"THE PREVALENCE OF PREMATURE ATHEROSCLEROSIS IN CHILDREN WITH BETA-THALASSEMIA MAJOR USING CAROTID DOPPLER ULTRASONOGRAPHY-A CROSS SECTIONAL STUDY"

BY

REG NO: BM0120016

Dissertation

Submitted to the
KAHER, Belagavi, Karnataka

In partial fulfilment
of the requirements for the degree of

M. D. (Doctor of Medicine)

IN

PAEDIATRICS

Jawaharlal Nehru Medical College,
KAHER, Belagavi, Karnataka

June / July - 2023

KLE Academy of Higher Education and Research
Belagavi, Karnataka

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LIST OF ABBREVIATIONS

CIMT - Carotid Intima Media Thickness

gm/dl – Gram per decilitre

ml/kg/year – Millilitre per kilogram per year

NTBI - non-transferrin-bound iron

DNA – Deoxy-ribonucleic acid

IMT – Intima – Media Thickness

SLE – Systemic Lupus Erythematosus

Cm – Centimeter

% - Percentage

mg – Milligram

gm - gram

WHO – World Health Organisation

Hb - Heamoglobin

HbE – Heamoglobin E

HbS – Heamoglobin S

IOL- Iron overload

b-thalassemia – Beta Thalassemia

IVS – Intervening Sequences

IVS II- Intervening Sequences II

G>C – Guanine replaced by cytosine

T>G – Thiamine replaced by Guanine

T>C – Thiamine replaced by Cytosine

G>A - Guanine replaced by adenosine

CD – Cluster of Differentiation

CD5 - Cluster of Differentiation marker 5

CD 47- Cluster of Differentiation marker 47

CD 30 - Cluster of Differentiation marker 30

Dr – Doctor

RBC – Red Blood Cell

PRBC – Packed Red Blood Cell

SBP – Systolic Blood Pressure

DBP – Diastolic Blood Pressure

α – Alpha

β - Beta

γ – Gamma

δ – Sigma

NTDT – Non- Transfusion Dependent Thalassemia

TDT - Transfusion dependent Thalassemia

MCV – Mean Corpuscular Volume

MCH – Mean Corpuscular Heamoglobin

MCHC – Mean Corpuscular Heamoglobin Concentration

HPLC – High Pressure Liquid Chromatography

RDW – Red Cell Distribution width

HSCT – Haemopoeitic Stem Cell Transplantation

HbF – Fetal Heamoglobin

JAK2 – Janus Kinase 2

LCI - labile cellular iron

LPI - Labile plasma iron

Fe2+ - Ferrous Iron

ROS - reactive oxygen species

MRI – Magnetic Resonance Imaging

LDL – Low density lipoprotein

HDL – High density lipoprotein

CCA – Common carotid Artery

ICA – Internal Carotid Artery

BMI – Body Mass Index

ABSTRACT

Background: For many years, the recommended course of treatment for Beta-Thalassemia major included regular blood transfusions and iron chelation therapy. Most of these individuals' problems have been caused by iron excess. According to the "Iron Hypothesis," iron is thought to be harmful to the cardiovascular system, hence encouraging onset and progression of atherosclerosis and placing these individuals at higher risk of experiencing clinical and/or subclinical thrombotic events.

Objectives: To ascertain prevalence of early atherosclerotic alterations and the contributing variables.

Setting: Pediatric thalassemia ward of Tertiary care hospital catering to rural population

Design: Prospective cross-sectional study.

Subjects: Children diagnosed with Beta – Thalassemia Major between 5-18 years

Outcome measures: Children with Carotid Intima Media Thickness (CIMT) greater than 0.07mm was considered as positive finding. Comparison CIMT with serum ferritin levels.

Results: In our study, we included 57 children with a confirmed diagnosis of Beta-Thalassemia Major, aged 5 to 18 years. Age, gender, transfusion regimen, and volume of blood transfused data have all been recorded, and a descriptive analysis has been done. Children undergo carotid doppler testing and pertinent investigations have been sent in order to evaluate CIMT. 10.5% of people had early atherosclerosis, according to our study. Significant risk variables include illness duration (p-value = 0.011), volume of blood transfused (p-value = 0.001), and serum ferritin (p-value = 0.001).

Conclusion: Beta-Thalassemia Major has significant changes in heamostatic processes, which puts it at high risk for developing thromboembolic events. With this study, we advocate for careful observation of individuals who are ageing so that derangements can be identified early and controlled with appropriate action.

Keywords:

Early atherosclerosis, Beta thalassemia, Carotid Intima Media Thickness, serum ferritin

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INTRODUCTION

Thalassemia is a diverse group of inherited anaemias that typically result from mutations that change the structure of haemoglobin. They impart from one generation to other generation in an autosomal recessive fashion. Nearly 240 million people in the world carry genes of beta thalassemia, of which 30 million are from India irrespective of the ethnicity, leading to ubiquitous distribution of the disease. (1)

Beta thalassemia is commonly characterised by ineffective erythropoiesis and haemolysis. Symptoms of the disease, typically arise when a new-born's normal physiologic anaemia fails to resolve. Severe beta thalassemia is characteristically identified between the ages of 6 months and 2 years.(1) The mainstay of management of severe beta thalassemia remains blood transfusion regularly given every 14 to 35 days, to sustain pre-transfusion haemoglobin beyond 9 to 10.5gm/dl. However, periodic blood transfusions expedite progressive iron overload. According to advocated transfusion regime (100-200ml/kg/year), when transfused it gives 116-232 milligram of iron/kg body weight.(2). In addition to this, the pathophysiology of imbalance in globin chains result in extensive premature destruction of red cells in bone marrow and extra medullary site.(2) This phenomenon is revered as "INEFFECTIVE ERYTHROPOEISIS" which has also been associated with increased iron absorption mainly in intestine which is caused by deficiency of hepcidin. The above two contributes to iron overload.(3)

It is envisaged that iron assimilation surpasses iron loss when production of red cell predecessors in bone marrow surmounts five times of healthy people. (2)

Absorption upsurges to 3-5 mg/day or more in poorly transfused people, bring about superfluous 1-2 grams of iron load year after year. When there is an iron overload, transferrin becomes saturated and there are iron species in the plasma that are not linked to transferrin. (NTBI, or non-transferrin-bound iron). In a study done by Cihan and his colleagues in 2017 which showed reactive oxygen species are formed as a result of this iron overload state in beta thalassemia major patients, which results in oxidative damage to proteins, lipids, and DNA (4) This oxidative DNA damage when happened in endothelial cells lead to vascular alterations, atherosclerosis and coronary Artery disease.(5) This results in early and subclinical arterial atherosclerosis, hypercoagulability, and thrombo-embolic consequences, which are currently the main causes of morbidity in these individuals. For these alterations in people at risk for cardiovascular disease, impaired endothelial function is a crucial biomarker.(6)

Chelation therapy, however, is employed in youngsters to eliminate the body's surplus iron. Chelation therapy usually started after 2 to 3 years of initiation of transfusion therapy, in order to prevent stagnation of tissue bound iron which takes years to eliminate once it gets accumulated. (2)Effective management of this disease require meticulous monitoring and a balance between iron overload and iron chelation and adverse effects of both.(7) Serum Ferritin is considered as a marker to monitor this balance of overload and chelation.

Suboptimal iron chelation leads to iron accumulation in tissues which leads to hepatic hemosiderosis, endocrinopathies, cardiomyopathy and cerebrovascular events. The risk of ischemic stroke in transfusion dependent thalassemia varies from 0.25% to 0.46%.(8),(9),(10) However, the risk of silent cerebral infarction is high up to 66%.(11) In atherogenesis, the vascular endothelium gradually thickens over the course of what is thought to be a lengthy, asymptomatic delay, causing morphological changes

in the artery wall. Along with more established diagnostic techniques like angiography and stress testing, measuring the intima (IMT) of the aorta, particularly the carotid artery, allows for the identification and quantification of early arterial wall alterations. It is a straightforward, precise, and non-invasive predictor of vascular abnormalities. Gradual augmentation in the CIMT is contemplated as a marker for early atherosclerosis and a predictor of upcoming cardiovascular events.(12)

Premature atherosclerosis is defined as Atherosclerosis which occurs in a person with certain risk factors like SLE, diabetes, smoking, hypertension, hypercholesterolemia, Beta-Thalassemia, Sickle cell anaemia, family history of early heart disease occurring at an earlier age [age less than 55years in males, less than 65 years in females] than that would occur in another person without risk factors.″(13–15)

Measurement of blood velocities in basal cerebral arteries using transcranial ultrasonography, which has been in use since 1982, can reveal the presence of early atherosclerotic changes. By evaluating Carotid intima-medial thickening, plaque presence, and vascular stenosis, this method is efficient in screening for stenosis, vasospasm, or modification in intracranial hemodynamic associated to occlusive carotid disease.(16) The normal CIMT thickness in children ranged in between 0.03 – 0.06cm in children aged between 6-18years of age.(17)

The current study seeks to identify the clinical and laboratory risk variables that are related with early subclinical atherosclerosis in individuals with beta-Thalassemia major by calculating their CIMT and finding its association with serum ferritin.

OBJECTIVES

PRIMARY OBJECTIVE:

- To study the prevalence of premature atherosclerosis in transfusion dependent Beta thalassemia major children.

SECONDARY OBJECTIVE:

- To find out the association between serum ferritin and atherosclerotic changes.

REVIEW OF LITERATURE

EPIDEMIOLOGY:

With 3% of the global population harbouring thalassaemic genes, thalassemia is the predominant monogenic illness.(18)

Beta thalassemia prevalence has historically been highest in the countries of Middle East, South Eastern countries of Asia and Mediterranean. However, Western Europe and North America are seeing an upsurge in the illness. This could be explained by immigration, the implementation of programmes for beta thalassemia, and higher survival rates.(19)

Every year since 2008, according to the WHO, more than 40000 children have been born with beta thalassemia, of which 25000 have the condition that necessitates transfusions.(20)

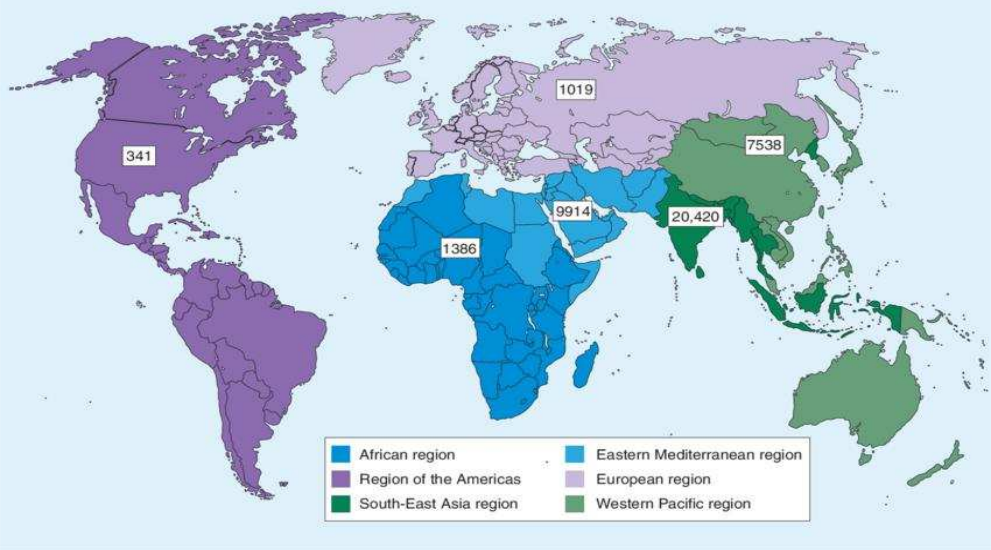


Figure 1 -World-wide Burden of Thalassemia(21)

BURDEN OF THALASSEMIA IN INDIA

The prevalence of beta-thalassemia in the Indian population is typically between 3 and 4 percent, translating to an estimated 30 to 40 million carriers in our 1.21 billion-strong multi-ethnic, culturally and linguistically diverse population, which also included 8% of tribal people according to the 2011 census of India.(22)

The frequency of beta thalassemia carriers varied irregularly in different districts of Gujarat (0-9.5%) and Maharashtra (1-6%), according to selected geographic micro mapping. In Maharashtra and Gujarat, there were 0.28 and 0.39 homozygosity rates per 1,000 live births, respectively.(23)

HbE variant is widespread in Eastern and North-eastern states of India, with prevalence >50% in various communities. However, it is now noticeable in various locations as a result of people moving there for employment as well as a shift in attitudes toward interstate and interreligious marriages, which has a negative impact on the number of consanguineous marriages. The highest frequency of HbS is found in the central Indian state of Madhya Pradesh, where >3000 new-borns are anticipated to be born each year with sickle cell disease. It is primarily found in scheduled caste and tribal populations. In the ethnic groups from several of India's western, eastern, and southern states as well as its central and western regions, the prevalence of HbS carriers has fluctuated from 1 to 35%. These Hb variations usually result in diseases of varying severity because they co-inherit with b-thalassemia. According to estimates of the number of new-borns born each year with these illnesses, India is home to at least 100,000 kids who have the beta thalassemia condition.(24)

52 mutations have been accounted for 97.5% of all the b-thalassaemic alleles, according to a countrywide meta-analysis on 17 chosen studies that included 8505 alleles. Between 44.8% in the north and 71.4% in the east, the most prevalent mutation, IVS 1-5(G>C), was found. The following uncommon mutations were discovered to be more prevalent in a select group of populations, including Prajapatis of Gujarat with CD5 (-CT), Gaud Saraswat, Brahmins in Goa and Karnataka with IVS II-837(T>G), CD 110 (T>C) Agris in Maharashtra, CD 47 (+A) Nicobarese in Car Nicobar, Andaman and Nicobar Islands, and Kachiya Patels with CD 30 (G>A). (25),(26),(27),(28)

BRIEF EVOLUTION OF THALASSEMIA

The first official description of thalassemia comes from Dr. Thomas Benton Cooley, an American Paediatrician from Detroit who in 1925 reported "a series of cases of enlarged spleen in children with peculiar changes".(29)

The word thalassemia took its beginning in 1932, it comes from the Greek word '*Thalassa'* meaning "of the sea" and was chosen as an overarching term as the condition was famous to be transcendent in individuals whose hereditary homes encompassed the Mediterranean Ocean. The term was coined by "Whipple and Bradfort".(30)

In this way a few creators all over the world included different subtle elements concerning clinical and research perspectives of the disease. While Camino Petros pointed out conclusively in 1936 that this was a familial condition acquired in an autosomal recessive mode.(31)

The abnormal haemoglobin structure was shown to be the cause in 1946. Anaemia results from the body's reaction of eliminating red blood cells. The body tries to produce red blood cells more quickly to make up for the loss, which results in various thalassemia problems such bone abnormalities and splenic enlargement.(32)

J. B. S Haldane, in 1948 inferred that population with beta-Thalassemia have an advantage of heterozygosity with respect to malarial infection; this was similarly conferred to that of sickle cell anaemia. Therefore, in areas of high malarial prevalence, such mutations would be selected and propagated, resulting in microcytic erythrocytes that are less vulnerable.(33)

In the 1970's, the preference of beta-thalassemia to influence Mediterranean population was recognised. Hence, pilot programmes for prevention were built to raise

awareness. Concurrently, red blood transfusions were the treatment of choice and complicated by blood borne diseases.(34)

Iron over-burden has been a prominent problem for beta-thalassemia major since blood transfusions were introduced. As a result, the use of Deferoxamine, a hexadentate Iron chelator, when it was approved for intravenous use in 1968, marked the introduction of Iron chelation therapy in Beta-Thalassemia Major. Deferoxamine has dramatically increased patients with iron overload's life expectancy and quality of life. Previously, these individuals did not make it through their adolescent years. In addition, it had been the "Gold Standard in Iron Chelation Therapy".(34),(35)

Over the past 20 years, oral iron chelators with higher compliance have been suggested and examined. Deferasirox received approval from the Food and Drug Administration in 2005 to be used as the first oral iron chelator in children older than 2 years. Deferiprone, a second oral iron chelator, was approved for use in humans on October 14, 2011, in the US.(35)

In 2008, Carelli and Gaziev proposed that transplantation with Haemopoietic stem cell from matched contributors has an 80-87% cure.(36)

In 2019, The clinical information of a review by Harrison prompted the restrictive endorsement of Lentiglobin BB305 quality treatment vector by European commission for gene therapy in beta-thalassemia major.(37)

TYPES OF THALASSEMIA

Haemoglobin in human RBC consists of a heme group and 4 globin chains, which consisted of 2 alpha globin and 2 beta globin chains. There are mainly 2 types of thalassemia, alpha and beta thalassemia and other less common types like gamma, delta. The deletion of a number of genes results in varied severity of thalassemia like trait, minor and major forms.(38)They are usually inherited in an autosomal recessive fashion.(1),(38)(39)

GENETICS OF THE DISEASE:

The synthesis of haemoglobin is directed by 2 gene clusters

α – locus -consisted of Embryonic gene + 2 adult α genes , located on chromosome 16

β – locus -consisted of Embryonic, Fetal Go and Aγ, Adult s and β genes, located on chromosome11

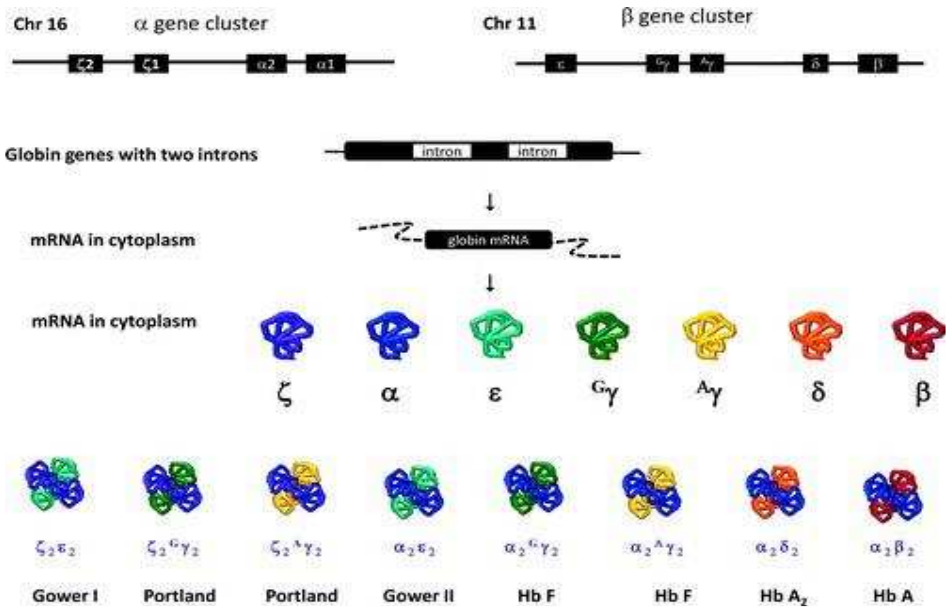


Figure 2. Genes involved in the synthesis of normal haemoglobin(40)

The hall mark of thalassemia is decreased synthesis of α and β globin chains. Heterogeneity arises because, the syndrome in any given individual represents the combination of 2 out of 30 or more mutations. It also includes syndromes of varying severity.

α-Thalassemia:

Alpha thalassemia arise due to deletion of alpha genes. Deletions can be either single or double. Double deletions can be either in cis forms or trans forms(38),(39),(41)

-Silent carrier (α or β)-Haematologically normal

-Thalassaemic trait (α or β)-Mild Anaemia with microcytosis and hypochromia.

-HbH disease (α-thalassemia)-Moderately severe haemolytic anaemia, Icterus and splenomegaly

-Hydrops Fetalis (α-thalassemia)-Death in utero caused by severe anaemia.

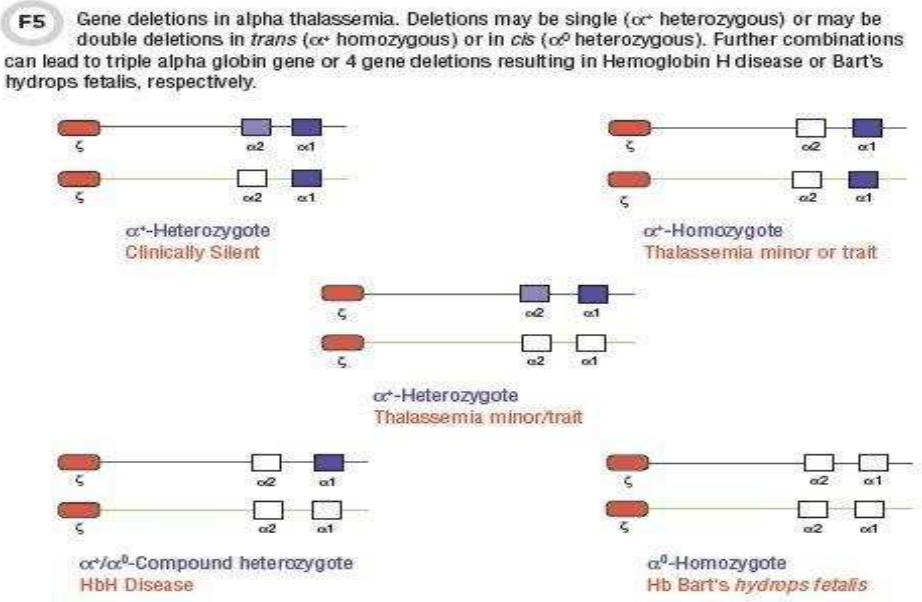


Figure 3. Types of Alpha Thalassemia(42)

β – Thalassemia:

Beta thalassemia arise due to point mutations in splicing point, Promotor region and stop codon or rarely by deletions in beta globin gene in chromosome 11. (1),(38),(43),(44)

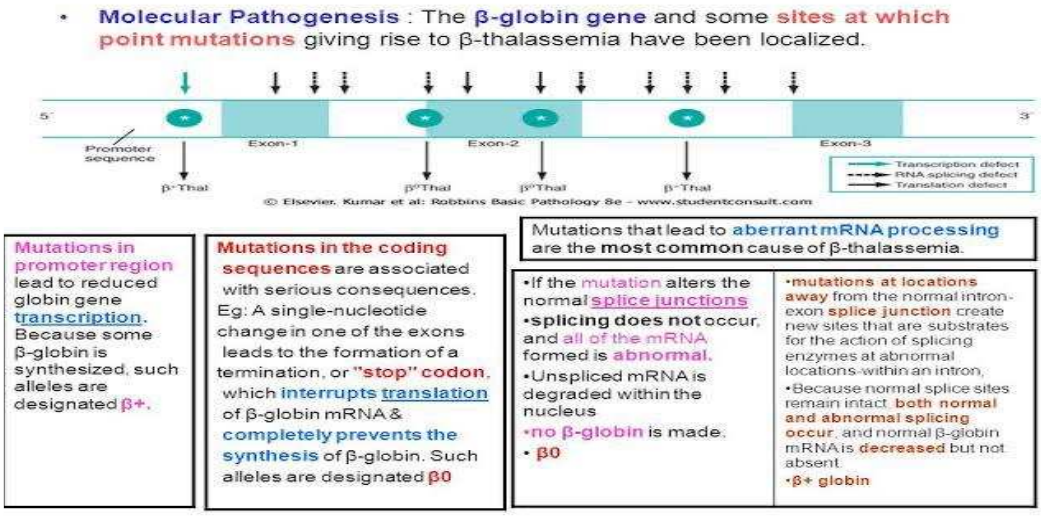


Figure 4-Types of Beta Thalassemia(42)

-Severe Beta Thalassemia (Cooley's Anaemia)-Severe Anaemia, Growth Retardation, Hepatosplenomegaly, Bone marrow expansion, Bony deformities

-Beta thalassemia Major-Transfusion dependant

-Beta thalassemia Intermedia- No regular transfusion dependant

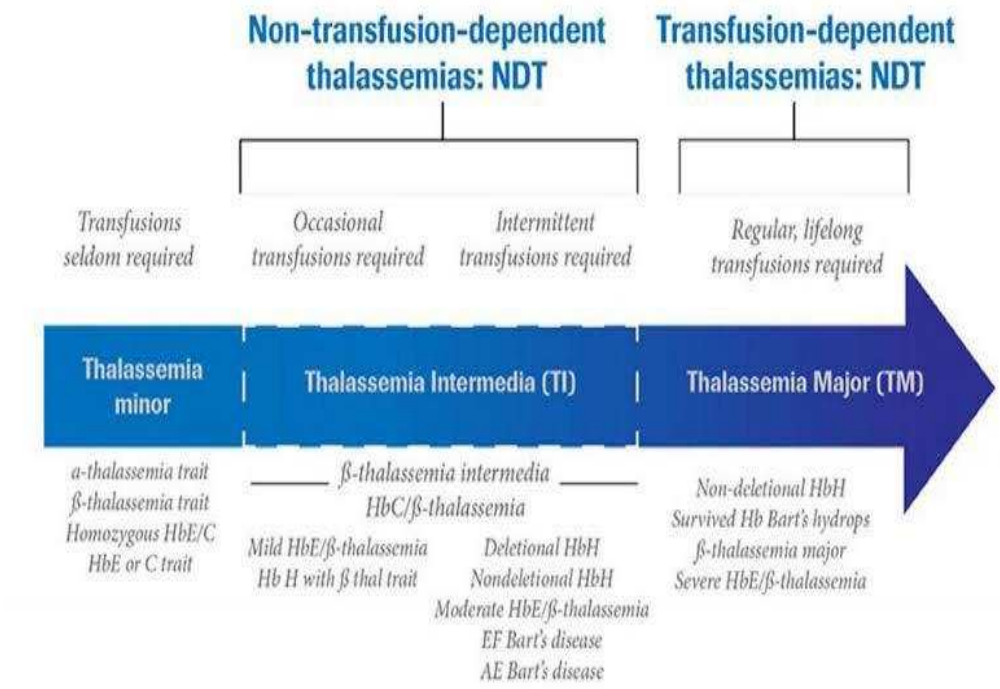


Figure 5-Clinical classification of thalassemia(45)

BETA-THALASSEMIA MAJOR

β - Thalassemia occurs when the quantity of normal β-globin chains decreases. They develop as a result of extremely diverse mutations that impact the β globin loci. Almost every conceivable abnormality influencing gene expression occur at transcriptional, post-transcriptional level and translational levels.(1) This results in a varied decrease in synthesis of β globin, ranging from a little shortfall as in mild β+ thalassemia alleles, to total absence seen in β° thalassemia. In β thalassemia, the unaffected α globin genes continue to produce regular α globin chains as usual, which causes an overabundance of mismatched α globin to build up in the erythroid precursors. Free α globin chains are unable to form functional tetramers; instead, they precipitate in the bone marrow's red cell precursors, leading to the development of inclusion bodies. By using optical and electron microscopy, it is possible to see these α chain inclusions in the peripheral red cells after splenectomy as well as in the erythroid precursors in the bone marrow. They are to blame for all thalassemia' poor erythropoiesis, which is caused by the severe intramedullary destruction of the erythroid precursors.

Therefore, peripheral haemolysis, inefficient erythropoiesis, and a general decrease in haemoglobin synthesis all contribute to anaemia in β thalassemia patients. Therefore, the phenotype will be influenced by variables that lessen the severity of chain imbalance and the quantity of chain excess in the red cell progenitors. This results in pallor, hepatosplenomegaly, and an enlargement of the bone marrow.(1),(6),(28),(37)

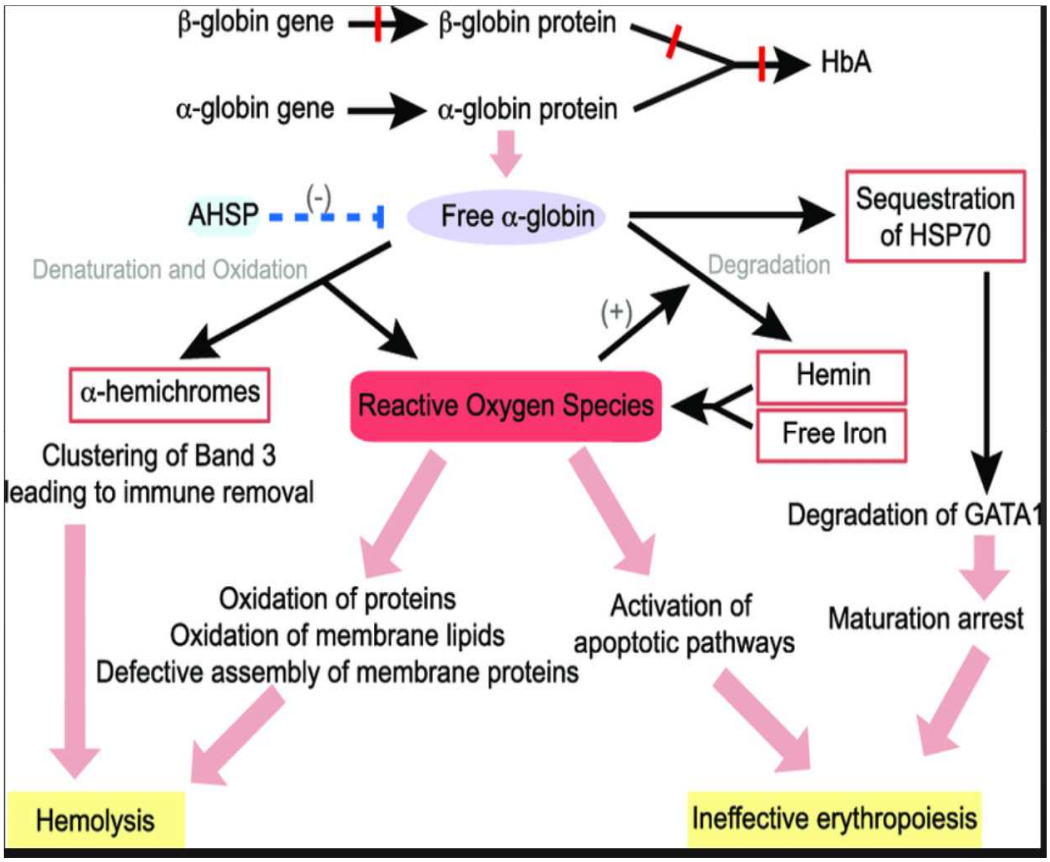


Figure 6 -Pathophysiology of Beta Thalassemia(46)

Clinical Features;

With failure to thrive, poor weight gain, skeletal abnormalities, and growth with developmental delay, severe beta thalassemia first manifests in the first year of life. Death from beta-thalassemia major, generally from heart failure, occurs eventually if untreated.

Ineffective erythropoiesis can also result in compensatory bone marrow growth, which can ultimately cause skeletal changes/deformities, bone pain, and craniofacial anomalies.

The main clinical signs are right upper quadrant pain brought on by gall stones, splenic infarction, and abdominal discomfort brought on by hypersplenism. Parents may have noticed the infant becoming progressively pale with protruding abdomen.

On clinical examination, child presents with

-Pallor, Poor growth, Inadequate food intake, Icterus, Palpable Splenomegaly ,Maxillary Hyperplasia, Dental Malocclusion, Cholelithiasis, Systolic Ejection Murmur in Severe anaemia

- characteristic chipmunk facies that arises due to Bone marrow expansion-characterised by bossing of skull, Prominent malar eminences, depressed nasal bridge. Due to the maxillary enlargement, the eyes have a mongoloid slant and the upper teeth are visible.

Haematologic diagnosis:

Based on symptoms investigations required for confirmation include

-Complete blood count-Beta thalassemia major is characterised by decreased Hb, Low Mean Corpuscular Volume of less than or equal to 80fl, Low Mean corpuscular Haemoglobin with less than 27pgs Increased red cell distribution width.(47)

Peripheral smear showing microcytosis, Hypochromia and Target cells.

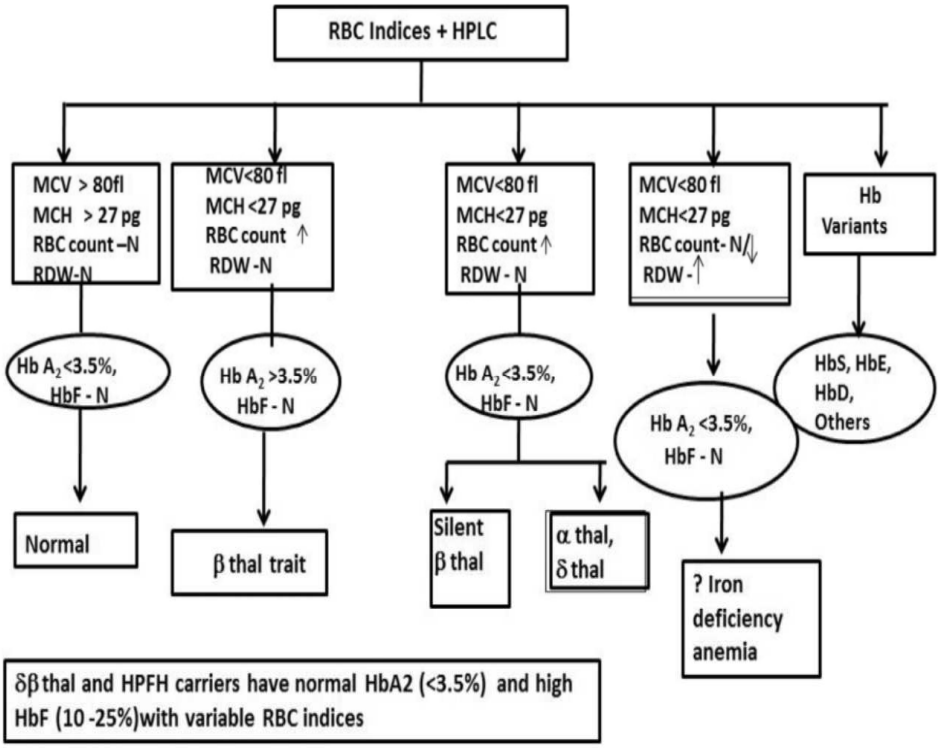


Figure 7 – Algorithm of diagnosis of thalassemia based on laboratory parameters(48)

-Haemoglobin Electrophoresis – Beta-thalassemia patients should have higher levels of haemoglobin A2 and slightly higher levels of haemoglobin F in their haemoglobin electrophoresis results. Beta Thalassemia confirmatory testing include haemoglobin electrophoresis or high-performance liquid chromatography.

Total iron binding capacity -Serum ferritin -Serum transferrin -Serum ferritin-DNA Analysis: testing is not routinely recommended, but it helps in the diagnosis of thalassemia and determine carrier status.

TREATMENT FOR THALASSEMIA

Possible therapeutic options available for thalassemia includes

-Conventional therapy **like regular blood transfusions** to treat anaemia and treating iron overloads with iron chelating agents such as deferoxamine, deferiprone, deferasirox.(49)(50)

-**Pharmacological induction of γ- gene** with agents like Hydroxy urea, Butyrates which helps in promoting effective erythropoiesis by raising level of HbF in the body.(51),(52)

-A life time therapy with **Haemopoietic stem cell transplantation**, where normal haemoglobin is produced by destroying the defective stem cells. A recent advancement in this arena included Autologous HSCT.(53)

- **Splenectomy carried out traditionally or as a supplement to transfusion treatment**. (54)

-**Gene therapy** - Using lentiviral vectors, exogenous beta or gamma genes are incorporated into the genome of autologous stem cells. This therapy is in PHASE 3 trials (55)

-**Gene editing** - the endogenous DNA of stem cells is altered using designer endonucleases.

-Agents such as RUXOLITINIB [JAK2 inhibitor], SOTATARCEPT [Activin 2A receptor fusion protein], LUSPATERCEPT [Activin 2B receptor fusion protein] are in PHASE 2A trials.(56),(57)

-Agents that improve iron dysregulation such as Mini hepcidins, TMPRSS6 inhibitors are in preclinical trials.(58)(59)

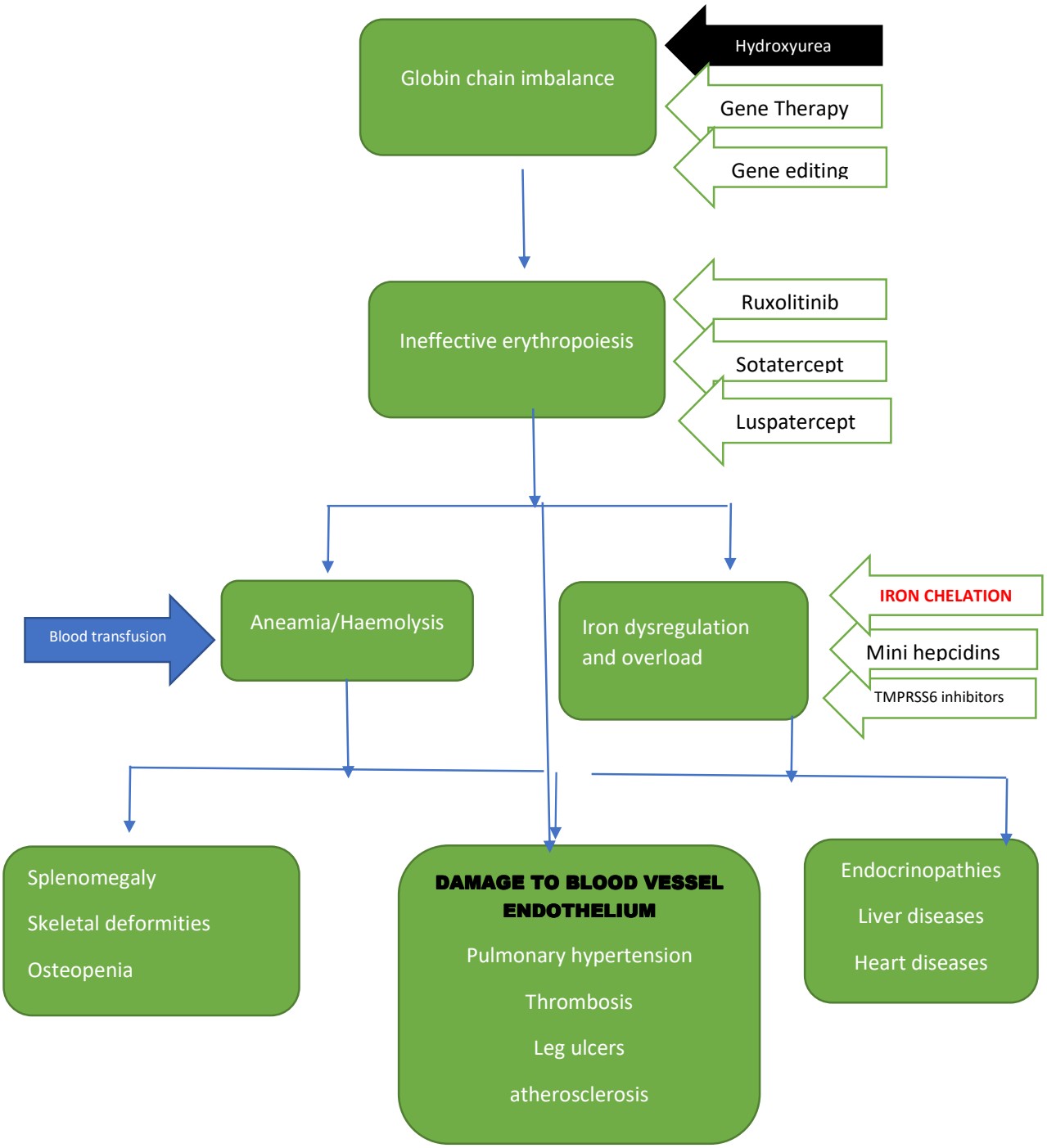


Figure 8- Recent advances in treatment of thalassemia(60)

Despite availability of many treatment modalities, regular blood transfusion therapy and iron chelation has been the standard treatment option in many centres in India.

However, this therapy leads to many complications due to iron overload, such as

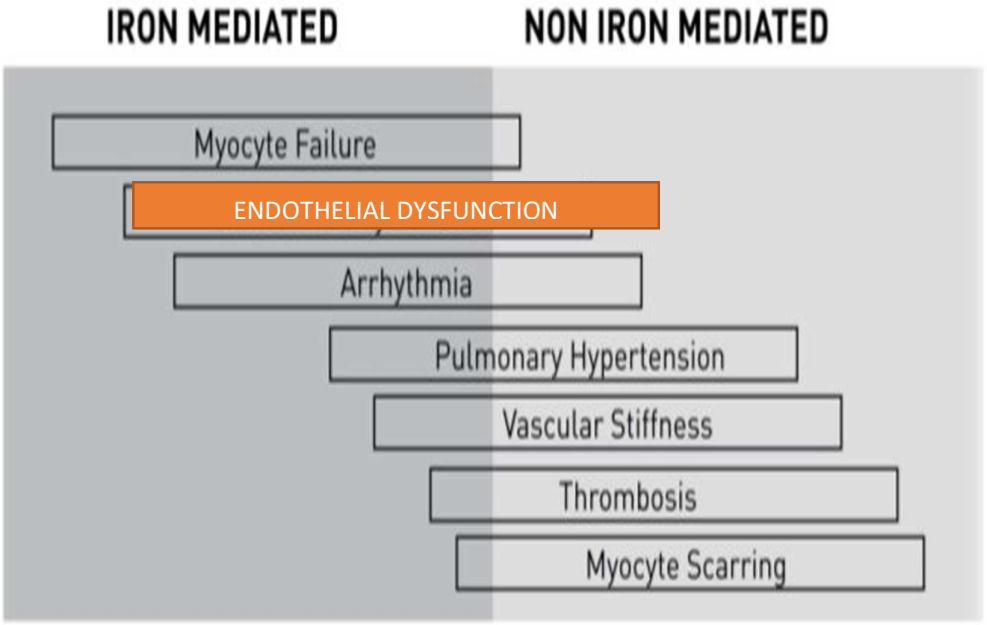


Figure-9 – Complications of Iron Overload(2)

IRON OVERLOAD AND ITS EFFECTS ON VASCULAR SYSTEM

Definition-

Premature atherosclerosis is defined as the occurrence of atherosclerotic changes in a child with risk factors like SLE, Diabetes mellitus, Hypertension etc., at an earlier age i.e. , age less than 55 years in male and 65 years in females compared to those without risk factors.(13–15)

PATHOPHYSIOLOGY OF IRON OVERLOAD IN BETA-THALASSEMIA MAJOR

Iron homeostasis is an intricate process, that maintains absorption at around 2 mg per day. Multiple molecules work together to carefully control this. Since humans lack a natural system for actively excreting iron, excess iron from blood transfusions is retained in bodily tissues and causes organ damage.(2),(38),(44) Phagocytoses of transfused red blood cells in the reticuloendothelial system leads to production of labile cellular iron (LCI), which attaches to plasma transferrin, consisting of two Fe3+ molecules. When the capacity of transferrin to bind iron exceeds 60–80%, non-transferrin-bound iron (NTBI) is produced in the plasma, accumulates in pancreatic, hepatic, pituitary, and cardiac cells. (44) However, Labile plasma iron (LPI), a highly reactive Fe2+ variant of NTBI, can enter cells through calcium channels that are not affected by the amount of intracellular iron present. The myocardial, liver, and endocrine glands are some examples of target organs where reactive oxygen species (ROS) generated by NTBI, labile plasma and cellular iron leads to cellular dysfunction, oxidative damage, fibrosis, apoptosis and necrosis. The varied loading rates of iron is organ specific which is seen by MRI. (47) Similar to this, the liver releases iron at a rate that is substantially higher than that of the heart and endocrine organs.

The iron content in each unit of packed RBCs for transfusion is 200–250 mg. Therefore, for a patient with Transfusion dependent Thalassemia [TDT], a typical transfusion regimen of 2-4 PRBCs / month introduces 4.8 to 12 gm / year of iron, which is higher than the iron consumed from diet i.e., 0.4 to 0.7 gm. The cardinal mechanism of IOL in TDT is due to multiple transfusions. It also occurs in the absence of routine RBC transfusions in NTDT, which occurs with increase in age; they also have lower

levels of serum ferritin due to selective accumulation of iron hepatocytes and portal system, secondary hepcidin suppression and increased intestinal absorption.

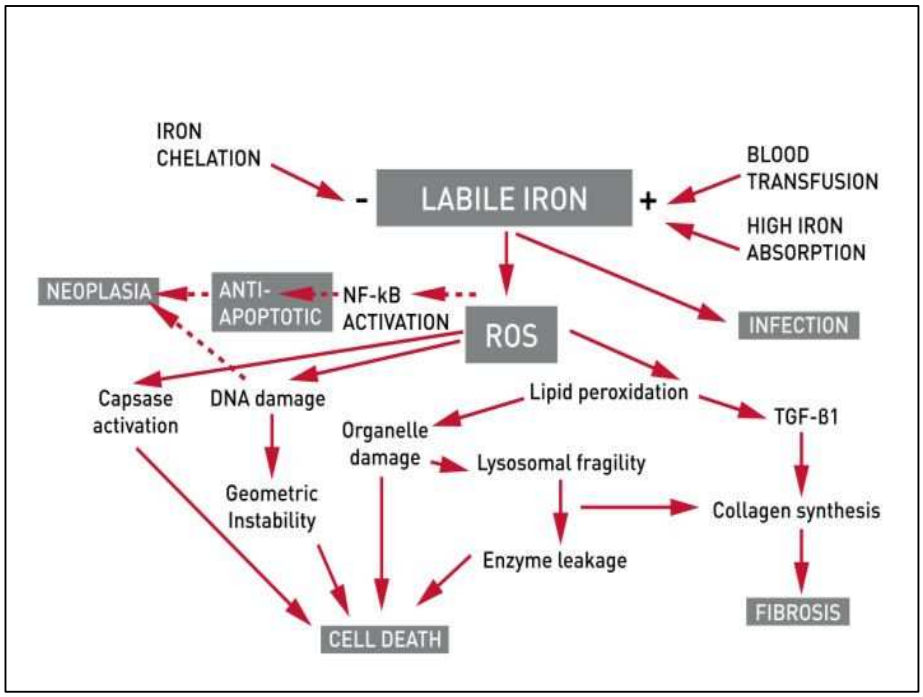


Figure 10- Pathophysiology of Iron in Beta Thalassemia(2)

Reactive oxygen species are formed as a result of iron overload brought on by repeated blood transfusions and increased intestinal absorption, which causes oxidative stress, organ malfunction, and tissue damage.(47) The most common cause of death from these cardiovascular problems, including hypercoagulability, thromboembolic complications, and early and subclinical atherosclerotic alterations.(61) A indicator of vascular health in persons at risk for cardiovascular disease is impaired endothelial function. Evaluation of endothelial function is thus a helpful prognostic tool.

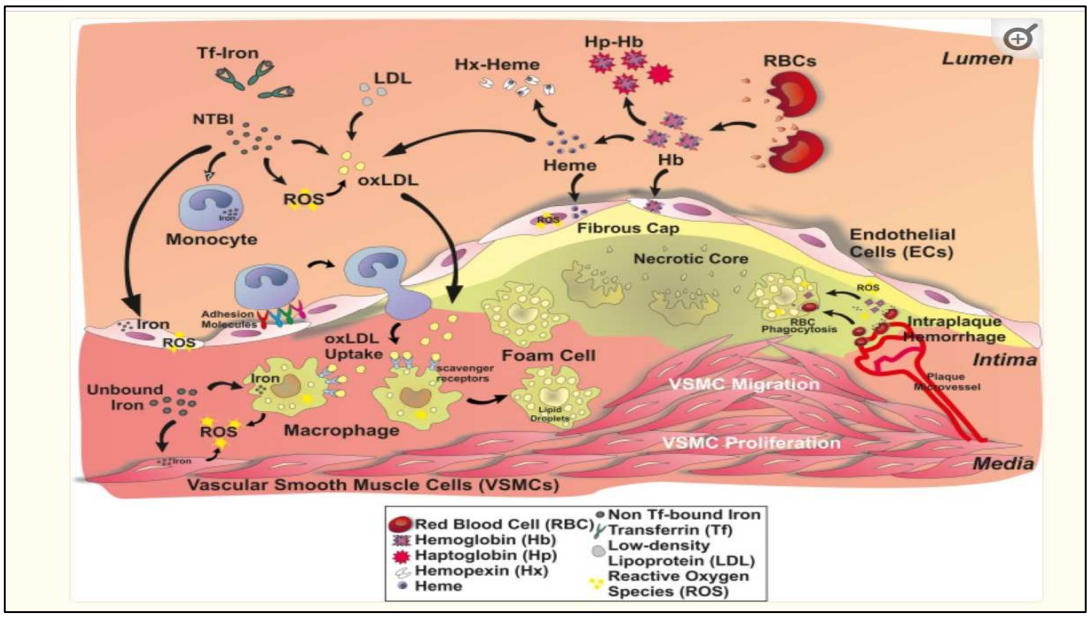


Figure 11- Formation of atherosclerotic plaque(62)

INVESTIGATIONS TO DECTECT THIS PREMATURE ATHEROSCLEROTIC CHANGES

The onset of vascular problems in thalassaemic individuals may be aided by effective methods to recognise early vasomotor dysfunction and concealed subclinical atherosclerosis as well as their risk factors. The extension of thalassemia patients' morbidity-free lifespan is facilitated by early detection of these problems. These complications can be identified by measuring

- Carotid intimal-medial thickness - The procedure measures the distance between the intima and media, the two innermost layers of the carotid artery.(12,63)
- The formula for calculating stiffness index is as follows: SBP and DBP stand for systolic and diastolic blood pressure, respectively. ln (SBP/DBP)/(δD/D), where δD is the difference between systolic and diastolic diameters, and D is the mean diameter(61)
- Young’s Modulus(61)- Any material's Young's Modulus is its capacity to withstand changes brought on by forces operating longitudinally. Young's modulus is frequently referred to as the elastic modulus.
- -Augmentation index- The Augmentation index, which measures arterial stiffness based on blood pulse-wave reflection and serves as a risk factor for cardiovascular disease, is widely used.(61)
- -Flow mediated vasodilatation- By comparing the brachial artery diameter at rest and during reactive hyperaemia, flow-mediated endothelium-dependent vasodilation was evaluated.-Arterial elastic properties.(61)
- Ultrasound imaging of both common carotid arteries up to their bifurcation, the carotid bulb, as well as the proximal 10 mm of the internal carotid artery on both

sides is used to quantify the carotid intimal medial thickness. Between the intima and media limits, a measurement is made. To calculate the CIMT for a given side, the mean values of CIMT from three locations are used.(63)

- It is necessary to seek for intimal plaques. A focal formation that encroaches into the artery lumen by at least 0.5 millimetres or 50% of the CIMT around it is referred to as a plaque.

BLOOD VESSELS AFFECTED IN BETA THALASSEMIA MAJOR

It has been demonstrated that iron excess results in endothelial dysfunction due to oxidative damage, which hastens and causes premature atherosclerosis(64),(65),(66),(67). Patients with high iron are known to experience thrombotic occlusions in coronary, cerebral, retinal and portal systems; (67) however, the mechanism behind such occurrences is currently poorly understood because to a lack of Indian research.

PREVENTION OF THESE PREMATURE ATHEROSCELEROTIC CHANGES

Iron overload, an unavoidable side effect of transfusion therapy, presents as difficulties with the heart, the endocrine system, the liver, and other organs. For example, cardiac iron overload leads to significant mortality and T2* magnetic resonance imaging can be used to detect it. (67) The effects iron poisoning on the cardiovascular system remains a mystery.(55)

These complications can be prevented by strict iron chelation, addition of antioxidants that prevent the evolution of premature atherosclerotic changes and strict monitoring of lipid profile in these children and controlling the LDL levels.

MATERIALS AND METHODS

- STUDY DESIGN: Cross sectional study

- STUDY PERIOD: 1YEAR,
    - JANUARY 2021-DECEMBER 2021

- PLACE OF STUDY: PAEDIATRIC WARD, KLE'S DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE

- INCLUSION CRITERIA:

  Children between 5-18years of age with confirmed diagnosis of Beta – Thalassemia Major.

- EXCLUSION CRITERIA:

  1. Children with Renal diseases.
  2. Children with hepatic diseases.
  3. Children with Diabetes Mellitus
  4. Children with Obesity
  5. Children with hypertension.
  6. Children with Hypercholesterolemia

- SAMPLE SIZE- The formula for calculating sample size is

$$n = \frac{p(100-p)\,Z^2}{(E)^2}$$

The required sample size is n, the percentage of occurrence of a condition or state is p, the percentage of maximum error is E, and the value corresponding to the level of confidence is Z. 19% of people have early atherosclerosis. sample size is provided by with a percentage maximum error of 10% at a 95% confidence range.

$$n = \frac{19\times 81\times (1.96)^2}{(10)^2}$$

$$n = 57$$

- Ethical Clearance- Prior to starting, the study received institutional ethics committee approval from JNMC, Belagavi.
- Sampling Method: Stratified Random Sampling (stratified as per age group).

Group 1:5-10years age group including 19 participants

Group 2:11-14years age group including 19participants

Group 3:15-18years age group including 19participant

METHODOLOGY

Total list of patients diagnosed with Beta Thalassemia Major and are enrolled in thalassemia ward is taken and all the children who met the inclusion and exclusion criteria are stratified into 3 groups based on their age and every 3rd patient from the list is enrolled into the study, with each group consisting of 19 participants. Parents of children who fulfilled the eligibility criteria are briefed about the nature of study. Also prior to enrolment informed consent taken.

1.A thorough history is taken, with particular attention paid to age, sex, the duration of the disease, the time and age at which transfusions began, the frequency of transfusions (such as the hypo/hyper/super regimen), and the frequency of chelation therapy. Patients are then classified as being adequately chelated, inadequately chelated, or not chelated at all based on serum ferritin levels and the frequency of chelation therapy.

2.To rule out obesity, evidence of heart failure, hepatic failure, and renal failure, anthropometric measurements were taken and recorded on WHO growth charts. To rule out the aforementioned systemic ailments, a general and systemic examination are conducted.

3.At the time of the study, lipid profiles, blood glucose levels, serum ferritin levels, and haemoglobin status were all measured in the laboratory.

4.In order to check for Carotid intima medial thickness, the presence of luminal plaques, and vessel stenosis, children are subjected to B-mode and color-coded duplex carotid doppler ultrasonography of the common carotid and internal carotid arteries by a single experienced radiologist using a GE Voulson S8 USG machine with high frequency probe 7.5Hz to 12Hz. The common carotid artery (CCA) is identified in the

transverse plane of the lower neck. It was then traced proximally until the clavicle blocked the transducer, and if possible, caudal angulations were made in an effort to assess the common carotid's origin. The CCA continued upward until it widens into the carotid bulb, at which point it split into external and internal carotid arteries. The transducer is then turned 90 degrees so that it is parallel to the CCA, the bifurcation, and the internal carotid artery [ICA]. The ICA is then followed distally until it is ideally lost behind the jaw. The veins were carefully examined for sub intimal lucency , atherosclerotic plaques that protruded into the lumen, and distance between vascular media and intima. The measurements of the carotid intimal medial thickness were then compared to age-specific reference values.

STATISTICAL ANALYSIS

For quantitative variables, the mean and standard deviation were used in the descriptive analysis, while frequency and proportion were used for categorical variables.

By visually inspecting histograms and normality Q-Q plots, all quantitative variables were examined for normal distribution within each category of explanatory variable. Additionally, the Shapiro-Wilk test was used to evaluate the normal distribution. A p value of >0.05 for the Shapiro-Wilk test indicated that the distribution was normal.

Using the Chi square test and Fisher's Exact test, categorical results were compared between research groups.

Using an independent sample t-test with two groups for regularly distributed quantitative parameters, mean values were compared between study groups. For normally distributed quantitative parameters, mean values were compared using an ANOVA with more than two groups.

A suitable post-hoc test (LSD/Bonferroni) was utilised to determine the statistical significance of pairwise comparisons if a statistically significant difference was discovered in the ANOVA.

For normally distributed Quantitative parameters Association between quantitative explanatory and outcome variables was assessed by calculating Pearson correlation

Statistical significance was defined as a P value 0.05. The statistical evaluation was performed using IBM SPSS version 22. (1)

IBM Corp. published in 2013. Version 22.0 of IBM SPSS Statistics for Windows. IBM Corp., Armonk, New York

RESULTS

57 children with beta-thalassemia major participated in our study, with a mean age of 12 years and 32 females and 25 males.

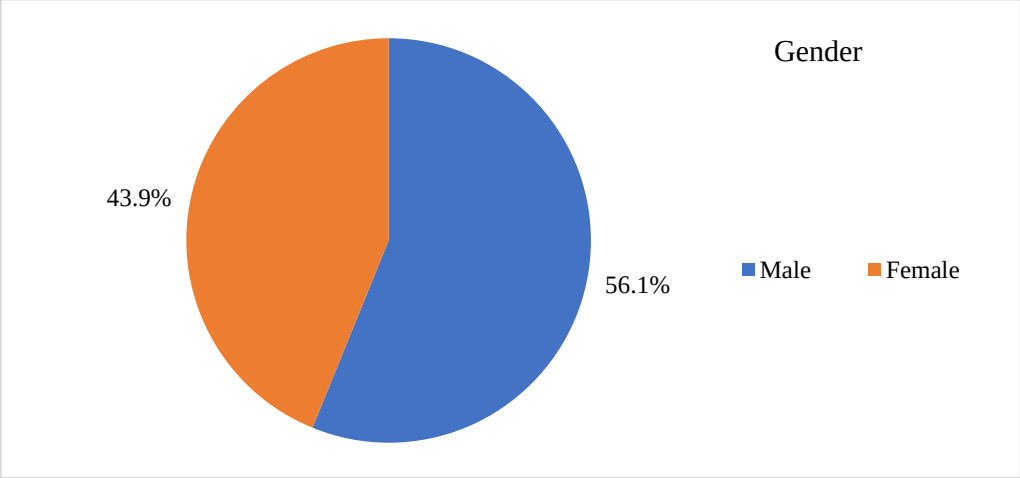
Table 1: Descriptive analysis of parameters in study population (N=57)

Parameter	Summary
Age In Yrs. [Mean ± SD, (Range)]	12.12 ± 3.9 (5-18)
BMI kg/m^2 [Mean ± SD, (Range)]	17.18 ± 3.27 (11.9-26)

Table 2: gender-specific descriptive analysis of the sample population (N=57)

Gender [n (%)]	Number and Percentage in study population
Male	32 (56.14%)
Female	25 (43.86%)

Figure 12: Gender distribution in the study population is shown in the pie chart. (N=57)

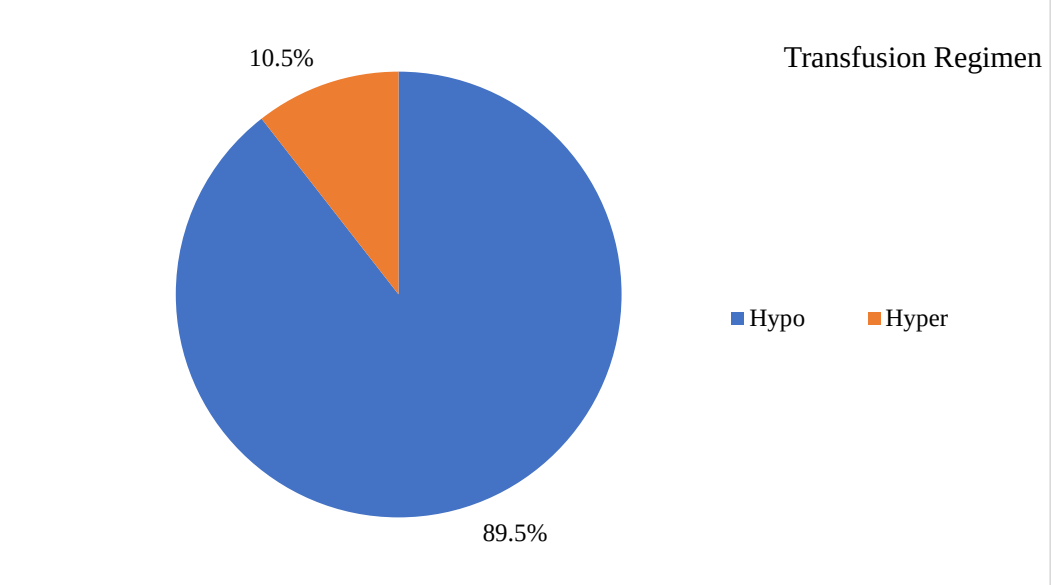


The average amount of blood transfused per child is 54918 ml, and their transfusion indices are 174 ml/kg/year .51 children on a hypo transfusion regimen and 6 children on a hyper transfusion regimen. Mean duration of chelation in study population is 112.79 months.

Table 3: Transfusion protocol in the study population - a descriptive analysis(N=57)

Transfusion Regimen	[n (%)]
Hypo transfusion	51 (89.47%)
Hyper transfusion	6 (10.53%)
Volume Transfused [Mean ± SD, (Range)]	54918.16 ± 41532.53 (8537-216734)
Transfusion Index [Mean ± SD, (Range)]	174.44 ± 45.89 (102-302)
Duration of Chelation in Months [Mean ± SD, (Range)]	112.79 ± 48.17 (22-214)

Figure 13: Pie chart of Transfusion Regimen in the study population (N=57)

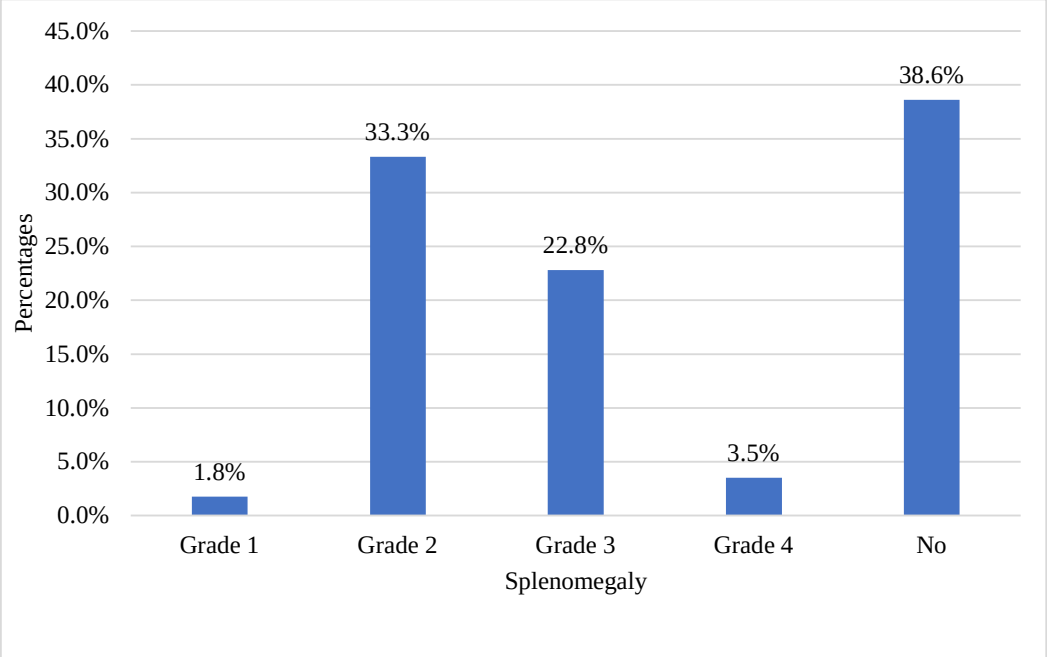


Study population consisted of 38.6% without splenomegaly, 1.75% with grade 1 splenomegaly, 33.33% with grade 2 splenomegaly, 22.81% with grade 3 splenomegaly and 3.51% with grade 4 splenomegaly.

Table 4: Splenomegaly in study population – a descriptive analysis (N=57)

Splenomegaly	[n (%)]
Grade 1	1 (1.75%)
Grade 2	19 (33.33%)
Grade 3	13 (22.81%)
Grade 4	2 (3.51%)
No	22 (38.6%)

Figure 14: Bar chart of splenomegaly in the study population (N=57)



Mean pre-transfusion haemoglobin in study population is 8.43gm/dl with minimum of 4.9gm/dl and maximum of 11.1gm/dl. Mean serum ferritin level of 2975ng/dl with minimum value of 635ng/dl and maximum value of 8451ng/dl. Minimum value of total bilirubin is 0.5mg/dl, Maximum value being 4.6mg/dl which is raised due to ongoing haemolysis. However, we noticed average parameters of study population was normal.

Table 5: laboratory parameters in study population – a descriptive analysis (N=57)

Hb [Mean ± SD, (Range)]	8.43 ± 1.27 (4.9-11.1)
Ferritin [Mean ± SD, (Range)]	2975.6 ± 1827.01 (635-8451)
TB [Mean ± SD, (Range)]	1.75 ± 0.94 (0.5-4.6)
DB [Mean ± SD, (Range)]	0.49 ± 0.18 (0.03-1.2)
SGOT [Mean ± SD, (Range)]	38.23 ± 21.27 (0.3-111)
SGPT [Mean ± SD, (Range)]	37.49 ± 28.03 (8-119)
ALP [Mean ± SD, (Range)]	234.98 ± 128.66 (28-617)
Urea [Mean ± SD, (Range)]	22.6 ± 7.86 (10-54)
Creatinine [Mean ± SD, (Range)]	0.49 ± 0.18 (0.2-1)

The average value of serum total cholesterol is 113.63mg/dl with minimum value of 72mg/dl and maximum value of 196mg/dl with 1 (1.75%) child above the normal value and 56 (98.25%) children having normal cholesterol profile. Whereas average triglycerides being 102.49 (46-192) with 54 (94.74%) having normal values and 3 (5.26%) children having higher values.

Mean LDL of study population is 64.19mg/dl with minimum of 34mg/dl and maximum of 128mg/dl. With 56 (98.25%) children in normal range and 1 child above the normal.

Mean HDL of study population 41.75 ± 11.49 (22-79)mg/dl with 27 (47.37%) with low values of HDL and 30 (52.63%) having higher values.

Table 6 : Lipid parameters in study population – a descriptive analysis (N=57)

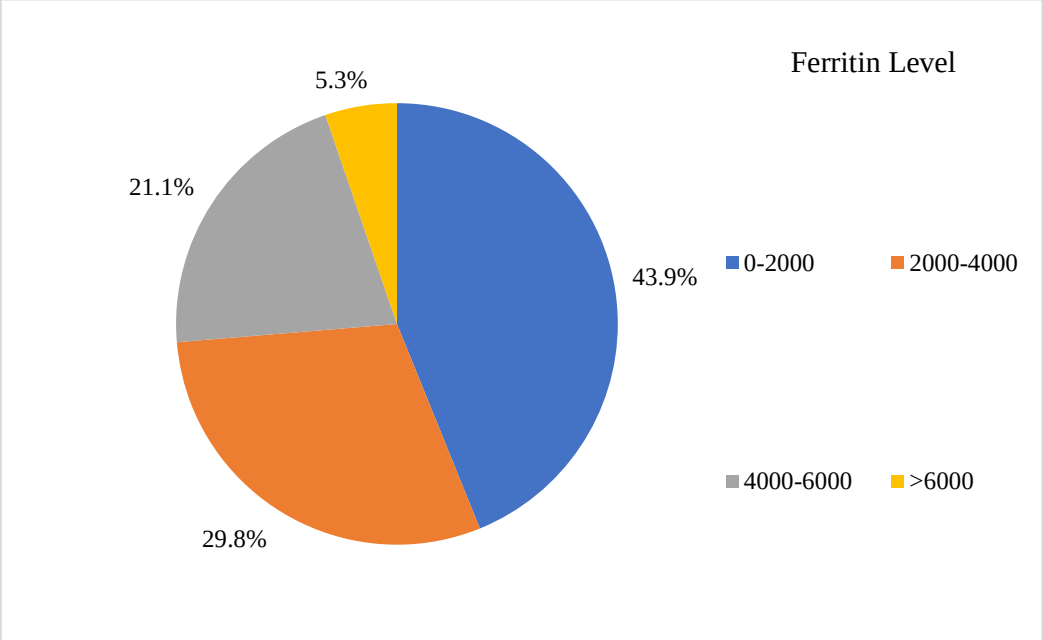
Total Cholesterol [Mean ± SD, (Range)]	113.63 ± 21.42 (72-196)
< 170	56 (98.25%)
≥ 170	1 (1.75%)
Total Triglycerides [Mean ± SD, (Range)]	102.49 ± 32.13 (46-192)
<150	54 (94.74%)
≥ 150	3 (5.26%)
LDL [Mean ± SD, (Range)]	64.19 ± 20.37 (34-128)
< 110	56 (98.25%)
≥ 110	1 (1.75%)
HDL [Mean ± SD, (Range)]	41.75 ± 11.49 (22-79)
< 40	27 (47.37%)
≥ 40	30 (52.63%)

As demonstrated in table -7 and figure- 15 our study group consisted of 25 children with serum ferritin level ranging between 0 to 2000ng/dl,17 children with ferritin level ranging from 2000-4000ng/dl,12 children with ferritin level of 4000 to 6000ng/dl and 3 children with ferritin level more than 6000ng/dl

Table 7 -Descriptive analysis of ferritin level in the study population (N=57)

Ferritin Level	Frequency	Percentages
0-2000	25	43.86%
2000-4000	17	29.82%
4000-6000	12	21.05%
>6000	3	5.26%

Figure 15 -Pie -Diagram depicting descriptive analysis of serum ferritin levels in the study population



All the children in study group are on chelation with Deferasirox at 20mg/kg/day, 3 are on dual chelation therapy with deferiprone and Deferasirox, 4 on dual therapy with desferoxamine and Deferasirox [Table 8]

Table 8: Drug of chelation in the study population – a descriptive analysis (N=57)

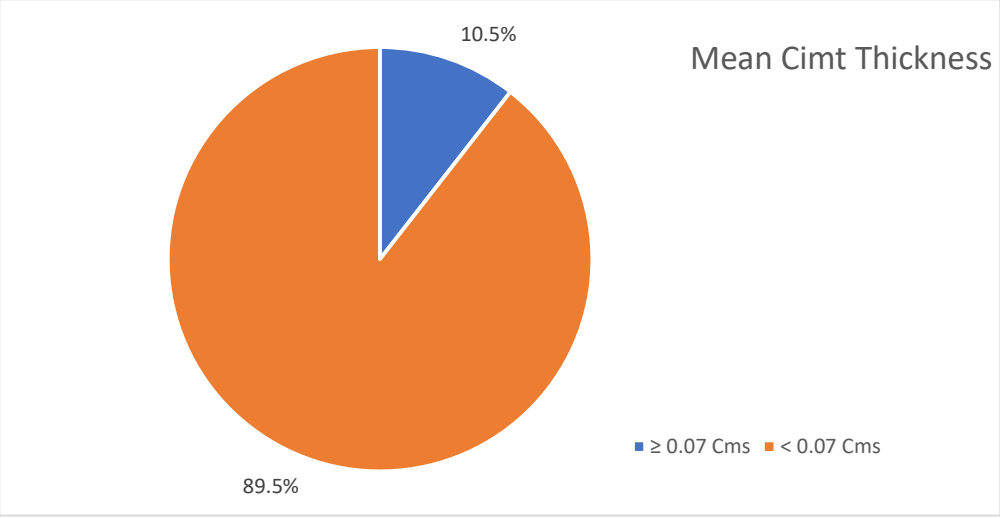
Drug of Chelation	Frequency	Percentages
Deferiprone3500mg, Desferoxamine1500mg+Deferasirox	2	3.5%
Deferiprone +Deferasirox	3	5.26%
Desferoxamine + Deferasirox	4	7.01%
Deferasirox	48	84.21%

Our study found that prevalence of premature atherosclerosis being 10.53% as depicted in table 9 and figure 16.

Table 9: Prevalence of atherosclerotic changes in the study population (N=57)

Mean CIMT Thickness [Mean ± SD, (Range)]	0.05 ± 0.01 (0.03-0.07)
≥ 0.07 CMS	**6 (10.53%)**
< 0.07 CMS	51 (89.47%)

Figure 16: Pie chart of Mean CIMT Thickness in the study population (N=57)



In children between the ages of 5 and 10 years, 11 to 14 years, and 5 to 18 years, respectively, our study showed mean transfusion indices of 172.53±47.09ml/kg/year,161.21± 42.79ml/kg/year, and 189.58± 45.53ml/kg/year. As shown in table 10, the study group had mean serum ferritin levels of 3104.74 ±1483.25,3638.63± 2422.6 in the ages of 11 to 14 and 15 to 18 years, respectively. There is no statistically significant difference in mean Transfusion Index & mean Ferritin between groups (P-value >0.05)

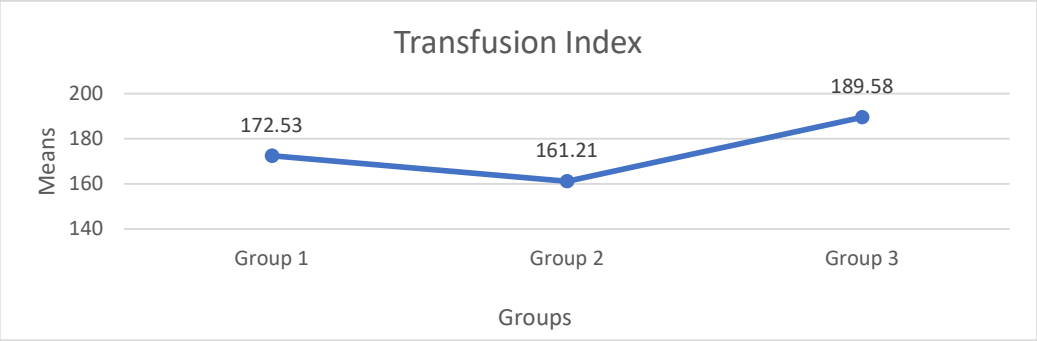
But there is statistically significant difference in mean of Mean CIMT Thickness between groups (at least one pair of groups) (P-value <0.05) which was represented in table 10 indicating the Age-related increases in serum ferritin levels and mean CIMT thickness, clearly noticed in group 2 and group 3 where with equal average of serum ferritin levels there is an increase in CIMT in group 3

Table 10-Comparison of mean Transfusion Index, Ferritin & CIMT thickness across group (N=57)

Parameter	**Groups**			**ANOVA F value**	**P Value**
	Group 1 [5-10Years]	**Group 2 [11-14 Years]**	**Group 3 [15-18 Years]**		
Transfusion Index	172.53 ± 47.09	161.21 ± 42.79	189.58 ± 45.53	1.89	0.160
Ferritin	2183.43 ± 1082.44	3104.74 ± 1483.25	3638.63 ± 2422.6	3.34	**0.043**
Mean CIMT Thickness	0.05 ± 0.01	**0.05 ± 0.01**	**0.06 ± 0.01**	4.07	**0.023**

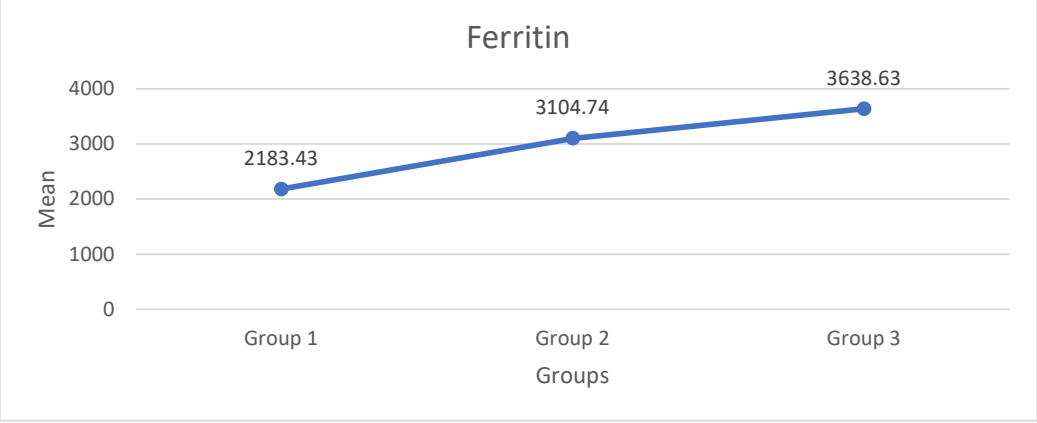
On a Pair-wise comparison-In comparison of **Transfusion Index** between individual groups, the mean difference of **Transfusion Index** between Group 1 Vs Group 2 (11.31), Group 1 Vs Group 3 (17.05) & Group 2 Vs Group 3 (28. 37) are statistically not significant with (P-value >0.05) as shown in table 11 and figure 17

Figure 17 – Line diagram of distribution of values of transfusion indices between groups



In **Ferritin**, The mean diffrance **Ferritin** between Group 1 Vs Group 2 (921.30) & Group 2 Vs Group 3 (533.8) are statistically not significant with (P-value >0.05), The mean diffrance **Ferritin** between Group 1 Vs Group 3 (1455.20) is statistically significant with (P-value <0.05) represented in table 11 and figure 18

Figure 18 – Line diagram of distribution of values of serum ferritin indices between groups



In **Mean CIMT Thickness**, The mean difference **Mean CIMT Thickness** between Group 1 Vs Group 2 (0.0035), Group 1 Vs Group 3 (0.0087) & Group 2 Vs Group 3 (0.0053) are statistically significant with (P-value <0.05) depicting age related increase in CIMT. (figure 19)

Figure 19 – Line diagram of distribution of values of mean CIMT between groups

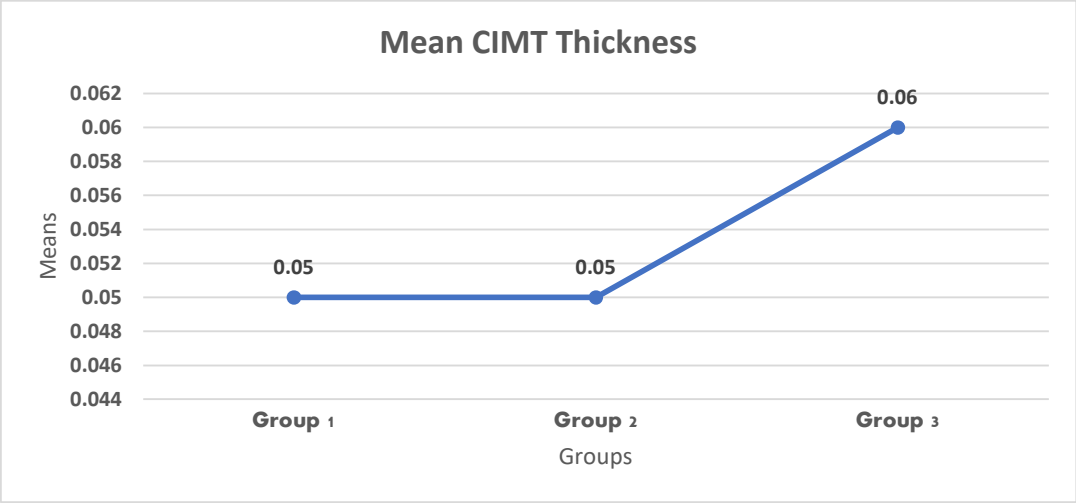


Table 11 – Pair wise comparison of transfusion index, Serum ferritin and Mean CIMT

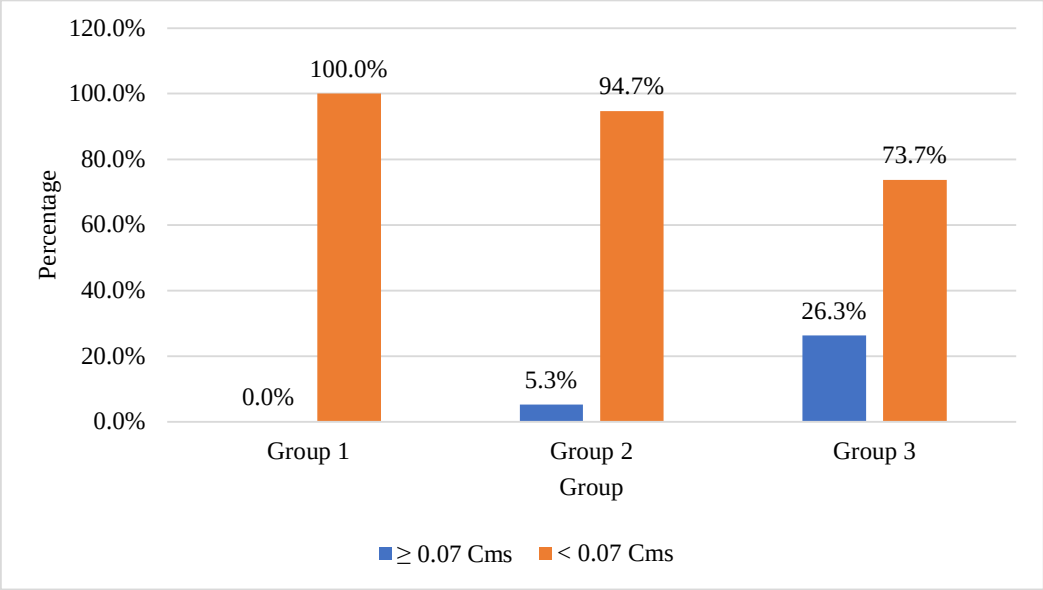
Pairs	Mean Difference	LSD (P-value)
Transfusion Index		
Group 1 Vs Group 2	11.31	0.443
Group 1 Vs Group 3	17.05	0.250
Group 2 Vs Group 3	28.37	**0.058**
Ferritin		
Group 1 Vs Group 2	921.30	0.111
Group 1 Vs Group 3	1455.20	**0.013**
Group 2 Vs Group 3	533.8	0.353
Mean CIMT Thickness		
Group 1 Vs Group 2	0.0035	0.264
Group 1 Vs Group 3	0.0087	**0.006**
Group 2 Vs Group 3	0.0053	**0.094**

With 5 children having intimal medial thickness greater than 0.07 cm, a prevalence of 26.32 percent, which is statistically significant (P = 0.02) for premature atherosclerosis in the age group of 15 to 18 years, Table 12 and Figure 19 demonstrate this.

Table 12-Comparison of mean CIMT thickness across group (N=57)

Mean CIMT Thickness	Group			Chi-Square	P-value
	Group 1 (N=19) [5-10Years]	Group 2 (N=19) [11-14 Years]	Group 3 (N=19) [15-18 Years]		
≥ 0.07 CMS	0 (0%)	1 (5.26%)	5 (26.32%)	7.82	0.020
< 0.07 CMS	19 (100%)	18 (94.74%)	14 (73.68%)		

Figure 20: Cluster bar chart of comparison of mean CIMT thickness across group (N=57)



As shown in Table 13 and Figure 20, 6 children on a hypo transfusion regimen had a carotid intima-medial thickness larger than 0.07 cm. This, however, lacked statistical significance.

Table 13: Comparison of Parameter between mean CIMT thickness (N=57)

Parameter	Mean CIMT Thickness		Chi-Square	P-value
	≥ 0.07 Cms (N=6)	< 0.07 Cms (N=51)		
Transfusion Regimen				
Hypo	6 (100%)	45 (88.24%)	0.79	0.374
Hyper	0 (0%)	6 (11.76%)		
Splenomegaly				
Grade 1	0 (0%)	1 (1.96%)	4.00	0.141
Grade 2	3 (50%)	16 (31.37%)		
Grade 3	2 (33.33%)	11 (21.57%)		
Grade 4	1 (16.67%)	1 (1.96%)		
No	0 (0%)	22 (43.14%)		

Figure 21: Cluster bar chart of comparison of transfusion regimen between mean CIMT thickness (N=57)

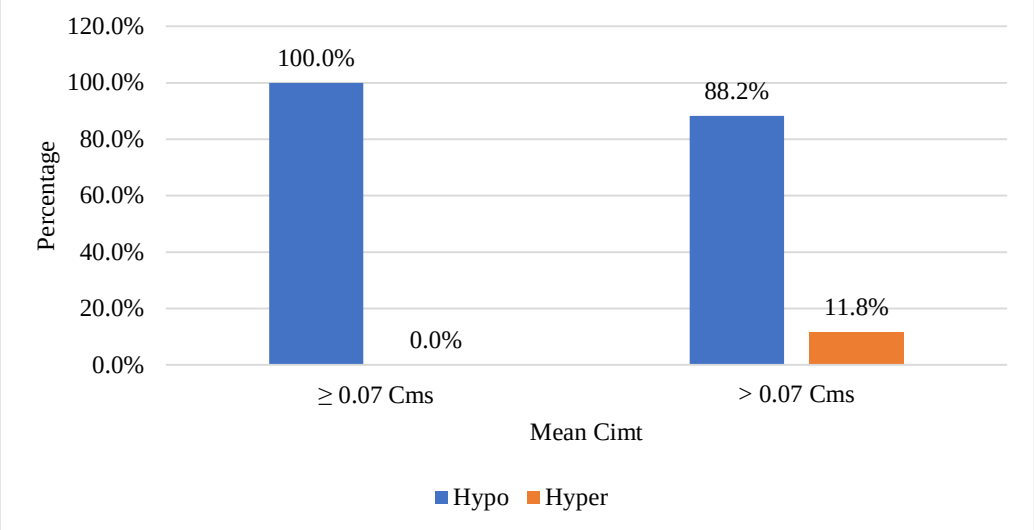


Table 13 demonstrates the key contributors to these atherosclerotic alterations, including serum ferritin levels greater than 6000ng/dl, volume transfused, duration of chelation, and direct bilirubin.

Table 13 Comparison of parameters between mean CIMT thickness(N=57)

Parameter	Mean CIMT Thickness (Mean± SD)		P value
	≥ 0.07 Cms (N=6)	**< 0.07 Cms (N=51)**	
Duration of the Disease[in months]	**181.67 ± 13.26**	**132.82 ± 45.08**	**0.011**
Age At Which Transfusion Started in Months	7.67 ± 6.06	7.24 ± 4.63	0.835
Volume Transfused In	**105696.17 ± 38380.66**	**48944.27 ± 37931.89**	**0.001**
Transfusion Index	182.33 ± 43	173.51 ± 46.54	0.660
Duration Of Chelation (Months)	**156.83 ± 19.13**	**107.61 ± 47.99**	**0.017**
HB	8.27 ± 1.33	8.45 ± 1.28	0.743
Ferritin	**6151 ± 1624.56**	**2602.02 ± 1456.8**	**<0.001**
TB	1.07 ± 0.43	1.83 ± 0.96	0.059
SGOT	42.5 ± 20.08	37.73 ± 21.54	0.608
SGPT	39 ± 22.59	37.31 ± 28.78	0.891
ALP	172 ± 73.98	242.39 ± 132.14	0.208
Urea	19.83 ± 5.15	22.92 ± 8.09	0.367
Creatinine	0.6 ± 0.14	0.48 ± 0.18	0.109
Total Cholesterol	109.67 ± 14.22	114.1 ± 22.17	0.636
Total Triglycerides	97 ± 27.94	103.14 ± 32.77	0.662
LDL	63.67 ± 23.13	64.25 ± 20.27	0.947
HDL	46 ± 16.2	41.25 ± 10.92	0.343

On a multivariate regression analysis of factors which were found significant are Serum ferritin levels as depicted in table 14.

Table 14: Multi variate logistic regression analysis of factors associated with Mean CIMT (Baseline = <0.07 Cms)

Parameter	Adjusted odds ratio	95% C.I.	P-value
Duration of the Disease Months	1.05	0.96 -1.16	0.311
Volume Transfused in ml	1.00	1.00 -1.00	0.498
Duration Of Chelation (Months)	1.01	0.96-1.07	0.672
Ferritin	**1.00**	**1.00 - 1.00**	**0.035**

Our study found that highest prevalence of premature atherosclerosis changes in Left Common carotid artery followed by left Internal carotid artery as showed in Table 15 and also that there is a linear relationship of CIMT with serum ferritin levels especially serum ferritin levels above 4000ng/dl.

Table 15 - Comparison of mean of CCA CIMT & ICA CIMT between Ferritin Level (N=57)

Parameter	Ferritin Level (Mean± SD)				F value	P Value
	0-2000	2000-4000	4000-6000	>6000		
CCA CIMT [Right]	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	10.48	<0.001
CCA CIMT [Left]	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	13.99	<0.001
ICA CIMT [Right]	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	12.24	<0.001
ICA CIMT [Left]	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	12.80	<0.001

A splenectomy was performed on 8 of the research group's children. Comparing splenectomy patients to non-splenectomised children, it was found that the transfusion index was lower in the former group, and the average serum ferritin levels in splenectomised children ranged from 3658ng/ml to 6372ng/ml. Additionally, the table demonstrated that mean CIMT is higher in Right CCA and Left ICA with a statistically significant value of 0.088 and 0.060, respectively.

Table 16: Comparison of mean of Parameter between underwent splenectomy(N=57)

Parameter	UNDERWENT SPLENECTOMY (Mean± SD)		P value
	Yes (N=8)	No (N=49)	
Transfusion Index in kg/year	148.5 ± 40.25	178.67 ± 45.72	0.085
CCA CIMT[RT]	0.06 ± 0.01	0.05 ± 0.01	**0.088**
CCA CIMT [LT]	0.05 ± 0.01	0.05 ± 0.01	0.168
ICA CIMT [RT]	0.06 ± 0.01	0.05 ± 0.01	0.127
ICA CIMT [LT]	0.06 ± 0.01	0.05 ± 0.01	**0.060**
Ferritin	3658.13 ± 1822.75	2864.17 ± 1821.94	0.258

However, our study did not find any statistically significant relationship of lipid parameters with premature atherosclerotic changes as depicted in table 17

Table 17 -Correlation of CCA CIMT & ICA CIMT with lipid parameters (N=57)

Characteristics	**lipide parameters**	**Pearson's Correlation Value (rs)**	P-value
CCA CIMT [Right]	Total Cholesterol	0.024	0.859
	Total Triglycerides	-0.062	0.645
	LDL	-0.026	0.149
	HDL	0.063	0.644
CCA CIMT [Left]	Total Cholesterol	0.064	0.634
	Total Triglycerides	-0.043	0.754
	LDL	0.051	0.706
	HDL	0.146	0.278
ICA CIMT [Right]	Total Cholesterol	0.094	0.485
	Total Triglycerides	-0.086	0.525
	LDL	-0.007	0.961
	HDL	0.121	0.370
ICA CIMT [Left]	Total Cholesterol	0.124	0.359
	Total Triglycerides	-0.035	0.798
	LDL	0.056	0.680
	HDL	0.134	0.320

DISCUSSION

Beta-Thalassemia Major is the most prevalent haemolytic anaemia in South India, with an incidence of 5–15%, according to research conducted in 2016 by Suresh Babu T V and Manjula Shantaram. This is due to the high in-breed frequency.(68) Due to repeated blood transfusions, children with Beta-Thalassemia Major are inclined to the development of subclinical atherosclerosis, which attributed to one of the major causes of morbidity in these children.

The main cause of death and one of the main causes of morbidity in children beta-thalassemia are cardiac problems caused by the growth of accelerated atherosclerosis .This aspect of cardiovascular issues in beta-thalassemia major have been rarely addressed so far. This could be attributed to epigenetic factors such as hyperlipidaemia , hypertension , age, family history, Diabetes mellitus and obesity and also to the gene-environment interactions as mentioned in their study by Nafady et al., in 2017.(69)

Patients with Beta-Thalassemia Major have been found to have elevated levels of iron in the body, longstanding deposition of iron causes alteration of endothelium of the vascular structures which eventually change the structure of arterial wall as quoted by Farmakis,2017 (70)such as increase in intima-media thickness, elastic properties of the arterial wall, formation of plaques in the lumen ultimately leading to stenosis of the vessel.(62,64,65)

Carotid doppler ultrasonography, which is non- invasive modality which has been in use world-wide helps in detecting these changes at the earliest.(71) Regular monitoring of carotid intima medial thickness in Beta thalassemia major showed a

progressive increase, which could be considered as the structural alarm in detecting premature atherosclerosis.

Although 57% of the male participants in the study conducted in Egypt by Sherief et al, (5)(2017) had thalassemia, there were no discernible differences between the two genders (P=0.306) (Sherief et al., 2017)(5). Another research by Gursel et al. (2012) (72) in Turkey, which revealed that 58% of the male participants had thalassemia with a P value of 0.37, also validated this finding (Gursel et al., 2012) (72). In line with the research done by Triana et al in 2021, where (54.3%) were male and (45.7%) were female(73).Similar finding was noticed in study done by Akhlagpoor S and Hoseini M.(74) Likewise, More than half of the participants in our research who had thalassemia (56.14%) were male. Gender did not, however, differ significantly.

The average age of Sherief et al (2017)(5) research study was 9.5 (SD 3.7) years old. The study by Gursel et al. (2012)(72) found that the average age was 10.84 (SD 4.38) years, similar finding was noted in a study done in 2014 by seham and his colleagues(75) . Triana et al in 2021 where mean age being 9 years, which was almost exactly the same as this finding(73). The average age of the thalassemia study participants is 12 (SD 3.9) years old. These findings suggested that premature atherosclerosis start as early as first decade in life in these children requiring a need for regular CIMT monitoring.

In terms of nutritional status, the average BMI in our research, which was 17.18(SD 3.27) kg/m2, is consistent with a study by Sherief et al. (2017)[5] that discovered individuals with low nutritional status had an average BMI of 18.1(SD 2.2) kg/m2. There was absolutely no correlation in terms of statistics (P=0.191.6). Similarly, Gursel et al. (2012)(72) reported an average BMI of 17.34 (SD 3.03) kg/m2, and they

observed no association between nutritional status and thalassemia (P=0.24).Our study contradicts the finding of correlation of bad nutritional status with organomegaly done by Triana et al(73)

Our study found that those with severe -thalassemia had a 10.53% risk of early atherosclerosis. This result was essentially less than that of the Egyptian research by Abaza et al., (2017)(71), Triana et al (73)where premature atherosclerosis incidence was reported to be at 19% and 17% and the average CIMT was 0.8 (SD 0.16) mm and 1.03mm respectively. According to Gursel et al (2012) (72)s study in Turkey, the CIMT level of the study group was substantially higher than that of the control group, measuring 0.56 (SD 0.06) mm as opposed to 0.48 (SD 0.05) mm, P=0.001. The average CIMT in thalassemia patients in our study was 0.73 mm, according to research by Tantawy et al. (2009)(76), which was higher than the average in the control group at 0.63 mm.

Our study finding of incidence of 26.32%in age group of 15 to 18 years infers that CIMT increases with increase in age so as the prevalence of premature atherosclerosis which concurs with a case – control study done by Ahmed Abdul-Mohsin Alshammary, Sabih Salih Al-Fetlawi , Zena Abdalameer Aljanabi in Iraq in 2018 (63) and a study done by Soltani et al. [64] This could be attributed to duration of the disease , increase in total volume of blood transfused which lead to long term deposition of cellular iron. According to the study, the CIMT grows linearly with age. This finding is consistent with that of Su et al.,(77) who also found that [IMT in millimetres = (0.009 age in years) + 0.35].

Our study is comparable with Doyon et al (17) and Abaza et al(71), where there is no effect of gender on CIMT measurement, and no significant variations between males and females.

Among our study population CIMT thickness of Left CCA is higher that Left ICA followed by Right which contradicts the finding of higher thickness in Right CCA CIMT in study done by Abaza (71)

Our study showed that CIMT was significantly high with increasing duration from the first blood transfusion (P =0.011).similar findings were observed in study done by Soltani et al.(78) and Ahmed Abdul-Mohsin Alshammary, Sabih Salih Al-Fetlawi , Zena Abdalameer Aljanabi in Iraq in 2018 (63)

Our study also found that CIMT thickness is not correlating with bilirubin which is an indicator of haemolysis. This opposes the finding in study of abaza et al(71) and goes in tune with study done by Tantawy and his colleagues (76) Dogan(79) and Gullu.(80)

The average LDL levels in this research were 64 (SD 20.37) mg/dl .This is similar to the study done by Maioli et al., (1984) and Sherief et al., (2017)(5) in Italy and Egypt respectively, where they stated the decrease in LDL level in thalassemia patients. The average HDL levels of half of the children in this study were also low, which was a prognostic factor in the risk of heart defects (Mansi and Aburjal, 2008) .(81). This is aligned with the study done by Ashar et al., (2015) in Pakistan, which was the first research to observe lipid level in β-thalassemia major patients. The results of our investigation, however, are at odds with the Egyptian researchers Abdelgawad et al(2019) .'s association of serum triglycerides and low-density lipoprotein (LDL) with CIMT findings.(82)

In this study, the average serum ferritin concentration was 2975.6(SD 1827.01)ng/ml. In contrast to a study done by Cheung, Chow, and Chan (2006)(67) that claimed ferritin level was not correlated to the degree of artery thickness and endothelial dysfunctions in thalassemia cases, the P value obtained was 0.001 (P0.05), indicating a significant relationship between serum ferritin and premature atherosclerosis in children with beta-thalassemia major. In individuals receiving iron chelation treatment, there was little association between ferritin level and liver iron accumulation (Cheung, Chow, and Chan, 2006)(67). In both the study and control groups, Gursel et al(2012) (72)'s study found no association between serum ferritin levels and CIMT.

Our study also found that Transfusion index in beta thalassemia major children who underwent splenectomy was low compared to non-splenectomised children. Whereas, Splenectomised children showed higher CIMT and serum ferritin values compared to latter ones. This contradicts the finding of study done by Abaza et al(71)

CONCLUSION

According to the findings of our study, there is a significant correlation between serum ferritin levels and carotid medial thickness, and there is a high prevalence of premature atherosclerosis changes that show that thromboembolic events are more likely to occur in people with beta-thalassemia major due to severe changes in haemostatic mechanisms. This study also showed that thalassemia children had subclinical carotid atherosclerosis even though their relative anti-atherogenic lipid profiles were more common. Age-related increases in carotid intimal medial thickness suggest the need for routine testing of beta- thalassemia children for early atherosclerosis alterations. This aids in the early identification of thrombosis-related problems in these children and the implementation of appropriate measures. With this study, we advocate for close monitoring of beta-thalassemia children so that these changes can be identified early and properly addressed. Additionally, to increase knowledge about cardiovascular diseases.

SUMMARY

One year cross sectional study was conducted from January 2021 to December 2021 in the Department of Pediatrics, KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgavi. A total of 57 children with confirmed diagnosis of Beta-Thalassemia Major were included in the study. The salient findings of study are summarised as follows.

- During the study 57 children aged 5 to 18 years, with confirmed diagnosis of Beta-Thalassemia Major were enrolled and stratified into three groups based on their age.
- Mean age of study participants was 12 years.
- Study population consisted of 32 females and 25 males.
- 51 children were on hypo transfusion regimen and 6 children were on hyper transfusion regimen.
- Average volume of blood transfused in study population was 54918ml with average transfusion index of 174ml/kg/year
- Average duration of chelation was 112months.
- Mean Haemoglobin of study population was 8.43gm/dl
- Mean serum ferritin values were 2975ng/ml
- Atherogenic profiles of study population were normal as per age, However half of the population has higher HDL values.
- Prevalence of premature atherosclerotic changes in our study population was 10.53%, However none of our patients had stenosis of vessel or presence of plaques.

- Our study highlights that with CIMT thickness increased with increase in age, whereas this relation is clearly noticed from 11years to 18years of age, with same average levels of serum ferritin in both the groups
- We have found that these atherosclerotic changes were well seen in left common carotid artery and left internal carotid artery.
- Our study found that factors like Duration of disease [p=0.011] , Total volume of blood transfused [p=0.001] ,duration of chelation [0.017] ,Serum Ferritin [p<0.001] as significant factors contributing to these changes.
- Our study highlights the importance of cardiovascular assessment in children with beta-thalassemia which were rarely addressed in the literature.

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CONSENT FOR PARTICIPATION IN RESEARCH

"THE PREVALENCE OF PREMATURE ATHEROSCLEROSIS IN CHILDREN WITH BETA-THALASSEMIA MAJOR USING CAROTID DOPPLER ULTRASONOGRAPHY**"**

Investigator: REG NO: BM0120016

Guide: DR._____

PROFESSOR

DEPARTMENT OF PEDIATRICS,

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

BELGAUM -590010.

You are hereby requested to involve your child in the above said research to be conducted at KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2021 to December 2021 by me.

Introduction

PURPOSE OF THE STUDY

The study aims to know the prevalence of premature atherosclerosis in children with beta-thalassemia major using carotid doppler ultrasonography.

Procedures involved: Blood sampling and carotid doppler ultrasonography

Voluntary participation

Your child's participation in this study is your voluntary decision. Whether to participate or not to participate will not affect your current or future relationship with the

KLES Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum. You are free to discontinue the participation in the study at any time for any reasons and you will not be paid any reimbursement for participation in the research.

Financial incentives for participation: Nil

Risk and benefits

There are no major risks involved, other than discomfort and pain caused during collection of biological sample.

Compensation:

Taking part in this study will not affect the cost of the treatment i.e, it will be similar to cost of standard procedure. In the event that you become injured as a result of taking part of the study, treatment will be offered to you or you will be given information about where to receive the medical care. But you/your insurance company will be responsible for the costs. However, no reimbursement, compensation or free medical care will be given.

Privacy and Confidentiality

The only people who will know that you are a research participant are member of the research team. No information about you or provided by you, during research will be disclosed to others without your written consent. When the results of the research are published or discussed in the conferences, no information will be disclosed that would reveal your identity. Any information obtained in connections with this study and that can be identified with you remain confidential and will be disclosed only with your permission.

Queries

If you have any queries you may contact

REG NO: BM0120016

Post Graduate Student

Department of Pediatrics

JNMC, Belagavi-590010

DR._____

PROFESSOR

DEPARTMENT OF PEDIATRICS,

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

BELGAUM -590010.

If you have any questions about your rights or research participation you may contact

Chairman ethical committee:

DR. HARSHA HEGDE

CHAIRPERSON, JNMC,

IEC & SCIENTIST D, ICMR,

NATIONAL INSTITUTE OF TRADITIONAL MEDICINE,

BELAGAVI-590010

STATEMENT OF CONSENT

I hereby voluntarily agree for my child participation in this study. I understand that even if I choose to allow my baby to take part in this study. I have the liberty to withdraw at any time. My signature below indicates that I have read or have been told about this entire consent form including the risks and benefits and have had all my questions answered. I will be given a copy of this consent form.

Signature of the Participant/LTI: _____

Date: _____

Name: _____

Signature of the authorized representative/ parent(if applicable)/LTI:

_____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Name: _____

Date: _____

Signature of investigator: _____

Name: _____

Date: _____

Place:_____

PROFORMA

SL NO-

DATE-

1) Name-

2) Age-

3) Gender-

4) Diagnosis:

5) Date of diagnosis:

6) Duration of disease:

TRANSFUSION DETAILS

1) Age at which transfusion started :
2) Frequency of transfusion :
3) Volume of transfusion :
4) Number of transfusions per year :
5) Transfusion index :

CHELATION THERAPY DETAILS

1) Drug of chelation :
2) Dosage of chelation :
3) Date of initiation of chelation therapy:

Has the child undergone Splenectomy :

If Yes, Date of splenectomy:

GENERAL EXAMINATION

Anthropometry:

1) Height	
2) Weight	
3) BMI	

Presence of 1) Icterus : Yes ☐ No ☐

2) Oedema : Yes ☐ No ☐

If Yes describe,

3)Peri Orbital puffiness :

Vitals : BP- PR- RR - SpO2:

Systemic Examination:

i. CVS :

ii. RS :

iii. CNS:

iv. Per abdomen:

INVESTIGATIONS

1. Heamoglobin :

2. Serum Ferritin :

3. Liver Function test –
    a. Total Bilirubin :
    b. Direct Bilirubin :
    c. S.G.O.T :
    d. S.G.P.T:
    e. A.L.P :

4. Renal Function test-
    a. Serum Urea :
    b. Serum Creatinine :

5. Random blood sugar :

6. Total Cholesterol :

7. Total triglycerides :

8. Carotid Doppler findings-

	Common carotid Artery		Internal Carotid Artery		Normal range
	Left	Right	Left	Right	
A. Carotid intima media thickness					
B. Presence of plaques in the lumen					
C. Stenosis of the blood vessel					

KEY TO MASTER CHART

CIMT	-	Carotid Intima Media Thickness
gm/dl	–	Gram per decilitre
ml/kg/year	–	Millilitre per kilogram per year
SLE	–	Systemic Lupus Erythematosus
Cm	–	Centimeter
%	-	Percentage
mg	–	Milligram
gm	-	gram
Hb	-	Heamoglobin
HbE	–	Heamoglobin E
HbS	–	Heamoglobin S
LDL	–	Low density lipoprotein
HDL	–	High density lipoprotein
CCA	–	Common carotid Artery
ICA	–	Internal Carotid Artery

S.NO	AGE in yrs	GENDER	DATE OF DIAGNOSIS	DURATION OF THE DISEASE	Transfusion Regimen	TRANSFUSION STARTED in months	VOLUME TRANSFUSED in ml	TRANSFUSION INDEX in /kg/yr	DRUG OF CHELATION	Additional drug of chelation	DATE OF INITIATION OF CHELATION	UNDERWENT SPLENECTOMY	HEIGHT in mts	WEIGHT in kgs	BMI	SPLENOMEGALY	HB	FERRITIN	TB	DB	SGOT	SGPT	ALP	UREA	CREATININE	TOTAL CHOLESTEROL	TOTAL TRIGLYCERIDES	LDL	HDL	CCA CIMT[RT]	CCA CIMT [LT]	ICA CIMT [RT]	ICA CIMT [LT]
GROUP 1																																	
1	9years	F	12-11-2013	102Months	Hypo	15months	54933	302	Defrijet 750mg	no	13-06-2018	No	1.04	21.4	19.79	grade 3	8	3905	1.1	0.4	71	94	212	14	0.2	100	138	48	24	0.05	0.04	0.04	0.04
2	5years	M	23-03-2016	48months	Hypo	12months	13,328	196	Defrijet 750mg	no	24-07-2020	No	1.08	17	14.57	grade 2	7.5	1657	1.1	0.5	30	22	150	16	0.4	93	84	41	35	0.05	0.04	0.05	0.04
3	5years	M	16-11-2016	54months	Hypo	6Months	8537	116	Defrijet 500mg	no	06-04-2020	No	1.16	16	11.89	garde 2	9.5	4256	1.4	0.6	34	40	324	23	0.4	156	132	62	76	0.056	0.06	0.064	0.062
4	8years	F	07-09-2013	92months	Hypo	4months	21242	212	Defrijet 1000mg	no	07-07-2013	No	1.24	25	16.26	grade 2	8.2	1186	2	0.6	23	18	419	23	0.3	82	48	37	35	0.05	0.04	0.04	0.04
5	5years	M	15-09-2016	56months	Hypo	4months	14400	180	Defrijet 500mg	no	16-08-2018	No	1.05	20	18.14	grade 3	9	2603	0.7	0.3	35	22	231	10	0.4	104	72	58	32	0.042	0.046	0.042	0.04
6	7years	F	06-06-2014	80months	Hypo	6months	41040	180	Defrijet 500mg	no	06-08-2015	No	1.4	38	19.39	grade 2	7.7	1889	1.1	0.5	31	14	136	15	0.7	92	112	50	36	0.04	0.046	0.042	0.04
7	7years	F	26-09-2014	76months	Hypo	8months	22937	112	Defrijet 1000mg	no	28-10-2018	No	1.28	32	19.53	grade 3	7.1	3251	1.6	0.5	37	30	219	21	0.3	92	78	52	32	0.052	0.048	0.04	0.04
8	6years	M	24-06-2015	67months	Hypo	5months	21705	114	Defrijet 500mg	no	06-04-2018	No	1.22	34	22.84	grade 2	8.8	2715	1.8	0.7	111	119	251	18	0.5	72	58	48	36	0.04	0.042	0.046	0.042
9	6years	F	21-05-2016	69months	Hypo	4months	22120	158	Defrijet 500mg	no	22-04-2018	No	1.15	25	18.9	No	8.4	1763	2.2	0.7	39	117	197	19	0.2	98	78	46	52	0.04	0.04	0.04	0.042
10	10years	F	21-08-2011	112months	Hypo	8months	49952	158	Defrijet 1000mg	no	12-10-2015	No	1.22	34	22.84	grade 2	9.9	952.2	3	0.6	36	39	360	38	0.4	100	91	66	44	0.04	0.042	0.04	0.04
11	9years	M	09-10-2013	100months	Hypo	5months	25132	136	Defrijet 500mg	no	12-09-2016	No	1.22	22	14.78	No	8.5	1389	1.5	0.7	33	12	551	23	0.5	102	192	44	30	0.046	0.04	0.048	0.04
12	9years	F	06-04-2013	102months	Hypo	6months	24324	206	Defrijet 500mg	no	04-04-2016	No	1.35	25	13.72	No	9.7	3642	0.9	0.3	29	28	221	22	0.4	108	78	62	46	0.042	0.046	0.048	0.046
13	10years	M	22-06-2011	112months	Hypo	8months	36432	198	Defrijet 1000mg	no	30-05-2013	No	1.06	20	17.8	No	8.5	1492	0.8	0.3	45	31	240	27	0.4	106	65	58	35	0.047	0.04	0.056	0.05
14	10years	F	04-02-2011	114months	Hypo	6months	22521	144	Defrijet 500mg	no	04-03-2013	No	1.07	17	14.85	No	9.7	1382	1.1	0.5	26	11	617	17	0.7	154	99	85	49	0.07	0.06	0.07	0.06
15	6years	M	30-10-2015	65months	Hypo	7months	15769	186	Defrijet 500mg	no	30-11-2016	No	1.18	19	13.65	No	8.4	1492	0.8	0.3	45	31	240	27	0.4	106	65	58	35	0.04	0.04	0.042	0.045
16	6years	M	06-10-2015	65months	Hypo	7months	18446	122	Defrijet 500mg	no	22-04-2017	**Yes**	1.22	28	18.81	No	8.2	1674	0.84	0.84	0.3	44	36	27	0.2	112	68	56	36	0.06	0.06	0.06	0.07
17	8years	M	06-06-2013	92months	Hyper	4months	36115	216	Defrijet500mg	no	02-06-2016	No	1.22	22	14.78	grade 3	10.7	3750	0.5	0.2	46	45	206	22	0.4	98	76	48	32	0.05	0.058	0.056	0.05
18	9years	F	03-12-2013	100months	Hypo	5months	33600	200	Defrijet 500mg	no	15-09-2015	No	1.19	20	14.12	grade 3	8	1658	1	0.4	23	15	221	25	0.4	108	112	64	44	0.046	0.048	0.044	0.046
19	7years	F	09-04-2015	85months	Hypo	3months	12602	142	Defrijet 500mg	no	16-03-2017	No	1.01	12.5	12.25	grade 3	8.7	829	1.9	0.7	29	31	482	31	0.4	116	102	62	56	0.036	0.032	0.034	0.036
GROUP 2																																	
1	13years	M	15-01-2009	159months	Hypo	1 month	70200	216	Defrijet1000mg	no	05-07-2011	No	1.3	25	14.79	grade 2	9.2	2922	1.5	0.4	22	15	120	25	0.6	106	112	56	50	0.042	0.04	0.04	0.04
2	11years	F	06-10-2011	126months	Hypo	6months	47318	124	Defrijet500mg	no	08-08-2012	No	1.24	36	23.41	No	7.7	1099	2.2	0.6	24	8	373	13	0.3	126	120	78	48	0.04	0.046	0.04	0.042
3	14years	M	15-04-2008	159months	Hypo	8months	17500	112	Defrijet 1500mg	sferoxam	16-08-2011	No	1.63	41	15.43	grade 2	8.5	1376	1	0.4	20	20	190	36	0.6	139	98	103	35	0.042	0.044	0.041	0.042
4	11years	F	16-01-2012	123months	Hyper	12months	20400	102	Defrijet 750mg	no	06-04-2013	No	1.29	20	12.02	grade 2	10.3	3221	1.9	0.5	56	57	406	15	0.7	114	86	86	28	0.042	0.046	0.042	0.04
5	13years	M	24-04-2009	153months	Hyper	5months	56347	156	Defrijet750mg	no	24-09-2010	No	1.36	28	15.14	grade 3	10.4	3466	3.2	0.6	45	32	191	28	0.8	116	98	72	44	0.04	0.042	0.041	0.043
6	12years	F	12-12-2009	147months	Hypo	4months	88200	210	Defrijet 1500mg	no	24-07-2011	No	1.45	35	16.65	grade 2	8.7	1836	2.4	0.4	23	14	222	19	0.4	122	114	62	60	0.04	0.04	0.04	0.04
7	12years	F	16-10-2008	144months	Hypo	4months	71688	206	Defrijet1000mg	no	17-01-2012	**Yes**	1.25	29	18.56	No	7.7	3696	1.8	0.6	46	44	446	29	0.6	120	118	58	38	0.056	0.05	0.056	0.053
8	14years	F	06-06-2008	165months	Hypo	3months	48038	108	Defrijet 500mg	no	11-10-2011	**Yes**	1.48	32	14.61	No	7.8	3762	1.8	0.6	48	46	441	28	0.6	120	116	56	36	0.058	0.05	0.056	0.054
9	14years	M	04-03-2009	156months	Hypo	12months	28080	150	Defrijet 500mg	no	04-11-2012	No	1.5	35	15.56	No	8	3652	1.4	0.6	33	36	66	31	1	106	66	54	34	0.062	0.064	0.062	0.06
10	11years	M	28-06-2010	128months	Hypo	4months	76032	220	Defrijet 500mg	no	24-10-2013	No	1.38	32	16.8	grade 2	7.4	1394	3	0.5	39	24	104	26	0.5	120	118	56	40	0.056	0.05	0.056	0.053
11	12years	M	24-10-2008	139months	Hypo	5months	53539	208	Defrijet 1000mg	no	21-08-2010	No	1.22	22	14.78	grade 3	8.1	4814	2.5	0.7	54	50	93	19	0.4	96	112	42	54	0.058	0.06	0.056	0.062
12	14years	F	14-10-2009	149months	Hypo	19months	60450	186	Defrijet 1000mg	sferoxam	26-10-2015	No	1.48	26	11.87	grade 3	5.5	3241	3.8	0.5	15	19	90	12	0.4	106	142	64	42	0.052	0.053	0.05	0.052
13	14years	M	01-06-2008	165months	Hypo	2months	44341	110	Defrijet 1000mg	no	06-09-2011	No	1.31	29	16.9	grade 4	6.3	4618	1.6	0.4	48	32	188	29	0.6	112	116	62	50	0.068	0.071	0.069	0.071
14	14years	M	20-04-2007	164months	Hypo	3months	67167	144	Defrijet 500mg	no	20-04-2015	**Yes**	1.46	33	15.48	No	9.3	6176	3.6	1.2	54	63	397	54	0.5	120	93	60	41	0.058	0.062	0.064	0.07
15	14years	F	14-01-2008	171months	Hyper	1month	28750	148	Defrijet1500mg	eriprone2	14-01-2010	No	1.5	39	17.33	grade 2	10.5	1710	1.9	0.7	22	11	270	16	0.4	113	126	54	52	0.042	0.044	0.042	0.042
16	13years	M	15-09-2009	150months	Hypo	9months	36600	122	Defrijet 750mg	no	12-11-2016	No	1.34	25	13.92	grade 2	8.2	1989	3.1	0.5	17	10	314	24	0.4	98	102	46	46	0.04	0.042	0.044	0.04
17	12years	M	11-10-2010	135months	Hypo	9months	43120	140	Defrijet1000mg	eriprone2	12-02-2013	Yes	1.35	28	15.36	No	8.3	4296	0.9	0.4	86	61	196	35	0.6	122	118	76	46	0.052	0.054	0.054	0.052
18	11years	F	22-04-2010	126months	Hypo	4months	61056	180	Defrijet 500mg	no	23-12-2013	No	1.28	32	19.53	grade 3	7.5	937	3.7	0.7	30	11	451	15	0.4	104	68	58	46	0.052	0.056	0.054	0.052
19	11years	F	01-04-2012	125months	Hypo	7months	20000	221	Defrijet 750mg	no	21-01-2015	No	1.15	19	14.37	grade 3	8.5	4785	1.7	0.5	22	38	217	20	0.2	106	112	62	42	0.042	0.044	0.042	0.042
GROUP 3																																	
1	16years	M	01-02-2005	188months	Hypo	4months	120000	160	Defrijet 1500mg	no	06-04-2007	Yes	1.65	50	18.37	No	7	5901	1	0.4	91	109	187	22	0.8	110	76	60	35	0.068	0.064	0.064	0.062
2	17years	F	12-10-2006	185months	Hypo	19months	116640	216	Defrijet 1250mg	Deferipr one 3000mg , Desferal inj 500mg	16-08-2008	No	1.46	36	16.89	grade 3	7.1	5734	1.4	0.5	29	30	63	14	0.7	112	92	82	30	0.072	0.069	0.068	0.068
3	16years	M	06-05-2007	185months	Hypo	7months	113760	158	Defrijet 1500mg	iprone 30	12-04-2010	No	1.36	48	25.95	grade 2	8.3	5122	0.5	0.2	32	18	239	18	0.8	110	116	34	76	0.07	0.072	0.07	0.07
4	15years	M	22-06-2006	174months	Hypo	7months	103192	186	Defrijet 1500mg	no	06-06-2009	No	1.42	38	18.85	grade 2	8.8	5060	0.7	0.3	22	21	264	18	0.6	112	120	78	44	0.068	0.071	0.068	0.07
5	15years	F	02-08-2006	177months	Hypo	3months	93452	196	Defrijet 1250mg	no	06-04-2009	No	1.52	32	13.85	grade 3	9.4	8451	1.3	0.4	78	75	132	18	0.4	84	46	38	36	0.068	0.066	0.072	0.076
6	18years	M	01-05-2003	204months	Hypo	8months	162792	228	Defrijet 1500mg	no	02-12-2006	No	1.4	42	21.43	grade 2	9.7	7921	0.9	0.03	46	58	146	22	0.5	128	92	88	40	0.068	0.07	0.072	0.07
7	16years	M	19-01-2006	180months	Hypo	12months	90720	216	Defrijet 1500mg	no	06-05-2008	No	1.34	28	15.59	No	8.2	727	2	0.5	33	30	262	19	0.5	142	190	106	36	0.05	0.052	0.048	0.056
8	15years	F	03-05-2006	130months	Hypo	24months	88374	206	Defrijet1250mg	no	06-10-2016	Yes	1.5	33	14.67	No	4.9	2657	1.6	0.5	82	113	193	26	0.4	146	78	98	40	0.05	0.052	0.052	0.056
9	15years	F	06-08-2006	170months	Hyper	9months	101388	210	Defrijet 1250mg	no	06-10-2008	No	1.38	34	17.85	No	11.1	3664	1.1	0.4	68	66	121	10	0.4	102	112	76	26	0.062	0.058	0.066	0.067
10	18years	M	06-02-2003	207months	Hypo	10months	216734	216	Defrijet 1500mg	no	16-08-2004	No	1.72	58	19.61	No	8.8	4260	2.5	0.4	15	10	211	33	0.6	94	57	38	79	0.056	0.052	0.052	0.052
11	16years	M	16-02-2006	184months	Hypo	8months	120960	192	Defrijet 1250mg	no	02-07-2008	No	1.46	42	19.7	grade 2	9.1	4594	1	0.3	59	61	271	27	0.4	122	84	82	40	0.048	0.042	0.048	0.042
12	18years	M	07-07-2004	207months	Hypo	9months	34675	112	Defrijet1500mg	no	18-06-2006	No	1.55	48	19.98	No	7.9	1058	2.3	0.7	24	18	113	11	0.5	150	120	86	36	0.044	0.04	0.042	0.04
13	16years	F	05-01-2005	187months	Hypo	7months	10040	180	Defrijet 1250mg	no	06-03-2008	No	1.28	36	21.97	No	7.5	635	0.6	0.2	21	34	154	22	0.4	196	132	98	36	0.04	0.042	0.046	0.048
14	15years	M	18-01-2006	163months	Hypo	5months	69630	102	Defrijet 1250mg	yes	04-08-2008	Yes	1.42	50	24.8	No	7	1103	1.7	0.6	14	23	28	30	0.4	150	148	128	22	0.048	0.046	0.042	0.042
15	15years	M	11-08-2006	176months	Hypo	3months	63936	120	Defrijet 1250mg	no	17-06-2010	No	1.55	36	14.98	grade 2	8.8	2158	3.7	0.3	28	23	219	17	0.5	90	76	36	36	0.046	0.044	0.04	0.042
16	17years	M	01-06-2004	184months	Hypo	20months	104781	162	Defrijet1500mg	no	02-02-2006	No	1.5	42	18.67	grade 2	5.8	4758	1.6	0.5	25	12	266	18	0.6	124	130	79	45	0.052	0.052	0.054	0.052
17	18years	M	01-06-2004	207months	Hypo	7months	40000	247	Defrijet1000mg	Deferipr one350 0mg, Desfero xamine 1500mg	02-01-2014	No	1.49	43	19.37	No	9.6	1239	1.1	0.4	20	22	92	24	0.4	96	104	44	48	0.044	0.04	0.042	0.04
18	17years	F	01-01-2003	193months	Hyper	2months	16800	277	Defrijet2000mg	no	04-07-2017	No	1.57	50	20.28	grade 4	10.9	3328	4.6	0.4	32	30	83	28	1	102	96	52	42	0.05	0.052	0.048	0.056
19	16years	M	06-10-2006	188months	Hypo	10months	28500	218	Defrijet1500mg	xyurea12	06-10-2006	No	1.52	35	15.15	grade 1	8.2	764	2	0.5	33	30	262	19	0.5	142	190	106	36	0.05	0.052	0.048	0.056