"ROLE OF HEMATOLOGICAL INDICES TO DISTINGUISH IRON DEFICIENCY ANEMIA FROM BETA THALASSEMIA TRAIT IN ANTENATAL SCREENING"

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Sub: Institutional Ethical Clearance for the study.

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(Dr. Smita Sonoli)
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LIST OF ABBREVATIONS USED

S.No	Abbreviation	Expansion
1.	**CBC**	Complete Blood Count
2.	**MCV**	Mean Corpuscular Volume
3.	**MCH**	Mean Corpuscular Hemoglobin
4.	**MCHC**	Mean Corpuscular Hemoglobin Concentration
5.	**RDW-CV**	Red Cell Distribution Width - Coefficient of Variation
6.	**RDWI**	Red Cell Distribution Width Index
7.	**MI**	Mentzer Index
8.	**RI**	Ricerca Index
9.	**PCV**	Packed Cell Volume
10.	**RBC**	Red Blood Cell
11.	**MPV**	Mean Platelet Volume
12.	**IDA**	Iron Deficiency Anemia
13.	**BTT**	Beta-Thalassemia Trait
14.	**BTM**	Beta-Thalassemia Major
15.	**MHA**	Microcytic Hypochromic Anemia

16.	**ACD**	Anemia of Chronic Disease
17.	**TSAT**	Transferrin Saturation
18.	**TIBC**	Total Iron Binding Capacity
19.	**HPLC**	High-Performance Liquid Chromatography
20.	**NESTROFT**	Naked Eye Single Tube Red Cell Osmotic Fragility Test
21.	**HbA**	Hemoglobin A
22.	**HbA2**	Hemoglobin A2
23.	**HbF**	Hemoglobin F
24.	**Serum Fe**	Serum Iron

ABSTRACT

TITLE: Role of Hematological Indices to Distinguish Iron Deficiency Anemia from Beta Thalassemia Trait in Antenatal Screening

BACKGROUND AND OBJECTIVES: Microcytic hypochromic anemia is a common finding in pregnancy, primarily due to iron deficiency anemia (IDA) or beta thalassemia trait (BTT). Differentiating these conditions is essential to prevent unnecessary iron supplementation in BTT and to provide genetic counseling. This study evaluates Red Cell Distribution Width (RDW) and Mentzer Index (MI) as cost-effective screening tools and compares them with the Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT), iron studies, and High-Performance Liquid Chromatography (HPLC) for confirmation.

METHODOLOGY: A hospital-based cross-sectional study was conducted on 75 primigravida women with microcytic hypochromic anemia. Hematological indices, including RDW and MI, were assessed. Cases were screened using NESTROFT, followed by iron studies (serum ferritin, serum iron, total iron-binding capacity, and transferrin saturation) for differentiation. HPLC was performed for definitive diagnosis of BTT.

RESULTS: IDA cases had higher RDW and MI values (>13), lower serum ferritin, and decreased transferrin saturation, while BTT cases had lower RDW and MI (<13) with normal or increased serum ferritin levels. NESTROFT showed high sensitivity in detecting BTT, while HPLC confirmed all BTT cases with elevated HbA2 levels (>3.5%). Statistical analysis revealed significant correlations ($p<0.05$) among RDW, MI, iron studies, NESTROFT, and HPLC results.

CONCLUSION: RDW and Mentzer Index are effective initial screening tools for distinguishing IDA from BTT. Iron studies aid in differentiation, as BTT cases maintain normal or high ferritin levels, whereas IDA cases show depleted iron stores. NESTROFT serves as a reliable secondary screening tool, while HPLC remains the gold standard for confirming BTT. Integrating these diagnostic approaches in antenatal care can improve early detection and appropriate management.

KEYWORDS: Microcytic anemia, Mentzer Index, NESTROFT, HPLC

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF FLOWCHARTS

INTRODUCTION

Anemia is characterised by Haemoglobin levels and Red Blood Cell (RBC) counts are below the average value for that particular age and sex. Anemia is one of the common problems in pregnant ladies and a major health burden in India. Iron Deficiency Anemia (IDA) is one of the commonest type of Anemia during pregnancy.

Worldwide, IDA is the most prevalent cause of microcytic anemia, affecting 52.2% of pregnant women in India.[1]

Thalassemia, a single gene disorder, is also a common problem in India with more than 200 million people being carriers of Beta Thalassemia gene in the world and about 30 million are in India.[2] Every year more than ten thousand children are born with Beta Thalassemia Major (BTM) in India accounting for 10% of Thalassemia major births worldwide.[3]

Morphologically Beta Thalassemia Trait (BTT) mimics IDA. Both the conditions are commonly seen in the antenatal women. India accounts for 10% of all thalassemia cases born globally each year. Certain communities like Sindhi's, Punjabi's, Gujarati's and Bengali's are more commonly affected with BTT.[3] Diagnosis of both these entities is often difficult due to its overlapping and similar features. [4]

Hence, accurately distinguishing these entities is vital for detecting BTT, delivering appropriate premarital counseling, and preventing excessive iron therapy in these individuals.

There are similarities in Red Cell Indices such as decreased haemoglobin, Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV).[5]

A diagnostic workup involving serum ferritin, TIBC, transferrin saturation percentage, serum iron and hemoglobin electrophoresis is essential for confirmation of these two conditions. However the gold standard tool for screening and detection of various Hemoglobinopathies are High Performance Liquid Chromatography (HPLC).[6]

Various other sophisticated tests such as cellulose acetate electrophoresis, isoelectric focusing in polyacrylamide or agarose gel, Immunological assays, structural analysis, and genotype methods may be used.[7] These tests are generally not available at many peripheral centres. Conducting such tests in outpatient settings, particularly in rural areas of our country, is often impractical and challenging for both patients and healthcare providers. Additionally, it places a significant financial strain on patients.[8]

However Electronic Cell Counters are readily available in many of the peripheral and Rural Centres which gives different Red Cell Indices. This research aims to analyze Red Cell Distribution Width (RDW) and Mentzer Index (MI) as potential markers for differentiating Microcytic Hypochromic Anemia (MHA) resulting from IDA and BTT.[9]

AIMS AND OBJECTIVES

OBJECTIVE OF THE STUDY:

- To differentiate IDA and BTT by RDW and MI.
- To find out the incidence of MHA in primigravida.
- Confirmation of BTT by Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) and HPLC.

Inclusion Criteria:

- Primigravida women carrying a singleton pregnancy, aged 18 to 49 years.
- Hemoglobin levels lower than 10 g/dL.
- Peripheral smear showing MHA.

Exclusion Criteria:

- Multigravida
- Haemoglobin more than 10gm/dl.
- Women with a history of blood transfusion in the previous three months.

REVIEW OF LITERATURE

Anemia is one of the common problems in pregnant ladies and a major health burden in India. Nutritional Anemia is one of the commonest type of Anemia during pregnancy.[10] IDA is the most common microcytic anemia during pregnancy, with a 52.2% prevalence rate.[1] Beta Thalassemia, a single-gene disorder, also affects many in India, with over 30 million carriers. Annually, more than ten thousand children in India are born with Thalassemia major, representing 10% of global cases.[3]

ANEMIA:

RBCs or haemoglobin level that is below normal. This condition reduces the blood's ability to carry oxygen to the body's tissues. There are several symptoms that might result from this decrease in oxygen delivery, which includes weakness, exhaustion, lightheadedness, and dyspnea. Depending on the degree of deficiency and the underlying cause, anemia can range in severity from mild to severe.[11]

Anemia is classified by the World Health Organization (WHO) according to haemoglobin concentration levels. When haemoglobin levels drop below 13 g/dL in men and below 12 g/dL in women who are not pregnant, anemia is diagnosed, according WHO standards. The threshold is lower in pregnant women (11 g/dL) because of the increased plasma volume during pregnancy.[11]

ANEMIA IN PREGNANCY:

Pregnancy-related anemia is prevalent and is an important health concern that can impact the health of both the mother and the fetus. It is characterized by a haemoglobin level below 11 g/dL during pregnancy and can result in different

complications if not adequately addressed. The frequency of anemia during pregnancy differs around the world, with developing nations experiencing higher rates.[12]

CAUSES OF ANEMIA IN PREGNANCY:

Many factors influence the issue of anemia in pregnant women, which is a major health concern. Examples of these factors are malnutrition resulting from insufficient intake of iron, folic acid, and vitamin B12.[13] Anemia in pregnancy often stems from a lack of iron, as expectant mothers need about 27 mg of iron daily for their growing blood volume, the developing fetus, and the placenta. Folate is necessary for DNA synthesis and cell division, with a recommended intake of 600 mcg during pregnancy; a shortage can lead to megaloblastic anemia.[14] Vitamin B12, essential for RBC production and nervous system development, requires a daily intake of 2.6 mcg during pregnancy, and deficiencies can cause anemia.[15]

Pregnancy also increases blood volume by around 50%, resulting in physiological anemia.[16] Genetic illnesses like sickle cell anemia and thalassemia can exacerbate anemia due to the increased physiological demands during pregnancy. Severe blood loss before delivery, caused by conditions such as placenta previa or placental abruption, can significantly reduce iron reserves.[17]

Besides, raised body demands during gestation period, Postpartum hemorrhage can also drastically reduce iron reserves and aggravate anemia. Infections such as hookworm and malaria can lead to anemia by causing severe blood loss and RBC destruction. These combined factors highlight the importance of effectively managing anemia during pregnancy to protect both maternal and fetal health.[17]

MATERNAL AND FOETAL COMPLICATIONS OF ANEMIA IN PREGNANCY:

Anemia in pregnancy can lead to several maternal complications. These include an increased risk of infections, low birth weight, postpartum depression, maternal death, and a higher chance of preterm delivery. Addressing anemia is crucial to mitigate these risks and ensure better health outcomes for the mother.

For the fetus, anemia in the mother can cause intrauterine growth restriction, preterm birth, and impaired cognitive and physical development. These complications underscore the importance of managing anemia during pregnancy to promote optimal fetal health and development.[18]

MORPHOLOGICAL CLASSIFICATION OF ANEMIA:

RBC are classified morphologically on the basis of MCV of RBC. There are three basic divisions within the morphologic classification system. Normocytic is defined as MCV 80-100 fL , Microcytic and Macrocytic are explained by MCV <80 fl and >100 fL respectively.

Adults with a MCV of less than 80 fL have microcytic cells. IDA and BTT are the most common conditions associated with moderate microcytic anemia. Other etiological causes include chronic illness anemia, lead poisoning, and sideroblastic anemia.[19]

IRON DEFICIENCY ANEMIA:

IDA is the most common MHA worldwide, affecting about 30% of the global population, primarily in developing countries.[11] It results from insufficient iron to synthesize haemoglobin, and it is prevalent in young children and women of

reproductive age, though it can occur in any age group. IDA is characterized by RBC morphological changes like microcytosis, hypochromia, anisocytosis, and poikilocytosis.[20]

In adults, IDA is usually caused by blood loss, while in children, a faulty diet is the primary cause. It represents a severe stage of iron deficiency where haemoglobin (or hematocrit) falls below normal levels, with no hemolysis and normal erythrocyte survival. Serum iron levels are typically low, and hypochromic red cells may be present even before anemia develops. A reduction in haemoglobin concentration is a late feature of iron deficiency.[20]

PATHOPHYSIOLOGY OF IDA:

Iron deficiency is the most common cause of anemia, with various reasons contributing to this condition. One major cause is inadequate iron consumption. A lack of iron in the diet over an extended period can lead to deficiency. Iron-rich foods such as beef, eggs, and green leafy vegetables are crucial, especially for pregnant women and young children, who need increased iron intake during rapid growth and development.

Blood loss due to menstruation is another common cause, particularly in women of reproductive age. Heavy menstrual flow and blood loss during childbirth can significantly deplete iron levels. Internal hemorrhage is another factor; medical conditions like stomach ulcers, colon polyps, or cancer can cause internal bleeding, leading to IDA. Regular use of pain medications, such as aspirin, can also result in stomach bleeding.

IDA is a widespread condition affecting individuals of any age, gender, or ethnicity. Intestinal problems or surgeries, such as those related to celiac disease or gastric bypass, can impair iron absorption. Certain groups are more susceptible, including pregnant women, those with poor diets, infants and children (especially those born prematurely or in growth spurts), and vegetarians lacking alternative iron-rich foods.

Understanding these risk factors is essential for preventing and managing IDA, ensuring that those at higher risk take necessary precautions to maintain adequate iron levels through diet and medical care.[21]

CLINICAL FEATURES OF IDA IN PREGNANCY:

Typical symptoms include fatigue, weakness, dizziness, palpitations, and shortness of breath, often accompanied by pale skin and mucous membranes. Signs specifically associated with iron deficiency include pica (craving substances like clay or ice), restless legs syndrome, glossitis (inflamed tongue), angular cheilitis (cracks at the mouth corners), and koilonychia (spoon-shaped nails).[21,23]

RISK FACTORS OF IRON DEFICIENCY ANEMIA IN PREGNANCY:

During pregnancy, the body's need for iron increases significantly. Pregnant women should consume 27 mg of iron per day, compared to 18 mg for non-pregnant women. This increased requirement is necessary to support the growing blood volume, the developing fetus, and the placenta.[22]

Many expectant mothers do not get sufficient iron from their diet, which should include iron-rich foods like lentils, beans, fish, chicken, red meat, and fortified cereals. Women who already have anemia before pregnancy are at a higher risk of

experiencing a worsening of their condition. Additionally, women expecting multiple fetuses, those with frequent pregnancies, and those with a history of heavy menstrual bleeding are more likely to develop IDA.[21,22]

Other additional risk factors are, Pregnant adolescents are particularly susceptible to IDA due to the demands of both their own growth and that of the fetus. Women from lower socioeconomic backgrounds often have limited access to dietary supplements, healthcare, and nutritious food, increasing their risk of developing IDA. Furthermore, gastrointestinal disorders such as celiac disease or inflammatory bowel disease can impair iron absorption, contributing to the risk of IDA during pregnancy.[22]

LAB DIAGNOSIS OF IRON DEFICIENCY ANEMIA IN PREGNANCY:

Laboratory evaluation starts with a Complete Blood Count, which typically shows reduced haemoglobin levels , low MCV, indicating microcytosis, and an elevated RDW due to size variability in RBCs. A peripheral blood smear commonly reveals microcytic hypochromic red cells with anisocytosis and poikilocytosis.[24,25]

Bone Marrow Findings:- Hypoplastic erythroid series, absent or markedly reduced sideroblasts on Iron's staining.[24,25]

Iron studies are crucial for confirmation, showing decreased serum ferritin levels (below 15 ng/mL), reflecting depleted iron reserves, reduced serum iron, increased TIBC, and low TSAT (below 16%).[24,25]

THALASSEMIA:

In 1925, Cooley and Lee described a severe form of anemia in early life, accompanied by bone abnormalities and splenomegaly. The term "thalassemia" was

coined by William L. Bradford along with George H. Whipple. "Thalasa" means sea in Greek, and "emia" refers to blood. Thalassemia is a major autosomal recessive hereditary haemoglobinopathy prevalent in Mediterranean, Far Eastern, and Southeast Asian countries. It is caused by genetic mutations of the haemoglobin (haemoglobin) genes, resulting in reduced production or total absence of one or more globin chains, with two main classes: alpha thalassemia and beta thalassemia.[26]

The most common form of thalassemia is beta thalassemia, subdivided into thalassemia major, intermediate, and minor/trait, caused by homozygous deletion of the beta-globin chain gene, leads to severe lifelong hemolytic anemia affecting multiple organs and causing significant morbidity and mortality. Intermediate thalassemia involves mutations in one or both globin genes, while BTT is the heterozygous form. Most BTT patients are asymptomatic or have mild anemia.[27]

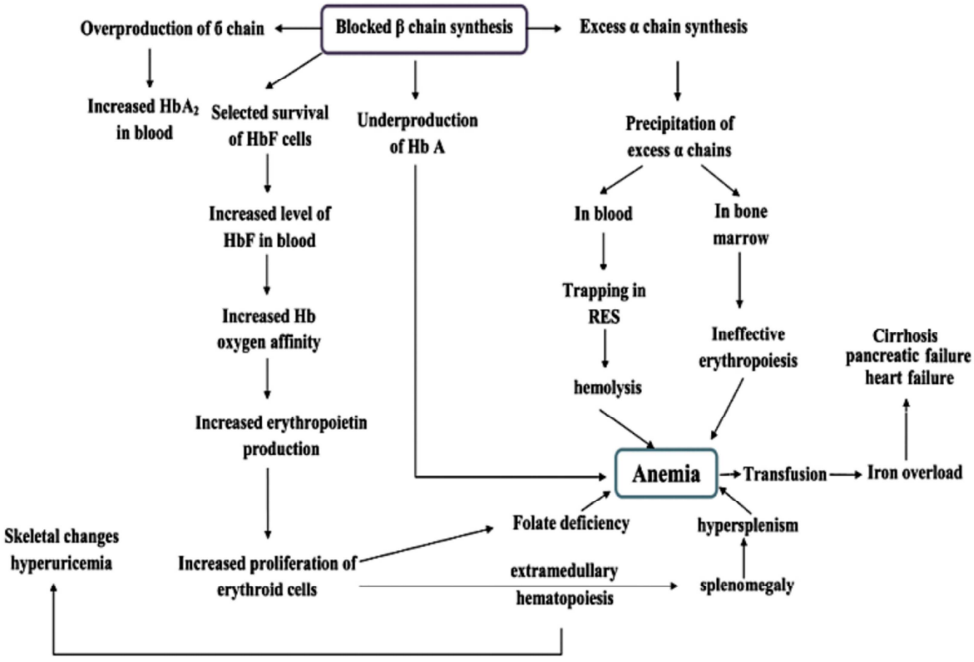
Women are carriers of Thalassemia. Hence they should be screened for Thalassemia Minor. Globally approx 5% people are thalassemic carriers.[28]

PATHOPHYSIOLOGY OF THALASSEMIA:

Unpaired polypeptide chains of haemoglobin are insoluble and can cause damage to RBCs. Globin chains (alpha and beta) combine to form a soluble tetramer that protects cells from harm. Normal globin production is regulated so that any new chain formed has a partner to pair with, ensuring balance and solubility.

In thalassemia syndromes, this control is disrupted, leading to the overproduction of one type of chain and underproduction of the other. This mismatch results in a concentration of unpaired chains, causing globin chain insolubility and precipitation. Thalassemias are classified as alpha or beta thalassemias based on

whether the genetic error or deletion occurs in the alpha or beta globin chain gene. Patients with alpha thalassemias have impaired alpha chain production, while those with beta thalassemias have impaired beta chain production. Each type of thalassemia can be heterozygous (minor) or homozygous (major).



FLOWCHART 1: PATHOPHYSIOLOGY OF THALASSEMIA[29]

CLINICAL FEATURES OF THALASSEMIA MAJOR IN PREGNANCY:

Pregnant women with thalassemia may experience fatigue, pallor, and splenomegaly due to varying degrees of anemia and extramedullary hematopoiesis. These manifestations can lead to bone changes, impaired growth, and iron overload.[30]

CLINICAL FEATURES OF THALASSEMIA MINOR IN PREGNANCY:

Thalassemia minor in pregnancy is often associated with minimal or no symptoms, as most carriers exhibit no noticeable health issues. They may have mild anemia and reduced RBC size (microcytosis). These characteristics are sometimes

misdiagnosed as IDA but can be differentiated through specific laboratory findings, such as a decrease in MCV and an increase in haemoglobin A2 and foetal haemoglobin levels, particularly in cases of BTT.[31,32,33]

In general, pregnancy outcomes for women with thalassemia minor are favorable for both mother and child. However, certain cases may experience challenges such as anemia, high blood pressure disorders like preeclampsia, or the need for blood transfusions. Newborn outcomes are typically positive, with most deliveries being uncomplicated, although there may be a slightly higher chance of neonatal intensive care unit (NICU) admission in pregnancies with additional maternal complications.[31,32,33]

RISK FACTORS FOR THALASSEMIA MAJOR AND MINOR IN PREGNANCY:

Thalassemia is an inherited haemoglobinopathy that poses significant challenges during pregnancy. Women with BTM often face complications such as anemia, iron overload, cardiac dysfunction, thromboembolism, alloimmunization, infections, endocrine disorders, and bone complications, all of which can adversely affect maternal and obstetric outcomes.[34]

Even carriers of the BTT have been linked to increased risks of obstetric and perinatal complications.[34] Furthermore, maternal BTM has been associated with a higher likelihood of long-term hematological morbidity in offspring, underscoring the importance of comprehensive prenatal care.[35]

Given these risks, pregnancies in women with thalassemia require meticulous preconception counseling and coordinated management by a multidisciplinary team to optimize outcomes for both mother and child.[36]

LAB DIAGNOSIS OF THALASSEMIA MAJOR AND MINOR IN PREGNANCY:

Laboratory diagnosis involves a CBC revealing microcytic anemia, and haemoglobin analysis through electrophoresis or HPLC to detect abnormal haemoglobin variants. DNA analysis may be employed to identify specific genetic mutations associated with alpha or beta and thalassemia- major or minor. Early and accurate diagnosis is crucial for managing thalassemia major or minor in pregnancy to mitigate potential complications for both mother and fetus.[33]

The other causes of MHA in pregnancy are;- Anemia of chronic diseases and sideroblastic anemia.

ANEMIA OF CHRONIC DISEASES IN PREGNANCY:

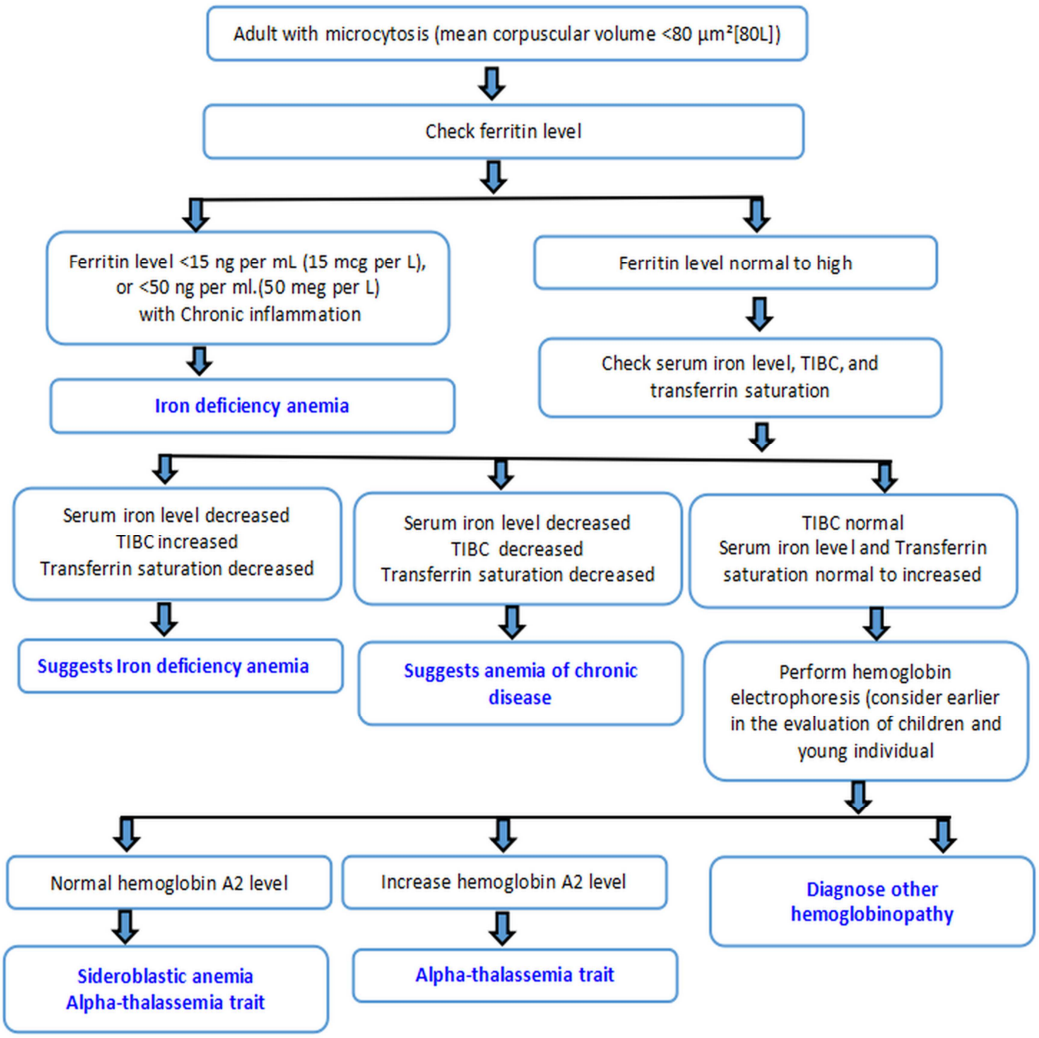
Anemia of chronic disease (ACD) during pregnancy is linked to prolonged inflammation or immune system activity, often caused by chronic infections, autoimmune conditions, cancers, or kidney issues. It generally manifests as mild to moderate anemia with symptoms such as fatigue and pale skin, typically without pronounced iron deficiency.[37]

Diagnostic findings include reduced serum iron, normal or increased ferritin, low TSAT, and elevated inflammatory markers like C- Reactive Protein. Differentiating Anemia of Chronic Deficiency from iron-deficiency anemia is crucial to ensure proper management.[37]

SIDEROBLASTIC ANEMIA IN PREGNANCY:

Sideroblastic anemia in pregnancy, although rare, presents substantial challenges. This condition is characterized by the presence of ring sideroblasts in the bone marrow and can be either congenital or acquired. Congenital cases are often linked to mutations in genes like ALAS2, which causes X-linked sideroblastic anemia, or SLC25A38, resulting in autosomal recessive sideroblastic anemia. Acquired forms are typically caused by factors such as alcohol use, certain medications (including antibiotics and chemotherapy), exposure to heavy metals, and deficiencies in vitamins like B6 or copper. [38,39]

Clinically, pregnant women with sideroblastic anemia exhibit typical symptoms of anemia, such as fatigue, pallor, and shortness of breath. In more severe cases, iron overload can lead to symptoms like enlarged liver or spleen, and in some cases, diabetes mellitus. Laboratory findings typically show microcytic, hypochromic anemia, with an elevated RDW. Diagnosis is confirmed through tests like iron studies, bone marrow examination to detect ring sideroblasts, and genetic testing to identify congenital forms.[38,39]



FLOWCHART 2: DIAGNOSING MICROCYTOSIS [40]

RED BLOOD CELLS:

Red blood cells are characterized by their biconcave disc shape, measuring approximately 7.8 micrometers in diameter, with variable thickness, thicker at the edges (2.5 micrometers) and thinner at the center (1 micrometer or less). Key quantitative measures of RBCs include hematocrit, haemoglobin, and the red cell count per unit volume. In healthy individuals, females have 4.0-5.0 million RBCs per cubic millimeter. haemoglobin levels range from 12-16 g/dL in females, while hematocrit values are 36%-46% in females.

Additionally, three indices describe the qualitative characteristics of RBCs: MCV, MCH, and mean corpuscular haemoglobin concentration (MCHC). MCV, indicating the average size of RBCs, ranges from 80-100 femtoliters (fL). MCH, which measures the average amount of haemoglobin per RBC, ranges from 27-32 picograms (pg) per cell. MCHC, representing the average concentration of haemoglobin in RBCs, ranges from 32-36 grams per deciliter (g/dL). These indices are crucial for understanding the health and functionality of RBCs.[41]

HEMOGLOBIN:

Hemoglobin is a tetramer made of two pairs of globin chains and heme, with two globin chains containing 141 amino acids and two with 146 amino acids. Adults have three types of haemoglobin: Haemoglobin A (96%), Haemoglobin A2 (3%), and Haemoglobin F (1%). The composition of hemoglobin A includes two alpha and two beta chains, whereas hemoglobin A_2 comprises two alpha and two delta chains, and hemoglobin F consists of two alpha and two gamma chains. The α-globin genes are on chromosome 16 and the β-globin genes are on chromosome 11.

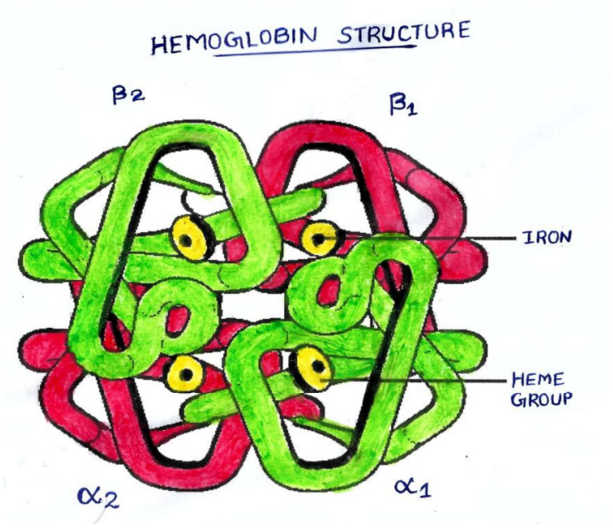


FIGURE 1: HEMOGLOBIN STRUCTURE[42]

MEAN CORPUSCULAR HAEMOGLOBIN (MCH):

MCH is a key RBC index that quantifies the average haemoglobin content per RBC and is an essential component of the CBC. It aids in the diagnosis of anemia, with typical values ranging from 27 to 33 picograms per cell. MCH, along with MCV and MCHC, was enhanced by the introduction of the Coulter Counter in the 1950s, which automated its calculation and improved accuracy and efficiency.[43]

MCH values help identify microcytic or hypochromic anemias, such as IDA or thalassemia, when low, and macrocytic anemias, associated with vitamin B12 or folate deficiencies, when high. However, MCH alone cannot reliably differentiate between IDA and BTT, as both conditions present with low values.[43]

Studies suggest that MCH has a sensitivity of approximately 70% and a specificity of 75%, making it a supportive but not definitive diagnostic tool. Therefore, it should be used in conjunction with other hematological indices for a more accurate diagnosis.[43]

MEAN CORPUSCULAR VOLUME (MCV):

MCV is a key hematological parameter used to assess RBC size and aid in anemia evaluation. Introduced by Maxwell Wintrobe in the 1930s as part of the CBC, along with MCH and MCHC, MCV was initially calculated manually using hematocrit and RBC counts.[44]

The introduction of automated technologies like the Coulter Counter in the 1950s and 1960s enabled direct measurement through electrical impedance and light scattering, improving accuracy and reliability. MCV is crucial for classifying anemias, with values below 80 fL indicating microcytic anemia, such as in iron

deficiency, and values above 100 fL suggesting macrocytic anemia, commonly associated with vitamin B12 or folate deficiencies.[44]

While MCV has moderate sensitivity (60–70%) and specificity (70–80%) for distinguishing IDA from BTT, it is not entirely reliable as a standalone diagnostic tool. Additionally, MCH does not effectively differentiate BTT from IDA, as both conditions can present with low values. Therefore, MCV and MCH should be interpreted alongside other hematological indices for a more accurate diagnosis.[44]

RDW COEFFICIENT OF VARIATION (RDW-CV):

RDW-CV is a crucial hematological measure that quantifies the variation in the size of RBCs, known as anisocytosis. The RDW-CV (coefficient of variation) is the most commonly used form of this measurement, calculated by dividing the standard deviation of the RBC volume by the MCV and multiplying by 100. This calculation provides insight into the degree of variation in cell size, which is helpful in diagnosing different types of anemia, including those caused by iron, folate, or vitamin B12 deficiencies. RDW values are usually reported as a percentage, with typical reference ranges between 11.0% and 15.0%.[45,46,47]

Clinically, RDW-CV is useful for differentiating causes of anemia. Elevated RDW-CV levels suggest the presence of both smaller and larger RBCs, which can be seen in early stages of iron deficiency or combined nutritional deficiencies. It is also valuable for identifying anisocytosis in conditions like myelodysplastic syndromes or liver disease. Furthermore, RDW-CV is a useful prognostic tool in various conditions, such as cardiovascular diseases and acute conditions like pneumonia.[45,46,47]

RDW-CV IN IRON DEFICIENCY ANEMIA DURING PREGNANCY:

In IDA, RDW-CV is notably elevated, reflecting a high degree of variability in RBC sizes. This variability arises due to the production of smaller, microcytic cells in response to iron deficiency. During pregnancy, this increased RDW-CV, often surpassing 18%, serves as a crucial diagnostic marker to differentiate IDA from other forms of anemia, as it highlights significant anisocytosis.[48,49]

RDW-CV IN BETA THALASSEMIA TRAIT DURING PREGNANCY

In BTT, RDW-CV remains relatively lower compared to IDA. The RBCs in BTT are consistently small (microcytic) and hypochromic, resulting in minimal size variation. This characteristic uniformity reduces RDW-CV values, which generally stay below 16%. Such differentiation is clinically significant, as RDW-CV in BTT does not reach the elevated levels typical of IDA, which can exceed 20%, especially during pregnancy.[48,49]

These variations make RDW-CV a valuable tool in distinguishing IDA from BTT, supporting accurate diagnosis and management in pregnant individuals

RDW-CV has a sensitivity of 78% for IDA and 58.3% for BTT, with specificity of 58.3% for IDA and 78% for BTT[48,49]

RBC INDICES USED TO DIFFERENTIATE IDA AND BTT:

MENTZER INDEX (MI):

The MI, developed by William Mentzer in 1973, is a straightforward calculation used to distinguish between IDA and BTT. It is derived by dividing the MCV by the RBC count. Values above 13 are indicative of IDA, while values below 13 suggest BTT.

This index is widely employed due to its ease of use and reliability, particularly in areas with high rates of thalassemia and iron deficiency. Its diagnostic accuracy has been extensively validated, with studies reporting sensitivities as high as 95% for identifying IDA and strong specificity for excluding thalassemia. The MI proves especially useful in resource-limited settings where advanced tests like haemoglobin electrophoresis or genetic analysis may not be accessible.

The MI is highly valued for its ability to differentiate between IDA and BTT, both of which present with similar clinical symptoms such as MHA. Research has shown that the index offers strong diagnostic accuracy, with a sensitivity of 91% and specificity of 83% for IDA, and 83% sensitivity and 91% specificity for BTT. These findings underscore the MI's usefulness in settings where more advanced, costly diagnostic tools like haemoglobin electrophoresis are not available. This makes it an ideal, cost-effective screening tool in regions with limited access to specialized laboratory facilities.[9,49,50,51]

OTHER INDICES USED TO DIFFERENTIATE IDA FROM BTT:

The MI and associated indices are widely used to differentiate between IDA (IDA) and beta-thalassemia trait (BTT). These indices, each with specific formulas and interpretations, assist clinicians in diagnosing anemia types. For example, the Shine and Lal Index, Green and King Index, and Srivastava Index, Ricerca index, Walter’s Index evaluate factors such as MCV, MCH, Iron studies haemoglobin levels, and RDWI to differentiate between IDA and BTT. Each index offers valuable insights for making early clinical distinctions, particularly in settings where advanced diagnostic tools like haemoglobin electrophoresis may not be readily accessible.

RICERCA INDEX (RI):

The Ricerca Index is a hematological index designed to differentiate IDA from BTT. It is calculated using the formula: RI = RDW / RBC count.[94] A value greater than 3.3 is indicative of IDA, whereas lower values may suggest BTT.[95] This index is often used alongside other diagnostic tools, such as the MI, to improve accuracy in distinguishing between these two conditions, which can present with similar hematological features. However, studies indicate that the Ricerca Index has a relatively lower sensitivity and specificity compared to the MI, making it less reliable as a standalone diagnostic tool but still useful in conjunction with other indices.[52]

RDW INDEX (RDWI):

The RDWI is a valuable hematological parameter for distinguishing between IDA and BTM, particularly in pregnant women, as both conditions present with microcytic anemia. It is calculated using the formula RDWI = (MCV × RDW) / RBC, where MCV, RDW, and RBC play a crucial role in determining the type of anemia. In IDA, RDWI values are typically above 220 due to a lower RBC count, smaller RBCs (low MCV), and greater variation in their size (high RDW), whereas in BTM, RDWI values are generally below 220, associated with a higher RBC count and more uniform cell size (normal or slightly reduced RDW). Accurate diagnosis is essential, as IDA requires iron supplementation, while BTM does not and may necessitate genetic counseling. Studies have demonstrated RDWI's high diagnostic accuracy, with 94.0% sensitivity and 88.0% specificity for detecting BTM, and 88.0% sensitivity and 86.0% specificity for identifying IDA. Another study reported 85% sensitivity and 83.3% specificity for IDA, and 83.3% sensitivity and 85% specificity for BTM, making RDWI a reliable and effective tool for differentiating these conditions when used alongside other diagnostic indices.[53,54]

MEAN CORPUSCULAR HAEMOGLOBIN (MCH):

The MCH is a hematological parameter that measures the average haemoglobin content in RBCs. While it is useful in assessing anemia, it cannot reliably differentiate between BTT and IDA, as both conditions can present with low MCH values. Studies indicate that MCH has a sensitivity of approximately 70% and a specificity of 75%, making it a supportive but not definitive tool for distinguishing between these two anemias (IDA & BTT). Therefore, MCH should be used in conjunction with other indices for a more accurate diagnosis.[55,56]

IMPORTANCE OF IRON STUDIES IN IDA AND BTT:

SERUM FERRITIN:

Serum ferritin is an iron storage protein primarily found in the liver, spleen, and bone marrow, where it sequesters iron in a non-toxic form, ensuring its availability for biological processes while protecting cells from oxidative damage. As a key marker of the body's iron status, serum ferritin levels are crucial for diagnosing various disorders, including anemia and inflammation. Levels below 12 ng/mL typically indicate iron deficiency, making it a valuable diagnostic tool. However, in pregnant women with BTM, serum ferritin levels are usually normal, even though iron levels, including ferritin, may resemble those seen in IDA. Despite normal ferritin levels, these women may still experience anemia due to impaired RBC production, a hallmark of BTM, which can result in more severe anemia than in a normal pregnancy. This often leads to misdiagnosis and unnecessary treatments, making serum ferritin an unreliable indicator for differentiating IDA from BTM.[57,58,59,60,61]

SERUM IRON:

During pregnancy, serum iron levels are influenced by conditions like IDA and beta-thalassemia. In IDA, declining serum iron levels result from depleted iron stores, which impair haemoglobin production and cause anemia, a common concern during pregnancy that often necessitates iron supplementation to maintain maternal and fetal health. Conversely, beta-thalassemia can lead to fluctuating serum iron levels, often associated with iron overload from increased absorption or frequent transfusions, though iron deficiency may also coexist. This complex interplay requires careful interpretation of iron profiles using markers such as ferritin and TSAT to ensure accurate diagnosis and management**.**[18,25,63,64]

TRANSFERRIN SATURATION (TSAT):

In pregnant women with IDA, TSAT levels are typically low, as it measures the proportion of transferrin bound to iron in the bloodstream. When iron stores are depleted, the body increases transferrin production to enhance iron transport, but due to insufficient iron availability, TSAT levels decrease. A TSAT value below 20% is a key indicator of iron deficiency and is commonly observed in IDA. As an essential component of iron studies, TSAT is often used alongside serum ferritin and iron levels to provide a comprehensive assessment of the body's iron status, aiding in the accurate diagnosis and management of iron-related disorders.[65,66,67]

TOTAL IRON BINDING CAPACITY (TIBC):

In pregnant women with IDA, TIBC levels typically rise as the body increases the production of transferrin, the iron-transporting protein, in response to low iron levels. Although there is insufficient iron available to bind to transferrin, the body

enhances its transport capacity to compensate for the deficiency. This elevated TIBC is a common marker of iron deficiency, as the body attempts to make more iron available for essential functions. Pregnancy itself also raises TIBC levels due to increased iron demands for both the mother and the developing fetus, but in IDA, this rise is more pronounced, reflecting a significant iron shortage. The normal range for TIBC in adults is generally 240–450 mcg/dL, but in pregnant women with BTM, TIBC levels usually remain normal. Studies indicate that TIBC and other iron-related markers in BTM are often similar to those seen in IDA, yet despite normal TIBC levels, anemia can still occur due to ineffective RBC production, a hallmark of BTM, which can lead to more severe anemia than what is typically expected during pregnancy.[68.69.70.71.72,73]

TABLE 1: LABORATORY FINDINGS TO DIFFERENTIATE IDA, BTT, ANEMIA OF CHRONIC DISEASE, AND SIDEROBLASTIC ANEMIA:[74,75]

PARAMETER	IDA	BTT	ANEMIA OF CHRONIC DISEASE (ACD)	SIDEROBLASTIC ANEMIA
MCV	Low	Low to normal	Low to normal	Low to high
MCH	Low	Low	Low to normal	Low
RDW	High	Normal	Normal or slightly high	High
SERUM FERRITIN	Low	Normal or high	Normal or high	High
SERUM IRON	Low	Normal or high	Low	High
TSAT	High	Normal or slightly high	Low	High
TIBC	High	Normal or high	Low	High
NESTROFT	Negative	Positive	Negative	Negative
HPLC	Normal	HbA2 > 3.5%	Normal	HbA2 Normal or Low
OSMOTIC FRAGILITY	Increased	Decreased	Normal	Variable

EPIDEMIOLOGICAL INSIGHTS INTO ANEMIA: DIFFERENTIATING IDA AND BTT:

Kaushal et al. conducted a cross-sectional study among 172 anemic pregnant women in Himachal Pradesh. They found that 50% had MHA with serum ferritin levels below 15 ng/mL. Mehrotra et al. analyzed 786 pregnant women in Andaman and Nicobar Islands, reporting an anemia prevalence of 50.9%. Among anemic cases, 86.7% exhibited microcytic hypochromic anemia. Shridevi et al. studied 600 pregnant women in Telangana, finding an anemia prevalence of 20%. Among anemic cases, 82.3% had microcytic hypochromic anemia.

A study by Al-Shammari et al. on 100 anaemic pregnant women found that 79% had IDA, confirmed by serum ferritin measurement, while 2% were diagnosed with Beta Thalassemia Trait (BTT) based on elevated haemoglobinA2 levels.[76]

Mangla D et al. reported a 70.27% (n-148) prevalence of anemia in their study population, with 68.92% (102 cases) identified as IDA and 1.35% (2 cases) as suspected BTT using hematological indices, where the MI classified 68.92% as IDA and 1.35% as BTT, while the RDWI identified 69.59% as IDA and 0.68% as BTT.[77]

The study conducted by Garg S et al. analyzed 255 patient samples, of which 114 cases (44.7%) had MHA, exhibiting symptoms of either BTT or IDA. The prevalence of BTT was found to be 13.3% based on Mentzer's Index and 12.9% based on RDW.[78]

The study by Kumar et al. included 350 patients, with 12.3% diagnosed as BTT (BTT) and 87.7% as IDA (IDA). MI (MI) had an accuracy of 89.6%, while RDW Index (RDWI) had the highest accuracy of 91.6%. HPLC confirmed BTT cases

with haemoglobinA2 > 3.5%, and NESTROFT served as a screening tool requiring confirmation with RBC indices or HPLC.[79]

Bhargava et al. examined 1353 cases of MHA, diagnosing 7.24% as BTT and 81.45% as IDA, with Ricerca Index achieving an overall accuracy of 88.42%, MI an accuracy of 88.08%, and RDWI the highest accuracy of 96.08%.[80]

Choudhary S et al. evaluated 830 samples, identifying 328 cases of MHA, of which 11% were diagnosed with BTT, with RDW detecting 58.3% and RDWI identifying 83.3% of these cases.[81]

Menakuru S et al. screened 800 pregnant women previously diagnosed with IDA using an iron study, revealing that 5.87% cases were actually BTT, identified through MI (<13) as an initial screening tool, followed by NESTROFT for further screening, and confirmed by HPLC.[82]

In a study by Yogalakshmi et al. Among 837 pregnant women screened, 74 cases (8.8%) were diagnosed as BTT. While increased RDW is common in IDA but less in BTT, the MI (<13) identified 78% of BTT cases, and haemoglobinA2 (>4%) measured via HPLC was the definitive diagnostic test, confirming 100% of cases.[83]

The retrospective study by Wahan et al.[84] on 40 microcytic anemia patients identified 19 cases (47.5%) as BTT using RBC indices, with the RDWI being the most effective (97.5% accuracy), followed by the MI (92.5% accuracy.[84]

Demir A et al. Investigated 63 children aged 2 to 16 years with microcytic anemia found that 58% were diagnosed with BTT, with RDWI correctly identifying 92% of cases, MI 76%, and RDW 59%.[85]

Mendiratta SL et al. assessed 1000 antenatal women, 7.9% were diagnosed with BTT, with NESTROFT correctly identifying 78.48% of BTT cases, MCV (<80 fL) detecting 73.42%, MCH (<27 pg) identifying 60.76%, and RBC count (>5 million/L) correctly diagnosing only 32.91% of cases.[86]

Gosavi M screened 441 pregnant women, 46.7% were anemic, 32.04% of anemic cases had MHA, and 9.2% were carriers of haemoglobinopathies, with 7.7% having BTT. The NESTROFT test showed an accuracy of 95.15% in detecting BTT, while HPLC remains the gold standard for confirming haemoglobinopathies in the present study.[87]

Singh V et al. conducted a study including 168 BTT cases, with 23.22% having coexisting IDA and 76.78% without IDA. RBC indices (MCV, MCH, MCHC) were significantly lower in BTT with IDA. The Mentzer index was 10.61 in BTT with IDA and 10.88 in BTT without IDA. HPLC, the gold standard, was used to confirm BTT by detecting elevated HbA2 (5.21 ± 0.82) levels.[88]

El-Shanshory et al. carried out a study on 2,118 relatives of β-thalassemia patients, finding that 35.84% had BTT, while IDA was diagnosed in 17.19%. Individuals with BTT had a higher RBC count of $5.28 \times 10^6/mm^3$ compared to $3.74 \times 10^6/mm^3$ in IDA, and a lower RDW of 15.30% compared to 16.89% in IDA. HPLC confirmed BTT based on HbA2 levels of 3.5% or higher, while serum ferritin levels were significantly lower in IDA, measuring 8.2 ng/ml in contrast to 68.73 ng/ml in BTT.[89]

Chakrabarti I et al., conducted research involving 500 antenatal mothers, 3.4% were diagnosed as BTT. NESTROFT detected 94.1% of BTT cases, with a specificity of 95.2%, PPV of 41.0%, and NPV of 99.8%. The test yielded 5.9% false negatives

and 4.8% false positives, including cases of Hb E trait, homozygous Hb E, and IDA. When considering both BTT and E-BTT, NESTROFT demonstrated an overall sensitivity of 95.0% and specificity of 95.8%. HPLC was used as the confirmatory test, showing HbA2 levels in BTT cases with a mean of 5.3%.[90]

Singh SP et al. conducted an analyses of 124 individuals revealing that 19.35% were normal, 70.16% with BTT, and 10.48% with iron-deficiency anemia**.** NESTROFT showed 97.7% sensitivity, 83.3% specificity, 95.5% PPV, 90.9% NPV, and 94.6% efficiency**.** False positives occurred in 16.7% of normal individuals and 23.08% of IDA cases**.** NESTROFT is a cost-effective, rapid screening tool**,** though HPLC remains the 100% gold standard for BTT detection, with HbA_2 > 3.5% confirming BTT**.**[91]

Sumera et al. performed a study involving 503 cases which revealed 50.3% had microcytosis. Among these, 69% were diagnosed with IDA, 29% with BTT, and 2% with other conditions. NESTROFT demonstrated a sensitivity of 93%, specificity of 88%, a PPV of 74%, and a NPV of 97%, with a false positivity rate of 13% in IDA cases. HPLC confirmed 100% of BTT cases based on HbA2 levels >3.5%, establishing HPLC as the gold standard for distinguishing BTT from IDA. [92]

Bain B et al. undertook a study on 696 antenatal patients with an MCV of less than 83 fL, revealing that 8.3% (58 cases) were diagnosed with BTT, Among these, the MI (MCV/RBC) correctly identified 34%, the England and Fraser Discriminant Function identified 19%, and the Srivastava Formula (MCH/RBC) identified only 5% of cases.[93]

MATERIALS AND METHODS

A cross-sectional, prospective study based in a hospital setting is being carried out, enrolling all eligible pregnant women who meet the inclusion criteria and visit the antenatal clinics at the Obstetrics Department of KLE's Dr. Prabhakar Kore Hospital Medical Research Centre. This study was conducted over a period of one year.

SAMPLE SIZE: 75 cases

Sample size for sensitivity:-

Where,

N is required sample

Sn is sensitivity

$n=z_{1\text{-}alpha/2}$ X Sn X (1-Sn) / L^2 X Prevalence

L= Absolute desired precision

$Z_{1\text{-}alpha/2}$ = initial value for given alpha

P=prevalence

Taking L=2 for alpha=0.001 or 1%, $Z_{1\text{-}alpha/2=2.58}$

p=52.2

Sensitivity= 64.2 = 0.642 (from previous study)

Substituting values in above equation;

$n=(2.58)^2$ X 64.2 X (100-64.2) / 2^2 X 52.2

n=15298.80/ 208.8= 73.26 (approx 75)

n=75 positive patients.

SAMPLING TECHNIQUE: Total of more than or equal to 75 Anemic patients with microcytic hypochromic blood picture in antenatal period shall be selected for the study using a non-probability sampling approach.

INCLUSION CRITERIA:

- All primigravida with singleton pregnancy between 18 years to 49 years of age.
- Haemoglobin less than 10gm/dl.
- Peripheral smear showing MHA.

EXCLUSION CRITERIA:

- Multigravida
- Haemoglobin more than 10gm/dl.
- Women transfused with blood in the past three months.

Information was recorded for all primigravida cases who attended the antenatal clinics at the Obstetrics Department, including their antenatal history, occupation, place of residence, familial occurrence of BTT, and history of prior transfusions.

Three milliliters of venous blood were drawn by a trained phlebotomist under strict aseptic precautions and collected in an ethylenediaminetetraacetic acid (EDTA) vacutainer. The samples were analyzed using the SYSMEX XN-3100 automatic cell counter, which operated based on fluorescence flow cytometry and hydrodynamic focusing, utilizing scatter and fluorescence signals to measure RBC count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hematocrit.

Peripheral blood smears were prepared, stained with Leishman's stain, and examined under a microscope at 100x magnification. The smears were evaluated for characteristic features of microcytic hypochromic anemia (MHA), along with

additional findings such as target cells and fragmented RBCs, which aided in morphological assessment. All cases of MHA that met the inclusion and exclusion criteria were included for further evaluation in the study.

With the use of automated analyzers such as the Cobas 6000, which relied on photometric and immunoassay principles, iron studies were conducted. These involved tests such as TSAT, TIBC, serum iron, and serum ferritin. These tests were used to categorize cases into IDA and non-IDA groups.

RBC indices such as RDW, RDWI, MI, and RICERCA were calculated from the complete blood picture report. The cases were grouped into IDA and BTT. All suspected cases of BTT based on RBC indices were further tested with NESTROFT and HPLC to confirm the diagnosis.

The NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) was performed for detecting hemoglobinopathies. It worked on the principle that red blood cells in individuals with hemoglobinopathies exhibited higher osmotic fragility when exposed to a hypotonic solution. The presence of a clear solution in the test tube signified a negative test, suggesting a normal result or the presence of IDA, whereas a turbid solution indicated a positive test, pointing towards hemoglobinopathy. NESTROFT proved to be an effective screening tool for distinguishing hemoglobinopathies from IDA in MHA cases.

Using ion-exchange chromatography, HPLC was conducted to differentiate IDA from BTT, facilitating the diagnosis of hemoglobinopathies and thalassemias. A rise in HbA2 and HbF levels confirmed all BTT cases.

STATISTICAL ANALYSIS:

SPSS version 27 was used to enter and perform the statistical analysis for the collected data. Cases were categorized into IDA and BTT groups, and the Chi-square test was conducted to assess the frequency between BTT and IDA. Sensitivity, specificity, positive predictive value, and negative predictive value of different indices along with the p-value were also calculated. A p-value of less than 0.05 was considered statistically significant.

FIGURE 2: Sysmex XN-Series Hematology Analyzer.

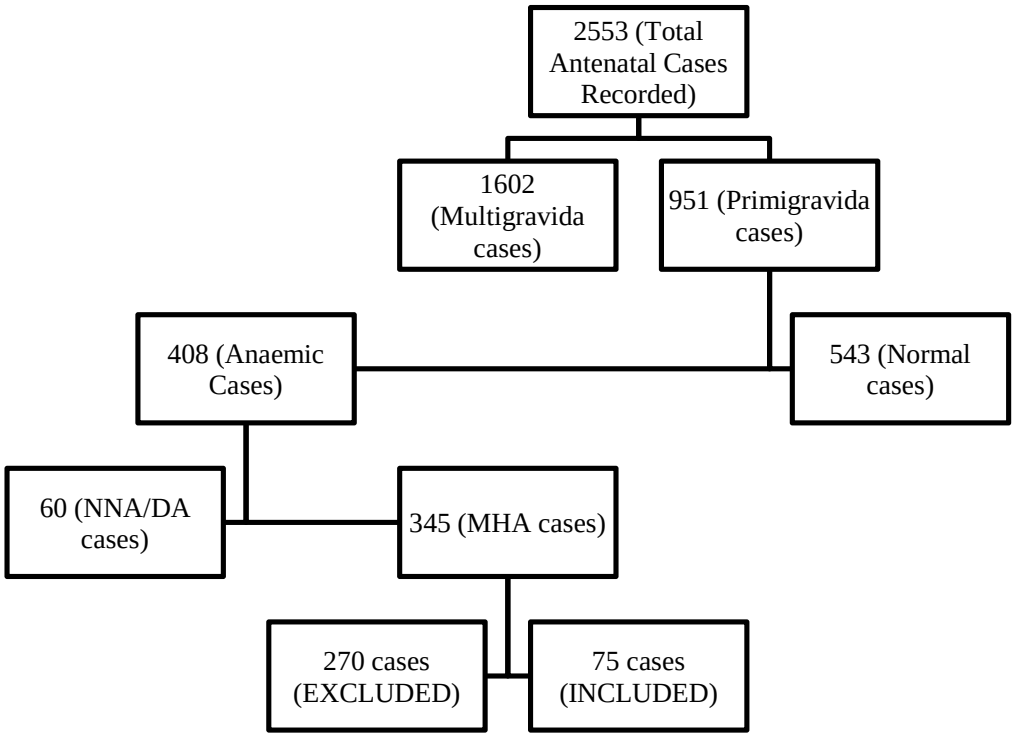
FIGURE 3: Cobas 6000 Analyzer Series

FIGURE 4: Bio-Rad D-10 HPLC System

RESULTS

This cross sectional study was done in KLE's Dr. Prabhakar Kore Hospital in primigravida for a period of 12 months with 75 anaemic patients having microcytic hypochromic blood picture were analyzed.

Out of the 2553 total antenatal cases recorded, 1602 cases (62.7%) were multigravida, while 951 cases (37.3%) were primigravida. Within the 951 primigravida cases, 543 (57.1%) were normal, whereas 408 (42.9%) were diagnosed with Anemia. Among the anaemic cases, 60 (14.7%) were classified as NNA/DA cases, while 345 (84.6%) were identified as MHA cases. Finally, within the MHA group, 270 cases (78.3%) were excluded, whereas 75 cases (21.7%) were included in the study.



FLOWCHART 3: SELECTION OF CASES

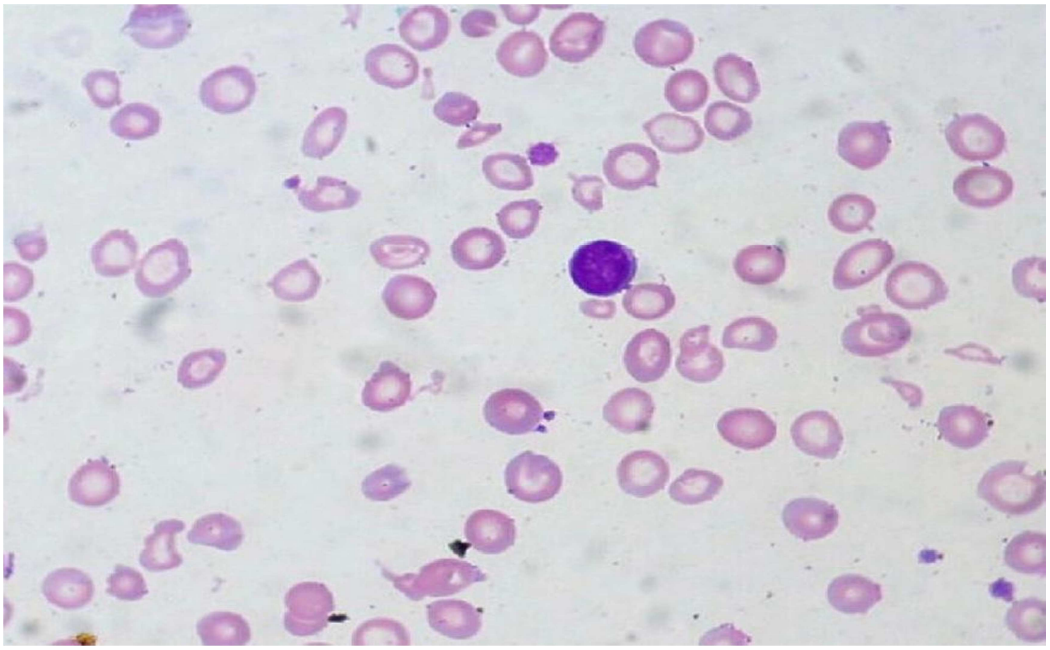
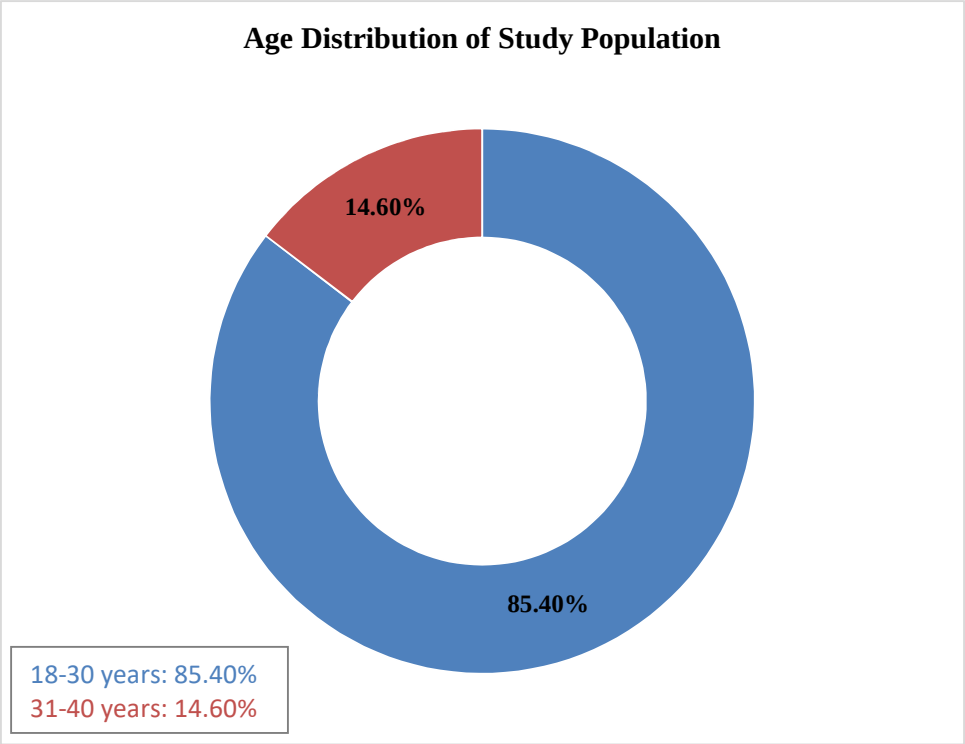


FIGURE 5: Photomicrograph showing Microcytic Hypochromic Anemia with Schistocytes (Leishman's stain; X 100)

TABLE 2: AGE DISTRIBUTION OF STUDY POPULATION:

Age Group	Number of Patients	Percentage
18-30 years	64	85.4%
31-40 years	11	14.6%

GRAPH 1: AGE DISTRIBUTION OF STUDY POPULATION:

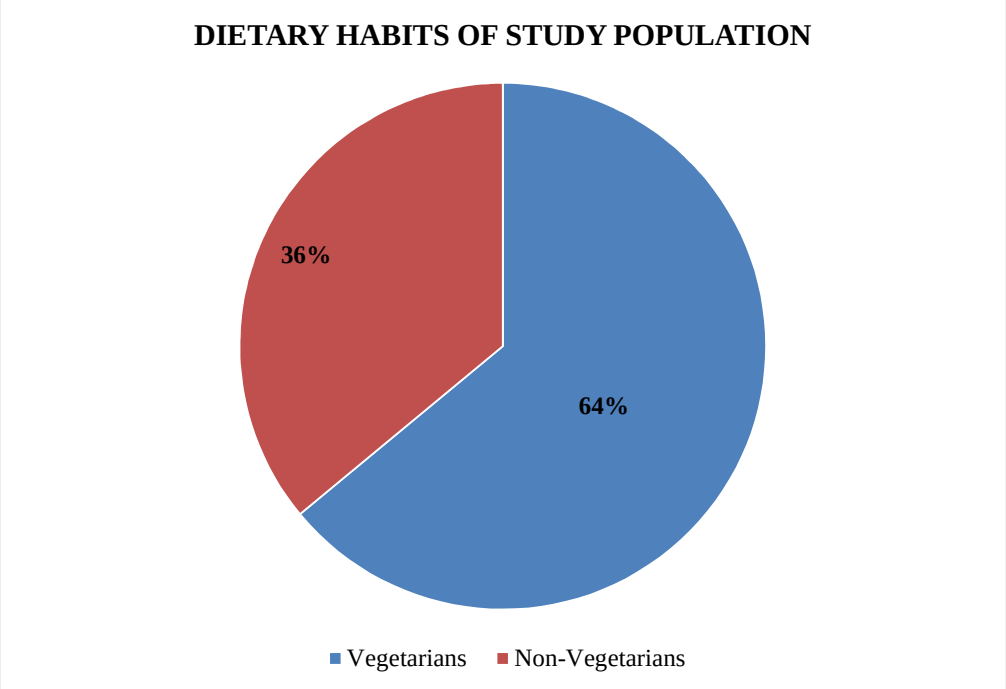


Majority of patients were between 18-30 years of age group (85.4%), whereas 14.6% were in the 4th decade.

TABLE 3: DIETARY HABITS AMONG STUDY POPULATION:

Dietary Habit	Number of Patients	Percentage
Vegetarians	48	64%
Non-Vegetarians	27	36%

GRAPH 2: DIETARY HABITS AMONG STUDY POPULATION:

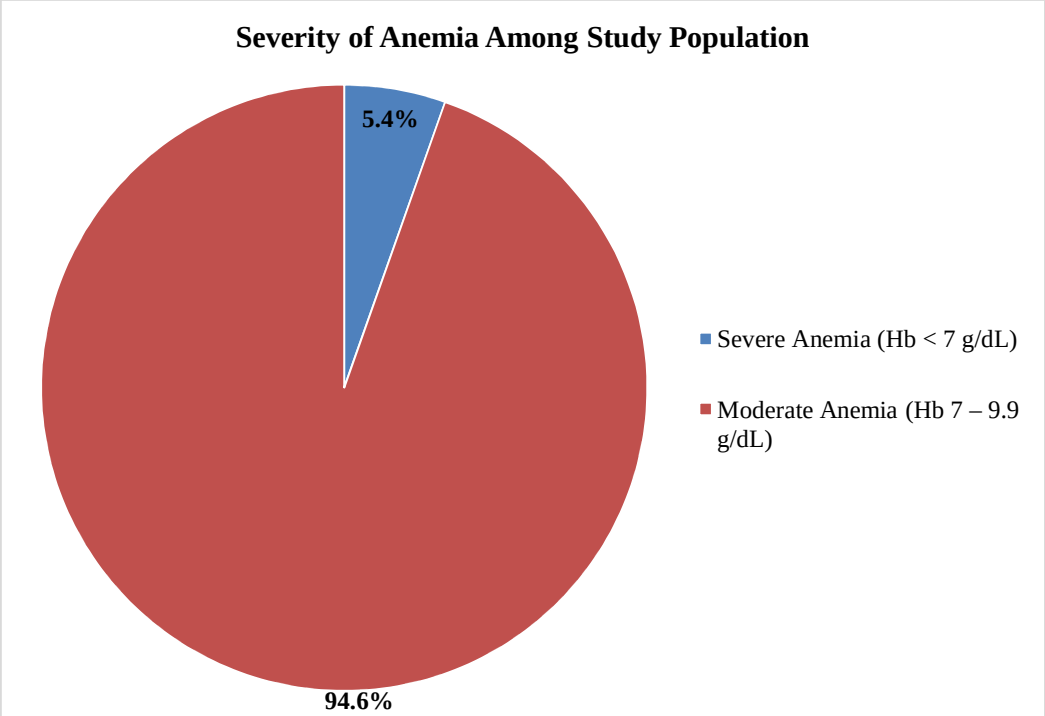


Majority of the patients were vegetarians (64%), rest of them were non vegetarians (36%).

TABLE 4: CLASSIFICATION OF ANEMIA SEVERITY AMONG STUDY PARTICIPANTS:

Anemia Severity	Number of Patients	Percentage
Severe Anemia (haemoglobin < 7 g/dL)	04	5.4%
Moderate Anemia (haemoglobin 7 – 9.9 g/dL)	71	94.6%

GRAPH 3: SEVERITY OF ANEMIA AMONG STUDY POPULATION:

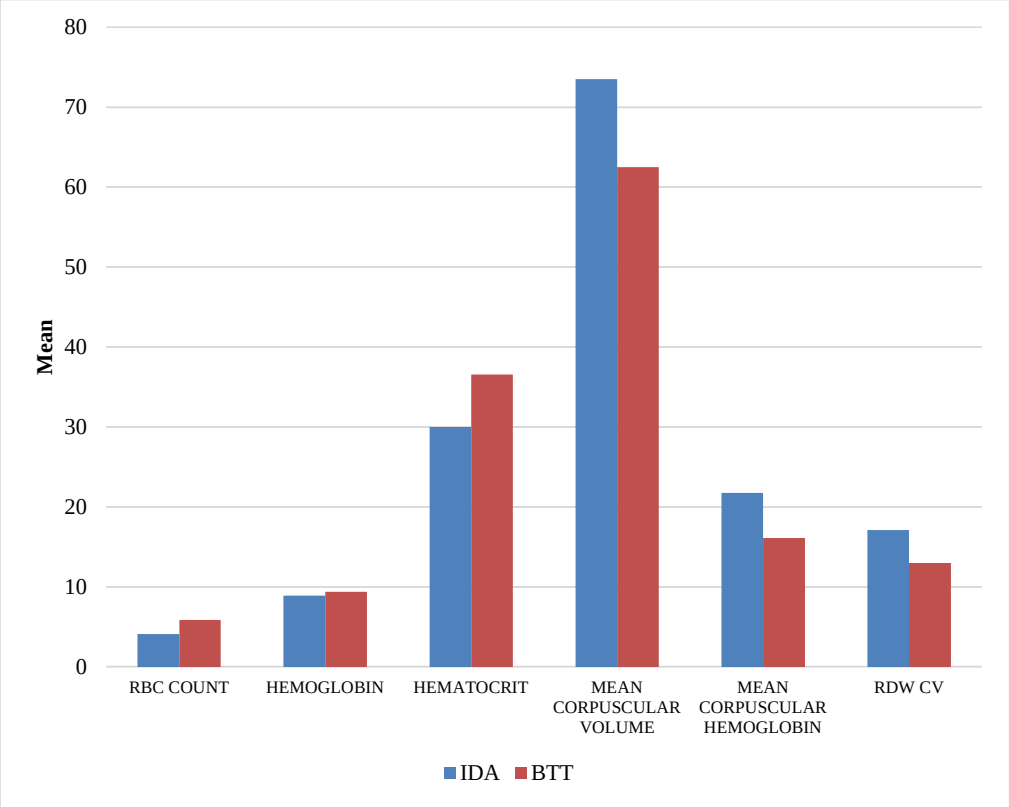


Based on WHO criteria for anemia in pregnant women. The study showed 94.6 % patients had moderate anemia, where as 5.4% patients had severe anemia.

TABLE 5: COMPARISON OF HEMATOLOGICAL PARAMETERS IN IDA AND NON-IDA (BTT) CASES:

Parameters	Diagnosis	Mean	Std. Deviation	t value	p value
RBC Count(millions/cumm) (4.2-5.4)	IDA	4.1	0.41	-4.97	0.12
	BTT	5.85	0.49		
haemoglobin(g/dl) (12.0-15.5)	IDA	8.88	0.93	-0.79	0.43
	BTT	9.4	0.28		
Hematocrit	IDA	30	2.78	-3.28	.002*
	BTT	36.58	3.51		
MCV (fl) (80-100)	IDA	73.48	5.08	3.04	.003*
	BTT	62.5	0.71		
Mean corpuscular haemoglobin(Pg) (27-32)	IDA	21.76	2.33	4.26	0.131
	BTT	16.1	1.84		
RDW (%) (14)	IDA	17.08	2.84	11.766	<0.001*
	BTT	13.0	0.14		

GRAPH 4: COMPARISON OF HEMATOLOGICAL PARAMETERS IN IDA AND BTT CASES:



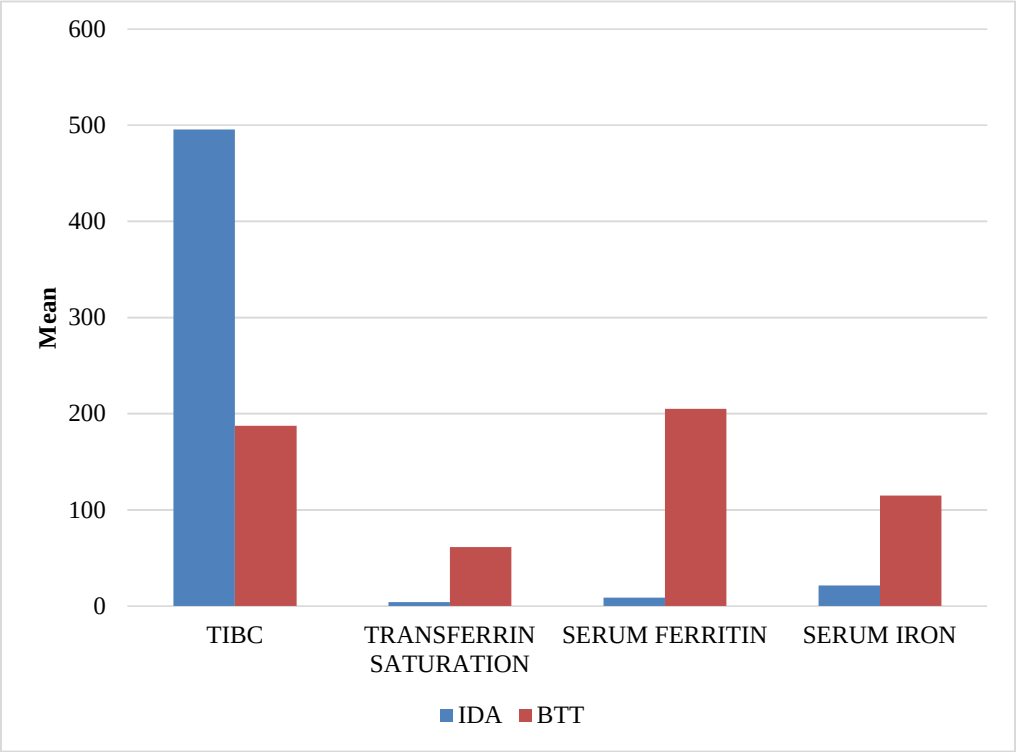
The RBC count 4.1 million/cumm and haemoglobin levels 8.88 g/dL are lower in IDA cases as compared to non-IDA cases which was 5.85 million/cumm and 9.4 g/dL, respectively, while MCV was 73.48 fl in IDA and 62.5 fl in non-IDA, MCH was 21.76pg in IDA and 16.1 pg in non-IDA, RDW was 17.08 % in IDA and 13.0% in non-IDA. Thus MCV, MCH and RDW was lower in non- IDA cases.

The p value of .002 for haematocrit, .003 for MCV and <0.001 for RDW are significant findings to differentiate IDA from BTT.

TABLE 6: COMPARISON OF IRON STUDIES IN IDA AND NON-IDA (BTT)CASES:

Parameters	Diagnosis	Mean	Std. Deviation	t value	p value
TIBC (µg/dL) (263–391)	IDA	495.85	56.54	43.59	<0.01*
	BTT	187.50	3.54		
TSAT (%) (16–50)	IDA	4.38	1.33	-40.39	<0.01*
	BTT	61.45	12.47		
Serum Ferritin (ng/ml) (15–200)	IDA	8.72	3.75	-21.13	<0.01*
	BTT	205.00	106.07		
Serum Iron (µg/dL) (56–168)	IDA	21.55	6.50	-18.84	<0.01*
	BTT	115.00	21.21		

GRAPH 5: COMPARISON OF IRON STUDIES IN IDA AND NON-IDA (BTT) CASES:



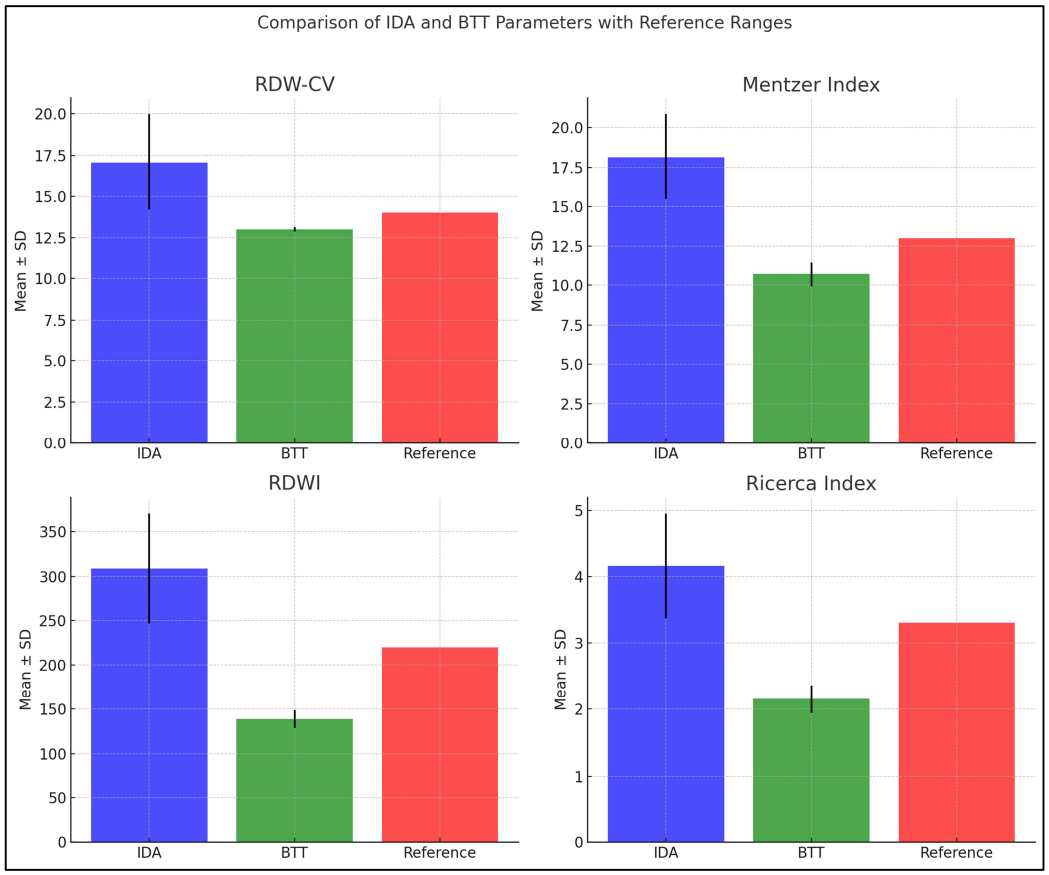
TIBC was 495.85 µg/dL which is higher in IDA cases compared to non-IDA cases being 187.5 µg/dL, while TSAT is 4.38% in IDA and 61.45% in non-IDA(BTT), serum ferritin 8.72 ng/mL in IDA and 205.0 ng/mLin non-IDA (BTT), and serum. iron 21.55 µg/dL in IDA and 115.0 µg/dL in non- IDA (BTT). Thus, TSAT, serum ferritin and serum iron is lower in IDA cases than the non-IDA cases (BTT).

The raised value of TIBC in IDA compared to BTT is significant. The IDA values of TSAT, serum ferritin and serum iron are comparatively very low than BTT. Hence the p value of TIBC value, TSAT, Serum ferritin and serum iron is less than 0.05%.

TABLE 7: COMPARISON OF VARIOUS INDICES IN IDA AND BTT CASES:

Parameter	IDA Mean ± SD 73 cases (n=75)	BTT (Mean ± SD) 2 cases (n=75)
RDW-CV (14)	17.08 ± 2.89	13.00 ± 0.14
MI (<13-BTT & >13-IDA)	18.15 ± 2.68	10.71 ± 0.78
RDWI (<220-BTT & >220-IDA)	308.98 ± 62.41	139.00 ± 9.90
Ricerca Index (<3.3-BTT & >3.3-IDA)	4.16 ± 0.79	2.15 ± 0.21

GRAPH 6: COMPARISON OF VARIOUS INDICES IN IDA AND BTT CASES:

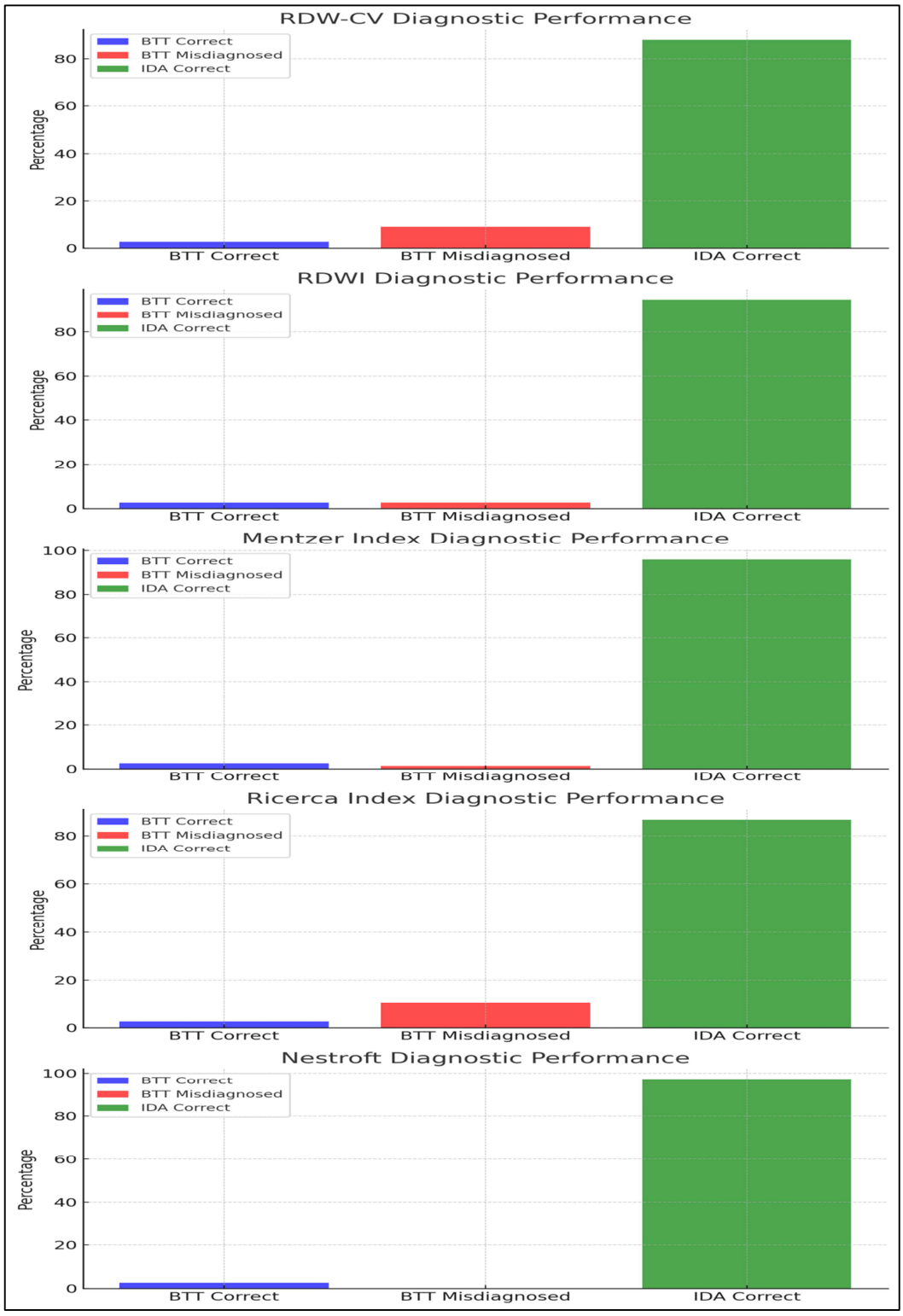


Various red cell indices differed significantly between IDA and BTT cases, with RDW-CV (17.08), MI (18.15), RDWI (308.98), and Ricerca Index (4.16) are higher in IDA cases compared to BTT, where RDW-CV is 13.00, MI is 10.71, RDWI is 139.00, and Ricerca Index is 2.15.

TABLE 8: DISTRIBUTION OF IDA AND BTT CASES USING DIFFERENT INDICES:

Method	Diagnosis	Correctly Diagnosed		Misdiagnosed		Total
		Number	Percentage	Number	Percentage	
RDW-CV	BTT	2	2.67%	7	9.33%	75
	IDA	66	88%	0	0%	
RDWI	BTT	2	2.67%	2	2.67%	75
	IDA	71	94.67%	0	0%	
MI	BTT	2	2.67%	1	1.33%	75
	IDA	72	96%	0	0%	
Ricerca Index	BTT	2	2.67%	8	10.67%	75
	IDA	65	86.67%	0	0%	
Nestroft	BTT	2	2.67%	0	0%	75
	IDA	73	97.34%	0	0%	

GRAPH 7: DISTRIBUTION OF IDA AND BTT CASES USING DIFFERENT INDICES:



The misdiagnosis made using Ricerca index was 10.67% followed by RDW-CV showing 9.33%, RDW-I in 2.67% and 1.33% in MI.

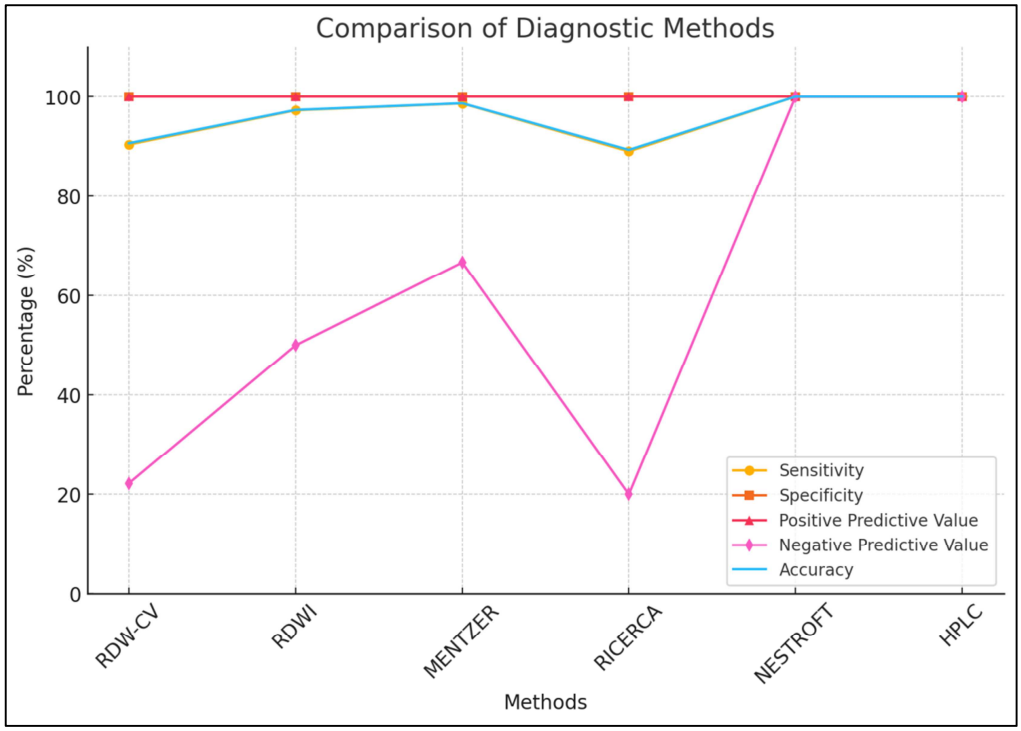
The misdiagnosis was seen mainly IDA cases. All the BTT cases were diagnosed correctly by RDW-CV, RDW-I, MI, Ricerca Index & NESTROFT.

NESTROFT has correctly diagnosed IDA cases, with 97.34% positivity, followed by the MI (96.0%), RDWI (94.67%), RDW (88.0%), and Ricerca Index (86.67%). Whereas Ricerca Index had the highest misdiagnosis rate (10.67%),followed by RDW-CV (9.33%) making it as the least reliable method.

Table 9: Comparison of sensitivity, specificity and accuracy of various Indices.

	Sensitivity	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**	**Accuracy**
RDW-CV	90.41%	100.00%	100.00%	22.22%	90.67%
RDWI	97.26%	100.00%	100.00%	50.00%	97.33%
MENTZER	98.63%	100.00%	100.00%	66.67%	98.67%
RICERCA	89.04%	100.00%	100.00%	20.00%	89.33%
NESTROFT	100.00%	100.00%	100.00%	100.00%	100.00%
HPLC	100.00%	100.00%	100.00%	100.00%	100.00%

Graph 8: Comparison of sensitivity, specificity and accuracy of various Indices.



The table compares different diagnostic tests for their effectiveness. The MI demonstrates high sensitivity (98.63%), specificity (100%), and accuracy (98.67%), followed closely by RDWI with 97.26% sensitivity, 100% specificity, and 97.33% accuracy. RDW-CV and RICERCA have slightly lower accuracy (~90%) and lower negative predictive values, meaning they may miss some true cases. HPLC (High-Performance Liquid Chromatography) is considered the gold standard for diagnosing haemoglobinopathies, offering 100% sensitivity, specificity, and accuracy. Notably, NESTROFT shows an almost identical performance to HPLC, making it a highly reliable and cost-effective screening tool. Given these findings, NESTROFT emerges as the best screening parameter, followed by the MI and RDWI for identifying potential cases effectively.

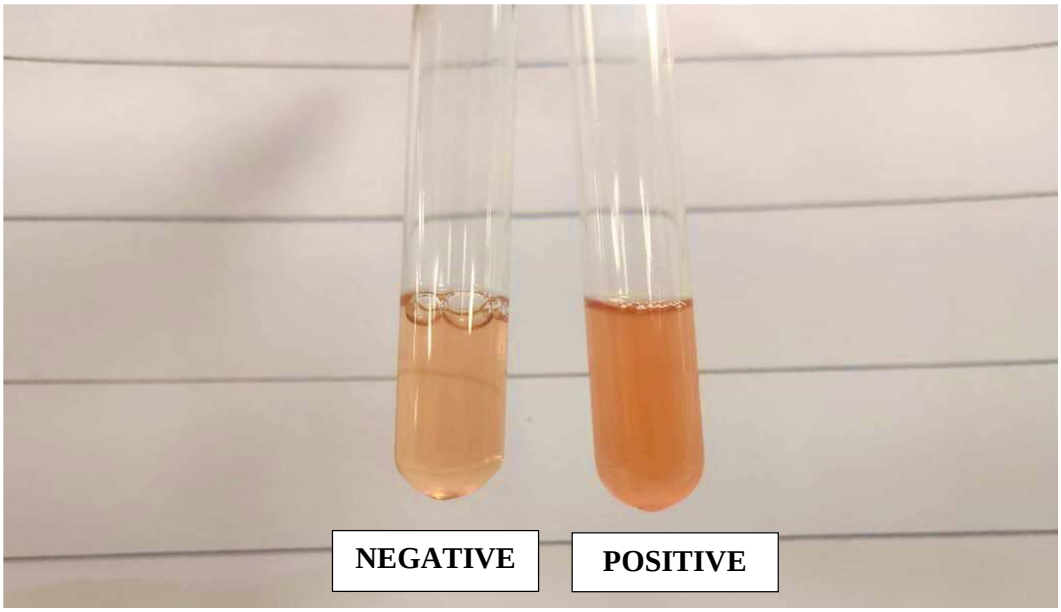
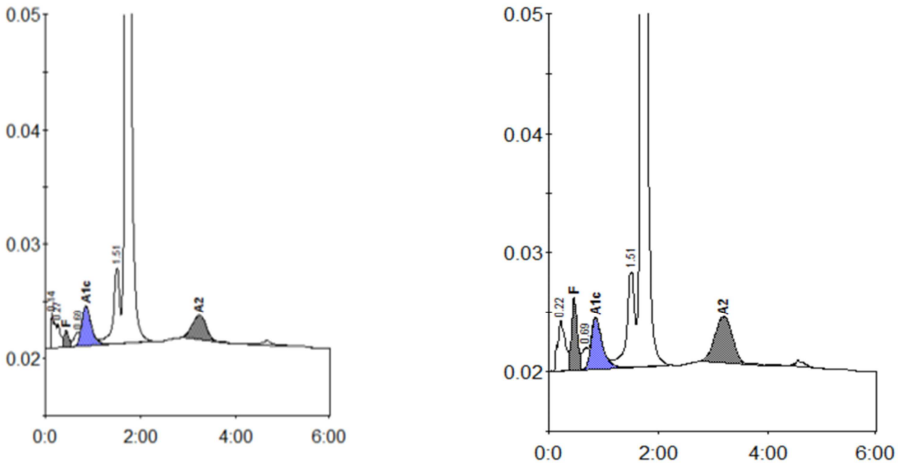


FIGURE 6: Left tube: Clear solution – Negative test, likely normal or iron deficiency anemia, Right tube: Turbid solution – Positive test, suggests beta-thalassemia trait.



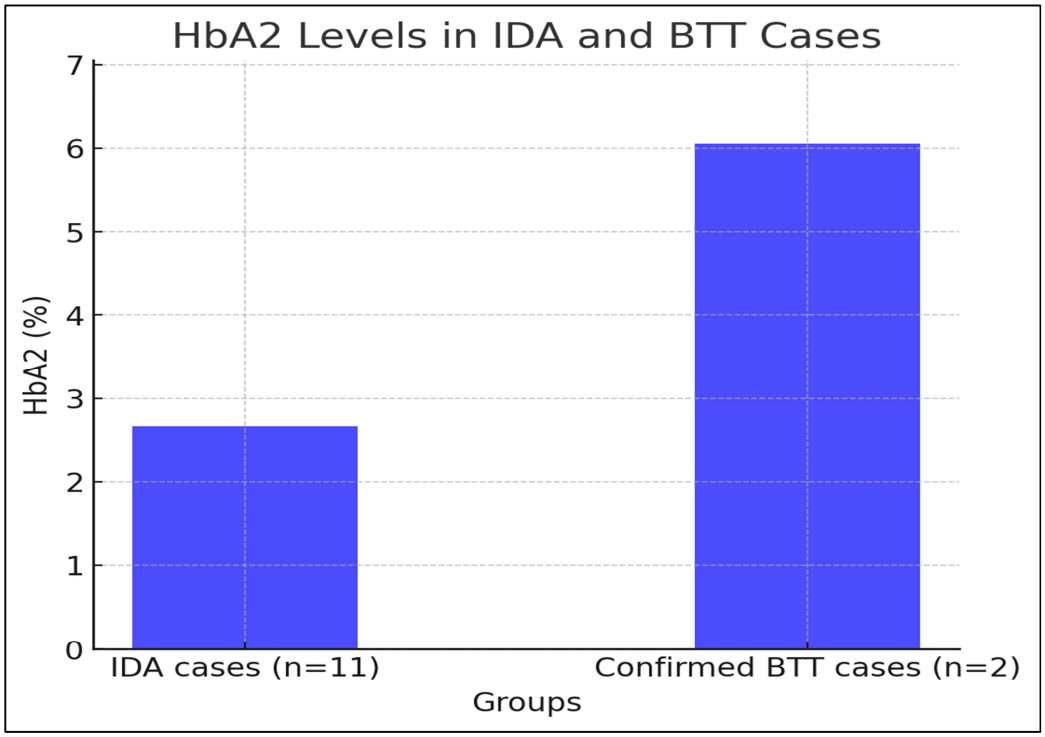
1. IRON DEFICIENCY ANEMIA | **2. BETA THALASSEMIA TRAIT**

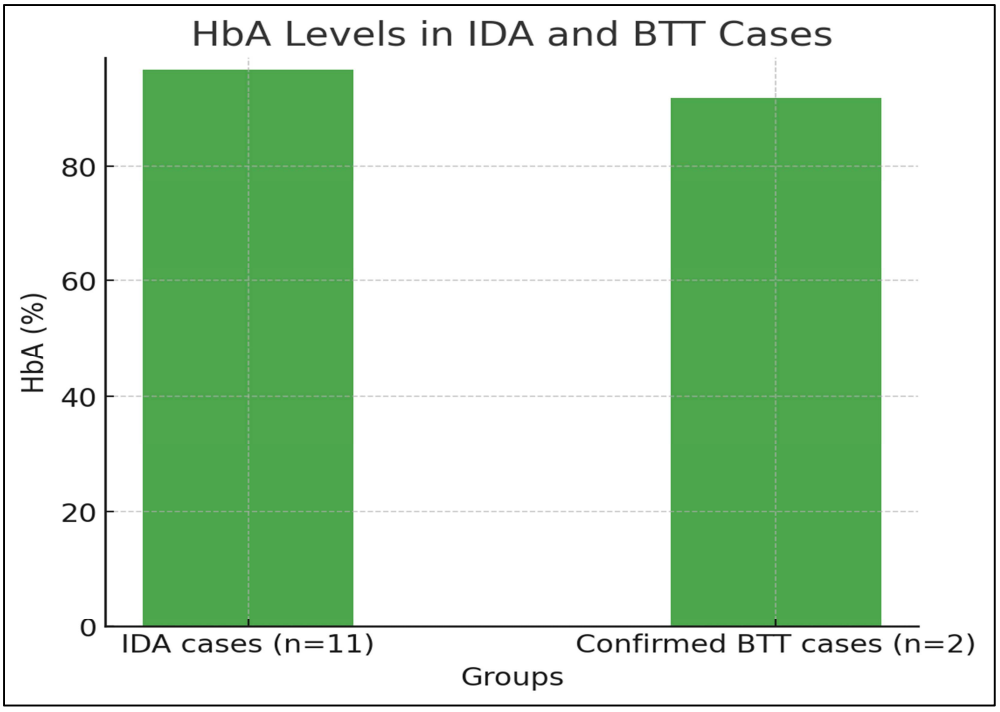
FIGURE 7: HPLC report 1 shows normal HbA2 (Iron Deficiency Anemia), HPLC report 2 shows increased HbA2 and HbF (Beta Thalassemia Trait)

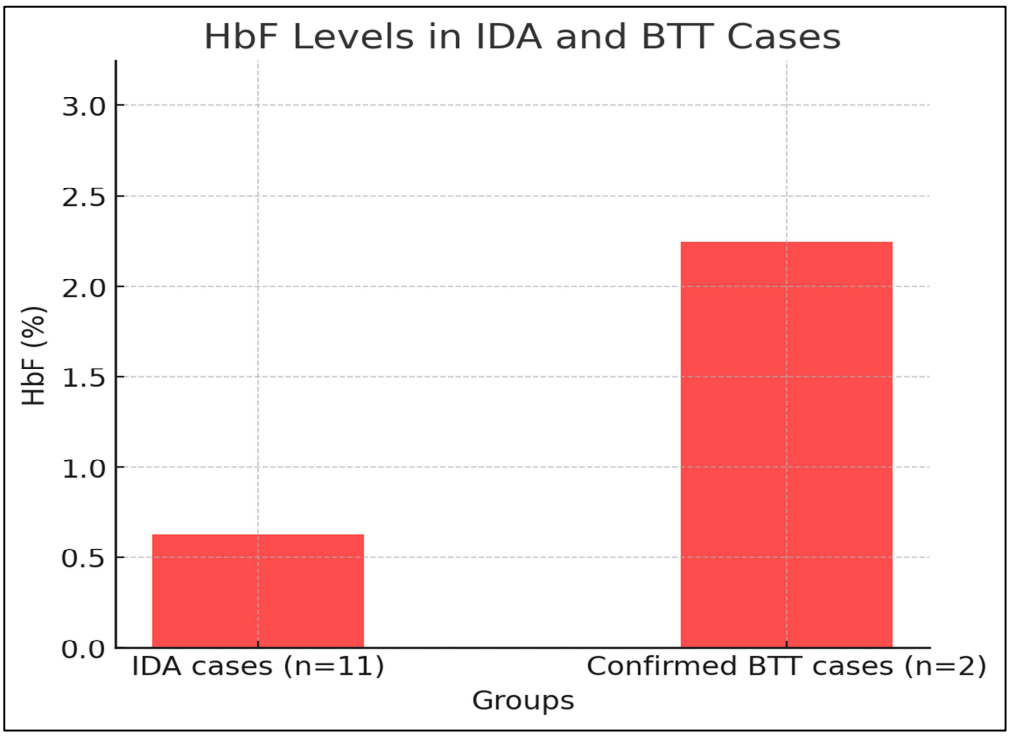
TABLE 10: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FINDINGS IN IDA AND CONFIRMED BTT CASES:

HPLC FINDINGS (Reference range)	haemoglobinA (94.3-98.5)	haemoglobinA2 (1.5-3.7)	haemoglobinF (0-2.0)
IDA cases: 11cases (n=75)	96.7 ± 0.5	2.67 ± 0.38	0.63 ± 0.21
Confirmed BTT cases: 2 caes (n=75)	91.7 ± 0.57	6.05 ± 0.21	2.25 ± 0.35

GRAPH 9: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FINDINGS IN IDA AND CONFIRMED BTT CASES:





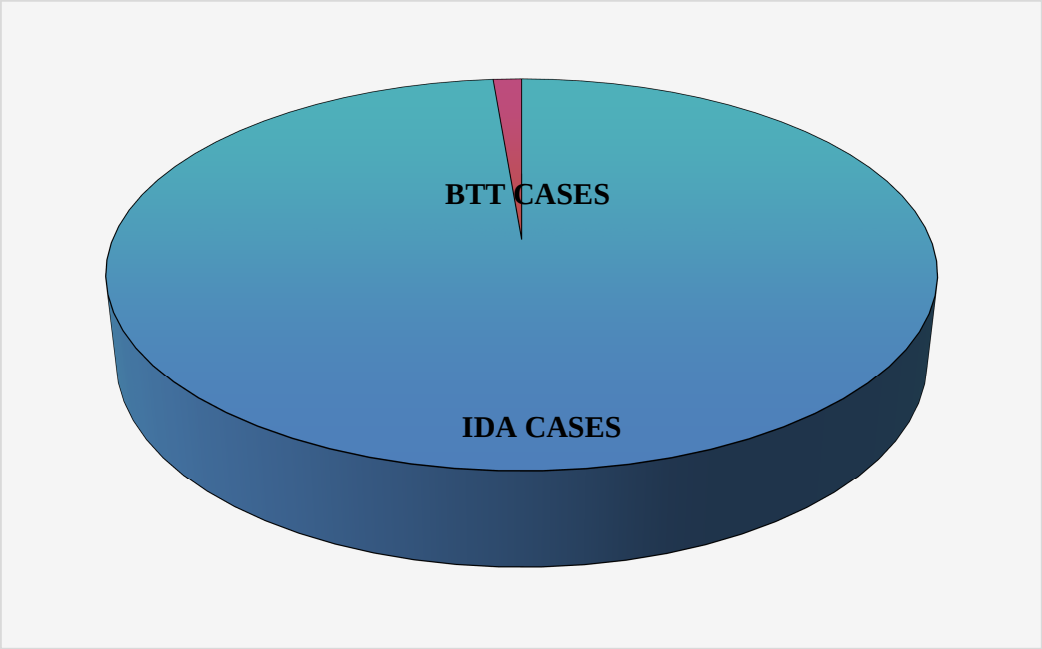


HPLC analysis confirmed that BTT (BTT) cases had elevated haemoglobinA2 6.05 ± 0.21%, and haemoglobinF 2.25 ± 0.35 % whereas IDA cases had haemoglobin A, haemoglobin A2 and haemoglobin F with in normal range.

TABLE 11: DISTRIBUTION OF IDA AND BTT CASES:

Category	Number of Patients	Percentage
IDA	73	97.4%
BTT	02	2.6%

GRAPH 10: DISTRIBUTION OF IDA AND BTT CASES:



The study included 97.4% with IDA and 2.6% with BTT cases.

After analysing the Hematological parameters, Indices, Iron studies, MI, Ricerca, RDW-I, RDW-CV, Nestroft and HPLC. It was found that majority of the cases were IDA (97.4%) Category and BTT were (2.6%).

DISCUSSION

TABLE 12:- COMPARISON OF AGE:

Study	Age Group 18-30 (%)	Age Group 31-40 (%)
Al-Shammari et al.[76] (n=100)	61%	39%
Present Study (n=75)	85.4%	14.6%

The present study had more younger participants (85.4%) than Al-Shammari et al.[76] study (61%), indicating demographic and sociocultural different practices.

TABLE 13:- COMPARISON OF ANEMIA SEVERITY (WHO CRITERIA) IN PERCENTAGE:

Authors	Mild Anemia (%) (haemoglobin 10-10.9 g/dL)	Moderate Anemia (%) (haemoglobin 7-9.9 g/dL)	Severe Anemia (%) (haemoglobin <7 g/dL)
Mangla D et al.[77] (n=148)	30%	50%	20%
Garg S et al.[78](n=255)	28%	52%	20%
Kumar et al.[79] (n=350)	32%	55%	13%
Bhargava et al.[80](n=1353)	29%	53%	18%
Choudhary S et al.[81] (n=328)	27%	51%	22%
Menakuru et al.[82] (n=800)	26%	50%	24%
Yogalakshmi E et al.[83] (n=837)	31%	54%	15%
Wahan et al.[84] (n=40)	33%	56%	11%
Present Study (n=75)	Not Reported	94.6%	5.4%

Our findings show that 94.6% of cases had moderate anemia, while 5.4% had severe anemia. No cases of mild anemia were found in our study.

Majority of the study show moderate type of Anemia followed by mild Anemia. Severe Anemia was rarely seen, but in our study moderate Anemia was common followed by severe Anemia. Mild Anemia cases were not seen in our study probably because of good antenatal care. In conclusion, all the studies show that moderate anemia is much more common.

TABLE 14:- COMPARATIVE STUDY OF HEMATOLOGICAL PARAMETERS:

Study	haemoglobin (g/dL) (Mean ± SD)		RBC (millions/cmm) (Mean ± SD)		PCV (%) (Mean ± SD)		MCV (fl) (Mean ± SD)		MCH (pg) (Mean ± SD)	
	IDA	**BTT**	**IDA**	**BTT**	**IDA**	**BTT**	**IDA**	**BTT**	**IDA**	**BTT**
Demir A et al.[85] (n=63)	9.47 ± 0.74	**10.88 ± 0.82**	4.47 ± 0.36	**5.4 ± 0.41**	NA	NA	61.38 ± 5.94	**61.66 ± 3.98**	19.95 ± 2.40	20.54 ± 2.03
Mendiratta SL et al.[86] (n=1000)	11.5 ± 1.68	11 ± 1.80	4.04 ± 0.57	4.60 ± 0.72	34.79 ± NA	35.25 ± NA	88.61 ± 8.21	75.94 ± 11.39	28.77 ± 3.39	24.83 ± 5.07
Kumar A et al.[79](n=350)	9.33 ± 1.77	10.89 ± 1.58	3.99 ± 0.65	5.22 ± 0.72	27.68 ± 4.78	32.68 ± 4.83	69.55 ± 6.51	62.64 ± 4.56	23.53 ± 3.66	20.89 ± 1.73
Gosavi M et al. [87](n=75)	9.36 ± 1.23	9.50 ± 1.09	3.75 ± 0.58	4.03 ± 0.69	27.37 ± 3.34	28.23 ± 3.32	74.11 ± 8.58	71.47 ± 10.78	25.46 ± 3.77	24.02 ± 4.67
Bhargava M et al.[80] (n=206)	9.60 ± 2.0	12.10 ± 0.40	4.10 ± 0.60	5.10 ± 0.60	NA	NA	70.30 ± 7.00	65.00 ± 4.60	26.90 ± 3.40	24.20 ± 3.40
Present Study (n=75)	8.88 ± 0.93	9.40 ± 0.28	4.10 ± 0.41	5.85 ± 0.49	30.00 ± 2.78	36.58 ± 3.51	73.48 ± 5.08	62.5 ± 0.71	21.76 ± 2.33	16.1 ± 1.84

In our study, we compared the haematological parameters from patients with IDA (IDA) and BTT (BTT) with those reported in earlier research by Demir A et al.[85], Mendiratta SL et al.[86], Kumar A et al.[79], Gosavi M et al.[87], and Bhargava M et al.[80] The haemoglobin level in IDA group was lower than BTT levels in all the studies including the present study except Mendiratta SL et al.[86] The raised haemoglobin levels in IDA compared to lower levels in BTT is because , more than 60% cases in this study included haemoglobin values of more than 10gm/dl which

included normocytic and microcytic anemia cases. Hence, in most of the studies in primigravida with MHA, the haemoglobin levels in IDA are lower compared to BTT.

The RBC counts and packed cell volume values are lower in IDA compared to BTT which correlates with the haemoglobin values. Hence, the findings of all the other studies correlates with the RBC count of our study.

The MCV and MCH in IDA group was higher than BTT levels in all the studies including the present study except Demir A et al. because this study group exhibited a wide range of MCV values, from 47 to 70 in IDA and 55 to 70 in BTT. Consequently, the mean and standard deviation were slightly lower in IDA than in BTT compared to other studies.

TABLE 15:- COMPARISON OF IRON STUDIES (IDA VS BTT):

Study	TIBC (µg/dL)		TSAT (%)		Serum Ferritin (ng/ml)		Serum Iron (µg/dL)	
	IDA	**BTT**	**IDA**	**BTT**	**IDA**	**BTT**	**IDA**	**BTT**
Singh V et al.[88] (n=168)	426.46 ± 58.01	336.55 ± 33.91	NA	NA	8.85 ± 2.89	107.01 ± 158.84	43.18 ± 19.99	91.91 ± 33.91
El- Shanshory et al.[89](n=2118)	NA	NA	NA	NA	8.20 ± 3.78	68.73 ± 49.33	NA	NA
Demir A et al.[85] (n=63)	431.9 ± 72.2	334.6 ± 49.6	4.52 ± 2.33	26.58 ± 1.10	4.36 ± 2.11	36.08 ± 7.19	20.85 ± 8.39	89.97 ± 38.7
Present study (n=75)	495.85 ± 56.54	187.5 ± 3.54	4.38 ± 1.33	61.45 ± 12.47	8.72 ± 3.75	205.0 ± 106.07	21.55 ± 6.5	115.0 ± 21.21

This study compares iron studies between IDA (IDA) and beta-thalassemia trait (BTT) and evaluate the differences from previous research. Total iron-binding capacity (TIBC) was significantly higher in IDA compared to BTT. This finding correlates with the other studies, such as Singh V et al.[88] and Demir A et al.[85]

In IDA, TIBC is higher because the body has less iron, so the transferrin used up is less compared to the free transferrin in circulation. So increase in the free transferrin, increases the TIBC. In BTT, which is a genetic condition and not caused by iron deficiency, the free transferrin is normal in circulation, thus keeping the TIBC in normal or slightly higher range.

TSAT, which indicates the proportion of iron bound to transferrin, was significantly lower in IDA compared to BTT . This is consistent with Demir A et

al.'s[85] findings in IDA and in BTT, indicating reduced iron availability in IDA, while BTT maintains better iron saturation.

Serum ferritin, an important marker of iron storage, was considerably lower in IDA compared to BTT. This trend is also observed in Singh V et al.[88] and Demir A et al.[85] The consistently lower ferritin levels in IDA confirm significant iron depletion, whereas higher ferritin levels in BTT indicate sufficient iron stores but altered RBC production.

Serum iron levels were also significantly lower in IDA compared to BTT , consistent with findings from Singh V et al.[88] in IDA and Demir A et al.[85] The reduced serum iron in IDA reflects the body's inability to maintain adequate iron levels, whereas in BTT, iron metabolism is not significantly impaired.

Overall while comparing, this study correlates with the other studies confirming that iron parameters serve as key differentiators between IDA and BTT. The significantly higher TIBC and lower TSAT, serum ferritin, and serum iron in IDA compared to BTT, emphasize the characteristic iron deficiency seen in IDA, while BTT maintains relatively stable iron levels.These differences reinforce the importance of iron studies in accurately distinguishing these conditions.

TABLE 16:- COMPARISON OF RBC INDICES IN IDA AND BTT

Author	RDW-CV (Mean ± SD)		RDWI (Mean ± SD)		MI (Mean ± SD)		Ricerca Index (Mean ± SD)	
	IDA	BTT	IDA	BTT	IDA	BTT	IDA	BTT
Demir A et al.[85] (n=63)	18.2 ± 2.5	14.5 ± 1.9	242 ± 45	204 ± 30	14.5 ± 2.2	11.2 ± 1.5	4.5 ± 1.0	3.2 ± 0.8
Mendiratta SL et al. [86](n=1000)	17.8 ± 2.9	13.9 ± 2.0	250 ± 50	198 ± 28	15.1 ± 2.5	10.9 ± 1.4	4.8 ± 1.1	3.0 ± 0.7
Al-Shammari et al.[76] (n=100)	19.0 ± 3.0	14.0 ± 1.8	235 ± 42	202 ± 27	14.8 ± 2.4	11.0 ± 1.3	4.6 ± 1.0	3.1 ± 0.6
Gosavi M et al.[87] (n=206)	18.5 ± 2.8	14.2 ± 1.7	245 ± 48	200 ± 29	14.9 ± 2.3	11.1 ± 1.5	4.7 ± 1.2	3.2 ± 0.8
Bhargava M et al. [80](n=1353)	17.9 ± 2.7	13.8 ± 1.9	248 ± 44	199 ± 31	15.0 ± 2.6	10.8 ± 1.4	4.9 ± 1.1	3.0 ± 0.7
Mangla D et al.[77] (n=148)	18.3 ± 2.6	14.1 ± 1.8	240 ± 46	201 ± 29	14.7 ± 2.2	11.0 ± 1.3	4.6 ± 1.0	3.1 ± 0.8
Garg S et al.[78] (n=255)	19.1 ± 2.5	14.3 ± 1.9	238 ± 43	198 ± 30	15.2 ± 2.3	11.2 ± 1.5	4.8 ± 1.1	3.2 ± 0.7
Choudhary S et al.[81] (n=75)	18.6 ± 2.4	14.0 ± 1.8	243 ± 47	203 ± 28	14.8 ± 2.5	10.9 ± 1.4	4.7 ± 1.0	3.0 ± 0.8
Present Study (n=75)	17.08 ± 2.89	13.00 ± 0.14	308.98 ± 62.41	139.00 ± 9.90	18.15 ± 2.68	10.71 ± 0.78	4.16 ± 0.79	2.15 ± 0.21

This study compares RBC indices in IDA (IDA) and beta-thalassemia trait (BTT) with findings from other studies.

RDW (RDW-CV) values were low in both IDA and BTT compared to earlier studies, suggesting a lower degree of variation in RBC size (anisocytosis) in the current study population.

RDW index (RDWI) values were high in IDA and low in BTT compared to previous research. The lower RDWI value for BTT in this study makes it easier to distinguish between IDA and BTT.

The MI, a commonly used tool for differentiating IDA from BTT, was found to be higher in IDA in this study than in earlier research. This suggests that the MI more effectively distinguishes IDA from BTT in the current population, making it a useful diagnostic tool.

The Ricerca Index, another marker for differentiating IDA from BTT . The ricerca index values were more than 3.3% in IDA and less than 3.3 in BTT. Hence the sensitivity is 89.04%.

Overall, these findings highlight important differences in RBC indices that may improve the accuracy of distinguishing and diagnosing IDA from BTT.

TABLE 17:- COMPARISON OF HPLC VALUES FROM DIFFERENT STUDIES:

Author	haemoglobinA (%)		haemoglobinA2 (%)		haemoglobinF (%)	
	IDA	BTT	IDA	BTT	IDA	BTT
El-Shanshory et al.[89] (n=2118)	NA	NA	2.54 ± 0.49	4.50 ± 0.59	NA	NA
Yogalakshmi E et al.[83] (n=837)	NA	NA	NA	5.5 ± 0.6	NA	NA
Mendiratta SL et al.[86] (n=1000)	NA	NA	2.74 ± NA	5.54 ± NA	NA	NA
Singh SP et al. [91](n=168)	NA	81.89 ± 2.25	NA	5.21 ± 0.82	NA	1.31 ± 1.06
Present Study (n=75)	96.7 ± 0.5	91.7 ± 0.57	2.67 ± 0.38	6.05 ± 0.21	0.63 ± 0.21	2.25 ± 0.35

This study compares haemoglobin (haemoglobin) levels in IDA (IDA) and beta-thalassemia trait (BTT) using high-performance liquid chromatography (HPLC) and examines differences from previous studies.

HaemoglobinA2 levels, which are crucial for diagnosing BTT, which were higher in BTT than in IDA. This finding is consistent with other studies, where an increase in haemoglobinA2 is a well-recognized characteristic of BTT, helping to distinguish it from IDA.

Fetal haemoglobin (haemoglobin F) was also found at a higher level in BTT compared to IDA. This study correlates with other studies. The variation in these

hemoglobin components plays an important role in differentiating IDA from BTT, as their pattern is distinctly different.

Overall, the findings of this study correlates with other studies, reinforcing the role of HPLC in accurately identifying and differentiating IDA from BTT. The clear differences in different types of hemoglobins and their levels observed in this study, further support the use of these parameters in routine diagnosis.

TABLE 18: COMPARISON OF HEMATOLOGICAL INDICES:

Author	RDW-CV		
	Sensitivity	**Specificity**	**Accuracy**
Kumar A et al.[79] (n=350)	55.6	83.7	39.3
Demir A et al.[85] (n=63)	96.0	32.0	30.0
Garg S et al. [78](n=255)	81.8	95.7	93.8
Choudhary S et al.[81] (n=830)	78.0	58.3	NA
Present Study (n=75)	90.41	100.0	90.67

Author	RDWI		
	Sensitivity	**Specificity**	**Accuracy**
Waha A et al.[84] (n=40)	95.0	100.0	97.5
Kumar A et al.[79] (n=350)	96.6	67.4	91.6
Demir A et al.[85] (n=63)	80.0	100.0	80.0
Mangla D et al.[77] (n=148)	98.07	66.3	NA
Bhargava M et al.[80] (n=1353)	70.41	98.37	96.08
Choudhary S et al.[81] (n=830)	85.0	83.3	NA
Present Study (n=75)	97.26	100.0	97.33

Author	Mentzer		
	Sensitivity	**Specificity**	**Accuracy**
Waha A et al. [84](n=40)	89.5	95.2	92.5
Kumar A et al.[79] (n=350)	92.3	76.7	89.6
Demir A et al.[85] (n=63)	62.0	86.0	48.0
Mangla D et al.[77] (n=148)	98.07	66.3	NA
Bhargava M et al.[80] (n=1353)	80.61	88.75	88.08
Garg S et al.[78] (n=255)	73.5	94.8	91.9
Present Study (n=75)	98.63	100.0	98.67

Author	Ricerca		
	Sensitivity	**Specificity**	**Accuracy**
Kumar A et al.[79] (n=350)	94.2	65.1	89.2
Bhargava M et al.[80] (n=1353)	80.61	88.75	88.08
Present Study (n=75)	89.04	100.0	89.33

Author	NESTROFT		
	Sensitivity	**Specificity**	**Accuracy**
Gosavi M et al.[87] (n=441)	84.21	96.25	NA
Mendiratta SL et al.[86] (n=1000)	78.48	94.14	NA
Chakrabarti I et al.[90] (n=500)	95.0	95.8	NA
Singh SP et al.[91] (n=124)	97.7	83.3	94.6
Sumera A et al.[92] (n=503)	93.0	88.0	94.6
Present Study (n=75)	100.0	100.0	100.0

The above tables explains about the hematological indices such as RDW-CV, RDWI, MI, Ricerca index, and NESTROFT test were evaluated for their sensitivity, specificity, and accuracy.

For RDW-CV, our study demonstrated a sensitivity of 90.41%, specificity of 100%, and accuracy of 90.67%, surpassing studies like Kumar A et al.[79] (55.6%

sensitivity) and Demir A et al.[85] (96% sensitivity but only 32% specificity). The significantly higher accuracy in our study highlights its diagnostic reliability.

RDWI in our study showed a high sensitivity (97.26%) and 100% specificity, with an accuracy of 97.33%. These results closely align with the Waha A et al.[84] study (97.5% accuracy) while outperforming studies such as Demir A et al.[85] (80% sensitivity) and Bhargava M et al. [80](96.08% accuracy).

For the MI, our study achieved the highest sensitivity (98.63%) and 100% specificity, with an accuracy of 98.67%, surpassing Waha A et al.[84] (92.5% accuracy) and Garg S et al.[78] (91.9% accuracy).

Regarding the Ricerca index, our study recorded 89.04% sensitivity, 100% specificity, and 89.33% accuracy, exceeding findings from Bhargava M et al.[80] (88.08% accuracy) and Kumar A et al.[79] (89.2% accuracy).

Finally, the NESTROFT test in our study achieved 100% sensitivity, specificity, and accuracy, outperforming all previous studies, including Singh SP et al.[91] (94.6% accuracy) and Sumera A et al.[92] (94.6% accuracy).

These findings highlight the NESTROFT test as the most reliable screening tool, followed closely by the MI. When comparing all the indices, NESTROFT demonstrated the highest diagnostic performance, with the MI being slightly less effective but still highly reliable.

TABLE 19:- COMPARISON OF IDA, BTT, AND OTHER CAUSES OF ANEMIA:

Author(s)	IDA (IDA) (%)	BTT (BTT) (%)	Other Causes of Anemia
Bain B et al.[93] (n=1000)	NA	8	NA
Yogalakshmi E et al.[83] (n=837)	NA	8.8%	NA
Bhargava M et al. [80](n=1353)	81.4%	7.2%	11.481.4%
Menakuru S et al.[82] (n=800)	94.1%	5.9%	NA
Al-Shammari et al. [76](n=100)	79%	2%	18
Present study (n=75)	97.3%	2.6%	NA

All the above mentioned studies had IDA which was the commonest cause for MHA followed by BTT except in two cases by Bhargava M et al.[80] and Al-Shammari et al. [76]where Anemia of chronic disorders was more prevalent than BTT. Hence in our study, predominant cases were IDA followed by BTT which reflects good antenatal care where Anemia of chronic diseases was adequately managed. The IDA in our study is probably because of nutritional and regional factors.

BTT was less common in the present study (2.6%) compared to Bain B et al.[93] (8%) and Yogalakshmi et al.[83] (8.8%). Bhargava et al. recorded 7.2%, while Al-Shammari et al.[76] had the lowest at 2%. These differences may be due to awareness and lesser degree of consanguinous marriages in North Karnataka.

These findings reinforce the need for larger, multi-center studies to better understand the underlying causes of anemia and improve diagnostic and management strategies. Implementing nutritional interventions and increasing awareness of hereditary anemias may help in reducing the burden of anemia in affected populations.

SUMMARY

A cross-sectional study conducted over 12 months at KLE's Dr. Prabhakar Kore Hospital analyzed 75 primigravida patients with MHA.

1. Majority (85.3%) were between the age group of 18-30 years, and 64% were vegetarians.
2. Majority of the patients were having moderate anemia (94.6%) and few cases had severe anemia (5.4%), with no cases of mild anemia.
3. Incidence of MHA in primigravida is 84.55%.
4. IDA was found in 97.4% of cases, while only 2.6% were BTT.
5. RBC count and haemoglobin levels were lower in IDA compared to BTT.
6. MCV and MCH and RDW is higher in IDA and lower in BTT.
7. MI & RDWI, RDW-CV and the ricerca index were Higher in IDA compared to BTT.
8. The Ricerca Index misdiagnosed few cases of IDA as BTT whereas all BTT cases were correctly diagnosed. The sensitivity and accuracy of ricerca index is low.
9. The TIBC is higher in IDA as compared to BTT.
10. The TSAT, serum ferritin and serum iron was significantly low in IDA and was high in BTT.
11. NESTROFT Sensitivity was 97.34%, making it the most effective screening tool in MHA to diagnose BTT.
12. HPLC sensitivity was 100%. There was increased levels of haemoglobinA2 and haemoglobin F in BTT by HPLC. HPLC is highly sensitive and gold standard for the diagnosis of BTT.

CONCLUSION

This study highlights the effectiveness of various hematological indices in differentiating IDA (IDA) from beta-thalassemia trait (BTT). NESTROFT and HPLC are the most reliable screening tool, followed by the MI. The findings emphasize the importance of combining indices with iron studies and HPLC for accurate diagnosis and optimal patient management.

LIMITATIONS OF THE STUDY

The study was limited by its small sample size and single-center design. Additionally, genetic analysis was not performed. Moreover, all anemic antenatal cases should have been included in the evaluation to identify variations in hematological indices and HPLC findings for other causes of anemia, including other haemoglobinopathies.

FUTURE SCOPE OF THE STUDY

Future research should focus on integrating genetic screening and molecular diagnostics in antenatal cases to accurately differentiate beta-thalassemia trait, prevent unnecessary iron therapy, and reduce thalassemia major cases through premarital and prenatal couple evaluation.

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ANNEXURES

ANNEXURE I

PROFORMA

PATIENT HISTORY:

Case no:

Name:

Age/Sex:

IP/OPD No:

Slide no:

BRIEF CLINICAL DETAILS:

LMP:

EDD:

PAST HISTORY:

- Diabetes Mellitus;-
- Hypertension;-
- Tuberculosis:-
- Blood transfusion history:-
- Jaundice:-
- Premature Delivery:-
- Fracture of long bones:-
- Growth retardation:-
- Gall stones:-
- Surgeries:-

FAMILY HISTORY:

- Anyone in the family diagnosed as Thalassemia.
- Any Sibling is transfusion dependent.

PERSONAL HISTORY

- Occupation
- Diet
- Is patient on any medication other than routine antenatal drugs.
- Other significant history

EXAMINATION

- Pallor----------☐
- Icterus----------☐
- Cyanosis----------☐
- Clubbing----------☐
- Lymphadenpathy----------☐
- Hepatospleenomegaly---☐
- Pedal edema----------☐

- Weight :-_____
- Height :-_____
- BP :-_____
- Pulse :-_____
- Temperature :-_____
- Respiratory Rate :-_____

Facial Features:-

Frontal Bossing:-

Malar Prominence:-

SYSTEMIC EXAMINATION

- CVS:-Murmurs/Arrhythmias/ Signs of CCF/_____
- RS:-

- Abdomen:- Hepatomegaly/ Splenomegaly/ Enlarged Gallbladder/_____

- CNS:-

LAB VALUES

SI NO	BLOOD TESTS	VALUE	INFERENCE
1	HEMOGLOBIN		
2	PERIPHERAL SMEAR		
3	MCV		
4	MCH		
5	MCHC		
6	RBC COUNT		
7	PCV		
8	RDW-CV		
9	MI		
10	PLATELET COUNT		
11	NESTROFF		
12	HPLC		
13	INFERENCE		

ANNEXURE II

INFORMED CONSENT FORM

"ROLE OF HEMATOLOGICAL INDICES TO DISTINGUISH IRON DEFICIENCY ANEMIA FROM BETA THALASSEMIA TRAIT IN ANTENATAL SCREENING"

Name of Student/Principal Investigator: Dr.

Name of Guide/Co Investigators: Dr.

Introduction: Anaemia is one of the common problems in pregnant ladies and a major health burden in India. Iron deficiency anaemia is one of the commonest type of anaemia during pregnancy. Thalassemia a single gene disorder is also a common problem in India with more than 200 million people are carriers of Beta Thalassemia gene in the world and about 30 million are in India.

Diagnosis of both these entities is often difficult due to its overlapping and similar features. There are similarities in Red Cell Indices such as decreased Hb , MCH and MCV. However the gold standard tool for screening and detection of various Haemoglobinopathies are High Performance Liquid Chromatography (HPLC)

A study is therefore being undertaken to assess the Red Cell Distribution Width (RDW) and Mentzer Index (MI). As the indicator for differentiating Microcytic Hypochromic Anaemia because of Iron Deficiency Anaemia and Beta Thalassemia Trait.

Explanation of procedure: During this study, blood shall be drawn by a trained Phlebotomist and the sample shall be run in Automatic Cell Counter "SYSMEX XN-3100".

Peripheral smear shall be prepared and stained with Leishman / Wright Stain.

RBC Indices will be analysed and Mentzer Index is calculated.

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

Possible benefits from participating in the study: .You will not get any benefits by participating in this study. The data gathered will help population at large.

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

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by Law.

Financial incentives: You will not receive any payment for participating in this study. Cost of investigations done during the course of study will be paid by the Principal investigator.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

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

If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

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

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"ROLE OF HEMATOLOGICAL INDICES TO DISTINGUISH IRON DEFICIENCY ANEMIA FROM BETA THALASSEMIA TRAIT IN ANTENATAL SCREENING"**. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE III

LEISHMAN'S STAIN

Principle: Romanowsky stains are made up of a combination of acid and basic dyes. The principle of staining depends on the differential pH of intracellular substances. Basic substances attract the acid stain while acidic substances attract the basic stain.

Preparation of Leishman's stain: Weigh out 0.2 gm of powdered dye and transfer it to a conical flask of 200 - 250 ml capacity. Add 100 ml of methanol and warm the mixture to 50°C for 15 minutes, occasionally shaking it. Allow the flask to cool and filter. It is then ready for use. The stain improves on standing.

1. **Procedure:**

1. Using a clean glass slide, blood films are prepared from EDTA anticoagulated blood.
2. The blood films are air- dried and the slides are placed on two parallel glass rods which are fixed across a sink.
3. The slide is flooded with stain for 2 minutes.
4. The slide is then diluted with double the volume of buffer for 5-7 minutes.
2. Mixing of stain and buffer is accomplished by a Pasteur pipette.
5. The slide is then washed in a stream of buffered water until it acquires a pinkish tinge.
6. Any excess stain on the underside of the slide is washed off & the slide is set upright to dry.
7. The slide is mounted using DPX.

Results:

Nuclei: Purple

Cytoplasm:

- Erythrocyte: Dark pink
- Reticulocyte: Grey blue
- Lymphocyte: Blue
- Monocyte: Grey blue
- Neutrophil: Pink / Orange
- Basophil: Blue

Granules:

- Neutrophil: Purple
- Eosinophil: Red- orange
- Basophil: Purple- black
- Toxic granules: Dark blue
- Platelet: Purple

ANNEXURE IV

AUTOMATED CELL COUNTER

Principle: The Beckman Coulter analyser works on the Coulter principle which states that particles pulled through an orifice, concurrent with an electrical current, produce a change in impedance that is proportional to the size of the particle traversing the orifice.

Parameters directly measured by the counter

- Haemoglobin: Cyanomethhaemoglobin method
- RBC count: Impedance principle
- Total count: Impedance principle
- Differential count: Volume- conductivity- scatter flow cell
- Platelets: Impedance principle
- MCV, MPV : Impedance principle

Parameters calculated by the counter

- Haematocrit, MCH, MCHC, RDW
- PDW, PCT.

ANNEXURE V

NESTROFT

Principle: Microcytic red blood cells are resistant to lysis when exposed to hypotonic solutions.

Preparation:

10% Buffered saline (pH - 7.4):

- Sodium chloride (NaCi) - 90 gm
- Disodium hydrogen phosphate (Na:HPO4) - 13.65 gm
- Sodium dihydrogen phosphate (NaH,PO,. 2H2O)- 2.43 gm
- Distilled water - 1000 ml.

1% Buffered saline:

- Prepared by diluting 10% buffered saline 1:10 (i.e. 10 ml of 10% buffered saline and 90 ml of distilled water.

0.36% Buffered saline:

- Take 36 ml of 1% buffered saline and 64 ml of distilled water.

Procedure:

- Take 2 ml of 0.36% Buffered saline in a clean test tube labelled as test.
- Take 2 ml of distilled water in a clean test tube labelled as control.
- Add 1 drop of the subject's blood to each of the test tubes labelled as test and control & mix well.
- Mix well & place on test tube stand for 30 minutes.
- After 30 minutes, both the test & control tubes are held against a black line on a white background.

Results:

Positive test: If the black line is blurred, then the test is considered as positive.

Negative test: If the black line is clearly visible, then the test is considered to be negative.

ANNEXURE VI

HPLC

Principle: HPLC depends on the interchange of charged groups on the ion exchange material with charged groups on the haemoglobin molecule. A typical column packing is 5 um spherical silica gel. The surface of the support is modified by carboxyl groups to have a weakly cationic charge. When a haemolysate containing a mixture of haemoglobins is adsorbed unto the resin, the rate of elution of different haemoglobins is determined by the pH and ionic strength of any buffer applied to the column. In automated systems, this is achieved by a continually changing salt gradient. Fractions are detected as they pass through an ultraviolet / visible light detector and are recorded on the integrating computer system. Analysis of the area under these absorption peaks gives the percentage of the fraction detected. The time of elution (Retention time) of any normal or variant haemoglobin present is compared with that of known haemoglobins, providing quantification of both normal haemoglobins & many variants.

ANNEXURE VII MASTER CHART

													IRON PROFILE					VARIOUS STUDY INDICES AND ITS IMPRESSION									Hb ELECTROPHORESIS			
SL NO	AGE	GENDER	OP NO.	TOTAL COUNT	DIFFERENTIAL COUNT (N/L/E/M)	RBC COUNT (millions/cumm)	PLATELET COUNT	HEMOGLOBIN (g/dl)	HEMATOCRIT (%)	MEAN CORPUSCULAR VOLUME (fl)	MEAN CORPUSCULAR HEMOGLOBIN (Pg)	PERIPHERAL SMEAR IMPRESSION	TIBC	TRANSFERRIN SATURATION	SERUM FERRITIN	SERUM IRON	PROVISIONAL DIAGNOSIS	RDW-CV	RDW-CV IMPRESSION (IDA>14/BTT<14)	MENTZER INDEX (MCV/RBC)	MENTZER IMPRESSION (IDA>13/BTT<13)	RDWI (MCV X RDW-CV/RBC)	RDWI IMPRESSION (IDA>220/BTT<220)	RICERCA INDEX (RDW/RBC)	RICERCA INDEX IMPRESSION (IDA>3.3/BTT<3.3)	NESTROFT	HbA	HbA2	HbF	FINAL DIAGNOSIS
1	24	Female	7171034	7.93	70/20/03/07	3.87	258	8.9	30.6	79.1	23	MHA	495	4.44	4	22	IDA	19.6	IDA	20.43	IDA	401	IDA	5.06	IDA	NEGATIVE	NA	NA	NA	IDA
2	29	Female	7284081	10.52	76/21/01/02	5	364	9.8	32.3	64.6	19.6	MHA	535	5.23	20	28	IDA	16.8	IDA	12.92	BTT	217	BTT	3.36	BTT	NEGATIVE	96.9	2.8	0.3	IDA
3	20	Female	7211474	13.82	76/18/01/05	4.09	283	9.1	30.5	74.6	22.2	MHA	485	3.3	4.6	16	IDA	17	IDA	18.23	IDA	310	IDA	4.1	IDA	NEGATIVE	NA	NA	NA	IDA
4	25	Female	7315428	8.42	72/24/01/03	4.01	344	7.8	28	69.8	19.4	MHA	415	6.02	3	25	IDA	18.9	IDA	17.4	IDA	328	IDA	4.7	IDA	NEGATIVE	NA	NA	NA	IDA
5	27	Female	7313989	7.96	70/23/01/06	4.05	279	9.1	31.1	76.8	22.4	MHA	450	3.33	4.5	15	IDA	15.1	IDA	18.96	IDA	286.3	IDA	3.72	IDA	NEGATIVE	NA	NA	NA	IDA
6	24	Female	7212549	7.72	/23/01/01	3.92	334	8	29	74.1	20.4	MHA	487	3.7	5.6	18	IDA	16	IDA	18.9	IDA	302.4	IDA	4.08	IDA	NEGATIVE	NA	NA	NA	IDA
7	32	Female	7320764	11.7	72/21/01/06	4.13	336	9.3	29.7	71.9	22.5	MHA	464	5.6	10	26	IDA	17.2	IDA	17.4	IDA	299.4	IDA	4.16	IDA	NEGATIVE	NA	NA	NA	IDA
8	22	Female	7248620	5.53	64/25/09/02	4.38	231	8.4	29.2	66.7	19.2	MHA	487	2.46	12	12	IDA	16.1	IDA	15.22	IDA	245.1	IDA	3.6	IDA	NEGATIVE	NA	NA	NA	IDA
9	21	Female	7316244	8.6	69/24/01/06	4.56	283	8.9	29.8	65.4	19.5	MHA	525	3.43	3.9	18	IDA	15.7	IDA	14.34	IDA	225.1	IDA	3.4	IDA	NEGATIVE	NA	NA	NA	IDA
10	27	Female	628476	10.1	76/19/01/04	4.89	213	9.5	31.7	64.8	19.4	MHA	510	3.14	8.3	16	IDA	16.7	IDA	13.2	IDA	221.3	IDA	3.4	IDA	NEGATIVE	NA	NA	NA	IDA
11	26	Female	7243646	11.18	72/22/01/05	4.08	311	9.1	30.9	75.7	22.3	MHA	490	4.69	8.5	23	IDA	16.3	IDA	18.5	IDA	302	IDA	3.9	IDA	NEGATIVE	NA	NA	NA	IDA
12	23	Female	7326178	14.35	67/23/07/03	3.66	390	8.7	28.8	78.7	23.8	MHA	480	3.33	12	16	IDA	15.8	IDA	21.5	IDA	339	IDA	4.3	IDA	NEGATIVE	NA	NA	NA	IDA
13	22	Female	7329742	9.68	70/23/02/05	4.35	320	9.4	32.7	75.2	21.7	MHA	518	3.67	5.9	19	IDA	16.5	IDA	17.2	IDA	285	IDA	3.7	IDA	NEGATIVE	NA	NA	NA	IDA
14	23	Female	7339152	5.65	61/31/02/06	3.82	306	7.7	24	62.0	20.1	MHA	486	3.75	7.9	18	IDA	17.8	IDA	16.2	IDA	288	IDA	4.6	IDA	NEGATIVE	NA	NA	NA	IDA
15	27	Female	7324241	10.2	74/17/02/07	4.34	235	9.8	32.6	75.1	22.6	MHA	495	3.43	4	17	IDA	19.6	IDA	17.3	IDA	339	IDA	4.5	IDA	NEGATIVE	NA	NA	NA	IDA
16	29	Female	6494490	11.9	73/20/02/05	3.70	239	8.7	27.9	75.4	23.5	MHA	520	4.42	7.9	23	IDA	17.0	IDA	20.3	IDA	346	IDA	4.5	IDA	NEGATIVE	NA	NA	NA	IDA
17	24	Female	7334680	10.1	62/31/01/06	4.43	443	9.3	32.3	73.1	21.0	MHA	390	4.62	8	18	IDA	17.4	IDA	16.5	IDA	287	IDA	3.9	IDA	NEGATIVE	NA	NA	NA	IDA
18	23	Female	7337131	4.51	72/19/04/05	4.37	238	9.8	34.1	78.0	22.4	MHA	437	4.81	6.7	21	IDA	16.6	IDA	17.8	IDA	296	IDA	3.7	IDA	NEGATIVE	NA	NA	NA	IDA
19	22	Female	7326867	9.38	68/25/01/06	3.85	413	9.6	30	78.9	24.9	MHA	439	6.38	7.8	28	IDA	21.3	IDA	20.4	IDA	436	IDA	5.5	IDA	NEGATIVE	NA	NA	NA	IDA
20	25	Female	5821191	7.5	73/20/02/05	4.10	180	8.0	28.3	68.9	19.5	MHA	620	4.19	3	26	IDA	15.9	IDA	16.8	IDA	267	IDA	3.8	IDA	NEGATIVE	NA	NA	NA	IDA
21	26	Female	7382596	10.4	75/20/01/04	4.28	268	8.4	27.6	64.5	19.6	MHA	510	7.84	9.5	40	IDA	18.6	IDA	15.0	IDA	280	IDA	4.3	IDA	NEGATIVE	NA	NA	NA	IDA
22	28	Female	7368876	8.8	69/22/02/07	3.79	252	8.8	29.6	78.1	23.2	MHA	495	3.84	7.8	19	IDA	15.9	IDA	20.6	IDA	327	IDA	4.1	IDA	NEGATIVE	NA	NA	NA	IDA
23	19	Female	7383917	7.05	62/30/02/06	4.54	246	9.6	31.1	68.5	21.1	MHA	485	5.36	7.8	26	IDA	14.4	IDA	15.0	IDA	217	BTT	3.1	BTT	NEGATIVE	96.71	2.58	0.71	IDA
24	26	Female	7335159	11	76/22/01/01	3.86	305	7.8	26.7	69.2	20.2	MHA	495	3.84	7.6	19	IDA	15.9	IDA	17.9	IDA	285	IDA	4.1	IDA	NEGATIVE	NA	NA	NA	IDA
25	30	Female	5120004	9.53	69/26/02/03	4.02	420	8.8	31.7	78.8	21.8	MHA	490	3.47	10	17	IDA	16.4	IDA	19.6	IDA	321	IDA	4.0	IDA	NEGATIVE	NA	NA	NA	IDA
26	23	Female	7387102	9.65	80/15/02/05	3.03	257	5.6	21.7	71.5	18.5	MHA	490	5.71	6.7	28	IDA	18.4	IDA	23.5	IDA	434	IDA	6.0	IDA	NEGATIVE	NA	NA	NA	IDA
27	26	Female	7298007	12.44	70/23/03/04	4.30	228	9.2	31.7	73.7	21.3	MHA	486	6.38	17	31	IDA	15.3	IDA	17.1	IDA	262	IDA	3.5	IDA	NEGATIVE	NA	NA	NA	IDA
28	39	Female	7340903	8.2	59/25/08/08	4.00	356	9.1	29.7	74.3	22.8	MHA	409	2.44	2.1	10	IDA	18.2	IDA	18.5	IDA	338	IDA	4.5	IDA	NEGATIVE	NA	NA	NA	IDA
29	23	Female	7342540	7.5	77/19/02/02	4.47	254	9.7	31	69.0	21.7	MHA	490	2.45	13	12	IDA	15.1	IDA	15.4	IDA	233	IDA	3.3	BTT	NEGATIVE	96.65	2.46	0.89	IDA
30	35	Female	7301371	9.43	78/15/02/05	3.67	238	9.3	29.3	79.2	25.3	MHA	550	2.91	9	16	IDA	12.6	BTT	21.58	IDA	271	IDA	3.4	IDA	NEGATIVE	97.36	2.22	0.42	IDA

31	23	Female	5435243	8.76	70/23/01/06	3.99	258	8.1	26.4	66.2	20.3	MHA	495	3.84	7.6	19	IDA	17.7	IDA	16.5	IDA	293	IDA	4.4	IDA	NEGATIVE	NA	NA	NA	IDA
32	24	Female	7259532	7.52	73/22/02/03	4.67	211	9.8	32.1	68.7	21.0	MHA	490	5.93	8.6	30	IDA	15.9	IDA	14.7	IDA	233	IDA	3.4	IDA	NEGATIVE	NA	NA	NA	IDA
33	28	Female	7345980	6.2	63/30/03/04	3.50	150	6.5	25.8	73.6	18.5	MHA	585	3.08	13	18	IDA	20.5	IDA	21.0	IDA	431	IDA	5.8	IDA	NEGATIVE	NA	NA	NA	IDA
34	34	Female	2314949	8.93	70/23/02/05	3.6	237	9.5	28.4	79.0	26.4	MHA	625	4.16	4	26	IDA	18.9	IDA	21.94	IDA	414	IDA	5.2	IDA	NEGATIVE	NA	NA	NA	IDA
35	31	Female	5601035	7.86	66/31/01/02	3.61	362	7.6	27.5	76.3	21.0	MHA	520	3.27	13	17	IDA	15.9	IDA	21.1	IDA	336	IDA	4.4	IDA	NEGATIVE	NA	NA	NA	IDA
36	21	Female	7364918	8.43	74/19/01/06	4.04	292	8.1	27.8	68.8	20.0	MHA	488	3.80	5.7	19	IDA	19.6	IDA	17.0	IDA	333	IDA	4.8	IDA	NEGATIVE	NA	NA	NA	IDA
37	22	Female	7370342	14.72	79/13/03/05	3.89	384	8.6	30.4	78.4	22.1	MHA	455	4.62	14	21	IDA	17.3	IDA	20.1	IDA	348	IDA	4.4	IDA	NEGATIVE	NA	NA	NA	IDA
38	32	Female	6748318	7.63	70/21/03/06	4.31	250	9.0	30.8	71.5	20.9	MHA	486	3.30	4.7	17	IDA	15.0	IDA	16.5	IDA	248	IDA	3.4	IDA	NEGATIVE	NA	NA	NA	IDA
39	24	Female	7373156	8.53	74/20/01/05	4.25	279	9.9	33.7	79.3	23.3	MHA	415	4.34	6.7	18	IDA	17.7	IDA	18.6	IDA	330	IDA	4.1	IDA	NEGATIVE	NA	NA	NA	IDA
40	24	Female	7346627	10.99	75/20/01/04	3.86	182	9.9	30	77.0	25.6	MHA	467	4.28	9.2	20	IDA	13.8	BTT	19.9	IDA	275	IDA	3.5	IDA	NEGATIVE	95.65	2.67	0.68	IDA
41	32	Female	7346824	9.01	70/23/02/05	3.71	237	9.7	29	78.0	26.1	MHA	525	5.33	12	28	IDA	13.4	BTT	21.0	IDA	281	IDA	3.6	IDA	NEGATIVE	95.68	3.48	0.84	IDA
42	27	Female	5262575	10.64	65/27/03/05	5.03	132	8.3	34.3	68.2	16.5	MHA	513	7.02	15	36	IDA	26.5	IDA	13.5	IDA	359	IDA	5.2	IDA	NEGATIVE	NA	NA	NA	IDA
43	25	Female	7348229	7.69	65/27/02/06	4.20	323	6.9	26.2	62.2	16.4	MHA	371	4.04	5.6	15	IDA	17.7	IDA	14.8	IDA	262	IDA	4.2	IDA	NEGATIVE	NA	NA	NA	IDA
44	27	Female	7134856	13.06	72/19/03/06	3.90	314	9.8	30.5	78.3	25.1	MHA	610	4.59	8	28	IDA	14.8	IDA	20.07	IDA	297	IDA	3.7	IDA	NEGATIVE	NA	NA	NA	IDA
45	26	Female	4987609	8.29	74/20/01/05	3.04	187	7.0	24.1	79.3	23.0	MHA	450	3.56	7.3	16	IDA	15.7	IDA	26.08	IDA	409	IDA	5.1	IDA	NEGATIVE	NA	NA	NA	IDA
46	24	Female	7350843	8.09	63/30/02/05	3.56	277	8.6	26.8	75.4	24.1	MHA	395	5.57	5.5	22	IDA	18.6	IDA	21.1	IDA	393	IDA	5.2	IDA	NEGATIVE	NA	NA	NA	IDA
47	34	Female	1842453	9.83	77/20/01/02	3.82	191	8.0	27.5	72.1	21	MHA	491	3.57	11	18	IDA	16.1	IDA	18.8	IDA	303	IDA	4.2	IDA	NEGATIVE	NA	NA	NA	IDA
48	28	Female	7350543	9.91	72/23/02/03	4.20	275	9.6	29.4	70.0	22.8	MHA	510	6.27	12	32	IDA	15.8	IDA	16.6	IDA	263	IDA	3.7	IDA	NEGATIVE	NA	NA	NA	IDA
49	20	Female	7300894	13.60	76/15/02/07	4.22	279	9.8	33.5	79.4	23.2	MHA	480	4.79	8.4	23	IDA	14.8	IDA	18.8	IDA	278	IDA	3.5	IDA	NEGATIVE	NA	NA	NA	IDA
50	22	Female	7248620	11.9	64/24/02/10	4.48	285	8.5	32.0	71.4	19.0	MHA	416	6.03	4	26	IDA	20.8	IDA	15.9	IDA	331	IDA	4.6	IDA	NEGATIVE	NA	NA	NA	IDA
51	25	Female	7248229	8.93	73/19/03/05	3.55	246	9.4	28	78.6	26.4	MHA	495	7.68	9.4	38	IDA	13.9	BTT	22.1	IDA	307	IDA	3.9	IDA	NEGATIVE	96.28	2.92	0.8	IDA
52	23	Female	6446391	11.11	75/20/02/03	4.49	221	8.8	31.9	71.2	19.6	MHA	454	3.74	7.8	17	IDA	15.6	IDA	15.8	IDA	247	IDA	3.4	IDA	NEGATIVE	NA	NA	NA	IDA
53	31	Female	7354763	5.12	62/27/02/09	3.94	371	6.5	26.7	67.8	16.4	MHA	510	3.53	5.6	18	IDA	28.6	IDA	17.2	IDA	492	IDA	7.2	IDA	NEGATIVE	NA	NA	NA	IDA
54	33	Female	4058736	8.71	73/23/01/03	4.17	184	9.5	32.5	77.9	22.8	MHA	496	3.85	7.9	20	IDA	22.3	IDA	18.6	IDA	416	IDA	5.3	IDA	NEGATIVE	NA	NA	NA	IDA
55	24	Female	5906501	11.57	73/20/02/05	4.20	278	9.6	32.2	76.7	22.8	MHA	489	3.68	7.5	18	IDA	16.5	IDA	18.2	IDA	301	IDA	3.9	IDA	NEGATIVE	NA	NA	NA	IDA
56	24	Female	7162200	13.09	62/28/04/06	3.80	315	8.7	26.2	69.0	22.9	MHA	400	6.75	10	27	IDA	15.8	IDA	18.1	IDA	286	IDA	4.1	IDA	NEGATIVE	NA	NA	NA	IDA
57	24	Female	5947246	13.96	78/18/02/02	3.33	318	8.0	26.4	79.1	24.0	MHA	575	3.30	15	19	IDA	14.1	IDA	23.7	IDA	334	IDA	4.2	IDA	NEGATIVE	NA	NA	NA	IDA
58	27	Female	6722080	6.81	65/27/03/05	5.06	280	9.6	34.5	68.2	18.9	MHA	488	2.56	13	11	IDA	16.8	IDA	13.4	IDA	226	IDA	3.3	BTT	NEGATIVE	NA	NA	NA	IDA
59	25	Female	7368780	8.67	74/20/01/05	4.16	261	9.8	32.8	78.9	23.5	MHA	580	3.97	13	23	IDA	23.7	IDA	18.96	IDA	449	IDA	5.6	IDA	NEGATIVE	NA	NA	NA	IDA
60	23	Female	7369253	11.98	74/13/04/09	4.21	218	9.7	33.2	78.9	23.0	MHA	489	5.32	18	26	IDA	15.6	IDA	18.7	IDA	292	IDA	3.7	IDA	NEGATIVE	NA	NA	NA	IDA
61	27	Female	7324241	10.2	74/17/02/07	4.34	235	9.8	32.5	75.1	22.6	MHA	650	1.23	7	8	IDA	19.6	IDA	17.3	IDA	339	IDA	4.5	IDA	NEGATIVE	NA	NA	NA	IDA
62	20	Female	7300623	18.6	76/18/02/04	3.76	371	9.1	28.1	74.7	24.2	MHA	470	6.38	7	30	IDA	14.3	IDA	19.8	IDA	284	IDA	3.8	IDA	NEGATIVE	NA	NA	NA	IDA
63	23	Female	7303042	8.94	72/25/01/02	4.20	319	9.5	33.4	79.4	22.6	MHA	490	5.71	7.9	28	IDA	13.7	BTT	18.9	IDA	258	IDA	3.2	BTT	NEGATIVE	96.34	3.1	0.56	IDA
64	25	Female	5271598	7.65	73/24/01/02	4.19	182	9.8	33.3	79.4	23.4	MHA	490	4.08	8.2	20	IDA	17.4	IDA	18.9	IDA	329	IDA	4.1	IDA	NEGATIVE	NA	NA	NA	IDA
65	32	Female	4058736	8.42	74/21/03/08	4.05	203	9.3	31.1	76.8	23.0	MHA	550	3.45	16	19	IDA	13.4	BTT	18.9	IDA	254	IDA	3.3	BTT	NEGATIVE	96.61	2.54	0.85	IDA
66	22	Female	7156844	10.5	74/18/06/06	3.71	308	7.9	28.1	75.7	21.3	MHA	670	4.78	7	32	IDA	21.3	IDA	20.4	IDA	434	IDA	5.7	IDA	NEGATIVE	NA	NA	NA	IDA
67	24	Female	7295883	7.1	56/33/02/09	4.42	295	8.9	31.5	71.3	20.1	MHA	510	2.94	9.4	15	IDA	16.3	IDA	16.1	IDA	262	IDA	3.6	IDA	NEGATIVE	NA	NA	NA	IDA
68	25	Female	6279679	10.1	77/18/02/03	4.54	389	9.2	32.6	71.9	20.2	MHA	487	5.54	9.5	27	IDA	16.4	IDA	15.8	IDA	259	IDA	3.6	IDA	NEGATIVE	NA	NA	NA	IDA
69	25	Female	7326369	10.68	62/30/02/06	4.47	353	9.6	31.7	70.9	21.5	MHA	487	3.90	5.8	19	IDA	17.9	IDA	15.8	IDA	283	IDA	4.0	IDA	NEGATIVE	NA	NA	NA	IDA
70	26	Female	4262679	8.28	65/24/06/05	4.62	400	8.0	28.0	60.6	17.3	MHA	478	5.86	13	28	IDA	19.6	IDA	13.1	IDA	257	IDA	4.2	IDA	NEGATIVE	NA	NA	NA	IDA
71	28	Female	6098841	12.91	62/29/02/07	4.44	398	9.8	34.2	77.0	22.0	MHA	478	5.65	9.5	27	IDA	14.4	IDA	17.3	IDA	249	IDA	3.2	BTT	NEGATIVE	97.34	2.25	0.41	IDA
72	28	Female	6800260	7.09	64/30/02/04	4.40	292	9.8	34.8	79.1	22.2	MHA	566	2.30	12	13	IDA	13.4	BTT	17.9	IDA	240	IDA	3.0	BTT	NEGATIVE	97.16	2.4	0.44	IDA
73	23	Female	7236957	8.48	77/17/02/04	3.45	282	9.2	26.5	77.0	26.7	MHA	480	3.75	4	18	IDA	15.8	IDA	22.31	IDA	352	IDA	4.5	IDA	NEGATIVE	NA	NA	NA	IDA
74	25	Female	7326566	8.3	77/18/02/03	6.2	480	9.2	39.06	63.0	14.8	MHA	185	70.27	280	130	BTT	13.1	BTT	10.16	BTT	132	BTT	2.0	BTT	POSITIVE	91.3	6.2	2.5	BTT
75	29	Female	4056734	8	73/24/01/02	5.5	450	9.6	34.1	62.0	17.4	MHA	190	52.63	130	100	BTT	12.9	BTT	11.27	BTT	146	BTT	2.3	BTT	POSITIVE	92.1	5.9	2	BTT