an absolute demarcation between diabetes and normal glycemic status. But this level is sensitive and specific to detect the patients at risk for developing retinopathy. An International Expert Committee has recommended the HbA1c level of more than 6.5% to diagnose diabetes[21]. The same has been affirmed by American Diabetes association[61]. But this diagnostic HbA1c test should be done by a standard method certified by the NGSP- National Glycohemoglobin Standardization Program[8] and traceable or standardized to the Diabetes Control and Complications Trial (DCCT) reference assay.[15,24,63]

Table 3.6 American Diabetic Association (ADA) criteria for the diagnosis of diabetes[22]

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[10]

1. HbA1c measures chronic hyperglycemia better than the two assessments of fasting glucose level or 2 hours OGTT

Chronic hyperglycemia is the biochemical hallmark of diabetes. But fasting glucose and 2 hour OGTT gives just a momentary glycemic status of that day. In contrast, HbA1c provides the glycemic status over a longer duration (3 months).

2. HbA1c is better associated with chronic complications than FPG

Diabetic glycemic levels have been proposed on the basis of their association with retinopathy. Multiple studies showed that FPG levels around 126 mg/dl and 2 hour PG around 200 mg/dl have been associated with higher nonproliferative diabetic retinopathy prevalence. Those studies also documented increased prevalence of retinopathy with HbA1c levels around 6.5%.[26,27,34]

3. Fasting is not needed for HbA1c assessment and acute perturbations do not affect HbA1c

Plasma glucose levels vary throughout the day. Multiple acute conditions could affect glucose homeostasis. Acute stress promotes neoglucogenesis and impairs the utilization of glucose. After exercise the glucose levels are decreased. Early morning and evening physical exertion might affect the fasting glucose levels. Smoking and certain drug intake could affect fasting glucose. Patient should not eat for 8 hrs before testing fasting glucose. So, without appropriate preparation fasting plasma glucose testing is less reliable for diagnosing diabetes. In contrary, HbA1c is not affected by those acute stressful events or duration of fasting. HbA1c can be measured at anytime of the day, irrespective of fasting.[19,21,35]

4. HbA1c has a greater pre analytical stability than plasma glucose

There are potential pre analytic errors in the measurement of glucose. Samples stored at room temperature before analysis will result in reduction in glucose levels due to in vitro glycolysis. Glucose concentration decreases 5 to 7 % per hour and the rate increases if the ambient temperature is high. So the results will show lower glucose levels than they are and diagnosis of diabetes can be missed. In contrast HbA1c values are relatively stable after collection**.**[15,21,61]

5. Biological variability of HbA1c is lower compared to FPG

The variability of HbA1c values is negligible compared to fasting glucose levels. With day to day or person to person, variation is less than 2% for HbA1c but 12 to 15% for FPG.[15,21]

6. Standardization of HbA1c assay is not inferior to standardization of glucose assay

The important concern about HbA1c was the poor standardization of the assays. An effective standardization program was made available to overcome this disadvantage[24,63]. The standardization of HbA1c assay minimized the laboratory based biases[17]. Moreover studies conducted

over various laboratories have clearly showed a significant laboratory based bias in glucose assessment, resulting in misclassification of glucose intolerance in 12% of subjects.[61]

7. Individual susceptibility to glycation might be an additional benefit for HbA1c assessment

Sometimes the HbA1c value could be lower or higher than the expected value in accordance with their glucose levels. The hemoglobin glycation index (HGI) is the difference between the observed and the predicted HbA1c levels. Depending on this index, the patients have been categorized as low, moderate or high HGI. Patients with high HGI have increased risk of micro and macro vascular complications even with better glycemic control. So the HbA1c assessment provides additional information about diabetes related complications.

8. Using the same biomarker for diagnosing and monitoring

HbA1c is used to monitor the degree of glycemic control. So the treatment strategies can be modified appropriately when the Hba1c values deviate from the target levels. When the HbA1c is used to diagnose diabetes, we already have a baseline HbA1c value. From that value further deviation from target can be assessed and managed effectively.

LIMITATIONS OF HBA1C

1. Diabetes is defined by high blood glucose and not by glycation of proteins

Diabetes is a clinical condition characterized by elevated plasma glucose concentration. High HbA1c level indicates higher level of glycation of proteins. Even though it occurs as a result of high plasma glucose, primary pathology has to be given importance over the secondary results. In addition, there will be delay in rising of HbA1c after an increase in plasma glucose level. So if we use HbA1c the diagnosis will be delayed compared to glucose measurement.

2. HbA1c is a poor marker of important pathophysiological abnormalities featuring diabetes

The pathophysiology of diabetes is better reflected by OGTT and 2 hour post glucose levels. The plasma glucose levels are at the peak during the postprandial state. Essential functioning of the pancreatic β cell is required during that time. The information about the postprandial state is provided only by the OGTT and 2 hour plasma glucose tests. HbA1c provides information about chronic hyperglycemia alone. Good β cell capacity is indicated by normal blood glucose 2 hrs after glucose load. Vice versa higher levels indicate impaired β-cell function. Studies show that HbA1c is a weaker correlate of insulin resistance and insulin secretion compared with FPG and 2 hrs PG.

3. Factors that influence HbA1c and its measurement[12,31,61]

1. Erythropoiesis: i)Increased HbA1c: ii)Decreased HbA1c:	• Iron deficiency • Vitamin B12 deficiency, • Decreased erythropoiesis. • Administration of , iron, vitamin B12, Erythropoietin, • Reticulocytosis, • Chronic liver disease.
2. Altered Hemoglobin: Genetic/chemical alterations in haemoglobin: haemoglobinopathies, HbF, methemoglobin - may increase or decrease HbA1c.	
3. Glycation: i)Increased HbA1c: ii)Decreased HbA1c: iii)Variable HbA1c:	• Alcoholism, • Chronic renal failure. • Aspirin, vitamin C and E, • Certain haemoglobinopathies • Genetic determinants.[30]
4. Erythrocyte destruction[15]: i)Increased HbA1c: ii)Decreased HbA1c:	• Increased erythrocyte life span: Splenectomy • Decreased erythrocyte life span: • Hemoglobinopathies, • Splenomegaly, • Rheumatoid arthritis • Drugs such as antiretrovirals and dapsone.
5. Assays i)Increased HbA1c: ii)Variable HbA1c: iii)Decreased HbA1c	• Hyperbilirubinaemia, • Carbamylated haemoglobin, • Alcoholism, • Large doses of aspirin, • Chronic opiate use. • Haemoglobinopathies.[61] • Hypertriglyceridaemia.

4. HbA1c has a poor sensitivity in diagnosing diabetes and that would change the epidemiology

OGTT identifies the asymptomatic diabetic cases and people with impaired glucose tolerance[18]. The diabetes preventive measures can be taken effectively in those patients. HbA1c cannot detect those cases.

Epidemiological studies in the general population showed that fasting (~50%) and 2 hours plasma glucose levels (~90%) detects the previously undiagnosed diabetic patients effectively when compared to HbA1c (~30 to 40%).[13,60]

5. 2 hrs PG and IGT are stronger predictors of CVD

Fasting PG is a poor predictor of diabetes associated mortality and CVD events. But the 2 hrs PG and HbA1c are better predictors. Increased mortality (~40%) was seen in patients with impaired glucose tolerance. These people are not detected by measuring FPG or HbA1c levels. Effective measures in these patients will prevent/decrease the progression to diabetes. Thereby, the mortality risk is reduced. This cannot be made possible by measuring HbA1c or FPG.[29]

6. Fasting is not essential to identify the defects in glucose metabolism

Excessive postprandial glucose excursion is the first sign of glucose homeostasis abnormality. And it predicts the cardiovascular outcome better, unlike fasting PG.

7. Standardization of HbA1c assay is very poor

Standardization of HbA1c assay has not been achieved worldwide yet. Inaccuracies in measurement are still a problem in many countries.[17,24]

8. HbA1c assay is unreliable and cannot be used in many situations[20,33]

HbA1c assays are affected by abnormality in Hemoglobin traits. Conditions affecting the life span of red cells will affect the HbA1c assay results[13]. These conditions will mislead the HbA1c assay results. There are ethnic differences and effect of aging[30] in the relation between blood glucose and HbA1c[11,12,29]. HbA1c cannot be used if different cut off points are considered in all these conditions.[36]

9. Day to day biological variability of plasma glucose might reveal disturbance of glucose metabolism

Pathophysiological processes of type 2 diabetes may result in day to day variability in plasma glucose levels. It is also reflected by the individual's dietary pattern and activities. HbA1c will not provide this information.

10. Individual susceptibility to glycation of hemoglobin is not relevant

As mentioned earlier individuals with high HGI are at risk for developing micro and macro vascular complications, even with good glycemic control compared to individuals with low HGI. This phenomenon is explained by postprandial glucose fluctuations. But HbA1c indicates only high mean exposure of hemoglobin to glucose. It does not provide information about the fluctuations in glucose levels.

11. Using the same biomarker for diagnosing and monitoring

Many patients who are diagnosed as diabetic based on their glucose levels had HbA1c less than 6.5%[23]. So if HbA1c is used, these individuals are not diagnosed and left untreated.[13,60]

12. Cost of the assay[23,24]

HbA1c assays are more expensive. And they are not available in many countries.[18]

EFFECTS OF IRON DEFICIENCY ANEMIA ON HbA1c LEVELS

This study aims to measure the HbA1c levels in iron deficiency anemia patients and to identify the changes in HbA1c levels after correcting the iron deficiency anemia. Although, there were multiple studies in the past investigating the relationship between iron deficiency anemia and HbA1c, the results were inconsistent.

Horton and Husiman[39] performed one of the earliest studies in evaluating the influence of iron deficiency anemia over HbA1c levels. They showed that in patients with iron deficiency anemia 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 anemia on HbA1c. In contrast to the earlier studies, HbA1c levels decreased as much as 17% after iron replacement according to these studies. It was postulated that there was a balance between serum glucose and HbA1c in normal individuals and if the serum glucose was maintained constant, a fall in hemoglobin could cause an increase in the Glycated fraction.[45]

Ford et al. conducted a study evaluating the influence of iron deficiency anemia 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 anemia 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 anemia should be retested or undergo another diagnostic method.[53]

A study conducted by Sinha et al. compared the HbA1c levels in iron deficiency anemia 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. But the study group belonged to a lower socio economic level. The cause of iron deficiency anemia in those patients is nutritional deficiency rather than malabsorption and bleeding and that could have affected the results.[56]

Brooks et al carried out a study in individuals with iron deficiency anemia. 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 anemia patients and it decreased after treatment. There was a postulation that the quarternary structure of the hemoglobin may be altered in iron deficiency anemia resulting in a higher rate of glycosylation of the β globin chain.[28]

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 anemia, red blood cell production will decrease leading to anemia 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 phenomenon shorter life span of red blood cells. [40]

Mitchell et al calculated the absolute amount of HbA1c using the mean cell hemoglobin in each red blood cell before and after 6 weeks of iron therapy. The HbA1c levels were 1.9pg and 1.95pg respectively,

which conveyed that there was no significant difference of iron therapy on HbA1c.[41]

It was postulated that the differences in HbA1c values seen in previous studies could have been a result of the post translational alterations of hemoglobin rather than the glycosylation. In cation exchange chromatography assay methods the modified hemoglobin would elute with the HbA1c and affect the readings. But that modification would not affect the readings in affinity gel assays because those assays are based on the binding between the glucose molecule on the β chain and the gel alone.[42]

Some studies showed that the supplementation with iron and vitamin B12 resulted in significant decrease in HbA1c values and altered red blood cell indices. It was said that in iron deficiency anemia the life span of red blood cell is normal but decreased in vitamin B12 deficiency anemia. The lower HbA1c values seen after treatment could be the result of increased bone marrow production of new red blood cells and release of immature cells.[43]

A study done in Pediatric patients with type 1 DM, with one third of them having iron deficiency anemia, showed an inverse relationship between HbA1c and hemoglobin levels. In diabetic patients with iron

deficiency anemia, higher HbA1c levels were found independent of glycemic control. HbA1c levels decreased with iron supplementation. This could be a result of young red blood cells that appear after iron supplementation which lead to a dilution effect and lowering of HbA1c values. They postulated that there was no correlation between other red cell indices and HbA1c. Rather than iron concentration, structural or affinity changes in hemoglobin are reflected in changes of HbA1c levels.[44]

Coban et al. carried out a study that compared the HbA1c levels in non patients with iron deficiency anemia to normal control groups. They observed that the mean HbA1c level in patients with iron deficiency anemia is higher than the control group. And after a 3 month course of iron therapy, the mean HbA1c level significantly decreased.[46]

Aslan et al. conducted a study on glycosylated hemoglobin for its potential use as a marker to differentiate thalassemia and iron deficiency anemia. They measured HbA1c levels in β thalassemia, iron deficiency anemia patients and normal healthy controls. The calculated mean HbA1c level was lower in β thalassemia minor compared to iron deficiency anemia patients. There was no difference noted between iron deficiency

anemia patients and control groups. They postulated that it could be due to normal red blood cell survival rate in iron deficiency anemia.[47]

Koga et al. studied about the link of menopause to iron deficiency anemia and HbA1c. In premenopausal women they evaluated the red blood cell indices and HbA1c, which showed that the RBC count is positively associated with glycosylated hemoglobin. In contrast hemoglobin, MCV and MCH is negatively associated with glycosylated hemoglobin. But none of the indices could be linked to HbA1c in the postmenopausal group.[48]

Harvey et al. carried out a study, which showed that the MCV and MCH indices are affected earlier than the total Hb and RBC count. Blood loss during menses can cause iron deficiency anemia with low MCV and MCH values in premenopausal women. It was postulated that it could be the cause for the elevated levels of HbA1c observed in them.[49]

In 2010, Koga et al. carried out a study in premenopausal women to find out the relation between the indices of iron metabolism and HbA1c. According to this study there was an inverse association between the H bA1c and the serum iron, serum transferrin saturation and serum ferritin. Higher HbA1c levels were observed in iron deficiency anemia group than in the normal iron state group. This study concluded that in

premenopausal women the regardless of anemia, iron deficiency increases HbA1c levels.[50]

Hashimoto et al. tried to find a correlation between glycemia and HbA1c in pregnant women. Most women in late pregnancy already have iron deficiency anemia. They conducted two studies in pregnant women included non diabetic and later diabetic pregnant women not supplemented with iron using the indices of erythrocyte and iron metabolism. In late pregnancy, HbA1c levels were significantly elevated in both the studies. There was found to be a negative correlation between HbA1c levels and mean corpuscular hemoglobin, serum transferrin and serum iron saturation. From this, they postulated that the increased HbA1c levels seen in late pregnancy was because of the presence of iron deficiency anemia at this stage.[51]

In 2012, Rafat et al. tried to analyze the influence of iron indices over HbA1c in pregnant women with iron deficiency anemia. They found a significant correlation between the indices of red blood cell and iron metabolism and HbA1c. The study indicated that in women with iron deficiency anemia HbA1c levels were elevated and it decreased after the iron supplementation.[52]

Kim et al. stated that iron deficiency anemia 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%.[32]

Observations on iron deficiency anemia 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.[53] Sharifi et al. concluded that there was a negative correlation between HbA1c and erythrocyte hemoglobin, which supported the postulation that iron deficiency increases glycosylation of hemoglobin[54].

Ng JM et al. conducted a study in type 2 diabetic patients with chronic kidney disease who are treated with parenteral iron and/or ESA (Erythrocyte Stimulating Agents). They observed a statistically significant decrease in HbA1c levels after treatment, regardless of glycemic control[55].

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 anemia patients, it will result in false high prevalence.[57]

THEORIES ON EFFECT OF IDA OVER HBA1C LEVELS

I. 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.

II. A) Life span of the red blood cells present in the circulation may be prolonged during anemic state resulting in higher HbA1c levels in iron deficiency anemia patients.

III. B) After treatment with iron there will be increased bone marrow red cell production and release of new immature red cells resulting in lower HbA1c levels.

IV. There was a postulation that there is a balance between hemoglobin concentration and HbA1c levels. So, if the serum glucose remained constant a decrease in hemoglobin concentration could cause an increase in the glycated fraction.

V. Use of different assay methods

MATERIALS AND METHODOLOGY

MATERIALS AND METHODS

Study Title

"A STUDY ON INFLUENCE OF IRON DEFICIENCY ANAEMIA OVER HBA1C LEVELS''

Aims and Objectives

Primary objective

To study the levels of HbA1c in iron deficiency anemia patients

Secondary objective

To study the changes in HbA1c level with the correction of iron deficiency anemia

Study centre

DEPARTMENT OF GENERAL MEDICINE,
ESIC MEDICAL COLLEGE & PGIMSR,
K.K. NAGAR, CHENNAI – 78

Study design

PROSPECTIVE INTERVENTIONAL STUDY

Study Period

18 months

Inclusion criteria

- All consented Iron deficiency anemia patients attending Medical OPD and medical wards in our hospital
- Age between 18 to 60 years

Exclusion criteria

- Age <18 years or >60 years
- Patients with diabetes/IFG/IGT
- Patients with chronic renal failure/ liver disease
- Patients with haemolytic anaemia
- Pregnancy
- Chronic alcoholism
- Known case of malignancy

Study population

All patients coming to the department of general medicine, ESIC medical college & PGIMSR, fulfilling the inclusion and exclusion criteria were enrolled in the study. An informed written consent was obtained from the patients.

Data collection

A detailed history was recorded along with complete clinical examination as in the proforma. Provisional diagnosis was made and this was subsequently revised after completion of the investigations.

Laboratory investigations

Samples were collected from all the participants to estimate complete blood count, blood urea, serum creatinine, serum electrolytes, blood sugar- FBS/PPBS/GTT, urine R/E, HbA1c level, anemia profile including serum ferritin, vitamin B12 and folic acid levels, based on standard tests available in our hospital. In addition, ECG, chest xray and ultrasonogram abdomen were done in necessary cases. The final data was entered onto Microsoft excel sheet 2007 version**.**

Study protocol

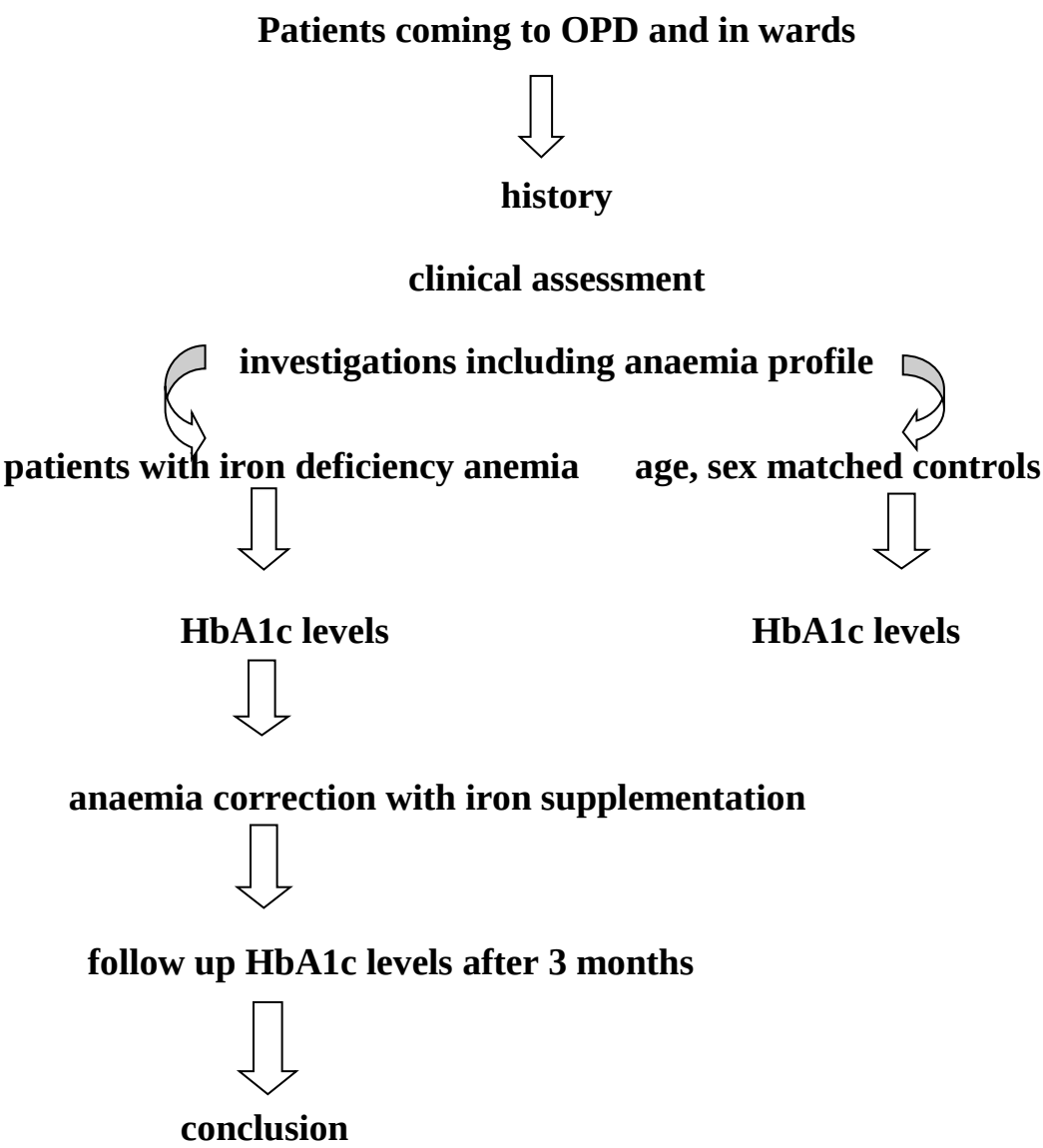
Patients with iron deficiency anemia based on WHO criteria cut off point and age, sex matched control patients were assigned for study. History, clinical assessment and investigations including serum ferritin, HbA1c were done.

NORMAL LEVELS

1.	Serum iron	:	50-150mic/dl
2.	Serum ferritin	:	50-200mic/l
3.	TIBC	:	300-360mic/dl
4.	Transferrin saturation	:	25-50%

HAEMOGLOBIN LEVELS TO DIAGNOSE ANAEMIA (g/dl)(WHO)

MEN	<13g/dl
WOMEN	<12g/dl



Collaborating Departments

Department of General Medicine, Department of Biochemistry and Department of Pathology

Ethical committee clearance	**:**	obtained
Consent	**:**	Individual written and informed consent
Conflict of interest	**:**	Nil
Sponsorship	**:**	No sponsorship

STATISTICAL ANALYSIS

The clinical parameters were compared and analyzed using Pearson chi square method. The diagnostic accuracy of all the parameters was then compared and interpreted with reference to clinical data.

In the present study, the statistical methods for quantitative data, descriptive statistics was presented by N, Mean, Standard Deviation and Range. For qualitative data, frequency count, N and percentage were put in a tabular manner.

To analyze the data, appropriate statistical tests were applied. The significance of difference between means in two groups was calculated using student t test and the significance of difference in proportions using

chi-square test. 2 x 2 tables were constructed for each variable and chi square value for degree of freedom calculated.

All the statistical analysis has been done by using statistical software SPSS (version 22). Other data, displayed by various tables and charts, by using Microsoft excel (windows 7).

* - Significant - $p < 0.05$

** - Very significant - $p < 0.01$

*** - Highly significant - $p < 0.001$

RESULTS
AND
ANALYSIS

OBSERVATION AND RESULTS

Table 5.1 AGE DISTRIBUTION

AGE (years)	CONTROL GROUP N (%)	STUDY GROUP N (%)
≤20	12(10)	5(4.2)
21-30	51(42.5)	40(33.3)
31-40	28(23.3)	41(34.2)
>40	29(24.2)	34(28.3)
TOTAL	120(100)	120(100)
Mean	32.06	34.067
SD	8.34	8.398
P value	0.064 Not Significant	

Interpretation

Mean age (±SD): in control group was 32.1 (8.34) and in study group was 34.1 (8.398).P value was 0.064 which is not significant i.e. the age distribution among the control and study group were equal.

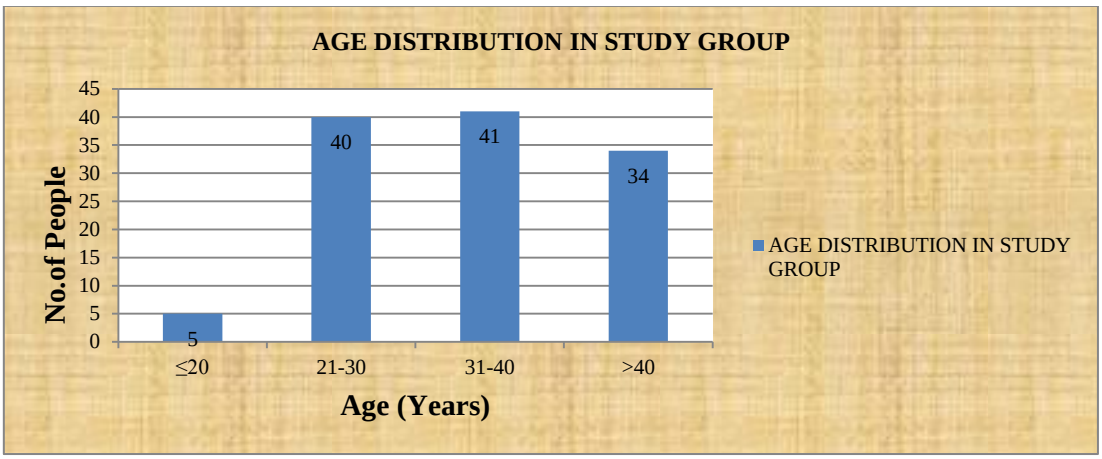


Figure 5.1: AGE DISTRIBUTION

Comments

About 67% of study subjects were in the age group of 21-40 years while 28% were aged 41-60 years. Minimum age: 19 years, maximum age: 49 years.

Table 5.2 SEX DISTRIBUTION

SEX	CONTROL GROUP N (%)	STUDY GROUP N (%)
MALE	56(46.7)	38(31.7)
FEMALE	64(53.3)	82(68.3)
TOTAL	120	120
P value	0.146 Not Significant	

Interpretation

Majority of the study subjects were females (68.3%) while the remaining 31.7% were males. It confirms the fact that iron deficiency anemia is more common in females. P value was 0.146 which is not significant i.e. sex distribution among the control group and study group were equal.

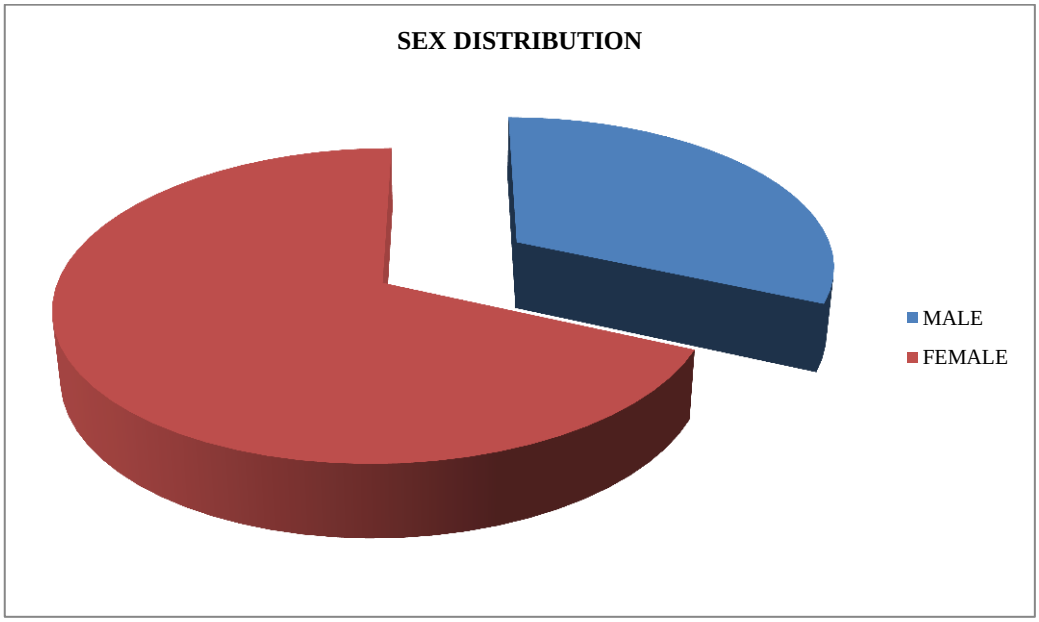


Figure 5.2: SEX DISTRIBUTION

Table 5.3 FBS DISTRIBUTION

FBS mg/dl	CONTROL GROUP N (%)	STUDY GROUP N (%)
≤80	11(9.2)	10(8.3)
81-90	66(55)	68(56.7)
91-110	43(35.8)	42(35)
N	120	120
Mean	88.508	88.525
SD	5.312	5.231
P value	0.98 not significant	

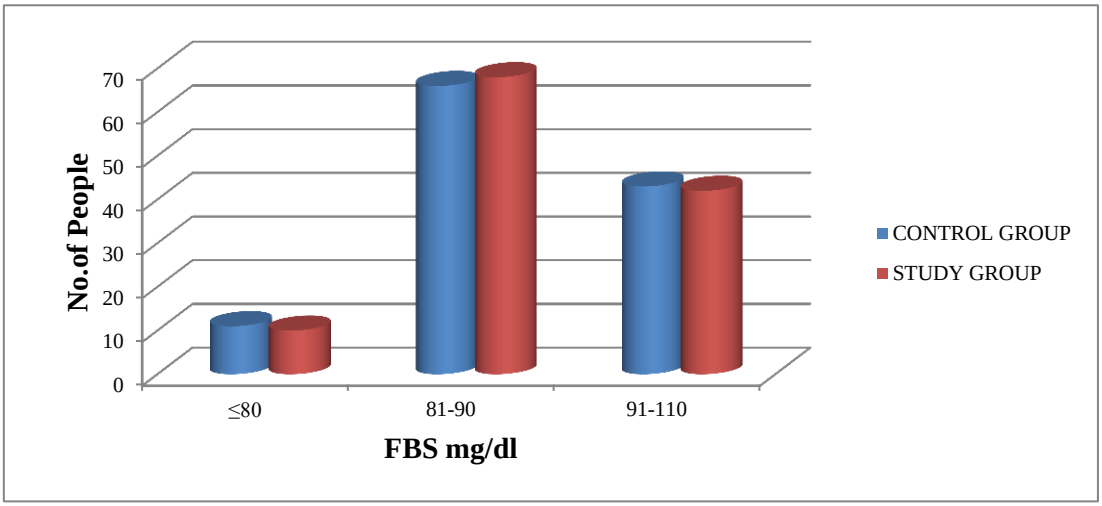


Figure 5.3: FBS DISTRIBUTION

Interpretation:

Mean (±SD): in control group was 88.508(5.312) and in study group was 88.525(5.231). P value was 0.98 which is not significant i.e. FBS distribution among the control and study group were same.

Table 5.4 PPBS DISTRIBUTION

PPBS mg/dl	CONTROL GROUP N (%)	STUDY GROUP N (%)
≤110	48(40)	57(47.5)
111-120	29(24.1)	21(17.5)
121-130	32(26.7)	32(26.7)
131-140	11(9.2)	10(8.3)
N	120	120
Mean	114.65	112.352
SD	12.522	13.725
P value	0.185 Not significant	

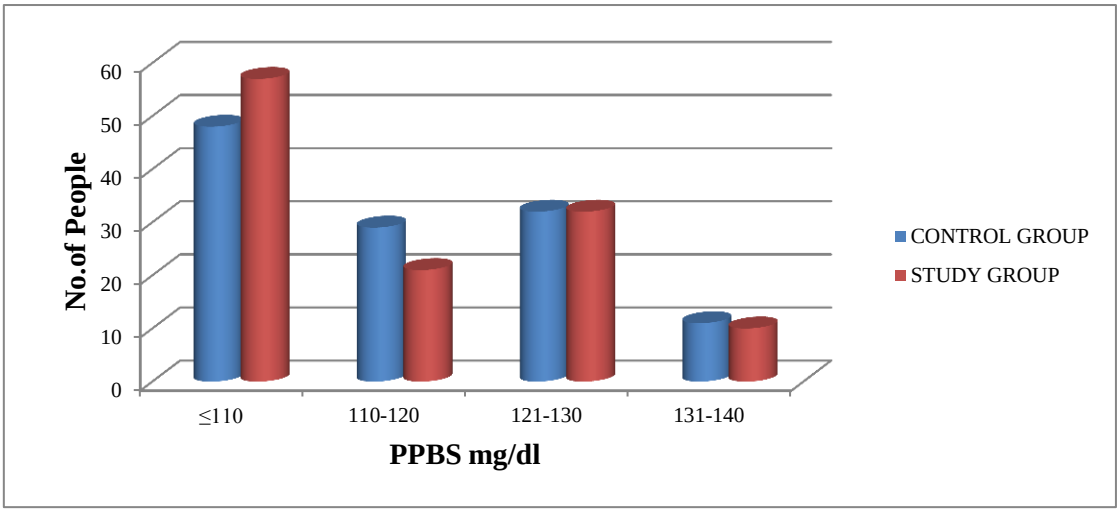


Figure 5.4: PPBS DISTRIBUTION

Interpretation

Mean (±SD): in control group was 114.65(12.522) and in study group was 112.352(13.725). P value was 0.185 which is not significant i.e. PPBS distribution among the control and study group were same.

Table 5.5 DISTRIBUTION OF HEMOGLOBIN IN CONTROL GROUP

HB (g/dl)	CONTROL GROUP N (%)
12-13	23(19.2)
>13	97(80.8)
N	120
Mean	13.408
SD	0.354

Interpretation

Mean (±SD): 13.408(0.354) gm/dl, minimum: 12.8gm/dl, maximum: 14gm/dl.

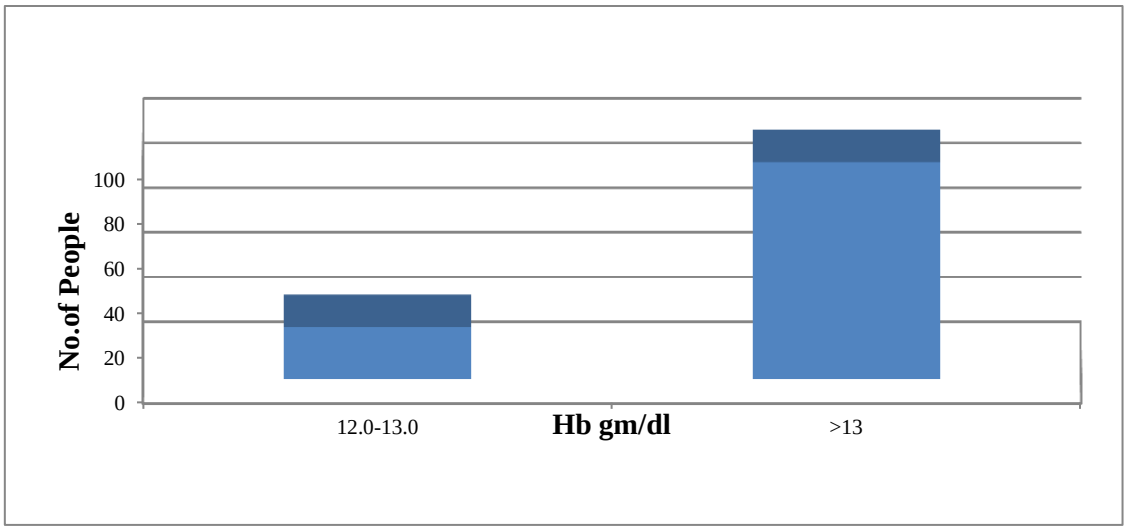


Figure 5.5: DISTRIBUTION OF HEMOGLOBIN IN CONTROL GROUP

Comments

About 80.8% of control subjects had Hb level more than 13gm/dl while 19.2% had Hb level between 12-13gm/dl.

Table 5.6 DISTRIBUTION OF HEMOGLOBIN IN STUDY GROUP PRE-CORRECTION

HB (g/dl)	STUDY GROUP PRE N (%)
<8	103(85.8)
8.0-8.9	17(14.2)
≥9	0(0)
N	120
Mean	6.778
SD	1.085

Interpretation

Mean (±SD): 6.778(1.085) gm/dl, minimum: 2.9gm/dl, maximum: 8.3gm/dl.

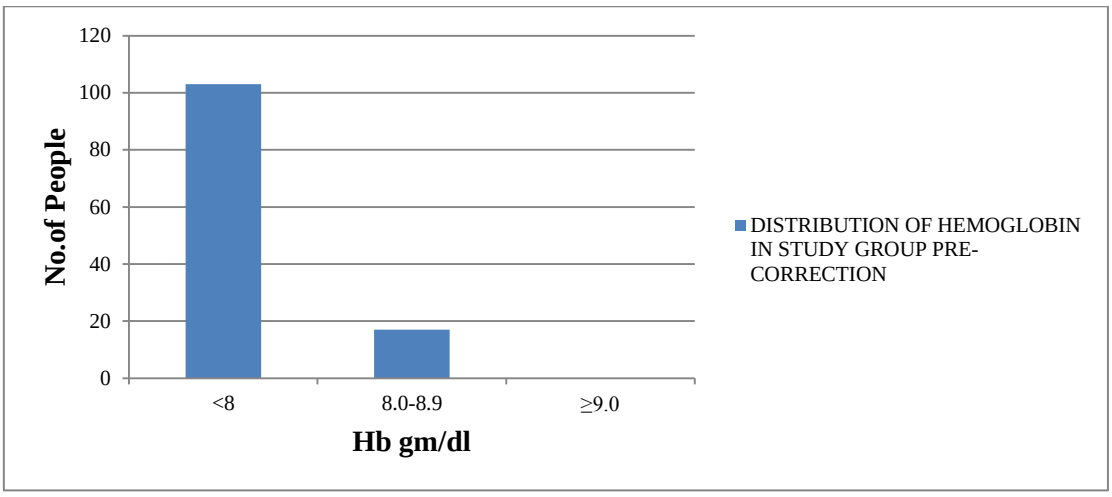


Figure 5.6: DISTRIBUTION OF HEMOGLOBIN IN STUDY GROUP PRE-CORRECTION

Comments

About 85.8% of study subjects had Hb level less than 8gm/dl while 14.2% had Hb level between 8.0-8.9gm/dl.

Table 5.7 DISTRIBUTION OF HEMOGLOBIN IN STUDY GROUP POST-CORRECTION

HB (g/dl)	STUDY GROUP POST N(%)
12-13	82(68.3)
>13	38(31.7)
N	120
Mean	12.659
SD	0.446

Interpretation

Mean (±SD): 12.659(0.446) gm/dl, minimum: 12gm/dl, maximum: 13.5gm/dl.

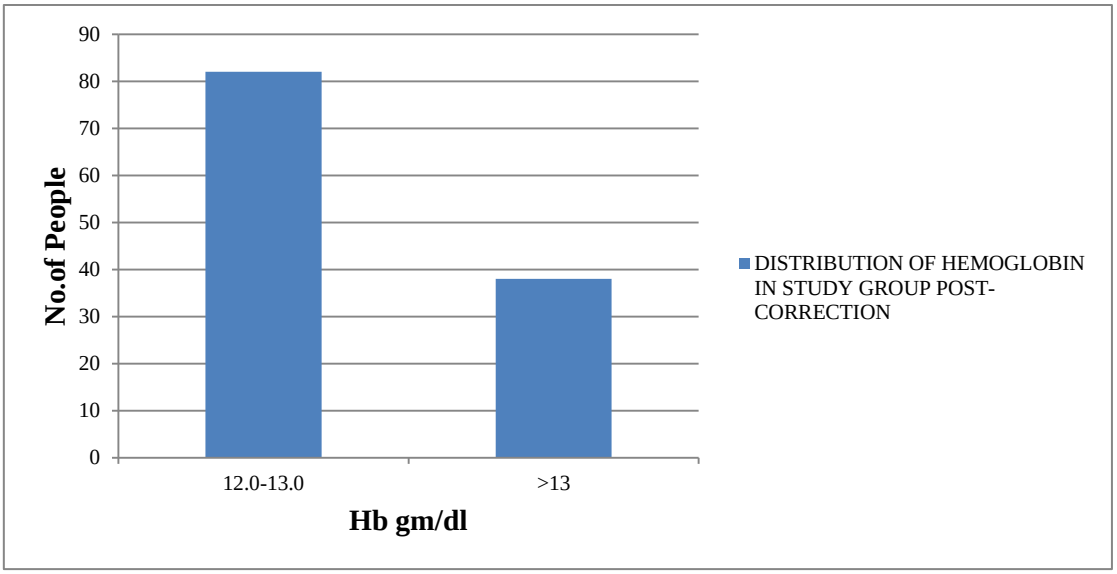


Figure 5.7: DISTRIBUTION OF HEMOGLOBIN IN STUDY GROUP POST-CORRECTION

Comments

After correction of anemia, about 68.3% of study subjects had Hb level between 12-13gm/dl, while 31.7% had Hb level more than 13gm/dl.

Table 5.8 DISTRIBUTION OF HEMOGLOBIN BETWEEN CONTROL AND STUDY GROUP PRE-CORRECTION

HB (g/dl)	CONTROL GROUP	STUDY GROUP PRE
≤13	23	120
>13	97	0
N	120	120
Mean	13.408	6.778
SD	0.354	1.085
P value	<0.001 Significant	

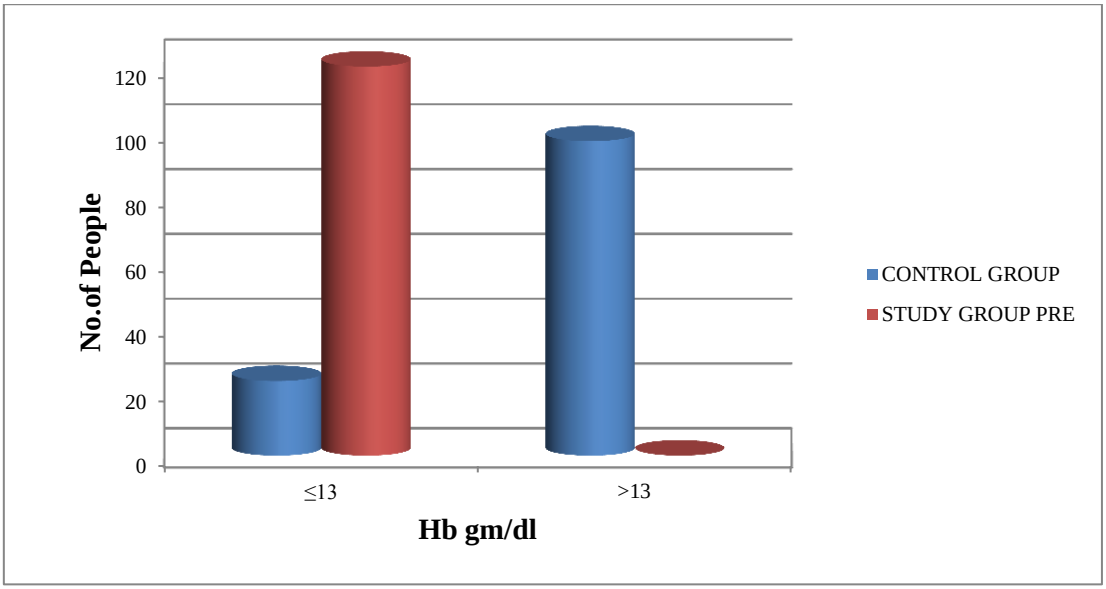


Figure 5.8: DISTRIBUTION OF HEMOGLOBIN BETWEEN CONTROL AND STUDY GROUP PRE-CORRECTION

Interpretation:

P value was less than 0.001 which is highly significant i.e. mean hemoglobin level in study group was significantly lower than the control group as expected.

Table 5.9 DISTRIBUTION OF HEMOGLOBIN IN STUDY GROUP PRE AND POST CORRECTION

HB (g/dl)	STUDY GROUP PRE	STUDY GROUP POST
≤13	120	82
>13	0	38
N	120	120
Mean	6.778	12.659
SD	1.085	0.446
P value	<0.001 Significant	

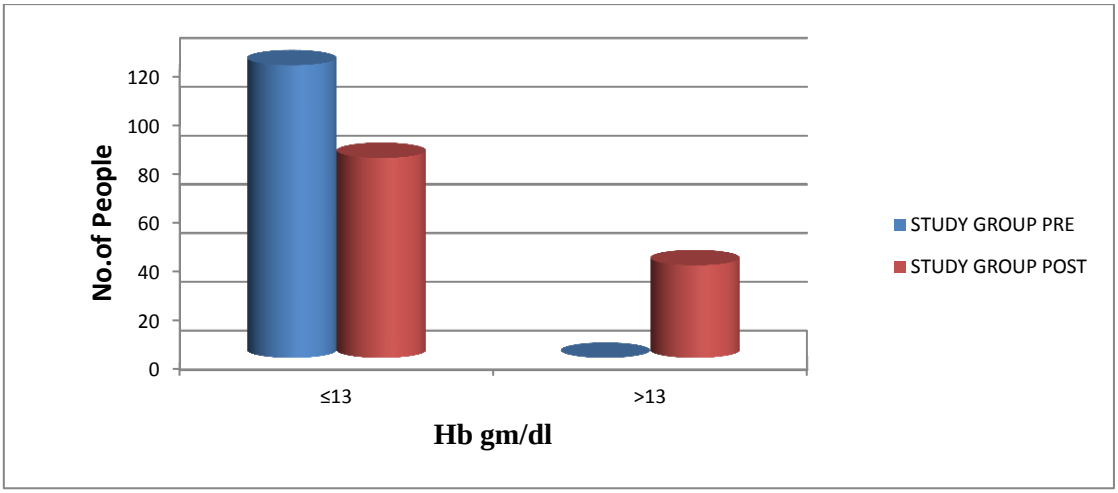


Figure 5.9: DISTRIBUTION OF HEMOGLOBIN IN STUDY GROUP PRE AND POST CORRECTION

Interpretation:

P value was less than 0.001 which is highly significant i.e. mean hemoglobin level had increased significantly in study subjects after iron treatment as expected.

Table 5.10 DISTRIBUTION OF MCV

MCV (fl)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<80	0(0)	120(100)	0(0)
80-90	45(37.5)	0(0)	44(36.7)
91-100	75(62.5)	0(0)	76(63.3)
N	120	120	120
Mean	91.316	64.46	91.349
SD	2.851	6.674	2.81
P value	<0.001 Significant		<0.001 Significant

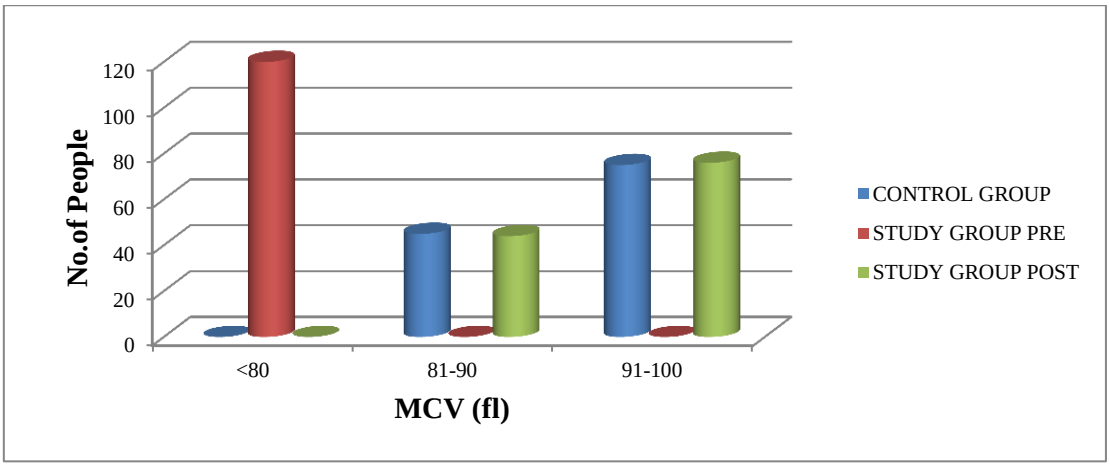


Figure 5.10: DISTRIBUTION OF MCV

Interpretation:

P value between control group and study group before anemia correction was less than 0.001 which is highly significant. It conveys that MCV was significantly lower in anemia group.

P value between study group pre and post correction was less than 0.001 which is highly significant. It indicates that MCV improved after iron treatment as expected.

Table 5.11 DISTRIBUTION OF MCH

MCH (pg/cell)	CONTROL GROUP N(%)	STUDY GROUP PRE N (%)	STUDY GROUP POST N(%)
<26	0(0)	119(99.2)	0(0)
26-28	50(41.7)	0(0)	49(40.8)
>28	70(58.3)	1(0.8)	71(59.2)
N	120	120	120
Mean	28.277	19.615	28.283
SD	0.836	3.018	0.833
P value	<0.001 Significant		<0.001 Significant

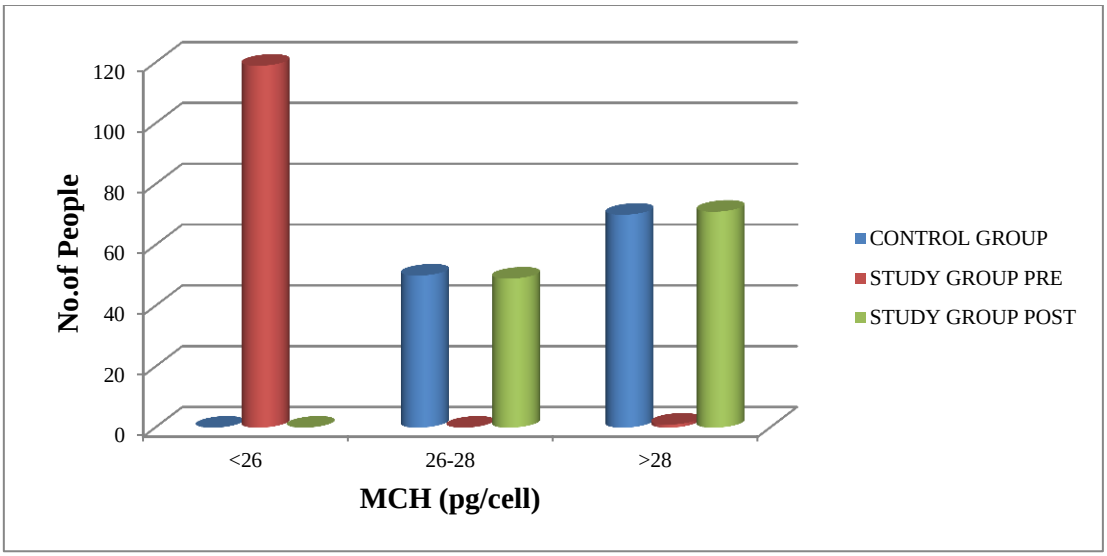


Figure 5.11: DISTRIBUTION OF MCH

Interpretation:

P value between control group and study group before anemia correction was less than 0.001 which is highly significant. It conveys that MCH was significantly lower in anemia group.

P value between study group pre and post correction was less than 0.001 which is highly significant. It indicates that MCH improved after iron treatment as expected.

Table 5.12 DISTRIBUTION OF SERUM IRON

SERUM IRON (mic gm/dl)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<30	0(0)	120(100)	0(0)
31-60	0(0)	0(0)	0(0)
61-90	3(2.5)	0(0)	4(3.3)
91-120	53(44.2)	0(0)	54(45)
>120	64(53.3)	0(0)	62(51.7)
N	120	120	120
Mean	117.167	21.257	117.147
SD	13.091	4.688	12.661
P value	<0.001 Significant	<0.001 Significant	

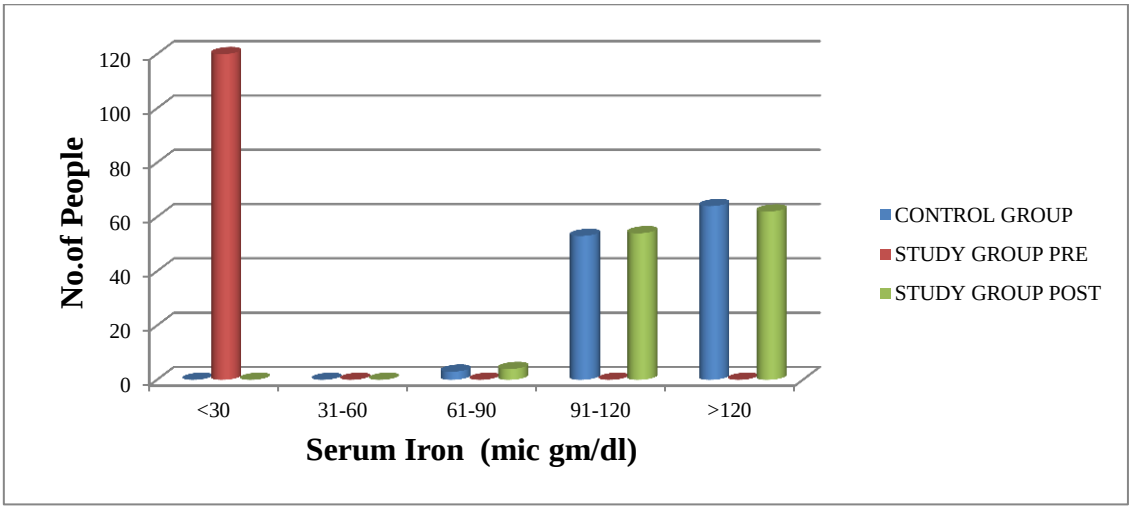


Figure 5.12: DISTRIBUTION OF SERUM IRON

Interpretation:

P value between control group and study group before anemia correction was less than 0.001 which is highly significant. It conveys that serum iron was significantly lower in anemia group.

P value between study group pre and post correction was less than 0.001 which is highly significant. It indicates that serum iron improved after iron treatment as expected.

Table 5.13 DISTRIBUTION OF SERUM FERRITIN

SERUM FERRITIN (g/L)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<15	0(0)	120(100)	0(0)
16-50	0(0)	0(0)	0(0)
51-150	0(0)	0(0)	0(0)
151-300	119(99.2)	0(0)	120(100)
>300	1(0.8)	0(0)	0(0)
N	120	120	120
Mean	232.264	6.871	237.239
SD	28.394	1.5	25.267
P value	<0.001 Significant	<0.001 Significant	

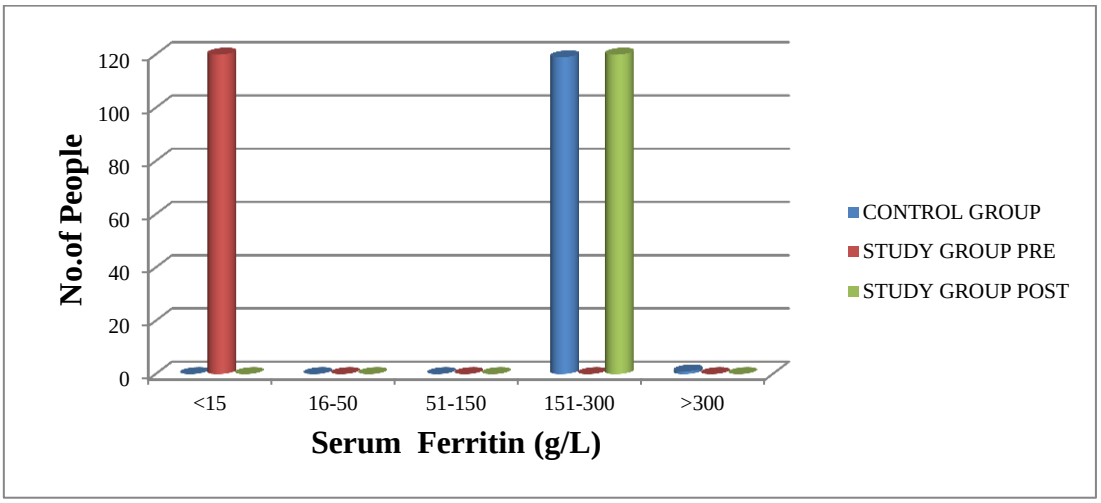


Figure 5.13: DISTRIBUTION OF SERUM FERRITIN

Interpretation:

P value between control group and study group before anemia correction was less than 0.001 which is highly significant. It conveys that serum ferritin was significantly lower in anemia group.

P value between study group pre and post correction was less than 0.001 which is highly significant. It indicates that serum ferritin improved after iron treatment as expected.

Table 5.14 DISTRIBUTION OF HBA1C BETWEEN CONTROL AND STUDY GROUP PRE-CORRECTION

HBA1C (%)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)
≤5	10(8.3)	104(86.7)
5.1-5.5	66(55)	16(13.3)
5.6-6.0	42(35)	0(0)
6.1-6.5	2(1.7)	0(0)
>6.5	0(0)	0(0)
Mean	5.446	4.619
SD	0.281	0.308
P value	<0.001 Significant	

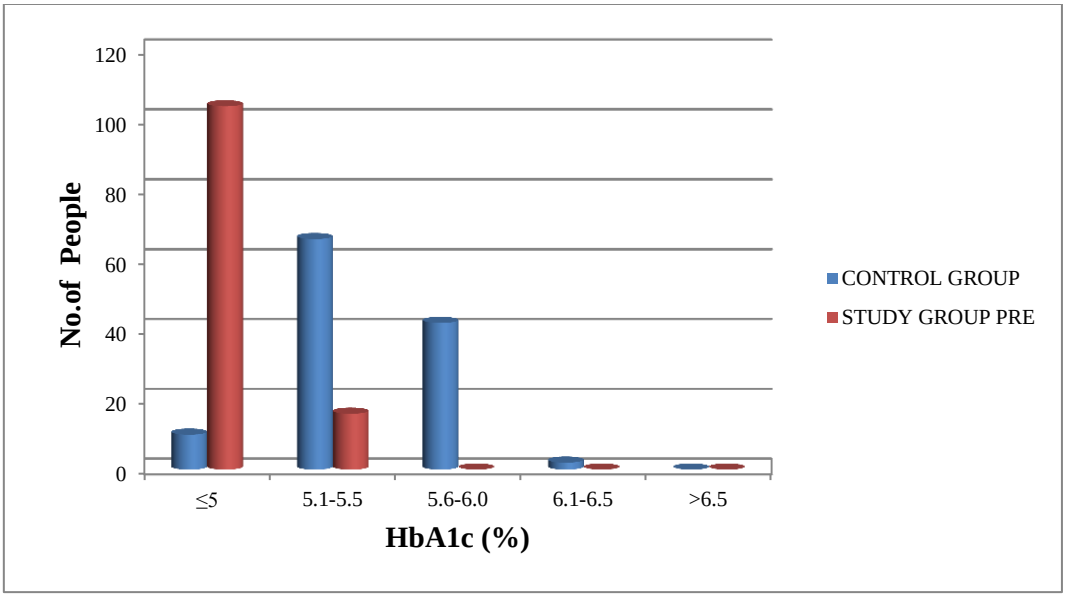


Figure 5.14: DISTRIBUTION OF HBA1C BETWEEN CONTROL AND STUDY GROUP PRE-CORRECTION

Interpretation

Mean HbA1c of iron deficiency anemia patients (4.619 ± 0.308) was significantly lower than control population (5.446 ± 0.281).

About 55% of control subjects had HbA1c level between 5.1-5.5% while 35% had HbA1c level between 5.6-6.0%.

About 86.7% of study subjects had HbA1c level between ≤5% while 13.3% had HbA1c level between 5.1-5.5%.

P value of HbA1c distribution between control group and study group pre-correction was less than 0.001 which is highly significant. It reveals that HbA1c was lower in anemia group.

Table 5.15 DISTRIBUTION OF HBA1C IN STUDY GROUP PRE AND POST CORRECTION

HBA1C (%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
≤5	104(86.7)	0(0)
5.1-5.5	16(13.3)	32(26.7)
5.6-6.0	0(0)	55(45.8)
6.1-6.5	0(0)	33(27.5)
>6.5	0(0)	0(0)
Mean	4.619	5.816
SD	0.308	0.323
P value	<0.001 Significant	

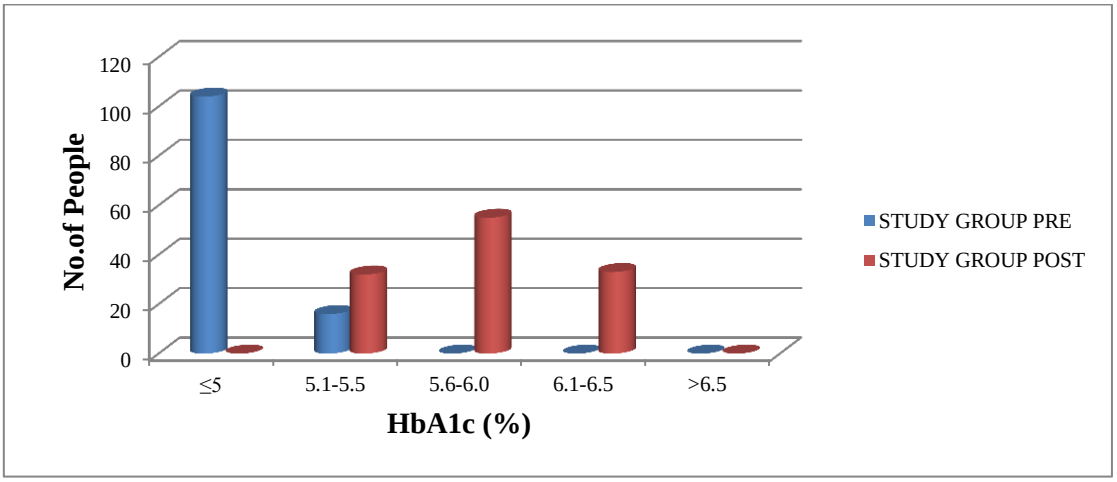


Figure 5.15 DISTRIBUTION OF HBA1C IN STUDY GROUP PRE AND POST CORRECTION

Interpretation:

The mean HbA1c level in the study group increased from 4.619(±0.308) % to 5.816(±0.323)% after correction of anemia

After correction of anemia about 45.8% of study subjects had HbA1c level between 5.6-6.0% while 27.5% had HbA1c level between 6.1-6.5%. P value of HbA1c in study group pre and post correction was less than 0.001 which is highly significant. It indicates that HbA1c increased after anemia correction.

Table 5.16 Mean Difference result in pre & post correction for Hb & HbA1c values

Parameters	Mean Difference
Hb (gm%)	5.88***
HbA1c (%)	1.20***

*** Highly Significant (P=0.0001)

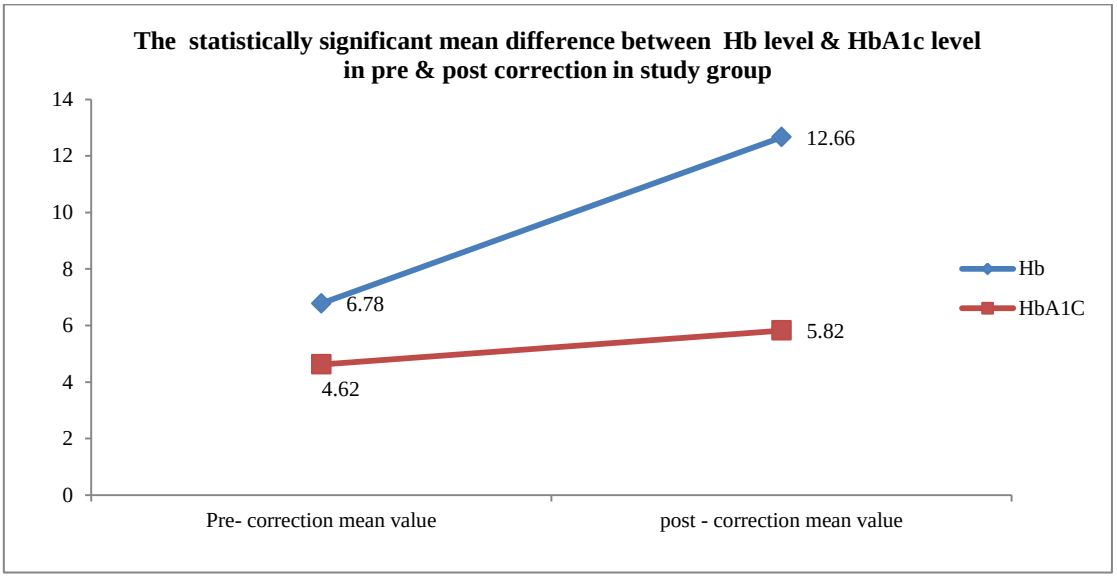


Figure 5.16: Mean Difference result in pre & post correction for Hb & HbA1c values

Interpretation

In this study HbA1c significantly increased after correction of anemia.

Table 5.17 Pre correction result for correlation:

Hb – Pre correction	HbA1C- pre correction	Interpretation
Pearson Correlation coefficient r	0.26	Positive, poor correlation
P value	0.005**	very significant
Sample size (n)	120	

Interpretation

In this study group, pre correction Hb & HbA1C showed positive, poor correlation (r = 0.26) which was statistically very significant (p= 0.005)

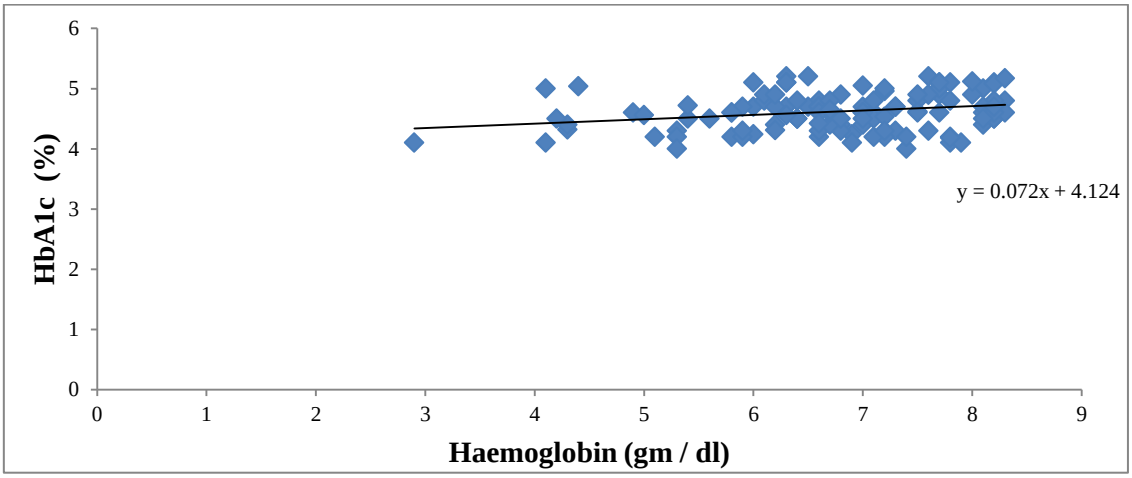


Figure 5.17: Correlation between Hb & HbA1C in study group before correction

Comments

In this scatter diagram, the trend line shows positive, poor and statistically significant correlation. For this line, Regression equation obtained, Y (HbA1C) = 0.072 (X = Hb) + 4.0124. That is, If we put the value of Hb (X) = 10gm %, the predicted HbA1C would be 4.73%. And for Hb = 12 gm %, the predicted HbA1C would be 4.88%. i.e. there was increase of 0.15% HbA1C level for each 2gm of Hb level.

Table 5.18 Post correction result for correlation

Hb -post correction	HbA1c- post correction	Interpretation
Pearson Correlation coefficient r	-0.15	Negative, poor correlation
P value	0.111	Not significant
Sample size	120	

Interpretation

In this study group, post correction Hb & HbA1C showed negative, poor correlation (r = -0.15) which was statistically not significant (p= 0.111)

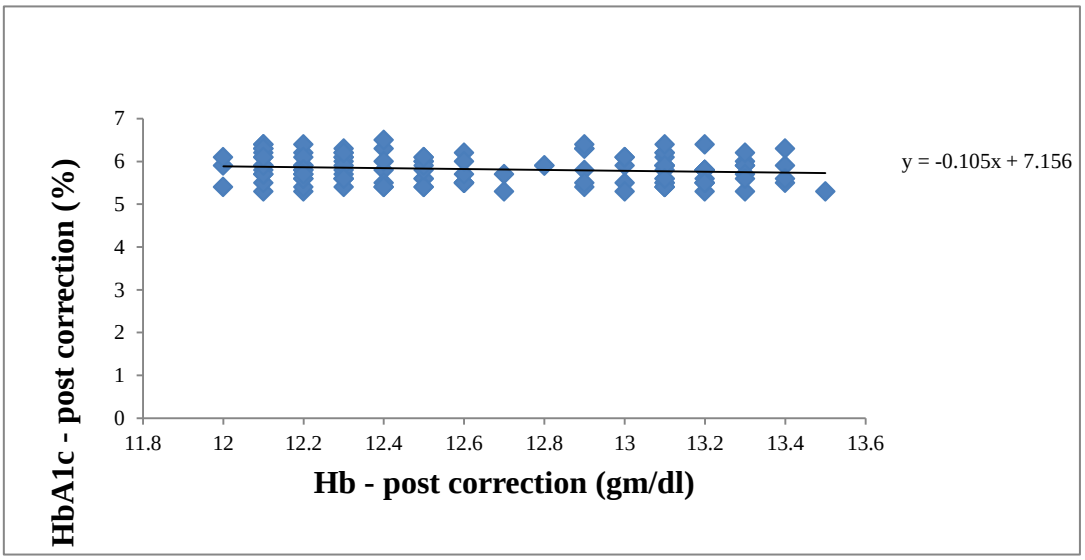


Figure 5.18: Correlation between Hb & HbA1c in study group after anemia correction

In this scatter diagram, the trend line shows negative, poor and statistically not significant correlation. For this line, Regression equation obtained, Y (HbA1C) = - 0.105 (X = Hb) + 7.156. That is, If we put the value of Hb (X) = 10gm %, the predicted HbA1C would be 6.11%. And for Hb = 12 gm %, the predicted HbA1C would be 5.9%. i.e. there was decrease of 0.21% HbA1C level for each 2gm of Hb level.

Inference

Sample size may not be enough to prove post correction correlation.

DISCUSSION

DISCUSSION

Iron deficiency is the commonest malnutrition. It is a major public health problem in both developing and developed countries.

Iron deficiency contributes to 50 percentage of anemia worldwide. Annually, about 8,41,000 deaths were attributed to iron deficiency anemia. Parts of Asia and Africa are affected more. These countries bear approximately 71 percentage of the global mortality burden.

In India, about 50% of anemia is attributed to iron deficiency. Children and women are the most vulnerable population. The factors contributing to iron deficiency anemia varies in different population.

Physiologically, HbA undergoes glycosylation in a slow and non enzymatic manner. The degree of glycosylation depends on the concentration of glucose. HbA1c is the predominant form of glycated hemoglobin. Glucose gets attached to the NH2 group in the terminal valine of the β-globin chains irreversibly.

Glycosylation process occurs throughout the life span (120 days) of red cells. Hence the measured glycohemoglobin levels reflect the glycemic status of the preceding 3 months.

HbA1c levels can be affected by multiple factors other than the plasma glucose level. Several conditions can result in falsely lower or higher values. Hemolytic anemia, hemoglobinopathies, uremia and chronic blood loss influence the HbA1c assays.

So far HbA1c has been used as a valuable tool in monitoring the glycemic control in diabetics. Recently American Diabetic Association and International Expert Committee recommended HbA1c for diagnosing diabetes. HbA1c level of 6.5% has been proposed as a diagnostic cut off point.

There were multiple studies investigating the relationship between iron deficiency anemia and HbA1c. But the results were inconsistent.

Our study aims to measure the HbA1c levels in iron deficiency anemia patients and to identify the changes in HbA1c levels after correcting the iron deficiency anemia.

Age distribution of the study population

In this study about 120 patients were allotted to the study group. The same number of age and sex matched controls were taken. The mean age group of the study population was 34.1±8.4 years. The minimum age was 19 years and the maximum was 49 years. About 67% of the study

subjects were in the age group of 21-40 years while 28% were aged 41-60 years. Thus in our study, the prevalence of iron deficiency anemia is more common in 2nd to 4th decade of life.

Sex distribution of the study population:

In this study, out of 120 patients 68.3% were females and 31.7% were males. It confirms the fact that women are more vulnerable to iron deficiency than men. The age and gender distribution of population in both the study and control groups were equal and comparable.

Hemoglobin distribution of the study population:

The mean hemoglobin of the study population was 6.8(±1.1)gm/dl. About 85.8 % of the study population had severe anemia i.e. less than 8 gm/dl. The minimum hemoglobin observed in the study population was 2.9 gm/dl and the maximum was 8.3 gm/dl.

The p value of unpaired t test between the study group hemoglobin and control group was less than 0.001 which is highly significant. It indicates that mean hemoglobin level in study group was significantly lower than the control group as expected.

The mean hemoglobin level in the study group increased from 6.8(±1.1)gm/dl to 12.7(±0.4)gm/dl after correction of anemia with iron. The minimum hemoglobin observed in the study population after iron treatment was 12gm/dl and the maximum was 13.5gm/dl.

The p value of paired t test in the study group hemoglobin before and after iron treatment was less than 0.001 which is highly significant. It indicates that mean hemoglobin level had increased significantly in study subjects after iron treatment as expected.

Red cell indices of the study population:

The mean MCV and MCH of the control and study group were 91.316(±2.851), 28.277(±0.836) and 64.46(±6.674), 19.615(±3.018) respectively. This shows that MCV, MCH were lower in the study group compared to the control group. The observed difference was statistically significant ($p<0.001$).

The mean MCV and MCH of the study group after iron treatment were 91.349(±2.81) and 28.283(±0.833) respectively. That was a statistically significant improvement.

Iron status of the study population

The mean serum iron and ferritin levels of the control and study group were 117.167(±13.091), 232.264(±28.394) and 21.257(±4.688), 6.871(±1.5) respectively. This shows that serum iron and ferritin levels were lower in the study group compared to the control group. The observed difference was statistically significant ($p<0.001$).

The mean serum iron and ferritin levels of the study group after iron treatment were 117.147(±12.661) and 237.239(±25.267) respectively. That was a statistically significant improvement.

HbA1c level of the study population

The mean HbA1c of the study population was 4.619(±0.308) %. About 86.7% of study subjects had HbA1c level ≤5% while 13.3% had HbA1c level between 5.1-5.5%. The mean HbA1c of the control group was 5.446(±0.281) %.

The p value of unpaired t test between the study group HbA1c and control group was less than 0.001 which is highly significant. It indicates that mean HbA1c level in study group was significantly lower than the control group.

The mean HbA1c level in the study group increased from 4.619(±0.308) % to 5.816(±0.323)% after correction of anemia with iron. After correction of anemia about 45.8% of study subjects had HbA1c level between 5.6-6.0% while 27.5% had HbA1c level between 6.1-6.5%.

The p value of paired t test in the study group HbA1c before and after anemia correction was less than 0.001 which is highly significant. It indicates that mean HbA1c level had increased significantly in study subjects after anemia correction.

Correlation between Hb & HbA1C

In this study group, pre correction Hb & HbA1C showed positive, poor correlation (r = 0.26) which was statistically very significant (p= 0.005). That is, when the hemoglobin decreases the HbA1c will also decrease and vice versa.

And post correction Hb & HbA1C showed negative, poor correlation (r = -0.15) which was statistically not significant (p= 0.111). That is, when the hemoglobin increases the HbA1c will decrease and vice versa, which is not significant statistically.

Similar to this study, in 2014 a study was conducted by Vishal Kalasker et al[59] on the effect of iron deficiency anemia on glycosylated

hemoglobin 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 anemia. But they concluded that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using concentration of HbA1c according to the American Diabetes Association (ADA) guideline.

In contrast to our study, a study done by Alap L. Christy et al[37] concluded that iron deficiency anemia elevates HbA1c levels in diabetic individuals with controlled plasma glucose levels. They postulated that iron deficiency anemia has a positive correlation with increased HbA1c levels.

A study done by Catherine Kim et al[32] concluded that iron deficiency shifted the HbA1c slightly upwards independent of fasting glucose level.

In non GDM mothers, Sasekala et al[58] conducted a descriptive cross sectional study. They showed that in anemic non GDM mothers the HbA1c levels are higher. So they advised to be cautious in interpreting the HbA1c and plasma sugar levels.

Alap L. Christy et al[38] conducted a study to evaluate the relationship between HbA1c and anemia in hypothyroid patients. They concluded that Nondiabetic hypothyroid individuals with anemia shows elevate A1C levels in prediabetes range. Hence care should be exercised while using HbA1C as a diagnostic tool for diabetes in such patients.

Study done by Van Heyningen et al[42] found out that there was no significant influence of iron deficiency anemia over HbA1c concentrations. They suggested that differences observed in previous studies could be due to the various laboratory methods used in estimating the HbA1c. Hansen et al also observed similar results. Contradicting the conclusion of Van Heyningen et al, Rai et al conducted a study using various assay methods to estimate HbA1c and found no significant alterations in HbA1c levels measured by those methods.

El-Agouza et al[45] reported that iron deficiency anemia patients had higher HbA1c levels and it decreased after treatment. They believed that there was a balance between hemoglobin concentration and HbA1c level. That is if the plasma glucose was maintained, the lower hemoglobin concentration would lead to rise in HbA1c levels.

In our study mean HbA1c of iron deficiency anemia patients (4.619 ± 0.308) was significantly lower than control population (5.446 ± 0.281) and it increased (5.816 ± 0.323%) significantly after iron treatment.

SUMMARY

Iron deficiency anemia is the most common nutritional anemia. It contributes to more than half of the global anemia burden. HbA1c was used to assess the glycemic control. But recent studies have recommended HbA1c to diagnose diabetes. HbA1c levels can be affected by multiple non glycemic parameters. This study was conducted to study the influence of iron deficiency anemia over HbA1c levels and to study the changes in HbA1c levels after correcting the iron deficiency anemia.

Our study confirmed that iron deficiency anemia was highly prevalent among women during the second to fourth decades of life.

Mean HbA1c of patients with iron deficiency anemia (4.619 ± 0.308) was lower, compared to healthy control group (5.446 ± 0.281) with p value less than 0.001(significant).In iron deficiency anemia patients mean HbA1c increased from 4.619 ± 0.308 % to 5.816 ± 0.323% after iron treatment with p value less than 0.001 which is statistically significant.

Among patients with iron deficiency anemia, hemoglobin and HbA1c showed positive poor correlation (r = 0.26) which was statistically very significant (p= 0.005). After anemia correction hemoglobin and HbA1c showed negative, poor correlation (r = -0.15) which was statistically insignificant (p= 0.111).

CONCLUSION

CONCLUSION

- The prevalence of iron deficiency anemia is more common in females during the second to fourth decades of life.
- HbA1c was lower in patients with iron deficiency anemia compared to healthy control group.
- After correction of anemia, HbA1c level increased significantly in iron deficiency anemia patients.
- Hemoglobin and HbA1c showed statistically significant positive correlation in patients with iron deficiency anemia.
- After correction of anemia Hemoglobin and HbA1c showed statistically insignificant negative correlation.
- Longer period of study and a larger sample size may be required to show a statistically significant positive correlation.
- Iron deficiency anemia has to be kept in mind before using the HbA1c to diagnose diabetes.

LIMITATIONS

LIMITATIONS

- The sample size of the study was small.
- The study period was short.
- The study should be done at the community level to prove a statistically significant correlation.

FUTURE PROSPECTIVES

FUTURE PROSPECTIVES

- Large scale trials over longer durations may give accurate information about the influence of iron deficiency anemia over HbA1c levels. This will increase the reliability of HbA1c in diagnosing diabetes.

- Further studies can be done in other markers of glycemic control like glycated albumin and fructosamine in iron deficiency anemia patients to assess their usefulness.

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ANNEXURES

ANNEXURES - I

PROFORMA

Name: Address:

Age: Occupation:

Sex: Socio economic status:

IP NO: Education:

DOA: DOD: Ward/unit:

PRESENTING COMPLAINTS:

DETAILED HISTORY:

EXAMINATION:

- General examination
- Vital signs

Pulse: Blood pressure:

Respiratory rate: Temperature:

Body mass index (BMI):

- Systemic examinations

CVS : RS :

Abdomen : CNS :

INVESTIGATIONS

- COMPLETE HEMOGRAM

  Hb (g/dL)

  TC (cells/cu.mm)

  DC

  RBC (millions/cu.mm)

  Platelets (Lakhs/cu.mm)

  PCV

- ANAEMIA PROFILE

  Peripheral smear

  Serum Iron

  Serum ferritin

  TIBC

  Transferrin saturation

  Vit B 12 and folic acid level

- HBA1C
- URINE ANALYSIS

  Albumin

  Sugar

  Deposits

- BLOOD SUGAR (MG/DL)—FBS/PPBS/GTT
- LIVER FUNCTION TEST

- RENAL FUNCTION TEST

  Blood urea (mg/dl)

  Serum creatinine (mg/dl)

  Serum Electrolytes
- ECG in all leads
- Chest x ray PA view
- USG Abdomen & pelvis
- DURING FOLLOW UP AT THIRD MONTH
  - History
  - Examination
  - Investigations
  - complete hemogram and anaemia profile
  - HbA1c levels

ANNEXURES – II

PATIENT CONSENT FORM

Study title: A STUDY ON INFLUENCE OF IRON DEFICIENCY ANEMIA OVER HBA1C LEVELS

Study centre : ESIC Medical College - PGIMSR

Participant name : Age :

Sex : IP No:

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to clarify all my queries and doubts and they have been answered to my satisfaction. Investigator explained very well about the procedure and I am made aware of the safety, advantage and disadvantage of the technique.

I understand that my participation in the study is purely voluntary and that I am free to withdraw at anytime without giving any reason. I have understood that the investigator, regulatory authorities and the ethics committee will have access to my health records both in respect to current study and any further research that may be conducted in relation to it, even if I decide to withdraw from the study. I have understood that my identity will not be revealed in anyway and information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.

Without any compulsion I am willing to give consent for the participation of myself in this study.

Date: Signature / thumb impression of patient

Place: Patient name:

Signature of the investigator:

Name of the investigator :

INFORMATION TO PARTICIPANTS

Investigator : Dr.K.Vijaya Durairaj

Study centre : ESIC Medical College & PGIMSR
K.K.Nagar, Chennai

Title : A STUDY ON INFLUENCE OF IRON DEFICIENCY ANEMIA OVER HBA1C LEVELS

You are invited to take part in this research study. We have got approval from the IEC. You are asked to participate because you satisfy the eligibility criteria.

Rights and confidentiality

The participation in this study is purely voluntary. You have every right not to participate in this study. All the data collected in this regard from you will be kept discretely and your name will not be revealed at any circumstances.

To contact

If you have any doubts and clarification required you can call the doctor, VIJAYA DURAIRAJ. at the 9600426353 mobile number at any time.

Signature / Thumb Impression of Patient
Patient Name:

Signature of the Investigator: _____

Name of the Investigator : _____

ANNEXURES – II

ஒப்புதல் படிவம்

பெயர்: வயது:

இனம்: காப்பீட்டு எண்:

நான் இரத்த பரிசோதனையின் □ லம் செய்யும் இந்த ஆய்வின் நோக்கத்தை புரிந்து கொண்டேன். மேலும் இந்த ஆய்வினை பற்றி சந்தேகங்களை ஏற்படும் நிலையில் எனது சந்தேகங்களை முழுமையாக விளக்கப் பெறலாம் என்பதையும் அறிந்து கொண்டேன்.

நான் எனது சொந்த விருப்பத்தின் பேரில் இந்த ஆய்விற்கு சம்மதிக்கிறேன். மேலும் எந்த நிலையிலும் நான் இந்த ஆய்விலிருந்து விலகிக் கொள்ளலாம் என்பதையும் அறிந்து கொண்டேன்.

மேலும் இந்த ஆய்விலிருந்து நான் விலகிக்கொண்டாலும் எனது உடல் பரிசோதனைகளின் முடிவுகளை இந்த ஆய்விற்கும் மற்றும் பின்வரும் ஆய்வுகளுக்கும் பயன்படுத்தலாம் என்பதையும் அறிந்து கொண்டேன்.

மேலும் அவசியமின்றி எனது பெயரோ, புகைப்படமோ எந்த நிலையிலும் வெளியிடப்படமாட்டாது என்பதையும் அறிந்து கொண்டேன்.

இவை அனைத்தும் தெரிந்தும் என்னுடைய சொந்த விருப்பத்தின் பேரில் நான் இந்த ஆய்விற்கு சம்மதிக்கிறேன்.

தேதி:

இடம்: கையொப்பம்

MASTER CHART

ANNEXURES – III

KEY WORDS TO MASTER CHART

FBS	-	Fasting Blood Glucose
PPBS	-	Post Prandial Blood Glucose
Hb	-	Hemoglobin
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
SIRON	-	Serum Iron
TIBC	-	Total Iron Binding Capacity
ISAT	-	Iron Saturation
FERRITIN		
HbA1c	-	Glycated Hemoglobin
C	-	Control Group
S	-	Study Group
S1	-	Study Group Pre Correction
S2	-	Study Group Post Correction

MASTER CHART

CONTROL GROUP														STUDY GROUP PRE CORRECTION												STUDY GROUP POST CORRECTION								
SLNO_C	AGE_C	SEX_C	FBS_C	PPBS_C	Hb_C	MCV_C	MCH_C	MCHC_C	SIRON_C	TIBC_C	ISATN_C	FERRITIN_C	HbA1C_C	AGE_S	SEX_S	FBS_S	PPBS_S	Hb_S1	MCV_S1	MCH_S1	MCHC_S1	SIRON_S1	TIBC_S1	ISATN_S1	FERRITIN_S1	HbA1C_S1	Hb_S2	MCV_S2	MCH_S2	MCHC_S2	SIRON_S2	TIBC_S2	ISATN_S2	FERRITIN_S2	HbA1C_S2
1	31	F	78	90	13.4	90.4	27.9	33.2	87.8	306	28.69	185.4	5.5	45	F	87	128	8.1	68.9	23.7	29.2	22.6	397	5.7	5.5	5	12.6	92.3	27.8	33.6	108.78	312	34.86	241.5	5.5
2	22	M	86	97	13.8	95.6	29.2	33.1	99.76	312	31.97	306.5	5.7	37	F	85	93	6.3	62.9	20.4	27.8	13.27	475	2.79	3.9	5.2	12.4	90.5	26.9	34.1	120.43	328	36.71	254.3	5.8
3	26	M	91	126	13.1	94.3	28.7	34.6	104.82	321	32.65	284.3	5.1	21	F	98	132	2.9	50.5	14.8	29.3	24.5	463	5.3	6.2	4.1	12.1	93.4	30.4	33.1	116.31	310	37.51	236.6	5.3
4	20	F	84	106	13.8	88.7	27.3	33.8	96.3	317	30.37	190.7	4.9	39	M	92	97	7.4	68.1	22.4	26.6	9.35	506	1.85	8.2	4	13.1	90.6	29.3	33.6	104.82	317	33.06	279.8	6.1
5	43	F	85	98	14	96.6	29.8	33.5	133.42	315	42.35	244.6	5	32	F	84	98	6.6	72.7	25.1	27.2	17.8	484	3.67	6.8	4.7	12.3	89.5	26.9	33.4	97.65	322	30.32	282.4	6
6	41	F	86	107	12.8	91.2	28.3	33.9	120.34	303	39.71	232.8	5.3	42	F	88	102	8.2	72.5	20.3	28	27.6	445	6.2	9.6	5.1	12.8	95.4	28.8	32.8	89.65	313	28.64	195.3	5.9
7	38	M	97	115	13	91.4	28.1	34.1	132.12	320	41.28	280.3	5.9	46	M	95	100.3	4.9	66.9	17.9	26.7	18.9	412	4.59	7.2	4.6	13	96	29.4	33.2	120.12	328	36.62	254.6	5.5
8	40	M	96	134	13.3	93.1	28.6	33.1	135.62	311	43.6	255.4	5.6	19	F	87	97	8	76	24.1	28.9	27	382	7.1	8.7	4.9	12.9	93.6	29.8	34.2	96.33	305	31.58	210.8	5.8
9	41	F	84	130	13.4	94.2	28.9	33.5	122.73	309	39.71	226.7	5.5	21	F	83	99	7.6	78.1	23.8	28.5	28.2	398	7.08	6.3	5.2	12.3	92.2	29.1	32.6	102.26	310	32.98	251.7	6.1
10	21	F	90	98	13.8	87.9	27.8	33.8	90.6	319	28.4	188.5	5	41	F	88	122	6.6	59.9	16.5	27.5	21.66	406	5.33	5.6	4.2	12.3	91.2	28.2	33.1	124.78	306	40.77	235.5	5.7
11	30	F	92	112	13	91.2	28.2	33.9	104.52	312	33.5	214.6	5.6	28	F	93	112	8.2	72	20.1	28	25.94	480	5.41	4.9	5.08	13.3	87.9	27.8	33.6	99.45	323	30.78	267.8	5.9
12	42	M	88	127	13.2	92.2	29.1	32.9	124.55	306	40.7	229.8	5.3	38	F	90	95	6.8	72.6	23	29.7	29.05	459	6.32	8	4.9	12.2	94.2	28.9	33.5	106.34	319	33.33	212.3	6.2
13	25	M	93	109	13.5	93.6	29.8	32.7	131.42	312	42.12	277.4	5.1	21	F	92	118	7.5	58.6	16.8	28.6	27.93	460	6.07	6.2	4.8	12.5	93.1	28.6	33.1	124.6	303	41.12	204.5	5.6
14	41	F	87	99	12.9	96	29.4	34.2	125.36	305	41.1	207.5	5.4	22	F	85	134	7	55.1	15.8	27.6	26.06	562	4.64	7.1	5.05	12.3	91.4	28.1	34.1	108.65	314	34.6	237.6	5.8
15	44	F	82	135	13.1	95.4	28.8	33.1	122.72	308	39.84	196.4	5.5	48	M	95	124	4.4	49	13.2	26.8	28.66	468	6.12	9.71	5.04	13.2	91.2	28.3	33.9	116.24	310	37.49	266.2	5.5
16	26	F	87	96	12.8	89.5	26.9	32.8	112.6	323	34.86	186.3	5.2	42	M	97	130	7.7	79	22.6	28.7	23.66	398	5.94	8.6	5.01	13	96.6	29.8	33.5	109.81	303	36.24	282.3	5.9
17	36	M	96	106	13.4	90.6	29.3	33.4	124.65	328	38	212.5	5.4	43	F	86	128	8.3	64.8	19.2	29.7	26.12	487	5.36	6.3	5.17	12.9	95.3	27.3	33.8	97.25	312	31.16	238.6	6.3
18	25	F	88	120	13.9	93.4	30.4	33.6	104.88	317	33.08	202.6	5.5	44	F	85	130	8.3	59.3	18	29.3	17.93	417	4.3	9.6	4.8	13.1	96.1	28.7	33.9	108.44	317	34.2	254.6	5.4
19	46	F	84	108	13.3	90.5	26.9	33.1	96.52	330	29.24	177.1	4.9	32	F	84	95	6.6	52.8	14.1	26.7	16.97	502	3.38	3.3	4.3	12.3	86.4	29.2	33.1	114.56	321	35.68	224.5	6.1
20	44	M	92	105	13.4	92.3	27.8	34.5	97.65	328	29.77	176.5	5.6	41	M	90	97	7.3	58.3	17.9	30.7	20.98	502	4.18	4.6	4.7	13.3	91.7	27.9	33.2	122.6	312	39.29	277.4	6
21	20	F	98	126	13	88.5	27.5	33.6	99.38	320	31.05	183.4	5.3	45	M	86	96	4.1	55.2	14.4	26.2	18.4	538	3.42	7.8	5	13.1	87.6	28.7	32.8	132.72	306	43.37	254.4	5.6
22	30	F	85	117	13.2	93.2	28.1	33.9	105.2	314	33.5	198.3	5.4	19	F	78	90	5.8	56.8	20.1	26.8	15.32	450	3.12	3.48	4.2	12.2	91.8	27.6	33.7	115.65	313	36.94	198.5	5.9
23	21	M	78	112	13.6	94.5	29.2	33.5	118.76	312	38.06	233.4	5.6	48	F	98	118	6.7	58	16	27.9	26.52	428	6.2	5.5	4.6	12.1	86.5	29.3	33.2	131.45	316	41.59	237.5	6.2
24	36	F	90	126	12.9	94.6	28.4	32.9	124.6	304	40.98	215.6	5.7	39	F	92	105	7	73.6	21.4	29	14.49	438	3.31	4.3	4.7	12.4	96.1	31.6	34.5	122.33	325	37.64	202.6	5.5
25	25	F	94	114	13.2	93.8	28.6	34.8	106.76	317	33.67	184.67	6	25	F	87	98	8.1	66.2	19.8	29.9	22.8	388	5.88	9.5	4.4	12.9	95.3	30.5	34.3	130.46	331	39.41	196.7	5.4
26	24	F	90	123	13.4	95.5	29.3	33.3	99.97	308	32.45	232.8	5.8	34	M	88	97	6.6	62.4	18	28.8	18.42	422	4.36	7.8	4.8	13.2	94.2	28.5	33.9	96.75	310	31.2	267.5	5.8
27	41	M	85	96	13.8	88.7	27.8	32.9	122.44	314	38.99	244.7	5.3	21	F	96	128	7.8	62.6	20	27.9	17.8	435	4.09	8.4	4.1	12.5	88.6	27.8	33.6	121.37	321	37.8	244.3	5.6
28	41	F	87	124	13.2	89.9	27.6	34.2	98.64	322	30.63	177.6	5.8	47	M	81	109	6.2	60	17	28.4	22	402	5.47	9.2	4.4	13.1	90.2	28.2	34.1	134.76	315	42.78	272.1	5.9
29	25	M	96	132	13.9	91.4	28.7	33.9	130.12	316	41.17	250.6	5.7	42	F	91	108	5.1	59.2	15.7	26.5	21.8	432	5.05	7.8	4.2	12.1	91.3	29.2	32.9	133.14	307	43.36	208.5	6.4
30	20	M	89	94	13.3	88.6	27.9	33.2	121.22	305	39.74	223.5	5.4	41	M	84	96	6.3	62	18.1	27.5	23	397	5.79	6.41	4.7	13.3	89.2	27.9	33.6	109.54	318	34.44	245.6	5.9
31	46	F	78	108	13.6	90.5	28.5	33.5	99.78	312	31.98	206.4	5.3	25	F	97	126	6	65	20.2	29.2	14.38	497	2.89	7.6	5.1	12.2	95.4	29.5	34.3	123.46	309	39.95	262.3	5.3
32	43	F	77	102	13.1	95.7	29.5	32.7	127.34	310	41.07	228.6	5.9	22	F	92	97	7.2	70	22.3	28	26.7	453	5.89	8.5	4.96	12.4	90.5	28.5	32.8	114.33	321	35.61	233.5	6.3
33	30	M	90	127	13.5	89.2	27.9	34.3	115.64	303	38.16	235.6	5.7	43	F	86	98	5.3	57	15.5	27.1	24.5	388	6.31	5.9	4.3	12.2	88.6	27.9	33.5	106.72	315	33.87	212.5	6.4
34	22	M	92	105	13.8	91.2	29.2	33.7	107.82	313	34.44	252.3	5.6	23	F	88	91	8.2	66.6	20.1	29.8	18.5	476	3.89	6.9	4.5	13.1	91.4	28.7	33.2	120.64	322	37.46	272.2	5.5
35	41	F	88	118	13	90	28.2	32.9	120.46	308	39.11	216.5	6.1	40	F	92	116	7.7	73.6	23.5	28	28.6	445	6.43	7.2	4.9	12.7	89.9	27.6	33.9	116.56	306	38.09	242.1	5.3
36	27	F	86	115	13.2	88.6	27.8	34.1	115.74	315	36.74	198.2	5.5	36	M	90	124	8.2	62	17.7	28.3	22.3	437	5.11	6.2	4.5	13.2	88.7	27.8	34.2	132.54	310	42.75	260.5	5.6
37	29	M	92	124	13.9	94.2	28.5	33.6	123.65	322	38.4	215.6	5.2	27	F	77	96	6.5	62.2	18.2	29.2	20.44	459	4.45	3.9	4.7	12.2	95.5	29.3	32.9	127.45	302	42.2	227.6	5.4
38	38	F	97	114	13.5	95.3	30.5	33.9	131.54	312	42.16	220.4	5.1	34	M	78	89	7.9	60.3	19.2	29.8	16.68	449	3.71	5.8	4.1	13.1	93.8	28.6	33.3	99.76	317	31.47	256.7	5.9
39	40	M	84	135	13.4	96.1	31.6	34.3	130.22	314	41.47	234.5	5.3	29	F	89	97	7.7	59.6	19.4	28.5	19.2	465	4.13	7.4	4.6	12.5	94.6	28.4	34.8	103.24	323	31.96	212.4	5.4
40	23	M	91	119	13.8	86.5	29.3	34.5	120.35	306	39.33	267.3	5.5	37	F	96	130	7.6	65	17.7	27.2	22.2	452	4.91	6.8	4.9	12.6	94.5	29.2	32.9	97.48	301	32.38	207.6	5.5
41	27	F	81	108	13.3	91.8	27.6	33.2	97.48	324	30.08	281.4	5.1	41	F	87	97	4.3	56.7	17.3	29.5	16.3	529	3.08	9.8	4.32	12.1	93.3	28.1	33.5	130.22	324	40.19	243.4	5.8
42	33	M	96	120	13.7	87.6	28.7	33.7	103.24	301	34.29	276.6	5.8	45	M	85	114	7.1	72	21.3	27.6	22.8	417	5.46	7.1	4.6	13.1	89.5	27.5	33.9	115.66	306	37.79	262.3	6.2
43	41	F	88	118	13.2	89.8	27.5	32.8	98.75	323	30.57	209.6	6.1	30	F	90	116	7.5	68	19.3	28.5	29.3	511	5.74	8.2	4.62	12.5	96.1	29.6	32.9	123.46	314	39.31	231.5	6.1
44	29	M	87	104	13.9	94.3	28.4	34.6	127.34	317	40.17	230.1	5.4	29	F	94	135	6.4	67	20.5	29	18.6	426	4.36	7.2	4.8	12.4	95.6	28.7	33.8	131.54	312	42.16	196.7	6
45	20	F	92	122	13.2	96.6	29.4	34.2	134.23	302	44.44	255.4	5.2	33	F	90	122	5.4	67	22.5	27.2	9.35	506	1.85	4.9	4.5	12.2	89.6	28.4	34.2	123.65	322	38.4	212.4	5.9
46	22	M	98	104	13.5	95.4	28.7	33.5	119.62	310	38.58	244.6	5.6	35	M	83	125	7.8	68	19.8	29.1	28.3	384	7.4	7.7	4.2	13.3	93.4	29.1	34.4	115.74	315	36.74	270.4	5.3
47	42	F	93	130	13	93.1	28.1	34.6	127.31	306	41.6	234.6	5	32	F	88	99	6.9	70	20.5	29.2	26.4	435	6.07	6.8	4.24	12.2	95.2	28.4	32.9	120.46	308	39.11	231.3	5.7
48	42	M	86	115	13.4	88.6	28.5	34.2	125.49	322	38.97	228.3	5.5	24	F	87	118	8.3	71.7	21.5	28.3	20.61	465	4.43	5.6	4.6	13.1	87.5	29.7	33.7	107.82	313	34.44	227.5	5.4
49	42	F	96	120	13.7	89.5	29.3	33.3	107.66	315	34.17	212.4	5.1	46	M	94	130	5	55	14.8	26.7	15.37	472	3.25	5.6	4.56	13	88.4	29.6	33.4	115.64	303	38.16	282.1	6.1
50	40	F	95	134	13.2	91.4	27.8	33.7	112.43	321	35.02	188.9	5.4	27	F	90	126	6	61.7	18.8	27.4	21.8	392	5.56	7.5	4.7	12.1	92.7	28.1	34.3	127.34	310	41.07	199.4	6.3
51	26	M	87	100	13.8	88.9	28.9	34.1	126.87	309	41.05	230.6	5.7	20	F	88	96	7.3	59.9	17.4	29	24.92	428	5.82	6.2	4.3	12.5	91.6	27.7	32.9	99.78	312	31.98	220.3	5.4
52	30	F	95	130	13.2	94.3	28.2	33.9	109.54	318	34.44	218.8	5.3	37	F	98	124	6.8	65	22.3	27.6	25.44	433	5.87	7.6	4.4	12.3	94.3	27.6	33.1	121.22	305	39.74	252.4	5.9
53	46	M	82	118	13.4	92.2	27.8	33.4	110.72	321	34.49	276.5	5.2	32	M	92	132	7.2	70	23.9	28.2	26.1	426	6.12	5.4	4.2	13.2	89.4	27.9	33.5	130.41	318	41	275.4	5.8
54	29	M	85	99	13.6	88.6	28.5	33.8	105.46	330	31.95	266.2	5.8	31	F	86	94	6.2	66.8	20.4	26.5	18.6	436	4.26	6.9	4.31	12	88.6	28.5	33.8	134.54	311	43.26	221.5	5.4
55	21	F	96	125	13.3	89.4	27.9	33.5	99.85	314	31.79	195.7	5.2	30	F	87	108	6.4	76.2	24.3	28.7	18.88	429	4.4	7.2	4.5	12.2	92.2	27.8	33.4	124.78	306	40.77	208.4	5.6
56	25	F	90	122	13.9	94.3	27.5	32.9	98.64	302	32.66	208.6	5.5	24	F	86	104	6.8	59.7	16.2	27.2	28.36	436	6.5	6.7	4.3	12.1	94.3	28.2	33.9	100.52	321	31.31	271.3	5.5
57	30	F	85	135	13.3	91.6	27.3	32.8	88.97	325	27.37	178.6	5.1	26	F	96	127	7.1	57.5	16.9	29.4	26.9	460	5.84	7.6	4.6	12.6	88.9	28.9	34.1	126.53	303	41.75	234.2	6.2
58	24	F	96	116	13.2	92.7	28.1	34.3	112.78	305	36.97	214.4	5.7	21	F	85	105	6.7	66.1	19.4	27.8	22.5	418	5.38	5.7	4.4	12.1	91.4	27.8	33.7	114.96	323	35.59	255.4	6.1
59	41	M	86	114	13.4	88.4	29.6	33.4	124.66	312	39.95	232.6	5.6	34	F	90	116	7.5	58.6	16.8	28.6	19.6	475	4.12	8.8	4.6	12.4	89.5	29.3	33.3	119.65	320	37.39	211.3	5.4
60	19	M	87	97	13.5	87.5	29.7	33.7	119.12	306	38.92	244.5	4.9	47	M	96	115	5.3	52	13	24.9	25.2	422	5.97	7.4	4.2	13.1	88.6	28.5	34.2	122.75	311	39.46	266.2	5.5

CONTROL GROUP														STUDY GROUP PRE CORRECTION											STUDY GROUP POST CORRECTION								
SLNO_C	AGE_C	SEX_C	FBS_C	PPBS_C	Hb_C	MCV_C	MCH_C	MCHC_C	SIRON_C	TIBC_C	ISATN_C	FERRITIN_C	HbA1C_C	AGE_S	SEX_S	FBS_S	PPBS_S	Hb_S1	MCV_S1	MCH_S1	MCHC_S1	SIRON_S1	TIBC_S1	ISATN_S1	FERRITIN_S1	HbA1C_S1	Hb_S2	MCV_S2	MCH_S2	MCHC_S2	SIRON_S2	TIBC_S2	ISATN_S2	FERRITIN_S2	HbA1C_S2
61	28	F	86	130	12.8	95.2	28.4	32.9	130.65	303	43.11	253.12	5.8	33	F	85	124	7.7	64.5	19.8	29.7	22.3	415	5.37	8.2	4.9	12.5	93.1	28.1	34.5	131.32	307	42.77	233.1	5.8
62	42	M	92	97	13.8	93.3	29.1	34.4	127.62	312	40.9	246.1	5.5	27	F	82	91	8.1	64.2	20	28.3	24.1	447	5.39	6.8	4.6	12.8	95.4	28.7	33.5	110.34	315	35.02	206.2	5.9
63	40	F	98	129	13.4	89.6	28.4	34.2	133.4	310	43.03	228.7	5.4	37	F	95	135	4.1	55	14.7	27	5.12	483	1.06	7.4	4.1	12.1	96.6	29.4	34.2	123.69	312	39.64	195.6	6.4
64	20	F	88	106	13.2	95.6	28.7	33.8	126.85	309	41.05	232.6	5.9	45	F	87	114	6.6	57.4	16.4	28.5	22.2	456	4.86	3	4.7	12.2	94.3	28.4	34.1	126.85	309	41.05	207.5	6.1
65	39	F	90	124	12.8	97	29.6	32.9	123.69	312	39.64	220.8	5.4	36	M	95	109	4.3	54.2	14.8	27.3	15.8	405	3.9	7.2	4.4	13.2	89.8	27.5	32.9	133.4	310	43.03	258.4	6.4
66	28	F	94	116	13	90.4	27.6	33.8	110.32	315	35.02	192.6	5.2	30	F	96	120	7	64.3	17.8	27.7	26.51	388	6.83	6.4	4.6	12.3	93	28.3	33.1	127.62	312	40.9	223.8	5.6
67	39	M	87	98	13.4	88.7	29.1	34.5	131.32	307	42.77	270.3	5.9	45	M	86	127	7.7	62.4	19.1	29.5	18.6	426	4.36	6.9	5.1	13.3	93.7	27.2	33.2	130.65	303	43.11	276.9	5.7
68	27	M	88	91	13.2	91.4	28.8	34.2	122.79	311	39.48	246.7	5.3	48	M	93	118	6.5	63	17.6	28.1	18.41	435	4.24	5.3	5.2	13	87.4	28.4	34.7	119.12	306	38.92	245.3	5.3
69	23	M	83	98	13.9	92.3	28.3	33.2	119.65	320	37.39	240.8	5.7	39	M	82	93	8.2	65	17.9	27.7	28.37	418	6.78	5.9	4.8	13.2	91.4	27.8	33.5	124.66	312	39.95	224.8	5.8
70	26	F	80	126	13.6	87.6	29.3	32.7	114.98	323	35.59	212.7	5.4	31	F	81	104	7.6	58	16.5	28.7	13.54	503	2.69	7	4.3	12.6	86.5	27.6	34.2	112.78	305	36.97	235.3	5.5
71	36	M	94	120	13.8	88.4	29.1	33.3	125.63	303	41.46	228.5	5.6	40	M	90	130	4.2	49	13.5	27.8	28.5	450	6.33	5.6	4.5	13.1	89.3	28.1	32.8	88.97	325	27.37	197.6	5.9
72	28	F	87	108	12.9	92.5	28.2	34.7	100.45	321	31.29	187.5	5.1	28	F	94	122	6.6	63	16.2	26	21.9	428	5.11	9.1	4.7	12.4	91.7	27.8	33.2	98.64	302	32.66	217.3	6.5
73	38	M	82	114	13.6	89.8	28.7	33.9	124.78	306	40.77	225.4	5.5	49	F	78	97	5.3	66.7	21.1	29.6	17.2	390	4.41	5.9	4	12.1	94.2	28.4	34.1	99.86	314	31.8	245.6	6.3
74	34	M	91	102	13.4	89	28.3	33.4	134.54	311	43.26	265.4	5.6	37	F	86	102	7.8	62.3	18	28.8	24.35	404	6.02	6.7	4.8	12.5	93.6	29.3	33.7	105.46	330	31.95	273.1	6
75	22	F	90	97	12.8	94.5	28.5	34.2	130.41	306	42.61	284.2	5.4	28	F	91	130	5.8	52.9	15.4	29.1	18.3	465	3.93	7.2	4.6	12	90.5	27.5	34.2	110.72	321	34.49	254.2	5.9
76	28	F	78	99	13.8	91.5	27.9	34.6	130.63	324	40.31	273.5	5.9	42	M	83	123	7.2	65.3	19.9	29.4	20.4	440	4.63	7.4	5	13.3	93.6	28.2	33.6	132.56	311	42.62	260.5	5.6
77	19	F	93	122	13	87.6	27.1	34.5	128.2	305	42.03	265.78	5.5	37	M	85	114	6.4	61.1	20.3	28.9	21.6	502	4.3	5.6	4.5	13.1	87.6	27.1	34.5	123.65	306	40.4	255.6	5.8
78	25	M	87	112	13.5	93.6	28.2	33.6	120.77	310	38.95	256.6	5.4	32	F	89	126	6.7	65.5	22.4	27.7	22.9	455	5.03	7.8	4.6	12.3	91.5	27.9	34.3	98.41	312	31.54	225.2	5.6
79	37	F	85	95	13.1	90.5	27.5	34.2	108.46	322	33.68	188.6	5.7	44	F	81	112	8.1	68	19.1	28.3	25.2	425	5.92	6.6	4.7	12.9	94.5	28.5	34.5	120.46	310	38.85	198.1	6.3
80	42	F	95	118	13.2	93.6	29.3	33.7	124.65	310	40.2	246.7	5.3	36	F	96	117	6.3	72	21.6	29	20.7	446	4.64	6.4	5.1	12.3	89.8	28.3	33.4	108.98	303	35.96	267.3	6.2
81	27	M	96	134	13.6	94.2	28.4	34.1	135.64	301	45.06	250.8	5.1	31	F	95	126	7.1	63.7	22.3	28.5	19.8	522	3.79	7.7	4.2	12.5	88.7	28.7	33.9	119.22	332	35.9	231.6	6
82	36	M	81	124	14	91.7	27.8	33.3	128.74	312	41.26	261.2	5.5	42	M	85	105	6.4	65.6	24.3	28.5	21.3	435	4.89	8.6	4.5	13.4	92.5	28.3	34.1	126.78	321	39.49	272.4	5.5
83	38	F	89	130	13.9	89.3	28.1	32.8	123.88	322	38.47	232.4	5.3	27	F	87	108	7	70.2	24	27.6	19.9	438	4.54	7.5	4.4	12.2	88.4	29.1	33.3	125.43	310	40.46	252.3	5.8
84	20	F	85	128	12.8	85.6	27.6	34.2	109.36	313	34.93	253.34	5.2	36	F	93	120	6.1	62.7	22.8	27.9	18.4	414	4.44	8	4.8	12.1	87.6	29.3	32.7	118.74	312	38.05	212.5	5.9
85	27	M	83	130	13.8	91.4	27.8	33.5	130.67	325	40.2	198.7	5.7	33	F	78	106	6.9	64.4	19.5	26.6	19.5	388	5.02	6.4	4.1	12.3	92.3	28.3	33.2	136.54	309	44.18	245.6	5.4
86	38	F	91	115	13.2	87.4	28.4	34.7	131.42	307	42.8	276.4	5.8	23	F	90	96	7.3	75	21.4	27.4	21.5	428	5.02	7.6	4.7	12.6	91.4	28.8	34.2	140.12	306	45.79	228.5	5.5
87	30	M	86	97	13.4	93.7	27.2	33.2	120.45	326	36.94	246.5	5.5	42	M	91	135	8.2	73.2	23.5	27.6	26.1	452	5.77	7.3	4.6	13.2	88.7	29.1	33.7	120.45	326	36.94	235.6	5.8
88	37	M	78	102	13.6	93	28	33.1	140.12	306	45.79	236.8	5.3	40	F	82	98	7.1	74.7	24.5	28.2	25.3	466	5.42	8.5	4.5	12.5	90.4	27.6	33.8	131.42	307	42.8	210.8	5.9
89	44	F	94	116	13.9	89.5	27.8	34.2	136.54	309	44.18	229.7	5.8	19	F	87	109	6	64.8	18.9	24.6	19.1	408	4.68	7	4.24	12.1	93.5	28.4	33.6	130.67	325	40.2	197.7	5.7
90	36	F	90	135	12.8	84.7	27.4	34.4	118.7	312	38.04	241.2	5.7	45	M	94	127	6.7	67.9	20.8	26.5	18.6	435	4.27	6.2	4.8	13.3	95.4	27.7	33.4	109.36	313	34.93	270.9	6.2
91	29	M	81	105	13.8	87.5	28.7	33.5	125.43	310	40.46	235.7	5.6	27	F	79	112	7.4	76.3	22.4	27.8	20.5	464	4.41	6.7	4.2	12.6	94.3	27.3	34.3	123.88	322	38.47	261.4	5.7
92	21	M	93	127	13.4	88.4	28.4	33.8	127.62	321	39.75	218.6	5.4	29	F	80	96	7.2	64	18.7	26.1	21.9	429	5.1	7.5	4.53	12.5	85.6	27.8	33.8	108.48	312	34.76	225.8	6.1
93	33	M	80	98	13.9	90.5	27.8	32.8	119.22	332	35.9	253.4	5.5	21	F	86	130	6.2	67.8	20.4	26.6	17.2	390	4.41	9.2	4.7	12.3	88.9	28.5	33.3	128.74	301	42.77	247.6	6.3
94	26	M	85	128	13.5	88.6	28.5	33.4	108.98	303	35.96	267.2	5.1	36	M	90	134	5.9	64.5	23.5	28.2	20.1	424	4.74	6.3	4.2	13.1	87.7	27.3	34.5	135.64	310	43.75	258.7	5.4
95	27	F	92	109	13	86.3	26.7	34.1	120.46	310	38.85	227.5	5.5	30	F	82	115	6.6	58.9	19.8	24.6	22.1	472	4.68	6.8	4.6	12.2	90.5	27.8	34.2	124.65	322	38.71	216.3	5.8
96	35	F	90	132	13.2	90.8	27.9	33.9	98.41	312	31.54	204.4	5.3	42	M	93	106	7.8	60.4	23.7	28.6	23.35	460	5.07	5.2	4.18	13.4	91.4	28.1	33.1	120.75	307	39.33	244.3	5.9
97	44	F	84	110	13.1	92.1	28.1	35.2	123.65	306	40.4	254.3	5.6	29	F	87	98	8	72.1	24.6	27.1	24.2	444	5.45	5.5	5.12	12.6	87.6	27.4	33.9	128.23	315	40.7	208.6	6
98	20	M	90	128	13.7	91.6	27.7	34.6	132.56	312	42.48	272.4	4.9	24	F	80	105	8.1	75.4	22.6	27.8	22.6	502	4.5	8.3	4.5	12.9	88.6	26.7	34.2	130.63	324	40.31	243.5	5.5
99	29	M	87	120	13.6	90.4	28.3	33.8	130.34	304	42.87	268.6	5.6	29	F	86	126	6.1	68.7	21.7	26.2	19.8	398	4.97	6.6	4.9	12.3	90.2	27.5	33.8	122.34	305	40.11	223.8	5.6
100	46	F	95	108	13.2	89.3	27.2	34.5	107.28	330	32.5	232.5	5.8	36	M	89	118	7	68.2	19.6	27.1	20.8	452	4.6	7.1	4.6	13.2	89.3	27.2	34.4	128.56	322	39.92	262.4	5.3
101	30	M	92	133	13.8	90.2	27.5	33.9	122.56	307	39.92	231.2	5.5	31	F	90	98	7.7	68.6	23.2	28.8	21.4	422	5.07	5.8	5	13.1	90.4	28.3	33.8	119.86	306	39.16	234.4	6.2
102	36	F	78	96	13	88.6	26.7	34.2	95.43	334	28.57	198.4	5.1	44	M	94	130	6.3	62.8	21.4	28.4	13.78	462	2.98	3.8	5.1	13	91.6	27.6	33.9	120.75	322	37.5	227.6	6.1
103	38	F	93	122	13.3	87.5	27.4	34.9	102.78	323	31.82	216.9	5.4	26	F	93	126	5.9	50.8	16.4	27.8	6.8	540	1.25	6.9	4.3	12.1	92.1	28.1	34.5	97.87	311	31.46	260.2	5.7
104	45	M	94	116	14	91.4	28.1	33.1	109.88	315	34.88	230.7	5.6	47	M	78	105	6.3	58.4	29.2	27.5	14.6	436	3.34	4.6	4.56	13.1	90.8	27.9	33.6	104.56	334	31.3	272.1	6.4
105	29	F	90	104	12.8	90.5	27.8	34.6	99.75	312	31.97	224.6	5.8	22	F	92	97	5.6	62	19.5	26.8	13.35	506	2.63	2.9	4.5	12.2	87.3	26.7	34.1	88.72	326	27.21	255.4	5.6
106	33	F	89	115	12.9	87.7	27.3	34.5	124.22	330	37.64	265.4	5.5	28	M	95	114	6.2	68.8	22.5	28.2	24.6	425	5.78	7.2	4.9	13.2	88.6	28.5	33.4	104.56	332	31.49	248.2	5.5
107	27	M	86	99	13.5	88.9	28.5	33.3	126.75	315	40.23	255.2	5.1	39	M	87	127	7.8	72.5	20.5	28	20.13	445	4.52	9.6	5.1	13.4	90.5	27.8	32.8	89.76	306	29.33	203.4	5.6
108	23	F	80	106	13.4	85.6	27.8	33.8	98.9	322	30.71	232.8	5.7	40	F	90	124	5.9	66.9	17.9	26.7	16.4	463	3.54	6.8	4.7	12.1	88.4	28.4	33.8	133.52	310	43.07	226.5	5.9
109	20	M	87	130	13.9	94.3	27.3	34.3	133.55	302	44.22	246.7	5	30	F	84	96	7.6	69.8	21.6	27.8	21.16	395	5.35	9.3	4.9	12.7	87.5	28.7	33.5	136.72	319	42.85	212.4	5.7
110	44	M	93	132	13.8	95.6	27.7	33.4	115.48	319	36.2	234.6	4.9	36	F	90	133	6.8	71.3	20.2	28.3	24.6	428	5.74	5.9	4.5	12	86.4	27.4	34.4	115.48	302	38.23	205.7	6.1
111	39	M	82	96	13.7	93.5	28.4	33.8	136.71	312	43.81	228.9	5.8	23	F	92	132	6.6	68.3	21.4	27.9	18.22	466	3.9	7.4	4.42	12.3	89.5	27.7	34.2	133.53	312	42.79	198.3	6.2
112	29	M	90	124	14	95.3	27.8	34.1	134.52	306	43.96	264.3	5.6	27	F	85	107	6.7	59.8	16.4	27.7	22.65	406	5.57	5.6	4.65	12.2	90.6	28.1	33.2	98.9	315	31.39	233.4	5.7
113	19	F	86	127	13.2	86.4	27.1	33.2	88.72	332	26.72	206.5	5.1	43	M	80	99	7.2	60.4	18.6	28.2	23.1	417	5.53	7.2	4.3	13.2	93.2	27.2	33.5	126.75	330	38.4	271.9	5.5
114	41	F	80	114	12.8	89.2	27.5	33.6	104.56	326	32.07	238.7	5.2	32	F	93	115	8.2	72	20.2	28.3	25.9	460	5.63	4.9	4.7	12.9	89.7	28.2	33.8	124.22	312	39.81	214.6	6.4
115	30	F	96	109	13	92.9	26.8	32.8	97.87	334	29.3	232.3	5.4	45	M	95	104	5.8	69.3	19.6	27.4	18.8	412	4.56	6.9	4.6	13.1	95.1	27.8	33.5	99.75	315	31.66	217.3	5.9
116	45	M	94	105	13.6	93.5	27.5	34.4	120.7	311	38.81	221.4	5.6	36	M	90	95	7.5	68.6	23	28.5	25.5	458	5.56	8	4.9	13.5	93.5	27.5	34.3	109.88	323	34.01	236.4	5.3
117	43	M	88	126	13.9	95.1	27.8	33.5	118.92	322	36.93	233.8	5.3	28	F	86	122	7.1	58.6	16.8	28.6	22.93	425	5.39	6.2	4.8	12.4	92.9	26.8	32.8	102.78	334	30.77	206.4	5.4
118	20	M	86	130	14	89.7	28.2	33.8	128.56	307	41.87	272.1	4.9	20	F	88	108	7	55.4	15.6	28.1	21.6	562	3.84	8.8	4.5	12.1	89.2	27.5	33.6	95.43	307	31.08	228.3	6.1
119	23	F	90	98	13.1	93.2	27.2	33.5	100.42	340	29.53	220.24	5.5	35	M	94	96	5.4	59	16.2	27.6	20.06	478	4.19	9.1	4.72	13.1	86.4	27.1	33.2	122.56	330	37.13	196.5	5.6
120	36	M	95	118	13.8	90.6	28.1	33.2	122.3	305	40.09	264.4	5.4	48	M	96	133	7.7	75.4	21.6	28.3	23.66	396	5.97	8.6	5.1	13.4	95.3	27.8	34.1	127.22	304	41.84	271.2	6.3