A Study Of Hematological And Biochemical Profile In Thalassemia Patients

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LIST OF ABBREVATIONS USED

EDTA ----------Ethylene Diamine Tetra Acetate.

SD----------Standard Deviation

Sr. Ca----------Serum Calcium

Sr. P----------Serum Phosphorous

Sr. Fe----------Serum Iron

Sr. TIBC----------Serum Total Iron Binding Capacity

Sr. ALP----------Serum Alkaline Phosphate

WHO----------World Health Organization

RBC----------Red Blood Cell

Hb----------Hemoglobin

HCT----------Hematocrit

MCV----------Mean Corpuscular Volume

MCH----------Mean Corpuscular Hemoglobin

MCHC----------Mean Corpuscular Hemoglobin Concentration

RDW (SD)----------Red cell Distribution Width (Standard Deviation)

RDW (CV)----------Red cell Distribution Width (Coefficient of Variation)

WBC----------White Blood Cell

ABSTRACT

Background:

Thalassemia is one of the most common hereditary disorders in Asia and most parts of the world and has drawn the attention of scientific research by many. It is accompanied with metabolic dysregulation, iron overload, chronic hypoxia and cell damage. All physiological changes result in ineffective erythropoiesis, hemolysis and anemia.

Most patients are dependent on either transfusion or bone marrow transplantation for survival. Regular transfusion and chelation therapy have improved the span and quality of their lives but have been known to cause alterations in the hematological and biochemical parameters.

Aim:

To assess the changes in hematological and biochemical parameters in thalassemia patients and timely correction of any deranged parameters, to prevent any severe complications and to improve quality of life.

Materials and Methods:

The study was done on a total of 35 thalassemia patients who were admitted for treatment and blood transfusions. Thirty-five healthy individual controls were matched by sex and age were included. The values of Total iron binding capacity (TIBC), Serum Iron (Fe), Serum Calcium (Ca), Serum creatinine, Serum phosphorus (P), Serum Alkaline phosphatise (ALP) and Serum Ferritin were estimated. Hemoglobin (Hb), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Platelet count, Red Cell Distribution Width-Standard Deviation (RDW-SD) and Red Cell Distribution Width-Coefficient of Variation (RDW-CV) were measured.

Results:

Study group having 35 cases matched with the control group with a mean age of 8.20 years and 8.74 years respectively. The male to female ratio was 1.9:1 in the study group. Hb, HCT, MCHC, TIBC, Serum Creatinine and Serum Phosphorous were reduced in thalassemia patients when compared with the control group with a significant p-value (<0.005). RDW(SD), RDW(CV), Serum Iron and Serum Ferritin showed a statistically significant increase (p<0.005) in the thalassemia patients. MCV, MCH, Platelet count, Serum Calcium and Serum ALP showed no significant difference between study and control groups.

Conclusion:

Our study demonstrates that repeated blood transfusions in thalassemia patients cause derangement of many hematological and biochemical parameters. Regular monitoring of these parameters is important for better management of thalassemia patients.

KEY WORDS: Thalassemia, Blood transfusion, Hematological, Biochemical Parameters.

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INTRODUCTION

In 1925 Cooley & Lee first described a form of severe anemia that occurs early in life and is associated with splenomegaly and bone changes. This anemia was first observed in the Mediterranean region, therefore it came to be known as "Cooley's anemia" or "Mediterranean anemia". The term "Thalassemia" was coined by Whipple.[1] "Thalassemia" is derived from Greek word "Thalassa" which means "the sea". Thalassemia is an autosomal recessive heterogeneous group of disorders. It is characterized by decreased production of one or more globin protein chains. Thalassemia is broadly divided into α and β subtypes. β thalassemia has emerged as a big public health problem in Asia and most parts of the world. It is one of the most common genetic disorder and has drawn the attention of scientific research by many.

Imbalanced globin protein synthesis is the key factor in determining the severity of the disease in thalassemia syndromes.[1] Thalassemia occurs because of the presence of the homozygous state of one of the thalassemia genes or hemoglobin (Hb) Lepore genes during infancy and childhood. It is accompanied by metabolic dysregulation, iron overload, chronic hypoxia and cell damage.[1,2] All of these physiological changes result in ineffective erythropoiesis, hemolysis and anaemia.[2]

Thalassemia patients are mainly dependent on regular blood transfusions for management. Regular transfusion combined with chelation therapy has enhanced the quality and span of their lives.[3] These days many patients are undergoing bone marrow transplantation as well.

Repeated blood transfusions in thalassemia patients have been known to cause alterations in their hematological and biochemical parameters. Hence this study was done to find out these

abnormalities in thalassemia patients in comparison with controls. Regular monitoring of hematological and biochemical parameters will help in providing timely interventions and prevent any complications that may arise due to changes in these parameters.

AIMS AND OBJECTIVES

To assess the changes in hematological and biochemical parameters in thalassemia patients and timely correction of any deranged parameters, to prevent any severe complications and to improve quality of life.

REVIEW OF LITERATURE

Thalassemias belong to a group of hemoglobinopathies, caused by mutations in genes responsible for α or β-globin chain synthesis. Abnormal globin chain production decreases the formation of Hb tetramers that affects the quantity and quality of hemoglobin resulting in hypochromia and microcytosis. However, production of the normal globin proteins takes place at the usual rate causing accumulation of α and β subunits. Imbalanced in α and β chain and accumulation of normal globin chains is responsible for the clinical phenotype manifested by thalassemia. The degree of impairment of production of the affected globin protein along with altered synthesis of other globin chains and coinheritance of other abnormal globin alleles play an important role in determining the clinical severity of the disease.

HISTORY:

The first description of clinical features of different types of thalassemias was published in the period between 1925 and 1940. Initially, it was thought to be a rare disorder restricted to Mediterranean ethnicities. In 1946, it was mentioned that the cause of thalassemia to be an atypical hemoglobin structure.[2] Dr. Cooley suggested that the disease was hemolytic in nature. In 1960, physicians treating thalassemia children started to transfuse them with fresh RBCs monthly.[2] This improved most of the symptoms and drastically enhanced the survival of these patients. To this day blood transfusion is the mainstay of treatment in thalassemia patients.

With the advent of molecular biology, many researchers have widely studied thalassemias. Methods developed in the last 25 years have enabled researchers to measure the number of globin chains synthesized and identify specific genetic mutations. Thalassemia is now

recognized as one of the most common genetic disorders affecting the world's population. It is a major health problem in many countries. Prevention is seen as an essential part of the management. Thus, many of these countries now have large screening and education programs to detect carriers. This has drastically reduced the number of individuals born with both homozygous and heterozygous forms of the disease.

GLOBAL LOAD AND EPIDEMIOLOGY OF THALASSEMIA:

Thalassemias are prevalent in a wide region reaching out from the Mediterranean region and Africa, all through the Middle East, the Indian subcontinent, Southeast Asia, and Melanesia to the Pacific Islands.[4]

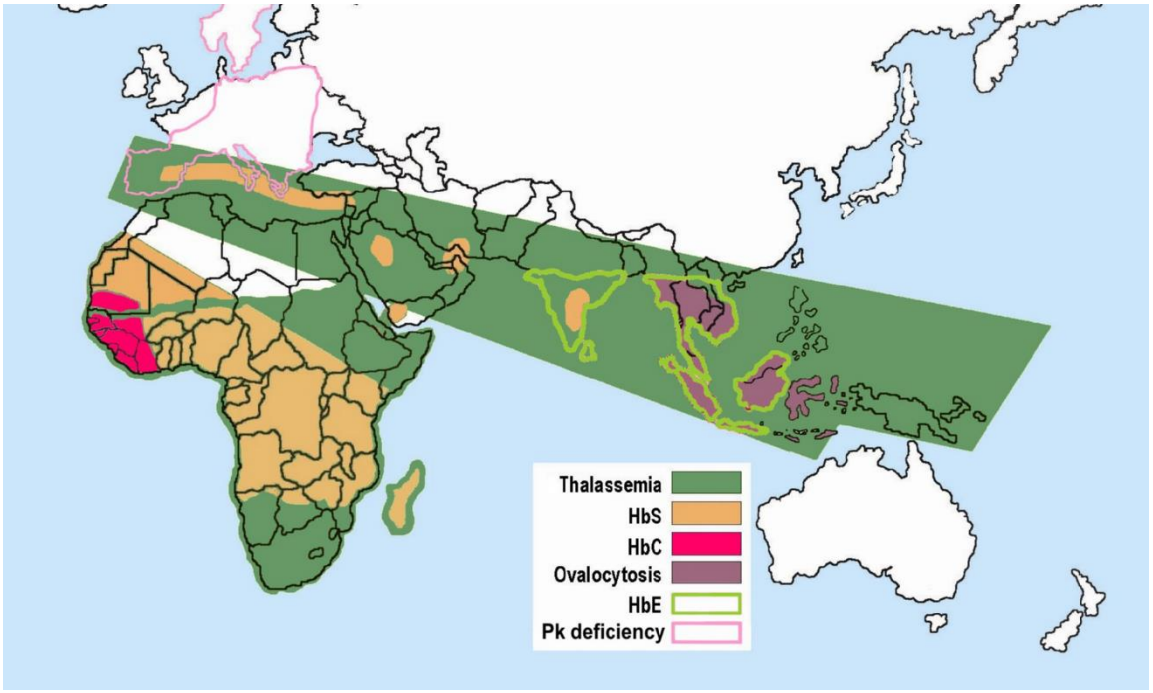


Fig 1: Global thalassemia distribution

Around 1-20 % of the population in these regions are β thalassemia carriers.[4] Milder types are prevalent in 10-20 % of the population in sub-Saharan Africa, to 40% or more in some Middle Eastern and Indian populations, to as high as 80% in northern Papua New Guinea and some parts of upper eastern India.[4]

Presently there are about 270 million thalassemia carriers in the world. Of these, 80 million are β-thalassemia carriers. According to some studies 300,000 to 400,000 babies are born with a serious Hb disorder every year (23,000 with β-thalassemia major) and up to 90% of these births happen in low-or middle-income nations.[4,5,6]

In India every year, approximately 10,000 children are born with thalassemia major. This constitutes about 10% of the total number of children born in the world every year. β-thalassemia is common in certain groups in India like Sindhis, Gujratis, Punjabis and Bengalis. Occurrence fluctuates from 1 to 17%.

Centers for care of thalassemia were started in the mid-1970s in Mumbai, Delhi and other cities. The International thalassaemic federation and Indian Red Cross Society play a crucial role in arranging voluntary blood donations and help in improving the care of thalassemics. More emphasis on thalassemia care has been taken by the Government of India in the 12th 5-year Plan. Many states provide blood transfusion and chelation therapy free of cost. Further, bone marrow transplantation and cord blood stem cell storage facilities are available in many centers which improves the health care of these patients.

HEMOGLOBIN STRUCTURE AND FUNCTION:

In 1960 Dr. Max Perutz demonstrated the 3-dimensional molecular structure of Hemoglobin using X-ray crystallography, for which he received the Nobel Prize in 1962. Hb is a globular protein comprised of four subunits. Each of these subunits has a polypeptide chain called globin and a heme group.

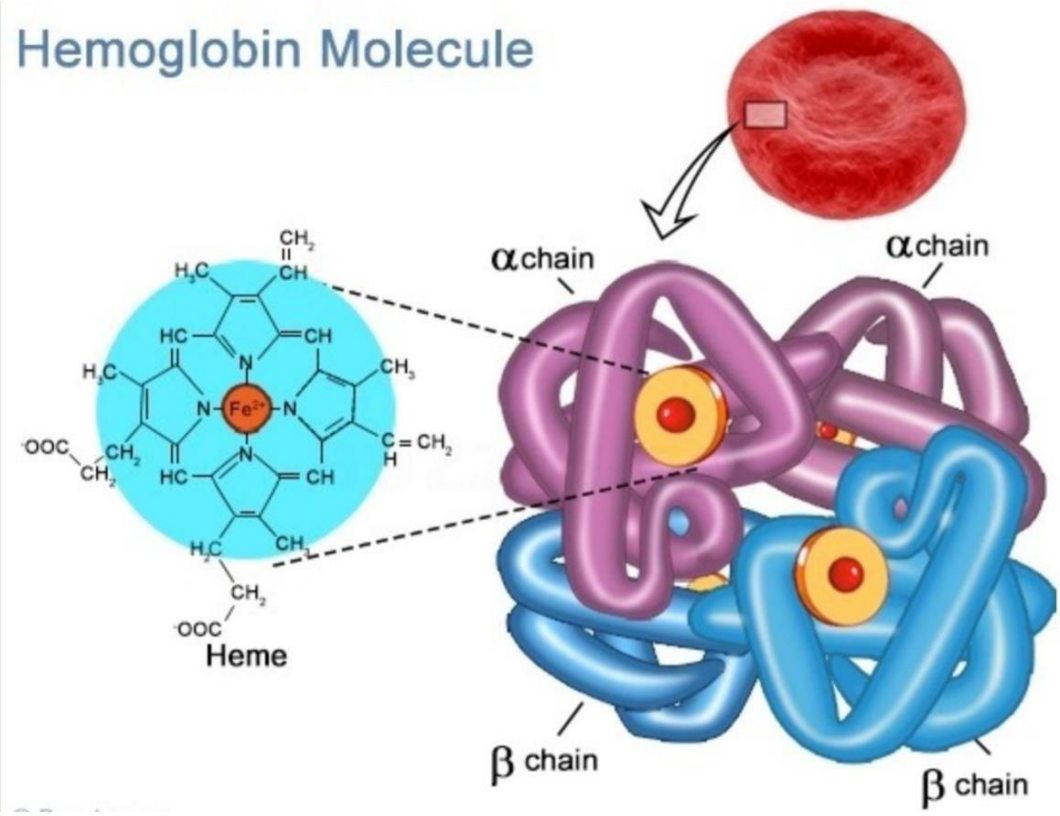


Fig 2: Structure of hemoglobin molecule

Different types of hemoglobins are produced during different phases of life- embryonic, fetal, and adult. Each of these types are composed of a tetramer of globin protein chains- two α-chains (141 amino acids) and two β chains (146 amino acids). In adults, HbA (α2 β2) is the major hemoglobin while HbA2 (α2δ2) is present in minor amounts.[5] HbF(α2γ2) is majorly fetal hemoglobin.[5]

Each globin protein chain encases one heme moiety, comprised of a protoporphyrin IX ring complexed with one iron molecule in the ferrous state (Fe2+). One heme moiety can bind to one Oxygen (O_2) molecule; one Hb molecule of can carry up to 4 O_2 molecules.[5]

The sequence of amino acids in different globin chains are homologous to one another. Each of these globin chain has a profoundly helical secondary structure. Because of their globular tertiary structure, the outer surface of the globin chain is rich in polar (hydrophilic) amino acids. This enhances the solubility of the globin chain. Nonpolar groups form the inner lining, making a hydrophobic pocket into which heme is embedded.[5] The tetrameric HbA contains two αβ dimers. α 1 β 1 links bind the α & β chains together. The entire tetramer is held together by interfaces (i.e., α 1 β 2 contacts) between the non-α chain of the one dimer and α-like chain of the other dimer.[5]

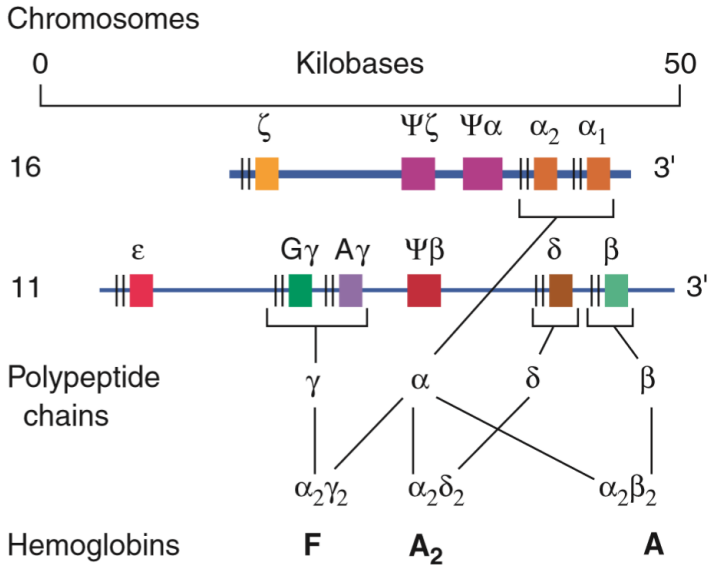


Fig 3: The globin genes. α-like genes (α,ζ) are encoded on chromosome 16; the β-like genes (β,γ,δ,ε) are encoded on chromosome 11. ζ and ε genes encode embryonic globins.[5]

The hemoglobin tetramer is highly soluble but individual globin chains are insoluble. The unpaired globin chain precipitates and form inclusions. These inclusions damage the red blood cell (RBC) membrane. Normally globin chain production is balanced. Each newly synthesized α or non-α globin chain has a partner with which it pairs. Reversible O_2 binding of Hb and solubility are the main properties deranged in most of the hemoglobinopathies. These properties depend mainly on the hydrophilic surface of amino acids.

Hb plays an important role in ensuring O_2 supply to all cells in the body. The primary function of Hb is O_2 transport from the lungs to the peripheral tissues. It also helps in the transport of carbon dioxide released from the peripheral tissues to the lungs.[6]

The various steps in O_2 and carbon dioxide (CO_2) transport are as follows:

- O_2 pickup: In the lungs, inhaled O_2 diffuses through the alveolar membrane and capillaries and binds to Hb in the RBCs. Since one Hb has 4 heme groups, it can carry 4 O_2 molecules.
- O_2 delivery: When Hb reaches the peripheral tissues, due to low pH encountered, there is a decrease in affinity for oxygen and oxygen is released which is taken up by the tissue. This phenomenon is also known as the Bohr effect.
- CO_2 pickup: Since Hb has a higher affinity for CO_2 than O_2, CO_2 molecules bind to deoxyhemoglobin. This phenomenon is known as the Haldane effect.
- CO_2 delivery: Dissociation of CO_2 occurs in the lungs in the presence of O_2. Hb is now free for O_2 pick-up again.

Classification of thalassemia syndromes:

Thalassemia syndromes are mainly classified into three types-

1. β thalassemia- This is the most common type all over the world. The different variants are:
    - Thalassemia major
    - Thalassemia intermedia
    - Thalassemia trait
    - Thalassemia minima
2. α Thalassemia- This type is mainly seen in South East Asian countries, China, Middle East, Europe and Indian subcontinent. The different variants are:
    - Hydrops fetalis
    - Hb H disease
    - α Thalassemia trait
3. Miscellaneous Thalassemic syndromes- These thalassemic syndromes are because of multiple combinations of β and α gene with other structurally abnormal Hb. These are usually asymptomatic and self-limiting. These are:
    - Hb S- Thalassemia
    - Hb E- Thalassemia
    - Hb D- Thalassemia
    - δ – β- Thalassemia
    - HPFH- Hereditary persistence of fetal hemoglobin
    - γ- Thalassemia
    - δ- Thalassemia

CLINICAL FINDINGS:

Clinical findings of thalassemia are related to anemia, chronic hemolysis, and ineffective erythropoiesis (Table-1). Combination of reduced HbA synthesis, ineffective erythropoiesis, and hemolysis results in anemia. The severity of anemia varies widely depending on the number of genes affected and the genes mutated. Hypoxia caused by anemia is exacerbated in some cases due to the presence of abnormal hemoglobins that have high oxygen affinity (HbH and Hb Bart's). These hemoglobins do not release oxygen readily to the tissues. Chronic hemolysis has several adverse effects. Splenomegaly is frequently present because the spleen is a major site of extravascular hemolysis. Occasionally, spleen can become overburdened by the process of erythrocyte destruction resulting in functional hyposplenism. In this case, the spleen's function as a secondary lymphoid tissue is compromised, leading to an increase in infections. Chronic hemolysis can also result in the formation of gallstones. Chronic demand for erythrocytes also has adverse effects. The bone marrow responds to the demand by increasing erythropoiesis, resulting in erythroid hyperplasia and in some severe thalassemias, bone marrow expansion, which causes thinning of calcified bones. Consequently, patients develop skeletal abnormalities and pathologic fractures. There is increased iron demand to support the erythropoietic activity that stimulates the absorption of more iron from gastrointestinal tract (GIT). This additional iron is not effectively incorporated into hemoglobin, so it accumulates in macrophages of the bone marrow, liver, and spleen. As this process continues, iron eventually accumulates in the parenchymal cells of various organs and adversely affects their function. Iron toxicity commonly affects organs like liver, pituitary, heart, and bone. This may result in cirrhosis, hypogonadism, growth failure, arrhythmias, cardiomyopathies, and pathologic fractures.[8] Ineffective

erythropoiesis in the bone marrow may be accompanied by extramedullary erythropoiesis in the liver and spleen.

Extramedullary erythropoiesis can produce masses large enough to cause compression syndromes. Pregnant women with thalassemia have physiological demands that affect the developing baby. to a greater extent than the mother. If the woman's O_2 concentration falls below 70 mmHg, developing fetus can experience diminished growth, premature birth, and even intrauterine death. Pregnant women who present with Hb 9-10 gm% around the time of delivery are often given blood transfusion to improve O_2 delivery. To avoid iron overload caused primarily by the combination of transfusion therapy and increased iron absorption, pregnant women should be given deferoxamine during and after the transfusion.[9]

Table 1: Clinical and laboratory findings in thalassemia:

Clinical Finding	Pathophysiology Laboratory	Finding
Anemia/hypoxia	↓ hemoglobin production/erythropoiesis Ineffective erythropoiesis Presence of high-affinity hemoglobins (HbH and Hb Bart's) ↑ extravascular hemolysis	↓ nRBC count, ↓ hemoglobin, ↓ hematocrit Microcytic / hypochromic RBCs ↓ MCV, ↓ MCH, ↓ MCHC Increased Reticulocyte count Anisocytosis and poikilocytosis Target cells, basophilic stippling, nRBCs, Bone marrow erythroid hyperplasia, ↑ RDW, Abnormal hemoglobin electrophoresis
Splenomegaly/ hemolysis	Splenic removal of abnormal erythrocytes Ineffective erythropoiesis	↑ Bilirubin ↓ Haptoglobin
Gallstones	↑ intravascular and extravascular hemolysis	↑ Bilirubin
Skeletal abnormalities	Expansion of bone marrow	Bone marrow erythroid hyperplasia
Pathologic fractures	Thinning of calcified bone	
Iron toxicity	Iron overload Multiple transfusions, Increased iron absorption	↑ Prussian blue staining in Bone marrow ↑ Serum iron/ferritin and ↓ TIBC

PATHOPHYSIOLOGY OF THALASSEMIA:

Normally equal amounts of α-chains and β-chains are synthesized by the maturing erythrocyte. In α and β thalassemia, synthesis of one of these chains is decreased or absent, resulting in an excess of the other chain.[7] If the α-chain is affected, there is an excess of β-chain

and vice versa. This imbalance in the synthesis of globin chains has several effects, all of which contribute to anemia in thalassemia.[7] Some of these effects are:

(1) Decreased total erythrocyte hemoglobin production.

(2) ineffective erythropoiesis.

(3) chronic hemolysis.

Excess α-chains are unstable and precipitate within the cell. These precipitates bind to RBC membrane, causing membrane damage and decreased RBC deformability.[7] Macrophages destroy the precipitate-filled erythrocytes in the bone marrow, resulting in ineffective erythropoiesis. Circulating erythrocytes with precipitates are pitted and/or removed by the spleen, causing chronic extravascular hemolysis.[7] Excess β-chains can combine to form Hb molecules with four β-chains known as HbH. This Hb has a high O_2 affinity and is also unstable. Thus, it is a poor transporter of O_2. In infants, when α-chains are decreased, excess γ-chains combine to form Hb molecules with four γ-chains. This is known as Hb Bart's. This hemoglobin also has a very high oxygen affinity. Thalassemia like conditions (e.g. HbE) having structural Hb variants can result in decreased synthesis of globin chains, giving the clinical picture of thalassemia.[7]

Ineffective erythropoiesis:

Anemia in thalassemia is due to ineffective erythropoiesis and shortened survival of the RBCs. Ferrokinetic and erythrokinetic studies have concluded that ineffective erythropoiesis contributes maximum to anemia because of the massive destruction of the erythroid precursors in the bone marrow.[2]

Haemolysis:

Destruction of abnormal RBCs causes anemia in thalassemia patients but is not as much important as ineffective erythropoiesis in determining the degree of severity of anemia. In various studies using Ashby or 51Cr-labelling methods, it has been observed that the survival time of RBCs of thalassemics ranged from 7 to 22 days. [10-16] There are two types of populations of RBCs, one is rapidly destroyed.[10,16] It has been mentioned in few studies that RBCs rich in HbF have longer life span while RBC population containing mostly HbA or α-chain precipitates are destroyed early.[2] Variations in the RBC membrane deformability, stability and the cellular dehydration of the RBCs are caused by accumulation of the extra α globin chains at the RBC membrane surface.[2,17,18] Decreased spectrin/band 3 ratio along with partial oxidation and faulty function of band 4.1 are also present.[2,19]

Response to anemia:

In response to chronic hypoxia, profound anemia stimulates increased production of erythropoietin. In pioneer studies, significant elevation in erythropoietin level was noted in the blood and urine of thalassemia patients with Hb of 7.0 g/dl or less.[2]

Erythroid expansion:

β thalassemia is characterized by ineffective erythropoiesis that leads to erythroid expansion that may be up to 10-30 times than normal.[2,20,21] This uncontrolled expansion of erythroid mass is the cause of most of the clinical features of thalassemia. Particularly important are bone deformities and formation of extramedullary tumor masses in few.[2] In young children, it also imposes an excessive metabolic burden. As a consequence growth of these children

becomes slow. There is poor muscular development, along with reduced body fat and body weight.[2] Exacerbation of the anemia takes place because of the shunting of blood through the massively expanded marrow in the presence of splenomegaly.[22] Profoundly anemic thalassemic children are in high output state. This may lead to cardiomegaly in these patients. Increased RBC precursor destruction also leads to elevated levels of urates in urine and serum uric acid levels compared to normal.

Hypersplenism and Splenomegaly:

The exact mechanism of splenomegaly in thalassemia is not clear. It has been mentioned that exposure of the splenic reticuloendothelial elements to abnormal RBCs in β thalassemia patients is the cause of progressive enlargement of the spleen.[2] This concept is supported by the observation that patients receiving regular blood transfusions since young age do not have many abnormal red cells in their circulation. Thus, these patients do not develop significant splenomegaly.[2] In 1963 it was observed that RBCs with inclusions appear in the peripheral blood only after splenectomy. This shows the importance of spleen in the pathophysiology of anemia in thalassemia.[2,24] Extramedullary hemopoiesis may also contribute to splenomegaly.

Splenomegaly leads to entrapment of all the formed elements of the blood, causing pancytopenia (thrombocytopenia, neutropenia and anemia). Shifting of a large quantity of the RBC mass towards the splenic sinusoids causes dilution of blood, and an increase in plasma volume.[2] Splenomegaly causes plasma volume expansion that worsens anemia and also poses a greater load on the myocardium. Vascular shunt mechanism across the vastly expanded bone marrow results in plasma volume expansion.

In a study done by Blendis *et al.*[14], it was observed that 9 to 40% of the total RBCs were entrapped in the splenic pool. There was extensive extramedullary hematopoiesis in the spleen. Interestingly, they also mentioned that the cause of splenomegaly in thalassemia may be the growth spurt in children. An enlarged spleen in thalassemia may cause many deleterious effects.[2,14]

Iron overload:

One of the most well recognized complication of thalassemia is generalized iron overload and deposition of iron in tissues and multiple organs.[12,25-28] Erratic absorption of iron from GIT as well as repeated blood transfusions carries excess iron into the body system. In patients who are inadequately transfused or those with intermediate thalassemias, enhanced absorption from the GIT is considered to be the predominant mechanism of iron overload.[2] On the other hand, in adequately transfused patients, the latter mechanism predominates as the major cause of iron overload.[2]

Mechanisms and rate of iron loading:

The iron stores of the body and the degree of erythropoiesis are the two prime factors that affect the degree of absorption of iron from the gut. One unit of blood contains approximately 200 mg of iron. Since there is no metabolic process by way of which iron can be excreted from the body, repeated blood transfusion rapidly increases the body iron stores.[2] Also because of ineffective erythropoiesis iron absorption from the gut rises exponentially.[2] Although it is a lifesaver in thalassemia patients, blood transfusion is the reason for many complications. Thereby amongst all the complications, iron overload is the most relevant.

Mechanisms of tissue damage because of iron overload:

Though iron is vital for many physiological processes in the body, excess of iron in the body generates toxic free radicals and causes extensive tissue damage. Normally iron is tightly bound to storage or transport proteins. For example, the catalytic effect of iron in free radical production can be prevented by the binding of plasma iron to transferrin.[27] However, when the transferrin gets saturated with the increasing levels of iron overload, plasma iron unbound from transferrin and becomes detectable in the blood and causes toxicity.[28-30] In patients with iron overload, iron is present in serum as well as in other tissues of the body.[28] Generally iron is firmly linked with haem protein and non-haem proteins like transferrin, ferritin and hemoglobin. Free iron can be released if an oxidant stress on iron-containing proteins is imposed. Important pathological consequences of iron overload is involvement of liver, cardiac system and endocrine system.[2]

CLINICAL CONSEQUENCES OF IRON OVERLOAD:

Iron overload is responsible for most of the complications of thalassemia. Heart, liver and various endocrine glands are commonly affected. Frequently thyroid, parathyroid, pituitary, pancreas and the gonads are affected. Sometimes they are not clinically evident initially and hence investigations are required for early detection. Monitoring of iron overload should be done in all thalassemia patients from time to time and appropriate treatment should be given. The dysfunction of the thyroid and parathyroid gland may be subclinical initially. So blood sugar estimation, thyroid function assessment and Serum calcium (Sr. Ca) should be monitored frequently.

Liver is affected due to various causes including repeated blood-borne infections and excessive iron deposition. Hence it is essential to advise liver function tests and viral markers frequently at least once in 6 months. It is also important to monitor the patients for growth failure and complications due to repeated infections.

Growth failure is seen in nearly all thalassemia children in our country. The mean age of attainment of sexual maturity is also delayed. Various causes have been attributed to growth retardation including poor compliance to regular blood transfusion, inadequate chelation, growth hormone deficiency secondary to pituitary hemosiderosis, defective hepatic biosynthesis of somatomedins and sex hormone deficiency and chronic hypoxia secondary to anemia. The need for treatment in subclinical hypothyroidism is debatable. Close monitoring of the patients is needed when treatment is considered as unnecessary. In overt hypothyroidism characterized by low levels of T4 along with signs and symptoms like physical and mental lethargies, cold intolerance, constipation and weight gain etc, treatment with L- thyroxine is considered. The use of intensive chelation therapy can reverse abnormal thyroid functions at an early stage.

70% of deaths in β thalassemia is due to cardiac complications including cardiac failure and arrythmias. Excess iron gets deposited in the heart especially in ventricular walls and the conduction system. When iron accumulates in the cardiac tissue, cells are damaged by free iron because of lipid peroxidation and lysosomal rupture. Cardiac complications in thalassemia children include overt cardiomyopathy, left atrial dilatation, aortic root dilatation and reduction in the internal dimension of left ventricle both in systole and diastole. Cardiac involvement can be detected early by evaluation of ferritin levels and by performing tests like ECG, echo etc., to evaluate cardiac functions. The best available method to assess the severity of cardiac evaluation is T2 weighted cardiac MRI but it is available only in certain centers in India.

MANAGEMENT OF THALASSEMIA MAJOR:

- Correction of anemia by packed cell transfusions
- Removal of excess iron by chelation therapy
- Management of complications
- Curative treatment: stem cell transplantation
- Future treatment: gene therapy
- Disease prevention can be done by genetic counseling, prenatal diagnosis and preimplantation genetics.

Transfusion therapy in thalassemia management:

The goals of treatment by blood transfusion is to correct the anemia and to suppress ineffective erythropoiesis. Regular packed cell transfusion is presently the mainstay of treatment.

Table 2: Types of transfusion regimen according to pretransfusion

Type of transfusion	Pre transfusion Hb	Mean Hb maintained
Palliative	<7g%	<8.5 g%
Hyper transfusion	>10g%	>12 g%
Super transfusion	>12 g%	>14 g%
Moderate transfusion	9 -10.5 g%	>12g%

The current recommendation is to maintain the mean post-transfusion Hemoglobin levels at 12gm%. Moderate transfusion is advised for patients with Hb levels of 9 to 10.5 g%. Post transfusion hemoglobin should not rise above 15 -16 g%.

Table 3: Complications of blood transfusion

Iron overloading	
Infections	- Viral infections (HIV, HCV, HBV, HTLV1, West Nile virus) - Bacterial infections - Parasitic infections - Creutzfeld – Jacob disease
Hemolytic reactions	-Acute hemolytic reactions - Delayed hemolytic reactions. - Autoimmune hemolytic anemia
Non hemolytic reactions	- Allergic and anphylactic rections - Febrile non hemolytic reactions - Transfusion-related acute lung injury - Graft versus host disease - Circulatory overload - Transfusion purpura

Chelation therapy:

Iron overload is the main problem encountered in the management of thalassemia. As there are no effective mechanisms for excretion of excess iron from the organs, the use of iron chelators is the only way for the removal of excess iron. The use of iron chelators is mainly aimed at maintaining the iron store of the body at low levels. The drugs used presently include desferroxamine, Deferiprone and deferasirox.

Desferrioxamine:

The dose is 30 -40mg/kg/day given subcutaneously over 8-10 hours for 6 nights a week using subcutaneous desferal infusion pump. Depot desferrioxamine is a newer modification of chelation therapy.

Deferiprone:

The dose is 75 -100 mg/kg/day in divided doses given orally. It is 70 -100% as effective as desferrioxamine and leads to effective reduction in both serum ferritin and tissue iron overload.

Deferasirox:

Newer oral iron chelator for the treatment of iron overload associated with chronic blood transfusion. The dose is 20 -40mg once daily and is adjusted upon the patient's response, serum ferritin and serum creatinine levels. It is found to be nearly five times more effective than subcutaneous desferrioxamine & ten times potent than deferiprone in animal studies.

As a general rule, chelation therapy should be started in patients with thalassemia major once they have received 10-20 transfusions or when serum ferritin levels rise above 1000µg/dl.[31]

Prospective and retrospective studies have shown that myocardial siderosis is reduced more effectively by deferiprone monotherapy.[32-34]

Intensive therapy with iron chelators seems to bring about improvement in glucose tolerance, abnormal thyroid function and other ill effects of iron overload in early stages.[35,36,37]

Future perspectives:

Newer drugs like PIH (pyridoxal isonicotynoyl hydrozone), Hydroxybenzyl ethylenediamine (HBED) and dimethyl HBED are promising as they are relatively non-toxic and effective. Pharmacological gene manipulations have been tried to increase the production of HbF and to prevent the precipitation of unpaired Hb chains.[38]

Indications for splenectomy:

- When the annual necessity of packed cell transfusion increases two-fold or more than the basic requirement. i.e. around 220 -250 cc per Kg.
- Decreased platelet count – It is comparatively a late indicator of hypersplenism.

All patients requiring splenectomy must be given Pneumococcal vaccine, meningococcal vaccine and H. influenza type B vaccine about 6 weeks preceding the splenectomy. Splenectomy is better avoided in children less than 5 years of age.

Stem cell transplantation:

It is the best available curative treatment available at present with an expectation of permanent cure and healthier forthcoming time for children with genetic disorders such as thalassemia.

PRENATAL DIAGNOSIS AND GENETIC COUNSELING:

Carrier detection, genetic counseling and prenatal diagnosis are the general basis of prevention of thalassemia. If both partners are carriers, genetic counseling should be done as they are at risk of having a thalassemic child. They should be informed about the risk of having genetically diseased children and the treatment options available.

In pregnancies at increased risk prenatal analysis is possible by DNA analysis of fetal cells obtained by amniocentesis or chorionic villi sampling. Identification of disease-causing mutation in parents is essential before prenatal testing is done. Currently, many studies are being undertaken to evaluate fetal cells and DNA in maternal blood for the presence of paternal

mutations. Preimplantation genetic analysis can be done in those families where disease triggering mutations have been recognized.

Preimplantation genetics:

A newer genuine method of prevention of disease would be preimplantation diagnosis that can be achieved by PCR. For this procedure 1-2 blastomeres from embryos are isolated. Alternately one may aspirate a polar body from the oocytes. Ideally, if the mutation resulting in the disease is eliminated, the rest of the blastomeres can be moved to the mother's womb allowing normal fetal development.[39] In the future there is an expected possibility to make available a comprehensive genetic screening of embryos that are fertilized and developed in vitro. Hence reduction or elimination of thalassemia may be possible by manipulation of the gamete with the help of biopsy taken from the embryo and PCR technique.[40]

Prevention of thalassemia:

Only 10 to 15% of Indian children suffering from thalassemia get optimum treatment. The cost of treatment for thalassemic child is around Rs.1,00,000 annually. Curative treatment in the form of bone marrow transplantation is not affordable by most patients. A thalassemic child endures much physical and emotional strain and the same is bore by the family as well as the economy of the nation. So, the goal has to shift from merely treating the patient to the prevention of such births. Prospective prevention that consists of population education, mass screening, genetic counseling, prenatal diagnosis and possibly preimplantation genetics, is the only effective method by way of which it is possible to cope successfully with thalassemia. A variety of screening tests have been used to perform mass screening in population. These include Mentzer's index, NESTROFT (Naked Eye Single Tube Red Cell Osmotic fragility test) etc.

However, none can estimate the confirmatory HbA2 estimation for the definitive identification of beta-thalassemia carriers. Fractions of Hb A, A2, F, H, E and other variants are measured by Hb Electrophoresis and High-performance liquid chromatography. Those who are confirmed to have thalassemia trait should be counseled for testing their partner. If both are tested to be positive, they need to be counseled regarding prenatal diagnosis in the first trimester with chorionic villi sampling and in the second trimester with amniocentesis. Hence when a baby is conceived by two thalassemia carriers, screening should be done in utero and if the fetus is affected, termination should be advised.

MATERIALS AND METHODS

Source of data:

The study was conducted on thalassemia patients who were admitted in the indoor of the Department of Pediatrics, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura. for blood transfusion and treatment. Total thirty-five (n=35) thalassemia cases were taken. The control group also comprised of thirty-five (n=35) subjects. The hematological and biochemical analysis was done in the Department of Pathology and the Department of Biochemistry, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

Study period: 1st December, 2017 to 30th June, 2019.

Subjects:

Study subjects were diagnosed cases of thalassemia between 3 and 14 years of age. All patients received multiple units of blood transfusion. Each unit comprised of 15 milliliter of packed cell transfusion per kilogram of bodyweight approximately. The cases were selected at random whoever had fulfilled the above criteria.

Control subjects were chosen from out-patients who attended the OPD of the Department of Pediatrics, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, for unrelated minor illnesses. They were between 4 to 13 years of age. None of the control subjects had any clinical symptoms or signs of anemia. The case and control groups were age and sex-matched. Controls were otherwise healthy & selected at random.

Sample collection and analysis:

- After taking informed consent, under aseptic precautions, venous blood sample

was collected from both cases and controls.

- Two ml of blood was taken in an EDTA vacutainer and immediately analyzed

for complete blood count, that included Hb, HCT, MCV, MCH, MCHC, RDW (SD and CV) and Platelet count using an automated 5-part differential hematology analyzer (SYSMEX XN-1000).

Fig 4: Automated Hematology Analyzer (Sysmex XN-1000)

- 4 ml of blood sample was taken in a plain vacutainer and was centrifuged @ 1500 rpm. It was then analysed for Sr. Iron, Sr. Ca, TIBC, Sr. Creatinine, Sr. Phosphorus, Sr. ALP and Sr. Ferritin using Biochemistry analyzer (VITROS® 5,1 FS Chemistry System).

Fig 5: Biochemistry analyzer (VITROS® 5,1 FS Chemistry System)

Table 4: The reference values for the studied Hematological parameters[22] are as follows:

S. No.	HEMATOLOGICAL PARAMETERS	REFERENCE RANGE
1.	Hb	13.5 ±2.0 gm/dl
2.	HCT	40.0 ±5 %
3.	MCV	86.0 ±9.0 fl
4.	MCH	29.0 ±4.0 pg
5.	MCHC	34.0 ±3.0 gm/dl
6.	Platelets	1.7-4.5 lakh/µl
7.	RDW-SD	42.5 ±3.5 fl
8.	RDW-CV	12.8 ±1.2 %

Table 5: The reference values for the studied Biochemical parameters[22] are as follows:

S. No.	BIOCHEMICAL PARAMETERS	REFERENCE RANGE
1.	Sr. Fe	Male: 49.0-188.0, Female: 37.0-177.0 µg/dl
2.	Sr. Ca	8.5-11.0 mg/dl
3.	TIBC	Male: 261.0-462, Female: 265.0-497.0 µg/dl
4.	Sr. Creatinine	0.66- 1.25 mg/dl
5.	Sr. Phosphorus	Adult: 39.0-117.0, Child: 65.0-210.0 mg/dl
6.	Sr. Alkaline Phosphate	Adult: 2.5-4.5, Child: 4.0-7.0 IU/L
7.	Sr. Ferritin	Male: 17.0-464.0, Female: 6.24-137.0 ng/dl

Inclusion criteria:

All known cases of thalassemia undergoing transfusions during the study period were included.

Exclusion criteria:

- Patients with all other possible causes of anemia and/or splenomegaly like iron, vitamin B12 or folic acid deficiency and other genetic disorders like hereditary spherocytosis, G6PD deficiency and other hemoglobinopathies were excluded.
- Children who received a blood transfusion in past for any reason.
- Patients who received blood transfusion within last 15 days.
- Patients below 3 years or above 14 years.

The nature and purpose of the study were carefully explained to the parents of the cases and controls before obtaining their consent.

The values of Total iron binding capacity (TIBC), Serum Iron (Fe), Calcium (Ca), Serum creatinine, Serum phosphorus (P) and Serum Alkaline phosphate (ALP), Serum ferritin were estimated. Hemoglobin (Hb), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet count, RDW-SD and RDW-CV levels were also measured.

STATISTICAL METHODS

Sample Size:

As in the study done by Karim Md *et al*[1], with average mean and standard deviation of TIBC 77 and 69 respectively, and 90% power in the study, the calculated minimum sample size was 30 per group.

By the following formula, $n= \frac{(z_\alpha + z_\beta) \times 2 \times p \times q}{d^2}$

Where,

$Z\alpha$ = Z value at α level

$Z\beta$ = Z value at β level

P = common proportion between two groups

q = 100p

d = difference between two groups.

Hence 35 cases and 35 controls were included in the study.

Statistical analysis:

The data obtained were entered in a Microsoft Excel sheet, and statistical analysis was performed using statistical package for the social sciences (Version 17). Results are presented as drawings, Mean ±standard deviation (SD), counts and percentages. Results were compared using independent t-test, Mann Whitney U test. For all tests, significance was achieved at $p<0.05$.

RESULTS

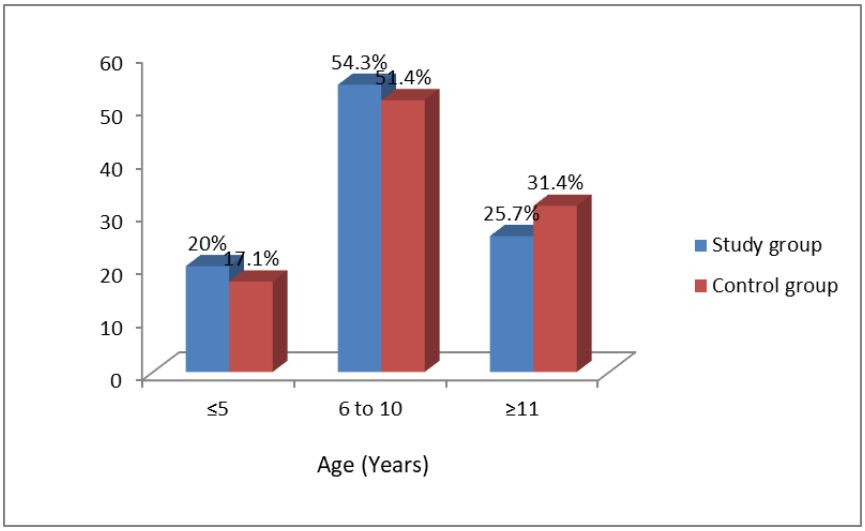
This study was done at the Department of Pathology, Biochemistry and Pediatrics, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka where thalassemia patients were being transfused and managed for clinical symptoms and manifestations of the disease. Hematological and biochemical analysis was done in the Department of Pathology and the Department of Biochemistry.

35 transfusion-dependent thalassemia patients were included in the present study for the hematological and biochemical evaluation. All 35 patients were found to be known cases of Thalassemia major. The control group was comprised of 35 children who had attended Pediatric OPD during the study period for minor illnesses. Here, we present an evaluation of the results of our study.

TABLE 6: AGE INCIDENCE IN STUDY AND CONTROL GROUPS

Age(Years)	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
≤5	7	20.0	6	17.1
6 - 10	19	54.3	18	51.4
≥11	9	25.7	11	31.4
Total	35	100.0	35	100.0

FIG 6: AGE INCIDENCE IN STUDY AND CONTROL GROUPS



In the present study, the age of the patients was within the range of 3 to 14 years In the control group age was within the range of 4 to 13 years. The maximum number of cases in both groups was between 6 and 10 years, amounting to 54.3% in the study group and 51.4% in the control group. (**Table 6, Fig 6**)

TABLE 7: COMPARISON OF AGE (YEARS) BETWEEN STUDY AND CONTROL GROUPS

Variables	Descriptive statistics				Test Statistics		
	Group	**N**	**Mean**	**± S.D.**	**Indepedent t-test**	**P value**	**Remarks**
Age	Study group	35	8.20	3.376	t=0.739	p=0 .463	IS
	Control group	35	8.74	2.737			

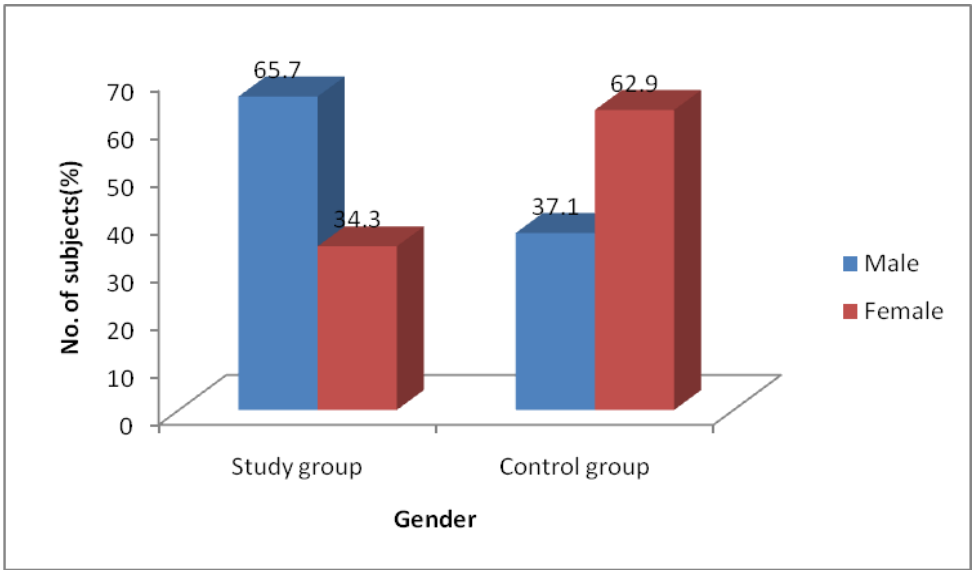
IS: Insignificant

In the present study, average age of patients in the study group and control group was 8.20 ±3.376 years (Mean±SD) and 8.74 ±2.737 years (Mean±SD) respectively which is statistically insignificant (p=0 .463). (**Table 7**)

TABLE 8: SEX INCIDENCE IN STUDY AND CONTROL GROUPS

Gender	Study group		Control group	
	No. of subjects	Percentage	No. of subjects	Percentage
Male	23	65.7	13	37.1
Female	12	34.3	22	62.9
Total	35	100	35	100

FIG 7: SEX INCIDENCE IN STUDY GROUP AND CONTROL GROUP



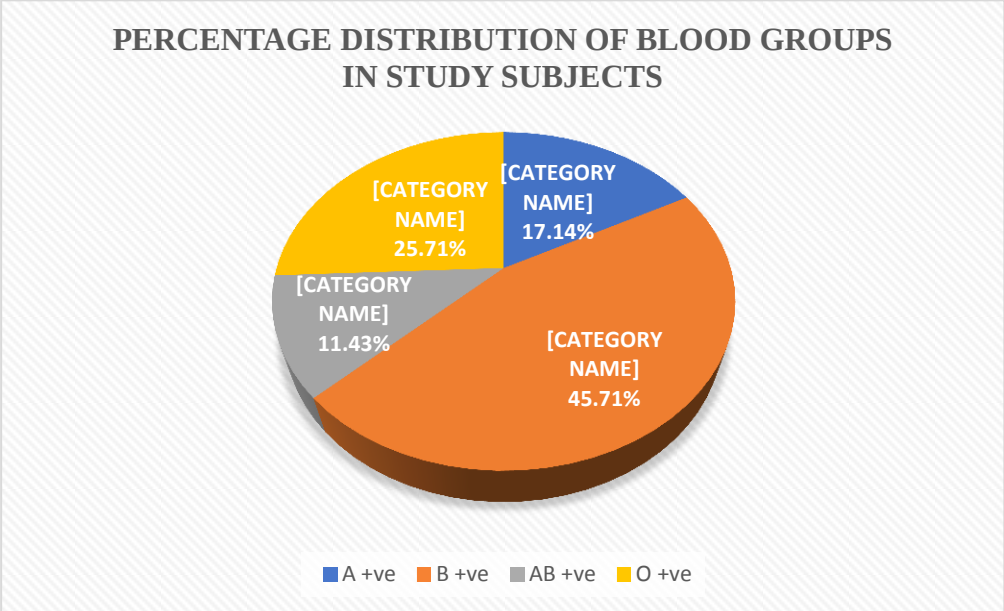
In study group, maximum number of patients were males (23 cases) amounting to 65.7%. while in control group maximum number of cases were females (22 cases) with a percentage of

62.9%. The male to female ratio was 1.9:1 and 1:1.9 in the study group and control group respectively. (**Table 8, Fig 7**)

TABLE 9: ABO-BLOOD GROUP DISTRIBUTION IN THE THALASSEMIA PATIENTS

Blood groups	Rh Factor	Number of subjects	Percentage
A	POSITIVE	06	17.14
B		16	45.71
AB		04	11.43
O		09	25.71
A	NEGATIVE	00	00
B		00	00
AB		00	00
O		00	00
Total		35	100

FIG 8: PIE CHART SHOWING ABO-BLOOD GROUP DISTRIBUTION IN THE

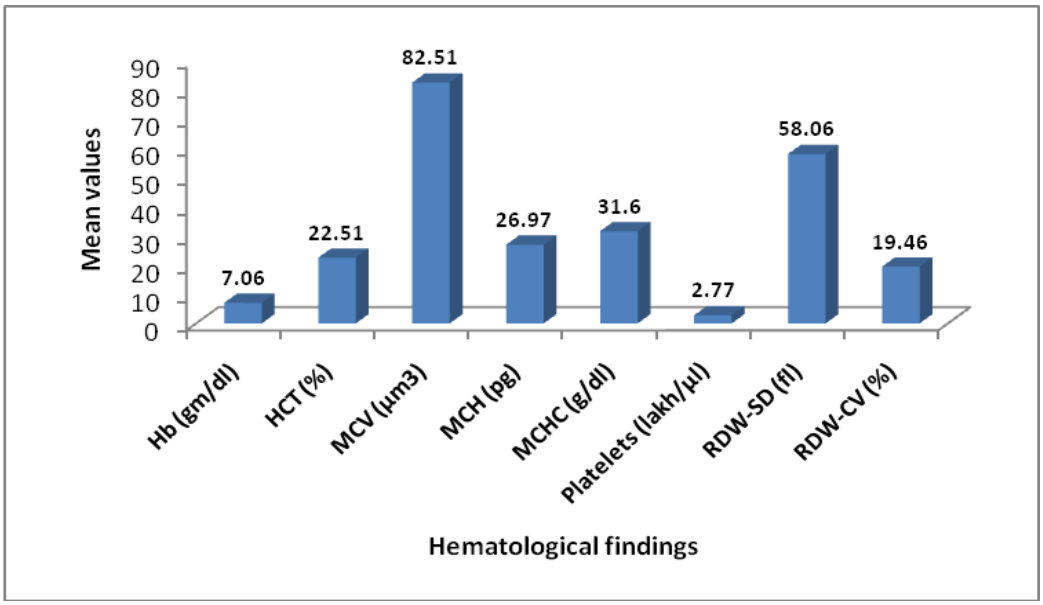


THALASSEMIA PATIENTS

TABLE 10: DESCRIPTIVE STATISTICS OF HEMATOLOGICAL FINDINGS IN STUDY SUBJECTS

Variables	Minimum	Maximum	Mean	Std. Deviation
Hb (gm/dl)	4	10	7.06	1.608
HCT (%)	10	31	22.51	4.985
MCV (µm3)	61	119	82.51	10.147
MCH (pg)	20	34	26.97	2.695
MCHC (gm/dl)	29	34	31.60	1.459
Platelets (lakh/µl)	1	7	2.77	1.457
RDW-SD (fl)	44	128	58.06	19.934
RDW-CV (%)	14	30	19.46	3.641

FIG 9: DESCRIPTIVE STATISTICS OF HEMATOLOGICAL FINDINGS IN STUDY SUBJECTS



On evaluation of hematological parameters in the study group, the following findings were noted:

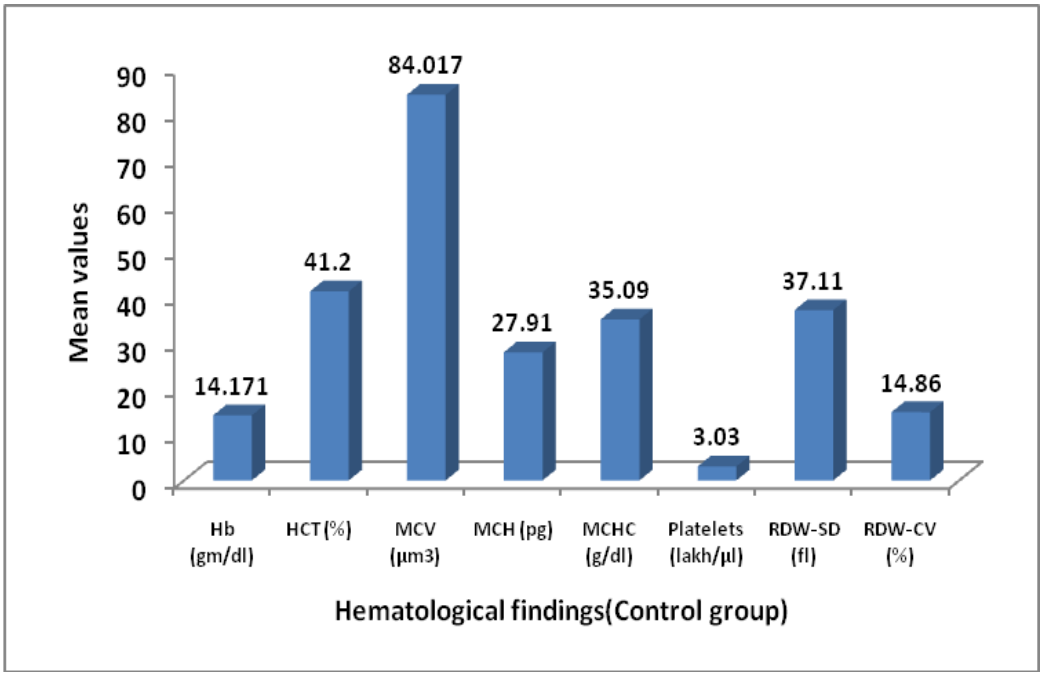
- Minimum Hb was 4.0 gm/dl and maximum was 10.0 gm/dl with an average of 7.06 ±1.608 (Mean ±SD).
- Minimum HCT was 10 % and maximum was 31 % with an average of 22.51 ±4.985 (Mean ±SD).
- Minimum MCV was 61 µm3 and maximum was 119 µm3 with an average of 82.51 ±10.147 (Mean ±SD).
- Minimum MCH was 20 pg and maximum was 34 gm/dl with an average of 26.97±2.695 (Mean ±SD).
- Minimum MCHC was 29 gm/dl and maximum was 34 gm/dl with an average of 31.60 ± 1.459 (Mean ±SD).

- Minimum platelet count was 1.0 lakh/µl and maximum was 7.0 lakh/µl with an average of 2.77±1.457 (Mean ±SD).
- Minimum RDW-SD was 44 fl and maximum was 128 fl with an average of 58.06 ± 19.934 (Mean ±SD).
- Minimum RDW-CV was 14 % and maximum was 30 % with an average of 19.46 ± 3.641 (Mean ±SD). (**Table 10, Fig 9**)

TABLE 11: DESCRIPTIVE STATISTICS OF HEMATOLOGICAL FINDINGS IN CONTROL GROUP

Variables	Minimum	Maximum	Mean	Std. Deviation
Hb (gm/dl)	12.0	16.0	14.171	1.3391
HCT (%)	40	47	41.20	7.045
MCV (µm3)	68.0	96.0	84.017	7.2614
MCH (pg)	25	32	27.91	1.669
MCHC (g/dl)	30	38	35.09	1.961
Platelets (lakh/µl)	2	5	3.03	.822
RDW-SD (fl)	33	44	37.11	2.709
RDW-CV (%)	12	22	14.86	2.487

FIG 10: DESCRIPTIVE STATISTICS OF HEMATOLOGICAL FINDINGS IN CONTROL GROUP



On evaluation of hematological parameters in the study group, the following findings were noted:

- Minimum Hb was 12.0 gm/dl and maximum was 16.0 gm/dl with an average of 14.171 ±1.339 (Mean ±SD).
- Minimum HCT was 40 % and maximum was 47 % with an average of 41.20 ± 7.045 (Mean ±SD).
- Minimum MCV was 68 µm3 and maximum was 96 µm3 with an average of 84.017 ±7.2614 (Mean ±SD).
- Minimum MCH was 25 pg and maximum was 32 gm/dl with an average of 27.91±1.669 (Mean ±SD).

- Minimum MCHC was 30 gm/dl and maximum was 38 gm/dl with an average of 35.09 ± 1.961 (Mean ±SD).
- Minimum platelet count was 2.0 lakh/µl and maximum was 5.0 lakh/µl with an average of 3.03±0.822 (Mean ±SD).
- Minimum RDW-SD was 33 fl and maximum was 44 fl with an average of 37.11 ± 19.709 (Mean ±SD).
- Minimum RDW-CV was 12 % and maximum was 22 % with an average of 14.86 ± 2.487 (Mean ±SD). (**Table 11, Fig 10**)

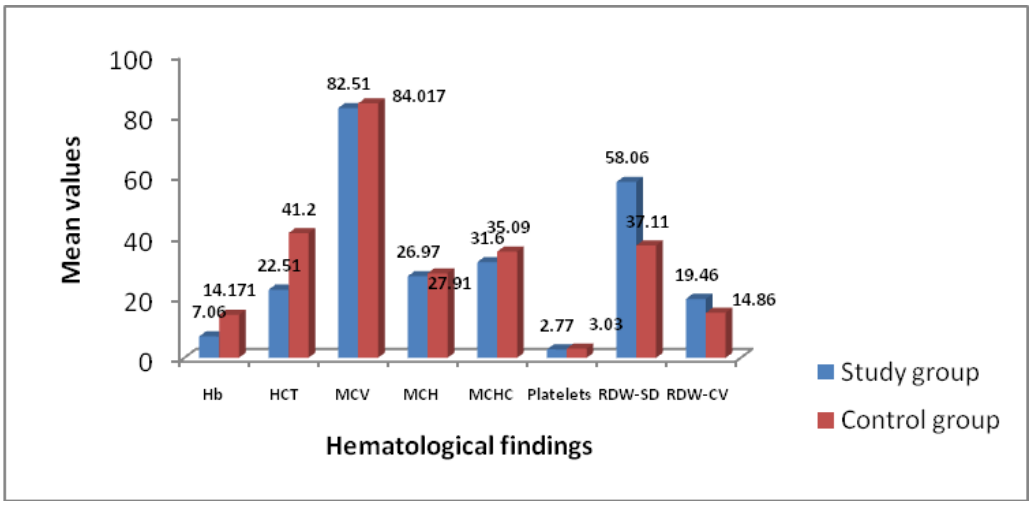
TABLE 12: COMPARISON OF HEMATOLOGICAL FINDINGS BETWEEN STUDY AND GROUPS

Variables	Descriptive statistics				Test Statistics		
	Group	N	Mean	± S.D.	Mann whitney U test/Indepedent t-test	p value	Remarks
Hb (gm/dl)	Study Group	35	7.06	1.608	t=20.115	p<0.001	HS
	Control Group	35	14.17	1.339			
HCT (%)	Study Group	35	22.51	4.985	U=35.000	p<0.001	HS
	Control Group	35	41.20	7.045			
MCV (µm3)	Study Group	35	82.51	10.147	t=0.713	p=0.479	IS

	Control Group	35	84.02	7.261			
MCH (pg)	Study Group	35	26.97	2.695	t=1.719	p=0.083	IS
	Control Group	35	27.91	1.669			
MCHC (g/dl)	Study Group	35	31.60	1.459	U=103.500	p<0.001	HS
	Control Group	35	35.09	1.961			
Platelets (lakh/µl)	Study Group	35	2.77	1.457	U=476.000	p=0.090	IS
	Control Group	35	3.03	.822			
RDW-SD (fl)	Study Group	35	58.06	19.934	U=1.500	p<0.001	HS
	Control Group	35	37.11	2.709			
RDW-CV (%)	Study Group	35	19.46	3.641	t=6.702	p<0.001	HS
	Control Group	35	14.86	2.487			

IS: Insignificant, HS: Highly significant

FIG 11: COMPARISON OF HEMATOLOGICAL FINDINGS BETWEEN STUDY AND GROUPS



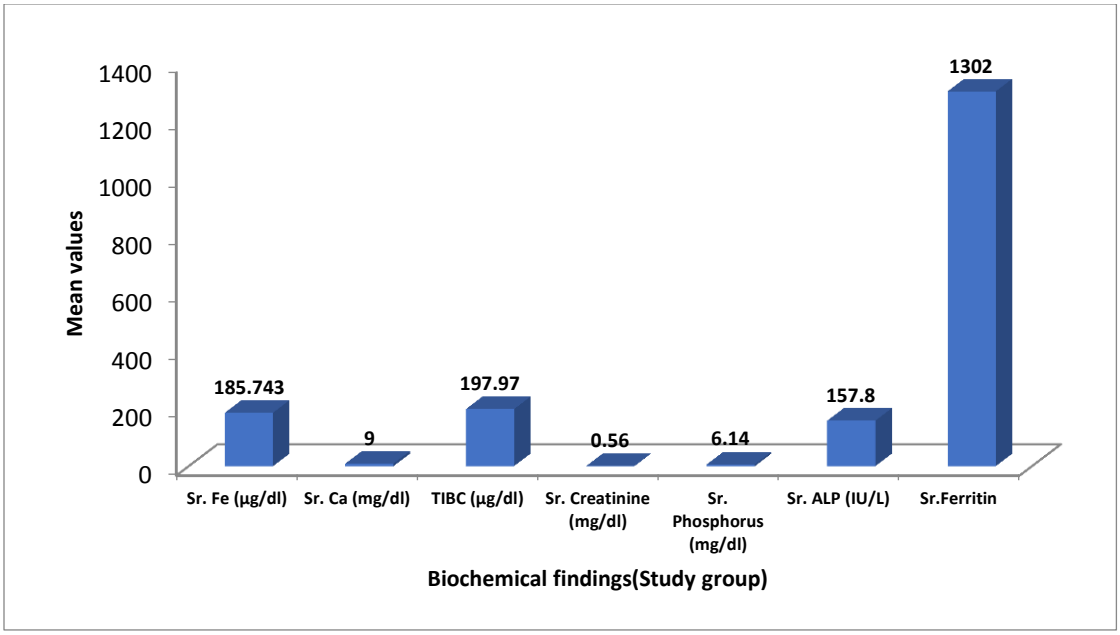
In the present study, on comparison of the hematological parameters between study and control groups, it was observed that the study group had decreased Hb and HCT in comparison to the control group. This decrease was statistically significant ($p<0.001$). There was a statistically significant decrease ($p<0.001$) in MCHC in the study group when compared with the control group. In the study group, both RDW-SD and RDW-CV were increased in comparison to the control group with a statistical difference of $p<0.001$ for both parameters. MCV ($p=0.4790$), MCH ($p=0.083$) and platelet count ($p=0.090$) showed no significant change in values, when compared between study and control groups. (**Table 12, Fig 11**)

TABLE 13: DESCRIPTIVE STATISTICS OF BIOCHEMICAL FINDINGS IN STUDY GROUP

Variables	Minimum	Maximum	Mean	Std. Deviation
Sr. Fe (µg/dl)	58	349	185.74	66.418
Sr. Ca (mg/dl)	5	10	9.00	1.000
TIBC (µg/dl)	85	315	197.97	62.442
Sr. Creatinine (mg/dl)	0.40	0.80	0.56	0.091
Sr. Phosphorus (mg/dl)	4	12	6.14	1.332

Sr. ALP (IU/L)	65	279	157.80	52.169
Sr. Ferritin	560	3200	1302.00	469.322

FIG 12: DESCRIPTIVE STATISTICS OF BIOCHEMICAL FINDINGS IN STUDY GROUP



On evaluation of biochemical parameters in the study group, the following findings were noted:

- Minimum Sr. Fe was 58 µg/dl and maximum was 349 µg/dl with an average of 185.74 ± 66.418 (Mean ±SD).
- Minimum Sr. Ca was 5 mg/dl and maximum was 10 mg/dl with an average of 9.0 ± 1.0 (Mean ±SD).
- Minimum TIBC was 85 µg/dl and maximum was 315 µg/dl with an average of 197.97 ± 62.442 (Mean ±SD).

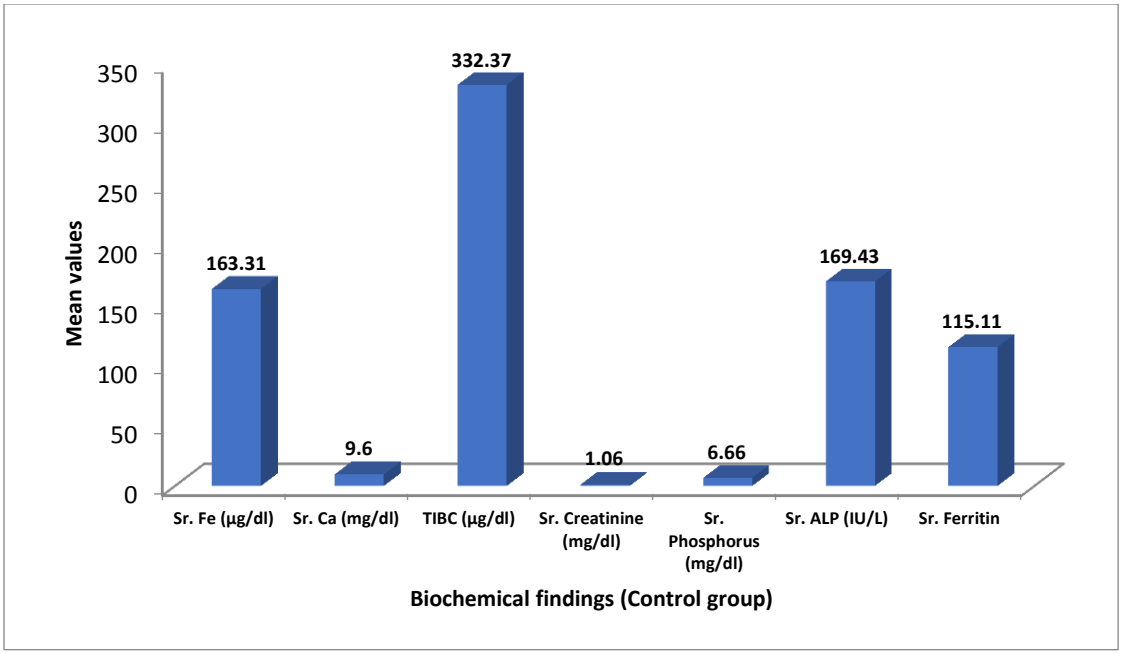
- Minimum Sr. Creatinine was 0.4 mg/dl and maximum was 0.43 mg/dl with an average of 0.43 ± 0.502 (Mean ±SD).
- Minimum Sr. Phosphorus was 4 mg/dl and maximum was 12 mg/dl with an average of 6.14 ± 1.332 (Mean ±SD).
- Minimum Sr. ALP was 65 IU/L and maximum was 279 IU/L with an average of 157.80 ± 52.169 (Mean ±SD).
- Minimum Sr. Ferritin was 560 ng/ml and maximum was 3200 ng/ml with an average of 1302.0 ± 469.322 (Mean ±SD). (**Table 13, Fig 12**)

TABLE 14: DESCRIPTIVE STATISTICS OF BIOCHEMICAL FINDINGS IN CONTROL GROUP

Variables	Minimum	Maximum	Mean	Std. Deviation
Sr. Fe (µg/dl)	112.0	199.0	163.31	23.607
Sr. Ca (mg/dl)	8.0	14.0	9.60	1.557
TIBC (µg/dl)	219.0	402.0	332.37	43.759
Sr. Creatinine (mg/dl)	1.0	2.0	1.06	.236
Sr. Phosphorus (mg/dl)	5.0	8.0	6.66	.802

Sr. ALP (IU/L)	105.0	223.0	169.43	25.663
Sr. Ferritin	60.0	176.0	115.11	28.034

FIG 13: DESCRIPTIVE STATISTICS OF BIOCHEMICAL FINDINGS IN CONTROL SUBJECTS



On evaluation of biochemical parameters in the control group, the following findings were noted:

- Minimum Sr. Fe was 112 µg/dl and maximum was 199 µg/dl with an average of 163.31 ± 23.607 (Mean ±SD).
- Minimum Sr. Ca was 8 mg/dl and maximum was 14 mg/dl with an average of 9.7 ± 1.557 (Mean ±SD).
- Minimum TIBC was 219 µg/dl and maximum was 402 µg/dl with an average of 197.97 ± 62.442 (Mean ±SD).

- Minimum Sr. Creatinine was 1.0 mg/dl and maximum was 2.0 mg/dl with an average of 1.06 ± 0.236 (Mean ±SD).
- Minimum Sr. Phosphorus was 5 mg/dl and maximum was 8 mg/dl with an average of 6.66 ± 0.802 (Mean ±SD).
- Minimum Sr. ALP was 105 IU/L and maximum was 223 IU/L with an average of 169.43 ± 25.663 (Mean ±SD).
- Minimum Sr. Ferritin was 60 ng/ml and maximum was 176 ng/ml with an average of 115.11 ± 28.034 (Mean ±SD). (**Table 14, Fig 13**)

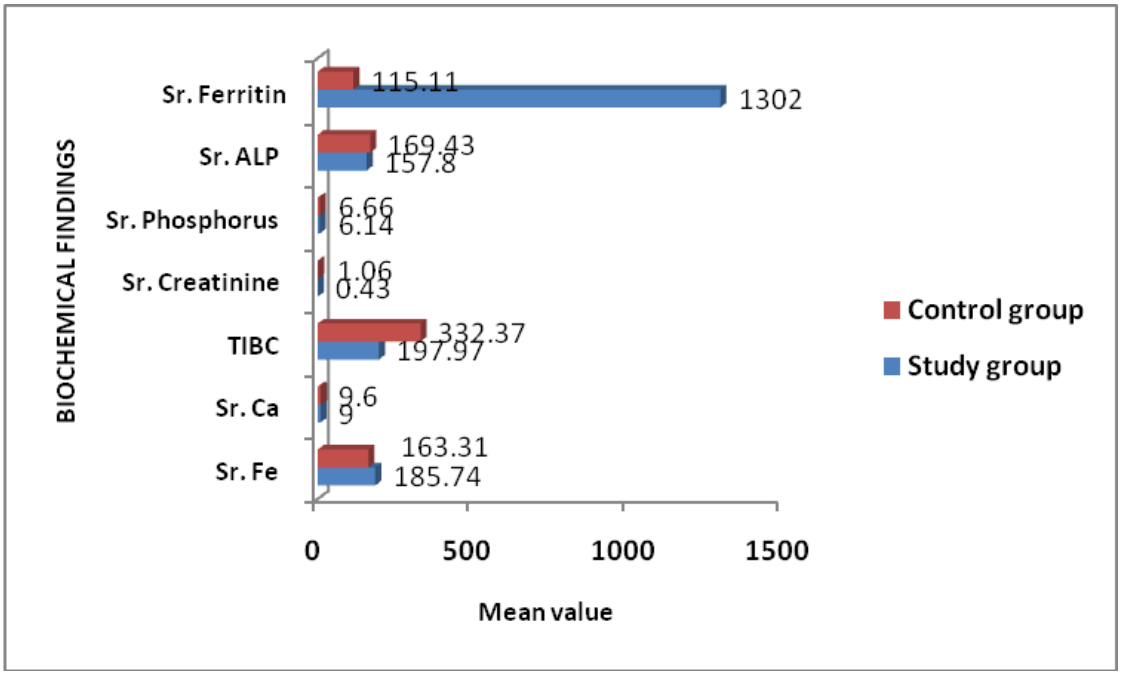
TABLE 15 : COMPARISON OF BIOCHEMICAL FINDINGS BETWEEN STUDY AND CONTROL GROUPS

Variables	Descriptive statistics				Test Statistics		
	Group	N	Mean	± S.D.	Mann whitney U test/Indepedent t test	P value	Remarks
Sr. Fe (µg/dl)	Study Group	35	185.74	66.42	t=2.12	P=0.04	S
	Control Group	35	163.31	23.61			

Sr. Ca (mg/dl)	Study Group	35	9.00	1.000	U=538.5	P=0.349	IS
	Control Group	35	9.60	1.557			
TIBC (µg/dl)	Study Group	35	197.97	62.442	U=65.0	P<0.001	HS
	Control Group	35	332.37	43.759			
Sr. Creatinine (mg/dl)	Study Group	35	0.56	0.502	U=249.5	P<0.001	HS
	Control Group	35	1.06	0.236			
Sr. Phosphorus (mg/dl)	Study Group	35	6.14	1.332	U=368.5	P=0.002	HS
	Control Group	35	6.66	0.802			
Sr. ALP (IU/L)	Study Group	35	157.80	52.169	t=1.183	P=0.242	IS
	Control Group	35	169.43	25.663			
Sr. Ferritin (ng/ml)	Study Group	35	1302.00	469.322	U=0.000	P<0.001	HS
	Control Group	35	115.11	28.034			

IS: Insignificant, HS: Highly significant

FIG 14: COMPARISON OF BIOCHEMICAL FINDINGS BETWEEN STUDY AND CONTROL GROUPS

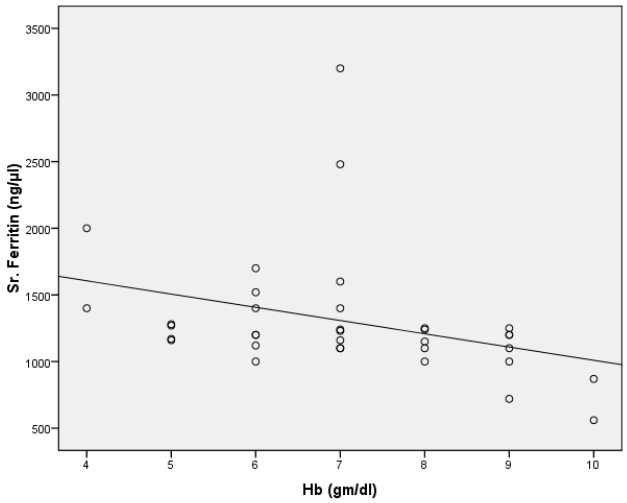


In the present study, on comparison of the biochemical parameters between study and control groups, it was observed that the study group had increased Sr. Fe and sr Ferritin in comparison to the control group. This increase was statistically significant with p=0.04 and p<0.001 respectively. There was a statistically significant decrease in TIBC (p<0.001), sr. creatinine (p<0.001) and sr. phosphorus (p=0.002) in the study group when compared with the control group. Sr. Ca (p=0.349) and sr ALP(p=0.242) showed no significant change in values when compared between study and control groups. (**Table 15, Fig 14**)

TABLE-16: CORRELATION BETWEEN SR. FERRITIN AND HEMATOLOGICAL PARAMETERS IN STUDY GROUP

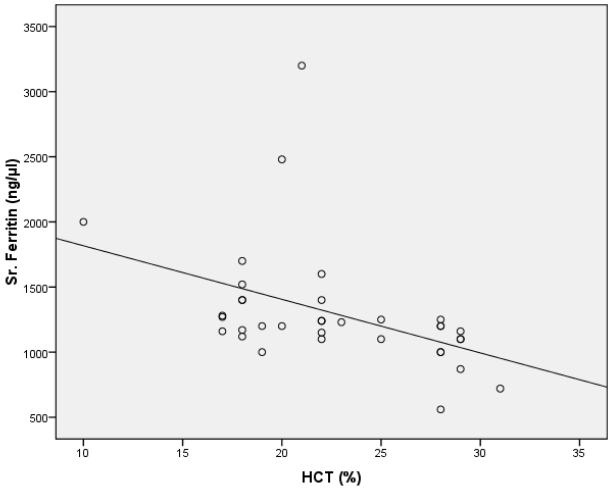
Correlation between Sr. Ferritin &	Correlation coefficient (r)	P value	Conclusion
Hb	r=-0.492	0.003*	Moderate positive correlation. Statistically significant
HCT	r==0.574	<0.001*	Moderate positive correlation. Statistically significant
MCV	r=-0.245	0.156	Moderate positive correlation. Statistically insignificant
MCH	r=-0.357	0.035*	Moderate Negative correlation. Statistically significant
MCHC	r=0.107	0.539	Moderate positive correlation. Statistically insignificant
Platelets	r=-0.534	0.001*	Moderate Negative correlation. Statistically significant
RDW-SD	r=-0.221	0.202	Moderate Negative correlation. Statistically insignificant
RDW-CR	r=-0.292	0.089	Moderate Negative correlation. Statistically insignificant

FIG 15 (SCATTER PLOT -1): CORRELATION BETWEEN SR. FERRITIN AND Hb



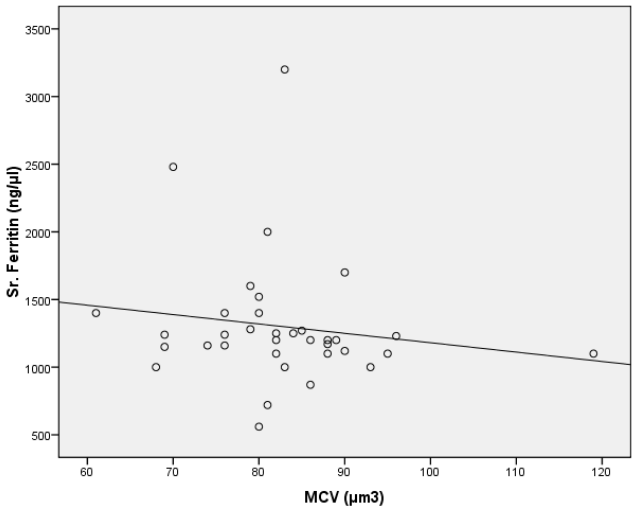
In the present study, statistically significant (p=0.003) moderate positive correlation was observed between Sr. ferritin and HB in the control group. **(FIG 15)**

FIG 16 (SCATTER PLOT -2): CORRELATION BETWEEN SR. FERRITIN & HCT



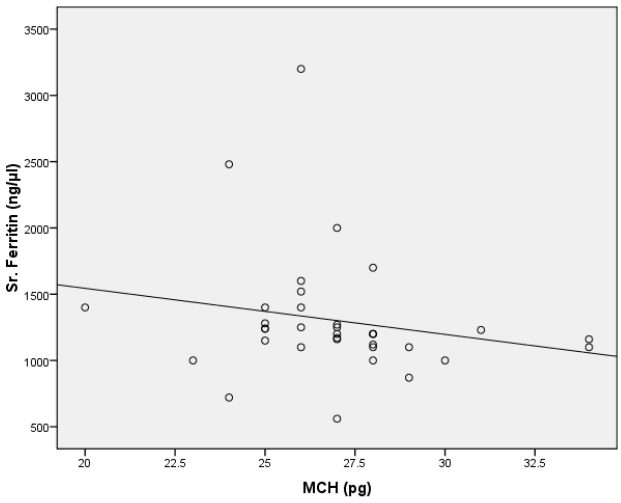
Statistically significant (p<0.001) moderate positive correlation was noted between Sr ferritin and HCT in thalassemia patients. **(FIG 16)**

FIG 17 (SCATTER PLOT -3): CORRELATION BETWEEN SR. FERRITIN & MCV



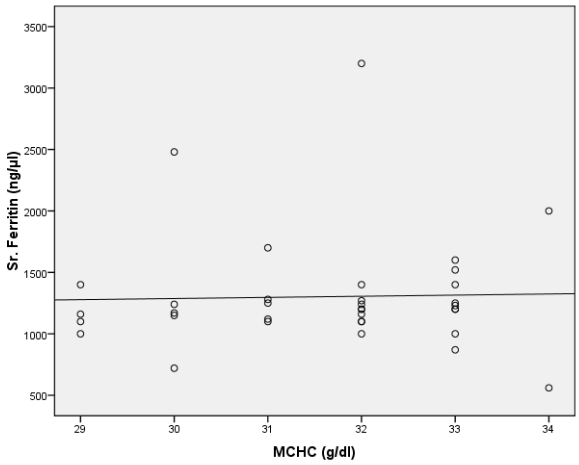
In the present study, a moderate positive correlation was observed between Sr ferritin and MCV in thalassemia patients. This correlation was statistically significant (p=0.156). **(FIG 17)**

FIG 18 (SCATTER PLOT -4): CORRELATION BETWEEN SR. FERRITIN & MCH



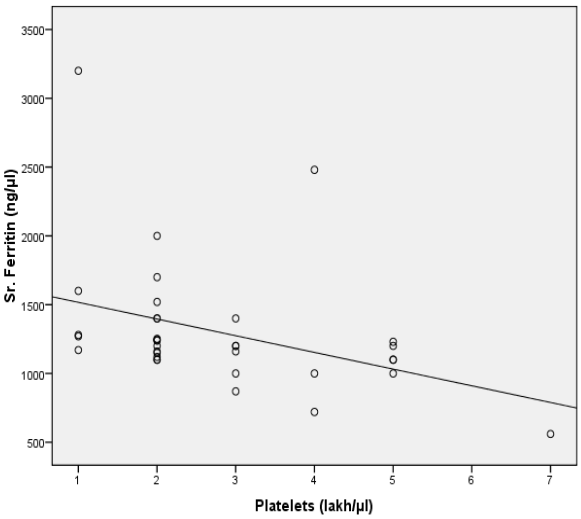
Statistically significant (p=0.035) a moderate negative correlation was noted between sr ferritin and MCH in thalassemia patients. Hence the values were inversely proportional to each other. **(FIG 18)**

FIG 19 (SCATTER PLOT-5): CORRELATION BETWEEN SR. FERRITIN & MCHC



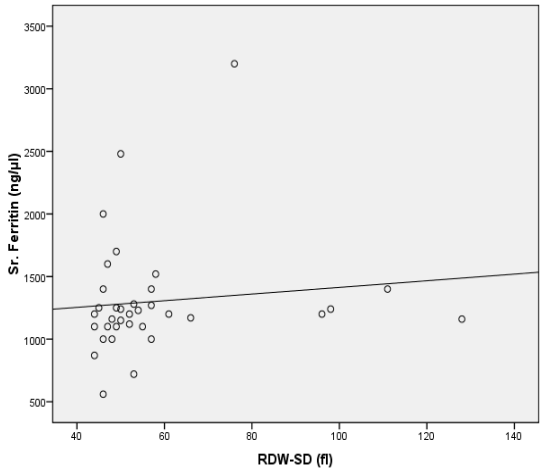
In the present study, a moderate positive correlation was observed between Sr ferritin and MCHC in the control group. However, this correlation was statistically insignificant (p=0.539). **(FIG 19)**

FIG 20 (SCATTER PLOT -6): CORRELATION BETWEEN SR. FERRITIN & PLATELET COUNT



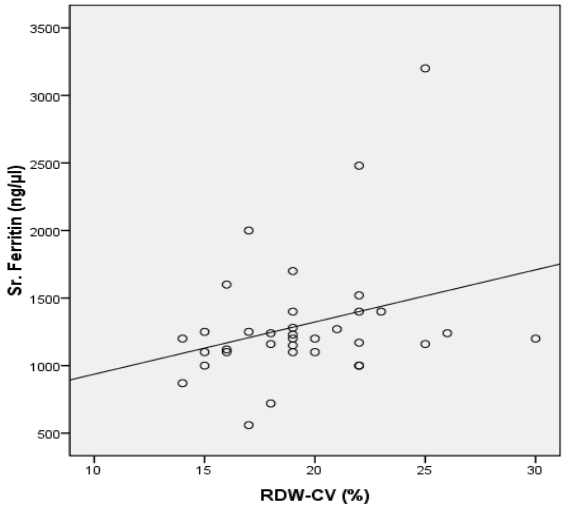
Statistically significant (p=0.001) moderate negative correlation was noted between Sr ferritin and platelet count in thalassemia patients. Hence the values were inversely proportional to each other. **(FIG 20)**

FIG 21 (SCATTER PLOT -7): CORRELATION BETWEEN SR. FERRITIN & RDW-SD



In the present study, a moderate negative correlation was observed between Sr ferritin and RDW-SD in the control group. However, this correlation was statistically insignificant (p=0.202). **(FIG 21)**

FIG 22 (SCATTER PLOT -8): CORRELATION BETWEEN SR. FERRITIN & RDW-CV

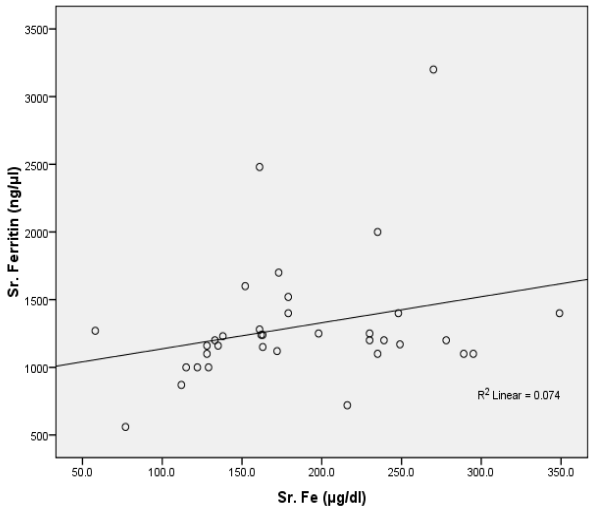


In the present study, a moderate negative correlation was observed between Sr ferritin and RDW-CV in thalassemia patients. However, this correlation was statistically insignificant (p=00.089). **(FIG 22)**

TABLE 17: CORRELATION BETWEEN SR. FERRITIN AND BIOCHEMICAL VARIABLES

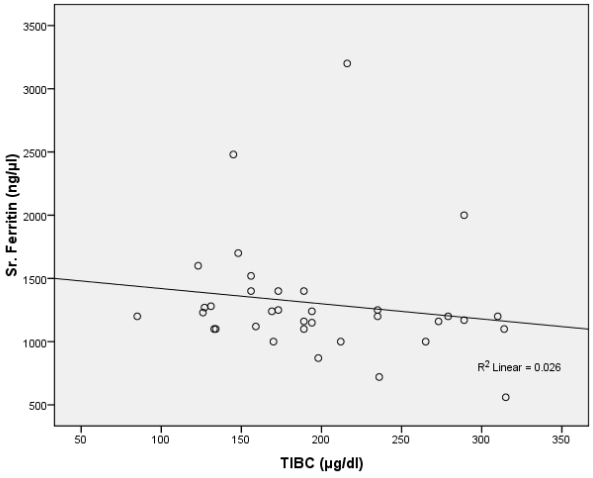
Correlation between Sr. Ferritin &	Correlation coefficient r	p value	Conclusion
Sr. Fe	r=0.310	p=0.070	Moderate positive correlation. Statistically insignificant
Sr. cal	r=0.028	p=0.875	No correlation. Statistically insignificant
TIBC	r=-0.347	p=0.041*	Moderate positive correlation. Statistically significant
Sr. Creatinine	r=0.037	p=0.832	No correlation. Statistically insignificant
Sr. Phosphorus	r=0.175	p=0.316	Moderate positive correlation. Statistically insignificant
Sr. ALP	r=-0.306	p=0.074	Moderate Negative correlation. Statistically insignificant

FIG 23(SCATTER PLOT -9): CORRELATION BETWEEN SR. FERRITIN & SR. Fe



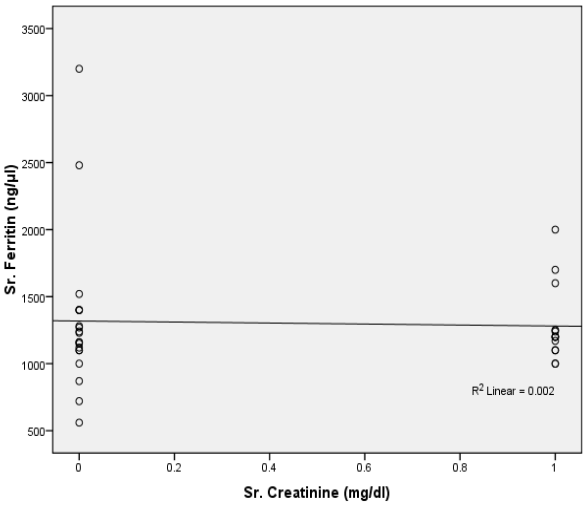
In the present study, a moderate positive correlation was observed between Sr ferritin and Sr Fe in the control group. However, this correlation was statistically insignificant (p=0.070) **(FIG 23)**

FIG 24 (SCATTER PLOT -10): CORRELATION BETWEEN SR. FERRITIN & SR. TIBC



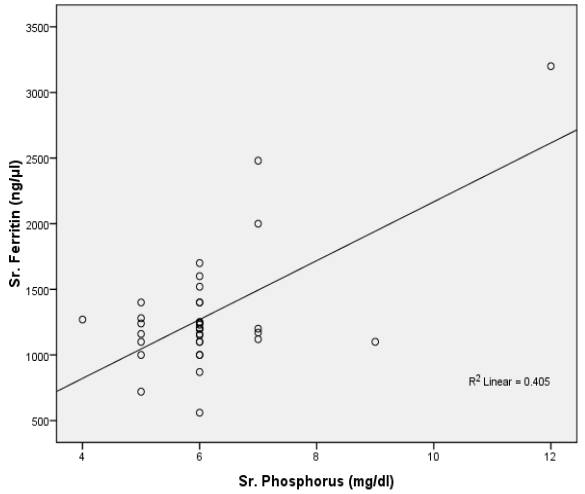
Statistically significant (p=0.041) moderate positive correlation was noted between Sr ferritin and Sr TIBC in thalassemia patients. **(FIG 24)**.

FIG 25 (SCATTER PLOT -11): CORRELATION BETWEEN SR. FERRITIN & SR. CREATININE



In the present study, there was no correlation between Sr ferritin and sr. creatinine in the thalassemia patients. **(FIG 25)**

FIG26(SCATTER PLOT -12): CORRELATION BETWEEN SR. FERRITIN & SR. PHOSPHORUS



In the present study, a moderate positive correlation was observed between Sr ferritin and Sr phosphorus in thalassemia patients. However, this correlation was statistically insignificant (p=0.316) **(FIG 26)**

DISCUSSION

Most patients with alpha thalassemia and beta-thalassemia trait are asymptomatic and need no or simple management while in case of beta-thalassemia major patients are managed with repeated blood transfusion.[22] Transfusion along with chelation therapy has dramatically improved the life expectancy of thalassemic children. However, frequent blood transfusions can lead to iron overload and derangement of many hematological and biochemical parameters. These deranged parameters, if not monitored regularly will lead to various complications that may decrease the quality of life and overall survival of thalassemia patients. In recent years, several authors have reported a high incidence of these complications, predominantly in patients diagnosed with thalassemia major.[5] Therefore monitoring of hematological and biochemical parameters in regularly transfused thalassemic patients is essential.

The present study was done on 35 thalassemic patients who underwent regular blood transfusions in the Department of Pediatrics, Shri B.M Patil Medical College and Research Center. All the study subjects were diagnosed with β thalassemia major.

In the present study, the mean age of the study subjects was 8.2 years. Similar findings were noted in studies done by Suman R L *et al.*[45], Logothetis J *et al.*[46] and De A *et al.*[47] In our study most patients belonged to the age group of 6-10 years and similar findings were observed in study done by Al-Kherbash H *et al.*[50] *and* Joseph N. *et al.*[51] where maximum cases were in the age range of 7-10 years and 5-10 years respectively. **(Table 18)**

Table 18: Age incidence in study subjects compared to other studies

Studies	Mean age ±SD
Present study	8.2 ±3.376
Suman RL *et al.*[45]	8.80 ±3.88
Logothetis J *et al.*[46]	10.3 ±4.2
De A *et al.*[47]	8.22

Since thalassemia is an inherited genetic disorder, most patients present early with symptoms of anemia leading to early diagnosis.[2]

In present study it was observed that majority of the patients in study group were having B +ve blood group. However in a study done by Sinha P A *et al.*[82] it was found that majority of the patients were O +ve followed by B +ve cases.

Majority of the patients in the present study are males (65.7 %). This finding is correlating with studies done by Tyagi S *et al.*[48], Patil S *et al.*[49], Al-Kherbash H *et al.*[50], and Joseph N. *et al.*[51] who also reported similar gender distribution in thalassemia affected children. **(Table 19)**

Table 19: Gender distribution in study subjects compared to other studies

Studies	Males (%)	Females (%)
Present study	65.7	34.3
Tyagi S *et al.*[48]	64.5	35.5
Patil S *et al.*[49]	60.4	39.6
Al-Kherbash H et al.[50]	53.2	46.8
Joseph N. *et al.*[51]	63.4	36.6

Hb level of thalassemia children is decreased in the present study with an average value of 7.06 ±1.608 (Mean ±SD). The decrease in Hb in comparison to control subjects is also statistically significant with p<0.001. This finding is correlating with studies conducted by Karim F *et al.*[1], Ayyash H *et al.*[53], Verma S *et al.*[54], De A *et al.*[47], Filiz *et al.*[60], Prakash A *et al.*[52], Sultan S *et al.*[55], Jameel T *et al.*[57] and Jain C *et al.*[59] who also observed decreased Hb level in thalassemia cases. Hb is usually low in cases of thalassemia because of defective Hb production and increased RBC destruction in the spleen leading to ineffective erythropoiesis. **(Table 20)**

Table 20: Hb value in study subjects compared to other studies

	Hb (Mean±SD)	p value
Present study	7.06 ±1.608	<0.001
Karim F *et al.*[1]	7.36 ±1.5	<0.001
Ayyash H *et al.*[53]	7.36 ±0.8	<0.001
Verma S *et al.*[54]	9.8 ±1.1	<0.001
De A *et al.*[47]	8.5 ±2.5	<0.001

Filiz *et al.*[60]	9.25 ±1.74	<0.001

In the present study HCT level in thalassemia children was decreased with an average of 22.51 ±4.985 (Mean ±SD). This decrease in HCT in comparison to control subjects is statistically significant with p<0.001. Similar findings were observed in studies conducted by Karim F *et al.*[1], Sultan S *et al.*[55], Munir B *et al.* and Filiz *et al.*[60] **(Table 21)**

Table 21: HCT value in study subjects compared to other studies

	HCT (Mean±SD)	p value
Present study	22.51 ±4.985	0.001*
Karim F *et al.*[1]	21.5 ±5.3	<0.001*
Filiz *et al.*[60]	27.07 ±4.65	<0.05*

In the present study, no significant change in MCV values (p=0.479) was observed between study and control groups. This finding is in correlation with a study done by Filiz *et al.*[60] who also noted no significant change in MCV in thalassemia patients. While Karim F *et al.*[1] observed a significant decrease in MCV in their study. **(Table 22)**

Table 22: MCV value in study subjects compared to other studies

	MCV (Mean±SD)	p value
Present study	82.51 ±10.147	0.479
Filiz *et al.*[60]	82.79 ±3.74	>0.05
Karim F *et al.*[1]	70 ±9.5	<0.05*

In the present study, no significant change in MCH values (p=0.083) was observed between study and control groups. This finding is in correlation with a study done by Filiz *et al.*[60] who also noted no significant change in MCH in thalassemia patients. While Karim F *et al.*[1] observed a significant decrease in MCH in a study done by them. (**Table 23**)

Table 23: MCH value in study subjects compared to other studies

	MCH (Mean±SD)	p value
Present study	26.97 ±2.69	0.083
Filiz *et al.*[60]	27.98 ±1.9	>0.05
Karim F *et al.*[1]	23.8 ±3.8	<0.05*

MCHC level of thalassemia children is decreased in the present study with an average value of 31.60 ±1.459 (Mean ±SD). The decrease in MCHC in comparison to control subjects is also statistically significant with p<0.001. Similar finding was observed in studies conducted by Verma S *et al.*[54], Jameel T *et al.*[57], Bushra M *et al.*[56], Bhushan *et al.*[61] and Guimaraes J *et al.*[62] However, Karim F *et al.*[1] did not find any significant decrease in MCHC in their study. (**Table 24**)

Table 24: MCHC value in study subjects compared to other studies

	MCHC (Mean±SD)	p value
Present study	31.60 ±1.459	<0.001*
Karim F *et al.*[1]	34.1 ±2.8	>0.05
Verma S *et al.*[54]	30.2 ±2.35	0.04*

Jameel T *et al.*[57]	35.2 ±3.0	<0.001*

Platelet count in thalassemia children is within the normal range in the present study with an average value of 2.77 ±1.457 (Mean ±SD) and showing no significant difference with the control group. Similar finding was observed in studies conducted by Naithani R *et al.*[63], Bushra M *et al.*[56], and Sultan S *et al.*[55] (**Table 25**)

Table 25: Platelet count in study subjects compared to other studies

	Platelet count (Mean±SD)	p value
Present study	2.77 ±1.457	0.090
Naithani R *et al.*[63]	2.26 ±1.23	NS

In the present study, RDW-SD level in thalassemia children is increased with an average value of 58.06±19.934 (Mean ±SD). The increase in RDW-SD in comparison to control subjects is also statistically significant with p<0.001. This finding is in correlation with studies done by Mahdi S. L *et al.*[72] and Munir B *et al.*[56] (**Table 26**)

Table 26: RDW-SD value in study subjects compared to other studies

	RDW-SD (Mean±SD)	p value
Present study	58.06±19.934	<0.001
Mahdi S. L *et al.*[72]	40.0 ±5.8	<0.0001

In the present study, RDW-CV level in thalassemia children is increased with an average value of 19.46 ±3.641 (Mean ±SD). The increase in RDW-CV in comparison to control subjects is also statistically significant with p<0.001. This finding is in correlation with studies done by Jameel T *et al.*[57], Filiz *et al.*[60], Rahman MMU *et al.*[64], Verma S *et al.*[54] and Bhushan *et al.*[61] (**Table 27**)

Table 27: RDW-CV value in study subjects compared to other studies

	RDW-CV(Mean±SD)	p value
Present study	19.46 ±3.641	<0.001
Jameel T *et al.*[57]	16.5 ±1.8	<0.001
Filiz *et al.*[60]	15.19 ±2.86	<0.05
Rahman MMU *et al.*[64]	14.16 ±0.46	<0.0001

Serum Fe level of thalassemia children is increased in the present study with an average value of 185.74 ±66.41 (Mean ±SD). The increase in Sr. Fe in comparison to control subjects is also statistically significant with p=0.04. Similar finding of Serum Fe in thalassemia children was found in studies done by Naithani R *et al.*[63], Livrea M. A *et al.*[66], De A *et al.*[47], Ghone, R.A *et al.*[67] and Salma O S *et al.*[73] (**Table 28)**

Increased Serum Fe in thalassemia patients is mainly because of transfusion-related iron overload. Also, the rate of iron absorption from G.I.T is approximately 3-4 times greater in β-thalassemic patients than normal. This may lead to iron accumulation about 2-5 gm per year.[45,74]

Table 28: Serum Fe value in study subjects compared to other studies

	Serum Fe (Mean±SD)	P value
Present study	185.74 ±66.41	0.04
Naithani R *et al.*[63]	190.0 ±89.0	<0.001
Livrea M. A *et al.*[66]	254.0 ±60.0	<0.001
De A *et al.*[47]	268.60	<0.01
Ghone R A *et al.*[67]	164.55 ±14.30	<0.001
Salma O S *et al.*[73]	180 ±94.9	<0.001

Serum Ca level of thalassemia children is within normal range in the present study with an average value of 9.00 ±1.00 (Mean ±SD). Similar finding was seen in studies done by Karim F *et al.*,[1] Sultan S *et al.*[55], Saboor M *et al.*[68], Salama O S *et al.*[75], Mahachoklertwattana *et al.*[73], Stefano *et al.*[74], and Eren and Yilmaz *et al.*[76] who found that serum calcium values were within normal range in thalassemia patients. (**Table 29**)

Table 29: Serum Ca value in study subjects compared to other

	Serum Ca (Mean±SD)	p value
Present study	9.00 ±1.00	0.349
Karim F *et al.*[1]	7.9 ±0.6	<0.05
Saboor M *et al.*[68]	8.83 ±0.58	0.04
Salama O S *et al.*[73]	8.9 ±1.4	0.176

In the present study a statistically significant decrease (p<0.001) was noted in the TIBC of thalassemia children with an average value of 197.97 ±67.442 (Mean ±SD). This finding is in correlation with studies done by Salama O S *et al.*[73], Livrea M. A *et al.*[66], De A *et al.*[47] and Ghone A R *et al.*[67] who also observed significantly decreased serum phosphorus levels in thalassemia patients. (**Table 30**)

Table 30: TIBC value in study subjects compared to other studies

	TIBC (Mean±SD)	p value
Present study	197.97 ±67.442	<0.001
Salama O S *et al.*[73]	246.3 ±131.8	<0.001
Livrea M. A *et al.*[66]	369 ±97	<0.001
De A *et al.*[47]	205.16	<0.001
Ghone A R *et al.*[67]	224.15 ±20.43	<0.001

Sr. Creatinine value of thalassemia children is decreased in the present study with an average value of 0.56 ±0.09 (Mean ±SD). The decreased in Sr. Creatinine in comparison to control subjects also statistically significant with p<0.001. Similar finding of platelet count in thalassemia children was found in studies done by Karim F *et al.*,[1] Bushra M *et al.*[56], Ayyash *et al.*[53] (**Table 31**)

Table 31: Sr. Creatinine value in study subjects compared to other studies

	Serum Creatinine (Mean±SD)	p value
Present study	0.56 ±0.09	<0.001
Karim F *et al.*[1]	0.4 ±02	<0.001
Ayyash *et al.*[53]	0.59 ±0.3	0.01

In present study, Sr. Phosphorus of thalassemia children is increased with an average value of 6.14 ±1.332 (Mean ±SD). The increase in Serum Phosphorus in comparison to control subjects is statistically significant with p=0.002. This finding is in correlation with the studies done by Salama OS *et al.*[73] and Costin G *et al.*[78] who also found significantly higher serum phosphorus in thalassemia patients. (**Table 32**)

Table 32: Serum Phosphorus value in study subjects compared to other studies

	Sr Phosphorus (Mean±SD)	p value
Present study	6.14 ±1.332	0.002
Salama OS *et al.*[73]	5.3 ±1.0	0.01
Costin G *et al.*[78]	5.8 ± 0.2	0.01

In the present study Sr. ALP did not demonstrate any significant difference (p=0.242) between patients and control groups. This finding is in agreement with studies done by Moulas A *et al.*[79], Soliman *et al.*[78], Mahachoklertwattana *et al.*[75], Salama OS *et al.*[73] and Asif M *et al.*[69] Rej R *et al.*[77] had mentioned in their study that iron overload leads to osteoblast poisoning leading to false results for ALP in thalassemia patients. **(Table 33)**

Table 33: Sr. ALP value in study subjects compared to other studies

	Sr. ALP (Mean±SD)	p value
Present study	157.80 ±52.169	0.242
Salama OS *et al.*[73]	194.1 ±37.1	0.055
Soliman *et al.*[78]	178±10	>0.05
Moulas A *et al.*[79]	99 +-13	>0.05
Asif M *et al.*[69]	450.99 ±16.05	0.52

In general, the body iron stores have been found to correlate with serum ferritin levels. However, being an acute phase reactant, single values of serum ferritin are not always reliable. Despite serial measurement serum ferritin is a simple and reliable method to evaluate iron deposition and efficiency of chelation therapy. To evaluate clinical relevance, need for treatment, timing and monitoring of chelation therapy, iron status should be assessed accurately.

In the present study Sr. Ferritin is elevated in the study group with a value of 1302.0 ±469.322 (Mean±SD). The increase in Sr. Ferritin is statistically significant in comparison to the control group with p<0.001. this finding is correlating with studies conducted by Karim F *et al.,*[1] Sultan S *et al.*[55]., Munir B *et al.*[56], Jain C *et al*[59]., Naithani R *et al.*[63], Guimaraes J *et al.*[62], Livrea M. A *et al.*[66], De A *et al.*[47], Ayyash *et al.*[53], Filiz *et al.*[60], Salma OS *et al.*[73] and Soliman *et al.*[78] **(Table 34)**

Table 34: Sr. ferritin value in study subjects compared to other studies

	Sr. ferritin (Mean±SD)	p value
Present study	1302.0 ±469.322	<0.001
Karim F *et al.*[1]	1249 ±59.2	0.005
Naithani R *et al.*[63]	3709.0 ±1625	<0.001
Livrea M. A *et al.*[66]	1866.0 ±996.0	<0.01
De A *et al.*[47]	1548.06	<0.001
Ayyash *et al.*[53]	7162.4 ±3297.3	<0.001
Filiz *et al.*[60]	1300 ±477.14	<0.001
Salma OS *et al.*[73]	881.4 ±245.1	<0.001
Soliman *et al.*[78]	880 ±46	<0.05

SUMMARY

A prospective study was done to assess the changes in hematological and biochemical parameters in thalassemia patients that can help in providing timely correction of any deranged parameter, prevent any severe complications and improve the quality of life in these patients The study was undertaken during the period of 1st December, 2017 to 30th June, 2019 in the Department of Pathology, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura. All patients received multiple units of blood transfusion. Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count, that included Hb, HCT, MCV, MCH, MCHC, RDW and Platelet count using an automated 5-part differential hematology analyzer (SYSMEX XN-1000). 4 ml of blood sample was taken in plain vacutainer and was centrifuged @ 1500 rpm and analyzed for Sr. Iron, Sr. Ca, TIBC, Sr. Creatinine, Sr. Phosphorus, Sr. ALP, Sr. Ferritin using Biochemistry analyzer (VITROS® 5,1 FS Chemistry System).

The salient features observed in this study are:

The age of the patients was within the range of 3 to 14 years. Maximum number of cases were between 6 and 10 years, amounting to 54.3%

Amongst thalassemia patients, the majority were males, 23 (65.7%) with a male to female ratio of 1.9:1.

Thalassemia patients showed a statistically significant ($p<0.001$) decrease in Hb, HCT, MCHC, TIBC, Sr. creatinine and Sr. phosphorus in comparison to the control group.

RDW-SD, RDW-CV, Sr. Fe and Sr. Ferritin were increased in thalassemia patients with a statistically significant difference of $p<0.05$ when compared with control group.

MCV ($p=0.4790$), MCH ($p=0.083$), platelet count ($p=0.090$), Sr. Ca ($p=0.349$) and Sr. ALP($p=0.242$) showed no significant change in values, when compared between study and control groups.

On the correlation of Sr. ferritin with biochemical parameters in thalassemia patients, moderate positive correlation was observed between Sr. ferritin and Sr. Fe, Sr. TIBC, Sr. Phosphorus.

Moderate negative correlation was noted between sr ferritin and MCH, platelet count, RDW-SD, RDW-CV.

There was no correlation between Sr. ferritin and sr. creatinine, Sr. calcium.

Hence regular blood transfusion in thalassemia patients causes derangement in their hematological and biochemical parameters. These abnormalities can lead to many complications in these patients, thus, decreasing the overall quality and span of life. Hence it is important to monitor these parameters in all thalassemia patients undergoing repeated blood transfusions for the management of anemia. Regular assessment of hematological and biochemical parameters will help in providing timely correction of any deranged parameters, prevent severe complications and improve the quality of life in these patients.

CONCLUSION

In a country like India, where thalassemia is highly prevalent in the general population and there is an ever-increasing load of patients we must focus on the prevention of thalassemia. Presently blood transfusion in conjunction with chelation therapy is the most popular treatment approach in symptomatic thalassemia cases. However, bone marrow transplantation should be advocated, if possible.

Thalassemia patients undergoing regular blood transfusions show significant changes in their hematological and biochemical parameters in comparison to non-thalassemic. Amongst hematological parameters, Hb, HCT, MCV, MCH, MCHC and RDW values are usually s like deranged in thalassemia. Also, biochemical parameters like Serum Fe, TIBC, Sr. Creatinine, Sr. Phosphorus, Sr. Ca, Sr. ALP and Serum Ferritin show significant changes in these patients. Therefore, along with Hb, the above-mentioned parameters should also be routinely evaluated in thalassemia patients to prevent associated complications.

It is very important to manage the general health of children with thalassemia and provide them better quality and longer span of life that is free of treatment-related complications so that they can stand with and can contribute to society and country's growth. Hence, we conclude that better management of thalassemia can be provided by continuous monitoring of the parameters evaluated in the present study.

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ANNEXURE-I

B.L.D.E.U.'s SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____ , S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that _____of ___BLDE____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr _____requesting my participation in the study. Apart from routine treatment procedure, the pre-transfusion, post-transfusion and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient/Guardian:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

ANNEXURE-II

PROFORMA

NAME	:	OP/IP No.	:
AGE	:		
SEX	:	D.O.A	:
RELIGION	:	D.O.D	:
OCCUPATION	:		
RESIDENCE	:		
Presenting Complaints	:		
Past history	:		
Personal history	:		
Family history	:		
Treatment history	:		

General physical examination:

Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphadenopathy	present/absent
Edema	present/absent
Built	poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE: WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

CBC

Serum Iron

Serum Calcium

TIBC

Serum Creatinine

Serum Phosphorus

Sreum Alkaline phosphate

Serum Ferritin

KEY TO MASTERCHART

S. No.----------Serial Number

OP No.----------Out Patient Number

IP No.----------In Patient Number

Sr. Ca----------Serum Calcium

Sr. P ----------Serum Phosphorous

Sr. Fe----------Serum Iron

Sr. TIBC----------Serum Total Iron Binding Capacity

Sr. ALP----------Serum Alkaline Phosphate

RBC----------Red Blood Cell

Hb----------Hemoglobin

HCT----------Hematocrit

MCV----------Mean Corpuscular Volume

MCH----------Mean Corpuscular Hemoglobin

MCHC----------Mean Corpuscular Hemoglobin Concentration

RDW (SD)----------Red cell Distribution Width (Standard Deviation)

RDW (CV)----------Red cell Distribution Width (Coefficient of Variation)

MASTER CHART

Study Group:

S. No.	Name	IP/OP No.	Age(yr.)	Sex	Blood Group	Hb (gm/dl)	HCT (%)	MCV (µm3)	MCH (pg)	MCHC (g/dl)	Platelets (lakh/µl)	RDW-SD (fl)	RDW-CV (%)	Sr. Fe (µg/dl)	Sr. Ca (mg/dl)	TIBC (µg/dl)	Sr. Creatinine (mg/dl)	Sr. Phosphorus (mg/dl)	Sr. ALP (IU/L)	Sr. Ferritin (ng/µl)
1	**Pragati Shivappa**	159797/18	14	F	AB +ve	7.6	21.6	69.3	24.7	29.8	2.18	98.4	26.4	163	9	194	0.5	6	65	1240
2	**Preetam S**	26613/18	8	M	A +ve	5.9	18	79.6	26.1	32.8	2.32	111.2	22.3	179	9.5	156	0.5	5.7	113	1400
3	**Channamalla. B.**	19019/18	12	M	B +ve	7.3	28.7	76.2	33.9	28.6	2.62	127.8	24.7	128	9	189	0.5	6.3	120	1160
4	**Vinay N Rajput**	18998/18	13	M	O +ve	9.3	28.3	82.5	27.1	32.9	5.49	61.1	20.3	230	10	235	0.7	5.9	180	1200
5	**Jahageer Murtaza**	26584/18	13	M	O +ve	5.4	17.7	88.5	27	30.5	1.43	65.5	21.8	249	9.9	289	0.7	6.9	105	1170
6	**Shreyansh S**	19054/18	3	M	O +ve	8.8	27.8	88.00	27.8	31.7	2.75	95.5	29.7	133	9.8	310	0.7	6.9	162	1200
7	**Vittal lamani**	158511/18	10	M	A +ve	6.5	20.1	86.3	28.3	32.8	1.9	44.5	14.1	239	9.1	279	0.6	5.5	105	1200
8	**Mehboob.M. D**	157984/18	12	M	A +ve	6.7	21.1	83.4	26.5	31.8	0.72	76.5	25.3	270	4.6	216	0.5	12.1	68	3200
9	**Satish Kumbar**	186439/18	8	M	O +ve	6.6	21.6	94.7	28.9	30.6	1.87	54.8	19.6	295	10.1	314	0.5	8.8	242	1100
10	**Bagesh Parashur**	26594/18	3	M	B +ve	9.6	28.3	79.9	27.1	33.9	6.64	46.4	17.2	77	9.7	315	0.5	6.4	185	560
11	**Vasudev Ramesh**	297737/18	4	M	B +ve	9.4	31	80.7	24.5	30.3	3.64	53.4	18.3	216	9.2	236	0.5	5.1	279	720
12	**Bhumi Dindappa**	18993/18	6	F	A +ve	7.8	27.8	68.3	22.7	29.3	2.98	56.8	22.3	122	7.8	265	0.5	5.3	169	1000
13	**Chinnamma P.**	26596/18	5	F	B +ve	5.4	17	74.3	27.1	31.8	2.19	47.9	17.9	135	8.7	273	0.5	5.3	142	1160
14	**Vaishali V C**	18992/18	7	F	B +ve	7.3	22.5	76	24.7	32.4	1.76	56.8	22.8	248	8.6	189	0.5	5.3	223	1400
15	**Asmita Ganu**	19008/18	4	F	O +ve	6.1	19.1	92.7	29.6	31.9	4.81	48.2	15.3	115	8.1	212	0.6	6.1	170	1000
16	**Shilpa G K**	19003/18	8	F	A +ve	5.9	18.7	89	28.1	31.6	3.33	51.8	19.1	278	9.3	85	0.8	5.8	137	1200
17	**Sarvesh**	22373/18	6	M	B +ve	9.3	29.3	87.7	27.8	31.7	4.95	46.7	15.9	289.0	9.5	134	0.6	5.5	206	1100
18	**Mallikarjun**	7934/18	7	M	B +ve	8	25.3	81.6	25.8	31.6	4.9	44.5	14.7	235	9.1	133	0.6	4.8	215	1100

19	**Mehak Hippargi**	7938/18	6	F	O +ve	7.4	22.7	96.2	31.4	32.6	5.48	53.6	18.9	138	7.9	126	0.5	5.6	151	1230
20	**Samarth**	22335/18	6	M	A +ve	7.1	21.6	79.1	26	32.9	0.77	47.1	16.2	152	9.6	123	0.6	5.8	152	1600
21	**Chetan C. Rathod**	19004/18	7	M	B +ve	5.4	17	85.4	27.1	31.8	1.48	57.4	21.2	58	8.9	127	0.5	4.4	129	1270
22	**Nandini**	7997/18	7	F	B +ve	5.8	18.5	90.2	28.3	31.4	2.23	51.5	15.7	172	8.7	159	0.5	7.3	164	1120
23	**Sunil**	22392/18	10	M	O +ve	7.9	25.2	83.7	26.2	31.3	2.35	45.3	14.9	198	7.6	173	0.7	6.1	126	1250
24	**Ningangauda**	18999/18	14	M	B +ve	5.8	18.5	90.2	28.3	31.4	1.74	48.8	19.3	173	8.2	148	0.6	6.4	223	1700
25	**Afsana Hippargi**	22339/18	8	F	B +ve	7.3	22.5	76	24.7	32.4	1.81	49.7	18.1	162	8.6	169	0.6	4.7	164	1240
26	**Akash shivu C**	26623/18	13	M	O +ve	4.3	17.6	61	19.9	29.3	3.42	45.8	18.9	349	9.2	173	0.5	5.9	161	1400
27	**Khushi Shivpur**	22340/18	3	F	AB +ve	9.3	28.1	83.1	27.5	33.1	3.71	46.2	22.3	129	9.4	170	0.6	5.8	192	1000
28	**Kasimsab**	229892/18	13	M	B +ve	6.7	20.3	69.9	24.4	30.5	3.86	49.7	21.5	161	9.5	145	0.5	6.8	258	2480
29	**Lokesh C.**	18995/18	3	M	AB +ve	9.6	28.8	85.7	28.6	33.3	2.58	43.7	13.7	112	8.3	198	0.5	6.4	172	870
30	**Shivani S madar**	25952/18	7	F	O +ve	5.3	16.9	78.6	24.7	31.4	1.27	52.6	18.9	161	7.9	131	0.4	4.7	162	1280
31	**Narendra Mali**	34064/18	11	M	AB +ve	7.6	21.6	69.3	24.7	29.8	2.26	49.7	19.4	163	9	194	0.5	6	65	1150
32	**Tipanna pujappa**	26592/18	9	M	B +ve	5.9	18	79.6	26.1	32.8	2.12	57.8	21.7	179	9.5	156	0.5	5.7	113	1520
33	**Paigamber Patel**	27741/19	10	M	B +ve	7.3	28.7	119	33.9	28.6	1.96	48.8	19.3	128	9	189	0.5	6.3	120	1100
34	**Siddartha shreesh**	26625/18	9	M	B +ve	9.3	28.3	82.5	27.1	32.9	2.19	49.2	17.3	230	10	235	0.7	5.9	180	1250
35	**Suraksha yellappa**	22372/18	8	F	B +ve	3.5	10.4	81.3	27.3	33.7	1.7	46.3	17.3	235	9.2	289	0.7	6.9	105	2000

Control Group:

S. No.	Pt. Name	OP/IP NO.	Age(yr.)	Sex	Hb (gm/dl)	HCT (%)	MCV (µm3)	MCH (pg)	MCHC (g/dl)	Platelets (lakh/µl)	RDW-SD (fl)	RDW-CV (%)	Sr. Fe (µg/dl)	Sr. Ca (mg/dl)	TIBC (µg/dl)	Sr. Creatinine (mg/dl)	Sr. Phosphorus (mg/dl)	Sr. ALP (IU/L)	Sr. Ferritin (ng/µl)
1	**Geeta patil**	250327/18	9	F	12.2	36.1	78.4	26.6	33.9	3.86	39	13.7	148	14	219	1.1	6.5	137	121
2	**Tanushree T**	346480/18	9	F	13.4	41.2	82.1	26.5	36.2	3.88	42.4	14.44	134	13.4	302	0.8	5.9	178	152
3	**Sachin S.**	211644/18	12	M	14.5	46.9	89.3	29.4	38.2	2.02	36.1	12.7	165	9.5	286	1.2	7.4	148	98
4	**Shivamma P**	11594/18	11	F	15.4	46.3	79.8	26.4	35.4	4.02	33.3	13.1	112	8.3	299	1.3	6.3	184	128
5	**Aryan**	144557/18	13	M	13.5	41.4	88.5	27.1	36.2	2.09	35.8	13.2	147	9.4	360	0.9	6.6	105	142
6	**Sadiya sheikh**	3368220/18	11	F	16.2	46.5	92.7	30.2	33.1	2.19	38.1	17.8	179	8.9	273	1.2	6.9	162	135
7	**Amrutha S.**	19383/18	11	F	12.2	41.1	91.3	27.4	36.2	4.63	39.7	14.5	195	8.9	296	0.8	6.4	184	122
8	**Shivani rugi**	151102/18	6	F	14.3	43.2	83.4	26.5	31.4	4.12	35.4	21.6	158	8.8	336	1.2	7.5	147	117
9	**Aryan kopad**	217885/18	8	M	14.8	42.1	94.7	28.9	38.4	2.83	39.8	19.9	184	9.4	378	0.94	6.7	164	127
10	**Chaitra**	169300/18	13	F	15.4	46.3	79.9	27.1	36.3	1.93	35.3	12.7	112	10.5	289	1.4	5.9	181	132
11	**Sadashiva**	151107/18	10	M	13.4	40.1	80.7	29.9	29.9	4.02	38.1	13.5	172	8.7	389	0.7	6.5	179	128
12	**Shrinidhi**	29095/18	4	F	14.7	41.2	68.3	26.4	36.4	3.71	35.3	12.4	194	10.3	299	0.9	7.2	169	90
13	**Kavitha P.**	39762/18	7	F	16.0	43.8	74.3	27.1	34.8	3.35	42.6	14.5	179	8.9	359	1.3	6.9	210	120
14	**Kaveri B K**	33636/18	12	F	13.3	39.8	76.0	28.3	32.8	3.92	36.1	12.9	149	9.4	385	1.8	6.4	163	137
15	**Rohit vasant**	17312/18	7	M	15.6	41.3	92.7	29.6	34.6	2.24	35.1	13.2	184	8.8	358	0.8	7.8	174	60
16	**Renuka kale**	250359/18	4	F	16.3	45.2	89.0	28.1	36.4	1.72	44.2	13.7	156	10.3	402	1.1	5.6	186	110
17	**Anushka B.**	29080/18	4	F	15.3	44.3	87.7	27.8	38.2	2.55	33.1	12.5	148	14	347	0.9	8.5	199	95
18	**Deepak kot**	276400/18	7	M	16.5	46.2	81.6	25.8	33.5	1.78	38.7	15.6	137	13.4	379	1.2	6.5	215	87
19	**Basavraj S**	325302/18	9	M	14.3	39.3	96.2	31.4	36.4	3.47	36.2	12.4	155	9.5	358	1.5	4.8	151	110
20	**Kavita shirol**	213367/18	10	F	14.3	41.1	79.1	28.4	32.9	2.34	38.6	13.5	149	8.3	359	0.8	6.4	152	84
21	**Jaishree M.**	335703/18	11	F	13.8	43.2	85.4	27.1	31.8	2.13	36.1	14.3	185	9.4	347	1.3	7.9	179	89
22	**Sachin banjat**	304871/18	12	M	13.7	41.1	90.2	28.3	34.5	3.23	34.2	13.6	169	8.9	371	0.7	6.4	173	77

23	**Basavraj S.**	309498/18	7	M	16.2	46.3	83.7	26.2	36.3	1.95	34.9	12.3	176	8.9	385	1.1	6.5	184	140
24	**Shilpa nagane**	303463/18	13	F	12.4	38.7	90.2	28.3	37.4	3.12	34.8	16.4	197	8.8	359	1.2	6.4	223	83
25	**Sadiya Naiko**	251932/18	10	F	12.3	40.1	76.0	24.7	35.3	3.91	32.9	15.8	163	7.9	329	1.2	7.2	158	120
26	**Shilpa sajjan**	301514/18	8	F	14.2	41.2	71.6	32.3	36.6	3.18	35.4	19.7	168	9.9	340	1.1	7.9	184	128
27	**Udaya kumar**	36645/18	9	M	13.8	43.2	83.1	27.5	32.8	2.83	38.1	20.1	173	9.5	374	0.8	6.4	192	168
28	**Shivapadma**	10777/18	9	F	14.2	4.1	69.9	29.3	37.3	2.89	36.9	14.6	199	8.5	298	1.2	7.8	157	89
29	**Basavraj jatti**	398680/18	4	M	13.8	40.3	85.7	28.6	34.9	4.11	37.2	14.5	158	9.4	276	1.1	7.3	147	152
30	**Nandini kava**	4421/18	6	F	15.4	43.7	78.6	29.7	35.2	3.13	39.4	13.8	173	8.6	367	1.3	7.4	162	78
31	**Sunil Bhosle**	226774/18	8	M	11.5	36.9	89.3	29.4	38.2	2.98	38.2	16.3	165	9.5	286	1.2	7.4	148	128
32	**Suman S**	218484/18	7	F	15.4	46.3	79.8	26.4	35.4	3.25	36.2	14.4	112	8.3	299	1.3	6.3	184	176
33	**Gorav Wali**	135466/18	5	M	13.5	41.4	88.5	27.1	36.2	2.63	38.3	16.2	147	9.4	360	0.9	6.6	105	139
34	**Nandini R**	37482/18	7	F	16.2	46.5	92.7	30.2	33.1	2.84	39.9	14.6	179	8.9	273	1.2	6.9	162	77
35	**Sangeeta pol**	240534/18	10	F	12.2	41.1	91.3	27.4	36.2	2.56	36.4	15.6	195	8.9	296	0.8	6.4	184	90