A STUDY ON

"EFFECT OF MICROCYTIC ANAEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NON- DIABETIC ADULTS"

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BRANCH -I

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DEPARTMENT OF GENERAL MEDICINE,

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BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"EFFECT OF MICROCYTIC ANAEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NON- DIABETIC ADULTS"** is a bonafide work done by Dr.S.Anburajan, post graduate student, department of General Medicine, Stanley Medical College & Hospital, Chennai - 600001, in partial fulfillment of the requirement for the award of degree of M.D General Medicine (Branch-I), carried out by him under direct supervision and guidance, during the academic year 2020 – 2023.

GUIDE

Prof. Dr.S.CHANDRASEKAR. M.D.
Unit Chief,
Department of General Medicine,
Stanley Medical College & Hospital,
Chennai - 600001.

PROFESSOR
DEPARTMENT OF GENERAL MEDICINE
GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL

HOD

Prof.Dr.S.PARIMALA SUNDARI M.D.,
Head of the Department,
Department of General Medicine,
Stanley Medical College & Hospital,
Chennai - 600001.

Professor and Head of
Department of Medicine,
Govt. Stanley Medical College & Hospital
Chennai - 600 001

Prof. Dr. P. BALAJI M.S., FRCS., Ph.D., FCLS.
Dean,
Government Stanley Medical College and Hospital,
Chennai.

DEAN
STANLEY MEDICAL COLLEGE
CHENNAI - 600 001

DECLARATION

I solemnly declare that the dissertation titled **"EFFECT OF MICROCYTIC ANAEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NON- DIABETIC ADULTS"** is a bonafide work done by me at Government Stanley Hospital, Chennai between March 2021 and September 2021 under the guidance and supervision of Prof. Dr. S. Chandrasekar, M.D. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any reward, degree or diploma to any other university or board either in India or abroad. This dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University, towards the partial fulfillment of requirement for the award of M.D. Degree in General Medicine (Branch – I).

S. Anburajan

Dr. S. Anburajan,

Post Graduate student,

M.D General Medicine,

Government Stanley Medical College

Register Number: 200120101002,

Chennai – 600001.

CERTIFICATE - II

This is to certify that this dissertation work titled **"EFFECT OF MICROCYTIC ANAEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NON- DIABETIC ADULTS"** of the candidate **Dr.S.Anburajan** with Registration Number - 200120101002 for the award of M.D. DEGREE in the branch of GENERAL MEDICINE (BRANCH-I), for the academic year 2020 - 2023. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains, from introduction to conclusion pages and result, shows 5 percentage of plagiarism in the dissertation.

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Prof.Dr.S.CHANDRASEKAR.M.D

Unit Chief,

Department of General Medicine,

Stanley Medical College & Hospital,

Chennai.

PROFESSOR
DEPARTMENT OF GENERAL MEDICINE
GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL

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Page | 11 INTRODUCTION HbA1c was introduced as a marker of the glycemic control status of the patient over the last 2-3 months and has evolved a long way as a diagnostic modality for diabetes approved by the WHO and American Diabetes Association (ADA). Many studies have discovered that the level of HbA1c can be altered by various other factors other than blood glucose levels. Few of such factors are the quantity of iron in the blood and the life span of the RBC. So, iron deficiency anaemia, which is the most common cause of the microcytic anaemia 1 plays a significant role in the level of HbA1c. According to World Health Organization (WHO), diabetes is among the top 10 leading causes of mortality worldwide 2 . According to the International Diabetes Foundation data 2021 3 , India has the 2 nd highest number of people with diabetes only next to China, than in any other country in the world. Thus, India was aptly named "the diabetes capital of the worldll 4 . By 2021, around 74.2 million Indians are affected by Diabetes, which comes to nearly 8.03% of India's adult Population. It is estimated that diabetes will affect nearly 125 million individuals by 2045 3 . Diabetes contributes to death of nearly half a million to 1 million Indians every year. Clinically evident diabetes becomes manifest around 42.5 years which corresponds to the peak of productive years of life. All these data promptly highlight the burden imposed by the diabetes on the Indian economy. This raises the issue of early diagnosis and prompt treatment of diabetes in order to prevent the development of complications and thereby improving

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ABBREVATIONS

ADA	American Diabetes Association
ADAG	A1c Derived Average Glucose
AGE	Advanced Glycation End Products
ATP	Adenosine Tri Phosphate
CBC	Complete Blood Count
CRP	C – Reactive Protein
DHS	Demographic And Health Survey
DIC	Disseminated Intravascular Coagulation
DM	Diabetes Mellitus
DMT	Divalent Metal Transporter
DNA	Deoxyribo Nucleic Acid
EPO	Erythropoietin
Hb	Hemoglobin
HbA1c	Hemoglobin a1c (glycosylated hemoglobin)
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
IDA	Iron Deficiency Anemia
LMW	Low Molecular Weight
MCHC	Mean Corpuscular Hemoglobin

	Concentration
MCV	Mean Corpuscular Volume
NFHS	National Family Health Survey
PS	Peripheral Smear
RBC	Red Blood Cell
RDA	Recommended Dietary Allowances
RDW	Red-Cell Distribution Width
RE	Reticulo – Endothelial Cells
SPSS	Statistical Package For Social Sciences
TfR	Transferrin Receptor
TIBC	Total Iron Binding Capacity
TRP	Transferrin Receptor Protein
TTP	Thrombotic Thrombocytopenic Purpura
WHO	World Health Organization

"EFFECT OF MICROCYTIC ANAEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NON- DIABETIC ADULTS"

ABSTRACT:

BACKGROUND: Many studies have shown that the level of HbA1c can be altered by various other factors apart from blood glucose values. One such factor is the quantity of iron in the blood. So, iron deficiency anemia, which is the most common of the microcytic anemias plays a significant role in the measurement of HbA1c. Thus, India being the diabetic capital of the world, along with microcytic anemia prevailing so common in the community, needs certain measures to prevent false reporting of diabetic cases.

METHODS: We did a case control study comprising of 30 cases and 30 controls. Anemic patients were taken as cases while age and gender matched non anemic patients were taken as controls. Mean HbA1c were measured in them and the difference is evaluated.

RESULTS: The difference between the mean HbA1c values of cases and controls is 1.39%. The HbA1c values were significantly higher in the cases group than the control group which is statistically significant with a 'p' value of < 0.001.

CONCLUSION: Based on our study we can safely conclude that microcytic anemia definitely has an impact on the HbA1c. As MCV decreases in patients with microcytic anemia, HbA1c tends to rise. Thus, microcytic anemia, if present must be corrected before making any diagnostic or therapeutic decision based on the HbA1c levels.

KEY WORDS: Hba1c, microcytic anemia.

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Introduction

INTRODUCTION

HbA1c was introduced as a marker of the glycemic control status of the patient over the last 2-3 months and has evolved a long way as a diagnostic modality for diabetes approved by the WHO and American Diabetes Association (ADA). Many studies have discovered that the level of HbA1c can be altered by various other factors other than blood glucose levels. Few of such factors are the quantity of iron in the blood and the life span of the RBC. So, iron deficiency anaemia, which is the most common cause of the microcytic anaemia[1] plays a significant role in the level of HbA1c.

According to World Health Organization (WHO), diabetes is among the top 10 leading causes of mortality worldwide[2]. According to the International Diabetes Foundation data 2021[3], India has the 2nd highest number of people with diabetes only next to China, than in any other country in the world. Thus, India was aptly named "the diabetes capital of the world"[4]. By 2021, around 74.2 million Indians are affected by Diabetes, which comes to nearly 8.03% of India's adult Population. It is estimated that diabetes will affect nearly 125 million individuals by 2045[3]. Diabetes contributes to death of nearly half a million to 1 million Indians every year. Clinically evident diabetes becomes manifest around 42.5 years which corresponds to the peak of productive years of life.

All these data promptly highlight the burden imposed by the diabetes on the Indian economy. This raises the issue of early diagnosis and prompt treatment of diabetes in order to prevent the development of complications and thereby improving

the quality of life of the population. Investigations for the early detection and management of diabetes are an ever evolving field of medicine. HbA1c is one such parameter which has become an important measure in the diagnostic criteria for diabetes.

Thus, India being the 2nd in diabetic population, along with microcytic anaemia prevailing so common in our community, there arises a need for certain measures to prevent false reporting and over treatment of diabetic cases.

Thus, the main aim of our study was to determine whether the presence of anaemia, especially microcytic hypochromic anaemia has any effect on the level of HbA1c levels. If so, then the anaemia needs to be corrected before taking any diagnostic or therapeutic decision.

Aim and Objectives

AIM AND OBJECTIVES

AIM:

- To analyse the effect of microcytic hypochromic anaemia on HbA1c levels in non-diabetic individuals.

OBJECTIVES:

- To estimate HbA1c levels among non-diabetic adults with microcytic anaemia.
- To compare it with HbA1c levels among age and gender matched non-diabetic controls and thereby establishing a correlation between MCV and the levels of HbA1c, if any.

Review of Literature

REVIEW OF LITERATURE

ANAEMIA:

1. DEFINITION:

It is defined as a reduction in the oxygen carrying capacity of the blood, which usually stems from a decrease in red cell mass to subnormal levels leading to inability to meet the physiological needs. The needs may vary according to age, sex, growth phases, altitude above sea level, pregnancy status and thus varying cut offs are used to define anaemia in various subgroups. Severity of anaemia is best considered in terms of blood hemoglobin concentration. The normal range of the hemoglobin concentration varies among the laboratories. WHO cut off of anemia is as follows[5]:

- Males - <13.0 g/dL
- Females - < 12.0 g/dL
- Pregnant females - < 11.0 g/dL

2. EPIDEMIOLOGY:

Anaemia is a major public health problem affecting populations in both rich and poor countries. It is generally seen in people of all age groups, but pregnant women and children were the most commonly affected population. Globally, iron deficiency remains the most significant contributor to the onset of anaemia as to the extent that Iron Deficiency Anaemia (IDA) and anaemia are often used synonymously[6]. Even the prevalence of anaemia has often been used as a proxy for IDA.

Though the exact estimation of proportion of iron deficiency anaemia cases is

done only in few countries, there is a generalized assumption that 50% of the cases are due to iron deficiency. This varies among different population groups and in different areas. The prevalence of IDA is on the raise recently. The risk factors contributing to this are dietary deficiency of iron, consumption of phytate rich diet which hinders with the absorption of dietary iron, and phases of life when iron requirements are especially high like pregnancy, early stages of growth, puberty not being given adequate iron supplementations. The presence of associated micronutrient deficiencies, like vitamins B12, folate, riboflavin, and copper adds on to the problem. Among the middle and old aged adults, occult malignancies causing chronic gastro-intestinal blood loss is a major risk factor.

Numerous other factors can be enlisted to be contributing significantly to anaemia:

- Poor purchasing power, illiteracy, ignorance regarding nutritional value of available cheap food.
- Cultural taboos, superstition.
- Discrimination faced by women eating only the 'last food' or the remaining food, heavy blood loss during menstruation.
- Parasitic infestations with hookworms, ascariasis, and schistosomiasis.
- Acute infections like malaria and chronic infections including cancer, tuberculosis, and HIV can also lower blood Hemoglobin concentrations.
- The presence of hemoglobinopathies in certain populations

As the optimal values of hemoglobin varies among people in different age groups and their physiologic states, WHO used various threshold of hemoglobin to classify individuals living at sea level as anaemic.

The threshold and the prevalence of anaemia are as follows:

Age or gender group	Haemoglobin threshold (g/l)
Children (0.50–4.99 yrs)	110
Children (5.00–11.99 yrs)	115
Children (12.00–14.99 yrs)	120
Non-pregnant women (≥15.00 yrs)	120
Pregnant women	110
Men (≥15.00 yrs)	130

Figure 1. Hemoglobin threshold used to define anaemia according to WHO

Estimates from various countries were combined to calculate the prevalence at the global level. This pooled data were also extended to the WHO region for women and preschool-age children by considering the weight given by these estimates to the population it represented. South East Asia and Africa tops the WHO regions in anaemia prevalence with values of 45.7% & 47.5% respectively while it is least in North America with only 1.4% of the total morbidity and mortality associated with iron deficiency. IDA accounts for nearly a million deaths annually worldwide.

INDIAN SCENARIO:

While WHO estimates provide a clue about the global picture, data from NFHS (National Family Health Survey -5)[7] provide estimates about the burden IDA poses on the country. In India, prevalence of anaemia is more in women than in men. About more than twice women are affected than men (57% vs. 25% respectively). Half of these anaemic women suffer from mild to moderate degree of anaemia.

Hemoglobin levels below which women and men are considered anaemic in

National Family Health Survey – 5 are (NFHS – 5):

- Non – pregnant women aged 15 – 49 years: < 11.0 g/dL.
- Pregnant women aged 15 – 49 years: < 12.0 g/dL.
- Men aged 15 – 49 years: < 13.0 g/dL.

Patterns observed in the trend of anemia based on background characteristics in NFHS – 5 are as follows:

- The overall prevalence of anaemia is consistently high (when compared to previous NFHS data), at more than 50 percent, in almost all of the subgroups of women. For men, the prevalence is above 20 percent in almost all of the subgroups.
- Anaemia varies by maternity status - 61 percent of women who are breastfeeding are anaemic, compared with 52 percent of women who are pregnant and 57 percent of women who are neither pregnant nor breastfeeding.
- The prevalence of anaemia generally decreases with schooling, from 59 percent among women with no schooling to 52 percent among women with 12 or more years of schooling. Across the same schooling groups, the prevalence of anaemia among men decreases from 32 percent to 19 percent.
- The proportion of anaemic women and men declines steadily as the wealth of the household increases (from 64% in the lowest wealth quintile to 51% in the highest wealth quintile among women and from 36% in the lowest wealth quintile to 18% in the highest wealth quintile among men).
- Women in urban areas are slightly less likely to be anaemic (54%) than those in rural areas (59%). The difference is larger for the prevalence of anaemia in men (27% in rural areas versus 20% in urban areas).

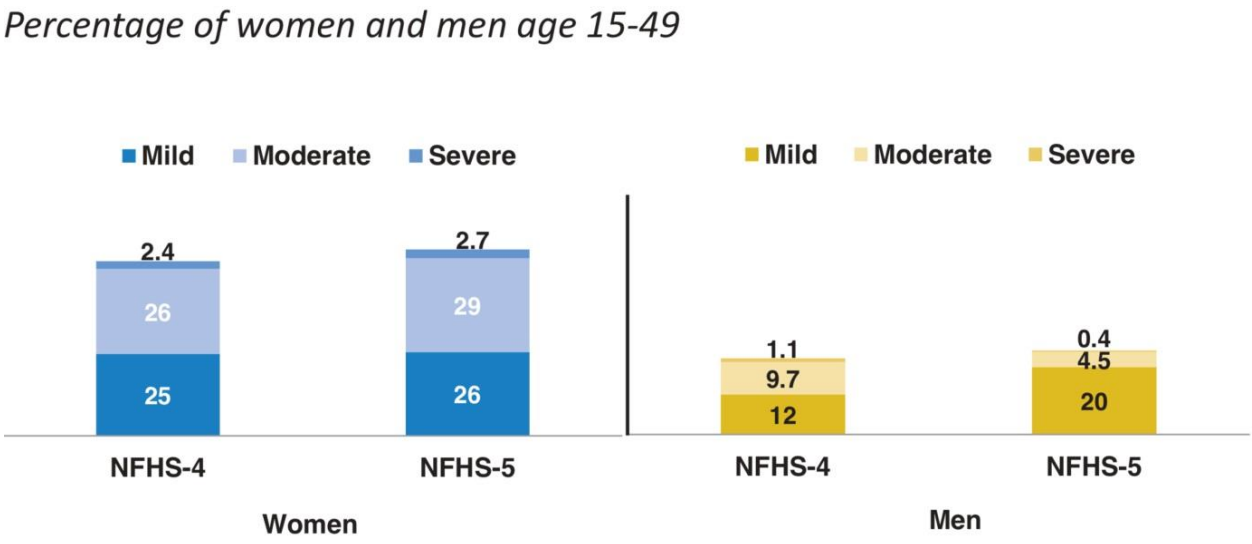


Figure 2. Trends in anaemia status.

The prevalence of anaemia among women is 60 % or more in Chhattisgarh, Bihar, Odisha, Gujarat, Jharkhand, Assam, Tripura and West Bengal. The prevalence is less than one-third in Lakshadweep (26%), and Nagaland and Manipur (29% each). The prevalence of anaemia is also very high in the union territories of Ladakh (93%), Jammu & Kashmir (66%), Dadra & Nagar Haveli and Daman & Diu (63%), and Chandigarh (60%).

Anaemia prevalence in men is highest in Ladakh (76%), West Bengal (39%), Jammu & Kashmir (27%), and Assam (36%), and it is lowest in Lakshadweep and Manipur (6% each) and Chandigarh (9%).

In Tamil Nadu, the prevalence of anemia among women aged 15 – 49 years is 53%. The prevalence is slightly lesser among pregnant women (48%) when compared with non – pregnant women (53%).

These statistical data from the global level to Indian level clearly portrays the burden of anemia, especially iron deficiency anemia on the community.

3. CLASSIFICATION:

Anaemia can be classified based on:

- Morphology
- Pathophysiology

MORPHOLOGICAL CLASSIFICATION:

The morphological classification of anaemia as normocytic, microcytic, or macrocytic often correlates well with the cause of anaemia. The degree of haemoglobinisation reflected in the colour of the cells (normochromic or hypochromic) and the shape of the cells are the other indices used to classify anaemia. The main advantages of this classification are that the classification is simple, is based on readily available red cell indices, for example, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), and forces the physician to consider the most important types of curable anemia: vitamin B12, folic acid, and iron-deficiency anemias. Such practical considerations have led to wide acceptance of this classification.

Visual inspection of peripheral smears can be used as a subjective measure of classifying anaemia while they are quantitatively expressed through the following RBC indices:

• Mean Corpuscular volume (MCV):

It is the average red cell volume, expressed in femtoliters (fL).

• Mean cell hemoglobin (MCH):

It is the average hemoglobin content (mass) per red cell, expressed in picograms (pg).

• Mean cell hemoglobin concentration (MCHC):

It is the average hemoglobin concentration in a given volume of packed red cells, expressed in grams per decilitre (g/dL). Lower MCH and MCHC show hypochromia.

• Red cell distribution width (RDW):

This signifies the coefficient of variation of red cell volume. Red cell volume distribution width (RDW) reflects the variation in the size of Red Blood Cells.

	Units	Men	Women
Hemoglobin (Hb)	g/dL	13.2–16.7	11.9–15.0
Hematocrit (Hct)	%	38–48	35–44
Red cell count	$\times 10^6/\mu L$	4.2–5.6	3.8–5.0
Reticulocyte count	%	0.5–1.5	0.5–1.5
Mean cell volume (MCV)	fL	81–97	81–97
Mean cell Hb (MCH)	pg	28–34	28–34
Mean cell Hb concentration (MCHC)	g/dL	33–35	33–35
Red cell distribution width (RDW)		11.5–14.8	

*Reference ranges vary among laboratories. The reference ranges for the laboratory providing the result should always be used in interpreting a laboratory test.

Figure 3. Adult reference values for RBC indices[5].

Thus anaemia is classified broadly based on the morphology of the RBCs, MCV and MCH as:

1. Microcytic Hypochromic Anaemia:
    - It is defined as MCV < 80 fL and MCH <27 pg.
    - It includes the following disorders:
        - ❖ Iron deficiency anaemia.
        - ❖ Lead poisoning.
        - ❖ Sideroblastic anaemia.
2. Normocytic Normochromic Anaemia:
    - It is defined by MCV 80-100 fL and MCH >27 pg.
    - It includes:
        - ❖ Haemolytic anaemia.
        - ❖ Anaemia of chronic disease.
        - ❖ Acute or Chronic blood loss.
        - ❖ Renal diseases.
        - ❖ Bone marrow failure (post-chemotherapy, infiltration by tumours, etc.).
3. Macrocytic Anaemia:
    - It is defined by MCV >100 fL
    - Some examples include the following:
        - ❖ Megaloblastic:
            - ✓ Vitamin B12 deficiency.
            - ✓ Folic acid deficiency.

- ❖ Non – Megaloblastic:
  - ✓ Alcoholism.
  - ✓ Liver disease.
  - ✓ Myelodysplasia.
  - ✓ Aplastic anaemia.

PATHOPHYSIOLOGICAL CLASSIFICATION:

Based on determination of the red cell mass, anemia can be classified as either relative or absolute. Relative anemia is characterized by a normal total red cell mass in an increased plasma volume, resulting in a dilution anemia, a disturbance in plasma volume regulation. Based on the pathophysiological process, which is best suited for relating disease process to potential treatment[8] anemia can be classified as:

I. <u>Absolute anemia (decreased red cell volume):</u>

A. Decreased red cell production:

1. Acquired:

- Pluripotential hematopoietic stem cell failure:
  - ➢ Autoimmune (Aplastic anemia):
    - ❖ Radiation-induced.
    - ❖ Drugs and chemicals (chloramphenicol, benzene).
    - ❖ Viruses (non–A-G hepatitis, Epstein-Barr virus).
    - ❖ Idiopathic.
  - ➢ Anemia of leukaemia and of myelodysplastic syndromes.
  - ➢ Anemia associated with marrow infiltration.
  - ➢ Post chemotherapy.

- Erythroid progenitor cell failure:
    - Pure red cell aplasia (parvovirus B19 infection, drugs, associated with thymoma, autoantibodies, etc.)
    - Endocrine disorders.
    - Acquired sideroblastic anemia (drugs, copper deficiency, etc).
- Functional impairment of erythroid and other progenitors from nutritional and other causes:
    - Megaloblastic anemias:
        - Vitamin B12 deficiency.
        - Folate deficiency.
        - Acute megaloblastic anemia because of nitrous oxide (N_2O).
        - Drug-induced megaloblastic anemia (pemetrexed, methotrexate, phenytoin toxicity, etc.)
    - Iron-deficiency anemia.
    - Anemia resulting from other nutritional deficiencies:
        - Vitamin A, C, E, B6, Riboflavin, Pantothenic acid, Niacin and Thiamine deficiency.
        - Trace metal deficiencies – Cu, Zn and Se.
        - Anemia of starvation.
        - Anemia of protein deficiency.
        - Alcoholism.
    - Anemia of chronic disease and inflammation.
    - Anemia of renal failure.

- Anemia caused by chemical agents (lead toxicity).
- Acquired thalassemias (seen in some clonal hematopoietic disorders).
- Erythropoietin antibodies.

2. Hereditary:

- Pluripotential hematopoietic stem cell failure:
  - Fanconi anemia.
  - Shwachman syndrome.
  - Dyskeratosis congenital.
- Erythroid progenitor cell failure:
  - Diamond-Blackfan syndrome.
  - Congenital dyserythropoietic syndromes.
- Functional impairment of erythroid and other progenitors from nutritional and other causes:
  - Megaloblastic anemias:
    - Selective malabsorption of vitamin B12 (Imerslund-Gräsbeck disease).
    - Congenital intrinsic factor deficiency.
    - Transcobalamin II deficiency.
    - Inborn errors of cobalamin metabolism (methylmalonic aciduria, homocystinuria, etc.).
    - Inborn errors of folate metabolism (congenital folate malabsorption, dihydrofolate deficiency, methyltransferase deficiency, etc.).

- Inborn purine and pyrimidine metabolism defects (Lesch-Nyhan syndrome, hereditary orotic aciduria, etc.).
- Disorders of iron metabolism:
    - Hereditary atransferrinemia.
    - Hypochromic anemia caused by divalent metal transporter (DMT)-1 mutation.
- Hereditary sideroblastic anemia.
- Thalassemias.

B. Increased red cell destruction:

1. Acquired:

- Mechanical:
    - Macroangiopathic (March hemoglobinuria, artificial heart valves).
    - Microangiopathic (disseminated intravascular coagulation [DIC]; thrombotic thrombocytopenic purpura [TTP]; vasculitis).
    - Parasites and microorganisms (malaria, bartonellosis, babesiosis, Clostridium perfringens, etc).
- Antibody mediated:
    - Warm-type autoimmune haemolytic anemia.
    - Cryopathic syndromes (cold agglutinin disease, paroxysmal cold hemoglobinuria, cryoglobulinemia).
    - Transfusion reactions (immediate and delayed).
- Hypersplenism.

- Red cell membrane disorders:
    - Spur cell hemolysis.
    - Acquired acanthocytosis and acquired stomatocytosis, etc.
- Chemical injury and complex chemicals (arsenic, copper, chlorate, spider, scorpion, and snake venoms, etc).
- Physical injury (heat, oxygen, radiation).

2. Hereditary:

- Hemoglobinopathies:
    - Sickle cell disease.
    - Unstable haemoglobins.
- Red cell membrane disorders:
    - Cytoskeletal membrane disorders (hereditary spherocytosis, elliptocytosis, pyropoikilocytosis).
    - Lipid membrane disorders (hereditary abetalipoproteinemia, hereditary stomatocytosis, etc).
    - Membrane disorders associated with abnormalities of erythrocyte antigens (McLeod syndrome, Rh deficiency syndromes, etc.).
    - Membrane disorders associated with abnormal transport (hereditary xerocytosis).
- Red cell enzyme defects (pyruvate kinase, 5′ nucleotidase, glucose-6-phosphate dehydrogenase deficiencies, other red cell enzyme disorders).

- Porphyrias (congenital erythropoietic and hepatoerythropoietic porphyrias, rarely congenital erythropoietic protoporphyria).

C. Blood loss and blood redistribution:

1. Acute blood loss.
2. Splenic sequestration crisis.

II. Relative (increased plasma volume):

A. Macroglobulinemia.

B. Pregnancy.

C. Athletes.

D. Post flight astronauts.

Specific therapeutic intervention depends on identifying the type of anemia as classified above but the limitation of such a classification is that, in most anemias, the pathogenesis involves several steps. For example, a decreased rate of production most often results in production of defective red cells with a shortened life span.

3. MICROCYTIC ANAEMIA:

Microcytic anaemia is characterised by the presence of small RBCs on a peripheral smear study and MCV < 80 fL on laboratory studies. There is increased central pallor i.e. more than 1/3rd of the diameter of the RBC.

List of conditions associated with microcytic anaemia[9]:

- ❖ Iron Deficiency anemia – most common cause.
- ❖ Thalassemia & Hemoglobinopathies:
  - o B-Thalassemia Major.

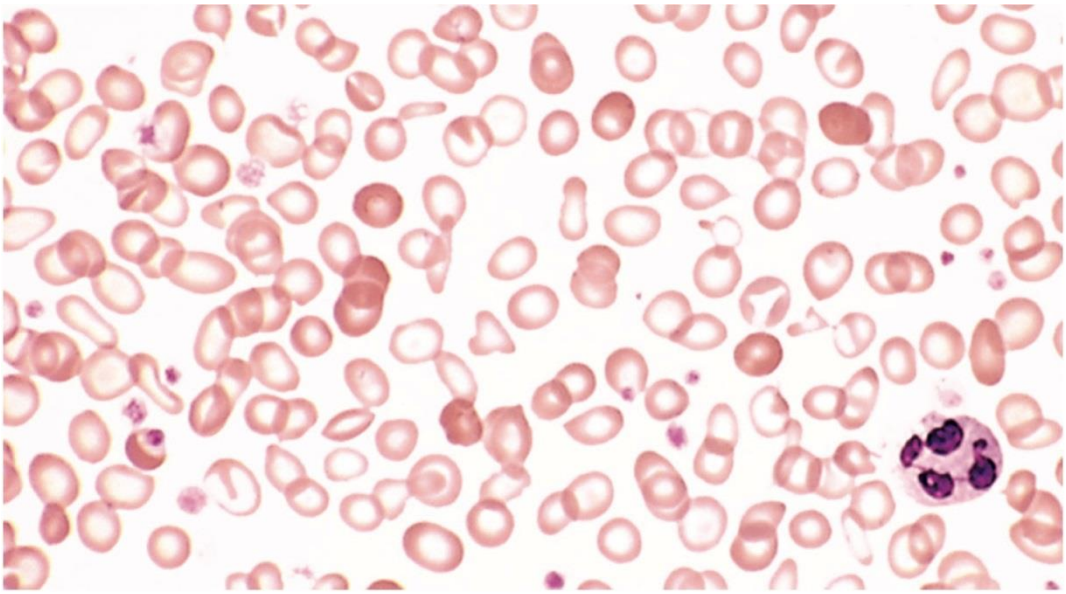


Figure 4. Peripheral blood picture of microcytic hypochromic anemia.

- B-Thalassemia Minor.
- δB - Thalassemia Major.
- α -Thalassemia Major.
- HbE trait.
- Hb H disease.
- Homozygous Hb E disease.
- Blockade of heme synthesis caused by chemicals:
  - ✓ Lead.
  - ✓ Pyrazinamide.
  - ✓ Isoniazid.

❖ Other disorders:

- Sideroblastic anaemia.
- Hereditary sex linked.
- Idiopathic acquired.

- Anaemia of chronic disease.
- Hb Lepore trait.

4. IRON AND ITS METABOLISM:

It is evident from the epidemiology that iron deficiency is the most common cause of microcytic anemia. Iron is a key element in the metabolism of nearly all living organisms. Iron is a component of heme, which is the active site of electron transport in cytochromes and cytochrome oxidase involved in mitochondrial energy generation. The heme moiety of hemoglobin and myoglobin binds O2, providing the means to transfer O2 from the lungs to tissues and to store it. Heme is also the active site of peroxidases that protect cells from oxidative injury by reducing peroxides to water or generate microbicidal hypochlorite in granulocytes. DNA synthesis requires the enzyme ribonucleotide reductase to convert ribonucleotides to deoxyribonucleotides. Neither bacteria nor nucleated cells proliferate when the supply of iron is insufficient. The factors underlying iron deficiency differ somewhat in various population groups and can be best considered in the context of normal iron metabolism.

A. DISTRIBUTION OF IRON IN THE AVERAGE PERSON:

1. Hemoglobin:

Hemoglobin, which is 0.34 per cent iron by weight, contains approximately 2 g of body iron in men and 1.5 g in women[10]. One mL of packed erythrocytes contains approximately 1 mg of iron. Because the life span of human erythrocytes is approximately 120 days, every day 1/120 of the iron in hemoglobin is recycled by

macrophages and returned to the plasma, from where it is largely delivered to marrow erythroblasts for incorporation into newly synthesized hemoglobin.

2. Storage compartment:

Iron is stored either as ferritin or as hemosiderin. The former is water-soluble; the latter is water-insoluble. The protein ferritin is composed of 24 similar or identical subunits arranged as 12 dimers forming a dodecahedron that approximates a hollow sphere with a cavity capable of storing up to 4500 Fe atoms as hydrous ferric oxide polymers. The ferritin subunits are of H (heavy) or L (light) type. H subunits have ferroxidase activity, thereby enabling ferritin to take up or release iron quite rapidly. Ferritin that is rich in H subunits takes up iron more readily, but retains it less avidly than does ferritin composed predominantly of L subunits. Much of the storage iron in liver and spleen is in ferritin containing mostly L subunits.

3. Myoglobin:

Myoglobin is structurally similar to hemoglobin, but it is monomeric rather than tetrameric: Each myoglobin molecule consists of a heme group nearly surrounded by polypeptide loops of the 154 amino acid protein. It is present in small amounts in all skeletal and cardiac muscle cells, where it may serve as an oxygen reservoir to protect against cellular injury during periods of oxygen deprivation and may scavenge nitric oxide and reactive oxygen species[11].

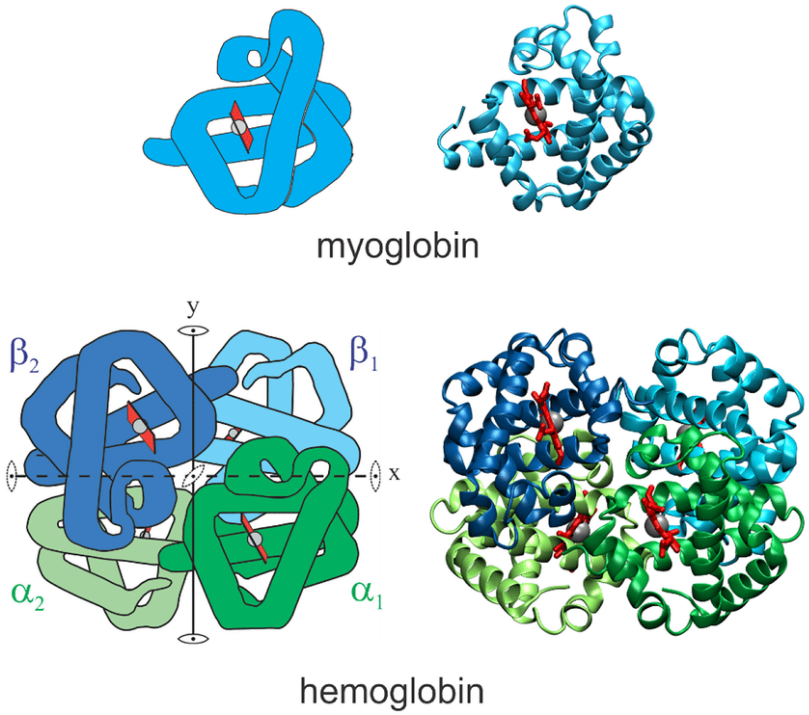


Figure 5. Structure of Hemogobin and Myoglobin

4. Labile iron pool:

The existence of a cellular labile iron pool was postulated from studies of the rate of clearance of injected ^{59}Fe from plasma[12]. Iron leaves the plasma and enters the interstitial and intracellular fluid compartments for a brief time before it is incorporated into heme or storage compounds. Some of the iron re-enters plasma, causing a biphasic curve of ^{59}Fe clearance 1 to 2 days after injection. The change in slope defines the size of the labile pool, normally 80 to 90 mg of iron. It is now sometimes considered to be equivalent to the chelatable iron pool[13].

5. Tissue iron compartment:

Tissue iron (exclusive of hemoglobin, ferritin, hemosiderin, myoglobin, and the labile compartment) normally amounts to 6 to 8 mg. This includes cytochrome and

other iron-containing enzymes. Although a small compartment, it is an extremely vital one and is sensitive to iron deficiency[14,15].

6. Transport compartment:

From the standpoint of its total iron content, normally about 3 mg, the transport compartment of plasma is the smallest but the most active of the iron compartments: Its iron, almost entirely carried by transferrin, normally turns over at least 10 times each day. This is the common pathway for interchange of iron between compartments.

B. IRON ABSORPTION:

1. Dietary iron:

TABLE 42–2. Recommended Dietary Allowances (RDAs) for Iron[16]

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		

*Adequate intake (AI).

Figure 6. RDA for Iron

Average adult men and women ingest 9 to 10 mg and 12 to 14 mg of iron daily, respectively. The amount of iron absorbed by a normal adult male need only balance the small amount that is excreted, mostly in the stool, approximately 1 mg/day. More

iron is needed during growth periods or after blood loss. In women, iron absorbed must be sufficient to replace that lost through menstruation or diverted to the fetus or milk during and after pregnancy In meat-eaters in Western countries, heme from hemoglobin and myoglobin normally comprises approximately 15 per cent of dietary iron but is much more efficiently absorbed than non-heme iron, and promotes the absorption of non-heme iron. The absorption of non-heme dietary iron is strongly affected by iron-binding components of food. Oxalates, phytates, and phosphates forms a complex with iron and retard its absorption, whereas simple reducing substances, such as hydroquinone, ascorbate, lactate, pyruvate, succinate, fructose, cysteine, and sorbitol, increase iron absorption. Iron-fortified cereals are major sources of iron in countries where fortification is practiced, but cooking in iron pots may also provide important exogenous iron.

Gastric acid secretion, the transit time, and mucus secretion all play roles in iron absorption. Red wine, contrary to popular belief, inhibits iron absorption, probably because of the presence of polyphenols. In mice, alcohol suppresses the response of hepcidin to iron, and this may contribute to iron loading that is seen in some alcoholic subjects.

2. Iron absorption:

Heme iron is absorbed directly into enterocytes by Heme transporter. While non heme iron, Following the reduction of ferric iron to ferrous iron, in part by duodenal cytochrome b (dcytb) reductase, ferrous iron is transported into the intestinal villus cell by the divalent metal transporter (DMT)-1. How iron transits within the enterocytes is not yet known. Basolateral export of ferrous iron is mediated by

ferroportin in association with hephaestin and plasma ceruloplasmin to oxidize iron to the ferric state. Ferric iron is taken up by plasma apotransferrin[16].

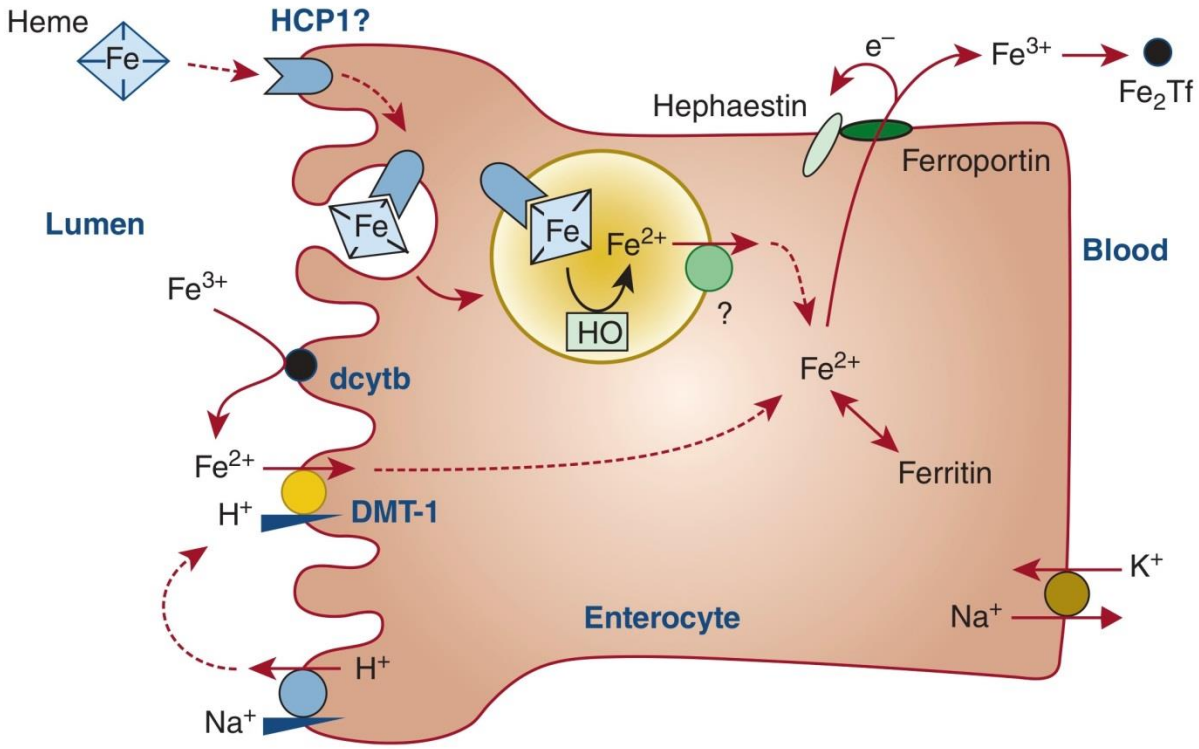


Figure7. Iron absorption and transport.

3. Iron recycling:

In humans, the destruction and production of erythrocytes generates most of the iron flux in and out of plasma (20 to 25 mg/day recycled in adults compared to 1 to 2 mg/day absorbed). Iron from other cell types is likely also recycled, but this source contributes little to iron flux and has not been studied. Destruction of aged erythrocytes and hemoglobin degradation occur within macrophages. This proceeds at a rate sufficient to release approximately 20 per cent of the hemoglobin iron from the cell to the plasma compartment within a few hours. Approximately 80 per cent of this iron is rapidly reincorporated into hemoglobin. Thus, 20 to 70 per cent of the

hemoglobin iron of nonviable erythrocytes reappears in circulating red cells in 12 days. The remainder of the iron enters the storage pool as ferritin or hemosiderin and then turns over very slowly. In normal subjects, approximately 40 per cent of this iron remains in storage after 140 days. When there is an increased iron demand for hemoglobin synthesis, however, storage iron may be mobilized more rapidly. Conversely, in the presence of infection or another inflammatory process (e.g., ulcerative colitis or malignancy), iron is more slowly reused in hemoglobin synthesis and is associated with anemia[17].

4. Iron transport:

Once an atom of iron enters the blood plasma from dietary iron absorption, it is virtually trapped in the body and cycles almost endlessly from the plasma to the developing erythroblast (where it is used in hemoglobin synthesis), thence into the circulating blood for approximately 4 months, and then to macrophages. Here it is removed from heme by heme oxygenase and released back into the plasma to repeat the cycle. The major function of the transport protein transferrin is to move iron from wherever it enters the plasma (intestinal villi, splenic and hepatic sinusoids) to the erythroblasts of the marrow and to other sites of use.

Diferric (holo) transferrin binds to the transferrin receptor (TfR)-1 on the cell surface and the holotransferrin–TfR1 complex forms clusters in pits on the cell membrane. The complex is then internalized by endocytosis. Within the cytosol the holotransferrin-TfR1 complex is in a clathrin-coated vesicle. The vesicles fuse with endosomes and become acidified to pH 5 which releases iron from transferrin. Iron depleted apotransferrin and TfR1 remain complexed as they return to the cell

membrane, where at neutral pH, apotransferrin separates from its receptor and is released to the interstitial fluid to re-enter plasma and take up more iron.

The TfR is a protein consisting of two subunits that are linked by disulfide bonds. Its aminoterminus is on the cytoplasmic side of the membrane, and its carboxyl-terminus is on the outer surface. Because of the role of TfR1 in the binding and endocytosis of diferric transferrin, control of TfR1 biosynthesis is a major mechanism for regulation of iron metabolism. Synthesis of TfR1 is induced by iron deficiency. Iron inhibits TfR1 synthesis by destabilizing TfR1 mRNA by a mechanism that involves the iron-responsive element (IRE)/iron-regulatory protein (IRP) regulatory system TFR1 binds to HFE, using a binding site that overlaps that of holotransferrin. According to a current model of iron sensing, high concentrations of holotransferrin would therefore displace HFE from its complex with TfR1, leaving HFE to signal to the BMP receptor complex to increase hepcidin transcription. This model is supported by studies in which the expression of HFE or its binding site on TfR1 is manipulated[18].

A second TfR, TfR2, also endocytic for holotransferrin, is not thought to be involved in delivering iron to cells but its hepatic expression is necessary for normal hepcidin expression and regulation. TfR2 influences the BMP complex and its signaling pathway to regulate hepcidin transcription but the molecular mechanism of this effect is not yet understood. TfR2 is also expressed in erythroid precursors where it interacts with the erythropoietin receptor and negatively modulates erythropoiesis, perhaps putting a brake on erythrocyte production during iron deficiency.

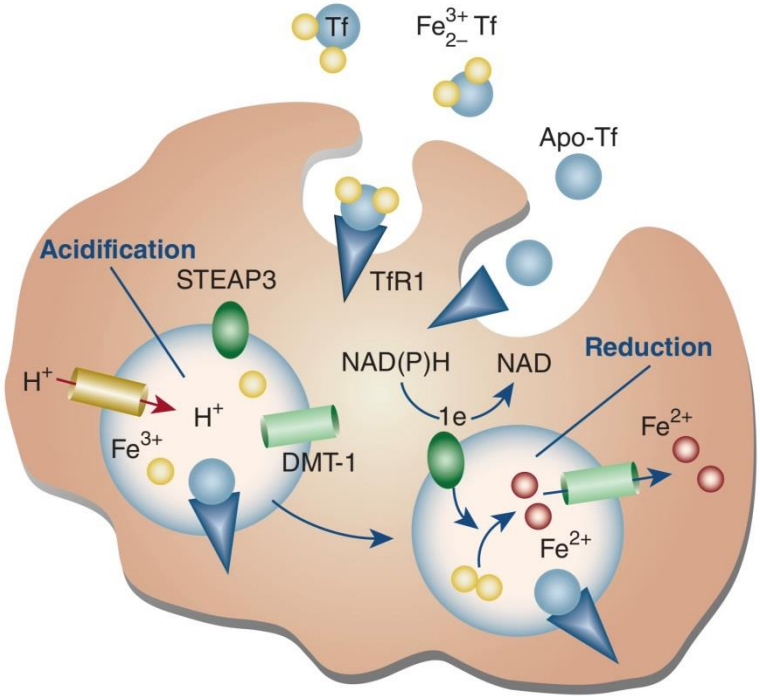


Figure 8. The transferrin cycle.

5. Iron excretion:

The body conserves iron with remarkable efficiency. Most iron loss occurs by way of desquamated intestinal cells in the feces and it normally amounts to approximately 1 mg/day, less than one-thousandth of total-body iron. Exfoliation of skin and dermal appendages and perspiration result in much smaller losses. Even in tropical climates, the loss of iron in sweat is minimal. Very small amounts of iron are lost in the urine. Lactation may cause excretion of approximately 1 mg iron daily, thus doubling the overall rate of iron loss. Blood loss by normal menstruation contributes to negative iron balance. Although total daily iron loss is normally approximately 1 mg for males, it averages approximately 2 mg for menstruating women. Persons with marked iron overload, as in hemochromatosis, may lose as much as 4 mg of iron daily, probably because of the shedding of iron laden cells, principally macrophages.

5. IRON DEFICIENCY ANEMIA:

ETIOLOGY:

Iron deficiency may occur as a result of chronic blood loss, diversion of iron to foetal and infant erythropoiesis during pregnancy and lactation, inadequate dietary iron intake, malabsorption of iron, intravascular hemolysis with hemoglobinuria, and diversion of iron to non-hematopoietic tissues like the lung, genetic factors, or a combination of these factors. Of these, gastrointestinal or menstrual blood loss is the most common. The average adult male has approximately 1000 mg of iron in stores, but on average, women have less than half of this amount. The average daily dietary intake of iron is 10 to 12 mg[19], but much of this is not absorbed, even when absorption is maximal. Blood loss of each millilitre of packed erythrocytes represents 1 mg of iron. Thus, chronic daily blood loss of greater than 5 mL will deplete iron reserves over weeks to months, and even if bleeding stops completely, the repletion of lost iron, including the restoration of iron stores (around 1000 mg in the average adult man), will take many months.

The sources of blood loss as a cause for iron deficiency anemia can be from:

1. ALIMENTARY TRACT:

- Esophagus:
  - Varices.
- Stomach and duodenum:
  - Ulcer.
  - Hiatus hernia.
  - Gastritis.

- Carcinoma.
- Varices.
- Angiodysplasia.
- Hemangioma.
- Leiomyoma (Ménétrier disease).
- Mucosal hypertrophy.
- Hypergastrinemia.
- Antral vascular ectasia.
- “Watermelon stomach”.

❖ Small intestine:

- Vascular ectasia.
- Tumors.
- Ulceration.
- Meckel’s diverticulum.

❖ Colon and anorectal:

- Hemorrhoids.
- Carcinoma.
- Polyp.
- Diverticulum.
- Ulcerative colitis.
- Angiodysplasia.
- Hemangioma.
- Telangiectasia.
- Amebiasis.

2. BILIARY TRACT:

- ❖ Intrahepatic bleeding.
- ❖ Carcinoma.
- ❖ Cholelithiasis.
- ❖ Trauma.
- ❖ Ruptured aneurysm.
- ❖ Aberrant pancreas.

3. GENITOURINARY TRACT:

- ❖ Menorrhagia.
- ❖ Uterine fibroids.
- ❖ Endometriosis.
- ❖ Carcinoma.
- ❖ Vascular abnormalities.

4. RESPIRATORY TRACT:

- ❖ Epistaxis.
- ❖ Carcinoma.
- ❖ Infections.
- ❖ Telangiectases.
- ❖ Idiopathic pulmonary hemosiderosis.

PREGNANCY AND PARTURITION:

Although physiologic decrease in hemoglobin concentration is an expected consequence of hemodilution associated with pregnancy, true iron deficiency frequently results in more severe anemia. In pregnancy, the average iron loss resulting

from diversion of iron to the foetus, blood loss at delivery (equivalent to an average of 150 to 200 mg of iron), and lactation is altogether approximately 900 mg; in terms of iron content, this is equivalent to the loss of more than 2 L of blood. Approximately 30 mg of iron may be expended monthly in lactation. Because most women begin pregnancy with low iron reserves, these additional demands frequently result in iron-deficiency anemia. Iron depletion has been reported in some 85 to 100 per cent of pregnant women. Iron-deficient mothers are likely to have smaller babies. The incidence of anemia and iron deficiency is lower in women who take oral iron supplementation, daily or intermittently. In regions with endemic malaria, iron supplementation may increase the risk of malaria and some recommend that it be combined with malarial prophylaxis[20].

Pool	Men	Women
Total	3450	2450
Functional		
Hemoglobin	2100	1750
Myoglobin	300	250
Enzymes	50	50
Storage		
Ferritin, hemosiderin	1000	400

Figure 6. Iron distribution in healthy adults (mg)

STAGES OF IRON DEFCIENCY[21]:

The progression to iron deficiency can be divided into three stages. The first stage is negative iron balance, in which the demands for (or losses of) iron exceed the body's ability to absorb iron from the diet. This stage results from a number of physiologic mechanisms, including blood loss, pregnancy (in which the demands for

red cell production by the fetus outstrip the mother's ability to provide iron), rapid growth spurts in the adolescent, or inadequate dietary iron intake. Blood loss in excess of 10–20 mL of red cells per day is greater than the amount of iron that the gut can absorb from a normal diet. Under these circumstances, the iron deficit must be made up by mobilization of iron from reticulo-endotheial (RE) storage sites. During this period, iron stores—reflected by the serum ferritin level or the appearance of stainable iron on bone marrow aspirations—decrease. As long as iron stores are present and can be mobilized, the serum iron, total iron-binding capacity (TIBC) and red cell protoporphyrin levels remain within normal limits. At this stage, red cell morphology and indices are normal.

When iron stores become depleted, the serum iron begins to fall. Gradually, the TIBC increases, as do red cell protoporphyrin levels. By definition, marrow iron stores are absent when the serum ferritin level is <15 μg/L. As long as the serum iron remains within the normal range, hemoglobin synthesis is unaffected despite the dwindling iron stores. Once the transferrin saturation falls to 15–20%, hemoglobin synthesis becomes impaired. This is a period of iron-deficient erythropoiesis. Careful evaluation of the peripheral blood smear reveals the first appearance of microcytic cells, and if the laboratory technology is available, one finds hypochromic reticulocytes in circulation. Gradually, the hemoglobin begins to fall, reflecting iron-deficiency anemia. The transferrin saturation at this point is <10–15%. When moderate anemia is present (hemoglobin 10–13 g/dL), the bone marrow remains hypoproliferative.

With more severe anemia (hemoglobin 7–8 g/dL), hypochromia and microcytosis become more prominent, target cells and misshapen red cells

(poikilocytes) appear on the blood smear as cigar- or pencil-shaped forms, and the erythroid marrow becomes increasingly ineffective. Consequently, with severe prolonged iron-deficiency anemia, erythroid hyperplasia of the marrow develops, rather than hypoproliferation.

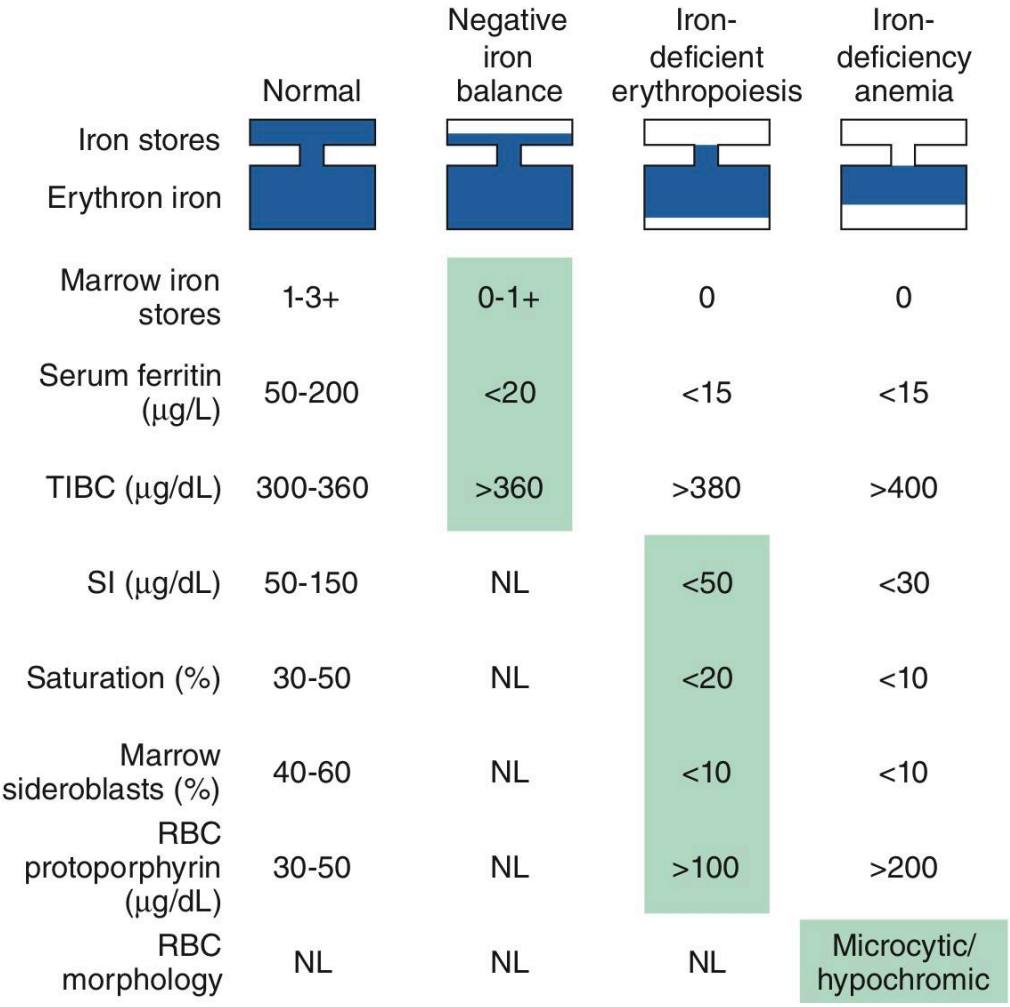


Figure 7. Laboratory studies in the evolution of iron deficiency.

LABORATORY IRON STUDIES:

1. Serum Iron and Total Iron-Binding Capacity:

The serum iron level represents the amount of circulating iron bound to transferrin. The TIBC is an indirect measure of the circulating transferrin. The normal range for the serum iron is 50–150 µg/dL; the normal range for TIBC is 300–360

μg/dL. Transferrin saturation, which is normally 25–50%, is obtained by the following formula: serum iron × 100 ÷ TIBC. Iron-deficiency states are associated with saturation levels < 20%. There is a diurnal variation in the serum iron. A transferrin saturation >50% indicates that a disproportionate amount of the iron bound to transferrin is being delivered to non-erythroid tissues[22]. If this persists for an extended time, tissue iron overload may occur.

2. Serum Ferritin:

Free iron is toxic to cells, and the body has established an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. As ferritin accumulates within cells of the RE system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the RE cells, although hemosiderin is less readily available. Under steady-state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores. The normal value for ferritin varies according to the age and gender of the individual. Adult males have serum ferritin values averaging 100 μg/L, while adult females have levels averaging 30 μg/L. As iron stores are depleted, the serum ferritin falls to <15 μg/L[22]. Such levels are diagnostic of absent body iron stores.

3. Evaluation of Bone Marrow Iron Stores:

Although RE iron stores can be estimated from the iron stain of a bone marrow aspirate or biopsy, the measurement of serum ferritin has largely supplanted these procedures for determination of storage iron. The serum ferritin level is a better

indicator of iron overload than the marrow iron stain. However, in addition to storage iron, the marrow iron stain provides information about the effective delivery of iron to developing erythroblasts. Normally, when the marrow smear is stained for iron, 20–40% of sideroblasts, which are the developing erythroblasts, will have visible ferritin granules in their cytoplasm. This forms the iron that is in excess than that is needed for synthesis of Hb. Conditions causing blockage of release of iron from storage sites, RE iron will be detectable, and there will be few or no sideroblasts. In the myelodysplastic syndromes, mitochondrial dysfunction can occur, and accumulation of iron in mitochondria appears in a necklace fashion around the nucleus of the erythroblast. Such cells are referred to as ring sideroblasts[23].

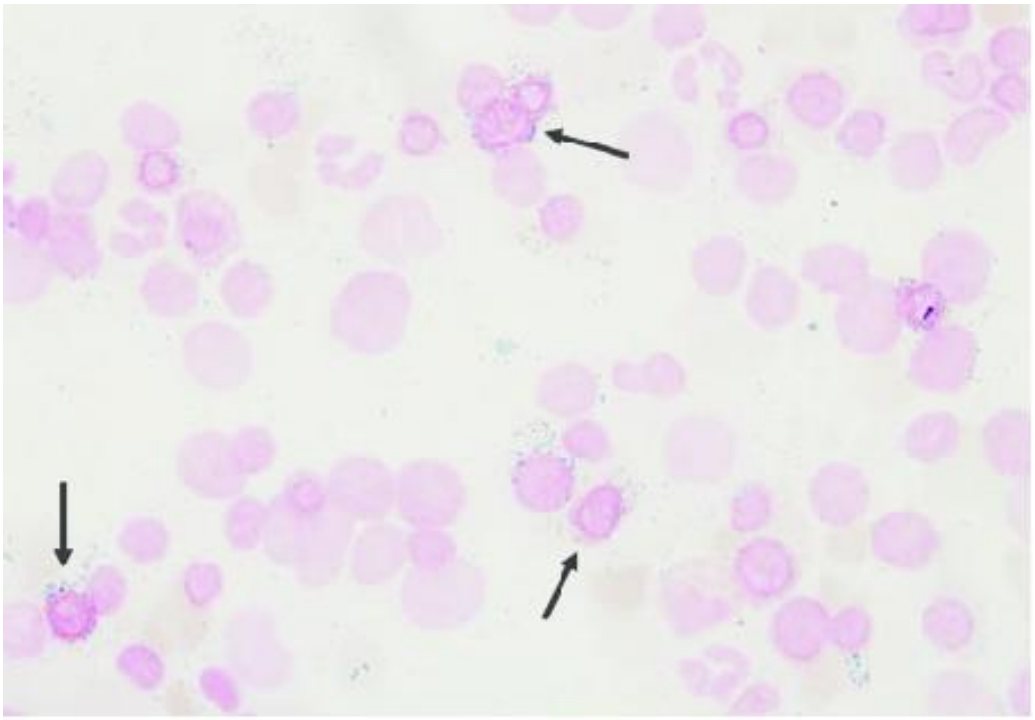


Figure 8. Iron stain of bone marrow aspirate demonstrating ringed sideroblasts.

4. Red Cell Protoporphyrin Levels:

Protoporphyrin is an intermediate in the pathway to heme synthesis. Under conditions in which heme synthesis is impaired, protoporphyrin accumulates

within the red cell. This reflects an inadequate iron supply to erythroid precursors to support hemoglobin synthesis. Normal values are <30 μg/dL of red cells. In iron deficiency, values >100 μg/dL are seen[24]. The most common causes of increased red cell protoporphyrin levels are absolute or relative iron deficiency and lead poisoning.

5. Serum Levels of Transferrin Receptor Protein:

Because erythroid cells have the highest numbers of transferrin receptors of any cell in the body, and because transferrin receptor protein (TRP) is released by cells into the circulation, serum levels of TRP reflect the total erythroid marrow mass. Another condition in which TRP levels are elevated is absolute iron deficiency. Normal values are 4–9 μg/L determined by immunoassay[25]. This laboratory test is becoming increasingly available and, along with the serum ferritin, has been proposed to distinguish between iron deficiency and the anemia of inflammation.

IRON THERAPY[26]:

- ORAL:

In the asymptomatic patient with established iron-deficiency anemia and an intact gastrointestinal tract, treatment with oral iron is usually adequate. Encouraging dietary intake of iron-rich foods is also useful. Such foods include oysters, kidney beans, beef liver, tofu, beef (chuck roast, lean ground beef), turkey leg, whole-wheat bread, tuna, eggs, shrimp, peanut butter, leg of lamb, brown rice, raisin bran (whole grain–enriched cereals), lentils, and beans. Various forms of oral iron supplements are available, ranging from simple iron salts to complex iron compounds with sustained release. Although the various preparations contain different quantities of iron, they are all adequately absorbed and are effective in treatment. Some come

with other compounds designed to enhance iron absorption, such as ascorbic acid. It is not clear whether the benefits of such compounds justify their costs. Typically, for iron replacement therapy, up to 200 mg of elemental iron per day is given, usually as three or four iron tablets (each containing 50–65 mg elemental iron) given over the course of the day. Ideally, oral iron preparations should be taken on an empty stomach, since food may inhibit iron absorption. Some patients with gastric surgery require special treatment with iron solutions because of the impaired retention capacity of the stomach. A dose of 200 mg of elemental iron per day should result in the absorption of iron up to 50 mg/d. This supports a red cell production level of about two to three times of normal, in a normally functioning marrow under appropriate erythropoietin (EPO) stimulus. However, as the hemoglobin level rises, EPO stimulation decreases, and the amount of iron absorbed is reduced. The goal of therapy is not only to repair the anemia, but also to provide stores of at least 0.5–1 g of iron. Sustained treatment for a period of 6–12 months after correction of the anemia will be necessary to achieve this.

- PARENTERAL:

Intravenous iron can be given to patients who are unable to tolerate oral iron; whose needs are relatively acute; or who need iron on an ongoing basis, usually due to persistent gastrointestinal or menstrual blood loss. Parenteral iron use has been increasing rapidly over the past several years with the recognition that recombinant EPO therapy induces a large demand for iron—a demand that frequently cannot be met through the physiologic release of iron from RE sources or oral iron absorption. The safety of parenteral iron has been a concern largely driven by the high adverse reaction rate to high-molecular-weight iron dextran. The newer iron complexes that

are available, such as ferumoxytol (Feraheme), sodium ferric gluconate (Ferrlecit), iron sucrose (Venofer), low-molecular-weight (LMW) iron dextran (InFed), ferric derisomaltose (Monoferric), and ferric carboxymaltose (Injectafer), have much lower rates of adverse effects. Ferumoxytol delivers 510 mg of iron per infusion; ferric gluconate 125 mg per infusion; LMW iron dextran up to 1500 mg per infusion; ferric carboxymaltose 750 mg per infusion; ferric derisomaltose 1000 mg per infusion; and iron sucrose 200 mg per infusion. Parenteral iron is used in two ways: one is to administer the total dose of iron required to correct the hemoglobin deficit and provide the patient with at least 500 mg of iron stores; the second is to give repeated small doses of parenteral iron over a protracted period. The latter approach is common in dialysis centers, where it is not unusual for 100 mg of elemental iron to be given weekly for 10 weeks to augment the response to recombinant EPO therapy.

The amount of iron needed by an individual patient is calculated by the following formula:

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

6. CLINICAL FEATURES:

The common symptoms of anaemia include:

- Fatigue
- Tiredness
- Effort intolerance
- Exertional dyspnoea
- Palpitations

- Giddiness
- Exertional angina

The most common sign of anaemia is pallor. Pallor is usually absent if the haemoglobin level is more than 9 g/dl, and usually present if the haemoglobin level is less than 6 g/dl. Other signs of anaemia include high output failure and congestive cardiac failure in late stages.

Certain clinical conditions carry an increased likelihood of iron deficiency. Pregnancy, adolescence, periods of rapid growth, and an intermittent history of blood loss of any kind should alert the clinician to possible iron deficiency. A cardinal rule is that the appearance of iron deficiency in an adult male or postmenopausal female means gastrointestinal blood loss until proven otherwise. Signs related to iron deficiency depend on the severity and chronicity of the anaemia in addition to the usual signs of anaemia - fatigue, pallor, and reduced exercise capacity. Cheilosis (fissures at the corners of the mouth) and koilonychia (spooning of the fingernails) are signs of advanced tissue iron deficiency. The diagnosis of iron deficiency is typically based on laboratory results.

7. HEMOGLOBIN:

Hemoglobin (Hb) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues. A sufficient hemoglobin level must be maintained to ensure adequate tissue oxygenation. When the hemoglobin level is low, the patient has anemia. An erythrocytosis is due to presence of too many red cells; this results in higher hemoglobin levels above normal[27].

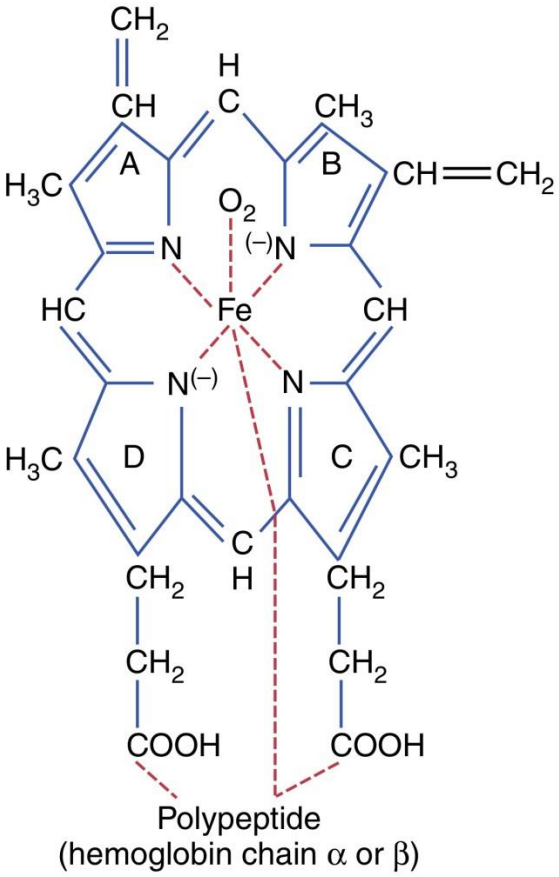


Figure 9. Structure of Hemoglobin.

Normal adult hemoglobin is comprised predominantly of HbA (α2β2) (97%) while HbA2 (α2δ2) (2.5%) and HbF (α2γ2) (0.5%) forming minor fractions. In 1958, Allen et al[19] discovered that Hb can be separated into at least three more minor components, which are more negative than Hb, by cation exchange chromatography. These fractions were separated based on their electrophoretic properties. These fractions moved fast because of their glycated nature. Further works by Rabhar et al[28] revealed that these minor hemoglobin fractions were elevated in diabetics and thus the temporal link was established.

HbA1c:

About 6% of total HbA is glycated in vivo by the blood glucose. This glycated hemoglobin fraction is termed HbA1. This has further sub-fractions of HbA1a1, HbA1a2, HbA1b and HbA1c. These fractions differ slightly from the major component HbA0 .They are defined as the 'fast hemoglobins' as they elude fast in chromatography and migrate fast in electrophoresis. HbA1c makes up the most of these fractions. HbA1c, also called as the glucohaemoglobin or the glycated hemoglobin, has a stable adduct of glucose which is linked to the N-terminal valine of the β chain by covalent bonds.

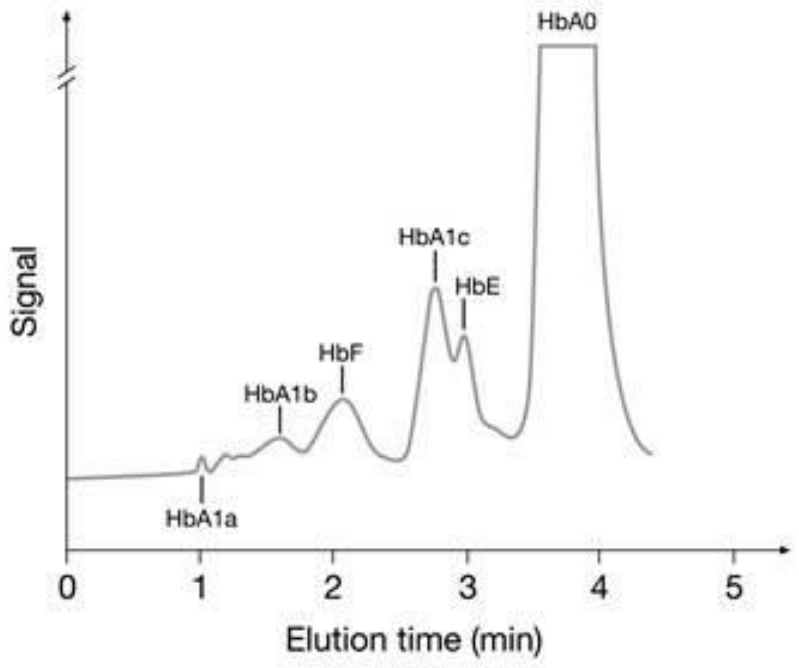


Figure 10. Hemoglobin fractionation in electrophoresis.

The processes involved in the formation of HbA1c are as follows[29]:

1) To the N-terminal portion of the β chain, a glucose molecule binds. This glucose molecule is always in the open chain format, forming an aldimine which is called as

Schiff base.

2) A more stable ketoamine is formed when this Schiff base undergoes an Amadori rearrangement. It is a non- enzymatic process which happens continuously in vivo.

Hemoglobin is also glycated at sites other than the N-terminus portion of the Beta chain for e.g., epsilon amino groups on lysine residues. These fractions also contribute to the total glycated hemoglobin.

The concentration of HbA1c depends predominantly on two factors:

- The concentration of glucose in the blood.
- The life span of the erythrocyte.

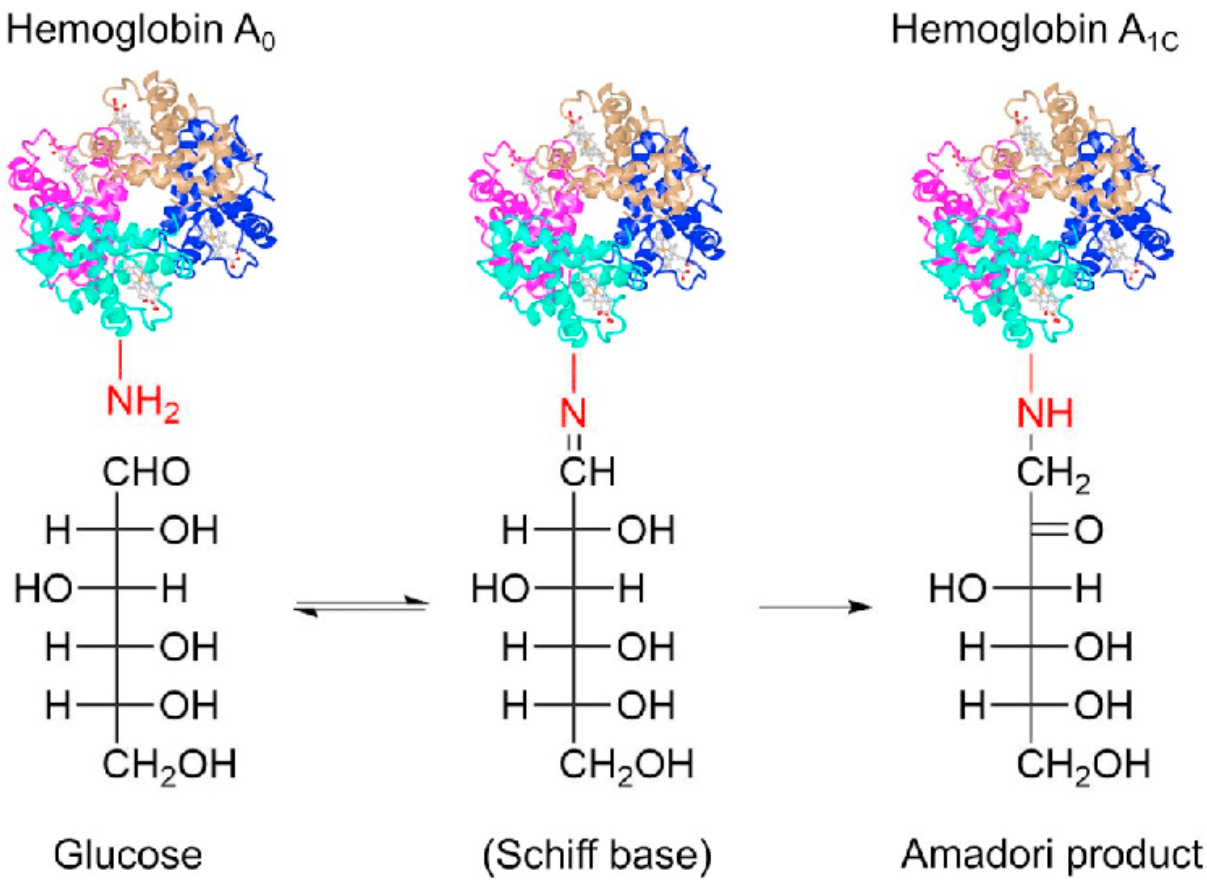


Figure 11.Amadori Reaction.

As the average life span of erythrocytes in the blood is 120 days, HbA1c represents the overall picture of the glucose levels over the preceding 8–12 weeks.

8. ADVANCED GLYCATION END PRODUCTS (AGE) IN DIABETES:

Glycation is a chemical process where the proteins are modified by reducing sugars. There is a possible association between hyperglcemia and a wide variety of tissue pathologies. Glucose forms a chemically reversible product with certain proteins called as Schiff's base. These reducing sugars then react with the amino groups of the long lived proteins, called Maillard reaction to produce non – enzymatic cross links. Formation of these cross-links occur at the end of the Maillard reaction leading to formation of Advanced Glycation End – products (AGE)[30].

AGEs are a group of complex, unstable, reactive compounds formed in excess in states of hyperglycemia. They alter the structural properties of tissue proteins and reduce their catabolism[31]. The level of these AGEs can be used as a surrogate marker for predicting the complications of diabetes, for example the blood levels of HbA1c correlate with the development of retinopathy.

All these mechanisms result in the production of reactive oxygen species leading to mitochondrial injury at the cellular level, thus resulting in tissue changes. Hyperglycemia produces super oxide anion from the endothelial cells, which may use up all the available nitric oxide, a potent endothelial derived vasodilator. Oxidative stress also interferes with endothelial relaxation and cell replication, all of which culminating in the vascular complications of diabetes mellitus.

ROLE OF HBA1C:

When the blood & urine sugar estimation allowed day-to-day monitoring of glucose levels, there raised a need for the objective assessment of long term control of

glycemic status. Then it was discovered that proteins like Hemoglobin (Hb) were non-enzymatically glycated in vivo depending on the blood sugar values. Measuring these glycated proteins especially hemoglobin and serum proteins gave a new dimension of monitoring in this field. The peculiarity in the measurement of these proteins is that they can quantitatively assess the glycemic control over the past few weeks or months complementing the day-to-day testing.

After the discovery of HbA1c and the process of protein glycation, there was a great surge in the number of small studies that were conducted correlating it to glucose measurements. All these studies lead to a conclusion that HbA1c, apart from being used as a measure of glycemic control could also be used as diagnostic criteria of diabetes. The A1C-Derived Average Glucose (ADAG) study[32] was conducted in 643 participants having a range of A1C levels entailing it as the first of its kind. It provided a validated relationship between HbA1c and average glucose levels. This could be applied to a variety of diabetic types and patient populations. HbA1c was recommended for clinical use in the 1980s and subsequently has become a milestone diagnostic tool for DM.

As HbA1c reflects the average plasma glucoses over a period of past 3 months, it can be measured at any time of the day irrespective of fasting status of the patient as it will have less bearing on the values measured. HbA1c is now the preferred test for assessing glycemic control in people with diabetes as it excludes the influence of day-to-day variability of glucose values, avoids the need for the person to fast and to have preceding dietary preparations. These advantages also make HbA1c an ideal tool for the early identification and treatment of diabetes which is being strongly advocated in

the recent years considering the potential complications and the socio - economic consequences of the disease. Recognized as the gold standard parameter of diabetic survey now, it was internationally standardized in the 1990s and 2000s. World Health Organization first made a mention of the promising utility of HbA1c in diabetes care in its 1985 report.

International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes, 2009[33] recommendations are as follows:

1. The HbA1c assay provides an accurate, precise measure of chronic glycaemic levels and correlates with the risk of diabetes complications.
2. The HbA1c assay has several advantages over laboratory measures of glucose.
3. Diabetes should be diagnosed when HbA1c is >6.5%.
4. If the clinical symptoms and plasma glucose levels of > 200 mg/dl (11.1mmol/l) are not present, diagnosis should be confirmed only after a repeat HbA1c test. A value of 5.7-6.4 % is considered as the high risk range. This holds true for pediatric population also.
5. People with HbA1c levels between 6.0 and 6.5% were at high risk for diabetes and might be considered for rigorous diabetes prevention interventions. If HbA1c testing is not available, previously recommended diagnostic methods remain acceptable.

FACTORS INFLUENCING THE MEASUREMENT OF HbA1c LEVELS:

1. Erythropoiesis:

I. Increased HbA1c:

i. Iron deficiency

ii. Vitamin B12 deficiency

iii. Decreased erythropoiesis

II. Decreased HbA1c:

i. Administration of erythropoietin

ii. Iron supplementation

iii. Vitamin B12 supplementation

iv. Reticulocytosis

v. Chronic liver disease

2. Altered Haemoglobin structure:

I. Genetic or chemical alterations in haemoglobin structure:

1. Haemoglobinopathies
2. HbF
3. Methaemoglobin (variable HbA1c).

3. Glycation of HbA1c:

I. Increased HbA1c:

1. Alcoholism
2. Chronic renal failure
3. Decrease in intra – erythrocyte pH.

II. Decreased HbA1c:

1. Aspirin
2. Vitamin C and E
3. Certain haemoglobinopathies
4. Increase in intra - erythrocyte pH.

III. Variable HbA1c:

1. Genetic determinants.

4. Erythrocyte destruction:

I. Increased HbA1c:

1. Increased erythrocyte life span: Spleenectomy.

II. Decreased HbA1c:

1. decreased life span of the erythrocyte:

- Haemoglobinopathies
- Spleenomegaly
- Rheumatoid arthritis
- Drug induced
  - Anti-retrovirals
  - Ribavirin
  - Dapsone.

5. HbA1cAssays:

I. Increased HbA1c:

1. Hyperbilirubinaemia
2. Carbamylated haemoglobin
3. Alcoholism
4. Large doses of aspirin
5. Chronic opiate use.

II. Decreased HbA1c:

1. Hypertriglyceridaemia.

III. Variable HbA1c:

1. Haemoglobinopathies.

Thus, conditions causing prolongation of RBC's life span lead to false

elevation of HbA1c and conversely any condition that shortens the RBC lifespan is likely to falsely reduce the HbA1c levels.

ADVANTAGES OF HbA1c:

- Does not require a period of fasting.
- Not affected by day to day variations in plasma glucose.
- Negligible biological variability associated with HbA1c.
- Proven relationship between HbA1c and the future risk of retinopathy.
- Simpler sampling and analysis requirements[34].

DISADVANTAGES OF HbA1C:

The high cost, not readily available, use of sophisticated instruments, need for skilled personnel and lack of standardization procedures among different assays are some of them. Apart from these, patient factors, kinetics of HbA1c, genetic, hematologic and illness-related factors are also known to influence the results[35]. The most common and important factors affecting HbA1c levels are hemoglobinopathies, all types of anaemia's and disorders associated with accelerated red cell turnover such hemolytic conditions.

The relationship between HbA1c and plasma glucose level is complex. Many studies have shown that HbA1c is a surrogate marker for the mean plasma glucose over the preceding weeks to months. Erythrocyte life span averages 120 days. The level of HbA1c in the blood is contributed by all the circulating erythrocytes, from the oldest (120 days old) to the youngest. However, recent plasma glucose levels over the recent 3– 4 weeks earlier contribute more to the level of HbA1c than does the long past plasma glucose levels (3–4 months earlier). Therefore, HbA1c is just an average of blood glucose levels during the preceding 120 days[36].

Plasma glucose levels in the preceding 30 days contribute to about 50% to the final HbA1c result, and plasma glucose levels from 90–120 days earlier contribute to just only 10% of the final value of HbA1c[37]. This explains the increase or decrease in the level of HbA1c with large changes in levels of blood glucose.

There is evidence that wide fluctuations can occur in HbA1c levels between individuals which is not related to glycemic status of the individual, suggesting that there are "low glycators" and "high glycators"[38,39].

FPG when used alone should be used with great caution as a measure of long-term glycemia. FPG tends to progressively underestimate HbA1c (and seven-point MPG) at increasing levels of plasma glucose. The data also suggest that the post-meal blood glucose contributes much to the HbA1c levels; however, all post-meal times are not equal in their contribution. Compared with the seven-point glucose profiles, post-breakfast blood glucose levels markedly overestimate the HbA1c levels, whereas post-lunch glucose levels show a relationship withHbA1c levels that is very much similar to that of mean plasma glucose. A previous study also showed that in patients with type 2 diabetes, post-lunch plasma glucose level is a better indicator of glycemic control than is FPG[40].

Materials and methods

MATERIALS AND METHODS

This study was conducted in a government tertiary care hospital at Chennai.

ETHICAL COMMITTEE APPROVAL:

Obtained.

PATIENT CONSENT:

Obtained.

DURATION OF THE STUDY:

6 months. From April 2021 to September 2021.

STUDY DESIGN:

Case control study.

STUDY POPULATION:

Non – Diabetic individuals aged 20-80 years.

INCLUSION CRITERIA:

Confirmed cases of microcytic anaemia as evidenced by:

1. Hb< 12 g/dl (women); < 13 g/dl (men).

2. MCV < 80 fl.

3. Peripheral smear showing microcytosis.

EXCLUSION CRITERIA:

1. Acute/Chronic blood loss.

2. Hemolytic anaemia.

3. Hemoglobinopathies.

4. Chronic kidney disease.

5. Pregnancy.

6. Established diabetes.

7. Impaired fasting glucose.

8. Impaired glucose tolerance.

9. Family history of diabetes.

10. Obesity**.**

SELECTION OF CASES:

Patients in the general medical ward meeting the inclusion and exclusion criteria were selected as cases for the study.

CONTROLS:

Age and gender matched subjects who did not have microcytic anaemia but still meeting the exclusion criteria were selected as controls.

SAMPLE SIZE:

Based on the reference study done by Alap L. Christy et al, Kasturba Medical College, Manipal University[41],

Formula:

$n = 2(Z_a+Z_B)^2 SD^2/(M_1-M_2)^2$

Where $Z_a = 1.96$ (statistical significant constant for 95% CI)

Z_B = 0.84 (80% power)

SD = 1.4 (Average Standard deviation of HbA1c among anaemic patients from previous study)

M_1 =6.87 (Mean HbA1c among anaemic patients from previous study)

M_2 = 5.65 (Mean HbA1c among non anaemic patients from previous study)

$(M_1-M_2)^2 = (6.87 – 5.65)^2 = (1.22)^2 = 1.4884$

On substituting in the formula

n = 2×7.84×1.96 / 1.4884

n = 20.65 = 21

Adding 10% non-response rate (i.e. 10% of 21= 2)

n = 23 (minimum sample size)

Therefore Sample size **n = 30 (1 group), n = 60 (2 groups).**

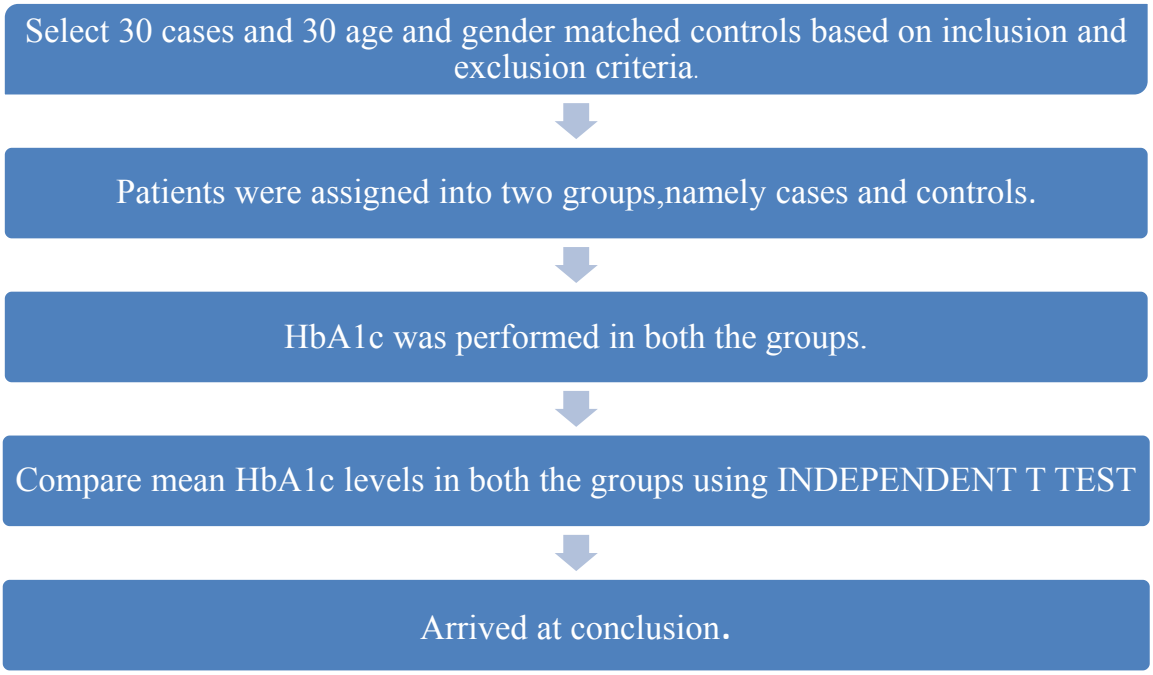
DATA COLLECTION AND METHODS:

A total of 30 cases and 30 controls from general medical ward and OPD were selected according to inclusion and exclusion criteria and patients were subjected to the following investigations:

1. Complete haemogram (CBC).
2. Peripheral smear study (PS).
3. Fasting blood glucose (FBG).
4. Post Prandial Blood Glucose (PPBG).
5. Glycosylated hemoglobin (HbA1c).

HbA1c levels were then compared between both the groups and its correlation with microcytic anaemia was calculated.

METHODOLOGY:



STATISTICAL ANALYSIS:

After collecting, the data was compiled and entered in Microsoft Excel Sheet. Analysis was done using Statistical software SPSS version 26. All continuous variables were expressed as Mean and Standard Deviation .All categorical variables were expressed as Percentages and Proportions. The test was considered significant if P value is <0.05, at 95% Confidence Interval.

ETHICAL CONSIDERATION:

Patients were given a patient information sheet and informed consent form that were verbally explained to the patients orally in a language they understand. Confidentiality is maintained.

CONFLICT OF INTEREST:

None to declare

SPONSORSHIP: No.

Observation and Results

OBSERVATION AND RESULTS

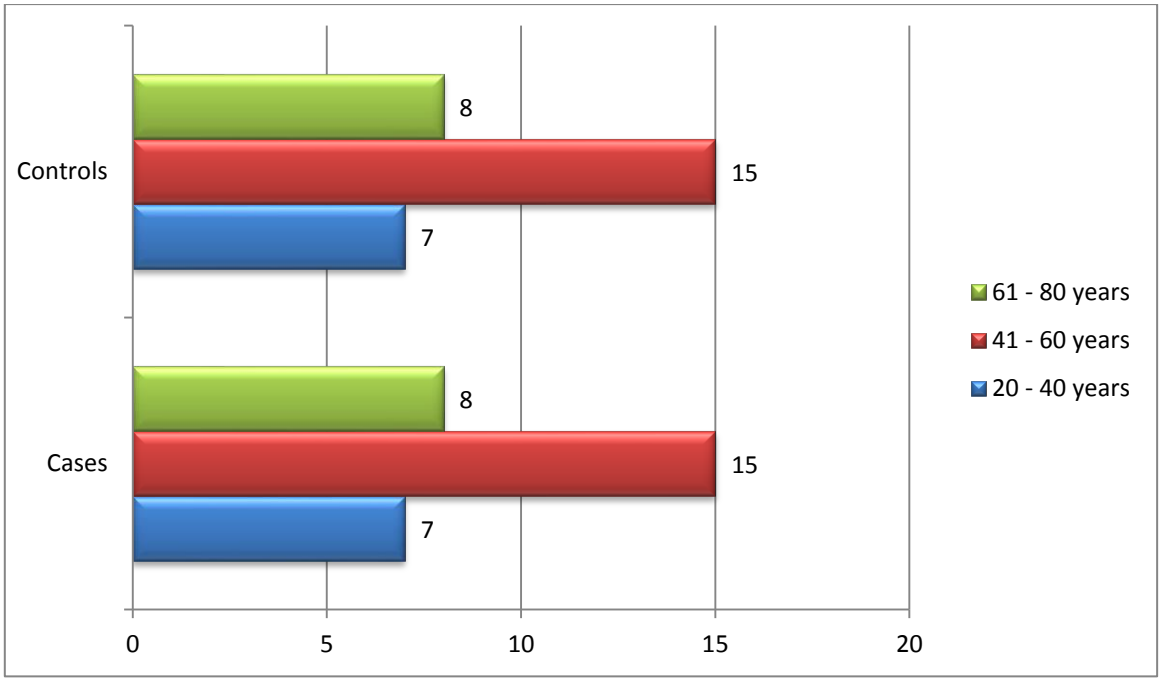
- The results obtained are analyzed using SPSS software version 26.
- For all practical purposes, anaemia defined in the analysis is to be considered as microcytic hypochromic anaemia as defined above and healthy as healthy age and gender matched controls.

1. AGE WISE DISTRIBUTION OF CASES AND CONTROLS:

			Age Group (in years)			**TOTAL**
			20 – 40	41 – 60	61 - 80	
Study Group	CASE	Count	7	15	8	30
		% within group	23%	50%	27%	100%
	CONTROL	Count	7	15	8	30
		% within group	23%	50%	27%	100%
TOTAL		Count	14	30	16	60
		% within group	23%	50%	27%	100%

Since the study was age and gender matched, the age wise distribution characteristics were similar in the case and control group. Patients were selected belonging to the age group of 20 – 80 years. About 50% of the patients in both case and control group belonged to the age group of 41 – 60 years, 27% belonged to 61 – 80 years and 23% belonged to 20 – 40 years. 30 participants were in the age group of 41 – 60 years, 16 participants were in the age group of 61 – 80 years and 14 were in the age group of 20 – 40 years.

BAR DIAGRAGM ILLUSTRATING THE AGE WISE DISTRIBUTION OF CASES AND CONTROLS:



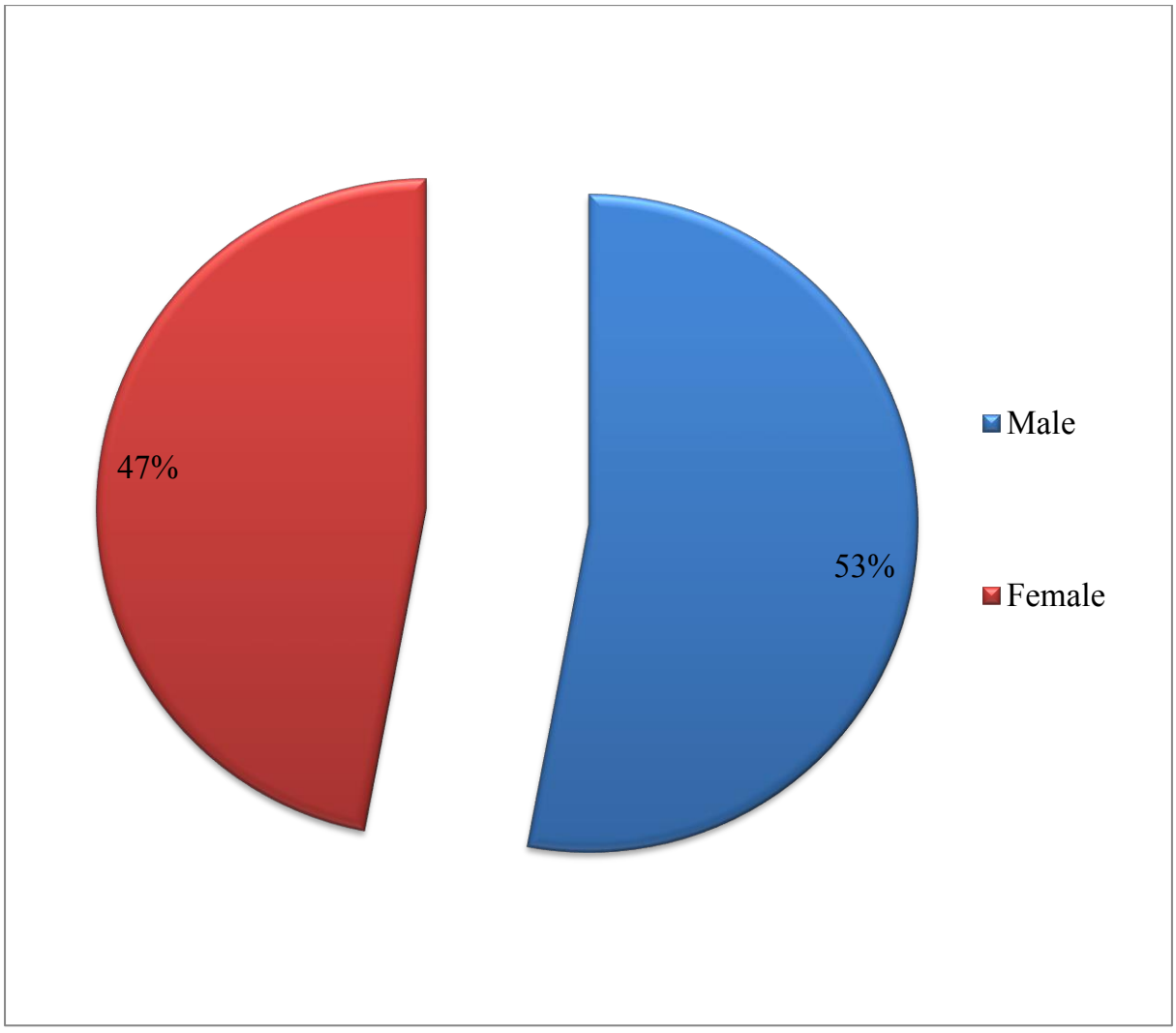
As illustrated above majority of the participants fall in the 41 – 60 years age group.

2. GENDER WISE DISTRIBUTION OF CASES AND CONTROLS:

			GENDER		TOTAL
			MALE	FEMALE	
Study Group	CASE	Count	16	14	30
		% within group	53%	47%	100%
	CONTROL	Count	16	14	30
		% within group	53%	47%	100%
TOTAL		Count	32	28	60
		% within group	53%	47%	100%

Since the study was age and gender matched, gender distribution is the same in control group as in the cases group. 53% of the participants were male and 47% were female. 32 of the 60 participants were male while 28 of the 60 participants were female.

PIE CHART ILLUSTRATING THE GENEDER DISTRIBUTION OF CASES AND CONTROLS:



As illustrated above, majority of the participants were male.

3. CASE AND CONTROL DEFINITION:

			Hemoglobin Group		TOTAL
			NON - ANAEMIC	ANAEMIC	
Study Group	CASE	Count	0	30	30
		% within group	0%	100%	100%
	CONTROL	Count	30	0	30
		% within group	100%	0%	100%
TOTAL		Count	30	30	60
		% within group	100%	100%	100%

Cases were anaemic patients, defined by a Hemoglobin level of < 12 g/dl for females and < 13 g/dl for males. Controls were non – anaemic subjects.

As illustrated above, patients in the case and control groups were fulfilling the inclusion and exclusion criteria and there were no overlap between the 2 groups with respect to their categorization as case or control.

4. DISTRIBUTION OF CASES ACCORDING TO SEVERITY OF ANAEMIA:

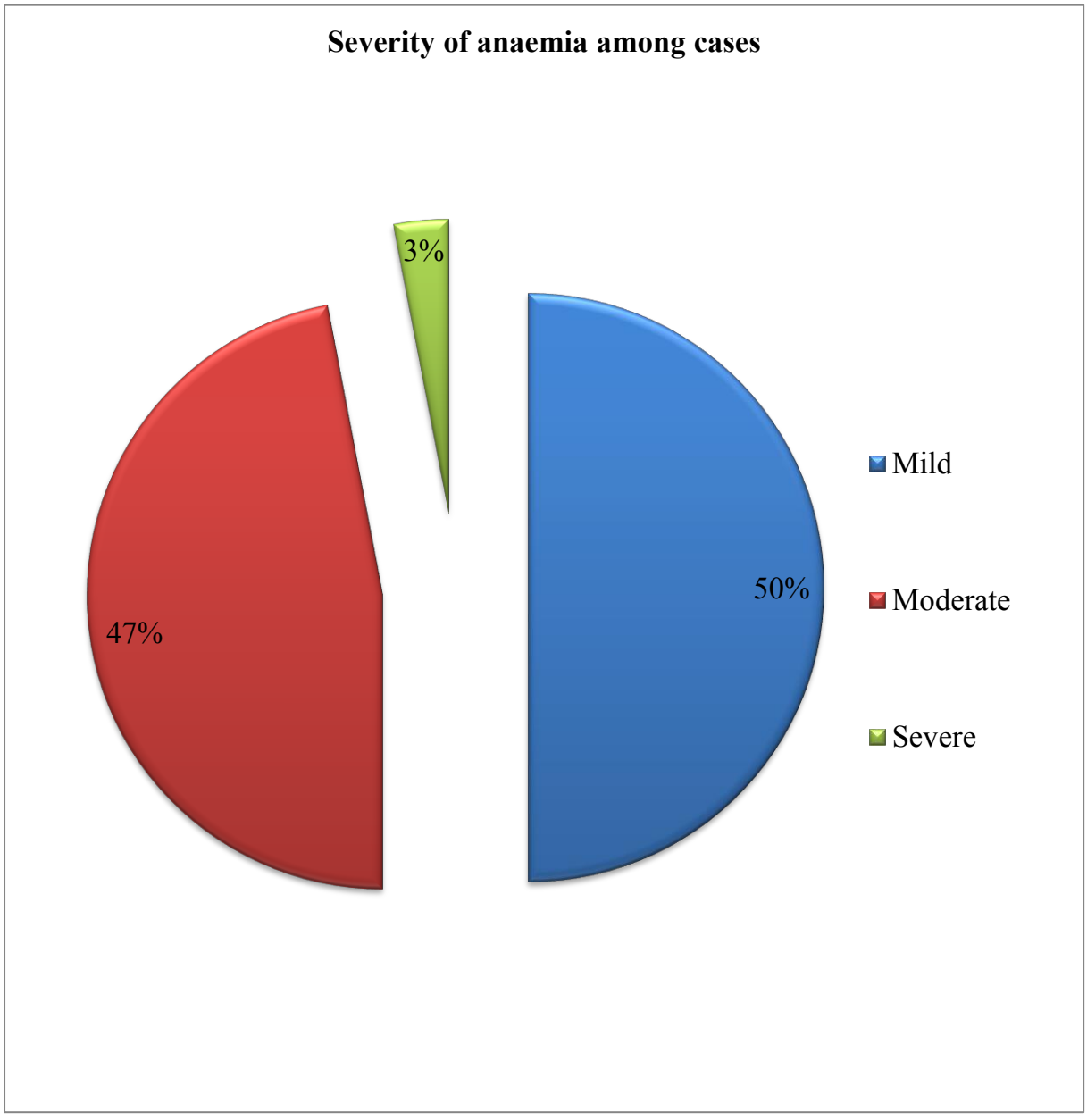
The severity of anaemia is classified as mild, moderate and severe based on the cut offs used in the DHS 7 statistics[42] as follows:

1. Mild anaemia: Hemoglobin count above 10.0 g/dL.
2. Moderate anaemia: Hemoglobin count between 7.0 and 9.9 g/dL.
3. Severe anaemia: Hemoglobin count less than 7.0 g/dL.

			Study group
			CASES
Severity Of Anaemia	MILD	Count	15
		% within group	50%
	MODERATE	Count	14
		% within group	47%
	SEVERE	Count	1
		% within group	3%
TOTAL		Count	30
		% within group	100%

Since the proportion of men in our study was higher, majority of the patients had mild anaemia. This trend was consistent with as observed in DHS 7, where mild anaemia was more prevalent among men than moderate or severe anaemia.

PIE CHART ILLUSTRATING THE PROPORTION OF SEVERITY OF ANAEMIA AMONG THE CASES:



5. CORELLATION BETWEEN MCV AND HbA1c LEVELS:

			HbA1c group		Number of participants
			NORMAL (< 6.5%)	ABNORMAL (> OR = 6.5%)	
MCV Group (fL)	50 - 60	Count	0	1	1
		% within group	0%	100%	2%
	61 – 70	Count	3	13	16
		% within group	19%	81%	27%
	71 – 80	Count	1	11	12
		% within group	8%	92%	20%
	81 - 90	Count	17	1	18
		% within group	95%	5%	30%
	Above 90	Count	12	1	13
		% within group	92%	8%	21%
Total		Count	33	27	60
		% within group	55%	45%	100%

ANALYSIS OF MCV VALUES AND HbA1c LEVELS:

As tabulated above, 2% of the participants had an MCV between 50 – 60 fL. Of these 100% of them had HbA1c > 6.5%. 1 participant had MCV between 50 -60 fL and had abnormal HbA1c.

27% of the participants had MCV between 61 – 70 fL. Of these 81% had HbA1c > 6.5% while 19% had HbA1c < 6.5%. 16 participants had MCV between 61 – 70 fL of which 13 had abnormal HbA1c and 3 had normal HbA1c.

20% of the participants had MCV between 71 – 80 fL. Of these 92% had HbA1c > 6.5% while 8% had HbA1c < 6.5%. 12 participants had MCV between 71 – 80 fL of which 11 had abnormal HbA1c and 1 had normal HbA1c.

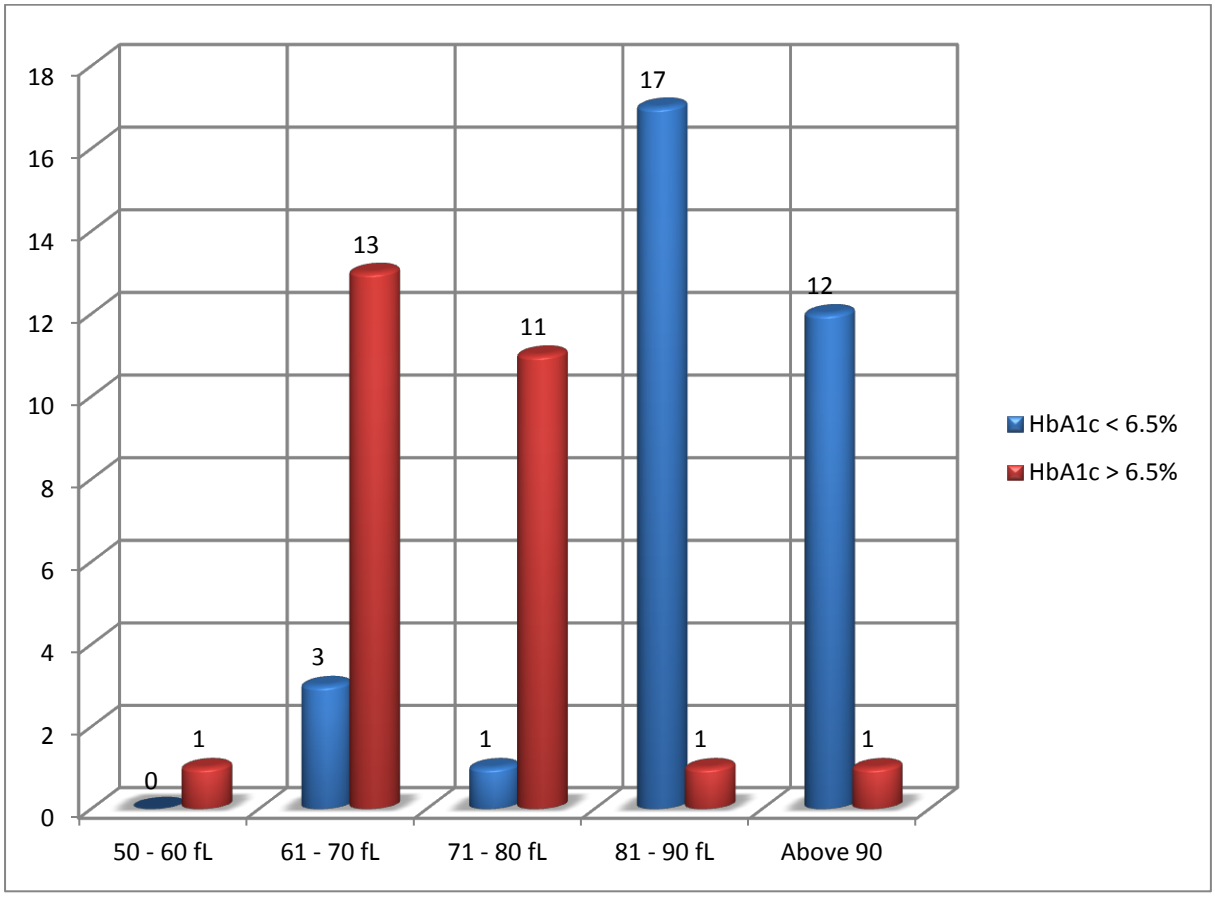
30% of the participants had MCV between 81 – 90 fL. Of these 5% had HbA1c > 6.5% while 95% had HbA1c < 6.5%. 18 participants had MCV in the range of 81 – 90 fL of which 1 had abnormal HbA1c and 17 had normal HbA1c.

21% of the participants had MCV above 90 fL. Of these 8% had HbA1c > 6.5% while 92% had HbA1c < 6.5%. 13 participants had MCV above 90 fL of which 1 had abnormal HbA1c and 12 had normal HbA1c.

The correlation between MCV and HbA1c level was analyzed using independent t test. **The Pearson Correlation was -0.705 which signifies a strong negative correlation between MCV and HbA1c with a 'p' value of 0.01 which is less than 0.05 and is statistically significant.** This implies that lower the MCV, higher will be the HbA1c which proves the fact that HbA1c can be elevated falsely in microcytic anemia.

BAR DIAGRAM DEPICTING THE CORRELATION BETWEEN

MCV GROUPS AND HbA1c LEVELS:



6. CORELLATION BETWEEN MCV AND HEMOGLOBIN LEVELS:

			Hb group		Number of participants
			NON ANAEMIC	ANAEMIC	
MCV Group (fL)	50 - 60	Count	0	1	1
		% within group	0%	100%	2%
	61 – 70	Count	0	16	16
		% within group	0%	100%	27%
	71 – 80	Count	0	12	12
		% within group	0%	100%	20%
	81 - 90	Count	17	1	18
		% within group	95%	5%	30%
	Above 90	Count	13	0	13
		% within group	100%	0%	21%
Total		Count	33	27	60
		% within group	55%	45%	100%

ANALYSIS OF MCV VALUES AND HEMOGLONBIN LEVELS:

As tabulated above, 2% of the participants had an MCV between 50 – 60 fL. 100% of them are anaemic. 1 participant had MCV between 50 - 60 fL and was anaemic.

27% of the participants had MCV between 61 – 70 fL. 100% of them had anaemia. 16 participants had MCV between 61 – 70 fL and all of them were anemic.

20% of the participants had MCV between 71 – 80 fL. 100% of them had anaemia. 12 participants had MCV between 71 – 80 fL and all of them were anaemic.

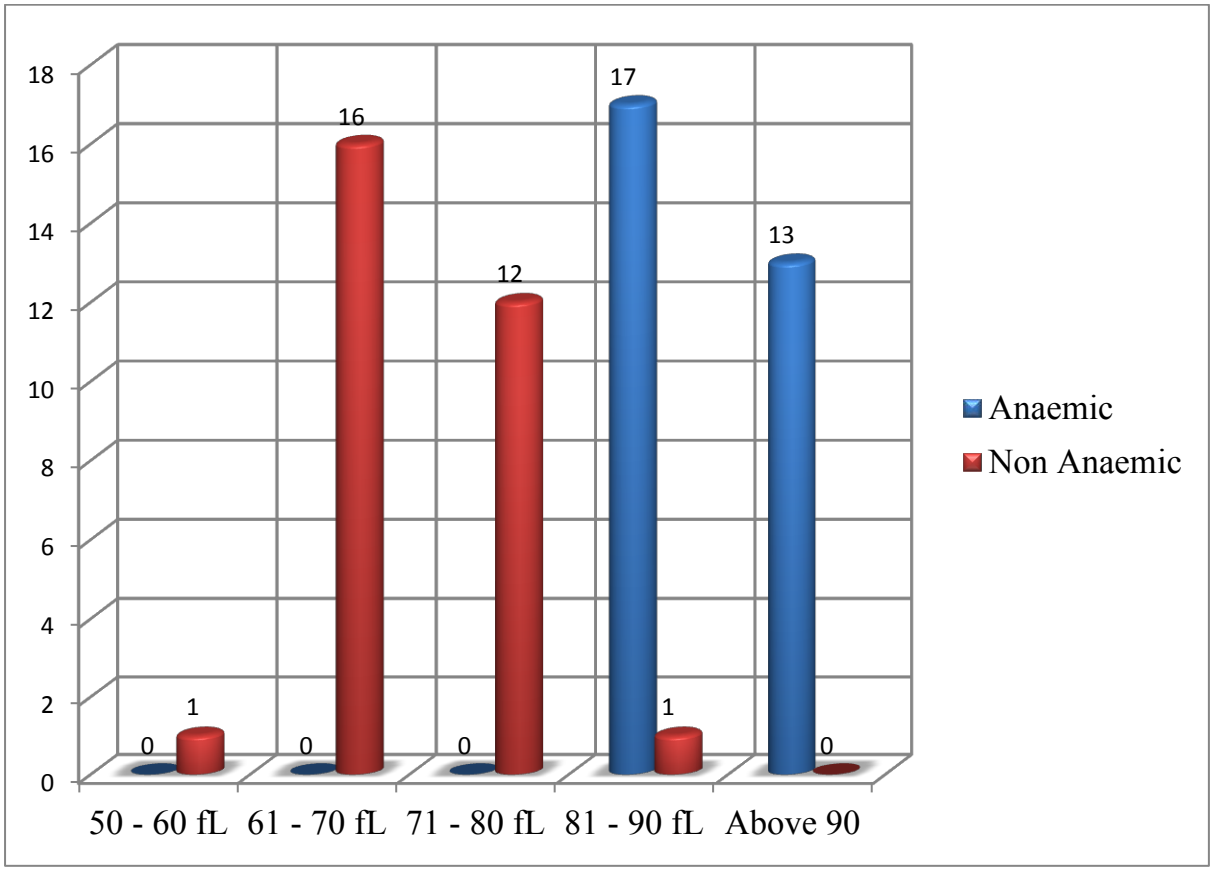
30% of the participants had MCV between 81 – 90 fL. Of these 5% had anaemia while 95% were non anaemic. 18 participants had MCV in the range of 81 – 90 fL of which 1 was anaemic and 17 were non anaemic.

21% of the participants had MCV above 90 fL. All of them were non anaemic. 13 participants had MCV above 90 fL.

The correlation between MCV and Hemoglobin levels were analyzed using independent t test. **The Pearson Correlation was 0.889 which signifies a strong positive correlation between MCV and Hemoglobin with a 'p' value of 0.01 which is less than 0.05 and is statistically significant.** This implies that people with lower MCV tend to have lower hemoglobin values.

BAR DIAGRAM DEPICTING THE CORRELATION BETWEEN

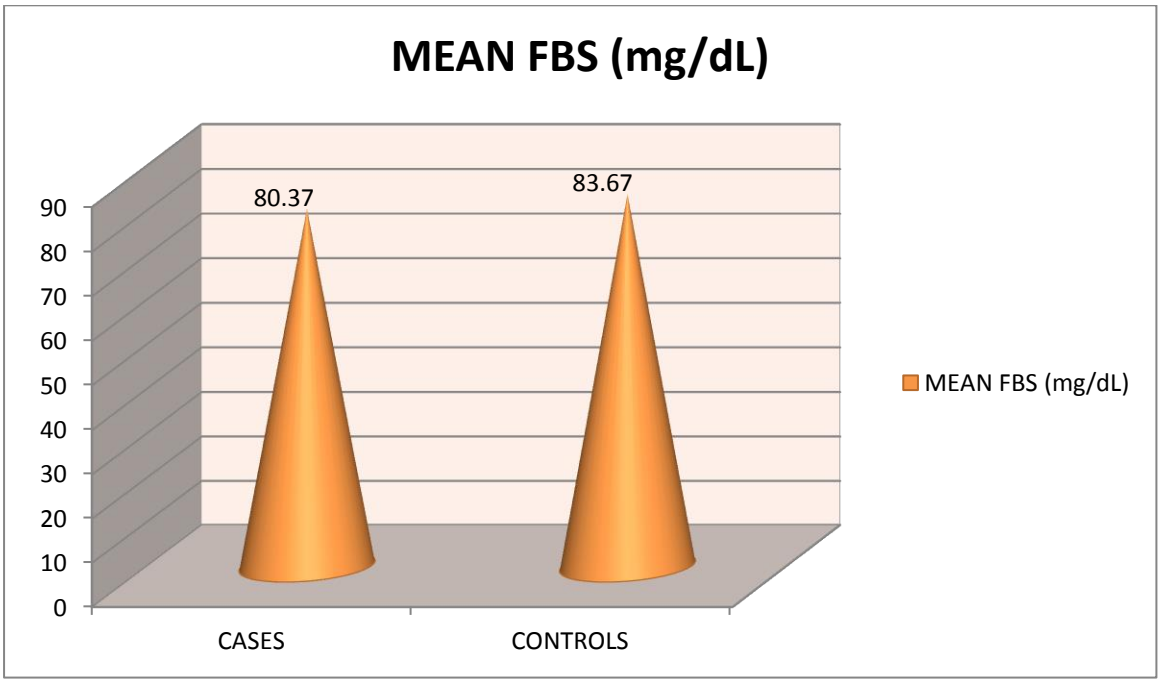
MCV GROUPS AND HEMOGLOBIN LEVELS:



7. GROUP STATISTICS:

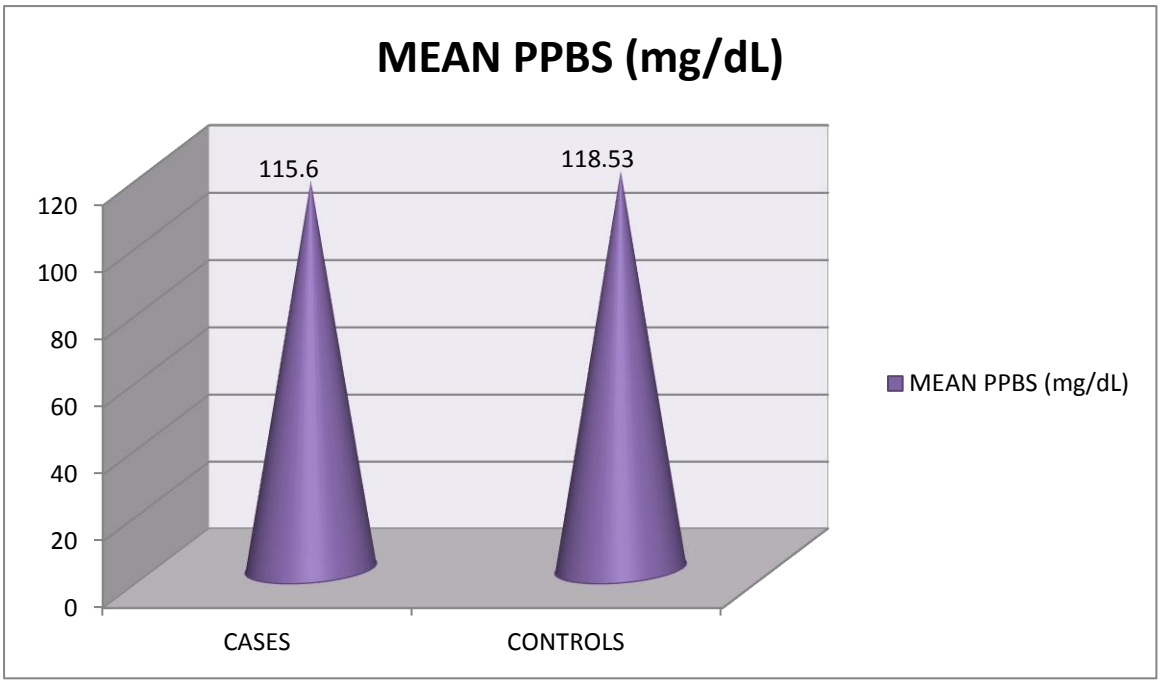
	GROUP	N	MEAN	STANDARD DEVIATION	STANDARD ERROR MEAN
FBS (mg%)	Case	30	80.37	7.963	1.454
	Control	30	83.67	8.766	1.601
PPBS (mg%)	Case	30	115.60	7.981	1.457
	Control	30	118.53	10.801	1.972

BAR DIAGRAM COMPARING THE MEAN FBS VALUES BETWEEN CASES AND CONTROLS:



- The mean FBS of the cases were 80.37 ± 7.963 mg/dL.
- The mean FBS of the controls were 83.67 ± 8.766 mg/dL.
- This implies that the cases and control group did not differ much in their mean FBS values.

BAR DIAGRAM COMPARING THE MEAN PPBS VALUES BETWEEN CASES AND CONTROLS:



- The mean PPBS of the cases were 115.60 ± 7.981 mg/dL.
- The mean PPBS of the controls were 118.53 ± 10.801 mg/dL.
- This implies that the cases and control group did not differ much in their mean FBS values.

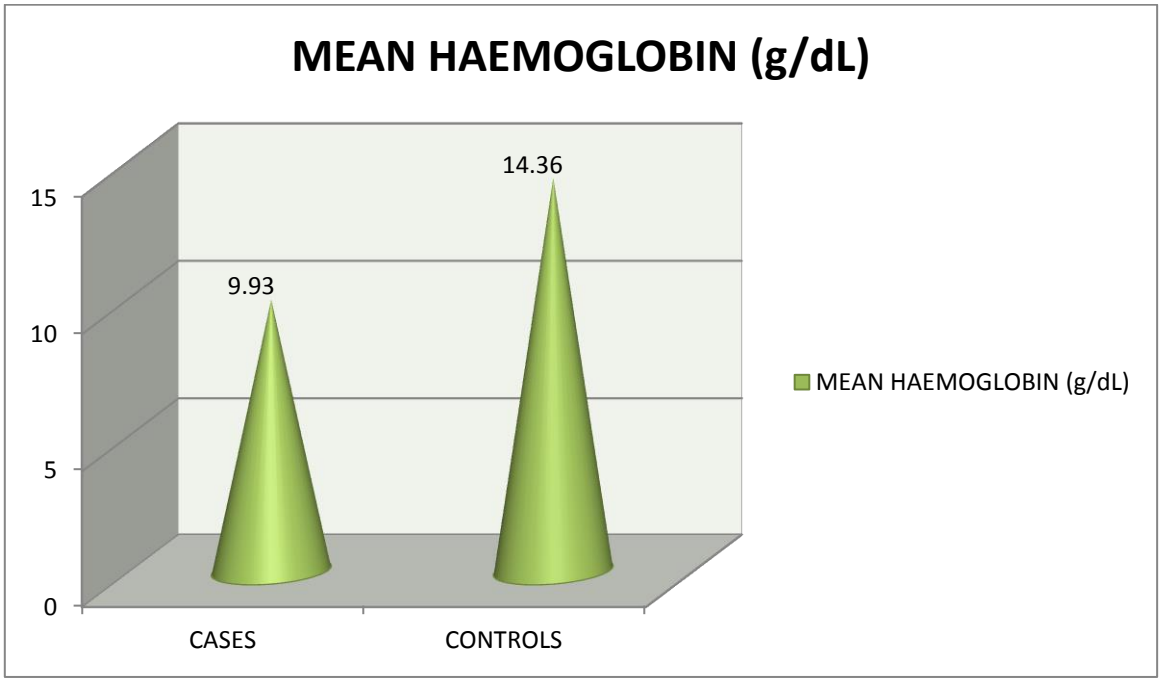
Through this analysis we can conclude that the effect of the well - known confounding factor – hyperglycemia has been eliminated in our study, as the study participants of the case and control group were similar in their characteristics with regards to their glycemic status.

8. GROUP STATISTICS:

	GROUP	N	MEAN	STANDARD DEVIATION	STANDARD ERROR MEAN	CALCULATED T-STATISTIC
Hb	CASE	30	9.93	1.148	0.210	-12.832
	CONTROL	30	14.36	1.341	0.245	
MCV	CASE	30	70.57	6.322	1.154	-12.992
	CONTROL	30	88.44	4.008	0.732	
HbA1c	CASE	30	6.80	0.532	0.097	12.732
	CONTROL	30	5.41	0.427	0.078	

The 'p' value of the calculated t-statistic was <0.001, which is statistically significant.

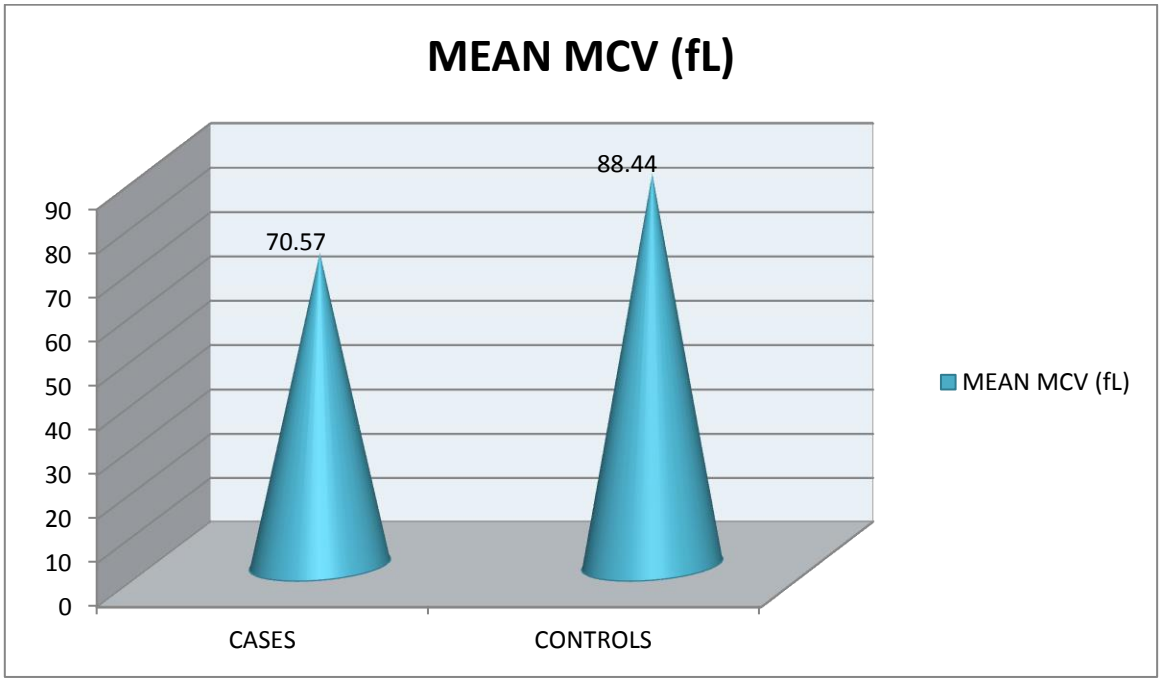
BAR DIAGRAM COMPARING THE MEAN HEMOGLOBIN LEVELS BETWEEN CASES AND CONTROLS:



- The mean hemoglobin level in the case group was 9.93 ± 1.148 g/dL.
- The mean hemoglobin level in the control group was 14.36 ± 1.341 g/dL.

The mean hemoglobin levels were significantly lower in the cases group than the control group with a difference of 4.43 g/dL of Hb. **This difference is statistically significant with a 'p' value of < 0.001, which proves that there is a definite difference in the Hb values between cases and controls**. This implies that there was no error in categorising patients as cases and controls.

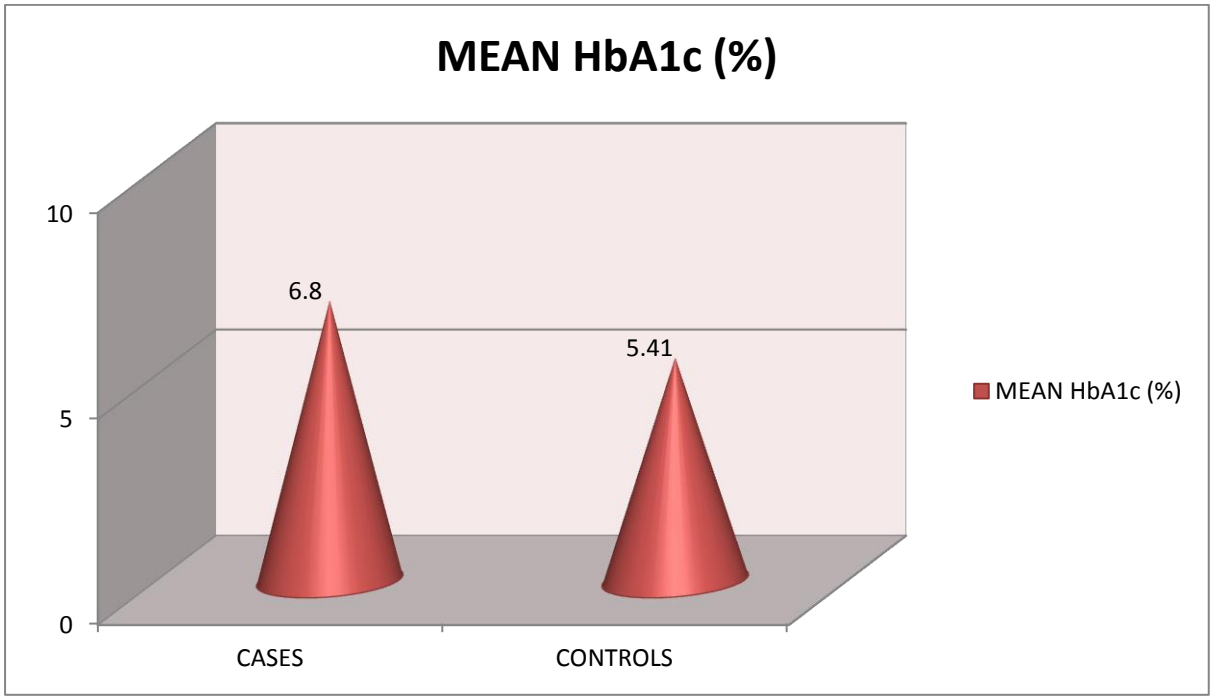
BAR DIAGRAM COMPARING THE MEAN MCV VALUES BETWEEN CASES AND CONTROLS:



- The mean MCV value in the case group was 70.57 ± 6.322 fL.
- The mean MCV value in the control group was 88.44 ± 4.008 fL.

The difference between the mean MCV values of the cases and controls was 17.87 fL. The MCV values were significantly lower in the cases group than the control group**. This difference in the mean MCV values between the two groups is statistically significant with a 'p' value of < 0.001**.

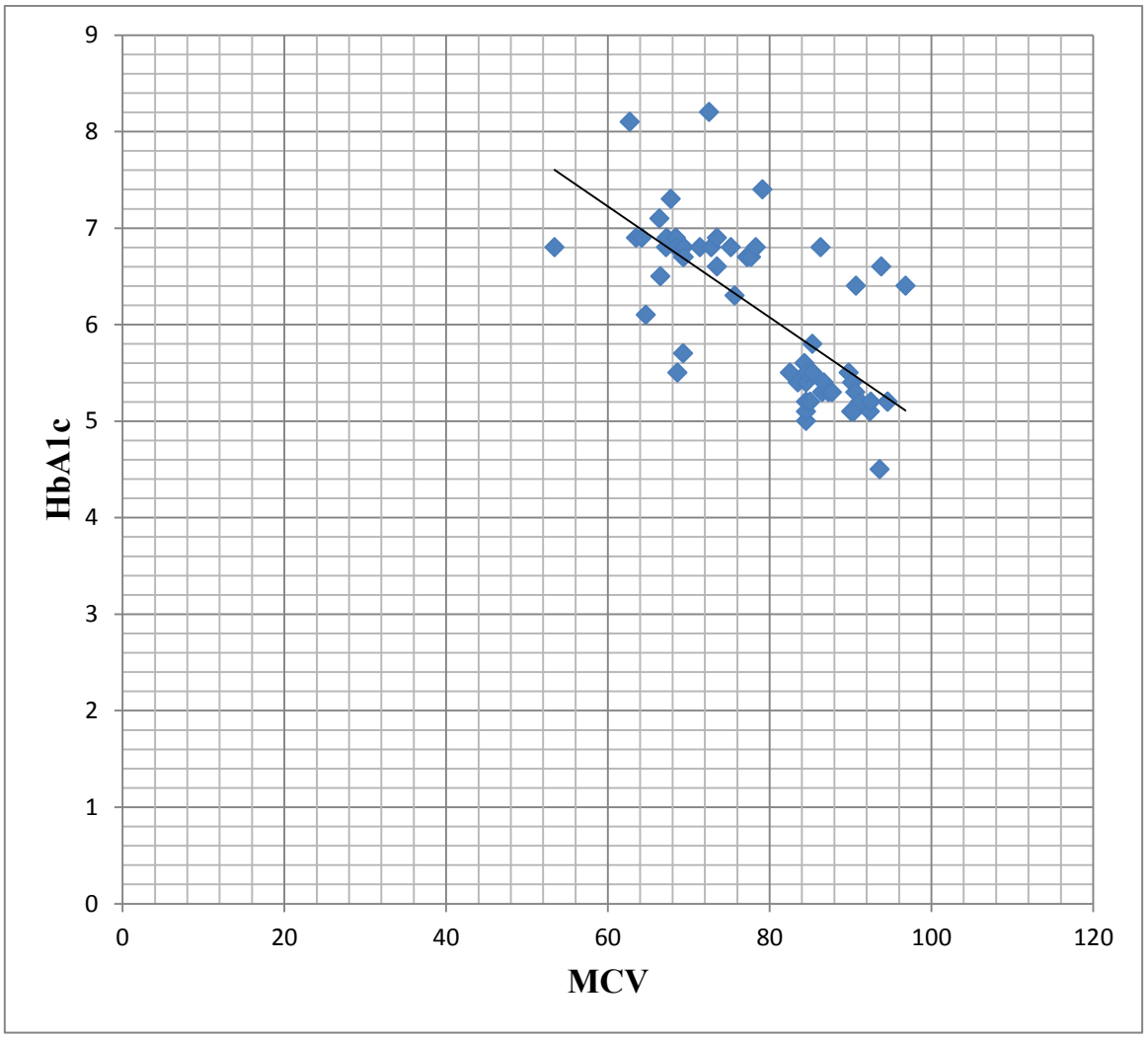
BAR DIAGRAM COMPARING THE MEAN HbA1c VALUES BETWEEN CASES AND CONTROLS:



- The mean HbA1c value in the case group was 6.8 ± 0.532 %.
- The mean HbA1c value in the control group was 5.41 ± 0.427 %.

The difference between the mean HbA1c values of cases and controls is 1.39%. The HbA1c values were significantly higher in the cases group than the control group. This difference is which is also statistically significant with a 'p' value of < 0.001. This concludes our study that a statistically significant difference in HbA1c levels exists between an anemic and non anemic patient for the same blood sugar levels.

SCATTER PLOT BETWEEN MCV AND HbA1c:



- This scatter plot signifies the negative correlation between MCV and HbA1c.

PEARSON CORRELATION

Correlations				
		MCV	Hb	HbA1c
MCV	Pearson Correlation	1	.889**	-.705**
	Sig. (2-tailed)		.000	.000
	N	60	60	60
Hb	Pearson Correlation	.889**	1	-.761**
	Sig. (2-tailed)	.000		.000
	N	60	60	60
HbA1c	Pearson Correlation	-.705**	-.761**	1
	Sig. (2-tailed)	.000	.000	
	N	60	60	60
**. Correlation is significant at the 0.01 level (2-tailed).				

- ✓ The Pearson correlation between MCV and HbA1c is **"-0.705"** which signifies **inverse correlation** between the two variables, thus completing the aim of the study.
- ✓ The Pearson correlation between MCV and Hb is "**0.889**" which signifies positive correlation between the two variables.

Discussion

DISCUSSION

A case control study was conducted in the department of internal medicine at Government tertiary care hospital, Chennai for a period of 6 months from March 2021 to September 2021.

30 cases and 30 controls were chosen from the general medical ward based on the inclusion and exclusion criteria as mentioned above. Fasting and post prandial blood sugars were performed to all cases and controls in order to ensure that all those chosen for the study were only non – diabetic adults. After getting informed consent from them, all patients were subjected to detailed history taking, physical examination and relevant laboratory investigations. HbA1c levels were obtained from both the groups and compared using the independent t test.

Iron deficiency anemia is the most common form of microcytic anemia and is the most prevalent anaemia in India and worldwide. Besides blood sugar, various other conditions such as hemolytic anemias, hemoglobinopathies, hyperbilirubinemias, iron, folate and vitamin B12 deficiency, acute and chronic blood loss, pregnancy, chronic liver disease and uremia have an impact on the levels of HbA1c. Recently, more concern has arisen in studying the effect of microcytic anemias like iron deficiency on HbA1c levels.

The earliest study that investigated the effects of iron deficiency anemia on HbA1c levels was conducted by Brooks et al.[43], in which HbA1c levels were assessed in 35 non-diabetic patients with iron deficiency anemia. Assessment of HbA1c values

was done before and after treatment with iron. The observation was that HbA1c levels in iron deficiency anemia patients were higher and the HbA1c values decreased after treatment with iron.

It was proposed that, iron deficiency alters the quaternary structure of the hemoglobin molecule. The glycation of the globin chain, in the relative absence of iron would occur more readily[44]. Sluiter et al[45], tried to provide an explanation for the above mentioned findings. They had proposed that the formation of glycated hemoglobin (HbA1c) within an erythrocyte was an irreversible process. Hence, the concentration of HbA1c within a single erythrocyte would linearly increase with the age of the red cell. They also found out that in patients with euglycemia, but with very young red cells, the HbA1c concentration was reduced.

Later, van Heyningen et al. reported that there were no differences in HbA1c concentrations compared to the non-diabetic patients with iron deficiency anemia before and after treatment with iron[46]. They also believed that the differences in HbA1c concentrations that were noted before and after iron supplementation were chiefly due to the differences in the laboratory methods used for measuring and calibrating HbA1c.

Raiet al. later investigated and came up with the conclusion that even though different methods are used to measure HbA1c, no difference was noted among the colorimetric methods, ion-exchange chromatography and affinity chromatography[47].

Hansen et al. also demonstrated that HbA1c levels tend to decreased upon treatment of the anemia[48] adding evidence to the study done by Sluiter et al., This was

thought to occur as a result of increased bone marrow erythropoiesis which was brought about by the treatment with iron, thus leading to production of new immature erythrocytes from the bone marrow. They also showed that HbA1cconcentrations was normal in iron deficiency, which would drop down to sub-normal levels after iron supplementation.

Further studies conducted by El-Agouza et al., and Cogan et al.[49] came up with the result stating that HbA1c levels were higher in patients with iron deficiency anemia and would decrease on iron supplementation[50]. They argued that, an elevated HbA1c level in iron deficiency anemia was due to the fact that, if the serum glucose remains constant, a decrease in the hemoglobin concentration increases the glycation of hemoglobin.

As is evident from the above studies, the mechanisms by which iron deficiency (microcytic) anemia affects HbA1c levels remains unclear. Since there are large variations in the study results, we conducted our own study to investigate the effects of microcytic anaemia on HbA1c levels. In our study, 30 non diabetic cases and 30 non diabetic controls were selected and grouped based mainly on hemoglobin and MCV values. Later HbA1c values were obtained from both the groups were compared using the independent t test. The correlation between MCV and HbA1c values were calculated using the Pearson co-efficient. **The 'p' value of the test was <0.001, which is statistically significant with a Pearson coefficient of "-0.705"**. So our study concludes that an inverse correlation exists between MCV values and HbA1c levels. So anemia, if present, must be corrected before any decision, whether diagnostic or therapeutic, is made based on the HbA1c levels.

Conclusion

CONCLUSION

Based on our study we can safely conclude that microcytic anemia definitely has an impact on the HbA1c. As MCV decreases in patients with microcytic anemia, HbA1c tends to rise. Thus, microcytic anemia, if present must be corrected before making any diagnostic or therapeutic decision based on the HbA1c levels.

Limitations

LIMITATIONS

- The sample size was small.
- Not all age groups are equally studied
- A confirmatory test for iron deficiency like serum ferritin was not applied in this study. A broad label of microcytic hypochromic anemia is used.
- Since being made in a government general hospital, the study population chiefly belonged to a lower socio-economic class. Here, the cause of microcytic anemia, the major cause being iron deficiency anemia is not just bleeding and mal-absorption, but also nutritional deficiency which may also have an impact on our study results. Other unknown variables such as racial, geographical factors also may influence our results.
- The study was a case control study. No effort was made at correcting anemia and henceforth the changes in the trend were not observed.

Further studies to confirm the roles of various other factors are needed to confirm our findings.

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Annexures

PROFORMA

Name: Age/Sex:
Address: Occupation:

SYMPTOMS:

Easy fatigability	
Abdominal pain	
Abdominal distension	
Dyspnea	
Pedal edema	
Previous blood transfusions	
Melena	

PAST HISTORY:

CAD	
CKD	
CLD	

PERSONAL HISTORY:

SMOKING	
ALCOHOL	

FAMILY HISTORY OF DIABETES:

GENERAL EXAMINATION:
Pallor
Icterus
Cyanosis
Clubbing
Pedal edema
Lymphadenopathy

BMI:

VITAL SIGNS:
PR BP
RR JVP
Temp-

SYSTEMIC EXAMINATION:
CVS:

RS:

ABDOMEN:

CNS:

INVESTIGATIONS:

1. COMPLETE HEMOGRAM:
   Hemoglobin:
   MCV:
   MCH:
   MCHC:
   Hematocrit:

2. PERIPHERAL SMEAR:

3. ORAL GLUCOSE TOLERANCE TEST (OGTT): Fasting:
   1hr:
   2hr:

4. HbA1c:

INFORMATION SHEET

We are conducting a study on **"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NONDIABETIC ADULTS"** among patients attending Government Stanley General Hospital, Chennai and for that your specimen may be valuable to us. The purpose of this study is to estimate HbA1c levels in non-diabetic adults with iron deficiency anemia and to compare it with HbA1c levels of non-diabetic controls.

We are selecting certain cases and if you are found eligible, we may be using your blood samples to do certain tests which in any way do not affect your final report or management.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator Signature of Participant

Date:

Place:

ஆராய்ச்சி தகவல் தாள்

சென்னை அரசு ஸ்டான்லி பொது மருத்துவமனையின் பொது மருத்துவத்துறையில் "நீரிழிவு நோய் அல்லாதவர்களில் குளூகோஸ் கூட்டப்பட்ட ஹீமோகுளோபின் ஏ1சியின மீது குறைந்த சிகப்பு ரத்த அணுக்களின் கொள்ளளவின் விளைவை ஆராய்தல்" பற்றிய ஆய்வு நடைபெறுகிறது.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதனால் தங்களது சிகிச்சையில் பாதிப்பு ஏற்படாது என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த ஆய்வில் தங்களுக்கு மருத்துவபரிசோதனை, இரத்தப் பரிசோதனை மற்றும் சிறுநீர் பரிசோதனை செய்யப்படும்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின்போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதை தெரிவித்துக்கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின்பேரில்தான் இருக்கிறது. மேலும் நீங்கள் எந்த நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளையும் நோயின் தன்மை பற்றியும் ஆராய்ச்சியின்போது அல்லது ஆராய்ச்சியின் முடிவின்போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

நாள் :
இடம் :

CONSENT FORM

Study Detail : **"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A1c IN NONDIABETIC ADULTS"**
Study Centre : Government Stanley Medical College and Hospital, Chennai.
Patient's Name :
Patient's Age :
Identification Number:

Patient may check (√) these boxes

a) I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ❑

b) I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ❑

c) I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access, However, I understand that my identity will not be revealed in any 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 this study. ❑

d) I understand that I don't have to spend money for anything regarding this study ❑

e) I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well-being or any unexpected or unusual symptoms. ❑

f) I hereby consent to participate in this study. ❑

g) I hereby give permission to undergo detailed clinical examination and blood investigations, as required. ❑

Patient's signature/left thumb impression Signature of Investigator

Patient's Name and Address:

Study Investigator'sName:
Dr.ANBURAJAN.S

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு : **நீரிழிவு நோய் அல்லாதவர்களில் குளுகோஸ் கூட்டப்பட்ட ஹீமோகுளோபின் ஏ1சியின் மீது குறைந்த சிகப்பு ரத்த அணுக்களின் கொள்ளளவின் விளைவை ஆராய்தல்.**

ஆய்வு நிலையம் : பொது மருத்துவத்துறை, அரசு ஸ்டான்லி மருத்துவக் கல்லூரி சென்னை - 1.

பங்கு பெறுபவரின் பெயர் :

உள்நோயாளி எண் :

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வில் தன்னிச்சையாகதான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக்கொள்ளவும் அதை பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கின்றேன். ☐

இந்த ஆய்வு தொடர்பாக நான் எதற்கும் பணம் செலவழிக்க வேண்டியதில்லை என்பதை நான் புரிந்துகொள்கிறேன் ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன், இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிகிறேன். ☐

இந்த இரு அறுவை சிகிச்சை முறைகளும் ஒப்புக்கொள்ளப்பட்ட முறைகள் என்பதையும் இதனால் உடலுக்கு எந்தவிதமான உபாதைகளும் இருக்காது என்பதை அறிந்துகொண்டு இந்த ஆய்வில் பங்குபெற முழு மனதுடன் சம்மதிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம்/
இடது கை பெருவிரல் ரேகை ஆய்வாளரின் கையொப்பம்

பங்கேற்பவரின் பெயர் : ஆய்வாளரின் பெயர்

இடம்: தேதி: மரு.அன்புராஜன்.**சு**

GOVERNMENT STANLEY MEDICAL COLLEGE & HOSPITAL,CHENNAI -01
INSTITUTIONAL ETHICS COMMITTEE

TITLE OF THE WORK	:	"EFFECT OF MICROCYTIC ANEMIA ON GLYCOSYLATED HEMOGLOBIN A1C IN NON- DIABETIC ADULTS"
PRINCIPAL INVESTIGATOR	:	DR. S. ANBURAJAN,
DESIGNATION	:	PG IN GENERAL MEDICINE,
DEPARTMENT	:	DEPARTMENT OF GENERAL MEDICINE

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 24.03.2021 at the Council Hall, Stanley Medical College, Chennai-1 at 11 am.

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

29/3/21

MEMBER SECRETARY,
IEC, SMC, CHENNAI.

MASTER CHART

Serial No	Category	Age	Gender	FBS	PPBS	Hb	MCV	HbA1c
1	CASE	46	F	80	125	10.3	68.4	6.9
2	CASE	54	F	74	114	10.5	69.3	6.7
3	CASE	54	M	83	105	11.9	79.1	7.4
4	CASE	48	F	86	108	10.6	72.8	6.8
5	CASE	37	M	92	120	8.5	72.5	8.2
6	CASE	73	F	75	109	8.9	67.8	7.3
7	CASE	54	M	67	104	10.4	78.3	6.8
8	CASE	56	F	75	124	10.5	73.5	6.9
9	CASE	67	M	81	114	9.7	53.4	6.8
10	CASE	32	M	76	107	9.5	66.5	6.5
11	CASE	56	F	89	121	9.4	68.6	5.5
12	CASE	37	M	74	113	11.9	71.4	6.8
13	CASE	73	F	94	121	9.6	64.2	6.9
14	CASE	38	M	79	119	9.4	75.7	6.3
15	CASE	63	M	84	117	11.3	68.4	6.8
16	CASE	28	M	85	129	8.9	69.3	5.7
17	CASE	48	M	72	132	11.3	77.3	6.7
18	CASE	38	F	79	123	11.5	77.2	6.7
19	CASE	57	F	83	105	10.5	75.2	6.8
20	CASE	59	M	63	118	10.4	67.2	6.8
21	CASE	61	M	75	109	9.7	77.7	6.7
22	CASE	47	F	84	104	9.4	64.7	6.1
23	CASE	73	M	89	115	6.2	62.7	8.1
24	CASE	67	M	89	126	9.5	66.4	7.1
25	CASE	27	F	95	122	10.3	63.5	6.9
26	CASE	63	F	73	107	9.5	69.4	6.8
27	CASE	59	F	85	114	8.6	67.3	6.9
28	CASE	43	M	76	118	10.5	73.5	6.6
29	CASE	56	F	85	105	9.5	69.4	6.7
30	CASE	54	M	69	120	9.6	86.3	6.8

Serial No	Category	Age	Gender	FBS	PPBS	Hb	MCV	HbA1c
1	CONTROL	46	F	98	135	12.8	82.5	5.5
2	CONTROL	54	F	90	121	13.6	84.5	5
3	CONTROL	54	M	88	125	15.8	92.5	5.2
4	CONTROL	48	F	86	104	12.6	85.3	5.5
5	CONTROL	37	M	72	129	16.5	96.8	6.4
6	CONTROL	73	F	95	106	13.5	86.7	5.4
7	CONTROL	54	M	84	108	15.5	90.7	6.4
8	CONTROL	56	F	81	105	13.6	84.5	5.1
9	CONTROL	67	M	88	115	15.4	91.2	5.2
10	CONTROL	32	M	95	122	14.5	87.7	5.3
11	CONTROL	56	F	92	127	14.6	84.5	5.4
12	CONTROL	37	M	95	127	16.2	93.8	6.6
13	CONTROL	73	F	75	134	13.2	84.3	5.6
14	CONTROL	38	M	70	135	16.1	90.1	5.1
15	CONTROL	63	M	95	105	12.6	85.3	5.5
16	CONTROL	28	M	77	100	14.5	90.4	5.1
17	CONTROL	48	M	79	120	16.4	93.6	4.5
18	CONTROL	38	F	87	128	12.6	83.5	5.4
19	CONTROL	57	F	77	115	13.5	87.3	5.3
20	CONTROL	59	M	95	128	15.5	92.4	5.1
21	CONTROL	61	M	81	132	14.8	90.6	5.3
22	CONTROL	47	F	73	113	13.5	89.8	5.5
23	CONTROL	73	M	85	120	16.6	94.6	5.2
24	CONTROL	67	M	72	120	14.5	94.6	5.2
25	CONTROL	27	F	80	127	12.6	85.3	5.8
26	CONTROL	63	F	75	105	13.9	86.5	5.3
27	CONTROL	59	F	70	124	12.5	84.5	5.2
28	CONTROL	43	M	94	115	14.9	90.2	5.4
29	CONTROL	56	F	76	101	13.2	84.6	5.5
30	CONTROL	54	M	85	110	15.4	85	5.2