The value of different red cell parameters in the diagnosis of microcytic hypochromic anaemia

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE MS DEGREE IN BIOTECHNOLOGY

Submitted by-

Raisa Akther
Session:2011-2012
Registration No:11276005
January, 2016

Department of Mathematics & Natural Sciences

Biotechnology Program

BRAC University

Bangladesh

http://www.bracu.ac.bd

DECLARATION

This to declare that the research work embodying the results reported in this thesis entitled **"The value of different red cell parameters in the diagnosis of microcytic hypochromic anaemia"**has been carried out by the under signed under joint supervision Professor Dr. Naiyyum Choudhury, Co-ordinator, Biotechnology and Microbiology program,Department of Mathematics and Natural Sciences,BRAC University and Dr. Hafizur Rahman, Senior scientific officer, Clinical Hematology Department , at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). It is further declared that the research work presented here is original and submitted in the partial fulfillment for the degree of Master of Science in Biotechnology, BRAC University, Dhaka and has not been submitted anywhere else for a degree or diploma.

Raisa Akther

Certified

Dr. Hafizur Rahman
Supervisor
Senior Scientific Officer,
Clinical Hematology Department
icddr,b.

Professor Dr. Naiyyum Choudhury
Supervisor
Biotechnology Program
Department of MNS
BRAC University.

COMMENTS OF THE GUIDE

Raisa Akther has worked on the subject "**The value of different red cell parameters in the diagnosis of microcytic hypochromic anaemia** " under my direct supervision. I have gone through the dissertation. It is up to my full satisfaction.

DR. HAFIZUR RAHMAN
SENIOR SCIENTIFIC OFFICER
DEPARTMENT OF CLINICAL HEMATOLOGY
ICDDRB,DHAKA

Acknowledgement

First and foremost, I express my deepest gratitude to the Almighty Allah for endowing me with health, patience, benediction, protection and mental power in all aspect of my life. During this year I have worked with many people for whom I have great regard, and I wish to extend my warmest thanks to all those who have helped me to complete the thesis.

I am overwhelmed to express my respect, sincere gratitude and heartfelt thanks to Dr. Hafizur Rahman, Senior Scientific officer, Clinical Hematology Department, icddr,b , Dhaka for his inspiration, constructive criticism, endearing company and specially for his scholastic guidance and his impeccable support to me in writing my thesis paper.

I would like to convey my indebtedness to Professor Dr. Naiyyum Chowdhury, Coordinator, Biotechnology and Microbiology, Department of Mathematics and Natural Sciences, BRAC University, for his inspiration, prudent advice, and affectionate guidance and for giving me the opportunity to work at icddrb under his supervision particularly.

I am grateful to Professor AA Ziauddin Ahmad, Chairperson, MNS department for allowing me to pursue my post graduate studies in the department of MNS and for his constant guidance and help throughout my entire period of study in the department.

I express my gratitude to Dr.Sharmin Zaman Urmee, Medical officer, icddr,b, Dhaka, for her valuable instruction, continuous encouragement and valuable suggestion pertaining to my work.

I am extremely grateful to my colleague Mr. Bikash Chandra Chanda, Senior Research officer, icddr,b, Dhaka who have contributed in various ways during this work. I am extremely grateful to my husband Sabbir Hasan, my friends Zubaida Marufee Islam and Anamika Vowmik for their active cooperation and enormous inspiration throughout my research work.

Finally, I like to express my outmost gratitude to my parents for their endless moral support and kind prayers during my thesis work.

Raisa Akther

Department of Mathematics and Natural Sciences

BRAC University, January,2016

Abstract

The most common causes of microcytosis are iron deficiency anemia (IDA) and Beta thalassemia trait (BTT). BTT is an important differential diagnosis of IDA in Southeast Asian countries including Bangladesh. A definitive diagnosis of BTT and IDA is based on the result of capillary electrophoresis and serum iron profiles. BTT often shows microcytosis, a normal or an increased red blood cell (RBC) count, and an elevated level of HbA_2, which provide the basis for laboratory screening and diagnosis of IDA was made on the basis of decreased ferritin values.
The purpose of this study was to explore the use of different red cell parameters with (RET – He) in diagnosis of BTT & IDA.

A total of 210 samples were obtained from specimen reception unit of Icddr,b irrespective of sex. Complete blood count was performed to all individuals. Hemoglobin electrophoresis and serum ferritin was performed to samples with MCV less than 80 fl.

Prevalence of BTT in this study was 16%, whereas IDA represented 16.6% of total 210 samples investigated. The lowest MCV values were seen in BTT compared with the IDA group. Red blood cell distribution width (RDW-CV) was the highest in IDA group followed by BTT group and then control group. Elevated RBCs count were seen in BTT than in IDA and normal group. A reduction in reticulocyte hemoglobin has been observed in other conditions besides iron deficiency, such as in thalassemia.

Microcytosis accompanied by a high RBC count, normal RDW and an elevated level of HbA_2 is suggestive of BTT. Microcytosis accompanied by a low ferritin value suggests iron deficiency. Besides measurement of the reticulocyte hemoglobin (RET-He) content is helpful in detecting early stages of iron deficiency prior to the development of anemia.

List of Abbreviations

IDA……Iron deficiency anaemia

BTT……Beta thalassemia trait

RBC……Red blood cell

Hb…… Hemoglobin

Hct…… Hematocrit

MCV…… Mean corpuscular volume

MCH…… Mean corpuscular hemoglobin

MCHC…… Mean corpuscular hemoglobin concentration

RDW – SD……Red cell distribution width standard deviation

RDW – CV……Red cell distribution width co efficient of varriation

RET…… Reticulocyte

RET- He…… Reticulocyte hemoglobin equivalent

CBC…… Complete blood count

EDTA……Eyhylene – diamine tetraacitic acid

g/dl……gram/ deciliter

fl……femto liter

Pg…… pico gram

ng/ml……nano gram/ Mililiter

List of Tables

List of Figures

Contents

1. Introduction

1.1 Introduction

The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia (IDA) and beta thalassemia trait (BTT). Other diagnoses to consider include anemia of chronic disease, lead toxicity and sideroblastic anemia.

Thalassemia is one of the major autosomal recessive hereditary hemoglobinopathies prevalent in the world population, particularly in Mediterranean belt, Far-eastern and South East Asian countries [1]. Thalassemias are a group of hemoglobinopathy caused by genetic mutations of the hemoglobin (Hb) genes, resulting in reduced production or total absence of one or more globin chains[2]. Thalassemia consists of two main classes, alpha thalassemia and beta thalassemia by their clinical manifestations and genetic background[3].

Beta thalassemia is the most frequent type of thalassemia which can be classified further into three forms: β thalassemia major, β thalassemia intermediate and β thalassemia minor/β thalassemia trait [4]. β-thalassemia major is commonly caused by homozygous deletion of the β-globin chain gene. It is clinically characterized by lifelong severe hemolytic anemia that eventually affects many organs and is associated with high morbidity and mortality[1]. Intermediate β thalassaemic individuals carry mutation in one or both of the β globin genes. β-thalassemia minor or β-thalassemia trait (BTT) is the heterozygous form. Most patients are asymptomatic and some patients have only mild anaemia[5]. Thalassemia occurs more often among certain ethnicities, including people of Italian, Greek, Middle Eastern, Asian and African decent[6].

In developing countries like Bangladesh where resources are limited, thalassemia is a major health burden. According to Bangladesh Thalassemia foundation, about 7% of the Bangladeshi population are thalassemia carriers which equals >10 million people and each year 7000 new babies born with thalassemia. BTT often shows microcytosis, a normal or an increased red blood cell (RBC) count, and an elevated level of HbA2, which provide the basis for laboratory screening [5]. HbA_2 is a normal Hb variant consisting of 2α chains and 2δ chains. HbA_2 has a function similar to that of HbA.

The percentage of HbA_2 varies depending on the assay but generally is in the range of 1.5-3.5%[2]. The majority of patients with BTT show elevated HbA_2 level and some authors have used an HbA_2 level of more than 4% to diagnose BTT [7].

Iron deficiency anaemia is the most common microcytic hypochromic anemia worldwide. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in young children and women of reproductive age but it can be found in people of any age-group[8]. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA) and most of those affected live in the developing countries like Bangladesh[9]. Iron deficiency anemia in adults is caused by loss of blood, while in childhood faulty diet is to blame. It is a severe stage of iron shortage in which haemoglobin (or haematocrit) falls below the normal range. There is no hemolysis, erythrocytes survive normally and serum iron level tends to be low[10].

Iron deficiency modulates the synthesis of HbA_2, resulting in reduced HbA_2 levels in patients with IDA[11]. Affected individuals show RBC morphological change of microcytosis, hypochromia, anisocytosis, and poikilocytosis. Percentage of hypochromic red cells may be high before the anemia develops. It is found that a reduction in Hb concentration is a late feature of iron deficiency [12].

Accelerated development, hormonal changes, malnutrition, and starting of menstrual periods in girls are the major causes of iron-deficiency anaemia during adolescence, which may also lead to impaired perception and learning difficulties [13]. The detrimental effects of anaemia on work productivity of adults and physical development of children are of major concern[14].

BTT is an important differential diagnosis of iron deficiency anemia (IDA). Thalassemia minor is an important hematologic condition because while the conditions observed in study of the blood may closely mimic those that are present in iron deficiency anemia, the therapy is radically different[10]. It is very important not to treat a patient with thalassemia with an iron supplement as this can lead to hemochromatosis (accumulation of iron in various organs, specially the liver). Thus

reliable and efficient diagnostic ways to distinguish between thalassemic and iron restricted microcytic hypochromic anemia are desirable.

1.2 Microcytic hypochromic anemia

1.2.1 Microcytosis

Microcytosis is usually encountered incidentally when a complete blood count (CBC) is performed for various reasons. The condition is defined as a mean corpuscular volume of less than 80 μm3 (80 fL) in adults and is often associated with anemia[15].

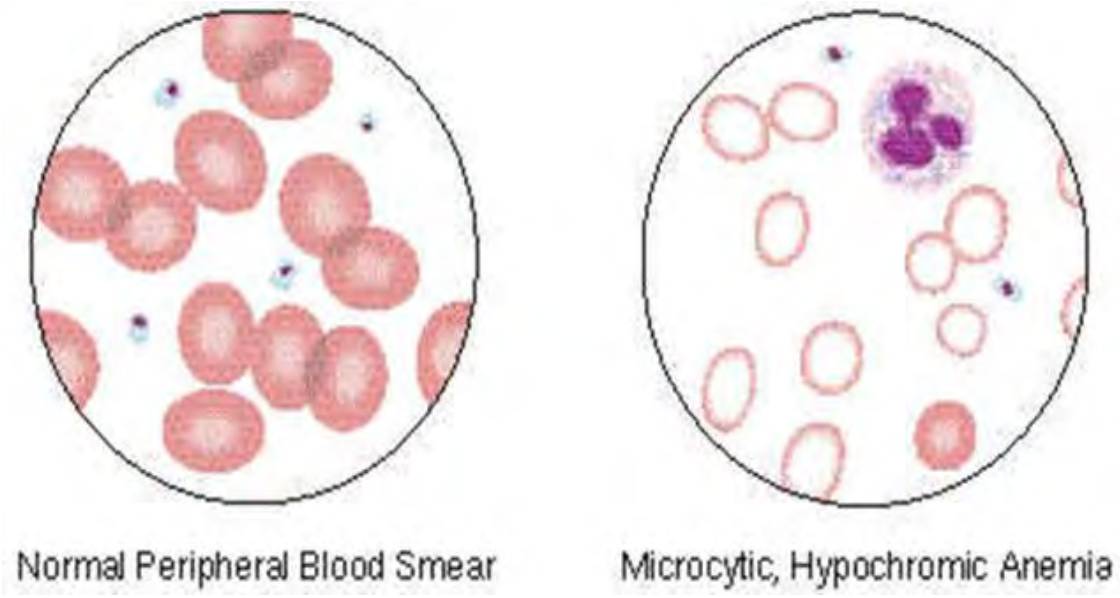

Figure1.1: Microcytic hypochromic anaemia in contrast to normal blood smear

Differential diagnosis: [16, 17]

The most common causes of microcytosis are iron deficiency anemia and thalassemia trait.

Table 1.1

Differential Diagnosis of Microcytosis		
Children and adolescents	Menstruating women	Men and non menstruating women
• Iron deficiency anemia • Thalassemia trait • Other hemo-globinopathies • Lead toxicity • Chronic inflammation • Sideroblastic anemia	• Iron deficiency anemia • Thalassemia trait • Pregnancy • Anemia of chronic disease • Sideroblastic anemia	• Iron deficiency anemia • Anemia of chronic disease • Thalassemia trait

Note: Listed in descending order of frequency

1.2.2 Specific causes of Microcytosis

1.2.2.1 Iron deficiency anemia

In Bangladesh, nutritional anaemia has long been identified as a serious public-health problem [18]. Moreover, many surveys conducted in the past stated that anaemia is a severe problem among all across age, population and geographic groups in Bangladesh. In 2004, another survey conducted by Nutritional Surveillance Project of Helen Keller International in collaboration with the Institute of Public Health Nutrition showed that 68% of under-five children were anaemic. The survey also

suggested that 40% of adolescent girls and 31% adolescent boys as well as 46% of non-pregnant and 39% of pregnant women were affected by anemia.[19]

Iron deficiency anemia occurs when the absorption of iron through dietary intake does not match the needs of the body. The mismatch occurs from inadequate dietary intake or increased needs, which usually cause only mild anemia or from blood loss or malabsorption, which can lead to more significant anemia.[15]

Causes of iron deficiency anemia[20]:

Iron deficiency is the most common cause of anemia. There are many reasons why a person might become deficient in iron. These include:

a) Inadequate iron intake

Eating too little iron over an extended amount of time can cause a shortage in your body. Foods such as meat, eggs, and some green leafy vegetables are high in iron. Because iron is essential during times of rapid growth and development, pregnant women and young children may need even more iron-rich foods in their diet.

b) Blood loss due to menstruation

In women of childbearing age, the most common causes of iron deficiency anemia are heavy menstrual bleeding and blood loss during childbirth.

c) Internal bleeding

Certain medical conditions can cause internal bleeding, which can lead to iron deficiency anemia. Examples include an ulcer in stomach, polyps (tissue growths) in the colon or intestines, or colon cancer. Regular use of pain relievers, such as aspirin, can also cause bleeding in the stomach.

d) Inability to absorb iron

Certain disorders or surgeries that affect the intestines can also interfere with how body absorbs iron. Even not intake of enough iron in diet, celiac disease or intestinal surgery, such as gastric bypass, may limit the amount of iron body can absorb.

Risk group for iron deficiency anemia[21]:

Iron deficiency anemia is a common condition and can occur in both men and women of any age and from any ethnic group. Some people may be at greater risk for iron deficiency anemia than others. These include:

- women of childbearing age or pregnant women
- people with poor diets
- people who donate blood frequently
- infants and children, especially those born prematurely or experiencing a growth spurt
- vegetarians who don't replace meat with another iron-rich food

Complications[21]:

Mild iron deficiency anemia usually doesn't cause complications. However, left untreated, iron deficiency anemia can become severe and lead to health problems, including the following:

1. Heart problems: Iron deficiency anemia may lead to a rapid or irregular heartbeat. Heart must pump more blood to compensate for the lack of oxygen carried in blood when anemia occurs. This can lead to an enlarged heart or heart failure.

2. Problems during pregnancy: In pregnant women, severe iron deficiency anemia has been linked to premature births and low birth weight babies. But the condition is preventable in pregnant women who receive iron supplements as part of their prenatal care.

3. Growth problems: In infants and children, severe iron deficiency can lead to anemia as well as delayed growth and development.

Treatment of IDA[21]:

Doctors normally treat the condition with iron supplements or changes to diet.

1.2.2.2 Thalassemia

Thalassemia is a major health problem, placing an immeasurable emotional, psychological

and economic burden on millions of people around the World[22]. Thalassemia, also called Mediterranean anemia, is a form of inherited autosomal recessive blood disorder characterized by abnormal formation of hemoglobin.[23]

Mutation in the genes encoding alpha chain and beta chain of hemoglobin are the primary cause of thalassemia resulting in the absence or inadequate synthesis of one of the globin chains. This anomaly in synthesis of globin chain can cause to the premature destruction of Red Blood Cells (RBCs), thus causing anaemia and other secondary effects, the typical symptoms of thalassemia.[23]

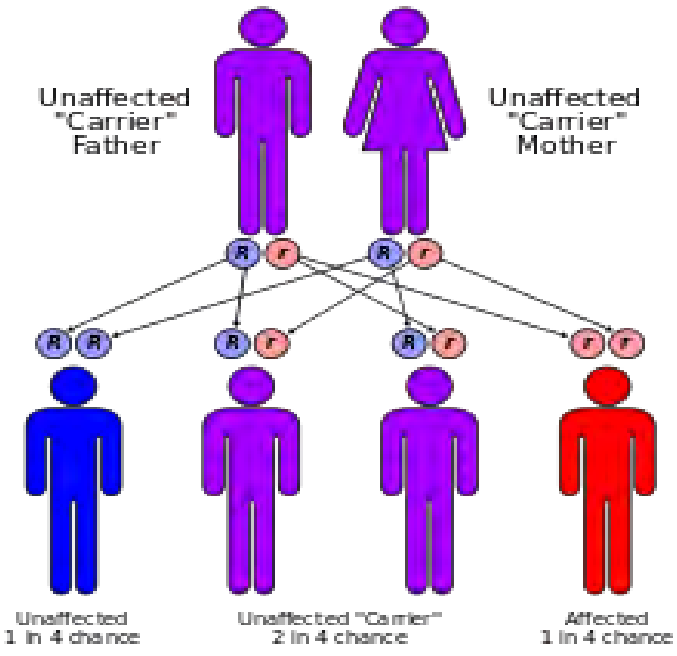

Figure 1.2 : Thalassemia has an autosomal recessive pattern of inheritance.

Pathophysiology[24]:

Normally, the majority of adult hemoglobin (HbA) is composed of four protein chains, two α and two β globin chains arranged into a heterotetramer. In thalassemia, patients have defects in either the α or β globin chain, causing production of abnormal red blood cells .

The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α-thalassemias, production of the α globin chain is affected, while in β-thalassemia, production of the β globin chain is affected.

The β globin chains are encoded by a single gene on chromosome 11; α globin chains are encoded by two closely linked genes on chromosome 16. Thus, in a normal person with two copies of each chromosome, two loci encode the β chain, and four loci encode the α chain.

Epidemiology:

The beta form of thalassemia is particularly prevalent among Mediterranean peoples and this geographical association is responsible for its naming.[25]Thalassemia resulted in 25,000 deaths in 2013 down from 36,000 deaths in 1990.[26]

In Europe, the highest concentrations of the disease are found in Greece, coastal regions in Turkey (particularly the Aegean Region such as Izmir, Balikesir, Aydin, Mugla, and Mediterranean Region such as Antalya, Adana, Mersin), in parts of Italy, particularly southern Italy and the lower Po valley. The major Mediterranean islands (except the Balearics) such as Sicily, Sardinia, Malta, Corsica, Cyprus, and Crete are heavily affected in particular. Other Mediterranean people, as well as those in the vicinity of the Mediterranean, also have high rates of thalassemia, including people from West Asia and North Africa. Far from the Mediterranean, South Asians are also affected, with the world's highest concentration of carriers (16% of the population) being in the Maldives.

Nowadays, it is found in populations living in Africa, the Americas, and in Tharu people in the Terai region of Nepal and India.[27] It is believed to account for much lower malaria sicknesses and deaths,[28] accounting for the historic ability of Tharus to survive in areas with heavy malaria infestation, where others could not. Thalassemias are particularly associated with people of Mediterranean origin, Arabs (especially Palestinians and people of Palestinian descent), and Asians.[29] The

Maldives has the highest incidence of Thalassemia in the world with a carrier rate of 18% of the population. The estimated prevalence is 16% in people from Cyprus, 1%[30] in Thailand, and 3–8% in population from Bangladesh,China,India,Malaysia and Pakistan. Thalassemias also occur in descendants of people fromLatin America and Mediterranean countries (e.g. Greece, Italy, Portugal, Spain, and others).

Classification of thalassemia: [31]

A) Alpha Thalassemia

People whose hemoglobin does not produce enough alpha protein have alpha thalassemia. It is commonly found in Africa, the Middle East, India, Southeast Asia, southern China, and
occasionallythe Mediterranean region.

Types of Alpha Thalassemia[32]

Alpha globin is made by four genes and one or more can be mutated or missing, so there are four kinds of alpha thalassemia:

a) One missing or abnormal gene makes a child a silent alpha thalassemia carrier. Silent alpha thalassemia carriers have no signs or symptoms of the disease, but are able to pass thalassemia on to their children.
b) Two missing or mutated genes is a condition called alpha thalassemia minor or having alpha thalassemia trait. Children with this condition may have red blood cells that are smaller than normal (microcytosis) and sometimes very slight anemia. People with alpha thalassemia minor usually don't have any symptoms at all, but can pass thalassemia on to their children. The two abnormal genes can be on the same chromosome (called the cis position) or one on each chromosome (called the transposition). If two genes on the same chromosome are affected, the person can pass along a two-gene defect to his or her child. This situation is much more common in people of Asian descent.
c) Three missing or mutated genes is called hemoglobin H disease. Signs and symptoms will be moderate to severe.
d) Four missing or mutated genes is a condition known as alpha thalassemia major or hydrops fetalis. This almost always leads to a fetus dying before

delivery or a newborn baby dying shortly after birth. However if this disease is suspected because of a history in the family, it can be diagnosed prenatally. Sometimes, if treatment is initiated before the baby is even born, the baby can survive.

B) **Beta-thalassemia**[31]

Beta-thalassemia is an autosomal recessive genetic condition in which the normal beta globin chains that make up hemoglobin are underproduced. It is found in people of Mediterranean descent, such as Italians and Greeks, and is also found in the Arabian Peninsula, Iran, Africa,Southeast Asia and southern China.

Types of Beta Thalassemia[31]:

There are three types of beta thalassemia that also range from mild to severe in their effect on the body.

a) Thalassemia Trait:

Beta-thalassemia trait is the heterozygous form of the disease. Individuals who have one abnormal beta globin gene have beta thalassemia trait (also known as beta thalassemia minor). In this condition, the lack of beta protein is not great enough to cause problems in the normal functioning of the hemoglobin. A person with this condition simply carries the genetic trait for thalassemia and will usually experience no health problems other than a possible mild to moderate microcytic anemia. In addition, the mean corpuscular volume can sometimes reach much lower levels than with iron deficiency anemia alone. The red blood cell count can help differentiate BTT and IDA because it is often in the high to normal range with beta-thalassemia trait. Ultimately, the diagnosis of beta-thalassemia trait is made when hemoglobin electrophoresis shows a slight increase in hemoglobin A2. Coexisting iron deficiency anemia can lower hemoglobin A2 levels.

As in mild alpha thalassemia, physicians often mistake the small red blood cells of the person with beta thalassemia minor as a sign of iron-deficiency anemia and incorrectly prescribe iron supplements.

b) Thallasemia intermedia:

In this condition the lack of beta protein in the hemoglobin is great enough to cause a moderately severe anemia and significant health problems, including bone deformities and enlargement of the spleen. However, there is a wide range in the clinical severity of this condition, and the borderline between thalassemia intermedia and the most severe form, thalassemia major, can be confusing. The deciding factor seems to be the amount of blood transfusions required by the patient. The more dependent the patient is on blood transfusions, the more likely he or she is to be classified as thalassemia major. Generally speaking, patients with thalassemia intermedia need blood transfusions to improve their quality of life, but not in orderto survive.

c) Thalassemia Major or Cooley's Anemia:

Beta- thalassemia major (also known as Cooley anemia) is the homozygous form. This is the most severe form of beta thalassemia in which the complete lack of beta protein in the hemoglobin causes a life-threatening anemia that requires regular blood transfusions and extensive ongoing medical care. These extensive, lifelong blood transfusions lead to iron-overload which must be treated with chelation therapy to prevent early death from organ failure.

Sign & Symptom:

Thalassemia can cause complications, including iron overload, bone deformities, and cardiovascular illness.

- Iron overload: People with thalassemia can get an overload of iron in their bodies, either from the disease itself or from frequent blood transfusions. Too much iron can result in damage to the heart, liver, and endocrine system, which includes glands that produce hormones that regulate processes throughout the body. The damage is characterized by excessive deposits of iron. Without adequate iron chelation therapy, almost all patients with beta-thalassemia accumulate potentially fatal iron levels.[33]

- Infection: People with thalassemia have an increased risk of infection. This is especially true if the spleen has been removed.[34]
- Bone deformities: Thalassemia can make the bone marrow expand, which causes bones to widen. This can result in abnormal bone structure, especially in the face and skull. Bone marrow expansion also makes bones thin and brittle, increasing the risk of broken bones.[35]
- Enlarged spleen: The spleen aids in fighting infection and filters unwanted material, such as old or damaged blood cells. Thalassemia is often accompanied by the destruction of a large number of red blood cells and the task of removing these cells causes the spleen to enlarge. Splenomegaly can make anemia worse, and it can reduce the life of transfused red blood cells. Severe enlargement of the spleen may necessitate its removal.
- Slowed growth rates: Anemia can cause a child's growth to slow. Puberty also may be delayed in children with thalassemia.
- Heart problems: Diseases, such as congestive heart failure and abnormal heart rhythms, may be associated with severe thalassemia.[36]

Management

Mild thalassemia: people with thalassemia traits do not require medical or follow-up care after the initial diagnosis is made.[37] People with β-thalassemia trait should be warned that their condition can be misdiagnosed as the more common iron deficiency anemia. They should avoid routine use of iron supplements; iron deficiency can develop, though, during pregnancy or from chronic bleeding.[38] Counseling is indicated in all persons with genetic disorders, especially when the family is at risk of a severe form of disease that may be prevented.[39]

Severe thalassemia: People with severe thalassemia require medical treatment. A blood transfusion regimen was the first measure effective in prolonging life.[37]

Medications

Multiple blood transfusions can result in iron overload. The iron overload related to thalassemia may be treated by chelation therapy with the medications

deferoxamine, deferiprone, or deferasirox.[40]These treatments have resulted in improved life expectancy in those with thalassemia major.[40]

Carrier detection

- A screening policy exists in Cyprus to reduce the incidence of thalassemia, which, since the program's implementation in the 1970s (which also includes prenatal screening and abortion), has reduced the number of children born with the hereditary blood disease from one of every 158 births to almost zero.[41]
- In Iran as a premarital screening, the man's red cell indices are checked first, if he has microcytosis (mean cell hemoglobin < 27 pg or mean red cell volume < 80 fl), the woman is tested. When both are microcytic, their hemoglobin A2 concentrations are measured. If both have a concentration above 3.5% (diagnostic of thalassemia trait) they are referred to the local designated health post for genetic counseling.[42]
- Large scale awareness campaigns are being organized in India both by government and non-government organizations in favor of voluntary premarital screening to detect carriers of thalassemia and marriage between both carriers are strongly discouraged.

Bone marrow transplant

Bone marrow transplantation may offer the possibility of a cure in young people who have an HLA-matched donor.[43]Success rates have been in the 80–90% range.[43] Mortality from the procedure is about 3%.[44] There are no randomized controlled trials which have tested the safety and efficacy of non-identical donor bone marrow transplantation in persons with β- thallassemia who are dependent on blood transfusion.[45]

If the person does not have an HLA-matched compatible donor, another method called bone marrow transplantation (BMT) from haploidentical mother to child (mismatched donor) may be used. In a study of 31 people, the thalassemia-free survival rate 70%, rejection 23%, and mortality 7%. The best results are with very young people.[46]

1.2.2.3 Anemia of chronic disease:

Anemia of chronic disease can be caused by chronic infections or inflammatory processes. Increased levels of cytokines cause a decrease in erythropoietin production, a decreased response to erythropoietin, and interference with iron metabolism. Although anemia of chronic disease is usually normocytic, about one fourth to one third of cases are mildly microcytic. The anemia is usually mild and not progressive. Additionally, although serum iron levels are decreased in anemia of chronic disease (similar to iron deficiency anemia), ferritin levels are increased because ferritin is an acute phase reactant. [33]

1.2.3 Diagnostic Strategy [15]

Serum ferritin measurement is the first laboratory test recommended in the evaluation of microcytosis. Low ferritin levels suggest iron deficiency. Once a presumptive diagnosis of iron deficiency anemia has been made, an underlying source for the deficiency should be determined. Iron deficiency anemia in adults is presumed to be caused by blood loss; the most common source of bleeding is the gastrointestinal tract. The possibility of gastrointestinal malignancy must be considered. If the serum ferritin level is not initially low, further evaluation should include total iron-binding capacity, transferrin saturation level, serum iron level, and possibly hemoglobin electrophoresis. Anemia of chronic disease is suggested with low iron levels and decreased total iron-binding capacity. Patients with beta-thalassemia trait usually have elevated levels of hemoglobin A2.

Diagnosing the Cause of Microcytosis:[15]

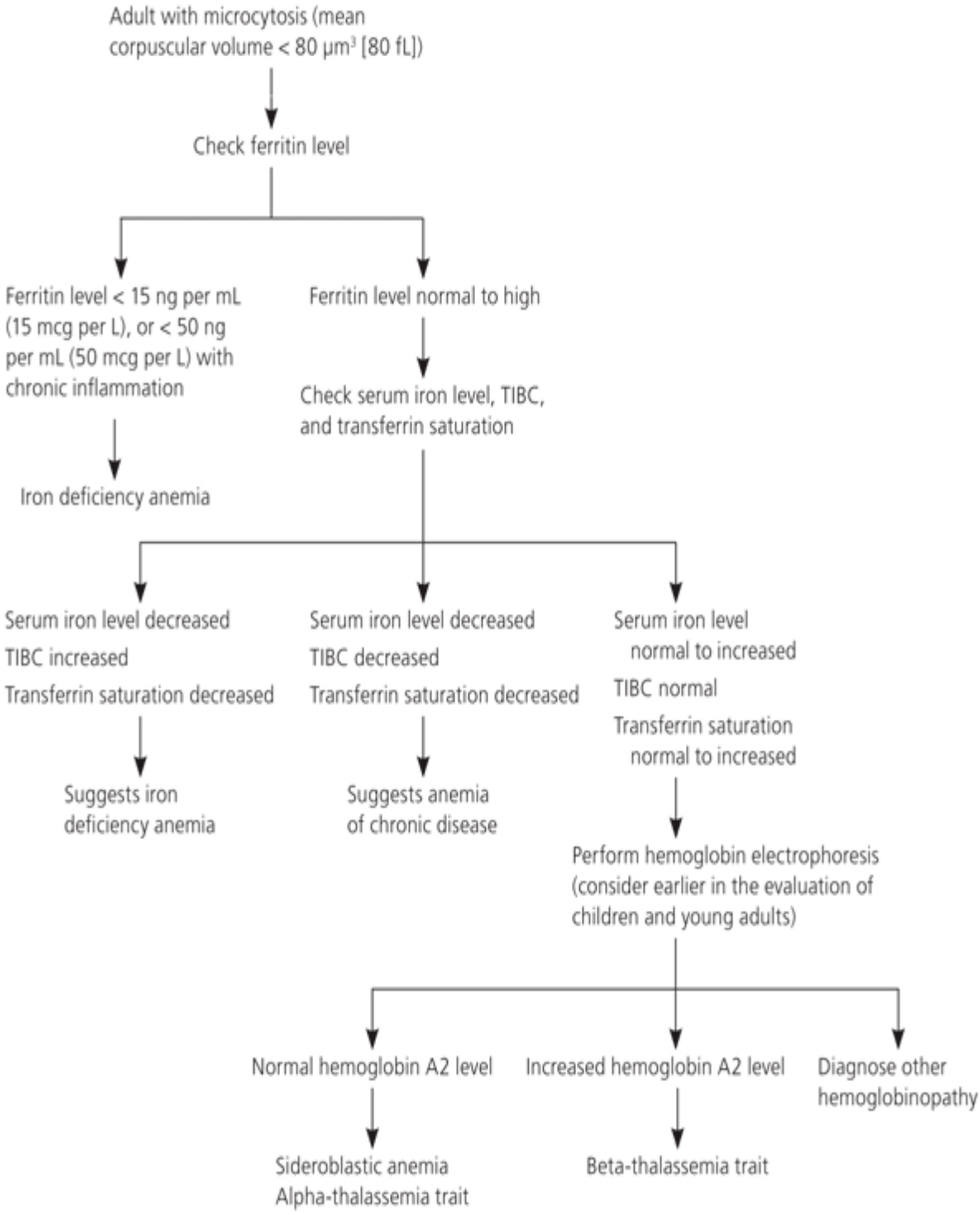

Figure 1.3 : suggested algorithm for diagnosing the cause of microcytosis in adults.

1.2.4 Laboratory Evaluation[17]

Laboratory tests that may help in differentiating the cause of microcytosis include red blood cell distribution width using the CBC, serum iron levels, serum ferritin levels, total iron-binding capacity (TIBC), transferrin saturation, hemoglobin electrophoresis, and occasionally reticulocyte blood count , recently included reticulocyte hemoglobin equivalent and peripheral blood smears.

CBC[47] :

- The complete blood count (CBC) is often used as a broad screening test to determine an individual's general health status and detect a wide range of disorders, including anemia, infection and leukemia.
- Parameters of particular interest through CBC was listed below:
  Hb, RBC, Hct, MCV, MCH, RDW–standard deviation (SD), RDW–coefficient of variation (CV), reticulocytes (%), Ret–hemoglobin equivalent (RET - He).
  - Red blood cell (RBC) count is a count of the actual number of red blood cells in a person's sample of blood.
  - Hemoglobin measures the total amount of the oxygen-carrying protein in the blood, which generally reflects the number of red blood cells in the blood.
  - Hematocrit measures the percentage of a person's total blood volume that consists of red blood cells.
  - Red blood cell indices are calculations that provide information on the physical characteristics of the RBCs:
    I. Mean corpuscular volume (MCV) is a measurement of the average size of a single red blood cell.
    II. Mean corpuscular hemoglobin (MCH) is a calculation of the average amount of hemoglobin inside a single red blood cell.
    III. Mean corpuscular hemoglobin concentration (MCHC) is a calculation of the average concentration of hemoglobin inside a single red blood cell.
    IV. Red cell distribution width (RDW) is a calculation of the variation in the size of RBCs.

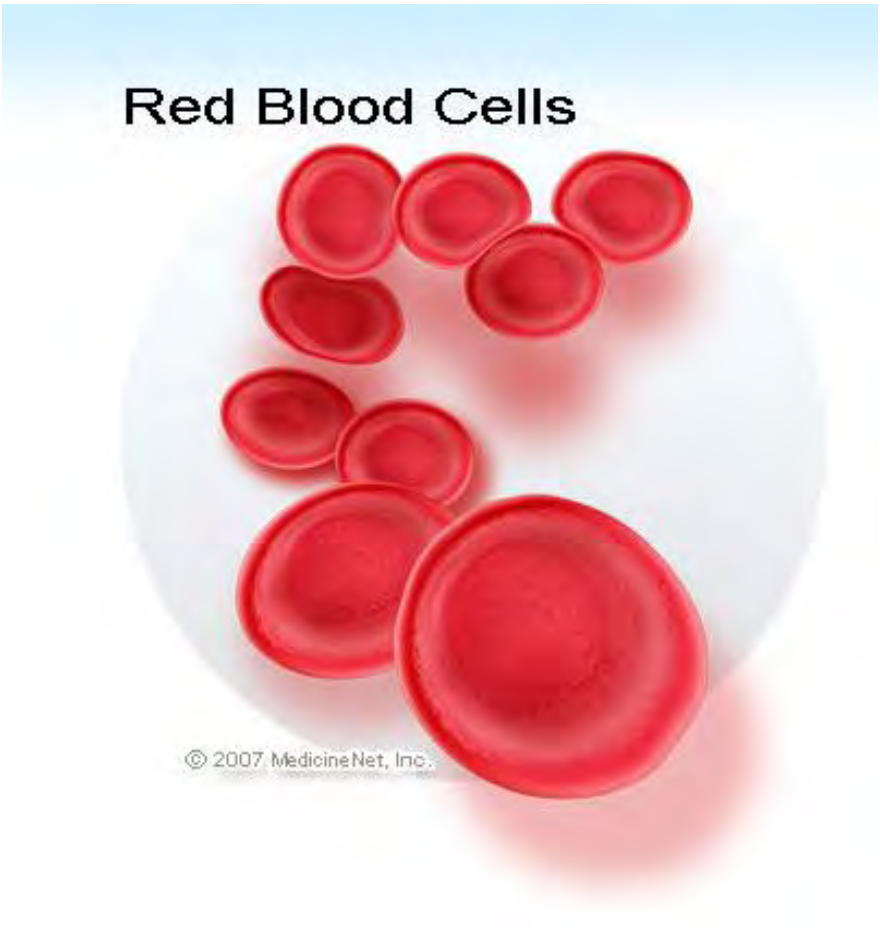

Figure 1.4 : Red Blood Cells

- Ret Hb Equivalent[48]:
  - Measuring the haemoglobin content of reticulocytes, RET-He or reticulocyte haemoglobin equivalent, is a way of diagnosing and monitoring iron deficiency anaemia.
  - The reference range for RET-He is approximately 28-35 pg [~1.77-2.22 fmol], below 28 pg [1.77 fmol] is considered iron deficient.
  - Benefit: The clinical usefulness of the Ret-He parameter has been proven and it is now an established parameter in advanced haematological analysis. "Reticulocyte haemoglobin content" is recommended in nephrology guidelines such as the European Best Practice Guidelines (EBPG), National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF KDOQI)

Ret-He:

- Indicates the trend of the current iron status.
- RET-He and RET# together let clinicians draw conclusions on both the quality and quantity of the young RBC fraction.
- Is an early marker for disease - earlier than clinical chemistry markers!
- Fast and inexpensive!
- RET-He is used for monitoring erythropoietin (EPO) and iron therapy. If the value increases it indicates the therapy is having a positive effect.

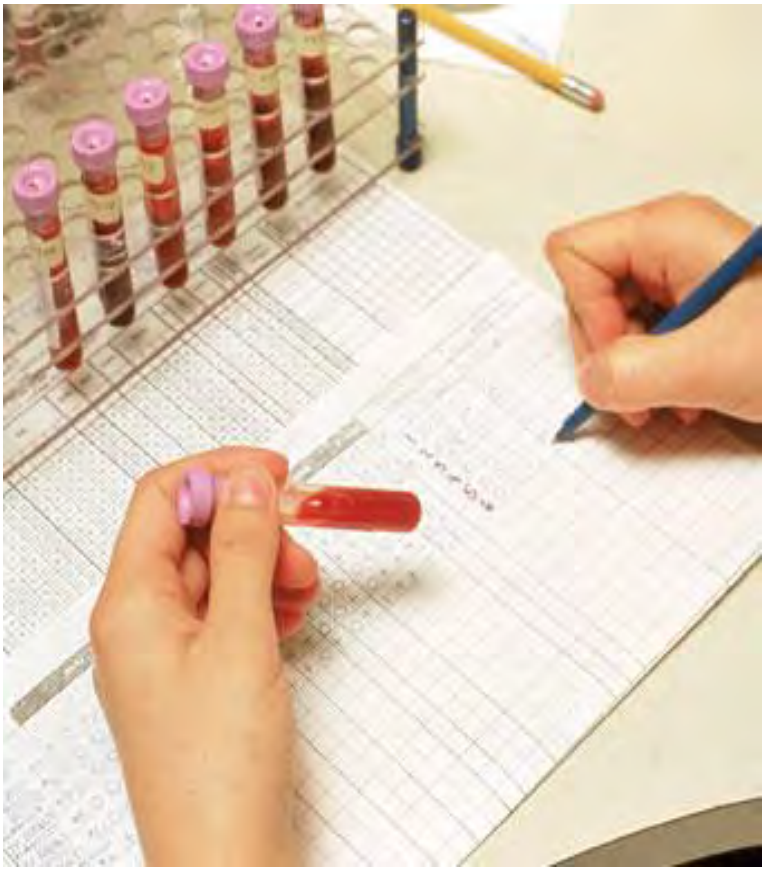

Figure 1.5 : Collection of blood in K_2EDTA vial

Normal Results of CBC: [49]

Blood counts may vary with altitude. In general, normal results are:

Red blood cell (RBC) count	
Men:	4.5-5.5 million RBCs per microliter (mcL) or 4.5-5.5 x 1012/liter (L)
Women:	4.0-5.0 million RBCs per mcL or 4.0-5.0 x 1012/L

Hematocrit (HCT)	
Men:	42%-52% or 0.42-0.52 volume fraction
Women:	36%-48% or 0.36-0.48 volume fraction

Hemoglobin (Hb)	
Men:	13-18 grams per deciliter (g/dL)
Women:	11.5-16.5 g/dL

- In general, a normal hemoglobin level is about one-third the value of the hematocrit.

Red blood cell indices	
Mean corpuscular volume (MCV)-Adults:	84-96 femtoliters (fL)
Mean corpuscular hemoglobin (MCH)-Adults:	27-32 picograms (pg)per cell
Mean corpuscular hemoglobin concentration (MCHC)-Adults:	32-36 grams per deciliter (g/dL)

Red cell distribution width (RDW)	
Normal:	11.5%-14.5%

Reticulocyte count	
Normal:	0.5%-1.5%.

Ret Hb Equivalent	
Normal:	28-35 pg

Ferritin[15]:

- Ferritin is a complex of iron and the binding protein apoferritin. Ferritin reflects true iron stores and is not susceptible to the short-term variations that occur with serum iron levels and TIBC. However, ferritin is also an acute phase reactant and can be elevated with liver disease, malignancy, and chronic renal disease. Iron deficiency anemia is likely if the ferritin level is less than 15 ng per mL (15 mcg per L) in an otherwise healthy person, or less than 50 ng mL (50 mcg per L) in a person with an underlying source of chronic inflammation. Iron deficiency can usually be excluded when the ferritin level is greater than 100 ng per mL (100 mcg per L).

Table: 1.2 Laboratory Tests in the Differential Diagnosis of Microcytosis[50]

TEST	SUGGESTED DIAGNOSIS			
	IRON DEFICIENCY ANEMIA	THALASSEMIA	ANEMIA OF CHRONIC DISEASE	SIDEROBLASTIC ANEMIA
Serum ferritin level	Decreased	Increased	Normal to increased	Normal to increased
Red blood cell distribution width	Increased	Normal to increased	Normal	Increased
Serum iron level	Decreased	Normal to increased	Normal to decreased	Normal to increased
Total iron-binding capacity	Increased	Normal	Slightly decreased	Normal

Hemoglobin Electrophoresis[51]

Hemoglobin electrophoresis test is a blood test done to check the different types of hemoglobin in the blood

- The electrophoresis process takes advantage of the fact that hemoglobin types have different electrical charges. During electrophoresis, an electrical current is passed through the hemoglobin in a blood sample, which causes the hemoglobin types to separate at different rates and form bands. By comparing the pattern formed with that of a normal blood sample, doctors can see the types and quantities of hemoglobin present in the blood sample.

The most common types of normal hemoglobin are:

- Hemoglobin A. This is the most common type of hemoglobin found normally in adults. Some diseases, such as severe forms ofthalassemia, may cause hemoglobin A levels to be low and hemoglobin F levels to be high.
- Hemoglobin F (fetal hemoglobin). This type is normally found infetuses and newborn babies. Hemoglobin F is replaced by hemoglobin A (adult hemoglobin) shortly after birth; only very small amounts of hemoglobin F are made after birth. Some diseases, such as sickle cell disease, aplastic anemia, and leukemia, have abnormal types of hemoglobin and higher amounts of hemoglobin F.
- Hemoglobin A2. This is a normal type of hemoglobin found in small amounts in adults.

Hemoglobin electrophoresis	
Hemoglobin A1:	96.5%-98.5% of total hemoglobin or 0.96-0.985 mass fraction
Hemoglobin A2:	1.5%-3.5% of total hemoglobin or 0.015-0.035 mass fraction
Hemoglobin F:	0%-1% of total hemoglobin or 0-0.01 mass fraction

An abnormal amount of normal hemoglobin or an abnormal type of hemoglobin in the blood may mean that a disease is present. Abnormal hemoglobin types may be present without any other symptoms, may cause mild diseases that do not have symptoms, or cause diseases that can be life-threatening.

After describing the diagnostic strategy of microcytosis, we can say that electronic cell counters have been used to determine red cell indices as a first indicator of β-TT and IDA. The purpose of using indices to discriminate anemia is to detect subjects who have a high probability of requiring appropriate follow-up and to reduce unnecessary investigative costs. Since 1970, a number of complete blood count indices have been proposed as simple, less time consuming and inexpensive tools to

determine whether a blood sample is more suggestive of β-TT or IDA[52]. Most of these articles include adults but very few data are available on children.

Previously, many discrimination indices have been reported using red blood cell (RBC) indices obtained by automated blood count. Many authors have calculated the sensitivity and specificity of these indices in the distinction between IDA and BTT . They proposed that the diagnosis could be established without having to resort to the more time-consuming methods such as transferrin saturation (TS), ferritin and hemoglobin A2 (HbA2) levels. But a definitive diagnosis of β-TT and IDA is based on the result of HbA_2 electrophoresis, serum iron levels, and a ferritin calculation [53]. However, none of these indices showed a sensitivity and specificity of 100% in prediction of IDA and BTT. Some showed considerable sensitivity for IDA or BTT, but not specificity[54].

It was noted that none of them was entirely satisfactory and none of these formulations was superior to RBC value obtained from automated analyzers in diagnosing these two conditions [26].So these indices are not very important today given the availability of Hb electrophoresis.A question arises that how many of physians memorize these formulas and use them in their daily practice in crowded outpatient settings. However, we do consider MCV and RBC which is easy to calculate. How many of them would be brave enough to not study Hb electrophoresis in a woman with mild hypochromic anemia unresponsive to iron therapy who was planning a pregnancy.Finally we can say that total body iron status, serum ferritin calculation and HbA2 level should be obtained for accurate diagnosis of IDA and BTT until more efficient tools are developed.

1.3 Aims and Objectives

a) This study provides a diagnostic strategy of microcytic hypochromic anaemia specially IDA & BTT.

b) The purpose of this study was to explore the diagnostic value of complete blood count (CBC) along with a new parameter (RET – He) in diagnosing BTT & IDA.

2 . Materials and Methods

2.1. Materials :

2.1.1 Chemicals :

I) For CBC :

- Cell pack DCL
- Cell pack DFL
- Sulfolyzer
- Lyser cell WNR
- Lyser cell WDF
- Flurocell WNR
- Flurocell WDF
- Flurocell RET
- Cell clean
- XN Check

II) For Hemoglobin Electrophoresis :

- Hemoglobin buffer
- Hemolysing solution
- Wash solution
- Capi clean
- Distilled water
- Normal Hb A2 Control

2.1.2 Euipments :

- Sysmex XN 1000
- Capillarys 2 Sebia
- Architect i1000SR immunoassay
- Centrifuge Machine
- Lab Rotator
- Rubber tit
- Pasteur pipette
- Micropipette
- Sample rack

2.2 Methods :

2.2.1. Selection criteria :

- No of cases : 210 adult subjects
- Age: 18 yrs – 55 yrs
- Sex: Both male and female

2.2.2 Exclusion criteria :

- Subject who received transfusion within 3 months
- Subject who receive iron therapy
- Age: subject below 18 yrs
- Hb level: <8.0 (gm/dl)
- Other abnormal Hb variant

2.2.3 Area of study :

Department of Clinical Hematology

Clinical laboratories

Icddr,b, Mohakhali, Dhaka

2.2.4 Period of Study :

January 2015 to October 2015

2.2.5 Following procedures were adopted :

- 250 adult subjects were selected irrespective of sex.
- The samples were obtained during the course of routine analysis. All samples were collected aseptically in specimen reception unit. Care was taken to avoid insufficient or excess anticoagulant, inadequate mixing of blood with anticoagulant, subject or specimen identification error and delay in transit to the laboratory.
- Routinely venous blood samples were collected from all subjects in k_2EDTA anticoagulated tube. It was used for the investigation of microcytosis for this study through CBC.
- All blood samples were analysed within 4 hours after collection.
- With the advent of automation in haematology, the first line of screening of IDA and thalassemia is possible through Complete Blood Count (CBC). At first for primary selection of subject, CBC was obtained for all samples. Samples were run on automated hematology analyser (sysmex XN-1000, Japan) on the day of collection (day 0) to avoid any changes in mean corpuscular volume (MCV) that may occur on sample storage in EDTA.
- Automated hematoly analyzer XN 1000 perform analysis based on the hydro dynamic focusing method, flow cytometry, semi conductor assay and SLS hemoglobin method.
- Parameters of particular interest were hemoglobin, RBC, Hct, MCV, MCH, RDW–standard deviation (SD), RDW–coefficient of variation (CV), reticulocytes (%), Ret–hemoglobin equivalent (He).
- The subjects with Hb value <8.0 g/dl (n = 21) were excluded in order to not confuse more severe IDA with BTT.

- We investigated for microcytosis and once microcytosis (defined as MCV less than 80 fl) is detected in samples, the following were performed:
    i. Measuring HbA_2 level by capillary zone electrophoresis (Capillary 2 Sebia) .
    ii. Diagnosis of BTT was made on the basis of HbA_2 levels more than 3.5%.
    iii. The subjects with abnormal Hb variant (n = 19) were excluded from the study.
    iv. For microcytic samples with normal Hb typing by capillary electrophoresis and HbA_2 level less than 3.5%, Iron deficiency anaemia was suspected. With proper consent taking from those suspected subjects, clotted blood sample was collected for measuring serum ferritin level by architect i1000SR immunoassay. Diagnosis of IDA was made on the basis of ferritin value lower than 15 ng/ml .

3. Results

3.1

After Analyzing CBC, according to MCV, selected blood samples were grouped into the following :

Group I: the normal MCV group (MCV > 80fl).

Group II: the microcytosis group (MCV < 80fl).

According to Hb electrophoresis results and serum ferritin level, group II was subdivided into the following :

Group IIA : the IDA group

Group IIB : the BTT group

3.2 Observation:

Age ranged from 18 to 55 years ([Table 3.1]). The study included 119 female and 91 male participants ([Table 3.1]).

TABLE 3.1

group	Age		
	Range	Mean	± SD
I	19-50	31.20	8.38
IIA	18-53	34	11.09
IIB	25-50	33.23	10.47

Table 3.1: Shows comparison between groups I, IIA, and IIB regarding age.

TABLE 3.2

Comparison regarding sex	G I	G IIA	G IIB	total
Female	73	26	20	119
Male	68	9	14	91
total	141	35	34	210

Table 3.2 : Shows comparison between groups I, IIA, and IIB regarding sex.

In this study, BTT represents 16% (n = 34) from total number of subjects, whereas IDA represents 16.6% (n = 35) ([Table 3.2]).

With respect to RBCs count, an elevated RBC count was detected in group IIB (5.48 ± 0.69 × 10^6 / mm^3) than group group I (4.85 ± 0.44 × 10^6 /mm^3) and IIA (4.66 ± 0.48 × 10^6 /mm^3) .([Table 3.3]).

TABLE 3.3

RBC	Number	Mean	SD	Max	min
I	141	4.85	0.44	5.83	4
IIA	35	4.66	0.48	5.87	3.88
IIB	34	5.48	0.69	7.06	4.55
	210				

Table : 3. 3 Comparison between groups I, IIA, and IIB regarding RBC concentration.

With respect to Hb level, boh group IIA (9.31 ± 0.81 g/dl) and IIB (10.58 ± 1.35 g/dl) showed reduced Hb concentration in contrast to group I (13.55 ± 1.41 g/dl). ([Table 3.4])

TABLE 3.4

Hb	Number	Mean	±SD	Max	min
I	141	13.55	1.41	16.7	11.5
IIA	35	9.31	0.81	10.5	8
IIB	34	10.58	1.35	13.8	8.6
	210				

Table : 3. 4 Comparison between groups I, IIA, and IIB regarding Hb concentration.

With respect to hematocrit value, boh group IIA (31.69 ± 2.6l%) and IIB (34.43 ± 4.35%) showed reduced Hct concentration in contrast to group I (41.08 ± 3.58%). ([Table 3.5])

TABLE 3.5

HCT	Number	Mean	±SD	Max	min
I	141	41.08	3.58	48.2	35.3
IIA	35	31.69	2.61	36.2	26
IIB	34	34.43	4.35	44.5	27.9
	210				

Table : 3.5 Comparison between groups I, IIA, and IIB regarding HCT value

Red blood cell distribution width (RDW - CV) was the highest in group IIA followed by group IIB and then group I (19.50 ± 3.43 %, 17.29 ± 2.13 %, 13.25 ± 2.11 % respectively) ([Table 3.6]).

TABLE 3.6

RwD-CV	Number	Mean	±SD	Max	min
I	141	13.25	2.11	26.2	11.8
IIA	35	19.50	3.43	28.5	13.6
IIB	34	17.29	2.13	24.9	13.6
	210				

Table :3. 6 Comparison between groups I, IIA, and IIB regarding RDW.

The least MCV values were seen in group IIB (63.02 ± 3.34 fl) compared with the group IIA (67.84 ± 4.40 fl). ([Table 3.7]

TABLE 3.7

MCV	Number	Mean	±SD	Max	min
I	141	85.05	3.20	91.9	80.1
IIA	35	67.84	4.40	73.9	60.1
IIB	34	63.02	3.34	69.4	55.4
	210				

Table : 3. 7 Comparison between groups IIA and IIB regarding MCV

With respect to MCH level, boh group IIA (20.03 ± 2.04 pg) and IIB (18.84 ± 1.38 pg) showed reduced MCH concentration in contrast to group I (27.84 ± 1.61 pg). ([Table 3.8]).

TABLE 3. 8

MCH	Number	Mean	±SD	Max	min
I	141	27.84	1.61	31.2	24.5
IIA	35	20.03	2.04	22.7	16.2
IIB	34	18.84	1.38	22.9	17.7
	210				

Table : 3.8 Comparison between groups I, IIA, and IIB regarding MCH concentration .

The least RET -He values were seen in group IIA (20.2 ± 3.34 pg) and IIB (20.31 ± 1.55pg) compared with the group I (29.44 ± 2.13 pg). ([Table 3.9])

TABLE 3.9

RET-He	Number	Mean	±SD	Max	min
I	141	29.44	2.13	33.2	21.6
IIA	35	20.20	3.34	28.4	14.6
IIB	34	20.31	1.55	24.7	17.8
	210				

Table 3.9 Comparison between groups I, IIA, and IIB regarding RET-He concentration

Raised Hb A_2 level was seen in group IIB (4.89 ± 0.58%) compared with group IIA (1.94 ± 0.18%) . ([Table 3.10])

TABLE 3.10

HbA_2	Number	Mean	±SD	Max	min
I	141	2.61	0.20	3.1	2.2
IIA	35	1.94	0.18	2.1	1.4
IIB	34	4.89	0.58	5.9	3.6
	210				

Table 3.10 Comparison between groups I, IIA, and IIB regarding Hb A_2 concentration

Electrogram of Group I, Group IIA and Group IIB by Capillarys 2 Sebia

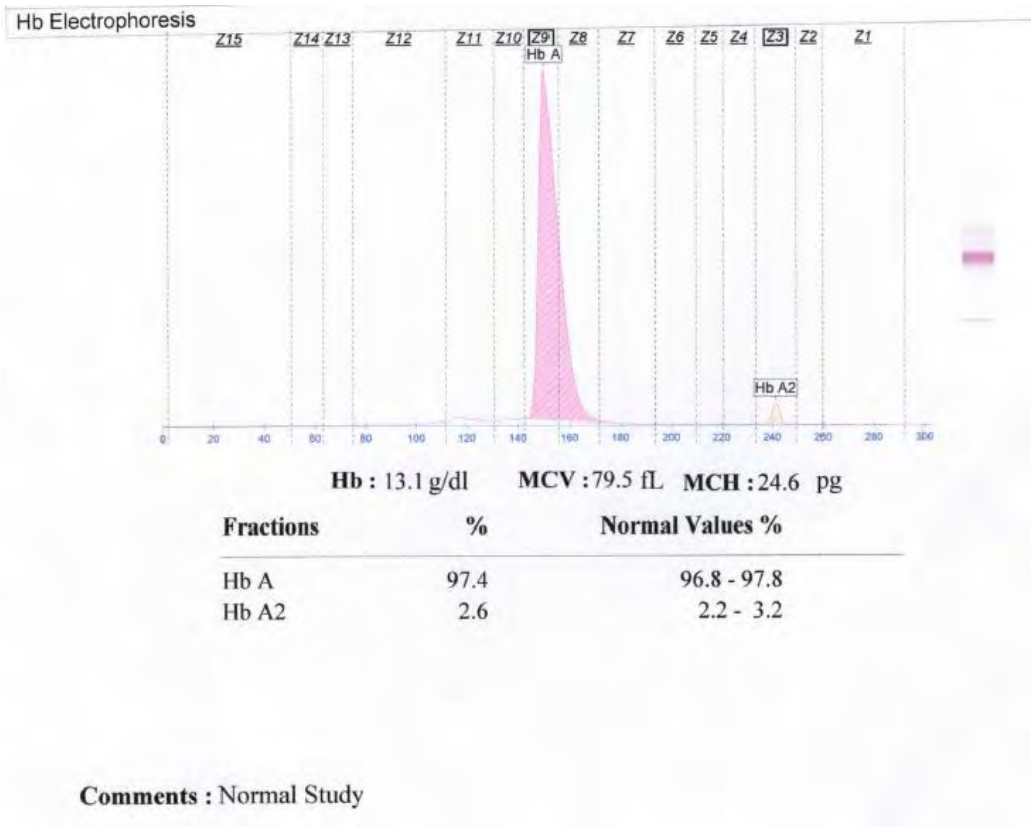

Figure 1.6: Electrogram of Group I(Normal study)

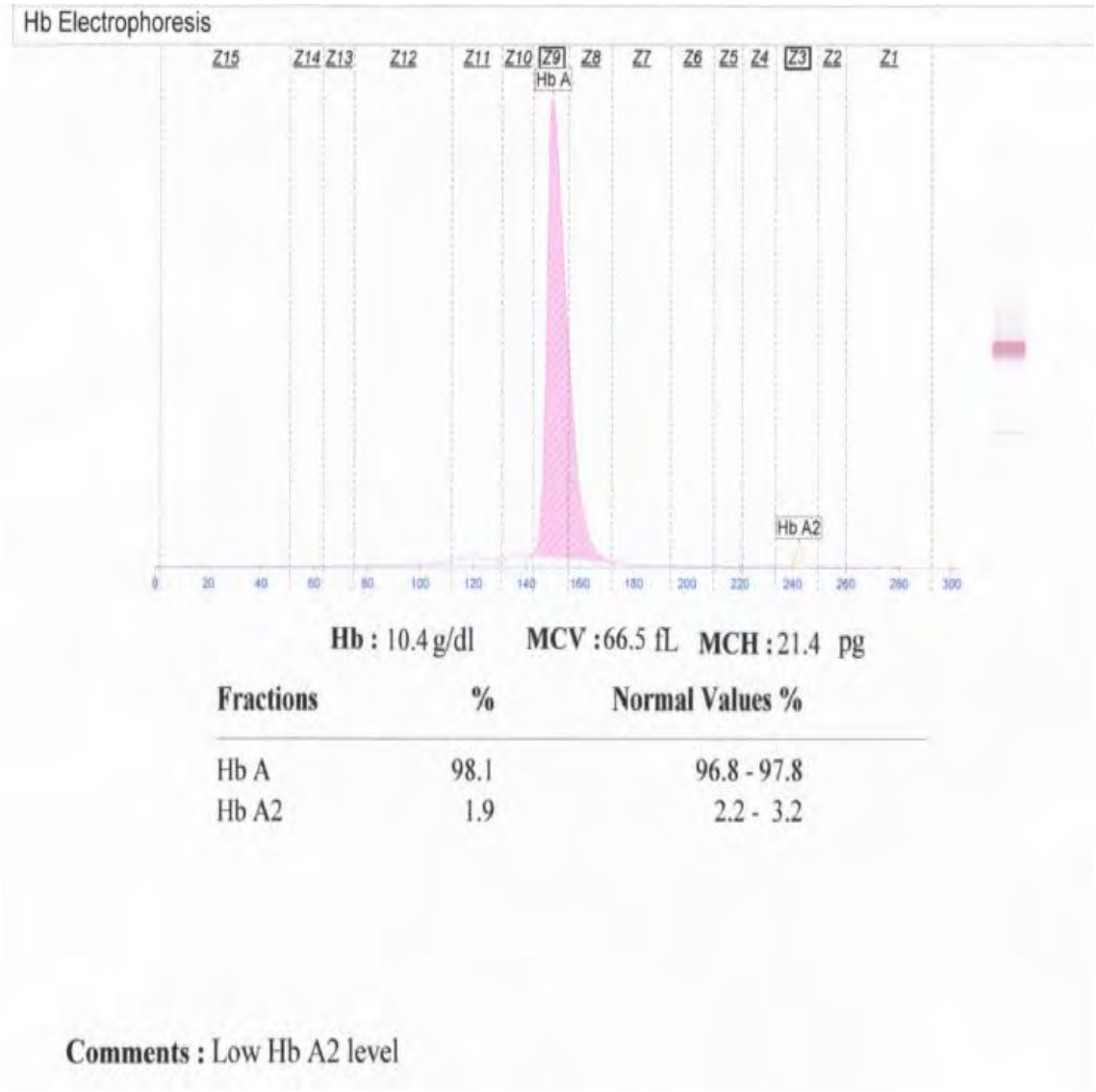

Figure 1.7: Electrogram of Group IIA(Low HbA_2 level)

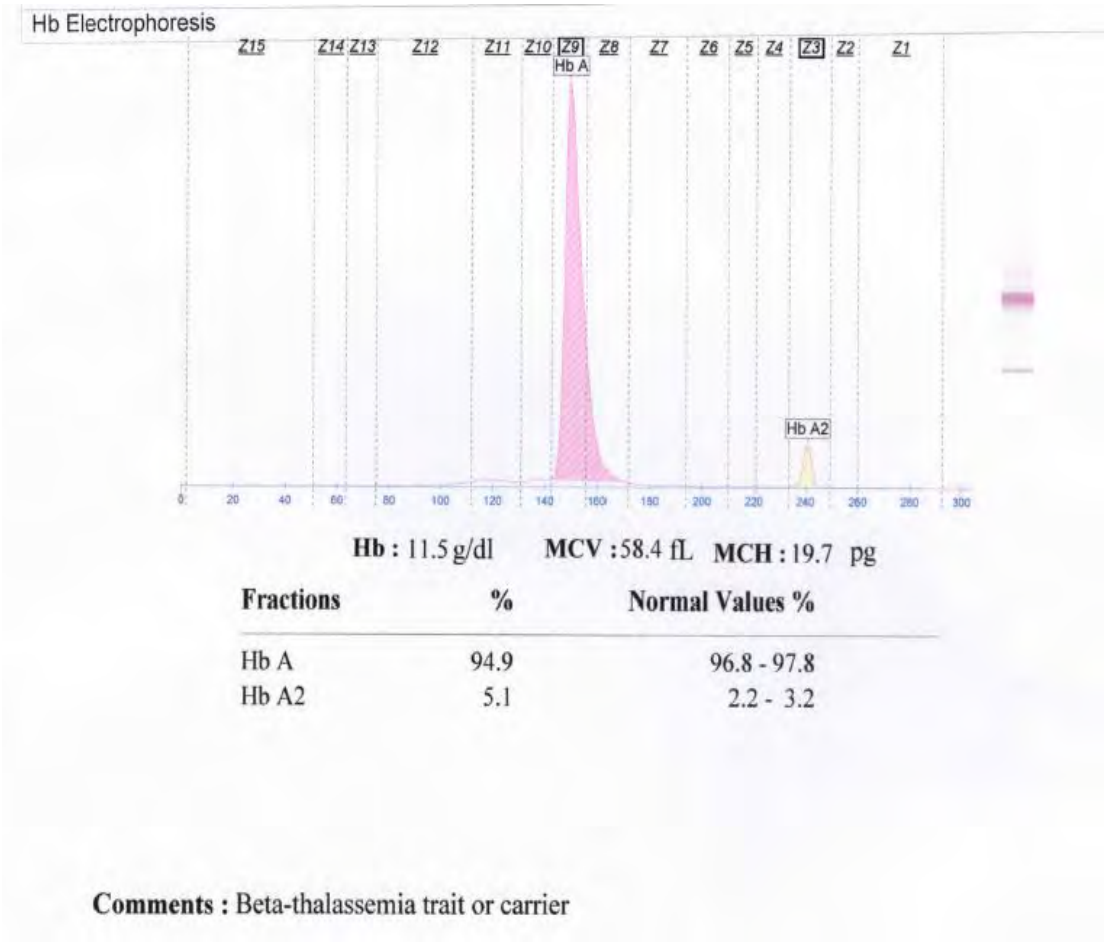

Figure 1.8: Electrogram of Group IIB(Beta-thalassemia trait or carrier)

Ferritin level of group IIA (5.63 ± 2.70 ng/ml) suggests iron deficiency anaemia.

TABLE 3.11

Ferritin	Number	Mean	±SD	Max	min
IIA	35	5.63	2.70	12.3	1.72

Table 3.11 Biochemical data of group IIA regarding Ferritin level

4. Discussion

IDA and β-thalassemia minor are recognized as the most important causes of hypochromia and microcytosis[55,56]. With respect to high prevalence of anemia among young couple, simple
and easy diagnosis of TT and IDA is crucial. Determination of cell blood count (CBC) by electroniccell counter is a first step in the diagnosis of TT and IDA in mass screening strategy. Both anemias are usually present with microcytosis mean corpuscular volume (MCV<80) and hypochromia mean corpuscular hemoglobin (MCH <27). the cell-count-based indices are easily available and reliable methods for detecting β-TT & IDA. Cell-count-based parameters particularly the MCV and RBC counts have good discrimination ability in diagnosing β-TT & IDA.

Of the 210 samples investigated in our study, microcytic samples were 69 samples representing 33.0% in all selected study subject. The reported prevalence of microcytosis in similar studies ranged from 5.4 to 45% according to the ethnic background of the study population.

In our study, BTT was found in 34 samples representing 16% of all 210 tested samples and 49.2% of all 69 microcytic samples. To avoid much more expensive, time-consuming, and complicated procedures for diagnosing these two disorders, researchers attempt to use either RBC indices such as MCV, MCH, and RDW, or formulas derived from these indices. This process helps to select appropriate
individuals for more detailed examination.

Comparable results with differences related to ethnic backgrounds of the studied groups were reported. In the Yemeni study by Al-Nood[57], BTT was detected in 31 patients representing 4.43% of all tested samples and 30% of microcytic samples. In an Indian study, Tiwari *et al.* [58]studied the prevalence of BTT in microcytic blood donor samples and it was 36% of all microcytic donor samples (18 from 50 microcytic samples). However, the study by Tiwari *et al.* [58]involved both deferred and actual blood donors on different ethnic group at Blood Bank of Uttarakhand,

Dehradun, Uttarakhand, India. In the study by Ali *et al.*[59], of the 181 patients studied, diagnosis of BTT was made in 10 patients (5.5%). This study was conducted in general Pakistani population.

In the study by Rahim *et al.*[60], totally 323 individuals (173 children and 150 adults) with microcytosis were investigated at Research Center of Thalassemia & Hemoglobinopathies, Ahwaz, Iran. Of the 323 patients, 153 (59 children and 94 adults) were diagnosed to have BTT representing 47.36% of microcytic samples. Comparing another study in India by Parthasarathy[61] who searched for BTT in 200 adult samples with microcytosis but not in healthy blood donors, BTT was found in 39 samples representing 19.5% of the total 200 microcytic samples. Parthasarathy used 80 fl as a cutoff for microcytosis in their study, which is similar what we used and could have attributed to their lower results.

Much lower prevalence of β-thalassemia minor was detected in other previous reports. Bolaman *et al.*[62] screened a total of 14200 couples before their marriage for BTT in Denzili, Turkey and found that carriers for b-thalassemia represent 2.2%. This was less than that found in our study, may be due to involvement of much more larger scale of population of different ethnic origin.

In our study, 35 of the 210 samples had IDA representing 16.66% of all tested samples and 50.7% of the 69 microcytic samples.

Another study by Tiwari *et al.*[58] who found IDA in 26 donors representing 2.81% of all tested samples and 52% of 50 microcytic samples.

In the study by Al-Nood [57] , iron deficiency accounted for only a small proportion (12/699 patients) representing 1.72% of all tested samples and 11.65% of microcytic samples, but he investigated samples in outpatient clinic not in blood donors.

Higher prevalence rate of IDA was detected by Al-Dabbagh *et al.*[63] who found that 16% of the studied Emirati healthy women had IDA (33/204) and 65.0% had ferritin values of less than 30.0 µg/l (133/204). Similarly, Parthasarathy [61] found IDA in 120 samples representing 60% of their study population.

IDA is well known to be the most common cause of microcytic anemia especially in underdeveloped countries. In our study, the prevalence of IDA was higher than that of BTT among our study population (16.6% and 16 %, respectively). In addition, our IDA group showed a highly significant reduction in their mean Hb levels when compared with the normocytic group, also the mean Hb of the BTT group showed highly significant difference compared with the normocytic group. This indicates that the BTT group and IDA group had lower Hb concentration.

In our study, a lower mean value of MCV for the BTT group than the IDA group was detected with highly significant statistical difference.

RBCs count showed a highly significant elevation in BTT than in IDA and also showed a highly significant elevation than in the normal group . previous study reported that RBCs count was the only parameter that had both sensitivity and specificity of 100% for differentiating both IDA and BTT. Tiwari *et al.* [58] also found that RBC count is the only parameter that had both sensitivity and specificity more than 80%. This difference may be due to involvement of deferred anemic donors in their study.

According to our study, RDW showed a highly significant difference between BTT and IDA . The highest RDW was found in IDA followed by the BTT reflecting more anisocytosis in IDA than BTT. Both Rahim *et al.* [60] and Parthasarathy [61] also found that RDW was higher in IDA than in BTT patients.

In previouly reportedmany study, the cutoff values of MCV 73fl or less, RBC count above 5.47×10^{6} /mm ^{3} , and RDW 14.5% or less were suggested to be associated with a high probability of BTT.

Controversy continues regarding the ideal red cell indices and their cutoff values for differentiating BTT and IDA. Kotwal *et al.* [64] conducted a study with 640 adult patients with microcytosis (MCV<80 fl), plotting receiver operator characteristic curves and recalculating the cutoff values for the Indian setting. The cutoff values of MCV less than 76 fl, RBC count at least 4.9×10^{12} /l, and RDW 18% or less were suggested to be associated with a high probability of BTT. However,

Parthasarathy[61] in India concluded that cutoff values of MCV below 76 fl, RBC count at least 4.9×10^6 /mm ^{3} , and RDW 18% or less were suggested to be associated with a high probability of BTT. Another Indian study [65] had cutoff values of MCV 78.0 fl or less, MCH 28 pg or less, and HbA_2 more than 3.8% for BTT diagnosis.

In the present study, the RBC count and MCV have greater sensitivity than RDW. Shalev *et al.*[66] reported that the combination of a high RBC count and low MCV is characteristic of BTT. It has been suggested that the RBC count is the most efficient single test for differentiating BTT and IDA [64 66 67]. Other studies showed superiority of RDW, followed by the RBC count and MCV in differentiating IDA and BTT[68] . Therefore, we think a combination of MCV, RDW, and the RBC count is more effective for identifying BTT and differentiating it from other nonthalassemic microcytosis; however, it should be noted that patients with BTT and concomitant iron, vitamin B_{12} , or folic acid deficiency, and double heterozygous dβ-thalassemics can have an elevated RDW [71,69,70]. Concomitant nutritional deficiency can also alter HbA_2 levels in BTT. Microcytosis accompanied by a high RBC count and normal RDW is suggestive of BTT.

In previous study measurement of hemoglobin in reticulocytes has proved useful for the diagnosis and follow-up of IDA [56], while its use in hemoglobinopathies has been less extensively elaborated[57-61]. In our study the least RET -He values were seen in IDA and BTT compared with the normal group .

These automated red cell parameters are routinely examined and offer a rapid and reliable method for BTT &IDA screening. Adequate utilization of these parameters can facilitate Primary identification of the majority of BTT & IDA cases at no additional cost to the healthcare system. Identifying BTT carriers and counseling them about the genetic implications of marrying another carrier is the most effective method for preventing β-thalassemia major.

Concluding remarks

Iron-deficiency anaemia, is the most common nutritional deficiency worldwide. This is a common health problem in rural women and young children of Bangladesh. The anaemia prevention and control strategies have focused on correcting this deficiency by routine iron supplementation.

Moreover, a thalassemic child and its family undergoes through a socio-economic strain in whole community. In thalassemia prevalent developing countries, carrier screening programs were successful in increasing the awareness about thalassemia among general mass as prevention is better than cure. The screening of thalassemia carriers in endemic areas remains a daily challenge for laboratory professionals. However, while Bangladesh is situated in a thalassemia prone region, hence it is imperative to consider thalassemia as an important health issue in this country. So the most effective approach to reduce the burden of the society and reduce disease incidence is implementation of carrier screening programmes offering genetic counselling and family screening, prenatal diagnosis and selective termination of affected fetuses.

As ß thalassemia minor and IDA are two common microcytic anemias, we conclude that genetic counseling for identification of thalassemia carriers in order to prevent the birth of thalassemia patients, differentiating BTT from IDA is warranted because the thalassemia heterozygote should not be given iron in a vain attempt to normalize MCV.

Previously reported discrimination indices using red blood cell (RBC) indices was not entirely satisfactory in diagnosing between these conditions. The above-described diagnosing system is aimed at screening for thalassemia & IDA in samples for which full blood count parameters have been technically and clinically validated prior to the interpretive process. Its main aim is to focus attention and efforts on those samples requiring further investigation for a complete diagnosis.The implementation of a system such as the one we have described will introduce an accurate differential diagnosis of IDA and BTT. Ferritin calculation and HbA2 level should be obtained for accurate differential diagnosis of IDA and BTT until more efficient tools are developed.

References

1.El-Harth, E.H., W. Kuhnau, J. Schmidtke, M. Stuhrmann, Z. Nasserallah and A. Al-Shahiri, 1999. Identification and clinical presentation of beta thalassaemia mutations in the eastern region of Saudi Arabia. J. Med. Genet., 36: 935-937.

2.Yang Z, Chaffin C, Easley P, et al. Prevalence of elevated hemoglobin A2 measured by the CAPILLARYS system. Am J ClinPathol 2009; 131 :42-48.

3.Langlois, S., et al., Carrier screening for thalassemia and hemoglobinopathies in Canada. J ObstetGynaecol Can, 2008. 30(10): p.950-71

4.Safizadeh, H., Z. Farahmandinia, S.S. Nejad, N. Pourdamghan and M. Araste, 2012. Quality of life in patients with thalassemia major and intermedia in Kerman-Iran (I.R.).Mediterr. J. Hematol. Infect. Dis., Vol. 4. 10.4084/MJHID.2012.058

5.Benz EJ. Clinical manifestations of the thalassemias. Available at: http://www.uptodate.com. [Last accessed on 2008 Feb 22].

6.Talsania, S., N. Talsania and H. Nayak, 2011. A cross sectional study of thalassemia in Ahmedabad City, Gujarat, (Hospital based).Indian Association of Preventive and Social Medicine, Gujarat.

7.Sirichotiyakul S, Maneerat J, Sa-nguansermsri T, et al. Sensitivity and specificity of mean corpuscular volume testing for screening for alpha-thalassemia-1 and beta-thalassemia traits. J ObstetGynaecol Res 2005; 31 :198-200.

8..AyselVehapoglu, Gamze Ozgurhan, AyşegulDoganDemir, SelcukUzuner, Mustafa AtillaNursoy, Serdar Turkmen, and ArzuKacan

9.Hematological Indices for Differential Diagnosis of Beta Thalassemia Trait and Iron Deficiency Anemia, 10 April 2014

10.Harthoorn-Lasthuizen EJ, Lindemans J, Langen-Huijsen MM. Influence of iron deficiency anaemia on haemoglobin A2 levels: possible consequences for beta-thalassemia screening. Scand J Clin Lab Invest 1999; 59 :65-70.

11.RALPH 0. WALLERSTEIN, M.D., and PAUL M. AGGELER, M.D.,Differentiating Between Thalassemia Minor and Iron Deficiency, San Francisco, May, 1955.

12.Balci YI, Karabulut A, Gurses D, Covut IE. Prevalence and risk factors of anemia among adolescents in Denizli, Turkey.Iran J Pediatr. 2012;22:77–81. [PMC free article] [PubMed]

13. Verdon F, Burnand B, Stubi CL, Bonard C, Graff M, Michaud A, et al.Iron supplementation for unexplained fatigue in non-anemic women: double blind randomised placebo controlled trial. BMJ. 326:1124. [PMC free article] [PubMed]

14. Ahmed F, Mahmuda I, Sattar A, Akhtaruzzaman M. Anemia and vitamin A deficiency in poor urban pregnant women of Bangladesh. Asia Pac J ClinNutr. 2003;12:460–6. [PubMed]

15. http://www.aafp.org/afp/2010/1101/p1117.html

16. Moreno Chulilla JA, Romero Colás MS, Gutiérrez Martín M. Classification of anemia for gastroenterologists. World J Gastroenterol. 2009;15(37):4627–4637.

17. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004;104(8):2263–2268.

18.Ahmed F, Mahmuda I, Sattar A, Akhtaruzzaman M. Anemia and vitamin A deficiency in poor urban pregnant women of Bangladesh. Asia Pac J ClinNutr. 2003;12:460–6. [PubMed

19. Helen Keller International The burden of anemia in rural Bangladesh: the need for urgent action. NutrSurveillProj Bull. 2006;16:1–4.

20.http://kidshealth.org/parent/medical/heart/ida.html#a_Treatment

21.http://www.mayoclinic.org/diseases-conditions/iron-deficiency-anemia/basics/causes/con-20019327

22.(Panos, 2005; Riewpaiboo etal. 2010)

23.^ Jump up to: ab*Mayo Clinic.* "Thalassemia". *Mayo Clinic*. Retrieved 17 October 2014.

24. Jump up^ Robbins Basic Pathology, Page No:428

25.^ Jump up to:a b John P. Greer JP, Arber DA, Glader B, et al. Wintrobe's Clinical Hematology

26.^ Jump up to:a b GBD 2013 Mortality and Causes of Death, Collaborators (17 December 2014)."Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.". Lancet 385: 117–71. doi:10.1016/S0140-6736(14)61682-2. PMC 4340604. PMID 25530442.

27.^*Modiano, G.; Morpurgo, G; Terrenato, L; Novelletto, A; Di Rienzo, A; Colombo, B; Purpura, M; Mariani, M; et al. (1991).* "Protection against malaria morbidity: Near-fixation of the α-thalassemia gene in a Nepalese population". *American Journal of Human Genetics 48 (2): 390–7.* PMC 1683029. PMID 1990845.

28.Jump up ^*Terrenato, L; Shrestha, S; Dixit, KA; Luzzatto, L; Modiano, G; Morpurgo, G; Arese, P (February 1988). "Decreased malaria morbidity in the Tharu people compared to sympatric populations in Nepal.". Annals of tropical medicine and parasitology 82 (1): 1–11. PMID* 3041928.

29.Jump up ^E. Goljan, Pathology, 2nd ed. Mosby Elsevier, Rapid Review Series.[page needed]

30.Jump up ^"Thalassemia" *(in Thai). Department of Medical Sciences,. September 2011. Archived from* the original *on 2011-09-25.*

31.http://www.stanfordchildrens.org/en/topic/default?id=beta-thalassemia-cooleys-anemia-in-children-90-P02330

32.http://kidshealth.org/parent/medical/heart/ida.html#a_Treatment

33.Thomas C, Kirschbaum A, Boehm D, Thomas L. The diagnostic plot: a concept for identifying different states of iron deficiency and monitoring the response to epoetin therapy. Med Oncol. 2006;23(1):23–36.

34.Jump up^ Cianciulli P (October 2008). "Treatment of iron overload in thalassemia". PediatrEndocrinol Rev 6 (Suppl 1): 208–13. PMID 19337180.

35.Jump up^ Vogiatzi, Maria G; Macklin, Eric A; Fung, Ellen B; Cheung, Angela M; Vichinsky, Elliot; Olivieri, Nancy; Kirby, Melanie; Kwiatkowski, Janet L; Cunningham, Melody; Holm, Ingrid A; Lane, Joseph; Schneider, Robert; Fleisher, Martin; Grady, Robert W; Peterson, Charles C; Giardina, Patricia J (March 2009). "Bone Disease in Thalassemia: A Frequent and Still Unresolved Problem". Journal of Bone and Mineral Research 24 (3): 543–557. doi:10.1359/jbmr.080505. ISSN 0884-0431.PMC 3276604.

36.Jump up^ "Thalassemia Complications". Thalassemia. Open Publishing. Retrieved27 September 2011.

37.^ Jump up to:a b Pediatric Thalassemia~treatment at eMedicine

38.Jump up^ Burdick CO; Ntaios, G.; Rathod, D. (March 2009). "Separating thalassemia trait and iron deficiency by simple inspection". Am. J. Clin. Pathol. 131 (3): 444; author reply 445. doi:10.1309/AJCPC09VRAXEASMH. PMID 19228649.

39.Jump up^ Harrison's Principles of Internal Medicine (17th ed.). McGraw-Hill medical. September 2008. p. 776. ISBN 0-07-164114-9.

40.Jump up^ Sabloff, M; Chandy, M; Wang, Z; Logan, BR; Ghavamzadeh, A; Li, CK; Irfan, SM; Bredeson, CN; et al. (2011). "HLA-matched sibling bone marrow transplantation for β-thalassemia major". Blood 117 (5): 1745–50.doi:10.1182/blood-2010-09-306829. PMC 3056598. PMID 21119108.

41.Jump up^ Sodani, P; Isgrò, A; Gaziev, J; Paciaroni, K; Marziali, M; Simone, MD; Roveda, A; De Angelis, G; et al. (2011). "T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients". Pediatric reports 3 (Suppl 2): e13.doi:10.4081/pr.2011.s2.e13. PMC 3206538. PMID 22053275.

42.^ Jump up to:a b John P. Greer JP, Arber DA, Glader B, et al. Wintrobe's Clinical Hematology

43.^ Jump up to:a b Gaziev, J; Lucarelli, G (June 2011). "Hematopoietic stem cell transplantation for thalassemia.". Current stem cell research & therapy 6 (2): 162–9.doi:10.2174/157488811795495413. PMID 21190532.

44.Jump up^ Sabloff, M; Chandy, M; Wang, Z; Logan, BR; Ghavamzadeh, A; Li, CK; Irfan, SM; Bredeson, CN; et al. (2011). "HLA-matched sibling bone marrow transplantation for β-thalassemia major". Blood 117 (5): 1745–50.doi:10.1182/blood-2010-09-306829. PMC 3056598. PMID 21119108.

45.Jump up^ Jagannath, Vanitha A (2014). "Hematopoietic stem cell transplantation for people with ß-thalassaemia major". Cochrane Database of Systematic Reviews 2014,(10): Art. No.: CD008708. doi:10.1002/14651858.CD008708.pub3. Retrieved18 October 2014.

46.Jump up^ Sodani, P; Isgrò, A; Gaziev, J; Paciaroni, K; Marziali, M; Simone, MD; Roveda, A; De Angelis, G; et al. (2011). "T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients". Pediatric reports 3 (Suppl 2): e13.doi:10.4081/pr.2011.s2.e13. PMC 3206538. PMID 22053275.

47.https://labtestsonline.org/understanding/analytes/cbc

48.https://en.wikipedia.org/wiki/Sysmex_Corporation

49. http://www.webmd.com/a-to-z-guides/complete-blood-count-cbc?page=4

50. Hematologic diseases. In: Wallach J. *Interpretation of Diagnostic Tests.* 8th ed. Boston, Mass.: Little Brown and Company; 2006:385–419

51. http://www.webmd.com/a-to-z-guides/hemoglobin-electrophoresis

52. W. C. Mentzer Jr., "Differentiation of iron deficiency from thalassaemia trait," The Lancet, vol. 1, no. 7808, p. 882, 1973. View at Google Scholar · View at Scopus

53.C. Thomas and L. Thomas, "Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency," Clinical Chemistry, vol. 48, no. 7, pp. 1066–1076, 2002. View at Google Scholar · View at Scopus

54.I. Shine and S. Lal, "A strategy to detect β thalassaemia minor," The Lancet, vol. 1, no. 8013, pp. 692–694, 1977. View at Google Scholar · View at Scopus

55Oski FA. Iron deficiency in infancy and childhood. *N Engl J Med* 1993; **329** :190-193.

56Olivieri NF. The beta-thalassemias. *N Engl J Med* 1999; **341** :99-109.

57Al-Nood H. Thalassemia trait in outpatient clinics of Sana'a City, Yemen. *Hemoglobin* 2009; **33** :242-246.

58Tiwari AK, Chandola I, Ahuja A. Approach to blood donors with microcytosis. British Blood Transfusion Society, Transfus Med 2010; **20** :88-94.

59Ali N, Moiz B, Bin Azhar W, Zaidi N, Memon R. Carrier detection for beta-thalassemia trait in general Pakistani population: a way forward. *Hematology* 2012l; **17** :237-240.

60Rahim F, Keikhaei B, *et al.* IDA and beta-TT differentiation. *Turk J Hemaol* 2009; **26** :138-145.

61Parthasarathy V. Search for beta thalassemia trait in India. *Turk J Hematol* 2012; **29** :427-429.

62Bolaman Z, Enli Y, Koseo Lu M, Koyuncu H, Aslan D. Prevalence of beta thalassemia trait in Denizli. *Turk J Haematol* 2001; **18** :85-88.

63Al-Dabbagh B, Shawqi S, Yasin J, Al Essa A, Nagelkerke N, Denic S. Half of the Emirati population has abnormal red cell parameters: challenges for standards and screening guidelines. *Hemoglobin* 2014; **38** :56-59.

64Kotwal J, Saxena R, Choudhry VP, Dwivedi SN, Bhargava M. Erythrocyte indices for discriminating thalassaemic and non-thalassemic microcytosis in Indians. *Natl Med J India* 1999; **12** :266-267.

65Bhukhanvala D, Seliya V, Shah A, Gupte S. Study of parents of β-thalassemia major children to determine cutoff values of hematological parameters for diagnosis of β-thalassemia trait and assessment of anemia in them. *Indian J Med Sci* 2013; **67** :117-122.

66Shalev O, Yehezkel E, Rachmilewitz EA. Inadequate utilization of routine electronic RBC counts to identify beta thalassemia carriers. *Am J Public Health*. 1988; **78** :1476-1477.

67Demir A, Yarali N, Fisgin T, Duru F, Kara AR. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. *Pedia Int* 2002; **44** :612-616.

68Batebi A, Pourreza A, Esmailian R. Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices. *Turk J Med Sci* 2012; **42** :275-280.

69Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. *Clin Chem* 2000; **46** :1284-1290.

70Niazi M, Tahir M, Raziq F, Hameed A. Usefulness of red cell indices in differentiating microcytic hypochromic anemias. *Gomal J Med Sci* 2010; **8** :125-129.

71 Rathod A, Kaur A, Patel V, *et al.* Usefulness of cell counter-based parameters and formulas in detection of β-thalassemia trait in areas of high prevalence. *Am J Clin Pathol* 2007; **128** :585-589.