VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN CHILDREN WITH THALASSEMIA MAJOR AND ITS CORRELATION WITH PULMONARY ARTERIAL HYPERTENSION AND SERUM FERRITIN

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LIST OF ABBREVATIONS USED

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

SD----------Standard Deviation Sr.

Ca---------- Serum Calcium

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

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

Hb.....Haemoglobin

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

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

MCH ---------- Mean Corpuscular Haemoglobin

MCHC ---------- Mean Corpuscular Haemoglobin Concentration

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

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

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

PAH ----------Pulmonary hypertension

BMP---------- Bone morphogenetic proteins

VEGF ---------- Vascular Endothelial Growth Factor

HIF ---------- Hypoxia Inducible Factor

PA ---------- Pulmonary artery

PAH ----------Pulmonary artery Hypertension

TI ---------- Thalassemia Intermedia

TM---------- Thalassemia Major

SCD ---------- Sickle Cell Disease

ABSTRACT

Background:

being one among most prevalent inherited diseases in Asia and the majority of the world, thalassaemia has attracted considerable scientific interest. Metabolic imbalance, iron overload, persistent hypoxia, and cell damage are also present. Haemolysis, anaemia, and poor erythropoiesis are the end results of all physiological alterations.

For survival, the majority of patients require either bone marrow transplantation or transfusion. Their lifespan and quality of life have increased thanks to regular transfusions and chelation therapy, but these treatments have also been linked to changes in haematological and biochemical markers.

Aim:

1. The aim of the study was to assess serum VEGF level in children with beta-thalassemia major as a marker of angiogenesis and as an indicator of pulmonary hypertension
2. And to correlate the VEGF levels with serum Ferritin and Pulmonary arterial Hypertension thalassaemic children.

Materials and Methods:

The study's sample size consists of 32 beta-thalassemia major individuals. The paediatric wing of the Shri B M Patil hospital in Vijayapura will be used to enrol patients with beta-thalassemia major. Based on clinical and haematological criteria, patients who have beta-Thalassemia major are diagnosed (CBC and haemoglobin electrophoresis). In the three weeks before to the trial, no patient

would have gotten a blood transfusion. The study did not include those who had additional hemoglobinopathies, cancer, or other causes of anaemia. All enrolled children's parents must offer their prior written approval for their child to take part in the study. All cases' histories will be collected in accordance with the proforma. Anthropometric measurements, vital signs, and a look for any problems were all part of the clinical examination.Laboratory investigations includes CBC, serum ferritin and serum VEGF. ECHOCARDIOGRAPHY will be done in all children in the study population. Then the data will be analysed for the correlation between serum VEGF, Ferritin and PAH

Sample collection and VEGF assay

Centrifuged for 10 minutes then sera will be separated and stored at −70°C. Thereafter, VEGF levels shall be measured by enzyme-linked immunosorbent assay using the VEGF ELISA kit according to the instruction manual.

Results:

In our study some of the salient features are,

Majority of the children are in the age group 6-10 years. 60% had stunting and about 51% were having acute malnutrition. Almost 60 % of them were having PAH. The VEGF levels were significantly higher in the study group which showed significant positive correlation with PAH and serum ferritin both with p value of <0.001

Conclusion:

In a country like India, where thalassaemia is highly prevalent in the general population and there is an ever-increasing load of patients we must focus on the prevention of thalassaemia. Presently blood transfusion in conjunction with chelation therapy is the most popular treatment

approach in symptomatic thalassaemia cases. Hence, we conclude that better management of thalassaemia can be done by frequent transfusions to maintain optimum Hb, along with that, adequate chelation and frequent evaluation of serum ferritin, VEGF and 2D echocardiography has to considered.

KEY WORDS: Thalassaemia, Blood transfusion, Haematological, Biochemical Parameters.

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INTRODUCTION

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

Imbalanced globin protein synthesis is the key factor in determining the severity of the disease in thalassaemia syndromes.[1] Thalassaemia occurs because of the presence of the homozygous state of one of the thalassaemia genes or haemoglobin (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, haemolysis and anaemia.[2]

Thalassaemia 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 thalassaemia patients have are known to cause abnormalities in their haematological, biochemical and cardio pulmonary parameters. Hence this study was done to find out these abnormalities in thalassaemia patients in comparison with

controls. Regular monitoring of haematological 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 haematological, biochemical parameters and echocardiography to look for PAH in thalassaemia patients and to correlate the VEGF levels, PAH and serum ferritin so that timely correction of any deranged parameters can prevent any severe complications and to improve quality of life.

Objectives:

1. The aim of the study was to assess serum VEGF level in children with beta-thalassemia major as a marker of angiogenesis and as an indicator of pulmonary hypertension

2. And to correlate the VEGF levels with serum Ferritin and Pulmonary arterial Hypertension thalassemic children.

REVIEW OF LITERATURE

Thalassaemias belong to a group of haemoglobinopathies, 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 haemoglobin 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 thalassaemia. 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 was published 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 thalassaemia to be an atypical haemoglobin structure.[2] Dr. Cooley suggested that the disease was hemolytic in nature. In 1960, Physicians treating thalassaemia 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 in thalassaemia patients.

With the advancements in the field of molecular biology, thalassaemia has been widely studied. Newer techniques in the past decades have made identification of the genes responsible for the production of globin chains and to measure the number of globin chains. Thalassaemia is the worlds most common genetic disorder. It is a major health problem in many countries.

Prevention is seen as an essential part of management. Because of the burden of the disease, several countries with high incidence of thalassaemia are carrying out mass screening and educational programs to detect the carriers and to perform prenatal diagnosis with genetic counselling to the parents who are carriers.

GLOBAL LOAD AND EPIDEMIOLOGY OF THALASSAEMIA:

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

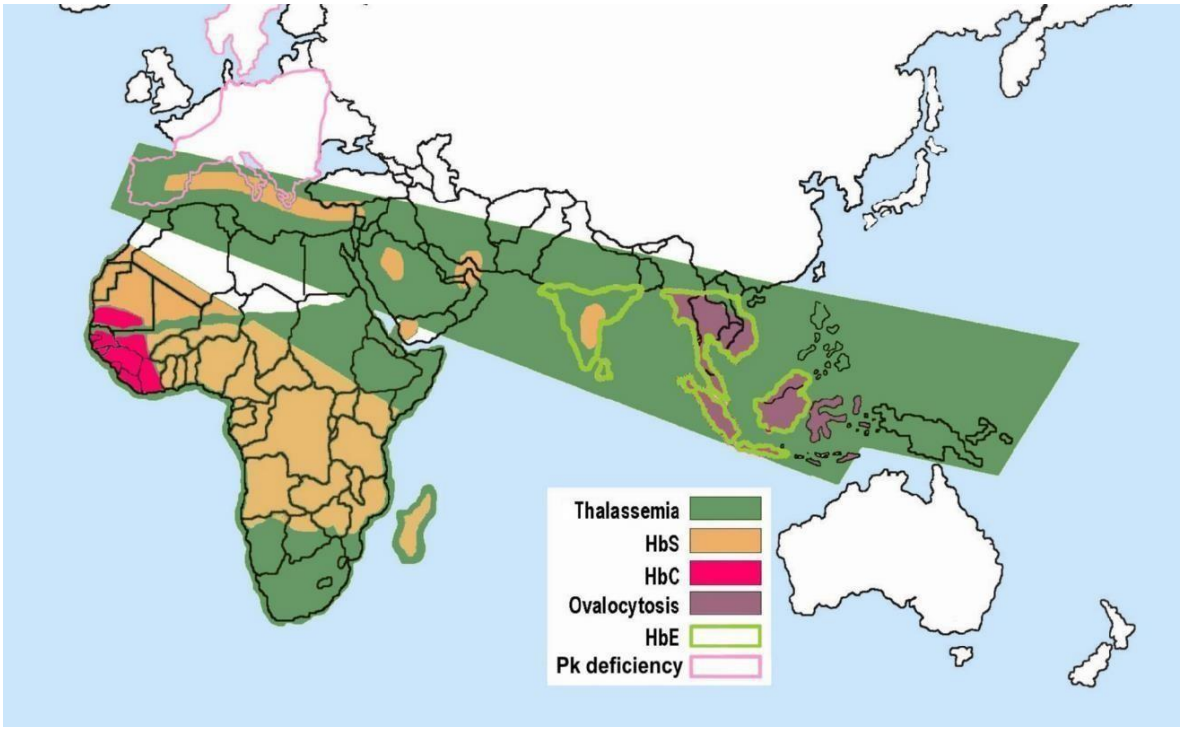

Fig 1: Global thalassaemia distribution

Around 1-20 % of the population in these regions are β thalassaemia carriers.[4] Milder types are prevalent in 10-20 % of the population in sub Saharan Africa, to >40% Middle Eastern and Indian population. In certain places like Papua New Guinea, and some some parts of north east india, prevalence is high as 80.[4]

Presently there are about 270 million thalassaemia carriers in the world. Of these, 80 million are β-thalassaemia carriers. According to some studies about a half a million babies are born who suffer with a serious haemoglobin disorder each year (among which, about 20,000 with β-thalassaemia major). Majority of those happen in low or middle income nations.[4,5,6]

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

Thalassaemia care centres were started in the 1970's in certain cities such as Mumbai and Delhi. Indian Red Cross Society and The International thalassaemic federation help thalassemia patients by arranging blood camps. Emphasis on thalassaemia care has been put forth by the Government of India through its twelfth five year Plan. In several states, blood transfusion and chelation are provided at no cost. Furthermore, several centres have started BMT and stem cell therapy facilities that helps managing the thalassemia patients.

HAEMOGLOBIN STRUCTURE AND FUNCTION:

In 1960 Dr. Max Perutz demonstrated the 3-dimensional molecular structure of

Haemoglobin 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.

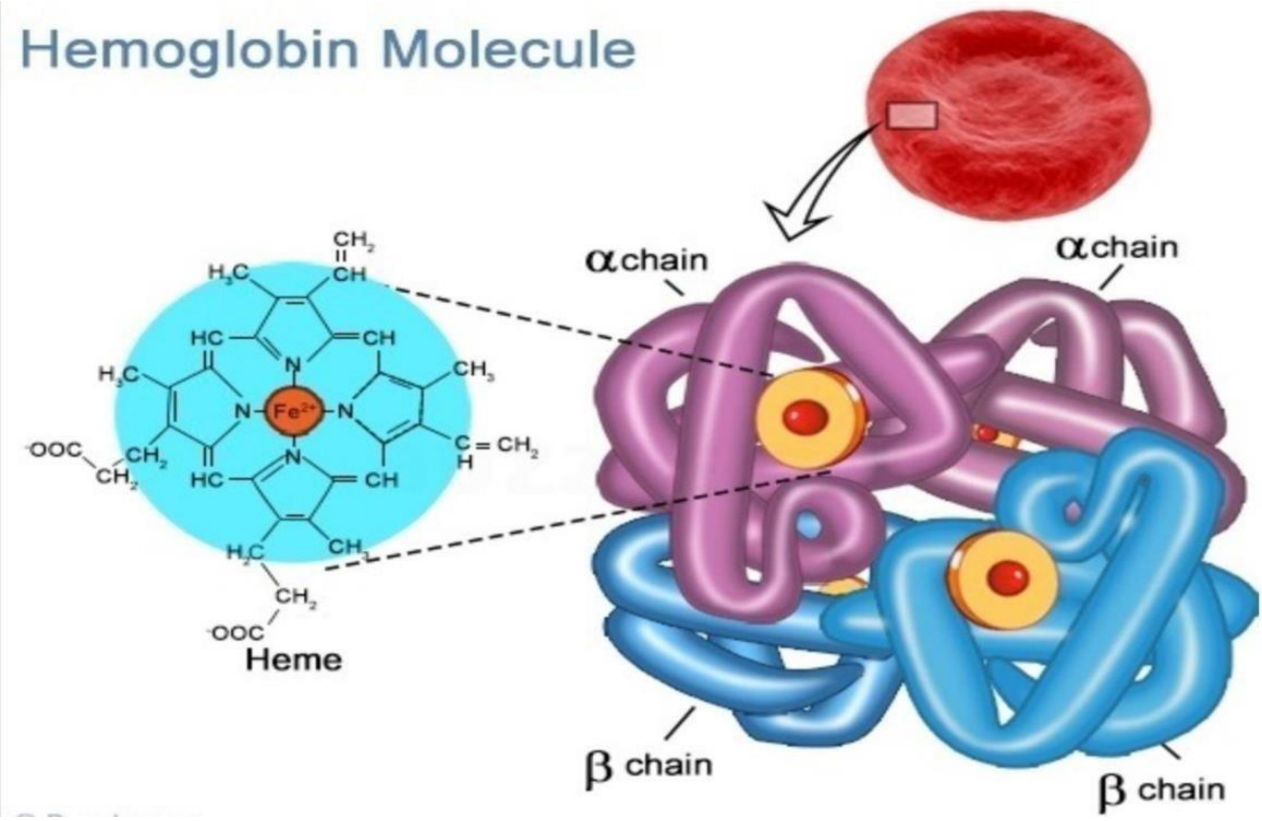

Fig 2: Structure of haemoglobin molecule

Different types of haemoglobins 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 αchains (141 amino acids) and two β chains (146 amino acids). In adults, HbA (α2 β2) is the major haemoglobin while HbA2 (α2δ2) is present in minor amounts.[5] HbF(α2γ2) is majorly fetal haemoglobin.[5]

Each globin protein chain encases one heme, comprised of a protoporphyrin IX ring fused with one ferrous molecule (Fe2+). One heme can bind to one Oxygen (O_2) molecule; one Hb molecule of can carry up to four oxygen O_2 molecules.[5]

amino acid sequence in different globin chains are homologous to one another. Each of these globin chains has 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, creating a hydrophoebic pocket in 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 surface (i.e., where α 1 β 2 contacts) between the non α chain of the one dimer and α like chain of the other dimer.[5]

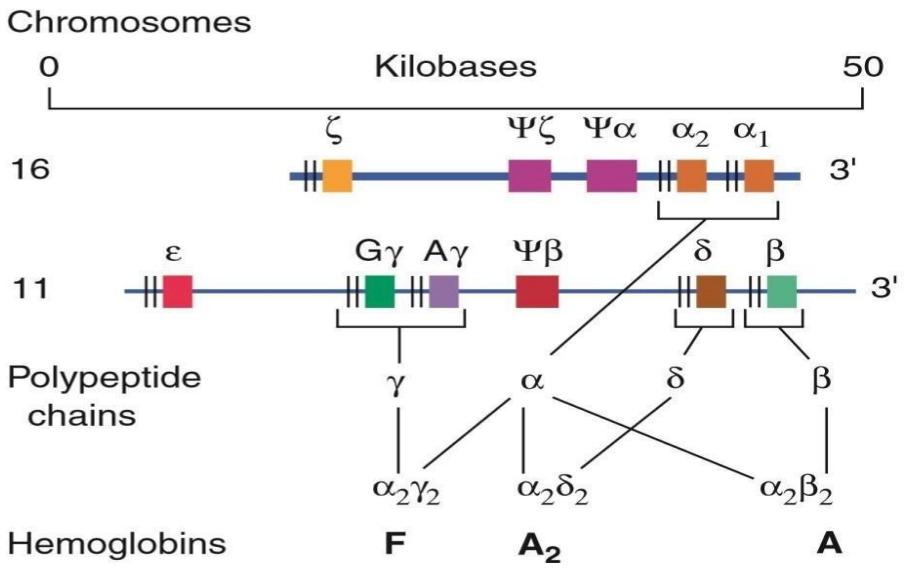

Fig 3: The globin genes. A like genes (α,δ) are encoded by chromosome 16; the β like genes (β,γ,δ,ε) are encoded by chromosome [11]. δ and ε genes encode embryonic globins.[5]

Individual globin chain is not soluble whereas HB tetramer is soluble. The unpaired globin chain forms inclusions by precipitation. 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 another globin chain with which it pairs. Reversible O_2 binding of Hb and solubility are the main properties deranged in most of the haemoglobinopathies. 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 deoxyhaemoglobin. 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 thalassaemia syndromes:

Thalassaemia syndromes are mainly classified into three types-

1. β thalassaemia- This is the most common type all over the world. The different variants are:
    - Thalassaemia major
    - Thalassaemia intermedia
    - Thalassaemia trait
    - Thalassaemia minima
2. α Thalassaemia- 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 Thalassaemia 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- Thalassaemia
    - Hb E- Thalassaemia
    - Hb D- Thalassaemia
    - δ – β- Thalassaemia
    - HPFH- Hereditary persistence of fetal haemoglobin
    - γ- Thalassaemia
    - δ- Thalassaemia

CLINICAL FINDINGS:

Thalassaemia is characterised by anaemia, chronic hemolysis, and ineffective erythropoiesis (Table-1). Anaemia is caused by of decreased HbA synthesis, faulty erythropoiesis, and

haemolysis. The severity of anaemia depends on the number of genes affected and the mutations in those genes. In some cases, hypoxia caused by anaemia is exacerbated by the presence of abnormal haemoglobins with abnormally elevated oxygen affinity such as (HbH). These haemoglobins do not readily release oxygen to the tissues. Chronic haemolysis has a number of negative consequences. As the spleen is the major site for extra medullary haematopoiesis, splenomegaly is very common finding in thalassemia.. The process of erythrocyte destruction can occasionally overburden the spleen, resulting in functional hyposplenism. Gallstones can form as a result of chronic haemolysis. Chronic demand for erythrocytes is also harmful. In response to the demand, the bone marrow increases erythropoietic activity, which results in erythroid hyperplasia and, in some severe thalassaemias, bone marrow expansion, which causes bone thinning. As a result, patients develop skeletal abnormalities as well as pathologic fractures. There is an increase in iron demand to support erythropoietic activity, which stimulates iron absorption from the gastrointestinal tract (GIT). Because this extra iron is not utilized entirely for erythropoiesis, it accumulates in other tissues such as marrow, liver, and spleen macrophages. As a result of this process, iron gradually builds up in the parenchymal cells of different organs and negatively impacts their functionality. Common organs impacted by iron poisoning include the liver, pituitary, heart, and bones. Cirrhosis, hypogonadism, growth retardation, arrhythmias, cardiomyopathies, and pathologic fractures may arise from this.8 Extramedullary erythropoiesis in the liver and spleen may also occur in conjunction with ineffective erythropoiesis in the bone marrow.

Spleenic Masses big enough to cause compression syndromes can be caused by extramedullary erythropoiesis. Thalassemia-affected pregnant women have physiological demands that have an impact on the growing foetus. more than the mother, actually. The growing foetus may undergo stunted growth, an early birth, or even intrauterine death if the mother's oxygen

saturation drops below 70 mmHg. To increase O2 delivery, blood transfusions are frequently administered to pregnant women who present with Hb 9–10 gm% around the time of delivery. Pregnant women should receive deferoxamine both during and after the transfusion to prevent iron overload, which is primarily brought on by the interaction of transfusion therapy and increased iron absorption.

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Table 1: Clinical and laboratory findings in thalassaemia:

Clinical Finding	Pathophysiology Laboratory	Finding
Anaemia/hypoxia	↓ haemoglobin production/erythropoiesis Ineffective erythropoiesis Presence of high-affinity haemoglobins (HbH and Hb Bart's) ↑ extravascular haemolysis	↓ nRBC count, ↓ haemoglobin, ↓ hematocrit Microcytic / hypochromic RBCs ↓ MCV, ↓ MCH, ↓ MCHC Increased Reticulocyte count Anisocytosis and poikilocytosis Target cells, basophilic stippling, nRBCs, Bone marrow erythroid hyperplasia, ↑ RDW, Abnormal haemoglobin electrophoresis
Splenomegaly/ haemolysis	Splenic removal of abnormal erythrocytes Ineffective erythropoiesis	↑ Bilirubin ↓ Haptoglobin
Gallstones	↑ intravascular and extravascular haemolysis	↑ 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 THALASSAEMIA:

Normally equal amounts of α-chains and β-chains are synthesized by the maturing erythrocyte. In α and β thalassaemia, 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 anaemia in thalassaemia.[7] Some of these effects are:

(1) Decreased total erythrocyte haemoglobin production.

(2) ineffective erythropoiesis.

(3) chronic haemolysis.

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 haemolysis.[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 haemoglobin also has a very high oxygen

affinity. Thalassaemia like conditions (e.g. HbE) having structural Hb variants can result in decreased synthesis of globin chains, giving the clinical picture of thalassaemia.[7]

Ineffective erythropoiesis:

Anaemia in thalassaemia is because of faulty erythropoiesis and shortened lifespan of the RBCs. Ferrokinetic and erythrokinetic studies have concluded that ineffective erythropoiesis contributes maximum to anaemia because of the massive lysis of RBC precursors in the marrow.[2]

Haemolysis:

Destruction of abnormal RBCs causes anaemia in thalassaemia patients but is not as much important as faulty erythropoiesis to determine the degree of severity of anaemia. In various studies using Ashby or 51Cr-labelling methods, it has been observed that the survival time of RBCs of thalassemia patients ranged from 7-22 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 cell 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]

Response to anaemia:

In response to chronic hypoxia, profound anaemia stimulates increased production of erythropoietin. In pioneer studies, thalassaemia patients with Hb of 7.0 g/dl or less[2] **,** showed significant elecation of EPO in blood and urine

Erythroid expansion:

β thalassaemia 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 erythroid expansion causes majority of the clinical features.. Particularly important are bone deformities and formation of extramedullary tumor masses in few.[2]in young children it causes severe metabolic burdern. As a result, growth of these children becomes slow. There is poor muscle development, along with reduced body fat and body weight.[2] Exacerbation of the anaemia takes place because blood shunts 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 urine levels of urates and serum uric acid levels compared to normal.

Hypersplenism and Splenomegaly:

The exact mechanism of splenomegaly in thalassaemia is not clear. It has been mentioned that exposure of the splenic reticuloendothelial elements to abnormal RBCs in β thalassaemia 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 much abnormal RBCs in circulation. Thus, these patients don't present with significant spleenomegaly.[2] In 1963 it was observed that RBCs with inclusions appear in the peripheral blood only after spleenectomy. This shows the role of spleen in the development of anaemia in thalassaemia.[2,24] Extramedullary hemopoiesis may also contribute to splenomegaly.

Splenomegaly leads to pancytopenia as a result of sequestration. 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 worsens anaemia by increasing plasma volume and increases load in the heart. Plasma volume expansion results from the vascular shunt mechanism.

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

Iron overload:

It is commonest complication of thalassaemia causing iron deposition in various tissues and deposition of iron in tissues and multiple organs.[12,25-28] Abnormal iron absorption from GIT and repeated blood transfusions carry excess iron into the body. Iron overload in patients with thalassemia intermedia and patients undergoing inadequate transfusions, is caused due to increased GI absorption of iron.[2]

Mechanisms and rate of iron overload:

Excess of iron in the body generates toxic free radicals and causes extensive tissue damage. Normally, in plasma iron is in a bound state to transport and storage proteins. For example, transferrin by binding to iron prevents its catalytic action in free radical production.[27] hence, in iron overload excess iron is not bound to transferring and becomes detectable in the blood casuing 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 proteins like transferrin, ferrittin and haemoglobin.

Free iron can be released if an oxidant stress on iron-containing proteins is imposed. Liver, heart and the endocrine organs are the most affected as a result of iron overload.[2]

Mechanisms of tissue damage due to iron overload:

Though iron is essential 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 in a bound state to transport or storage proteins. For example, transferrin by binding to iron prevents its catalytic action in free radical production.[27] hence, in iron overload excess iron is not bound to transferring and becomes detectable in the blood casuing 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 haemoglobin. 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 PRESENTATION OF IRON OVERLOAD:

Liver, cardia and endocrine organs are usually affected. Frequently thyroid, parathyroid, pituitary, pancreas and the gonads are affected. Sometimes they are not clinically significant initially but investigations are required to detect early. Monitoring of iron overload should be done in all thalassaemia patients from time to time and appropriate treatment should be given. Parathyroid and thyroid dysfunction maybe subclinical in the beginning. Hence serum calcium,

thyroid function tests and blood sugar must be checked often to diagnose any underlying endocrine dysfunction.

Repeated blood borne infections and iron overload causes insult to the liver. So it is necessary to do liver function tests at least once in 6 months. And it is also important to keep monitoring growth.

In India, growth retardation is observed in most of the patients. sexual maturity age is also delayed. Growth retardation can be brought on by a number of factors, such as poor compliance with routine transfusions, inadequate chelation, growth hormone deficit brought on by pituitary hemosiderosis, somatomedins and sex hormone deficiency, and chronic hypoxia brought on by anaemia. It is debated whether subclinical hypothyroidism requires medication. When treatment is deemed unnecessary, patients must be closely monitored. Treatment with L-thyroxine is an option in cases of overt hypothyroidism, which is defined by low T4 levels as well as signs and symptoms include fatigue, weight gain, cold intolerance, constipation, and others. The use of intensive chelation therapy can reverse abnormal thyroid functions at an early stage.

Cardiovascular complications, such as cardiac failure and arrhythmias, account for 70% of thalassaemia deaths. Excess iron accumulates in the cardia, particularly in myocardium and conduction system. free iron accumulates in cardiac tissue then it damages cells through lysosomal rupture and lipid peroxidation . Overt cardiomyopathy, left atrial dilatation, aorta root dilatation, left ventricular systolic and diastolic dysfunction are all cardiac complications in thalassaemia children. Cardiac involvement can be detected early by evaluating serum ferritin and performing cardiac function tests such as ECG, echo, and so on. T2 weighted cardiac MRI is gold standard method for cardiac evaluation, but it is only available in a few centres..

PULMONARY HYPERTENSION IN THALASSEMIA:

Definition of pulmonary hypertension: Secondary PAH is emerging as a significant cause of mortality and morbidity in patients with hemolytic anemia.[42–44] ph is defined as a mean pulmonary artery pressure of ≥ 25 mmHg at rest or≥ 30 mmHg during exercise. In our study, we have classified the ph as mild, moderate and severe. i.e. (25-39, 40- 59, ≥60mmHg respectively)

Prevalence and risk factors: PAH is becoming more common in thalassemia and contributes to heart failure and death.43,44 Studies of both thalassemia intermedia and thalassemia major show that adolescents frequently have undetected PAH with a prevalence of 60-75% reported.51, In one study, 60% of thalassemia intermedia patients had a pulmonary systolic pressure greater than 30 mmHg with preserved ejection fraction.63,64

The degree of anaemia, the number of transfusions, and serum ferritin levels did not appear to correlate with risk in one study of patients with thalassemia major, but a more recent report discovered ferritin to be a strong indicator of an elevated TRV in patients with both thalassemia major and thalassemia intermedia. Larger studies are required to resolve these discrepancies. Inadequately transfused thalassemia patients may be more susceptible to the onset of PAH because careful adherence to chronic transfusion and chelation therapy to prevent iron overload minimises the risk of heart failure and prevents PAH..

Pathophysiology:

Platelets, the coagulation system, erythrocytes, and endothelial cells, as well as inflammatory and vascular mediators, all play a role in the aetiology of PAH in thalassemia. Although overlap in mechanisms contributing to vasculopathy and PAH is expected in all forms

of thalassemia, the pathophysiology of PAH in patients with TI is fundamentally different from that of TM. Hemolysis is most likely a driving force in non transfused TI patients, whereas iron overload and oxidative stress play a larger role in TM patients on transfusion therapy. There will be concurrent, varying degrees of involvement from the long-term effects of splenectomy, red cell membrane disease, coagulation abnormalities, poor nitric oxide bioavailability, excess arginase activity, platelet activation, oxidative stress, iron overload, and chronic hemolysis.

Endothelial dysfunction, which increases hypercoagulability and in situ thrombus formation within the pulmonary artery walls and leads to morphological and functional changes in pulmonary vasculature, is brought on by tissue injury, a disrupted arginine-nitric oxide pathway, and oxidative stress (Fig. 1). By releasing both erythrocyte arginase[51] and cell-free haemoglobin at the same time, hemolysis shuts off the arginine-nitric oxide pathway. [42,44,52,52] Nitric oxide and its necessary substrate, arginine, are both quickly depleted[57,62].

Additionally, intravascular hemolysis may promote a procoagulant condition. Because of the decreased nitric oxide bioavailability in hemolytic diseases, nitric oxide loses its typical capacity to suppress platelet activation, tissue factor expression, and thrombin generation[54,63]. Additionally, free heme stimulates the production of endothelial tissue factor[63], opening up a further pathway via which hemolysis heightens anomalies in coagulation. Hemolysis, iron overload, and cycles of ischemia and reperfusion all contribute to the pathogenesis of PAH and worsen oxidative stress, which is another important pathophysiologic process. Through additional processes, iron overload, which is found in transfused thalassemia patients, impacts ph. It causes left and right cardiac hemosiderosis, interstitial pulmonary fibrosis, and interstitial pulmonary fibrosis[59], all of which contribute to cardiac dysfunction and influence pulmonary vascular resistance. [59] Further

vasoconstriction and an increase in pulmonary vascular resistance are caused by chronic anaemia and hypoxia.

Finally, thalassemia may cause changes in the glutathione buffering system that prevent RBC from tackling oxidant stress, predisposing them to hemolysis. Nitric oxide bioavailability is ultimately affected by hemolysis and oxidative stress, which results in the clinical symptoms of PAH (Fig. 2). [52–54] We have found that the severity of PAH in sickle cell disease (SCD) and biomarkers of hemolytic rate, including levels of arginase activity in the plasma, are related to a decrease in RBC glutamine levels and abnormalities in RBC glutathione metabolism. In the process of producing NADPH, glutamine, a key precursor in nicotinamide adenine dinucleotide phosphate (NADPH) biosynthesis, is converted to the glutathione substrate glutamate. Thus, glutamine serves as an antioxidant. Additionally, glutamine acts as a precursor in the citrullinearginine pathway, which produces arginine from scratch. The bioavailability of arginine and nitric oxide may be impacted by its ph depletion. Glutamine treatment is being looked into for its potential to reduce oxidative stress in individuals with thalassemia and after first showing promise in SCD[62].

PAH is thought to be a disease process involving altered arginine metabolism or decreased bioavailability, according to mounting data. [57,62] It is discovered that dysregulated arginine metabolism, just like in SCD[62], also occurs in thalassemia patients[60], pointing to a condition of decreased arginine bioavailability in both hemoglobinopathies. [38,39]This supports a more significant, genotype-independent function for arginine bioavailability in the aetiology of vasculopathy.

Patients with thalassemia contain high levels of the arginase in their RBC. [42] The excess arginase activity reported in thalassemia is caused by hemolysis, hypoxia, and elevated thrombin

levels. This is an important issue as enhanced arginase activity has been linked to the pathophysiology of PAH in several disease conditions. [31,37,38,43]

L-arginine is converted into l-ornithine and polyamines by arginase, which are necessary for collagen synthesis and the proliferation of smooth muscle cells. Therefore, arginase activation promotes abnormal arterial wall remodelling and neointima formation, which contributes to the structural alterations seen in hemoglobinopathies patients' lungs. The arginases trigger a mechanism that contributes to a proliferative vasculopathy by shifting ornithine metabolism.

hemolysis-associated PAH is becoming a significant factor in thalassemia, especially for patients. TI.[54,22] Red cell apoptosis, increased free aberrant nitric oxide, anaemia, and plasma haemoglobin both nontransfused tissues and blood contain oxidising affected by transfusions.[50] A 202 TM patient study concluded that heart failure is prevented by strict adherence to chronic transfusion and chelation therapy to prevent iron overload, and stops PAH.[44,48,60] Even though more aggressive transfusion programmes might offer more security from the PAH,[44,60,] development

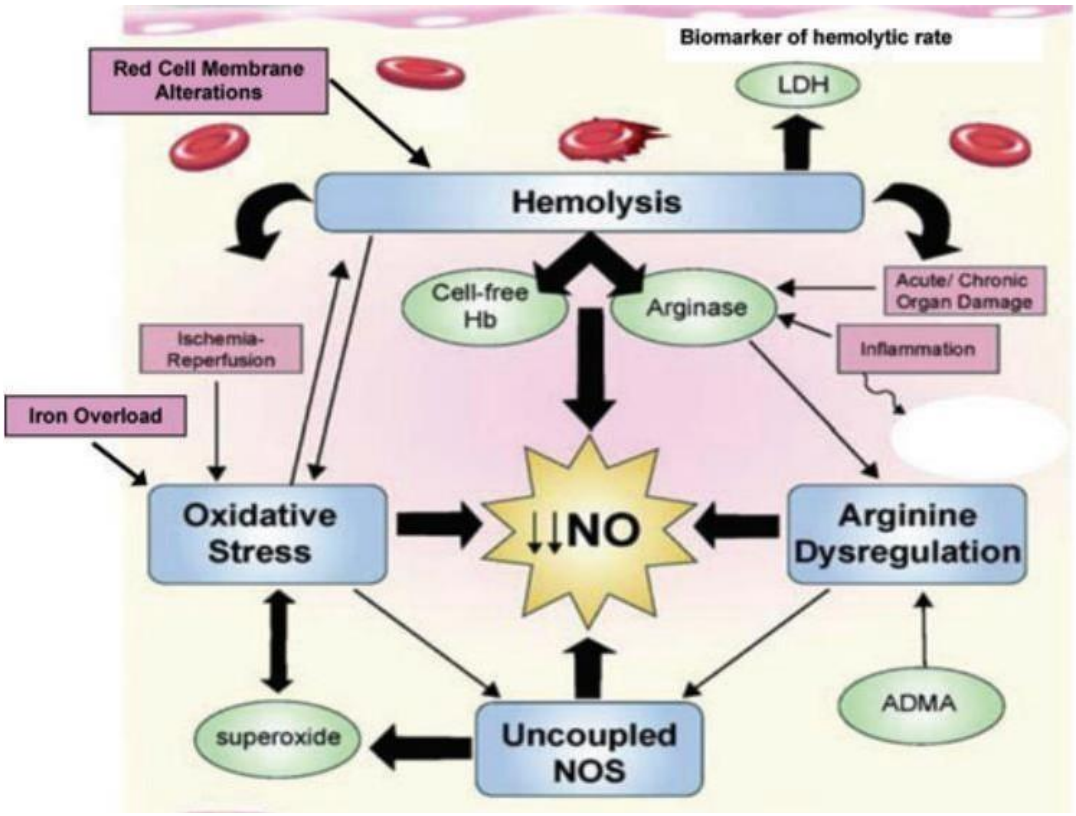


Fig 4: mechanisms of pulmonary hypertension caused by vasculopathy. Pulmonary hypertension manifests in the clinical setting of hemolytic illnesses like thalassemia due to the complex vascular pathophysiology of these conditions.

Clinical features of Pulmonary hypertension:

In chronic hemolytic diseases, the early stages of PAH may be asymptomatic or associated with minor symptoms. Exertional dyspnea, one of the primary symptoms PAH, overlaps with chronic anaemic symptoms frequently postponing clinical diagnosis till late in the illness. Additionally, hemolytic anemia's multiorgan consequences could make exercise challenging. Tolerance not affected by elevations in pulmonary arterial resistance[55] Hemolytic anemia's cofounding consequence of asthma may also exist. Hypoxemia, which may additionally an early

sign of restricted lung disease could be proof of PAH. cardiac dysfunction is usually due to iron overload, chronic anaemia, hypoxia, liver illness, and a hypercoagulable condition, as well as more specific PAH symptoms including syncope, angina, and lower limb edema are rare and are associated with severe and advanced pulmonary hypertension.

ROLE OF VEGF IN PULMONARY HYPERTENSION:

Judah Folkman et al discovered tumour angiogenesis factor, a factor secreted by tumours that causes angiogenesis, in 1970.[80] Senger et al discovered a vascular permeability factor secreted by guinea pig and hamster tumours in 1983. Ferrara and Henzel discovered a similar factor in bovine pituitary follicular cells in 1989, which they purified, cloned, and named VEGF. [81] Tischer et al discovered a similar VEGF alternative splicing in 1991. [82]Christinger and De Vos obtained the crystal structure of VEGF in 1996 and 1997, first at 2.5 resolution and then at 1.9 resolution.

Recent studies have suggested that the hypoxia is the most important factor regulating the VEGF upregulation along with various other factors, hence causing angiogenesis and in turn ph. HIF transcriptionally activate various genes which regulate the vascular tone and angiogenesis via VEGF, metabolism, and proliferation in pulmonary vascular cells after prolonged exposure to low oxygen levels. Furthermore, recent research has focused on the potential role of HIFs and the underlying molecular mechanisms in the dysregulation of the innate and adaptive immune systems in PAH.

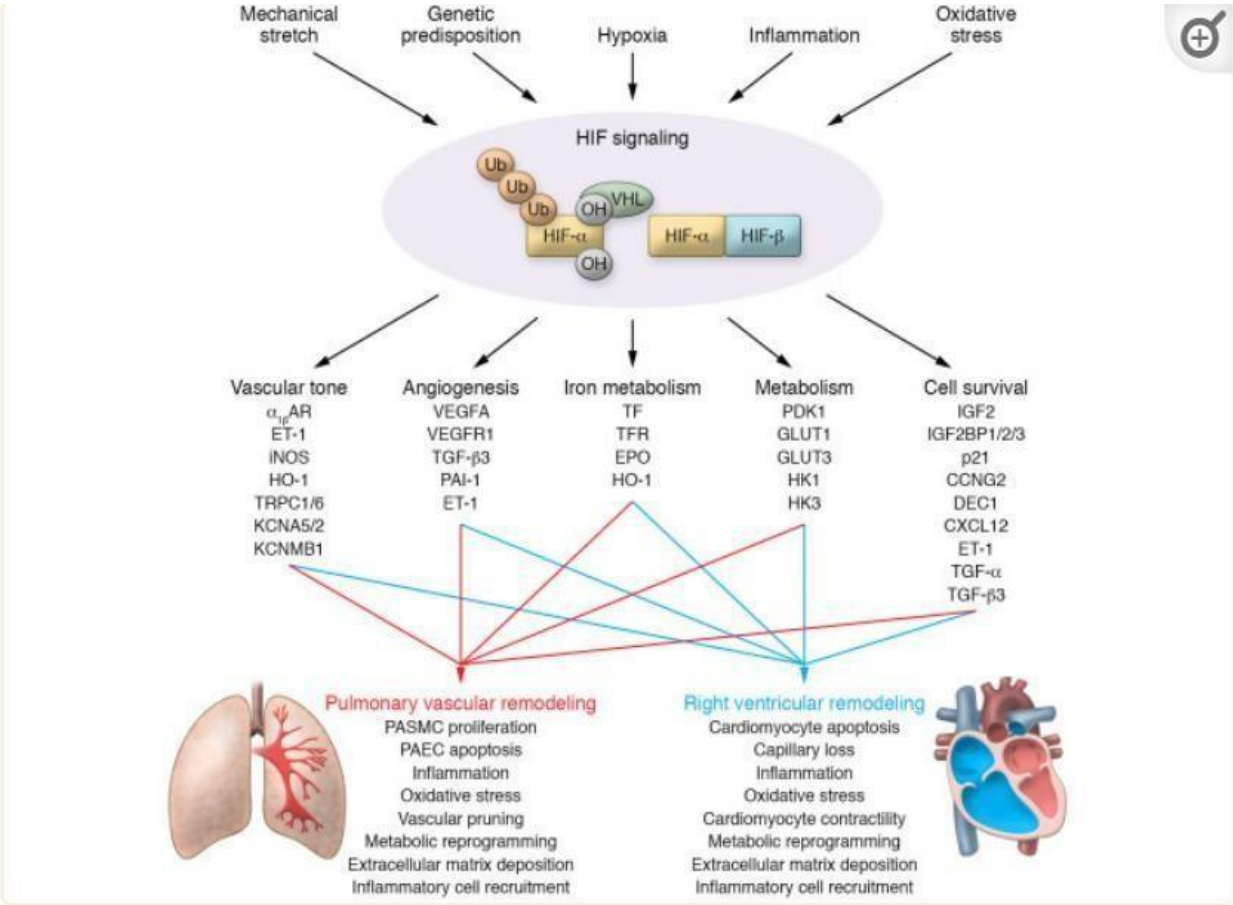


Fig 5 : HIF signaling pathway through VEGF and various vasoactive agents. Hypoxia and oxidative stress stimulate HIF 1alpha signaling which in turn acts on HRE gene and stimulates release of several vasoactive agents such as VEGF, TGF, EPO. This causes pulmonary vascular remodelling and right ventricular remodelling leading to PAH

HIF pathway knockout mouse models have given important insights into the adaptation of the pulmonary vasculature to hypoxia insult leading to PAH. Previous research has demonstrated that HIF genes are required for embryological development and biallelic deletion of most of those genes is incompatible with life. In mice, deletion of PHD 2 is non compatible with life whereas PHD1/PHD3 deletion, mice were viable [83] .When HIF1a, HIF 2a, or HIF 1b are completely deleted in mice, several developmental abnormalities result in embryonic death. Mice that have either a worldwide heterozygous deletion of Hif1a or Hif2a, however, mature and do not display homeostatic characteristics, making them ideal for researching the function of HIFs in disease. For instance, mice with a heterozygous deletion of HIF1a have lower RV hypertrophy and ph in response to hypoxia [(84)]. On the other hand, mice with a heterozygous deletion of Hif2a are totally shielded against PAH induced by hypoxic insult [85]but did not survive long-term hypoxia [86].

Right ventricular failure is the leading cause of mortality in PAH patients[87]. When PAH strikes, the RV remodels to maintain contractility, as evidenced by right ventricular wall thickening and as well as moderate dilatation. However, the RV's compensatory mechanisms fail eventually in persistent pressure overload, and the right ventricle fails.

Under physiological circumstances, the right ventricle has considerably higher levels of HIF-1 expression than the LV [88] The RV HIF-1 is expressed at a higher level in cases of severe PAH and certain animal models such as hypoxia exposed, pulmonary artery banded rats[89]. RV HIF-1 expression is elevated in PE rats, and RV hypertrophy and PAP are positively linked with it [88]. Interestingly, despite a large rise in PAP, mice with Hif2a mutations causing function gain, show RV hypertrophy but without dilatation, suggesting that RV function is retained [89]. Gain-of-

function mutations in HIF-1 are linked to better outcomes and retained RV function in individuals with corrected tetralogy of Fallot. For instance, severe polycythemia and dilated cardiomyopathy enhance the death rate in PHD2 global inducible deletion mice [90]. Similar to humans, mice with PHD2 deletion which are EC specific develop spontaneous PAH resulting in severe RightVentricular failure when HIF-2 activation occurs .

MANAGEMENT OF THALASSAEMIA MAJOR:

- Adequate anaemia correction by PCV transfusions
- Chelation- to tackle iron overload
- Prevention and management of the complications associated with the disease process and the treatment itself
- Curative treatment: stem cell transplantation
- Gene therapy is promising, however further research is needed.
- Prenatal diagnosis and counselling the parents who are carriers for thalassemia can helps prevent the burden of thalassemia.

Blood transfusion in thalassaemia management:

The mainstay of management of thalassemia is frequent transfusion.

Table 2: Types of blood transfusion regimen according to pretransfusion HB

Type of blood transfusion	Pre transfusion Hb	Mean Hb maintained
Palliative Transfusion	<7.0gm%	< 8.5gm%
Hyper-transfusion	>10.0gm%	>12.0gm%
Super-transfusion	>12.0gm%	>14.0 gm%
Moderate transfusion	9.0gm% -10.5 g%	> 12.0gm%

Recent recommendation is to maintain the mean post-transfusion Haemoglobin at 12.0gm%. Moderate transfusion is advised for patients with Hb levels of 9.0 to10.5gm%. Post transfusion haemoglobin shouldn't be >15.0-16.0 g%.

Table 3: Complications associated with blood transfusion

Iron overloading	
Infections	- Viral infections (HIV, HCV, HBV, HTLV1, West Nile virus) - Bacterial - Parasites - Creutzfeldt–Jacob disease
Haemolytic reactions	-Acute haemolytic reactions Delayed haemolytic reactions. - Autoimmune haemolytic anaemia
Non haemolytic reactions	- Hypersensitivity and anaphylaxis - Febrile non-haemolytic reaction - TRALI (transfusion related acute lung injury) - Graft versus host reactions - Volume overload

Chelation:

Iron overload is one of the most common hurdles in management of thalassaemia. Iron chelation is the only way to tackle the problem as there is no other effective mechanism to excrete the iron out. The main aim is to lower the iron levels in the body. Some of the most commonly used drugs are desferoxamine, Deferiprone and deferasirox.

Deferoxamine:

30 to 40 mg/kg/day given subcutaneously with help of infusion pump over 8-10 hours

It is given for 6 days in a week a night.

Deferiprone:

75 -100 mg/kg/day in 2 divided doses per-orally. It is 70 -100% as effective as deferoxamine and effectively controls the serum ferritin and iron stores in the body.

Deferasirox:

It is a recent iron chelating agent used for iron overload due to long term Blood transfusion.it is 5 times more potent than deferiprone and subcutaneous desirox. Given at a dosage of 20 to 40mg/kg/day.

In general, chelation has to started after 10 to 20 transfusions or when serum ferritin >1000mcg/dl. [31]

Deferiprone monotherapy reduces myocardial siderosis more effectively, according to various prospective and retrospective studies.[32-34]

Intensive chelation, helps in improving glucose tolerance, thyroid dysfunction and endocrine effects of iron overload in thalassemia, in early stages.[35,36,37]

Future perspectives:

Recently few new drugs have been introduced such as, PIH (pyridoxal isonicotynoyl hydrozone), Hydroxybenzyl-ethylenediamine (HBED) and dimethyl-HBED have shown promising results, as they have lesser side effects. pharmacological gene manipulations increase the production of fetal hemoglobin HbF and help prevent the precipitation of unpaired hemoglobin chains.[38]

Indications for splenectomy:

- Decreased platelet count – It is comparatively a late indicator of hypersplenism.
- When the annual requirement of packed RBC transfusion increases by two-fold or more than the basic requirement. i.e. about 220 to 250mL /Kg

Prior to splenectomy about 6 weeks, all patients must be given following vaccines, Pneumococcal, meningococcal and H. influenza type B vaccine.

Splenectomy is not preferred in children <5 years of age.

Stem cell transplantation: it is the best available treatment promising permanent cure.

PRENATAL DIAGNOSIS AND GENETIC COUNSELING:

The general foundation of thalassaemia prevention is detection of carriers, prenatal diagnosis and genetic counselling. If both partners are carriers for thalassemia, they should seek genetic counselling because they are at risk of having a thalassemic child. They shall be informed with the risks of the thalassemic children and the available treatment options

In pregnancies at increased risk prenatal analysis is possible by amniocentesis or chorionic villi sampling. Identifying the mutation in parents is the essential . 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:

Preimplantation diagnosis is a PCR based test which is the most recent development in prevention of thalassemia. For this procedure 1-2 blastomeres from embryos are isolated. Alternately one may aspirate a polarbody from the oocytes. If the disease-causing mutation is removed, the remaining blastomeres is implanted into mother, allowing normal foetal development.[39] As a result, thalassaemia reduction or elimination may be possible through gamete manipulation using a biopsy taken from the embryo and the PCR technique.[40]

Prevention of thalassaemia:

Only 10 to 15% of Indian children suffering from thalassaemia are optimally managed. It costs upto 1,00,000Rs per year to treat a child with thalassaemia. Bone marrow transplantation, which is a cure, is not affordable to most of the patients. A child with thalassaemia endures much physical and emotional stress 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 prevent such births.. For mass screening various types of screening tests have been used such as Mentzer's index, NESTROFT (Naked Eye Single Tube Red Cell Osmotic fragility test) etc.

Fractions of Hb A, A2, F, H, E and other variants are measured by Hb Electrophoresis and High-performance liquid chromatography. Those with thalassaemia trait must be counseled for

testing their partner, in case the partners both are confirmed as carriers, they should be counselled regarding the possibilities of having a thalassaemic child and prenatal diagnosis. Such as, in the chorionic villi sampling in first trimester and amniocentesis in the second trimester. Hence, in case of the fetus is affected, the parents can have the option to terminate the pregnancy.

MATERIALS AND METHODS

Source of data:

The study was conducted on thalassaemia 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) thalassaemia cases were taken.

Study period: 1st December, 2020 to 30th June, 2022.

Subjects:

Study subjects were all diagnosed cases of thalassaemia. 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. And the sample was drawn before transfusion.

Sample collection and analysis:

- After taking informed consent, under aseptic precautions, venous blood sample was collected from patients
- Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count, that included CBCusing an automated 5-part differential haematology 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. Ferritin using Biochemistry analyzer (VITROS® 5,1).

- 2ML OF sample was centrifuged and the serum was stored in -70 celsius. Later with which Serum VEGF estimated was done using KRISHGEN VEGF ELISA KIT.

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

S. No.	HAEMATOLOGICAL 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

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. VEGF	35 ± 23.5 Pg/ml
3.	Sr. Ferritin	Male: 17.0-464.0, Female: 6.24-137.0 ng/dl

Table 6: PAH grading

	Grades of PAH	**Pulmonary pressure**
1	**Mild PAH**	**25 – 39mmHg**
2	**Moderate PAH**	**40-60mmHg**
3	**Severe PAH**	**>60mmHg**

Inclusion criteria:

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

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 32 per group.

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

Where,

Z^{α} = Z value at α level

Z_{β} = Z value at β level

P = common proportion between two groups

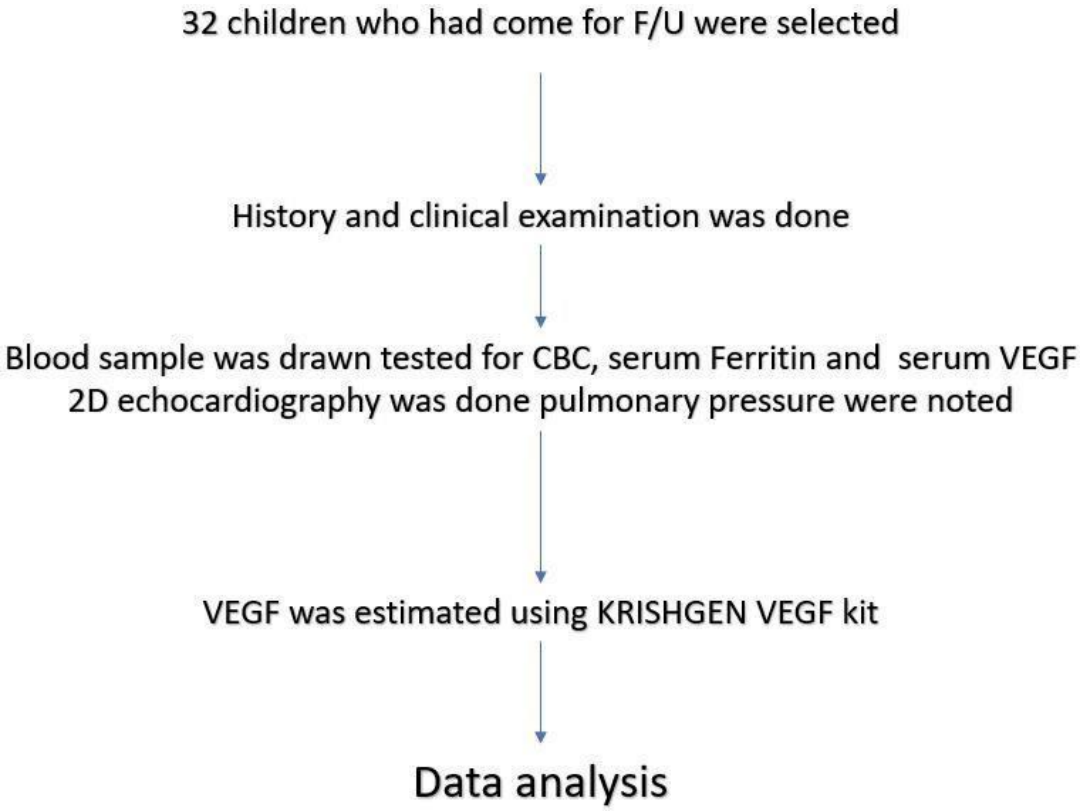
q = 100p d = difference between two

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 physiology, 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 thalassaemia patients were being transfused and managed for clinical symptoms and manifestations of the disease. Haematological and biochemical analysis was done in the Department of physiology, Pathology and the Department of Biochemistry.

35 transfusion-dependent thalassaemia patients were included in the present study, although the sample size was 32. They evaluated for the haematological, biochemical and 2D echocardiographic evaluation. All 35 patients were found to be known cases of Thalassaemia major. Here, we present an evaluation of the results of our study.

TABLE 7: AGE DISTRIBUTION IN STUDY POPULATION

Age (in years)	No of cases	Percent
≤ 5	15	42.9
6-10	10	29.4
> 10	10	26.4
Total	35	100.0
Mean & Sd	8.23 ± 4.8	

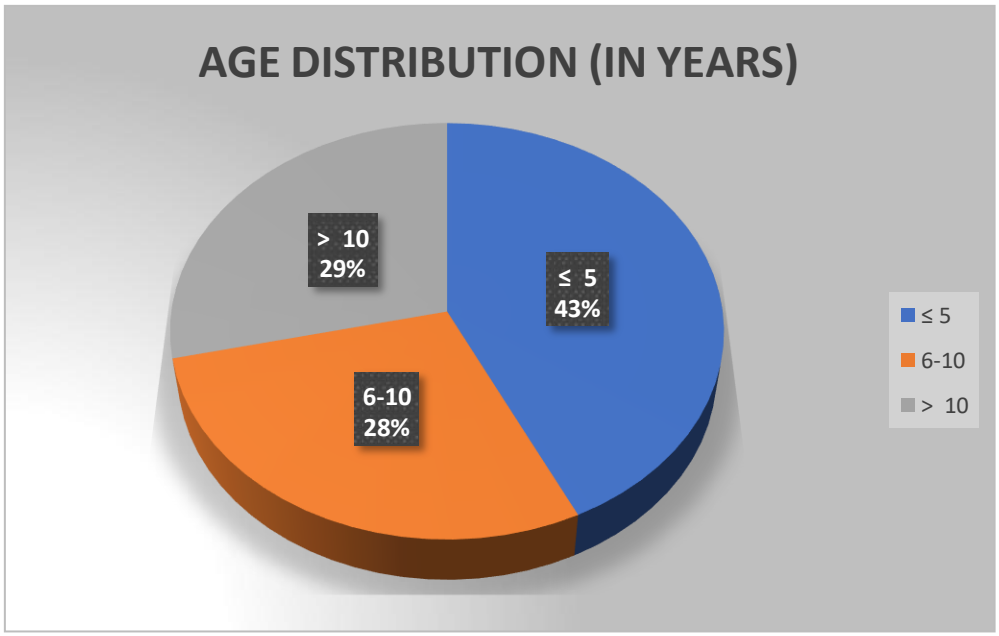


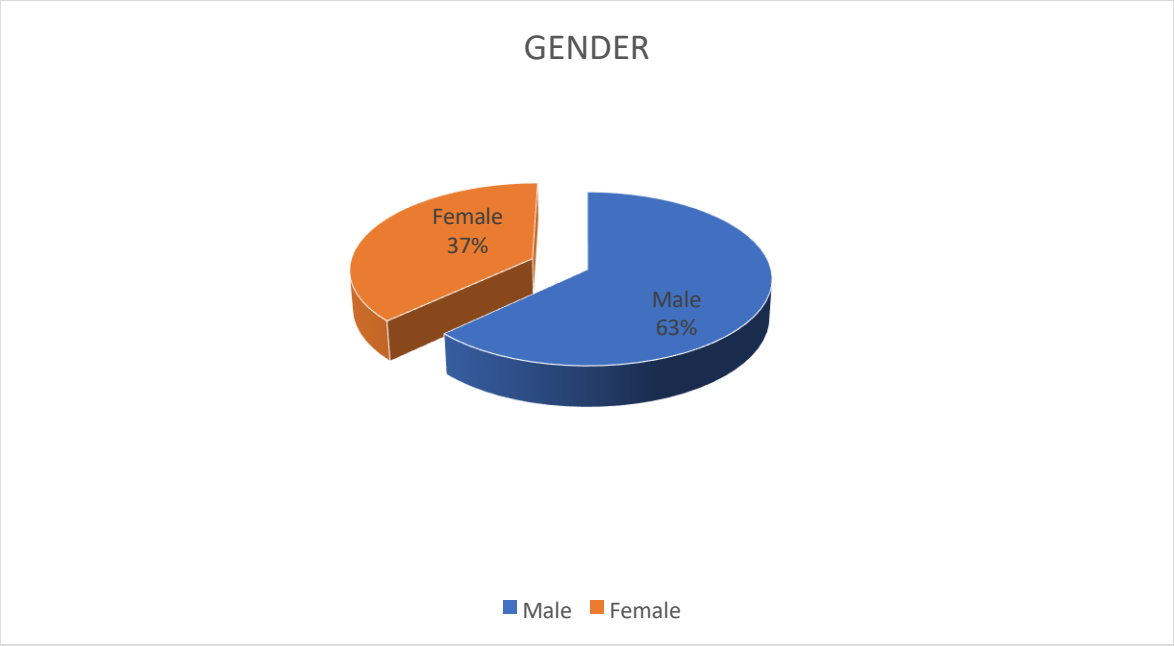
FIG 6 : distribution of age in the study population

In the present study, the age of the patients was within the range of 2 to 17 years. The maximum number of cases was under 5 years which constituted of 42% (**Table 7, Fig 6**)

TABLE 8: SEX INCIDENCE IN STUDY AND CONTROL GROUPS

Gender	No of cases	Percent
Male	22	62.9
Female	13	37.1
Total	35	100.0

FIG 7: GENDER DISTRIBUTION IN STUDY POPULATION

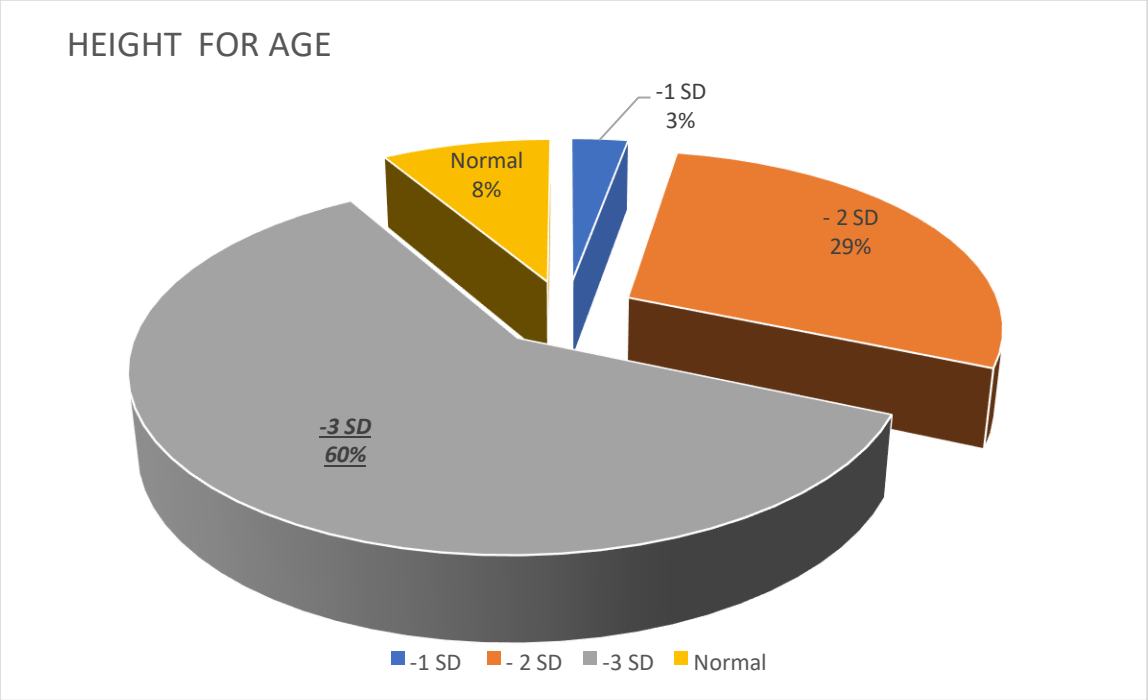


Males comprised of the most number i.e 63% of the study population and females 37%(**Table 8, Fig 7**)

TABLE 9: HEIGHT FOR AGE IN THE STUDY POPULATION

Height for age	No of cases	Percent
-1 SD to -2SD	1	2.9
- 2 SD TO -3SD	10	28.6
<-3 SD	21	60.0
Normal	3	8.6
Total	35	100.0

FIG 8: DISTRIBUTION OF HEIGHT FOR AGE IN THE STUDY POPULATION



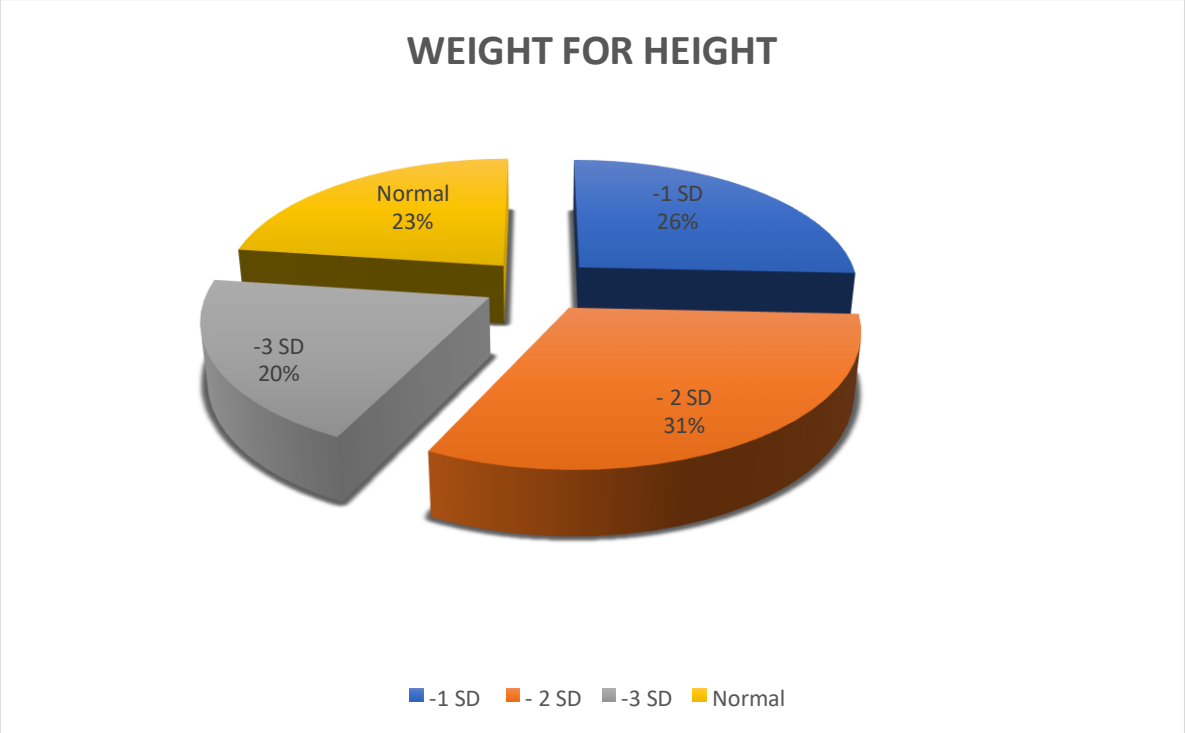
In our study population of 35, majority of the children i.e. 60% were below the <3SD of Height for age, 29 % of the children were < 2SD. Standard deviation was as per IAP growth charts for 5 to 18 years, and WHO growth charts for < 5 years. (**table 9, Fig 8)**

TABLE 10: VARIATION OF WEIGTH FOR HEIGHT

Weight for height	No of cases	Percent

-1 SD	9	25.7
- 2 SD	11	31.4
-3 SD	7	20.0
Normal	8	22.9
Total	36	100.0

FIG 9: VARIATION OF WEIGTH FOR HEIGHT

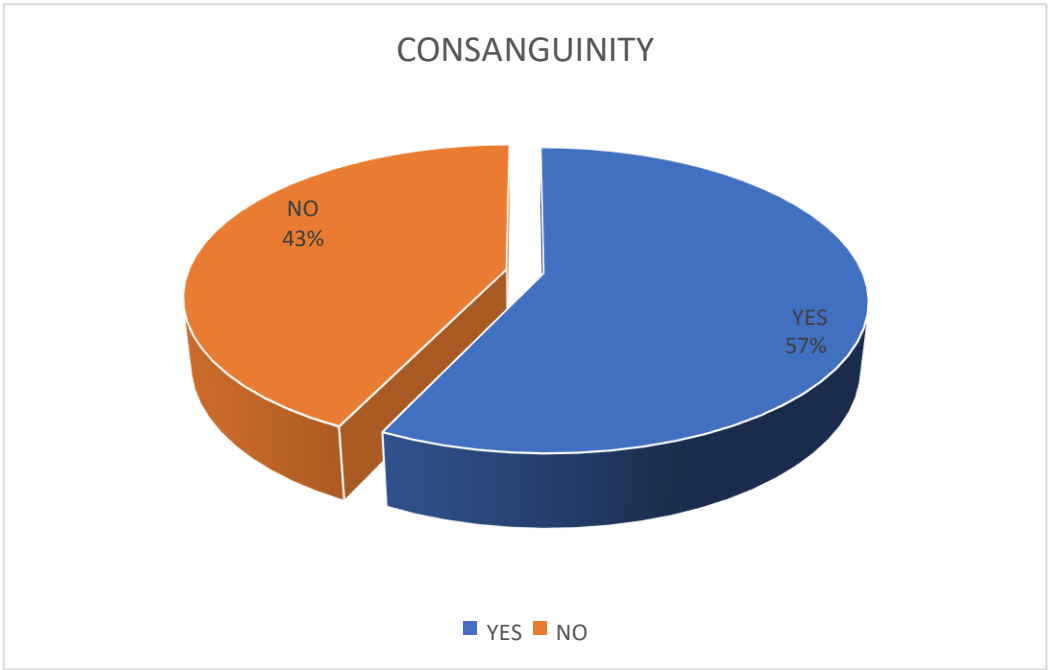


In our study population, the weight for height variable, was significantly comprised of <2SD i.e. 31%, then < 1SD i.e. 25%. Among 35 children, 7 (20%) were having weight for height < 3SD. (**Table 10, Fig 9**)

TABLE 11: DESCRIPTIVE STATISTICS OF CONSANGUINITY HISTORY IN THE STUDY POPULATION

CONSANGUINITY	No of cases	Percent
YES	20	57.1
NO	15	42.9
Total	35	100.0

FIG 10: DESCRIPTIVE STATISTICS OF CONSANGUINITY HISTORY IN THE STUDY POPULATION



In the study population, 57% of the patients had h/o consanguinity(**Table 11, Fig 10**)

TABLE 12: DESCRIPTIVE STATISTICS OF BLOOD TRANSFUSION HISTORY IN THE STUDY POPULATION.

Transfusion	Mean	Minimum	Maximum
Age at first transfusion (in months)	8.11 ± 2.87	5.00	18.00
Frequency of transfusion (in weeks)	4.94 ± 3.23	2.00	18.00
Months of transfusion	90.63 ± 57.59	12.00	244.00
Total Transfusions	56.03 ± 49.76	3.00	224.00

The above table shows the blood transfusion history of the patients.

Frequency of transfusion varied from 2 weeks to 18 weeks, and mean (4.94 weeks) (**TABLE 12)**

Age of first transfusion varied from 5 to 18 months, with average of approx., 8 months (**TABLE 12)**

Total months of transfusion varied from 12 to 244 months. On average approx., 90 months (**TABLE 12)**.

Total number of transfusions varied from 3 to 224, average being 56 transfusions (**TABLE 12)**

TABLE-13: DESCRIPTIVE STATISTICS OF PRESENCE OF SPLENOMEGALY, HEPATOMEGALY AND SPLENECTOMISED PATIENTS IN THE STUDY POPULATION.

Parameters		No of cases
Splenomegaly	YES	18
	NO	6 (excluding splenectomy cases)
Hepatomegaly	YES	27
	NO	2
SPLENECTOMY	YES	10
	NO	25

In the study population,

Splenomegaly was present in approx., 54% of all patients.

Hepatomegaly was present in 80% of all patients.

30% of the patients had undergone splenectomy. **(TABLE 13, FIG 11)**

Table 14: DESCRIPTIVE STATISTICS OF HAEMATOLOGICAL PARAMETERS IN THE STUDY POPULATION.

Parameters	Mean	Minimum	Maximum
HB	5.92 ±1.16	3.60	8.40
PCV	21.51 ±8.72	12.00	31.00
TC	7128 ± 2681	3240.00	16480.00
PLATELET	147577 ± 85082	47000.00	413000.00
FERRITIN	1017.77 ± 601.27	132.00	2400.00
Gradient	34.29 ± 13.80	19.00	68.00
VEGF	832.80 ± 1420.63	106.20	7117.00

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

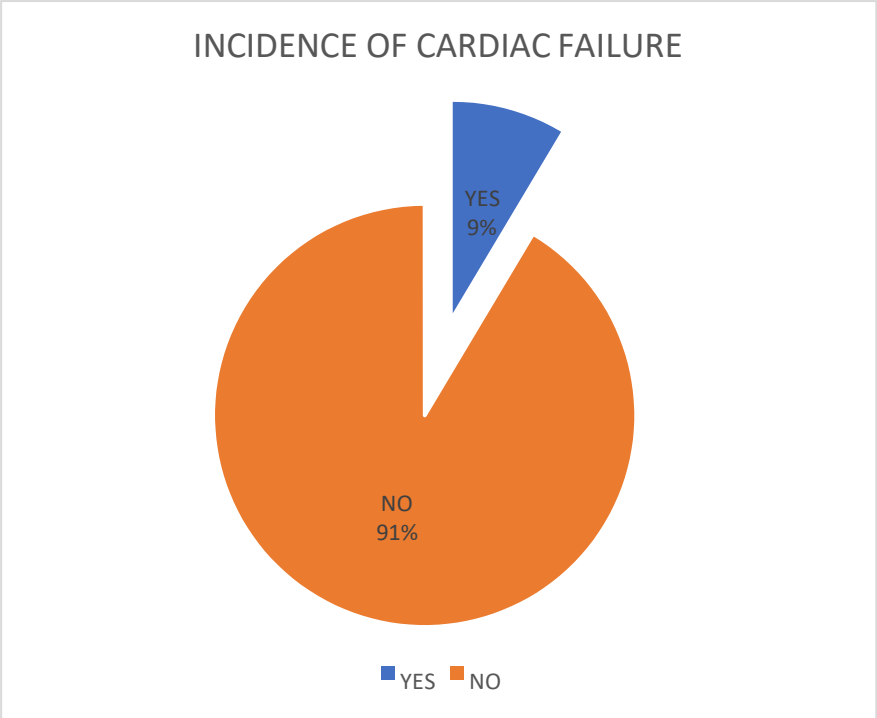
- Minimum Hb was 3.60 gm/dl and maximum was 8.40 gm/dl with an average of 5.92 ±1.16 (Mean ±SD).
- Minimum HCT was 12 % and maximum was 31 % with an average of 21.50 ± 8.52 (Mean ±SD).
- Minimum PLATELET was 47,000/10^{-6} L and maximum was 4,13,000/10^{-6} L with an average of 1,47,577 ± 85,082 (Mean ±SD).
- Minimum FERRITIN was 132 and maximum was 2400 with an average of 1017.77 ± 601.27 (Mean ±SD).
- Minimum PA PRESSURE was 19mmHg and maximum was 68mmHg with an average of 34.29 ± 13.80 (Mean ±SD).
- Minimum VEGF was 106.2 pg/mL and maximum was 7117pg/mL with an average of 832.80 ± 1420.63(Mean ±SD).

TABLE 15: DESCRIPTIVE STATISTICS OF INCIDENCE OF CARDIAC FAILURE

CARDIAC FAILURE	No of cases	Percent
YES	3	8.6

NO	32	91.4
Total	35	100.0

FIG 12: DESCRIPTIVE STATISTICS OF INCIDENCE OF CARDIAC FAILURE



In the present study, incidence of cardiac failure among the patients was 8.6% i.e. 3 patients of 35, suffered from cardiac failure (**TABLE 17, FIG 12)**

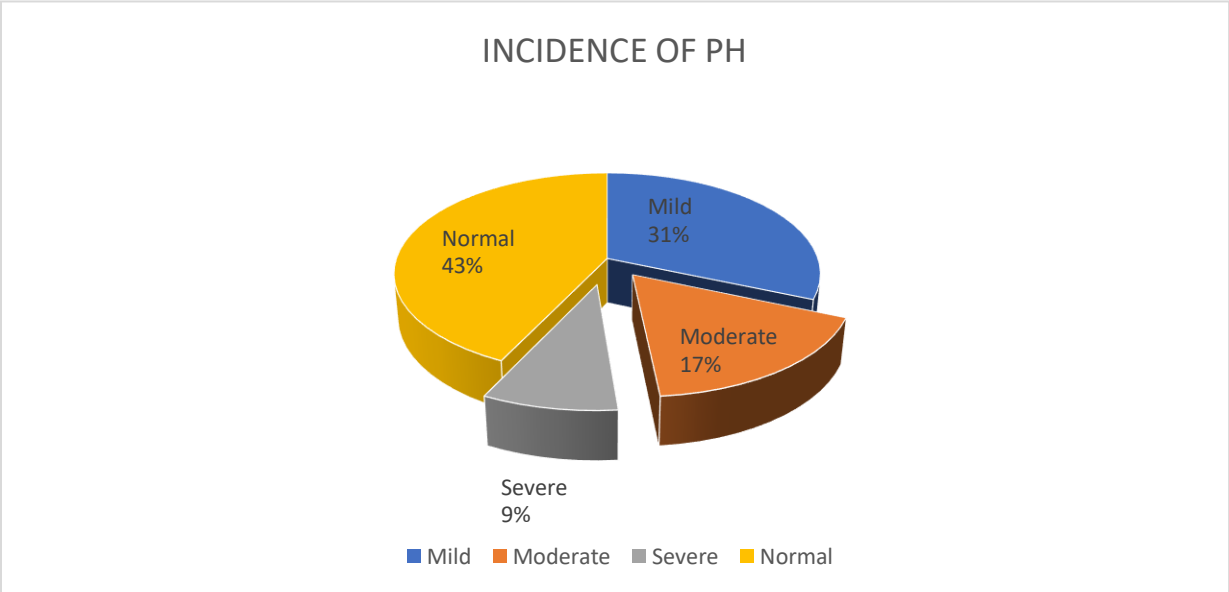
TABLE 16: DESCRIPTIVE STATISTICS OF GRADES OF PULMONARY HYPERTENSION AMONG THE STUDY GROUP

PULMONARY ARTERIAL HYPERTENSION	No of cases	Percent

Mild	11	31.4
Moderate	6	17.1
Severe	3	8.6
Normal	15	42.9
Total	35	100.0

In the present study, 43% were having no PAH 31% have mild PAH, 17% have moderate PAH and 9 % had severe PAH **(TABLE 18, FIG 13)**

FIG 13 DESCRIPTIVE STATISTICS OF GRADES OF PULMONARY HYPERTENSION



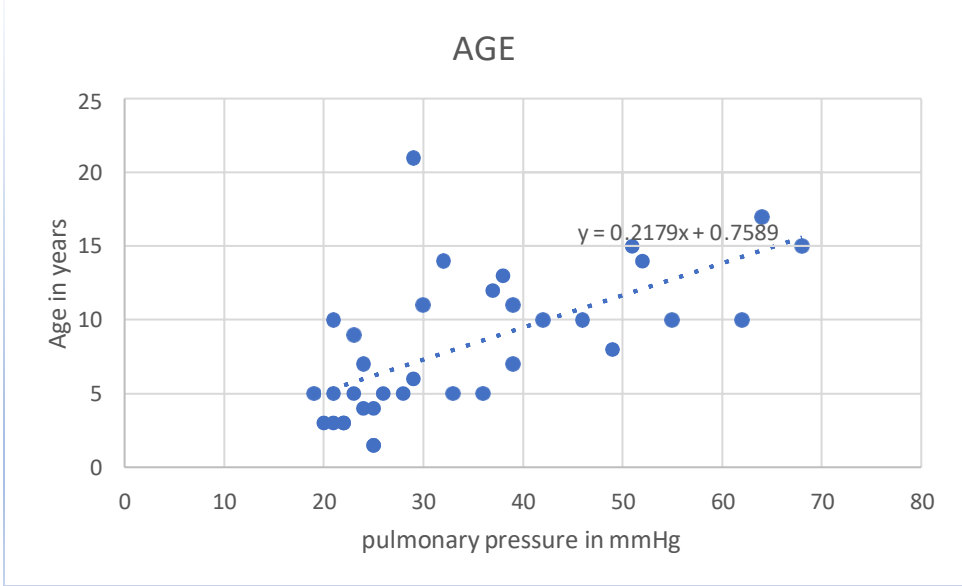


FIG 14 SCATTERPLOT OF AGE VS PULMOANRY PRESSURE

The above chart shows the scatterplot of age vs pulmonary pressures, moderate ph ie(>40mmHg) is more prevalent after age of 10 years

TABLE 17: CORRELATION BETWEEN FERRITIN, PA PRESSURE AND VEGF

Variables	Pearson's Correlation		
	r Value	P Value	Significance
Ferrittin & PA PRESSSURE	0.849	P<0.001	Sig
Ferrittin & VEGF	0.618	P<0.001	Sig
PA PRESSURE & VEGF	0.758	P<0.001	Sig

In the present study, we found strongly positive correlation between:

Ferritin and PA pressure, i.e. p<0.001 (**TABLE17, FIG 14)**

Ferritin and VEGF, i.e p< 0.001 (**TABLE19, FIG 15)**

PA pressure and VEGF, i.e. p< 0.001 (**TABLE19, FIG 16)**

FIG 15: SCATTER DIAGRAM SHOWING CORRELATION BETWEEN S FERRITIN AND PA PRESSURE

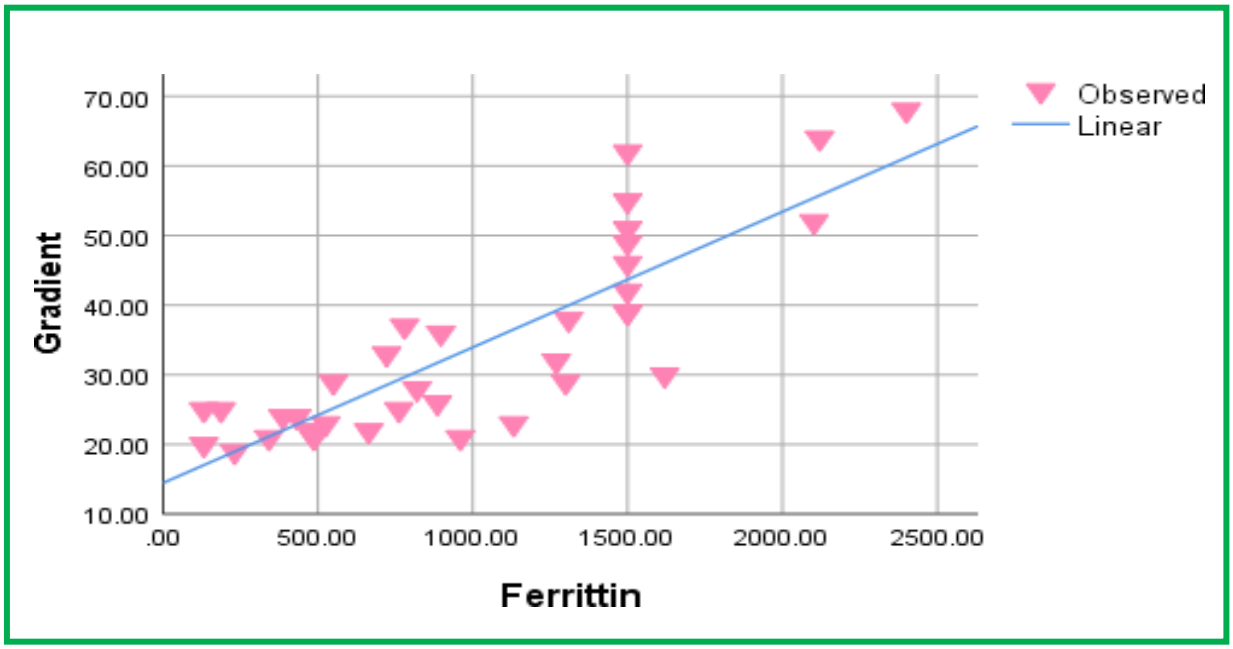


FIG 16: SCATTER DIAGRAM SHOWING CORRELATION BETWEEN S FERRITIN (IN mg/dL) AND VEGF (in pg/mL)

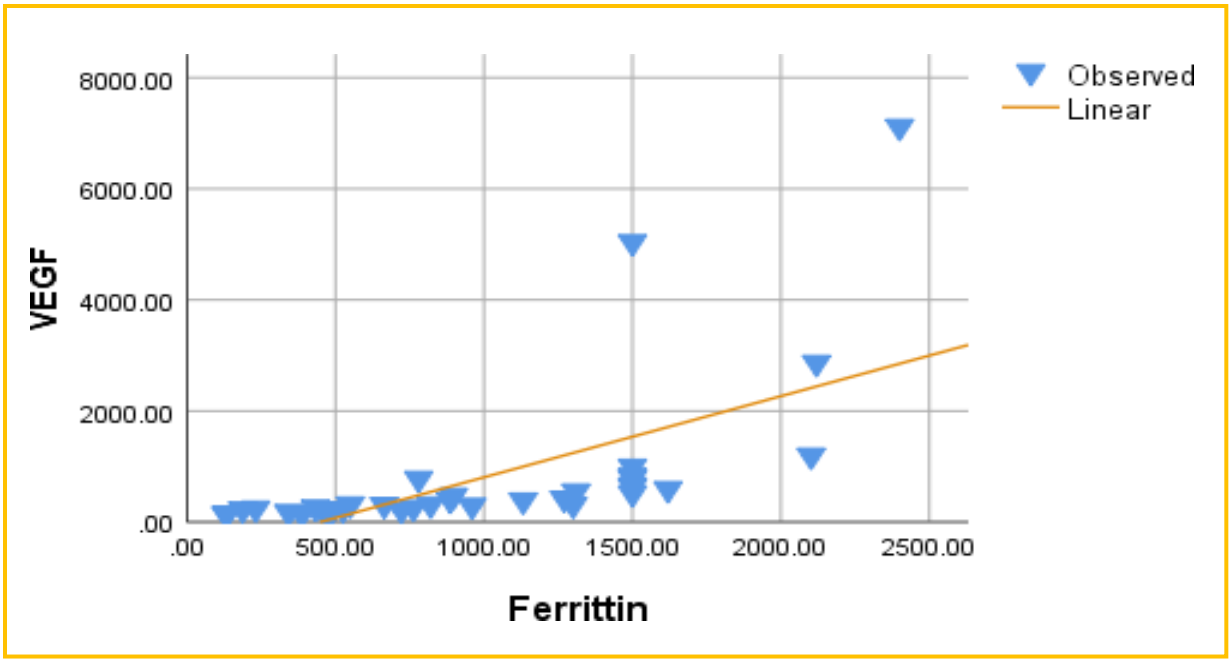


FIG 17: SCATTER DIAGRAM SHOWING CORRELATION BETWEEN PA PRESSURE AND VEGF

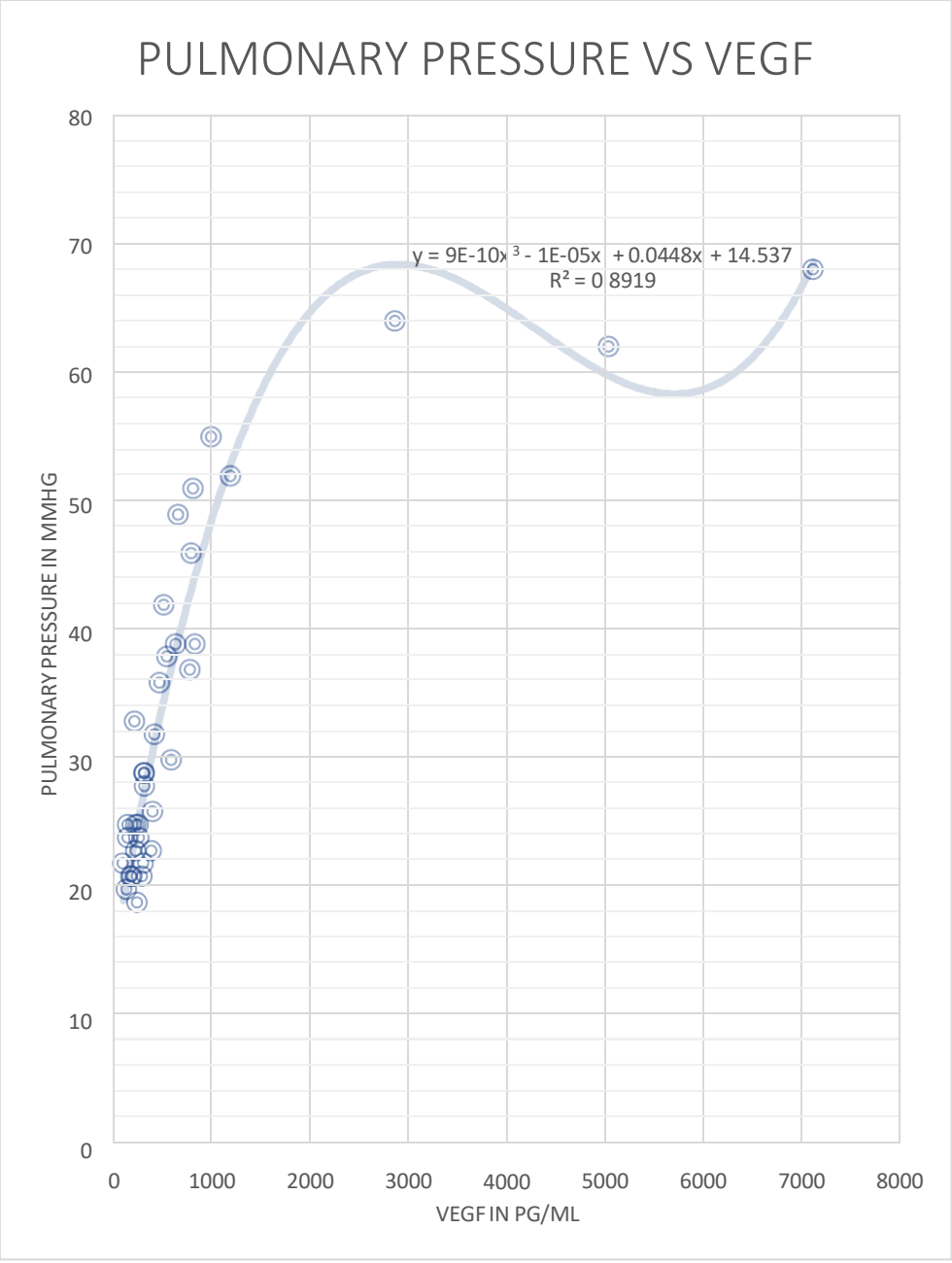


TABLE 18: CORRELATION BETWEEN BLOOD TRANSFUSION AND VEGF.

Transfusion		VEGF		Kruskal-Wallis H test	
		Mean	Std. Deviation	P Value	Sig
Age at first transfusion in Yrs	≤ 5	448.79	217.93	0.854	NOT Sig
	6-10	974.11	1662.04		
	> 10	525.64	328.17		
Frequency of transfusion in weeks	2-4	1179.55	1710.02	P<0.001	Sig
	5-7	257.20	100.45		
	≥ 8	228.07	63.33		
Months of transfusion	≤ 60	249.77	99.71	P<0.001	Sig
	61-120	1069.68	1507.83		
	> 120	1552.46	2085.88		
Total Transfusion	≤ 50	273.86	135.72	P<0.001	Sig
	51-100	991.33	1291.30		
	> 100	2464.54	2771.53		

In the present study, as per the Kruskal-wallis H test for correlation between VEGF and blood transfusion related parameters are as follows:

1. Frequency of transfusion and VEGF, are strongly correlating with $p<0.001$
2. Age of first transfusion and VEGF, correlation is not significant, $p>0.005$ (observed $P=0.854$)
3. Months of transfusion and VEGF, had strong correlation $p<0.001$
4. Total number of transfusion and VEGF were also strongly correlated, $p<0.001$ **TABLE 20**

TABLE 19:PEARSON'S CORRELATION BETWEEN BLOOD TRANSFUSION AND VEGF.

Variables	Pearson's Correlation		
	r Value	P Value	Significance
VEGF & Age at first transfusion	-0.098	0.575	Not Sig
VEGF & Frequency of transfusion	-0.313	0.067	Not Sig
VEGF & Months of transfusion	0.431	P<0.01	Sig
VEGF & Total Transfusion	0.552	P<0.001	Sig

In the present study, as per the Pearson's correlation between VEGF and blood transfusion related parameters are as follows:

1. Frequency of transfusion and VEGF, correlation was not significant, p=0.067, r= -0.313
2. Age of first transfusion and VEGF, correlation is not significant, p>0.005 (observed P=0.575), r= -0.098
3. Months of transfusion and VEGF, had strong correlation p<0.001, r = 0.431
4. Total number of transfusion and VEGF were also strongly correlated, p<0.001, r = 0.552 (**TABLE 19)**

DISCUSSION

Majority of patients with alpha thalassaemia and beta thalassaemia trait are asymptomatic and need nil or simple intervention, whereas beta-thalassaemia major patients undergo repeated blood transfusion.[22] Blood Transfusion along with proper chelation therapy has dramatically improved the life expectancy of thalassaemic children and improved the quality of life. However, frequent blood transfusions result in iron overload and derangement of several haematological, biochemical parameters and cardio-pulmonary status. These deranged parameters, if not monitored regularly will lead to several complications that may hinder the quality of life and overall survival of thalassaemia patients. In recent years, several authors have reported a high incidence of these complications, predominantly in patients diagnosed with thalassaemia major.[5] Therefore monitoring of haematological 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 thalassaemia 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.*[91], Logothetis J *et al.*[92] and De A *et al.*[93] 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.*[96] *and* Joseph N. *et al.* where maximum cases were in the age range of 7-10 years and 5-10 years respectively. **(Table 20)**

Table 20: Age incidence in study subjects compared to other studies

Studies	Mean age ±SD
Present study	8.23 ± 4.8
Suman RL *et al.*[91]	8.80 ±3.88
Logothetis J *et al.*[92]	10.3 ±4.2
De A *et al.*	8.22

Since thalassaemia is an inherited genetic disorder, most patients present early with symptoms of anaemia leading to early diagnosis.

Majority of the patients in the present study are males (62.9 %). This finding is correlating with studies done by Tyagi S *et al.*[94], Patil S *et al.*[95], Al-Kherbash H *et al.*[96], and Joseph N. *et al.*[97] who also reported similar gender distribution in thalassaemia affected children. **(Table 21)**

Table 21: Gender distribution in study subjects compared to other studies

Studies	Males (%)	Females (%)
Present study	62.9	37.1
Tyagi S *et al.*[94]	64.5	35.5

Patil S *et al.*[97]	60.4	39.6
Al-Kherbash H et al[96]	53.2	46.8
Joseph N. *et al.*[97]	63.4	36.6

Hb level of thalassaemia children is decreased in the present study with an average value of 5.92 ±1.16 (Mean ±SD). This finding is correlating with studies conducted by Karim F *et al.*[1], Ayyash H *et al.*[99], Verma S *et al.*[100], De A *et al.*[93], Filiz *et al.*[106], Prakash A *et al.*[98], Sultan S *et al.*[101], Jameel T *et al.*[103] and Jain C *et al.*[105] who also observed decreased Hb level in thalassaemia cases. Hb is usually low in cases of thalassaemia because of defective Hb production and increased RBC destruction in the spleen leading to ineffective erythropoiesis. **(Table 22)**

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

	Hb (Mean±SD)
Present study	5.92 ±1.16
Karim F *et al.*[1]	7.36 ±1.5
Ayyash H *et al.*[99]	7.36 ±0.8
Verma S *et al.*[100]	9.8 ±1.1
De A *et al.*[93]	8.5 ±2.5
Filiz *et al.*[106]	9.25 ±1.74

In the present study HCT level in thalassaemia children was decreased with an average of 21.51 ± 8.72 (Mean ±SD). Similar findings were observed in studies conducted by

Karim F *et al.*[1], Sultan S *et al.*[101], Munir B *et al.* and Filiz *et al.*[106] **(Table 23)**

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

	HCT (Mean±SD)
Present study	21.51 ± 8.72
Karim F *et al.*[1]	21.5 ±5.3
Filiz *et al.*[60]	27.07 ±4.65

Platelet count in thalassaemia children is within the normal range in the present study with an average value of 1.477 ± 0.85 (Mean ±SD). Similar finding was observed in studies conducted by Naithani R *et al.*[99],

Bushra M *et al.*[102], and Sultan S *et al.*[101] (**Table 24**)

Table 24: Platelet count in study subjects compared to other studies

	Platelet count (Mean±SD)
Present study	1.477 ± 0.85
Naithani R *et al.*[109]	2.26 ±1.23

In the present study Sr. Ferritin is elevated in the study group with a value of 1017.77 ±601.27 (Mean±SD). This finding is correlating with studies conducted by Karim F *et al.*,[1] Sultan S *et al.*[101]., Munir B *et al.*[102], Jain C *et al*[105]., Naithani R *et al.*[109], Guimaraes J *et al.*[108], Livrea

M. A *et al.*[112], De A *et al.*[93], Ayyash *et al.*[99], Filiz *et al.*[106], Salma OS *et al.*[119] and Soliman *et al.*

(Table 25)

Table 25: Sr. ferritin value in study subjects compared to other studies

	Sr. ferritin (Mean±SD)
Present study	1017.77 ±601.27
Karim F *et al.*[1]	1249 ±59.2
Naithani R *et al.*[109]	3709.0 ±1625
Livrea M. A *et al.*[112]	1866.0 ±996.0
De A *et al.*[93]	1548.06
Ayyash *et al.*[99]	7162.4 ±3297.3
Filiz *et al.*[106]	1300 ±477.14
Salma OS *et al.*[119]	881.4 ±245.1
Soliman *et al.*[78]	880 ±46

In the present study, we observed that the Minimum VEGF was 106.2 pg/mL and maximum was 7117pg/mL with an average of 832.80 ± 1420.63. (TABLE 26)

Table 26: Sr. VEGF value in study subjects compared to other studies

	VEGF levels (Mean±SD)
Present study	832.80 ± 1420.63.

Sameh S. Fahmey1 *et al.*	1241.5±632.9

In the present study, we have observed that the VEGF levels correlation with frequency of transfusions and age of first transfusion were not significant, (p= 0/067 and 0.575 respectively). Which is correlating with the study conducted by Sameh S. Fahmey1 *et al,* where they found negative correlation with frequency of transfusion p=0.02. (TABLE 27)

Table 27: CORRELATION BETWEEN VEGF AND BLOOD TRANSFUSION PARAMETERS.

Variables	Pearson's Correlation		
	r Value	P Value	Significance
VEGF & Frequency of transfusion	-0.313	0.067	Not Sig
VEGF & Age at first transfusion	-0.098	0.575	Not Sig
VEGF & Months of transfusion	0.431	P<0.01	Sig
VEGF & Total Transfusion	0.552	P<0.001	Sig

In the present study, we have found that there is strongly positive correlation between VEGF levels with, serum ferritin and ph, with p<0.001 in either of the cases. And also there is positive correlation between ferritin and pulmonary artery pressure. Which correlates with the findings of the study conducted by Papaioannou, A.I., Zakynthinos, E., Kostikas, K. et al where they found that correlation between VEGF and ph with p=0.01

Z D DU *et al,* where they found 22 of 33 thalassemia patients hacing ph, i.e. 66%.

Table 28: CORRELATION BETWEEN VEGF WITH FERRITIN AND PA PRESSURE.

Variables	Pearson's Correlation		
	r Value	P Value	Significance
Ferrittin & PA PRESSSURE	0.849	P<0.001	Sig
Ferrittin & VEGF	0.618	P<0.001	Sig
PA PRESSURE & VEGF	0.758	P<0.001	Sig

In the present study we have found that 17 out of 35 have ph, i.e. accounting to 63% which is correlating with the study conducted by Z D DU *et al,* where they found 22 of 33 thalassemia patients hacing ph, i.e. 66%.

Table 29: INCIDENCE OF PAH IN THE STUDY SUBJECTS

PULMONARY ARTERIAL HYPERTENSION	No of cases	Percent
Mild	11	31.4
Moderate	6	17.1
Severe	3	8.6
Normal	15	42.9
Total	35	100.0

SUMMARY

A prospective study was done to assess the changes in haematological and biochemical parameters in thalassaemia 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 December 2020 to July 2022 in the Department of Pediatrics, 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. All the patients underwent 2D echocardiography and pulmonary pressures were noted. Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count, that included Hb, total counts, differential count and Platelet count using an automated 5-part differential haematology analyzer (SYSMEX XN-1000). 4 ml of blood sample was taken in plain vacutainer and was centrifuged @ 1500 rpm and analyzed Sr. Ferritin using Biochemistry analyzer. 2ml of blood was centrifuged and the serum was storage in -70c and later tested for VEGF with KRISHGEN VEGF kit.

(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 17 years. Maximum number of cases were between 6 and 10 years, amounting to 28%

Amongst thalassaemia patients, the majority were males (62.9%) with a male to female ratio of 1.9:1.

Majority of the children i.e. 60% were below the <3SD of Height for age as per the IAP charts and WHO chart

In our study population, the weight for height variable, 31% were between -2SD and -3SD. 20% were <-3SD.

There was no correlation between VEGF and transfusion frequency and age of first transfusion

Among all the study population, 57% had h/o consanguinity

Frequency of transfusion varied from 2 weeks to 18 weeks, and mean (4.94 weeks)

Age of first transfusion varied from 5 to 18 months, with average of approx., 8 months

Total months of transfusion varied from 12 to 244 months. On average approx., 90 months

Total number of transfusions varied from 3 to 224, average being 56 transfusions

In the present study, we found strongly positive correlation between:

Ferritin and PA pressure, i.e. $p<0.001$

Ferritin and VEGF, i.e $p< 0.001$

Hence regular blood transfusion in thalassaemia patients causes derangement in their haematological, biochemical parameters and chronic hypoxic changes. 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 thalassaemia patients undergoing repeated blood transfusions for the management of anaemia to maintain optimum HB with proper chelation therapy. Regular assessment of haematological 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 thalassaemia is highly prevalent in the general population and there is an ever-increasing load of patients we must focus on the prevention of thalassaemia. Presently blood transfusion in conjunction with chelation therapy is the most popular treatment approach in symptomatic thalassaemia cases.

Thalassaemia patients undergoing regular blood transfusions show significant changes in their serum ferritin, VEGF and pulmonary hypertension especially after the age of 10years.

Hence, we conclude that better management of thalassaemia can be done by frequent transfusions to maintain optimum HB, along with that, adequate chelation and frequent evaluation of serum ferritin, VEGF and 2D echocardiography has to considered.

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B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/No-09/2021
(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956) Date- 22/01/2021
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Vascular Endothelial growth factor levels in children with thalassemia major & its correlation with pulmonary arterial hypertension & its serum ferritin.

Name of PG student: , Dr Nandakishore.B.Kulkarni, Department of Paediatrics

Name of Guide/Co-investigator: Dr S V Patil, Professor of Paediatrics

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

5

ANNEXURE I

RESEARCH INFORMED CONSENT FORM

BLDEA'S SHRI B M PATIL MEDICAL COLLEGE AND HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

TITLE OF THE PROJECT : **" VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN CHILDREN WITH THALASSEMIA MAJOR AND ITS CORRELATION WITH PULMONARY ARTERIAL HYPERTENSION AND SERUM FERRITIN "**

GUIDE : **DR. S.V PATIL, MD**

PROFESSOR,

DEPARTMENT OF PEDIATRICS

CO- GUIDE

Dr SUMANGALA PATIL, MD

PROFESSOR AND HEAD

DEPARTMENT OF PHYSIOLOGY

PG STUDENT : **Dr. Nandakishore B Kulkarni**

PG DEPARTMENT OF PEDIATRICS
(MD PEDIATRICS)

PURPOSE OF RESEARCH:

I have been informed that the present study will help in assessing the pulmonary function impairment in Thalassemia casesadmitted to Shri B.M. Patil Medical College.

PROCEDURE:

I understand that after having obtained a detailed clinical history, thorough clinical examination and relevant investigations, a final follow up of Thalassemic children.

RISK AND DISCOMFORTS:

I understand there is no risk involved and that the baby may experience some pain and discomforts during the examination. This is mainly the result of the condition and the procedures of this study are not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation in the study will have no direct benefit to me other than the potential benefit of the research and education.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality. Information of sensitive personal nature will not be part of the medical record, but will be stored in the investigations research

file.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs will be used only with special written permission. I understand that I may see the photograph before giving the permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time; Dr. NANDAKISHORE B KULKARNI at the department of Pediatrics is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice.

I also understand that Dr. NANDAKISHORE B KULKARNI may terminate my participation in the study after he has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to my baby resulting directly from baby's participation in this study, if such injury were reported promptly, the appropriate treatment would be available to the baby. But, no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to_____the purpose of the research, the procedures required and the possible risks to the best of my ability.

_____ _____

Dr. NANDAKISHORE B KULKARNI Date

(Investigator)

ANNEXURE-II

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

PARENTS / GUARDIAN CONSENT STATEMENT:

We confirm that Dr. NANDAKISHORE B KULKARNI, is doing a study on **"VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN CHILDREN WITH THALASSEMIA MAJOR AND ITS CORRELATION WITH PULMONARY ARTERIAL HYPERTENSION AND SERUM FERRITIN"**

Dr.NANDAKISHORE B KULKARNI , has explained to us the purpose of research and the study procedure. We are willing to give as much as information required for the study and consent for investigations and the possible discomforts as well as benefits. We have been explained all the above in detail in our own language and we understand the same.

Therefore we agree to give consent for baby's participation as a subject in this research project.

_____ _____

(Parents / Guardian) Date

_____ _____

(Witness to signature) Date

ANNEXURE-III

PROFORMA

Name –

IP no –

DOB - Age

weight -

Sex –

Address –

Others –

Frequency of transfusion:

Age at which transfusion started

Months of transfusion

Total number of transfusions

GENERAL phYSICAL EXAMINATION:

weight….....Kg

HR : RR : BP:

HC :

LENGTH/HEIGHT:

HEIGHT FOR AGE:

WEIGHT FOR HEIGHT:

SYSTEMIC EXAMINATION:

CVS:

RESPIRATORY SYSTEM:

GASTRO – INTESTINAL SYSTEM:

SPLEENOMEGALY:

HEPATOMEGALY:

CNS:

INVESTIGATIONS:

CBC:

Serum VEGF:

SERUM FERRITIN:

ECHOCARDIOGRAPHY: PULMONARY PRESSURE

KEY TO MASTERCHART

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

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

Hb..... Haemoglobin

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

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

MCH ---------- Mean Corpuscular Haemoglobin

MCHC ---------- Mean Corpuscular Haemoglobin Concentration

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

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

Tx..... Transfusion

PAH ----------Pulmonary Arterial Hypertension

Gradient---------- Pulmonary Arterial pressure

NAME	IP	AGE	SEX	HEIGHT	WEIGHT	HT FOR AGE	WT FOR HT	CONSANGUINITY	AFFECTE	TX FREQ	1ST TX AGE	MONTHS OF TX	TOTAL TX	CVS	RS	PA	splenomegaly	hepatomegaly	SPLENECTOMY	CNS	HB	PCV	TC	PLATELET	FERRITIN	CARDIAC FAILU	RE	2D ECHO	gradient	PAH	VEGF
PRAMOD	47390	5	M	104	15	-2	-1	YES	1	6	7	53	18	N	N		yes	yes	no	N	6.	1	634	17600	82	NO		N	2	NO	323.
SURAKSHA	2238	10	F	110	16	-3	-1	YES	1	2	8	112	72	N	N		no	yes	YES	N	5.	1	324	5400	150	YES		N	6	SEVERE	504
MARIAM HIPPARGI	159745	3	F	84	10	-3	-1	YES	2	6	10	26	12	N	N	N	yes	yes	no	N	6.	2	924	12400	47.	NO		N	2	NO	106.
SUNIL	164519	10	M	126	25	-2	N	NO	0	4	9	111	72	N	N		no	yes	YES	N	7.	3	786	10400	150	NO		N	4	MOD	798.1
VIJAY KUMAR	82177	15	M	142	32	-3	-1	YES	0	4	10	170	117	N	N		no	yes	YES	N	7.	3	1132	8700	150	NO		N	5	MOD	81
SATISH KUMAR	249461	14	M	133	25	-3	-2	YES	0	4	9	163	94	N	N		no	yes	YES	N	5.	2	568	9300	127	NO		N	3	MILD	428.5
VARSHA SINGI	95910	10	F	116	16	-3	-2	YES	0	6	18	102	51	N	N		yes	yes	no	N		2	575	16700	96	NO		N	2	NO	298.
AFSANA	135809	10	F	117	20	-3	N	YES	2	2	15	105	88	N	N		yes	yes	no	N	5.	1	438	7200	150	NO		N	5	MOD	1003.2
DANESHWARI PUJARI	24261	13	F	142	30	-2	-2	NO	0	3	7	149	64	N	N		no	yes	YES	N	6.	2	567	10100	131	NO		N	3	MILD	554.9
SINCHANA	69240	4	F	97	16	-2	N	YES	0	4	8	40	20	N	N		yes	yes	no	N	6.	2	476	21000	76.	NO		N	2	NO	26
PREETAM	2283	11	M	112	22	-3	1	YES	0	3	5	127	88	N	N		yes	yes	no	N	5.	2	739	14320	150	NO		N	3	MILD	83
SHIVANI	7424	3	F	83	13	-3	1	NO	0	6	8	28	13	N	N	N	yes	yes	no	N	6.	2	834	17600	66.	NO		N	2	NO	312.4
NOORJAN	7360	5	M	102	15	-2	N	YES	0	4	7	53	32	N	N		yes	yes	no	N	5.	1	724	18300	72.	NO		N	3	MILD	222.
GANESH MADAR	69355	4	M	82	11	-3	-3	NO	1	4	6	42	15	N	N		yes	yes	no	N	5.	1	470	7300	38	NO		N	2	NO	156.4
SHREYAS MAGI	4084	6	M	94	15	-3	N	NO	0	3	12	60	34	N	N		yes	yes	no	N	5.	1	579	9100	55	NO		N	2	MILD	323.2
LAXMAN KUNDARGI	7284	1.5	M	80	10	N	-2	YES	0	10	5	13	4	N	N		no	yes	no	N	6.	2	665	21000	18	NO		SMALL ASD	2	NO	23
YUVRAJ CHAVAN	46236	5	M	104	16	-2	N	NO	0	6	10	50	18	N	N		yes	yes	no	N	5.	2	895	17500	48	NO		N	2	NO	201.5
AKASH	2348	21	M	171	58	N	-1	NO	0	8	8	244	142	N	N		no	yes	YES	N	8.	3	639	23100	130	NO		N	2	MILD	319.
TIPPANNA	42486	10	M	114	18	-3	-3	YES	1	4	5	115	58	N	N		yes	yes	no	N	4.	1	487	6100	150	NO		N	4	MOD	52
SIDDHARTH GADAGI	2385	7	M	106	12	-3	-3	YES	2	2	6	78	58	N	N		no	yes	YES	N	7.	2	745	5400	150	NO		N	3	MILD	64
SAKSHI	5459	5	F	95	16	-3	1	NO	0	3	12	48	38	N	N		yes	yes	no	N	5.	1	346	6200	89	NO		N	3	MILD	477.8
MALLIKARJUN	2229	9	M	122	29	-2	2	YES	0	3	6	102	63	N	N		yes	yes	no	N	5.	1	737	8800	113.	NO		N	2	NO	392.3
VEERESH	55372	1.5	M	79	12	N	N	NO	0	18	6	12	3	N	N		no	yes	no	N	7.	2	875	17400	13.	NO		N	2	NO	154.
MANJUNATH	55382	8	M	95	14	-3	-3	NO	0	4	9	87	35	N	N		yes	yes	no	N	4.	1	887	6400	150	NO		N	4	MOD	667.1
ARCHANA	2453	5	F	105	15	-3	-2	NO	0	4	8	52	14	N	N		yes	yes	no	N	5.	1	358	8900	52.	NO		N	2	NO	238.8
VASUDEVA	63208	7	M	104	15	-3	-3	NO	0	6	5	79	22	N	N		no	yes	no	N	7.	6	664	22600	43.	NO		N	2	NO	26
SHILPA	2289	11	F	122	24	-3	-3	YES	0	3	8	124	82	N	N		yes	yes	no	N	4.	1	569	16400	162	NO		N	3	MILD	596.
MALLIKARJUNA	46218	3	M	80	10	-3	2	YES	1	6	9	27	10	N	N		no	NO	no	N	5.	1	731	13900	13.	NO		N	2	NO	142.8
SHIVANANDA	46216	5	m	109	15	-2	-2	YES	1	12	9	51	16	N	N		no	yes	no	N	5.	2	946	32600	23.	NO		N	1	NO	247.2
TANU	2635	3	F	79	9	-1	N	YES	0	8	7	29	8	N	N		NO	NO	NO	N	8.	2	984	41300	34.	NO		N	2	NO	184.
KASHIMSAB	23755	15	M	158	40	-2	-2	NO	0	3	6	174	146	N	N		no	YES	YES	N	4.	1	1648	4700	240	YES		LOw ejecti	6	SEVERE	711
ASHMITA	2398	5	F	106	17	-2	-3	YES	0	5	5	55	24	N	N		yes	YES	no	N	5.	1	332	29500	88	NO		N	2	MILD	407.2
BHOOMIKA	55645	14	F	124	23	-3	-2	NO	0	2	8	160	124	N	N		NO	YES	YES	N	4.	1	635	9400	210.	NO		N	5	MOD	1198.
NABILAL	3674	12	M	118	20	-3	-3	NO	0	3	10	134	78	N	N		yes	YES	no	N	5.	1	984	25300	78	NO		N	3	MILD	78
AKASH	2348	17	M	127	27	-3	2	YES	0	2	7	197	224	N	N		N	Y	YES	N	3.	1	1052	14600	212	YES		LOW EJECTI	6	SEVERE	287

VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN CHILDREN WITH THALASSEMIA MAJOR AND ITS CORRELATION WITH PULMONARY ARTERIAL HYPERTENSION AND SERUM FERRITIN

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