"EFFICACY OF COMBINATION THERAPY WITH ORAL IRON CHELATORS IN CHILDREN WITH TRANSFUSION DEPENDANT B-THALASSEMIA MAJOR ON ASSESSING CARDIAC FUNCTION BY ECHOCARDIOGRAPHY AND TISSUE DOPPLER IMAGING- A HOSPITAL BASED SINGLE BLIND RANDOMIZED CONTROLLED TRIAL"

BY

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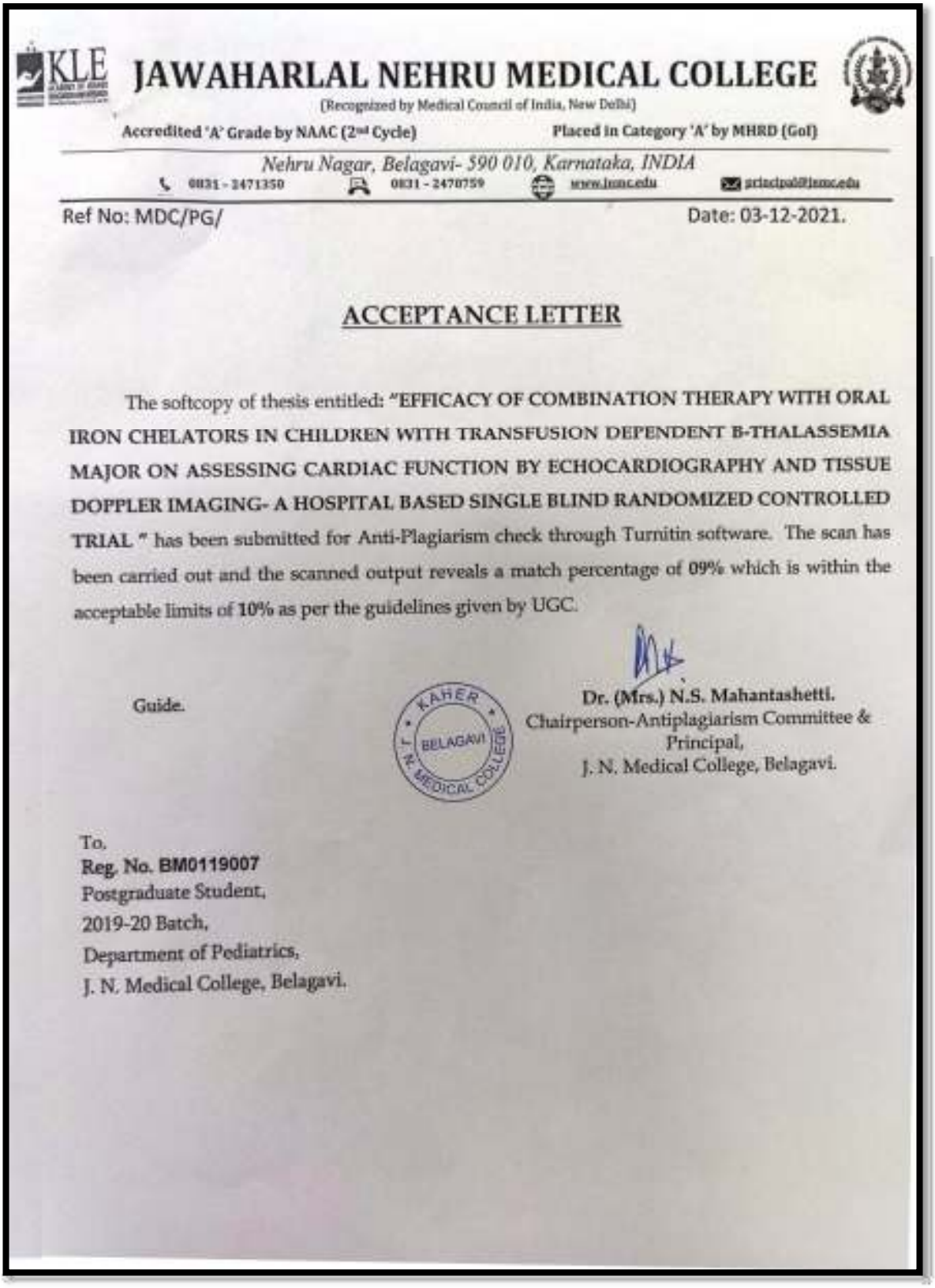

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LIST OF ABBREVIATIONS

B	-	Beta
/cumm	-	Per cubic millimeter
ACE	-	Angiotensin-Converting Enzyme
AF	-	Atrial Fibrillation
BT	-	Blood Transfusion
BTM	-	β Thalassemia Major
CBC	-	Complete Blood Count
DFO	-	Desferrioxamine
DFP	-	Deferiprone
DFX	-	Deferasirox
ECG	-	Electrocardiogram
ECHO	-	Echocardiography
fl	-	Femto litre
TDI	-	Tissue Doppler Imaging
gm/dl	-	Grams per decilitre
g%	-	gram percent
Hb	-	Haemoglobin
HbA	-	Haemoglobin A
HbA2	-	Haemoglobin A2
HbC/β	-	Haemoglobin C/ beta
HbD	-	Haemoglobin D
HbE	-	Haemoglobin E
HbE/β	-	Haemoglobin
HbF	-	Fetal Haemoglobin

HbS/ β	-	Haemoglobin S/beta
HBsAg	-	Surface antigen for Hepatitis B virus
HCV	-	Hepatitis C virus
HF	-	Heart Failure
HIV	-	Human Immunodeficiency Virus
HLA	-	Human Leucocyte Antigen
HPLC	-	High Performance Liquid Chromatography
HSCT	-	Hematopoietic Stem-Cell Transplant
i.e.,	-	That is, LVEDV - Left Ventricular End Diastolic Volume
LIC	-	Liver Iron Concentration
LPI	-	Labile Plasma Iron
LVEF	-	Left Ventricular Ejection Fraction
LVESV	-	Left Ventricular End Systolic Volume
LVIDd	-	Left Ventricular Internal Diameter end diastole
LVIDs	-	Left Ventricular Internal Diameter end systole
LVF	-	Left Ventricular Failure
m/k/d	-	milligram per kilogram per day
MAPSE	-	Mitral valve Annular Plane Systolic Excursion
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
mrna	-	messenger Ribonucleic Acid
NTDT	-	Non Transfusion Dependant Thalassemia
PAH	-	Pulmonary Artery Hypertension
pg	-	picogram

RBC	-	Red Blood Cell
RDW	-	Red Cell Distribution Width
RCT	-	Randomized Controlled Trial
SD	-	Standard Deviation
SF	-	Serum Ferritin
T2*MRI	-	T2 * weighted Magnetic Resonance Imaging
TAPSE	-	Tricuspid Annular Plane Systolic Excursion
TDE	-	Tissue Doppler Echocardiography
TDT	-	Transfusion Dependant Thalassemia
TIF	-	Thalassemia International Federation
TM	-	Thalassemia Major
vs.	-	versus
USG	-	Ultrasound
WBC	-	White Blood Cell
WHO	-	World Health Organisation
α2	-	Alpha 2

ABSTRACT

Background and objectives: Deferiprone when used in combination with Deferasirox, has proven efficacy for reducing serum ferritin levels and the prevention and control of iron overload. This study is aimed at assessing the efficacy of combination therapy with oral iron chelators (Deferasirox and Deferiprone) in children with transfusion-dependent Beta-thalassemia using Echocardiography and Tissue Doppler Imaging.

Methodology: This hospital based single blind Randomized Controlled Trial was done between July 2020 to May 2021. A total of 40 patients with abnormal echocardiography findings in the age group of 10-18 years on Deferasirox Monotherapy underwent baseline ECHO-Tissue Doppler Imaging, S. ferritin levels and USG assessment of liver and spleen size. 20 participants were randomly assigned to the intervention group and were administered Deferiprone for 6 months. Both intervention and control groups continued to receive Deferasirox and cardiac functions, serum ferritin and liver & spleen size were reassessed after 6 months of intervention.

Results: Mean age of children in interventional group was 15.95 ± 2.26 as compared to 14.95 ± 2.63 in control group. Majority of the children in both the intervention group (65%) and control group (60%) were male with a male to female ratio of 1.6:1. The Mean pre-transfusion Hemoglobin (g/dl) measured at 6 months was higher in intervention group with mean of 9.16 ± 0.81 as compared to 8.32 ± 1.18 in control group (p= 0.0118*). Mean S. Ferritin levels recorded at 6 months after treatment in intervention group was comparable with that of control group (3610.80 ± 1785.21 vs 3030.70 ± 1737.52; p=0.3043). The difference in mean liver size from baseline to 6

months post-intervention in intervention group (0.83;p= 0.0139*) and in control group (-0.88; p= 0.0494*) was significantly high. Difference in mean spleen size from baseline to 6 months post-intervention was significantly high in intervention group (1.28; p= 0.0079*). Mean FS after 6 months of treatment in intervention group was higher in comparison to that of control group (34 ± 6.33 vs 28.4 ± 7.04; p= 0.0118*). LVIDd at baseline was significantly higher in intervention group as compared to that of control group (4.33 ± 0.48 vs 4.06 ± 0.33; p= 0.0486*). MAPSE of Septal wall (MVSSAE) showed significant improvement from baseline to 6 months (p= 0.0494*) in intervention group and we also observed a statistically significant reduction of MVLI-E velocity from baseline to 6 months post-randomization in the control group (t=-2.3472; p= 0.0299*) indicating early diastolic dysfunction in control group. We also recorded that with decline in serum ferritin levels at 6 months, LVEF (p=0.0010*)., MVLSAE (p=0.0050*). and MVLI- E (p= 0.0120*) velocity at 6 months seemed to significantly improve in the intervention group.

Conclusion and interpretation: Treatment with combination oral iron chelators i.e. Deferiprone and Deferasirox for 6 months was effective in improving cardiac functions, in reducing liver and spleen size and caused increase in hemoglobin levels. Tissue Doppler Echocardiography can detect early myocardial diastolic and systolic dysfunction than global dysfunction by Conventional ECHO.

Keywords: Beta thalassemia; Combination oral chelation; Tissue Doppler Imaging; Echocardiography; Deferiprone

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF PHOTOGRAPHS

INTRODUCTION

"Thalassemia refers to a group of blood disorders, which are the most common inherited hemoglobinopathies characterized by partly or completely decreased synthesis of normal globin chains and ineffective erythropoiesis.[1] They have an inheritance of autosomal recessive in nature and are the commonest single gene disorders globally, more prevalent in the Mediterranean, Indian Sub-continent, Southeast Asia and Africa.[1] Based on the involvement of globin chains, Thalassemia has been classified as alpha and beta-thalassemia.

Thalassemia is a heterogenous disorder with varied phenotypes, ethnicities accounts for the global public health problem.[2] It is estimated that around 3,00,000-4,00,000 children are born in the world with some hemoglobin disorder every year, out of which around 1,00,000 children with thalassemia are contributed by India.[3] Thalassemia is more commonly seen in low and middle income countries. Worldwide 56,000 conceptions are having thalassemia disorder, out of which 30,000 have Thalassemia major.[4] Northern India is having high carrier rate of about 3-15% when compared to Southern India which is 1-3%. [3]

β-thalassemia's are characterized by partly or completely beta globin chains suppression and excessive production of alpha globin chain. β-thalassemia's are more common than α-thalassemia's. Based on the extent of production of globin chains, manifestations range from mild anemia to transfusion dependance.

The age of presentation in β-thalassemia major will be usually between 6-24 months with Hepato-splenomegaly, mild jaundice and severe microcytic anemia.

Regular blood transfusions and chelating agents are the mainstay treatment in thalassemia major, which will increase the life expectancy of the patient.

In recent years, since the blood transfusions facility has increased especially in patients with transfusion-dependent thalassemia major, the associated iron overload leads to significant complications. The accumulation of cardiac iron in thalassaemia children is the single largest risk factor for cardiac dysfunction. High levels of non-transferrin bound iron in the bloodstream over time cause heart dysfunction, arrhythmias, and, if not treated, fibrosis. An important role in development of iron cardiomyopathy is due to dysregulation of calcium homeostasis.(5)

Diagnosis of cardiac dysfunction is made with ECG, Echocardiography (ECHO), Doppler Echocardiography, T2*MRI. Cardiac T2* MRI is the gold standard non-invasive modality for monitoring patients with thalassaemia major due to its advantage such that it allows early estimation of cardiac iron burden but also helps in identifying consistently the preclinical changes in ejection fraction. Because of its high cost, limited availability, and lack of expert interpretation, it is inconvenient for mass screening, especially in underdeveloped countries like India.(6)

Non-expensive imaging modalities like Tissue Doppler echocardiography have been used as an early diagnostic test to detect regional systolic and diastolic myocardial dysfunction based on abnormal myocardial velocities, even before overt heart failure or cardiomyopathy sets in. As a result, in beta thalassemia children and adolescents, this modality is critical for detecting asymptomatic cardiac insufficiency before it is diagnosed by conventional echocardiography.(7)

The mainstay of treatment for cardiac iron overload is iron chelation therapy. Desferoxamine, Deferiprone, and Deferasirox are the three iron chelators that are currently in use. To prevent iron overload complications, it is used as a monotherapy or in combination.

Parenteral desferoxamine has previously been used successfully in combination with the oral iron chelators deferiprone or deferasirox, but at the expense of repeated painful injections and poor compliance.

When compared to monotherapy alone, combination therapy with the iron chelators DFP and DFX was more effective in lowering serum ferritin levels. T2* MRI results were inconclusive.[8]

Combined chelation results in a constant presence of chelating agent in the patient's circulation, preventing free Labile Plasma Iron (LPI) entry into cells and thus protecting from reactive oxygen species that are mostly responsible for organ damage, resulting in better & enhanced chelation and a decrease in total body iron as measured by Serum Ferritin (SF) levels at regular intervals.[9]

The SF measurement correlates with the body's iron stores. It is relatively simple and inexpensive to determine routinely, especially in Thalassemia. Major children are receiving multiple blood transfusions, and those on iron chelators are being monitored for response to treatment.

Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one), also known as L1, is an orally active chelator that belongs to the bidentate hydroxypyridinones family. It is a small lipophilic molecule that can enter cardiac myocytes and aid in iron removal. A

large body of evidence suggests that the use of deferiprone is associated with lower cardiac iron stores when compared to other chelators.[10] [11] [12]

In a large clinical observational study conducted by Janet L. K., Ami Belmont, and colleagues, patients treated with deferiprone showed a significant improvement in cardiac T2* MRI over time, including those with significant cardiac iron loading (T2* 8 to 20 ms), whereas deferoxamine showed no significant improvement.[12]

Deferasirox (DFX) is an orally active trident molecule that was initially developed as a once-daily oral monotherapy for the treatment of transfusion-dependent iron overload and is now approved as first-line monotherapy.[13] Pennell DJ, Porter JB, Cappellini MD, et al. published a study in 2010 that demonstrated the efficacy of Deferasirox in reducing and preventing cardiac iron overload in patients with β-thalassemia.[14]

Combination DFO/DFP and DFP/DFX are equally efficacious in reducing cardiac iron overload by increasing mean LVEF and in reducing SF levels as for better tolerability and compliance offered.[10]

There have been very few studies on Tissue Doppler Imaging and 2D-echocardiography to assess early myocardial dysfunction in asymptomatic Thalassemia Major children receiving combination oral iron chelators. As a result, this study aimed to assess the efficacy of combination oral iron chelators on assessing cardiac functions as done by TDI and was likely the first of its kind.

OBJECTIVES

The objectives of this study were;

PRIMARY:

To assess the efficacy of combination therapy with oral iron chelators (Deferasirox and Deferiprone) in children with transfusion-dependent Beta-thalassemia using Echocardiography and Tissue Doppler Imaging.

SECONDARY:

1) To determine the effect of combination therapy on S. Ferritin levels.
2) To evaluate the effect of combination therapy on liver and spleen size reduction.

REVIEW OF LITERATURE

The thalassemias are a group of hemolytic anaemias due to inherited defects in the haemoglobin production. They belong to a heterogeneous cluster of single-gene disorders more common in some parts of the world, more prevalent in the Mediterranean, Indian Sub-continent, Southeast Asia and Africa.[15]

Beta-Thalassemia:

β thalassemia is a major health burden globally and also is one of the commonest autosomal recessive disorders worldwide. 95 % of the thalassemia births worldwide were from Middle Eastern, Asian and Indian regions.[16] Thalassemia is commonly seen in people of African origin. Cyprus (14%), Sardinia (12%) and South-East Asia had the highest reported incidences.[4]

HISTORICAL

In the 20th century beginning, European physicians noticed an anaemia syndrome with enlargement of spleen in infancy. The first clinical description of thalassemia is given by Detroit paediatricians Thomas B.Cooley and Pearl Lee. Cooley and Lee had described four Italian children with anaemia, discolouration of the skin, sclera enlargement of spleen and liver. Red blood cells of these children have increased resistance to hypotonic solutions and the peripheral blood smear showed moderate leukocytosis with nucleated erythrocytes. They used to have hemolytic facies with prominent cranial and facial bones.

Previously chronic childhood anaemias were named under a group called as Von Jaksch's anaemia, later it was separated by Cooley and Lee and named it as "Erythroblastic anaemia or Cooley's anaemia.[17]

Whipple and Bradford coined the term "thalassemia" in 1932, derived from the Greek words "thalassa" (black sea) and "mia" (blood). Based on the severity, Valentine and Neel classified the milder forms of Cooley's anaemia as 'Thalassemia minor' and the more severe entity as 'Thalassemia major'. In 1925, it was described that the disease was running in the families by Rietti and told to be familial condition. In 1936, Lehndorff first proposed that the condition is also inherited.

Mukerjee reported the first case of beta-thalassemia from India in 1938 from Calcutta. In 1935, Sheldon described the severe pathologic sequelae associated with iron overload.

Wolman in 1964 was the first to suggest that chronic blood transfusion may be able to prevent many of the problems of the disease. Lesky, et al and Model, et al. in 1974 were able to initiate clinical trials with desferrioxamine, an iron chelator, in an attempt to promote the excretion of large percentage of transfusional iron overload. Deferiprone an oral iron chelator was discovered in 1981. In 1982 Dr. E Donald Thomas performed the first bone marrow transplantation on a thalassemic patient. The first bone marrow transplantation in India was successfully done by Dr M. Chandy at Christian Medical College, Vellore.

Table 1: Historical landmarks in the field of hemoglobin and thalassemia.[16]

Year	Landmark	Author
1628	Circulation of the blood	Harvey
1862	Oxygen binding pigment is named 'hemoglobin'	Hoppe – Seyler
1866	Fetal blood is alkali-resistant	Korber
1913	Structure of haem	Kuster
1925-1927	Molecular weight of hemoglobin	Adair, Svedberg
1925	Description of 'thalassemia'	Cooley, Lee
1932	Term 'thalassemia' first used	Whipple & Bradford
1937-1944	Inheritance of thalassemia	Caminopetros, Neel, Valentine,Silvestroni
1944-1946	Sickle-cell thalassemia	Silvestroni, Bianco
1948	Alkali-resistant hemoglobin in thalassemia	Vecchio
1949	Malaria hypothesis	Haldane
1955	HbA2 raised in some thalassemia	Kunkel & Wallenuis
1957	Ineffective erythropoiesis in thalassemia	Sturgeon & Finch
1958	Adult hemoglobin controlled by two gene Loci	Smith & Torbert
1959	Three – dimensional structure of hemoglobin	Perutz
1959	α andβ thalassemia hypothesis	Ingram and Stretton

1960-1963	Structure of α, β, γ and δ chains	Konigsberg et al., Schroeder et al., Jones et al., Braunitzer et al., Hill et al.,
1962	Chelation therapy – Desferrioxamine	Sephton – Smith
1964	High level transfusion for thalassemia	Waldman
1965	Imbalanced globin synthesis in α andβ Thalassemia	Weatherall, Clegg
1966	Consequences of globin imbalance	Nathan, Gunn
1970	Genetics of α thalassemia	Na-Nakron and Wasi
1973	Dominantly inherited β thalassemia	Weatherall et al
1974	Liver iron level controlled by Desferrioxamine	Barry et al
1979	Restriction – fragment length polymorphism for prenatal diagnosis	Kan and Dozy
1979	Stop – codon mutation in β globin mRNA	Chang and Kan
1979	β Thalassemia due to gene deletion	Orkin et al
1980-1981	Globin genes sequenced	Lawn et al., Spritz et al., Barralle et al
1981	Mutations in β thalassemia cloned in DNA	Spritz et al., Westaway and Williamson.

DEFINITION:

"Thalassemia syndromes are a group of hereditary blood disorders characterised by decreased or absent globin chain synthesis, resulting in decreased haemoglobin in red blood cells, decreased RBC production, and anaemia."[(18)] It is inherited in an autosomal recessive pattern.

Table 2: β thalassemia can be classified into following categories.[(5)]

BETA-THALASSEMIA:- 1. Thalassemia major 2. Thalassemia intermedia 3. Thalassemia minor
4. β thalassemia with associated Hemoglobin anomalies o HbC/ β thalassemia o HbE/ β thalassemia o HbS/ β thalassemia
5. Hereditary persistence of fetal hemoglobin and β thalassemia
6. Autosomal dominant β thalassemia
7. β thalassemia associated with other manifestations:- o β thalassemia-trichothiodystrophy o X-linked thrombocytopenia with thalassemia.

Thalassemia major & Thalassemia intermedia are the 2 phenotypes β-thalassemia that exhibit as either genetic homozygous or heterozygous compound. Children with thalassemia major usually show clinical symptoms by the age of two and frequently require frequent blood transfusions to survive. Thalassemia intermedia

manifests in late ages and does not require regular blood transfusions. Except in the rare dominant forms, heterozygous β-thalassemia manifests as a clinically silent carrier state. HbC/thalassemia and HbE/thalassemia manifest in a wide spectrum of severity phenotypes.[19]

Epidemiology

β-thalassemia is a global health problem and the commonest hemoglobinopathies. Mutations, base substitutions or insertion of nucleotides in β-globin genes will cause β-thalassemia. β-thalassemia is more common in certain geographical areas/populations because mutations are relatively population specific.

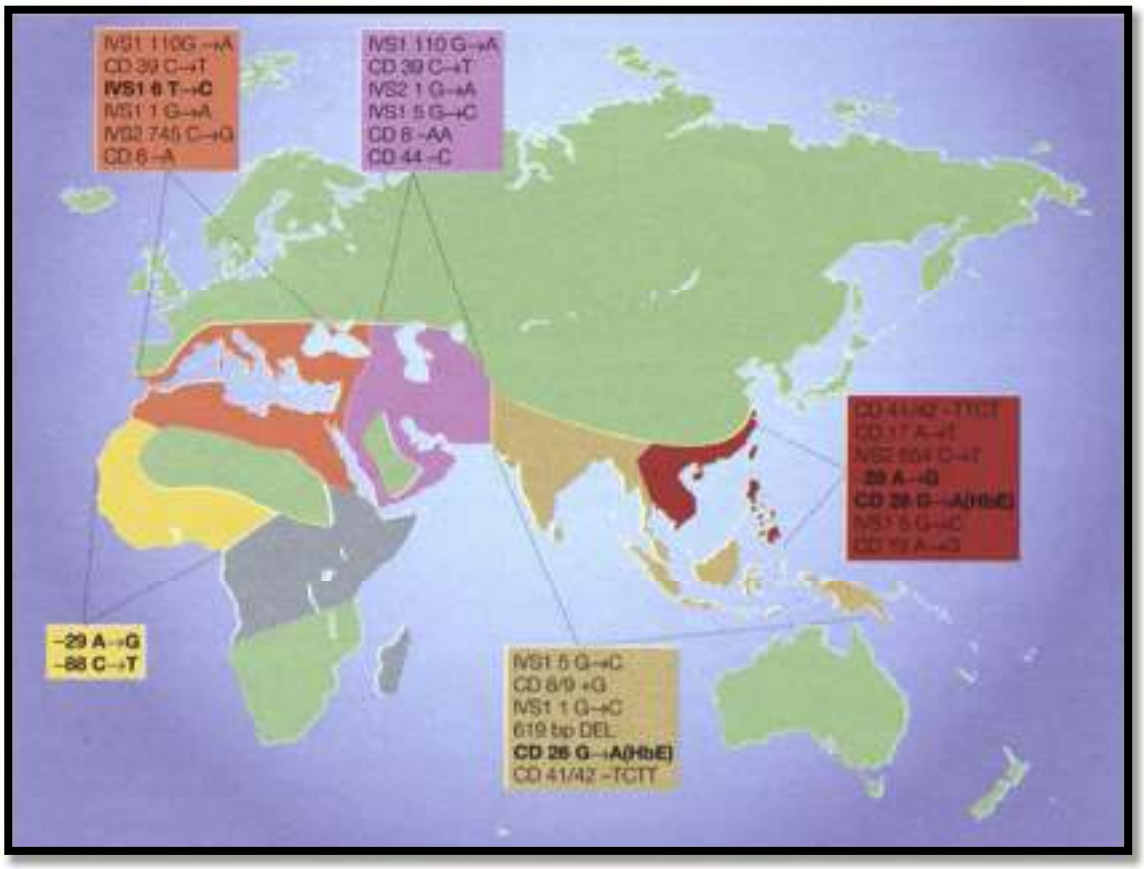


Figure 1: The approximate distribution of the β-thalassemia worldwide (20).

There are an estimated 270 million carriers of abnormal haemoglobins and thalassemias worldwide, with 80 million carriers of ß-thalassemia. According to recent surveys, between 300,000 and 400,000 babies are born each year with a serious haemoglobin disorder (23,000 with ß-thalassemia major), with up to 90% of these births occurring in low- or middle-income countries.[21]

The incidence of symptomatic β thalassemia major individuals throughout the world is estimated as 1 in 100,000. As per Thalassemia International Federation (TIF) throughout the world, only 2,00,000 β-thalassemia patients are present and taking treatment on registration regularly. HbE/β thalassemia is the most frequently encountered combination of β thalassemia with structural haemoglobin variant or abnormal haemoglobin and has a high prevalence in Southeast Asia, with a carrier frequency of 50%.[5]

Thalassemia is now present in almost all the nations of the world due to migration of people and intermarriage between varied ethnic groups. 1.5% of the global population has a carrier state for β-thalassemia. Secondary to migration of population between different regions, marriage between different ethnic groups, races, thalassemia is prevalent in almost every country. In India, the overall beta-thalassemia carrier rate is estimated to be 4.05 percent, with an annual incidence of 11,316 - thalassemia homozygotes births.[19]

INDIA

β-thalassemia is the commonest single-gene haemoglobinopathy in Indian population. India comprises ten per cent of the total world thalassemia births every year. Grow K et al. The β – thalassemia carrier frequency varies from 3.0 to 17.0% In India.[22]

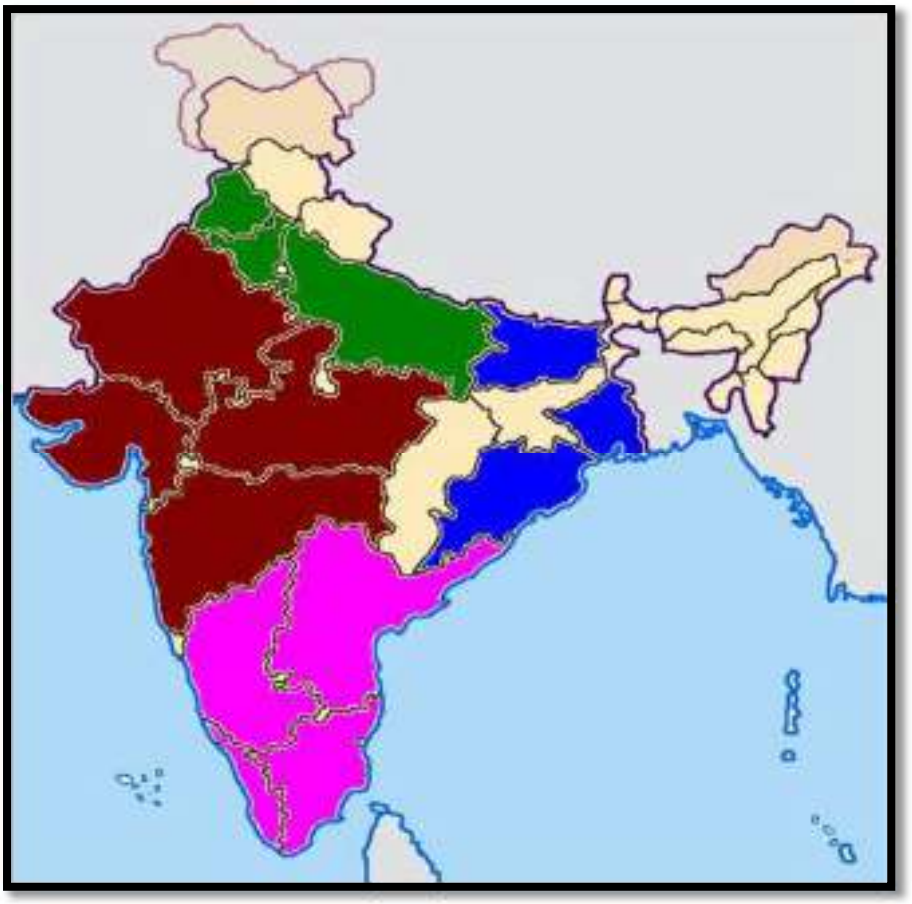


Figure 2: Map showing distribution of beta thalassemia in India.[23]

In India, as per WHO update on β-thalassemia, an overall carrier frequency of 3–4% was seen, which would render the carrier rate between 35.6 and 47.5 million nationwide. Prenatal diagnosis and detection of carrier status are required to decrease the mutant load in the gene pool.

In a non-Mediterranean region, the first case of Thalassemia was reported from India. It was reported in 1983 that beta-thalassaemia has been observed in different castes and communities like Gujaratis, Sindhis, Marathas, Khojas, Bori Muslims, Jains, Baniyas and Punjabis.

India has a high rate of consanguineous marriage accounting for an estimated 10.4% of world population, as they follow their specific caste. Due to more

consanguineous marriages and the lack of premarital checkup in India, it leads to many disorders. One such disease is thalassemia, seen almost in every community of India.[23]

Hereditary Transmission

Beta thalassemia's show autosomal recessive inheritance pattern. Affected child parents will be heterozygotes and contain a single copy of pathogenic gene mutation in the beta-globin chain. 25 % chances of children getting affected if parents are heterozygotes, 50% chances of asymptomatic carrier, & 25% chances of not getting affected & carrier.

Thalassemia syndromes are classified based on the blood transfusion requirement, clinical severity into

A. Transfusion Dependent Thalassemia's (TDTs)
B. Non-Transfusion Dependent Thalassemia's (NTDTs)

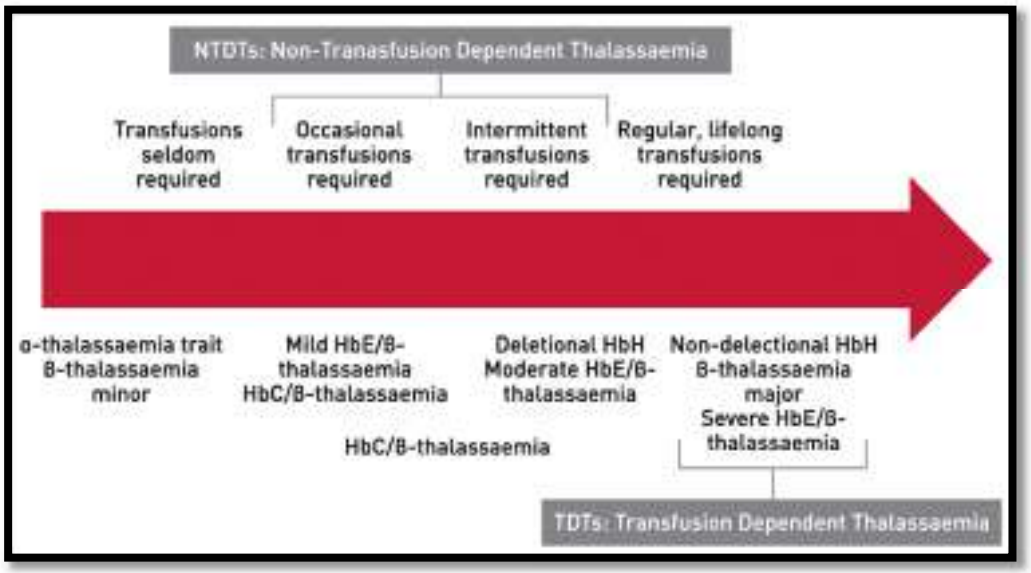


Figure 3a: Thalassemia syndrome phenotypic classification based on clinical severity and transfusion requirement.[5]

TDT's would be requiring regular blood transfusions, iron chelating agents to avoid iron overload. They will be presenting at an early age with increasing pallor, abdominal distension and respiratory distress.(5)

Phenotypic heterogeneity

β-thalassemia is having three forms

1. 1. Thalassaemia Major, also known as "Cooley's Anaemia" or "Mediterranean Anaemia," is a type of anaemia that occurs in the Mediterranean region.
2. "β-thalassaemia carrier - Thalassaemia Intermedia & Thalassaemia Minor"
3. β-thalassaemia trait / heterozygous β-thalassaemia.(19)

PATHOPHYSIOLOGY:

Decreased or lack of β-globin chain production with corresponding excessive synthesis of α-chains is the primary defect seen in β-Thalassemia Major.

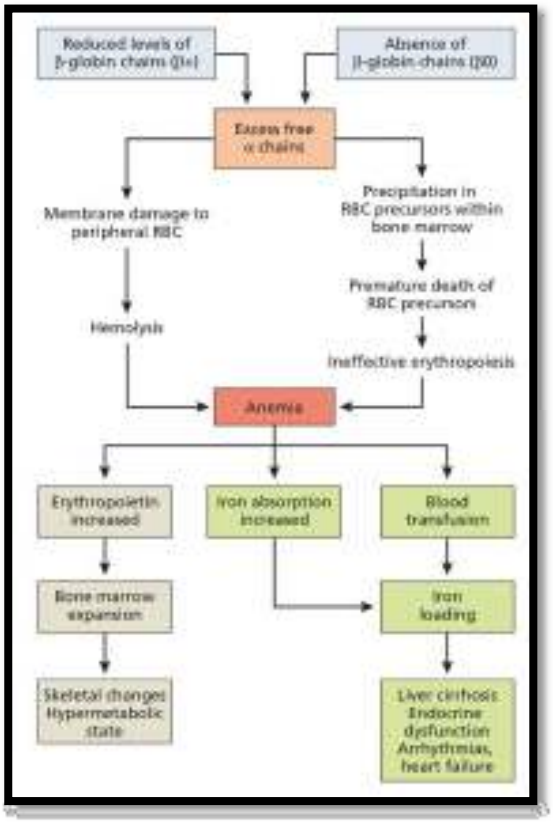


Figure 3b: Pathophysiology in Beta Thalassemia Major

Direct significant changes are

- Decrease in haemoglobin synthesis.
- Globin chain synthesis imbalance.

Increased levels of erythropoietin is the first response to anaemia and ineffective erythropoiesis, causing severe erythroid hyperplasia, skeletal deformities, osteoporosis and contributes to splenomegaly.

CLINICAL DIAGNOSIS

β-thalassemia major usually presents between 6-24 months of life & is characterized by

1. Severe microcytic anaemia
2. Hepatosplenomegaly
3. Jaundice.

Failure to thrive, increasing pallor will be seen. Repeated fever episodes secondary to infection, and progressive spleen & liver enlargement causing abdominal distension. In cases of improper treatment or unavailability of transfusion facilities, the clinical picture will be short stature, pallor, jaundice, muscle weakness, hepatosplenomegaly, skeletal deformities secondary to bone marrow expansion.[18]

Skeletal complications like bone deformities of legs and craniofacial variations characterized by thalassemic facies (skull bossing, prominent malar eminence, depressed nasal bridge and maxillary hypertrophy, which causes exposure of the upper teeth).

In affected children, the risk of developing post-transfusion iron overload is very high after the age of 10-11 years. Growth retardation and sexual maturation delay are two complications of iron overload. Later stages of disease involve the heart (dilated cardiomyopathy, arrhythmias, and eventually heart failure), endocrine (diabetes mellitus, parathyroid, thyroid, hypogonadism, and pituitary insufficiency), and liver (fibrosis and cirrhosis).

Hematologic Diagnosis

β-Thalassemia major is considered by decreased Hemoglobin parameters (<7 g/dl). RBC indices have microcytic hypochromic anemia, MCV between > 50 < 70 Femto litre and MCH between > 12< 20 pg, normal RDW. [5,19]

Peripheral blood smear

RBC will show moderate to severe anisocytosis, teardrop cells, microcytic hypochromic and nucleated RBC's. Degree of low haemoglobin levels will be correlated with the number of erythroblasts and will be more in number in post-splenectomy patients. Erythroblasts are not usually seen in carriers.[19]

HPLC-Electrophoresis

HPLC electrophoresis is diagnostic and advised both for the child and parents to look for transmission of the genes. β-thalassemia type will vary depending on the Hb pattern. HbA will be absent in ß0 thalassemia homozygotes and HbF institutes 92-95% of total Hb. HbA levels will be in between 10 - 30% and HbF in range of 70 - 90% In homozygotes of β-thalassemia and ß+/ ß0 genetic compounds. HmA2 varies in homozygotes of β-Thalassemia and is higher in children with β-thalassemia minor.

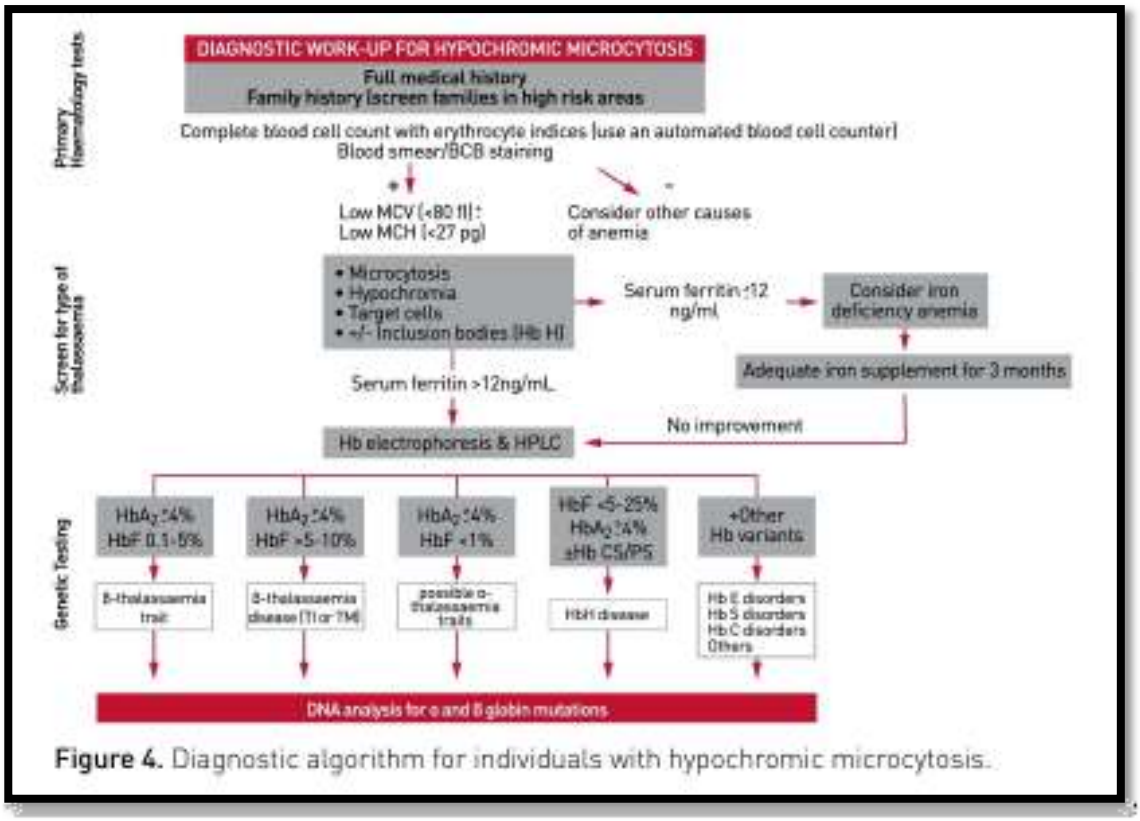


Figure 4. Diagnostic algorithm for individuals with hypochromic microcytosis.

TREATMENT:

Beta Thalassemia requires lifelong treatment. Main stay of therapy includes regular blood transfusion. If no regular transfusions and chelation therapy available, the majority of children could not survive until the age of 20- 23 years.

Backbone of Clinical Management of β-thalassemia:

1. Blood Transfusion
2. Iron Chelation
3. Multidisciplinary Care - mainly but not limited to:
    a. Heart
    b. Liver
    c. osteoporosis
    d. Endocrine
    e. Infection.

1. Dietary restrictions and supplements: High caloric intake is recommended along with consumption of folic acid, calcium, zinc and multivitamins (especially vitamin D).
2. Hydroxyurea: The ability of hydroxyurea to induce γ-globin is its most important action in β-haemoglobinopathies, reducing the frequency of transfusion in TDT-β-Thalassemia Major. It is recommended to begin with a lower dose of 10–15 mg/kg/day and gradually increase to a typical dose of 15–30 mg/kg/day (the maximum dose is 35 mg/kg/day) in 2.5–5 mg/kg/day steps. [24]

Haemoglobin levels should be maintained at least 9 to 10 g per deciliter with regular transfusion therapy, which reduces rate of hepatosplenomegaly, skeletal deformities ameliorates growth and development.[19]

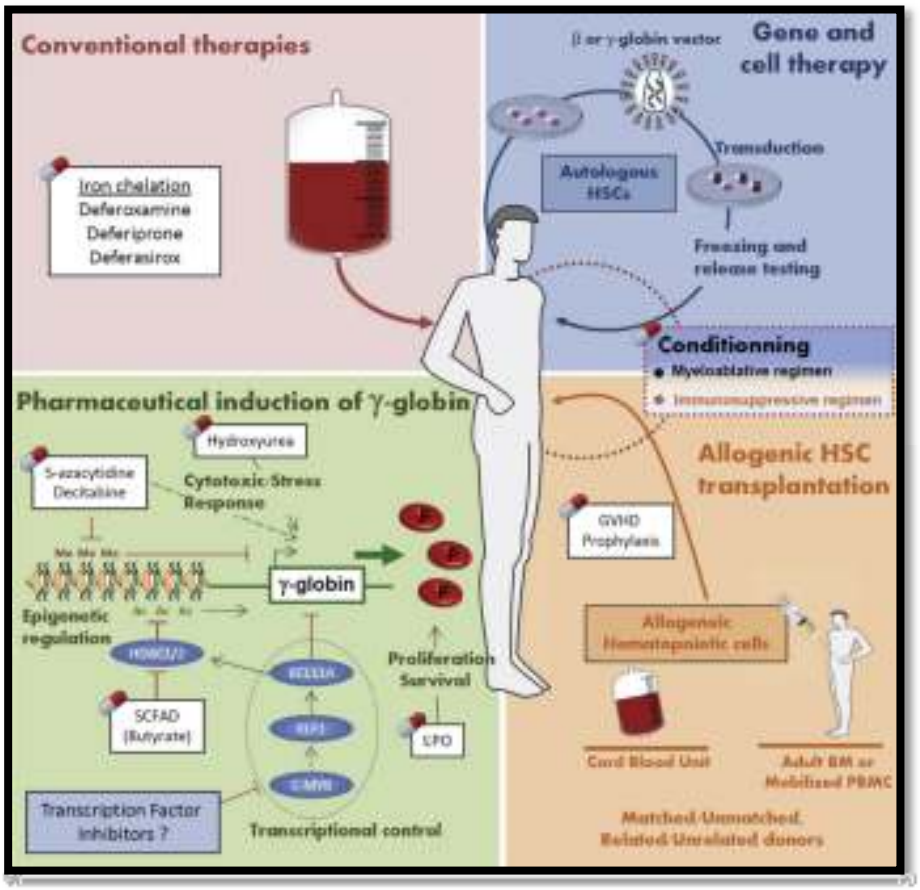


Figure 5: Treatment modalities in Thalassemia major

Enrolled patients	Myeloablative regimen	Mobilization protocol	Drug product
TDT 9 (3 adults, 6 children)	Treosulfan and Thiotepa	G-CSF and Plerixafor	Autologous HSCs genetically modified with GLOBE lentiviral vector encoding for the human β-globin gene
TM 18 (between 12 and 35 years of age)	Busulfan	G-CSF and Plerixafor	Autologous HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human βA-T87Q-globin gene
TDT 4 (between 5 and 35 years of age)	Busulfan (adjusted based on daily PK monitoring)	G-CSF and Plerixafor (after 3 months of enhanced transfusion)	Autologous HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human βA-T87Q-globin gene
TDT non β0/β0 β0, ≤ 50 years of age Estimated enrollment: 20 pts	Busulfan	G-CSF and Plerixafor	Autologous HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human βA-T87Q-globin gene
TDT β0/β0, β0/IVS-I-110, or IVS-I-110/IVS-I-110 ≤ 50 years of age Estimated enrollment: 18 pts	Busulfan	G-CSF and Plerixafor	Autologous HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human βA-T87Q-globin gene
Estimated enrollment: 45 participants including SCD and other hematological disorders	Busulfan	NA	CTX001: autologous CD34+HSPCs modified with CRISPR-Cas9 at the erythroid lineage specific enhancer of the BCL11A gene
TDT ≥ 18 and ≤ 40 years of age Estimated enrollment: 15 participants	Busulfan	NA	SB-400: autologous CD34+ HSPCs genetically modified with ZFN technology at the erythroid specific enhancer of the BCL11A gene

Figure 6a: Gene editing and gene therapy options for β-thalassemia. [25]

Enrolled patients	Drug product	Route of administration	Target	Sponsor/center
16 TDT 30 NTDT	Sotatercept (ACE-011)	Subcutaneous	Ineffective erythropoiesis	Celgene Multicenter international sites
31 TDT 33 NTDT	Luspatercept (ACE-536)	Subcutaneous	Ineffective erythropoiesis	Acceleron Pharma Multicenter international sites
336 TDT	Luspatercept (ACE-536)	Subcutaneous	Ineffective erythropoiesis	Celgene and Acceleron Pharma Multicenter international sites
145 NTDT	Luspatercept (ACE-536)	Subcutaneous	Ineffective erythropoiesis	Celgene and Acceleron Pharma Multicenter international sites
30 TDT	Ruxolitinib	Oral	Ineffective erythropoiesis through inhibition of JAK2	Novartis Pharmaceuticals Multicenter international sites
100[a] TDT	LJPC-401	Subcutaneous	Iron metabolism through synthetic human hepcidin	La Jolla Pharmaceutical Company Multicenter international sites
192[a] TDT and NTDT	PTG-300	Subcutaneous	Iron metabolism through synthetic human hepcidin	Protagonist Therapeutics
36[a] NTDT	VIT-2763	Oral	Iron metabolism through ferroportin inhibition	Vifor Multicenter international sites
36[a] NTDT	IONIS TMPRSS6-LRx	Subcutaneous	Iron metabolism through TMPRSS6 inhibition	Ionis Pharmaceuticals
TDT and low-risk MDS	SLN124	Subcutaneous	Iron metabolism through TMPRSS6 inhibition	Silence Therapeutics

Figure 6b: Pharmacological treatment options for β-thalassemia. [(25)]

CARDIAC DYSFUNCTION IN THALASSEMIA

INTRODUCTION: Cardiac dysfunction is mainly due to cardiac iron accumulation which forms the single major risk factor in thalassemia patients receiving repeated blood transfusions resulting in a combination of constant state of high cardiac output & increased absorption of iron from intestines.

One such major complication involving cardiac iron overload is Heart failure.

Table 3: Classification of cardiovascular complications in Beta-Thalassemia Major. [5]

1. Iron overload complications	2. Non-iron overload complications
a. Reversible myocyte failure. b. Arterial changes due to loss of vascular compliance. c. Arrhythmia, including heart block.	a. Cardiac function changes due to restriction / diastolic dysfunction / fibrosis. b. Pulmonary artery hypertension (PAH) c. Thrombotic stroke due to AF. d. Arterial changes - loss of vascular compliance. e. Arrhythmia – especially Atrial Fibrillation (AF), later in life.

INCIDENCE: Incidence of cardiac dysfunction is increasing nowadays because of improvement in treatment modalities, availability of blood transfusion facilities along with chelating agents and management of complications at different levels of care. Based on different studies, 11.4% to 15% is the incidence rate of Iron overload Cardiomyopathy in β-Thalassemia.[26]

PREVALENCE IN DIFFERENT STUDIES

Age & year of birth are the characteristics upon which prevalence is determined. 37% of 97 patient cohort born before 1976 had heart disease as determined by need for inotropic support or anti-arrhythmic drugs as studied by Oliveri et al.[27]

In a survey conducted in US in 2004 by Cunningham MJ et al, 10% [35/341] of TM patients were on cardiac medications. [28] The prevalence of Heart Failure (HF) in an Italian cohort, reported by Borgna-Pignatti C et al, by 15 years of age was 5% in patients born between 1970-1974 and 2% between 1980 and 1984 cohort groups.[29]

The incidence of HF at 1st T2* MRI scan conducted in a worldwide survey conducted in 2012 by Carpenter et al, was 3.1% [107/3445] (30). Alternately, the prevalence rate of detectable left ventricular dysfunction is greater than that of clinically manifest HF. In a study conducted by Tanner MA, 11.4% (19/167) Italian patients, had left ventricular dysfunction.[31]

In a recent Italian study by Marsella M, 70% of cases accounted for mortality resulting from cardiac iron overload which continued to attribute to the leading cause of death in Thalassemia patients.[32]

ETIOLOGY:

A) GENETIC FACTORS

B-Thalassemia Major with HF showed a great attribution to HLA-DQA1*0501 allele while HLA-DRB1*1401 allele is protective against heart failure.[33]

B) ACQUIRED FACTORS CAUSING CARDIAC DYSFUNCTION IN BETA-THALASSAEMIA

1- Endocrine disorders	Hypothyroidism Hypogonadism Hypo-parathyroidism
2- Nutritional deficiencies	Carnitine, Thiamine, Selenium Vitamin D deficiency
3- Infections	Hepatitis B, Hepatitis C infection
4- Acute Myocarditis	

PATHOGENESIS:-

The pathophysiology of high output state in β-thalassemia is complex. Abnormal haemoglobin with increased oxygen affinity (HbF) along with decreased levels of 2, 3- bisphosphoglycerate in transfused blood from chronic anemia causes prolonged tissue hypoxia resulting in high cardiac output state.

Cardiac output elevation results from:-

a) Hepatic cirrhosis secondary to iron overload or blood-borne viral infections like Hepatitis B/E
b) Extensive haematopoietic tissue & intra-splenic shunts that leading to bone marrow expansion & splenomegaly.

Labile plasma iron existing in circulation due to iron overload conditions, when transferrin is saturated, enters cardiomyocytes via voltage gated L-type Calcium channel in ferrous form is stored as: Ferritin, haemosiderin, labile cellular iron.

Peroxidative damage by reactive oxygen species formed from labile cellular iron results in cardiomyocyte apoptosis & cardiac dysfunction.(33)

Dysregulation of calcium homeostasis via ryanodine channel, is an important factor contributing to iron-overload cardiomyopathy.

In affected patients, myocardial iron overload eventually leads to congestive heart failure, which is the leading cause of death in this population. According to diastolic dysfunction, which develops before systolic dysfunction and overt heart failure, restrictive cardiomyopathy usually occurs before dilated cardiomyopathy. Left-sided heart failure is more common than right-sided heart failure in clinical terms. Right ventricular dysfunction, on the other hand, appears to occur earlier in asymptomatic TM patients.(26)

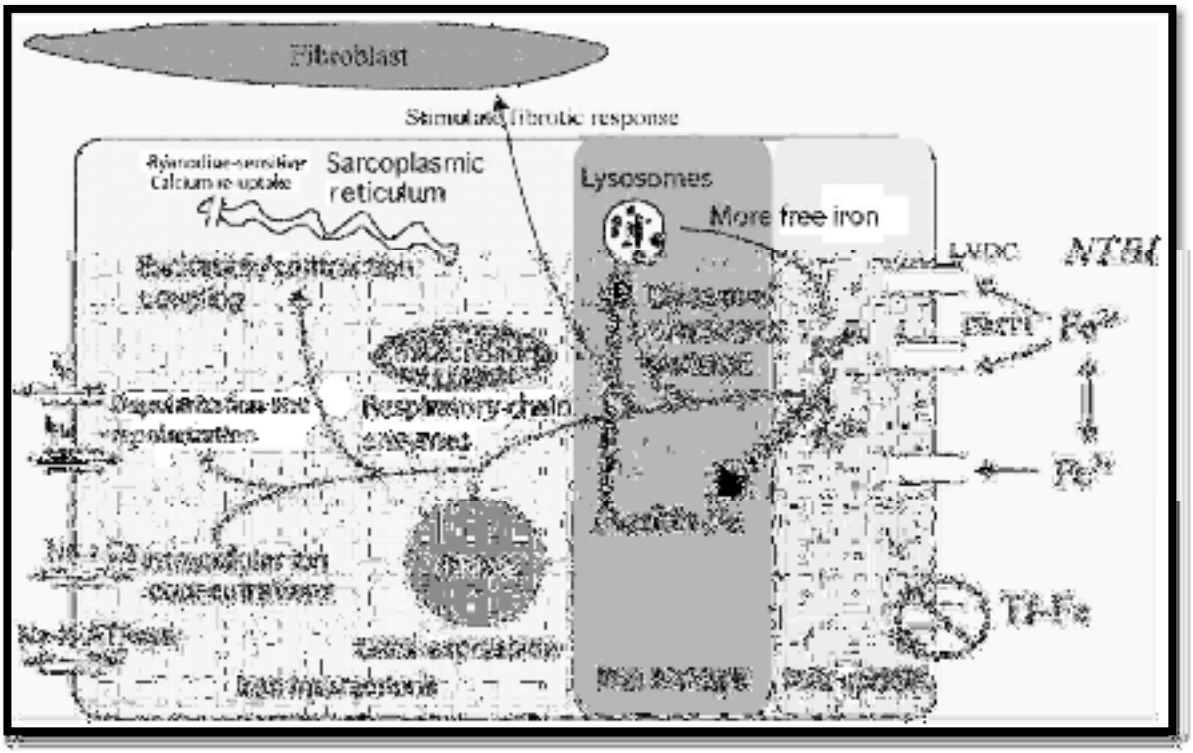


Figure 7: Etio-pathogenesis in Thalassemia Major.(5)

In a cohort of 251 individuals with thalassemia major, an increased percentage of allele e4 of apolipoprotein E, which is associated with a hereditary deficiency of antioxidant capabilities, was linked to the prevalence of left ventricular failure.

In patients with thalassemia, myocarditis appears to have a role in the development of cardiomyopathy. At a mean age of 15±3 years, 4.5 percent of patients with thalassemia major exhibited a clinical picture consistent with acute myocarditis, and histology confirmed the diagnosis in more than half of the cases. [33] Acute heart failure developed in 23.4 percent of patients, the majority of whom died within a year after diagnosis, whereas chronic heart failure developed in 27.6 percent of cases in a mean of 3 years; the remainder patients recovered completely.[34]

Elevated levels of autoantibodies due to viral infections in transfusion dependant thalassemia seems to be of particular importance as progression of left ventricular dysfunction secondary to myocardial remodeling results from persistence of viral genome with viral antigens exerting direct cytotoxic effects, extracellular matrix and cytoskeleton injury leading to chronic inflammation.

Multifactorial causation involved in the evolution of LVF (dilated type) in β-thalassemia include immune-mediated inflammatory along with inherited components. Also, possible intrusion of other aggravating factors like mal-adaptive and eccentric myocardial hypertrophy with excessive cytokine storm.[34]

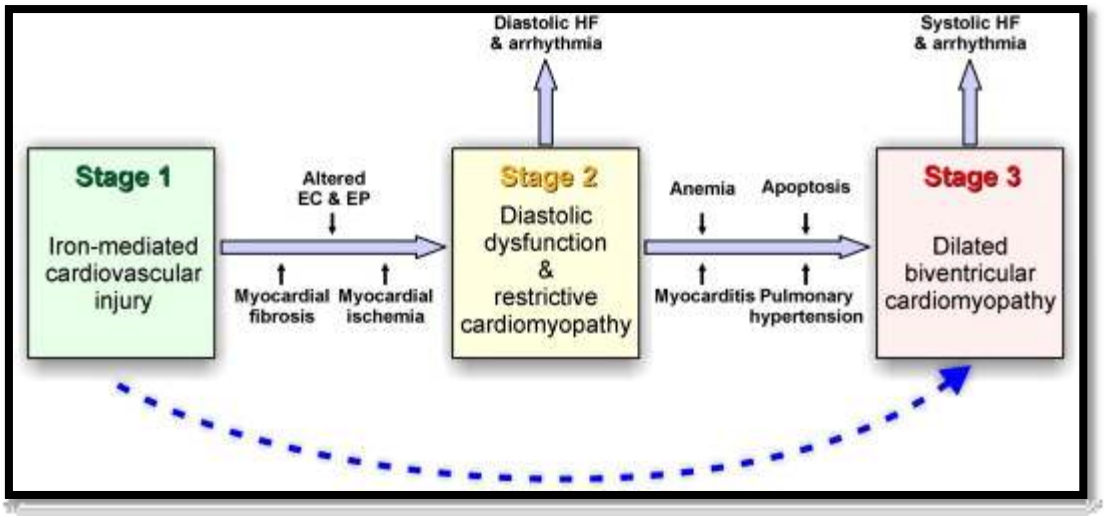


Figure 8: Development & Progression of Iron-overload Cardiomyopathy

CLINICAL FEATURES:

Signs & symptoms are related to the degree of ventricular involvement.

1. Early symptoms may include exertional dyspnea due to anaemia.
2. In later stages, patients develop heart failure symptoms like dyspnoea with severe exercise limitation, dependant edema (with both postural and diurnal variation), weight gain. Signs of left heart failure include pitting lower limb edema, hypotension, rales or crackles and decreased breath sounds on auscultation, S3 gallop.
3. Symptoms of right-sided congestive heart failure include exertional dyspnea with orthopnea, increased fatiguability, weight gain, abdominal distension, loss of apetite, lower limb (dependant site) edema. Clinical signs include neck vein distension (raised JVP), tender hepatomegaly with hepato-jugular reflux, B/L piiting lower limb edema, ascites. Indicates advanced disease with a poor prognosis. Acute decompensation, especially right heart failure mimics that of an acute abdomen usually mistaken for either for cholangitis/ biliary obstruction.[5]

4. Arryhthmias- especially Paraoxysmal atrial fibrillation presenting as palpitations, syncope.

DIAGNOSIS OF CARDIAC IRON OVERLOAD IN THALASSEMIA CHILDREN

A) **MARKERS:**S. Ferritin

-N- terminal pro-B-type Natriuretic Peptides (NT-ProBNPs). [34]

B) **ELECTROCARDIOGRAPHY (ECG)**

C) **IMAGING:**-Conventional & Doppler Echocardiography (includes TDI Tissue Doppler Imaging)

-Cardiac MRI-T2* sequence Imaging.

LV diastolic dysfunction mostly precedes systolic dysfunction in iron-overload cardiomyopathy, its early identification is of utmost importance.

T2*MRI has been considered as gold standard investigation as predictor of Cardiac Iron load and helps in diagnosis of early myocardial dysfunction in TDT-Beta-Thalassemia Major children. However, it is expensive, not available across many centers and interpretation always needs an expert. Hence, recent advances in ECHO like Tissue Doppler Imaging technique has found to be both sensitive and specific in prognosticating the presence of myocardial iron load in thalassemia patients.[35]

ECG

Non- specific findings are common, such as depolarisation changes in :-

- T-waves & ST segments of the anterior chest leads
- T wave axis and QT interval.

Abnormal findings include:-

a. P-waves showing bi-atrial enlargement.
b. First degree heart block, conduction disturbance in the form of bundle branch block may be seen.

Regular monitoring of non-specific findings of ECG should be started in childhood to detect new onset ECG abnormalities during follow-up such as increase in right heart forces that reflect the development of pulmonary artery hypertension.

- Ambulatory monitoring of ECG for cardiac arrhythmia is via Holter ECG recording for ≥ 24 hours.

The standard method for detecting and investigating cardiac

- Exercise ECG / Stress EKG is standard method for patients at risk for developing cardiac arrhythmias or for assessing functional capacity.[5]

ECHO

Echocardiography is hugely available, low-cost and simple. Basic easiest measurements of chamber dimensions can provide quick valuable data on cardiac status and clinical progress.

Data should include the following :

I. Chamber Dimensions o LV in diastole & systole. o Pulmonary artery and Aortic root. o Ventricular wall thickness. o LV and RV dimensions/ volumes. o Atrial dimensions & areas.	**II. Cardiac Function** o LV ejection fraction by standardized methods that should include: Teicholz and Simpson's methods. o Diastolic function. o Mitral Doppler. o Tissue Doppler annular velocities. o Pulmonary vein Doppler profiles
III. Doppler flow assessments o Tricuspid regurgitant jet velocity o Pulmonary artery flows, acceleration/ diastolic jet velocity	**IV. Morphology** o Structure and function of valves. o Exclusion of thrombus in right atrium in patients with implanted lines. o Chamber morphology. o Presence of shunts or foramen ovale.[5]

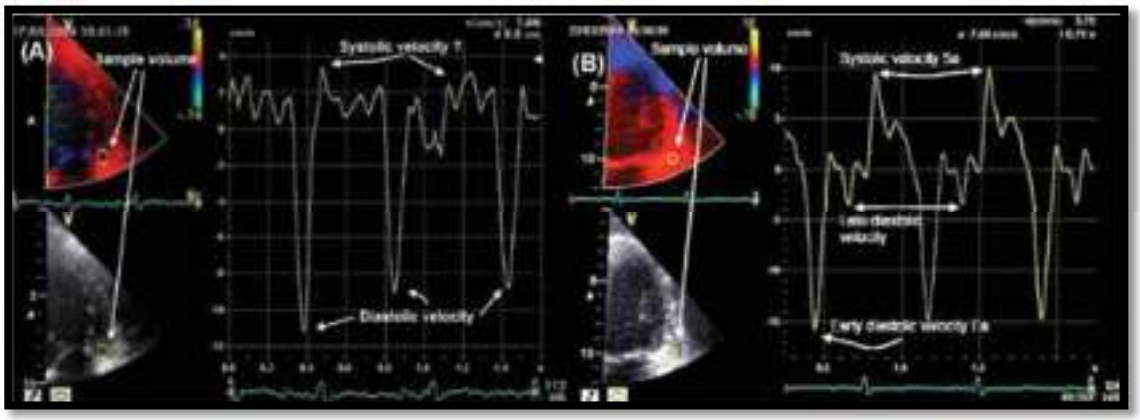


Figure 9: Sample of ECHO done on a thalassemia child.

TDI

Doppler echocardiography is a non-invasive imaging modality that provides information on distinctive hemodynamics.

"The Doppler principle states that the frequency of reflected ultrasound is altered by a moving target, such as red blood cells" [36]

Currently, Doppler echocardiography consists of 3 modalities:

1) Pulsed wave (PW) Doppler
2) Continuous wave (CW) Doppler
3) Colour Doppler Imaging.

Regional systolic & diastolic myocardial dysfunction is diagnosed earlier than global dysfunction in thalassemia patients by Tissue Doppler ECHO.

Some of the echocardiographic indices of diastolic and systolic function measured using TDE are as follows:

A) Mitral/ Tricuspid flow velocities across inflow, lateral and septal walls. [35]

a) E: Peak of early diastolic flow velocity
b) A: Late diastolic flow velocity
c) E/A ratio- ratio of 'E' wave to 'A' wave (E/A ratio >1 <2). [37, 38]

Mitral flow mean velocity of 1 m/sec (0.8-1.3 m/sec). [39]

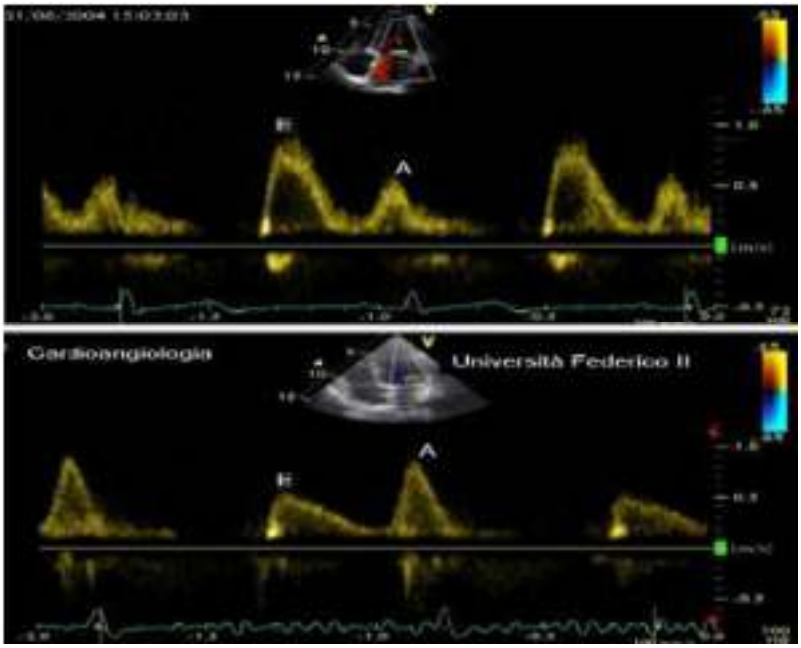


Figure 10: Measurement of doppler trans-mitral indices of diastolic function. E- early diastolic filling velocity, A- late diastolic filling velocity. [37]

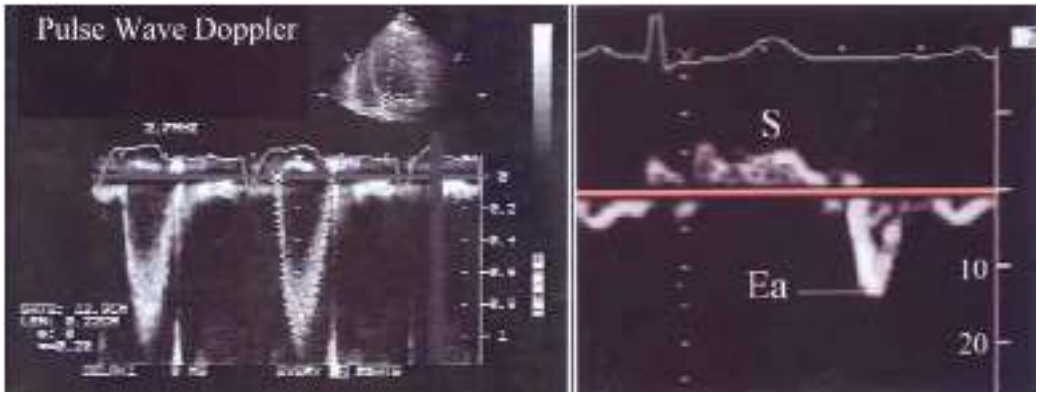


Figure 11: Myocardial velocity obtained with TDI- PW mode showing positive wave 'S', early diastolic wave 'E' and late diastolic wave 'A' of base of lateral wall. [36]

B) **LV systolic function** mainly with:-

a) LVEF (Left Ventricular Ejection Fraction): myocardial systolic function index (EF >55%). [37, 38]

b) FS: Fractional Shortening of LV (FS > 25%).

Severity Indices		Normal	Mild	Moderate	sever
Echo	E/A	1-1.5	<1	1-1.5	2<
	DT(ms)	160-240	240<	160-240	<160
	E'(cm/s)	10<	<10	<7	<7
	E/E'	≤8	<8	8-14	15<
LVEF		55≤	45-55	35-45	≤35

Table 4:- Normal levels of mitral flow velocity and LVEF indices by echocardiography. [40]

c) Mitral Annular Plane Systolic Excursion (MAPSE) by M-mode ECHO provides for relatively load-independent index of total LV long-axis/ longitudinal systolic performance.[41]

LVEDV- LV end diastolic volume &

LVESV- LV end systolic volume

LVIDd- LV internal diameter end diastole

LVIDs- LV internal diameter end systole (help to determine MAPSE)

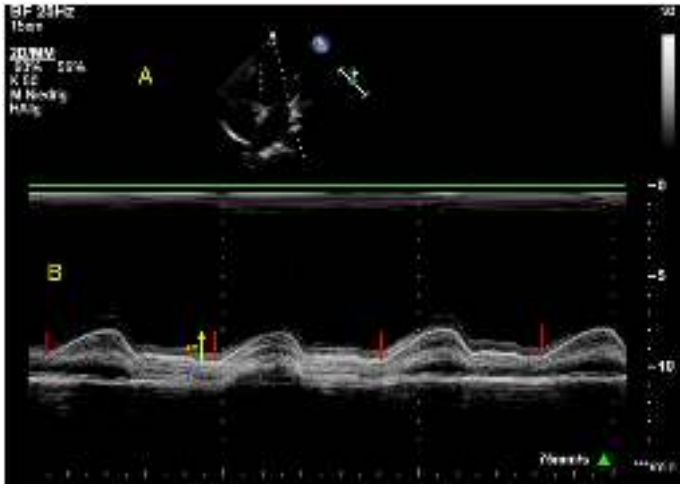


Figure 12: Apical 4-chamber view with M-mode image of the lateral MAPSE. The absolute longitudinal displacement is measured in cms shown as the red line. The yellow arrow marks the upper and lower measure points. [41]

C) **Tricuspid Annular Plane Systolic Excursion (TAPSE):** used as a non-invasive measurement to study RV systolic function in children**.** Decline in TAPSE has been shown to be associated with poor prognoses with PAH and HF. [42]

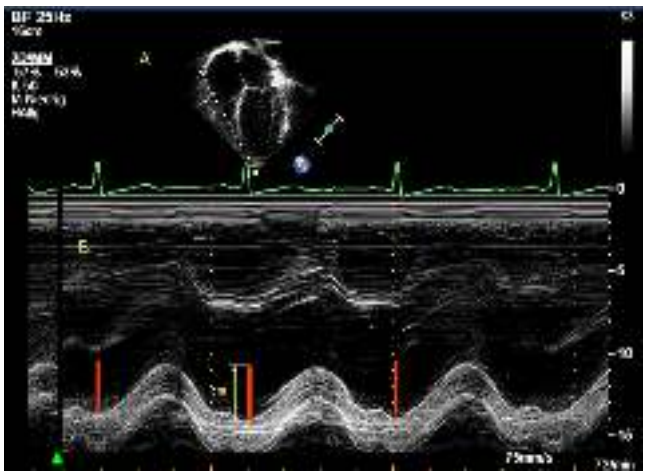


Figure 13: Apical 4-chamber view with M-mode image TAPSE. The absolute longitudinal displacement is measured in cms shown as the red line. The yellow arrow marks the upper and lower measure points. [42]

T2*MRI

Cardiac T2* MRI represents the gold standard investigation as it allows estimation of cardiac iron overload and in detecting preclinical changes in EF early. [30]

T2* scan provides a very precise estimation of cardiac & hepatic iron overload. Hence, has become a useful tool for the evaluation of children with thalassemia major and for the therapeutic guidance and follow-up of iron chelation therapy.

T2* MRI reference values:-

Normal: >25 ms
Mild to moderate iron load: 8 - 20 ms
Severe iron load: <8 ms).

Kremastinos DT, conducted a multi-center study that evaluated 652 patients with thalassemia major to prognostic value of myocardial T2*, hepatic T2* and SF. The cardiac T2* MRI values accurately assessed the development of HF, arrhythmias within a year and was superior to hepatic T2* and serum ferritin levels.[33]

Studies recommend follow-up evaluation at 24, 12, and 6 month intervals for low, standard, and high risk patients.[5] However it is expensive, not widely available in centres across our country, needs an expert for reporting.

INDICATION OF ECHO-TDI IN CHILDREN

For early diagnosis of cardiac dysfunction in TDT patients despite good global ventricular function. [35]

TREATMENT

Therapeutic strategy aims to reduce the risk of heart complications & to mostly maintain a pretransfusion Hb of at least 10 g/dl.

The main goal must be to ensure regular iron chelation therapy as well as to screen & even detect subclinical cardiac dysfunction [43] through T2* MRI or even ECHO-TDI as it may have great success since cardiac iron deposition clearance is an especially slow process which usually requires 3 or more years.

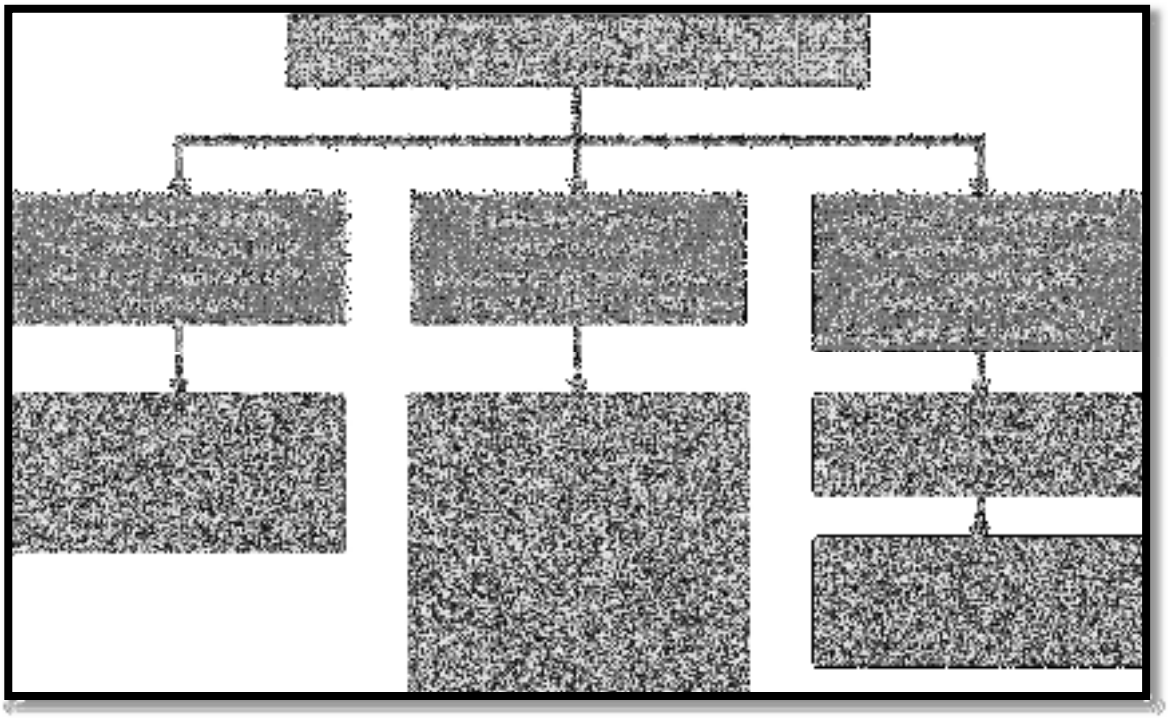


Figure 14: Treatment plan for thalassemia major children with or at high risk of developing Iron overload Cardiomyopathy.[5]

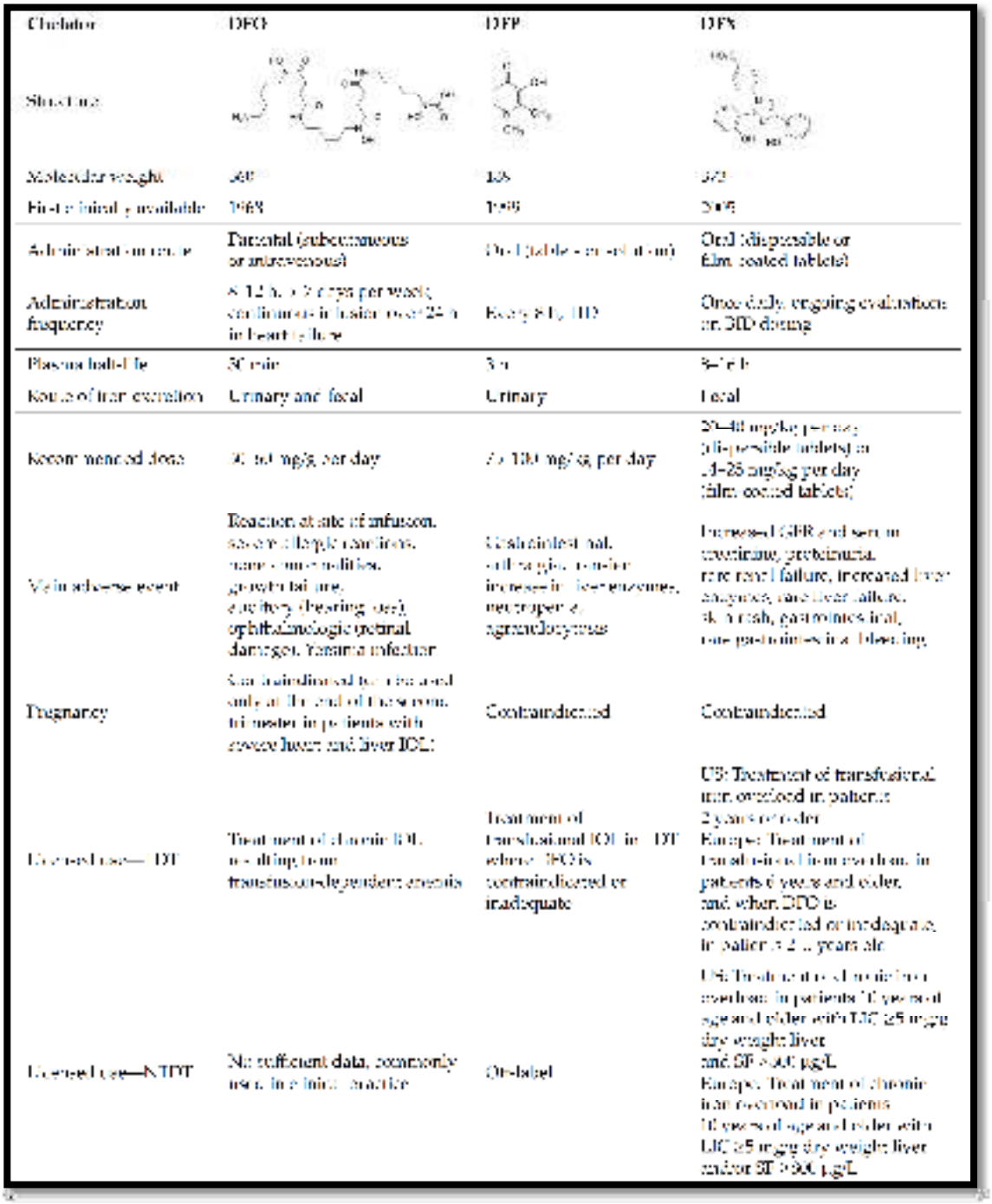
IRON CHELATORS

Chelating agents are a cornerstone in the treatment of thalassemia major who are on regular blood transfusions. Early initiation of chelating agents will avoid complications secondary to iron deposition in various tissues.

Principles of iron chelation therapy:

1) Prevention Therapy:- by controlling body iron to safe levels at all times.
2) Rescue Therapy:- where iron overload has already set in, before toxic levels are reached.
3) Emergency Therapy:- in presence of heart failure requiring urgent modification and/or intensification of treatment.
4) Dose adjustment of Therapy:- to monitor for under-chelation or over-chelation regularly.
5) Adherence to Therapy:- Especially difficult with DFO infusions to provide continuous protection from labile iron. The goal is to encourage adherence to chelation therapy and provide psycho-social support to the children and their family.[5]

Figure 15: Characteristics of iron chelators in thalassemia.[44]



Chelator	DFO	DFP	DFX
Structure			
Molecular weight	560	139	372
First clinically available	1968	1998	2005
Administration route	Parental (subcutaneous or intravenous)	Oral (dispersible or film coated tablets)	Oral (dispersible or film coated tablets)
Administration frequency	8–12 h, 3–7 days per week; continuous infusion over 24 h in heavy iloads	Every 8 h, TID	Once daily; ongoing evaluation on DFO dosing
Plasma half-life	20 min	3 h	8–16 h
Route of iron excretion	Urinary and fecal	Urinary	Fecal
Recommended dose	20–60 mg/kg per day	75–100 mg/kg per day	20–40 mg/kg per day (dispersible tablets) or 14–28 mg/kg per day (film coated tablets)
Major adverse event	Reaction at site of infusion, severe allergic reactions, auditory abnormalities, growth failure, auditory (hearing loss), ophthalmologic (retinal damage), Yersinia infection	Gastrointestinal, cytopenias, increase in liver enzymes, neutropenia, agranulocytosis	Increased GFR and serum creatinine, proteinuria, rare renal failure, increased liver enzymes, rare liver failure, skin rash, gastrointestinal, rare psychoses in children
Pregnancy	Can be administered to the second and third trimester in patients with severe heart and liver IOL	Contraindicated	Contraindicated
Licensed Use—EU	Treatment of chronic IOL in conditions with transfusion-dependent anemia	Treatment of transfusional IOL in thal when DFO is contraindicated or inadequate	US: Treatment of transfusional iron overload in patients 2 years and older. Europe: Treatment of transfusional iron overload in patients 6 years and older, and when DFO is contraindicated or inadequate in patients 2–5 years old
Licensed Use—NTDT	No sufficient data, commonly used in clinical practice	Off-label	US: Treatment of chronic iron overload in patients 10 years of age and older with LIC ≥5 mg/g dry weight liver and SF >300 µg/L. Europe: Treatment of chronic iron overload in patients 10 years of age and older with LIC ≥5 mg/g dry weight liver and/or SF >300 µg/L

- **DESFERRIOXAMINE (DFO)**

Desferrioxamine is the chelator of choice for iron overload in Thalassemia Major longtime. The drug effectively chelates both cardiac & hepatic iron and can reverse myocardial iron overload complications, especially when administered in higher doses as a 24-hour continuous infusion.

A major limitation of its use is that due to its very low oral bioavailability and also short half-life, it must be given either as intravenous (IV) or subcutaneous (SC) continuous infusion, typically over 8-24 hours for 5-7 days/week. This is both painful and time-consuming for patients, which often impedes adherence.[12]

Combination therapy with DFO at 40-50 m/k/day dosing with DFP at 75-100 m/k/day dosing is the best choice to remove myocardial iron and stabilize ventricular functions.[5]

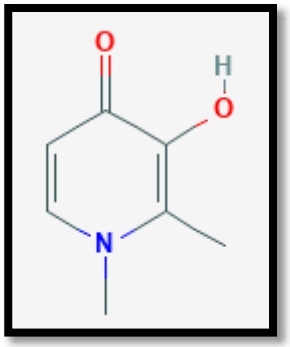
ORAL IRON CHELATORS

- **DEFERIPRONE (DFP)**

The established efficacy regarding cardiac iron clearance makes deferiprone an attractive chelation choice especially in the presence of cardiac iron loading or overt iron-related cardiac disease. Its most concerning adverse effect is a risk of agranulocytosis necessitating frequent blood count monitoring. Studies have generally shown good rates of adherence with DFP, with an altogether compliance ranging from 79 – 98%.[12]

STRUCTURE AND PROPERTIES

DFP (1,2-dimethyl-3-hydroxypyrid-4-one), designed by modifying siderophore chemistry, is a bi-dentate chelator, requiring three molecules of the drug to bind each ferric ion.



MOA

Deferiprone is absorbed rapidly, reaching a peak blood level by 45 minutes after consumption. The drug is removed from blood with 85% conversion to a glucuronide, which is incapable of binding iron. The amount of iron chelated is related to the area under the curve of the free drug, which depends mainly on the speed of glucuronidation. The deferiprone–ron complex is excreted in the urine. [45]

PHARMACOKINETIC PROPERTIES

Oral absorption of deferiprone is rapid with a distribution of 1.6 L/kg in patients with thalassemia. The complex is primarily excreted in the urine as a metabolite, with very little faecal elimination. It is primarily metabolized by UGT 1A6 to 3-O-glucuronide, which lacks iron binding capacity. Its plasma half-life is short (1.5 – 2.5 hours), necessitating three times daily dosing. Deferiprone rapidly scavenges NTBI.[12, 45]

THERAPEUTIC USES

Used for chelation in chronic iron overload conditions like Transfusion Dependent Thalassemia Major, Hemolytic anemias, Aplastic anemia, Myelodysplastic syndromes, Acute iron poisoning, Siderosis causing hepatic cirrhosis.

1. EFFECTS ON MYOCARDIAL IRON:-

The effectiveness of deferiprone with regard to cardiac iron chelation is of particular interest, as cardiac disease from iron overload has the highest frequency for cause of death in TDT patients. Deferiprone is a small lipophilic molecule that is capable to enter myocardial cells, facilitating cardiac iron removal. An extensive body of data support that the use of deferiprone is associated with lower cardiac iron stores compared with other chelators. [5, 11, 12, 45]

Berdoukas V in 2009 conducted large observational study, where a major improvement in cardiac T2* over time was seen among those treated with deferiprone, including those with significant myocardial iron over-loading (T2* 8 to < 20 ms), while no significant change was seen with deferoxamine. [46]

The use of DFP also has been associated with a reduction in iron-related cardiac morbidity and mortality. In cross-sectional analyses done by Anderson LJ, LVEF was drastically higher among cases receiving deferiprone compared with those receiving deferoxamine.[47] Another study showed that treatment with deferiprone was associated with greater improvement in right ventricular function compared with deferoxamine.[48]

2. EFFECTS ON FERRITIN:-

A prospective cohort study done in 2013 demonstrated reduction of SF levels with DFP monotherapy administered at a dose of 75mg/kg/day in 3 divided doses. At higher baseline SF i.e. > 2,500 µg/L, this dose had greater efficacy.

A study conducted in Thailand by Viprakasit V in 2013 which included thalassemia children >2 years of age as 45% study population, showed great decline in SF levels after 1 year of DFP therapy at 79mg/kg/day. Here efficacy was determined based on baseline SF values. Those with SF >3,500 µg/L had a very significant reduction in SF values at 1 year. [49]

EFFECTS ON LIVER IRON:-

Fischer R in 2003, conducted a non-randomised prospective trial with DFP where 28% increase in LIC with DFP at two years and by 68% at the end of three years. [50]

A study by Viprakasit V in 2013 involving children with thalassemia, decrease in LIC was observed in those who showed a clinical response by decline in SF levels and especially in those with a higher baseline LIC. [49]

DOSAGE

Recommended daily dose is 75 to 100mg/kg/day in 3 divided doses. The drug is available as 250mg and 500 mg capsules and an oral solution (100 mg/mL) was approved for use in late 2015. Both forms have been shown to have similar efficacy.[12] Starting with a lower dose and then increasing it has been found to reduce the risk of gastrointestinal side effects.

ADVERSE DRUG EVENTS

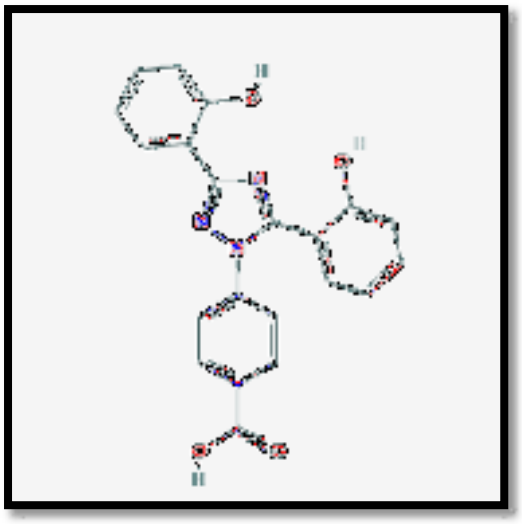
1. GI symptoms like nausea, vomiting, and abdominal pain,
2. Neutropenia (Absolute Neutrophil Count (ANC), <1.5×109/liter)
3. Agranulocytosis (ANC, <0.5×109/liter)
4. Rise in aminotransferases (AST, ALT)
5. Arthropathy
6. Zinc deficiency
7. Rare adverse effects-
8. Neurological- stroke, cognition dysfunction, nystagmus, ataxia, dystonia
9. Ophthal- central scotoma (rare)

- **DEFERASIROX (DFX)**

DFX was developed as a once-daily dosing, oral monotherapy for the treatment of iron overload in TDT. It was licensed as first-line monotherapy for patients with thalassaemia major worldwide. A reduction in both hepatic and cardiac iron, relatively equivalent to that seen with deferoxamine, has been demonstrated in the majority of patients when treated with appropriate doses of DFX. Adverse effects include hepatic transaminitis, decreased renal function, proteinuria, and rash. Adherence is generally better with the DFX dispersible tablet. [5, 12]

STRUCTURE AND PROPERTIES

Deferasirox is a tri-dentate oral chelator, requiring two molecules of the drug to bind each ferric ion.



MOA

Easy access of DFX into cardiomyocytes is due to its inherent property of being lipid soluble. It is metabolized into acyly-glucuronide compound while retaining its ability to bind iron and is excreted mostly in faeces.[5]

PHARMACOKINETIC PROPERTIES

The tablet is dispersed in water or apple juice using a stirrer of non-metallic nature like plastic/glass. It is ingested as a drink, once a day, ideally before a meal. Tmax occurs in 1 to 4 hours with 70% bioavailability. DFX is ~99% bound to albumin and is metabolized into acyly-glucuronide compound by glucuronidation, followed by biliary excretion. 84% of the parent drug and its metabolites are excreted in faeces and urinary excretion is as low as ~8%. The plasma half-life of unbound DFX is 8-19 hours, and that of DFX–iron complex is 7-18 hours, thus providing 24hr protection from plasma labile iron. ≈ 90% of DFX exists in the free form and 10% as iron bound complex. [5, 52]

THERAPEUTIC USES

Indicated as treatment of choice in TDT due to chronic iron over-load from repeated blood transfusions and in Non-transfusion Dependant Thalassemia when LIC is 5mg Fe/g dry weight and SF >300 µ/l.

1. EFFECTS ON MYOCARDIAL IRON:-

In a study conducted by Darr S., in 2009, in a group of patients with high risk for HF receiving DFX showed stability of LV ejection fraction and also absence of clinical signs of HF rendering its effectiveness in prophylaxis for HF, especially in patients with T2* values of 5-10 ms due to its prolonged plasma half life exerting actions over 24 hours against labile plasma iron.[52]

A prospective study established the efficacy of DFX in improving cardiac T2* MRI from 5-20 ms, while 41% patients at baseline, had severe myocardial iron loading <10 ms.[5, 53]

2. EFFECTS ON SERUM FERRITIN:-

There exists a dose-dependent effect on SF.[5] Cappellini M. D. et al conducted a prospective RCT study where the effects were compared between 296 TM patients on DFX vs 290 TM patients on DFO. It was discovered that with increase in DFX dose from 20mg/kg/day to 30 mg/kg/day, SF levels declined with average fall of 1,249 µg/L over one year, decreasing progressively over time. [5, 54]

3. EFFECTS ON LIVER IRON:-

Cappellini M.D., in the RCT study, assessed iron balance with DFX by serial Liver Iron Concentration at DFX dose of 20mg/kg/day with mean LIC stable over a

year. Negative iron balance is seen with 30mg/kg/day dose with a decline of 8.9 mg/g dry weight in mean LIC over a year. Hence, negative iron balance is increased over a year as DFX dose is escalated.[5, 54]

DOSAGE

- An initial dose of 20 mg/kg is recommended for TM children who have received at least 10-20 transfusions and are presently receiving standard blood transfusion at the rate of 0.3-0.5 mg of iron/kg/day.
- With transfusion rate >0.5 mg/kg/day or in children clinically requiring decrease in iron loading, dose 30 mg/kg/day is recommended.
- With transfusion rate <0.3 mg/kg/day, 10-15 mg/kg dose may be sufficient.

DFX in children can be started by two years of age.[5]

ADVERSE EFFECTS

1. GI symptoms:- nausea, vomiting, abdominal pain, diarrhoea.
2. Increased creatinine
3. Increased hepatic enzymes
4. Proteinuria
5. Pyrexia
6. Headache
7. Cough
8. Rash (with urticaria)
9. Ear infection

COMBINATION ORAL CHELATION THERAPY WITH DEFERIPRONE AND DEFERASIROX

Chelation intensity can be increased in patients who do not respond well to a single medication by increasing the length of exposure to the chelator, increasing the dose to the highest tolerated level, or adding a second chelator. Continuous chelation is the result of combined chelation.[9]

Because the LMW orally absorbed DFP and DFX rapidly access intracellular iron in the cytosol and organelles, the idea to combine two oral iron chelators came from non-overlapping toxicity profiles and access to separate intracellular iron pools. Another advantage of the investigated combination (DFP and DFX) is that DFX has a long plasma half-life, levels are maintained within the therapeutic range for 12-18 hours, and LPI binding is achieved with once-daily treatment. DFP penetrates cells to chelate internal iron from lysosomes and mitochondria because it has the smallest molecular weight of the three chelators. As a result, the combination DFP/DFX regimen was found to be much more successful in cardiac patients.[9]

Several research had looked at the efficacy of combining DFP and DFO, but only a few had looked at the efficacy of combining DFX and DFP.

In 2015, 96 -TM patients with severe iron overload were enrolled in a prospective open-label RCT to compare the safety, effectiveness, compliance, treatment satisfaction, and quality of life (QoL) of two regimens: deferiprone (DFP) and deferoxamine (DFO) against DFP and deferasirox (DFX). Patients were given either DFP with DFO (arm 1) or DFP with DFX (arm 2). The difference between two groups in serum ferritin (SF), liver iron content (LIC), cardiac MRI, and quality of life were used as efficacy objectives (QoL). At 12 months, SF and LIC in both arms were

significantly lower, and mean cardiac T2* was higher than baseline. In conclusion, the DFP/DFX combination improved cardiac T2*, treatment compliance, and patient satisfaction while causing no additional side effects. (55)

Research including 16 patients who were given a combination of DFX and DFP revealed promising outcomes, with all patients experiencing a significant rise in their mean LVEF and three of them experiencing a reversal of cardiac problems. They were tracked for a period of two years. The examination of efficacy measures revealed a statistically significant reduction in total body iron load, as measured by serum ferritin, LIC, and MRI T2* indices. When compared to the toxicity of monotherapy with each medicine, the incidence of adverse events was low. In 2/4 of the patients, cardiac dysfunction was reversed, and the mean LVEF rose considerably. They came to the conclusion that combined oral chelation in thalassemia has the potential to be simple and effective. (56)

STUDIES

Bornaun et al., conducted a prospective study between Jan 2015 till September 2015 where 84 children with Thalassemia Major were assessed by conventional ECHO and PW-TDI. Group 1 data was compared with data of Group 2 comprising 85 age & sex-matched healthy controls. 49/85 patients underwent Cardiac T2* MR imaging. Group 1 (TM patients) had significantly lower values for E'/A' ratio and ejection time (ET) and significantly higher values of early diastolic velocity-E over late diastolic velocity (A), IVRT and RV magnetic perfusion imaging (MPI) than Group 2 concluding that RV diastolic dysfunction occurs earlier than systolic dysfunction in TM children, concluding that RV dysfunction occurs early and hence TDI measurements can be used to screen the same. (57)

Ibrahim M, Azab A, et al. conducted a multicentre case-control study between January 2014-2015 on 100 BTM patients below 18 years old to assess early detection of myocardial dysfunction by Tissue Doppler Imaging (TDI) before overt HF or cardiomyopathy develops. Statistically significant difference between patients and controls regarding "Aa" and "S" of the septal wall of basal mitral annulus, "Ea" of the lateral wall of the mitral annulus & 'S' of the basal tricuspid annulus. Hence, TDI is superior to conventional ECHO. [7]

Gomber Sunil, Jain Prachi et al., in 2016 conducted a prospective comparative study on the efficacy and safety of oral iron chelators (Deferiprone and Deferasirox) in 49 TDT children who received Deferiprone or Deferasirox monotherapy or combination of same doses for 12 months. SF values decreased from a mean of 3140.5 ng/mL to 2910.0 ng/mL in deferiprone monotherapy group, 3859.2 ng/mL to 3417.4 ng/mL in deferasirox alone group and from 3696.5 ng/mL to 2572.1 ng/ mL in the combination group thus proving that the combination oral iron chelator therapy was more efficacious in causing fall in serum ferritin levels compared to monotherapy (P=0.035 and 0.040, respectively). No significant adverse drug reactions were noticed in either groups, establishing its efficacy.[8]

Dabirian M, Jalalian R, et al. conducted a prospective cross-sectional study where 50 TM patients aged ≥ 15 years underwent T2* MRI and TDI results were obtained. Efficacy of tissue Doppler echocardiography was obtained. P value < 0.05 was considered statistically significant. Patients divided into groups; normal amount of iron in the heart tissue, mild, moderate and severe. Good similarity between results of T2* MRI and tissue Doppler echocardiography.[40]

TREATMENT OF CARDIAC FAILURE IN THALASSEMIA

The best option for clearing cardiac iron and stabilising ventricular function is to use a combination of deferiprone 75-100 mg/kg and deferoxamine 40-50 mg/kg/day. [58]

The following is a summary of the recommendations [13] :-

1. As long as the patient has acceptable urine production, continued deferoxamine medication at 50 mg/kg/day should be provided. As soon as the patient is able to tolerate oral drugs, deferiprone at 75 mg/kg/d, divided TID, should be added.

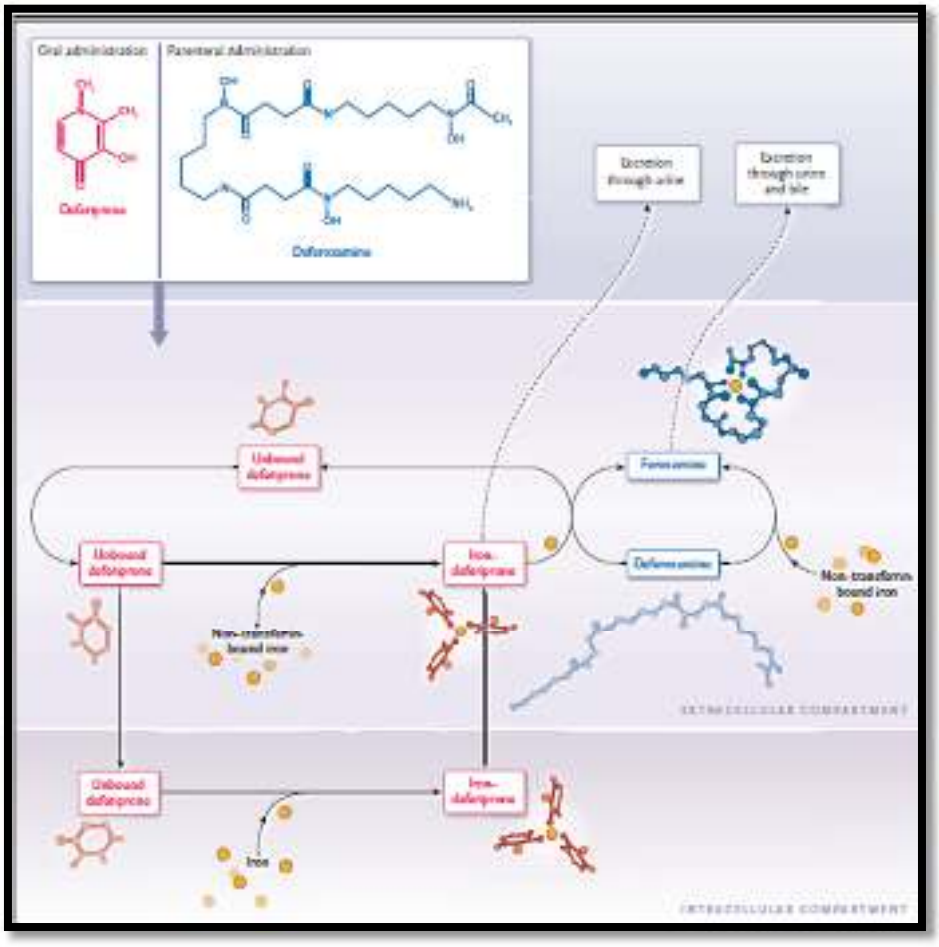


Figure 16: Shuttle therapy with DFO-DFP combination therapy. [45]

2. Because iron-mediated oxidative stress is exacerbated by pressor medicines, they should be used with caution. People with thalassemia usually have lower diastolic and mean blood pressures than other patients.
3. While gentle diuresis might help relieve congestive symptoms, excessive diuresis can lead to acute renal failure. Patients with thalassemia who are in heart failure frequently have restrictive physiology and rigid vasculature, making them vulnerable to hypovolemia.
4. Patients with liver injury from iron overload, hepatitis C, or passive congestion may have decreased synthetic function and low oncotic pressures, making furosemide drips easier to titrate than bolus diuretics in the acute setting. Albumin replacement is beneficial.
5. Controlling arrhythmias can be tricky. Because of its broad spectrum of action and low influence on cardiac function, amiodarone treatment is the medication of choice in the acute context.
6. Because both deferoxamine and deferiprone are mostly removed by the kidney, maintaining urine output is essential. If kidney function fails despite the best medical care, dialysis should be started right away.
7. Biochemical indicators of heart failure (BNP or pro-N-terminal BNP) can be helpful. In decompensated heart failure, values are high and decline in response to treatment. According to the evidence, delaying hospital release in decompensated heart failure until BNP is re-evaluated is a good idea.
8. Transplantation of the heart is still considered a last option. If organ function can be maintained, iron cardiomyopathy is generally fully reversible.

Myocardial dysfunction is best treated with a combination of medications (see Table 5), including angiotensin-converting enzyme inhibitors (ACE inhibitors). These drugs, together with beta-blockers and aldosterone antagonists, have been demonstrated in controlled trials to reduce mortality in individuals with cardiomyopathy and the onset of heart failure in those with asymptomatic left-ventricular dysfunction.

CLASS	AGENT	DOSING
ACE inhibitor	Ramipril	1.25 - 10 mg once daily
	Captopril	6.25 - 50 mg three times daily
	Enalapril	2.5 - 20 mg twice daily
	Lisinopril	2.5 - 40 mg once daily
	Perindopril	2 - 16 mg once daily
ARB	Losartan	25 - 150 mg once daily
	Valsartan	20 - 160 mg twice daily
	Candesartan	4 - 32 mg once daily
Beta-adrenoceptor blocker	Bisoprolol	1.25 - 10 mg once daily
	Carvedilol	3.125 - 50 mg twice daily
Aldosterone antagonist	Spironolactone	12.5 - 50 mg twice daily
	Eplerenone	25 - 50 mg once daily

Table 5: Drugs and dose regimens commonly used to treat cardiac dysfunction, including heart failure in thalassaemia patients. [5]

DIAGNOSIS AND MANAGEMENT OF PULMONARY HYPERTENSION

TR and pulmonary insufficiency jets offer systolic and diastolic pressure values for the pulmonary artery, respectively. TR velocity less than 2.5 m/s indicates a negative screening test, 2.5 – 3.0 m/s indicates a borderline result, and TR velocity greater than 3 m/s indicates a positive result. TR velocities that are borderline or abnormal should prompt a reassessment of transfusion methods to see if inefficient erythropoiesis is sufficiently controlled.

- In the case of obstructive sleep apnea, continuous positive airway pressure should be administered.
- In the case of nocturnal desaturation without airway obstruction, a nasal cannula may be sufficient.
- In patients with severe pulmonary hypertension, chronic anticoagulation is the treatment of choice for thromboembolic illness and should be regarded as prophylactic against thrombosis in situ.
- Shortening transfusion intervals often reduces early pulmonary hypertension in thalassaemia major patients by decreasing proinflammatory cytokines like PLGF.
- Hydroxyurea has been shown to be beneficial in non-transfused thalassaemia syndromes and is helpful in select patient populations.
- Sildenafil has been shown to be useful in limited studies in patients who have failed to respond to more conservative treatments and is generally well tolerated.

CARDIOVASCULAR COMPLICATIONS IN THALASSEMIA

1. Complications of iron excess

a. Myocyte failure that is reversible.
b. Arrhythmia, including heart block
c. Arterial alterations, such as a reduction in vascular compliance.

2. Complications of non-iron overload

a. Pulmonary hypertension
b. Arrhythmia, especially Atrial Fibrillation (AF) in later life.

c. AF-related thrombotic stroke.

d. Changes in cardiac function as a result of limitation, diastolic dysfunction, or fibrosis

e. Loss of vascular compliance due to arterial changes. [5]

MATERIALS AND METHODS

This study was conducted at the Paediatrics Department of KLES Dr. Prabhakhar Kore Hospital & Medical Research Center in Belagavi from July 2020 to May 2021.

Study design: The study design was a Hospital based Single Blind Randomized Controlled Trial

Study duration and period: This study was carried out from July 2020 to May 2021.

Place: The research was carried out in the Department of Paediatrics at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, a tertiary care teaching hospital affiliated with J.N Medical College, Belagavi.

Source of data: Registered cases of transfusion-dependent beta-thalassemia in the Department of Pediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi

Sample size: A total of 40 registered cases of transfusion-dependent beta-thalassemia with abnormal conventional ECHO findings were studied.

Sampling procedure: The minimum sample size formula based on prevalence is

n = sample size

z_α is associated with the level of significance

z_β is associated with the power of the test.

For a 5% level of the significance z_α =1.96 and for an 80% power of the test, z_β =0.84.

Ref: (8).

$\bar{X}_1$ is the mean 'S' (Systolic Myocardial Velocity) of the intervention group (14.8)

$\bar{X}_2$ is the mean of the control group (12.6).

The standard deviation of "S," the intervention group, is denoted by s1 (2.63)

s2 is the control group's standard deviation (1.30).

The sample size obtained with these values is 14 per group.

To make the study more confirmative, the sample size will be increased to 20 per group. Hence, all 40 children fulfilling the eligibility criteria for the study will be enrolled.

SELECTION CRITERIA

Inclusion Criteria: Transfusion dependent Beta-Thalassemia Major Patients between 10-18 years of age, on single oral iron chelator (Deferasirox) with abnormal 2D-ECHO findings.

Exclusion criteria:

1. Patients on more than 1 oral iron chelator
2. Congenital heart disease
3. Other haemoglobinopathies like sickle cell disease
4. Rheumatic heart disease
5. Chronic infections like TB, HIV, HEP-C, HEP-B
6. Raised serum transaminase levels (>5 times the upper normal limit)
7. History of allergy to either drugs.
8. Splenectomized patients.

Ethical clearance: The study was initially approved by the J.N Medical College, Belagavi, Ethical and Research Committee. Clinical trial registration done (CTRI/2020/07/026771).

Informed Consent: Before enrolment in the study, the parents of children who met the inclusion criteria were briefed on the nature of the study, and written informed consent was obtained from the parents (Annexure I).

Method of collection of data: Children between the ages of 10 and 18 who are enrolled in the thalassemia day-care centre of KLE Dr Prabhakar Kore Charitable Hospital & Medical Research Centre, which is affiliated with KLE Academy of Higher Education and Research's J N Medical College, BELAGAVI, undergoing bi-annual conventional echocardiography and who meet the inclusion criteria were chosen. After a thorough history was taken, informed consent was obtained from the parents after explaining the purpose of the study. The data from the participants was entered into a structured proforma.

Out of 84 patients between the age group of 10-18 years, 40 patients (excluding 4 splenectomised patients and 1 death due to COVID-19 infection) with abnormal conventional 2D-ECHO findings were randomized by computer generated sequence into two groups of 20 patients each in intervention group and control group after undergoing baseline Tissue Doppler Imaging and ECHO evaluation performed by Pediatric Cardiologist who was blinded to the treatment allocation in the study, along with Liver and Spleen size assessment by palpatory method and Ultrasound and investigations like HIV, Surface antigen for hepatitis B virus (HBs-AG), Complete Blood Count, serum ferritin levels, liver function tests, renal function tests, USG.

Children who are with congenital heart diseases, rheumatic heart disease, chronic infections like HIV, TB, HEP-C, already on more than 1 oral iron chelator, with other haemoglobinopathies like sickle cell disease or those splenectomised were excluded.

Group 1 (Interventional group) received combination of oral iron chelators, Deferasirox at the dose 30mg/kg/day Q24hourly and Deferiprone at the dose 75mg/kg/day Q8hrly for 6 months and group 2 (control group) received only Deferasirox. Over the course of 6 months, patients were monitored using laboratory parameters i.e. CBC, twice every month to look for leucopenia/neutropenia, decreased pre- transfusion hemoglobin. Further, S. Ferritin, Liver function tests and Renal function tests were monitored every 3rd month for toxicity. USG assessment of liver and spleen size at baseline and 6th month.

ECHO and TDI were performed at 6 months in both the groups to re-assess cardiac functions. Reduction in S. Ferritin values along with Liver and Spleen sizes was also assessed in both groups.

Cap Deferiprone (brand name -Kelfer 500mg) was administered to the intervention group participants at 75mg/kg/day in 3 divided doses. Tab Deferasirox (brand name – Defrijet 500mg) was administered at 30mg/kg/day Q24H to both the groups according to the calculated dosing.

The parents and children were counselled to take deferiprone capsule after meals and were advised not to lie down for 1 hour to prevent oesophagitis. Side effects associated with deferiprone like gastritis, oesophageal burn, vomiting, GERD, abdominal pain, joint pains and fever were explained.

If the above symptoms were noted, the child was prescribed Tab Rantac (Ranitidine 150mg) to take orally, and drink plenty of water. The patients were asked to continue their multivitamins and folic acid. If fever is present, to review the child with blood counts to evaluate for Neutropenia, a serious complication of Deferiprone.

Antibiotics to be started early, dose of Deferiprone to be adjusted to prevent further worsening until complete clinical and laboratory recovery.

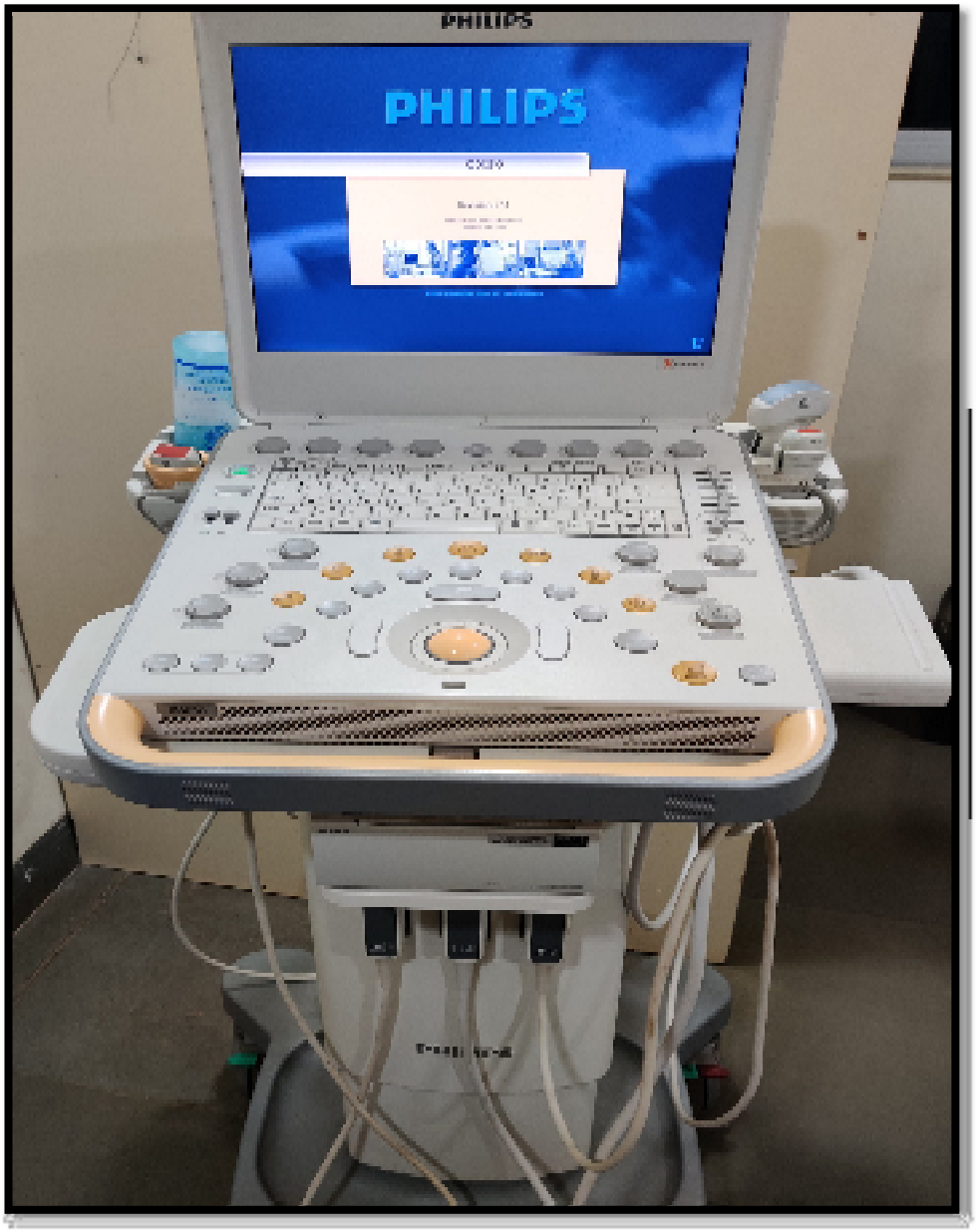
Children enrolled were receiving blood transfusion every 2-4 weekly as per their pre transfusion hemoglobin.

INVESTIGATIONS: Under all aseptic precautions, blood was drawn and the following investigations were done.

1. HIV, HCV (at baseline)
2. Surface antigen for hepatitis B virus (HBs-AG) (at baseline)
3. Complete blood count (every 2 weekly for 6 months)
4. Serum ferritin concentrations (at baseline, 3rd month and 6th month)
5. Renal function examinations (at baseline, 3rd month and 6th month)
6. Liver function tests (at baseline, 3rd month and 6th month)
7. USG Abdomen (at baseline and 6^{th} months)
8. ECHO and TDI (at baseline and 6^{th} month)

HIV, HCV, HBsAG was done at the time of enrolment, as it was a part of the exclusion criteria. Complete blood count estimation was done at the time of every admission to assess pre-transfusion Hemoglobin as a part of the guidelines and also to monitor for neutropenia/agranulocytosis known to be seen with deferiprone therapy. Renal function tests were done at the time of enrolment to look for any renal

dysfunction because deferasirox is known to cause renal toxicity. Liver function tests were done to monitor for deferiprone toxicity and serum ferritin levels to monitor efficacy of oral iron chelation. USG Abdomen was done at the time of enrolment and 6 months later to assess reduction in liver and spleen size post chelation therapy



Photograph 1: Echocardiogram - philips cx 50 revision 3.1.1 software

ECHO-TDI: Transthoracic echocardiography was performed on a PHILIPS CX 50 REVISION 3.1.1 SOFTWARE machine equipped with 3 and 8 MHZ transducers based on the patient's age and tissue velocity imaging capabilities. M-mode, 2D, and Doppler echocardiography were all forms of conventional echocardiography. The parameters were averaged over three cardiac cycles, and all measurements were carried out in accordance with the American Society of Echocardiography's guidelines.

Left Ventricle Ejection Fraction (LVEF) was calculated using Simpson's biplane method, LV end-diastolic volume (LVEDV) with end-systolic volume (LVESV) was calculated using 2D echocardiography from parasternal long axis view along with LV fractional shortening (LVFS). Trans-mitral LV filling velocities like peak of early diastolic flow velocities (E), late diastolic flow velocities (A), the ratio of E/A and systolic myocardial velocity (S) across Mitral valve Inflow, Lateral and Septal walls were measured using PW-Doppler echocardiography.

To determine total LV systolic longitudinal function, M-mode left ventricular (LV) longitudinal function was assessed using the mitral annular plane systolic excursion (MAPSE) across lateral and septal walls. [38]

TAPSE (Tricuspid Annular Plane Systolic Excursion) was measured to assess RV systolic function using M-Mode echocardiography in an apical 4-chamber view, with the cursor placed at the free wall of the tricuspid annulus. [39]

In our study, TDI-echocardiography was performed at the time of enrolment and again, after 6 months of treatment, in both intervention group and control group.

SAMPLE COLLECTION: Blood will be collected from the venous route while securing an IV line. One EDTA bulb containing 1 millilitre will be sent for CBC. Another plain bulb containing 4ml will be sent for urea, creatinine, serum ferritin, liver enzymes (SGOT, SGPT).

- Estimation of Hemoglobin was done by Cyanomethaemoglobin method on Mindray CAL 80 analyser, before the commencement of the study.

- Estimation of ferritin levels was done by electrochemiluminescence immunoassay (ECLIA) on Cobas analyser (COBAS E 601) before the commencement of the study.

- Urea – urease UV method
- Creatinine – enzymatic method(IFCC-IDMS)
- SGPT,SGOT – U.V without P5P method

Photograph 2: Beckmann coulter used for analysis of complete blood picture

Photograph 3: COBAS analyser used for estimation of serum ferritin

FOLLOW UP : The children were treated and followed up to 6months of treatment to evaluate the efficacy and compliance of deferiprone in intervention group. The child was examined per abdomen for liver and spleen size assessment, evaluated for laboratory parameters as mentioned earlier and lastly reassessed with ECHO-TDI at 6 months.

The study is focused on comparison of two groups. Data analysis was done using SPSS version 20.00. For the continuous quantitative variables mean and standard deviation will be calculated. The inter group continuous variables will be compared using suitable tools of statistics like unpaired student's t test. Two quantitative variables, within a group, will be compared using student's paired t test.

The categorical data will be expressed in terms of rates, ratios and percentages. The association between the outcome, clinical and demographic characteristics will be tested using Chi-square test or Fisher's exact test.

Pearson correlations were used to find correlations between values of ECHO, TDI and serum ferritin levels.

Discrete variables will be represented by median.

Nonparametric tests will be used for comparing discrete variables.

Suitable graphs will be used to depict the comparison.

For all the tests the value of p less than 5% (0.05) will be considered significant

OUTCOME VARIABLES

The children were monitored for the following parameters.

1. Improvement of cardiac function post 6 months combination iron chelation therapy as assessed by ECHO-TDI.
2. Effect of combination therapy on reduction of S. Ferritin levels.
3. Effect of combination therapy on reduction of Liver and Spleen size.

Table 6: Reference values of ECHO-TDI

Left Ventricle Ejection Fraction (LVEF)	>55% (37,38)
Fractional Shortening (FS)	>25%
Mitral Valve Flow Velocities	0.8-1.3 m/sec (39)
E/A ratio	>1 to <2 (37,38)
MAPSE (Mitral Annular Plane Systolic Excursion)	1.2 - 1.6 cm
TAPSE (Tricuspid Annular Plane Systolic Excursion)	>2cm

RESULTS

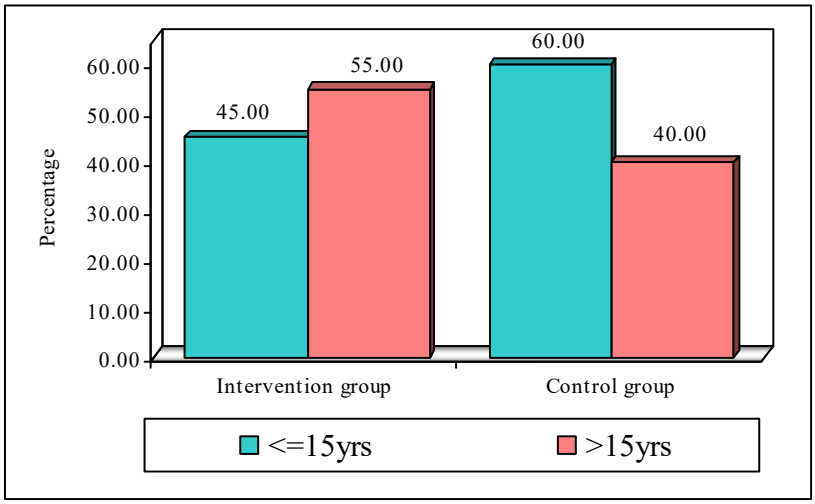
A hospital-based single-blind Randomized Controlled Trial was conducted From July 2020 to May 2021 in the Department of Paediatrics at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre in Belagavi. A total of 45 cases of transfusion-dependent beta-thalassemia children between the age group of 10-18 years with abnormal conventional 2D-ECHO findings were enrolled in the study, amongst which 4 splenectomised patients were excluded and 1 patient death due to COVID-19 infection. All thalassemia patients in our centre undergo bi-annual conventional echocardiography. Total of 40 patients underwent baseline ECHO-Tissue Doppler Imaging evaluation performed by Pediatric Cardiologist who was blinded to the treatment allocation in the study along with S. ferritin levels, USG assessment of liver and spleen size amongst other blood investigations and then were randomly assigned based on computer generated randomization sequence into intervention group with 20 patients who received Tab. Deferiprone 75mg/kg/day Q8hrly for 6 months and the other 20 children formed the control group. Both groups continued to receive Tab Deferasirox at 30mg/kg/day Q24hourly dosing for 6 months and were evaluated for ECHO-TDI cardiac functions, serum ferritin and liver & spleen size at 6 months.

The data was analysed using SPSS version 20.00 and the final results were tabulated and interpreted as below.

Table 7: Distribution of children according to Demographic data

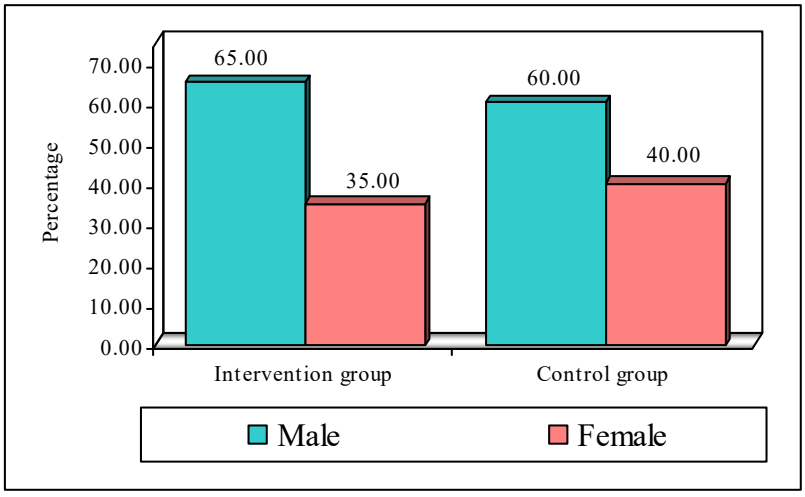
Profile	Intervention group	%	Control group	%	Total	%	χ^2	p-value
Age groups								
<=15yrs	9	45.00	12	60.00	21	52.50	0.9020	0.3420
>15yrs	11	55.00	8	40.00	19	47.50		
Mean	15.95		14.95		15.45			
SD	2.26		2.63		2.47			
Gender								
Male	13	65.00	12	60.00	25	62.50	0.1070	0.7440
Female	7	35.00	8	40.00	15	37.50		
SES								
Lower class	1	5.00	0	0.00	1	2.50	3.2820	0.5120
Lower middle	3	15.00	2	10.00	5	12.50		
Middle class	5	25.00	3	15.00	8	20.00		
Upper middle	5	25.00	4	20.00	9	22.50		
Upper class	6	30.00	11	55.00	17	42.50		
Mothers education								
Illiterate	5	25.00	5	25.00	10	25.00	2.1540	0.5410
High school	14	70.00	12	60.00	26	65.00		
PUC	0	0.00	2	10.00	2	5.00		
Degree	1	5.00	1	5.00	2	5.00		
Fathers education								
Illiterate	3	15.00	5	25.00	8	20.00	6.1000	0.1920
High school	11	55.00	4	20.00	15	37.50		
PUC	2	10.00	2	10.00	4	10.00		
Degree	4	20.00	8	40.00	12	30.00		
University	0	0.00	1	5.00	1	2.50		
Location								
Urban	6	30.00	8	40.00	14	35.00	0.4400	0.5070
Rural	14	70.00	12	60.00	26	65.00		
Total	20	100.00	20	100.00	40	100.00		

Graph 1: Comparison of intervention group and control group with age groups



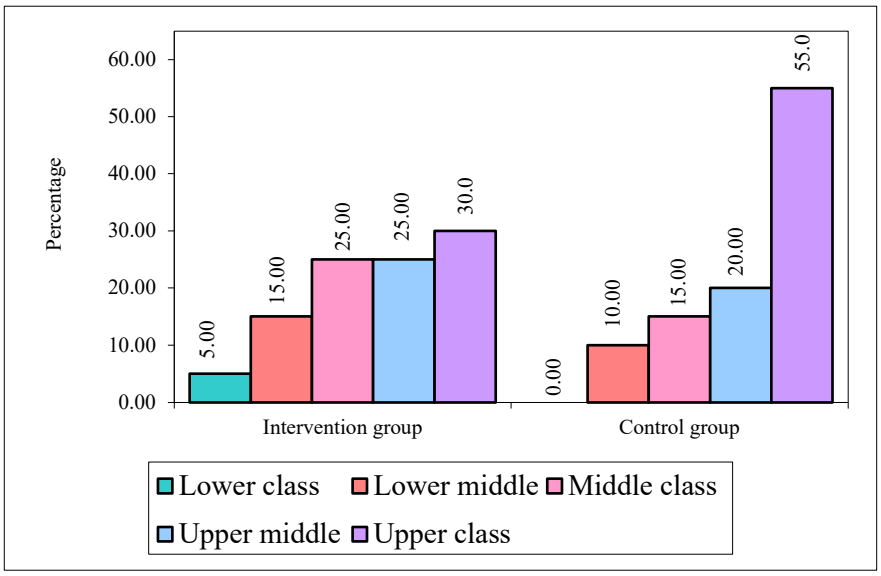
In this study, 45% children were aged less than 15years of age in the interventional group as compared to 60% in control group, 55% children aged more than 15 years of age in interventional group as compared to 40% in control group. The mean age of children in interventional group was 15.95 ± 2.26 as compared to 14.95 ± 2.63 in control group. No significant difference was seen in distribution of children according to age (p= 0.3420).

Graph 2: Comparison of intervention group and control group with gender



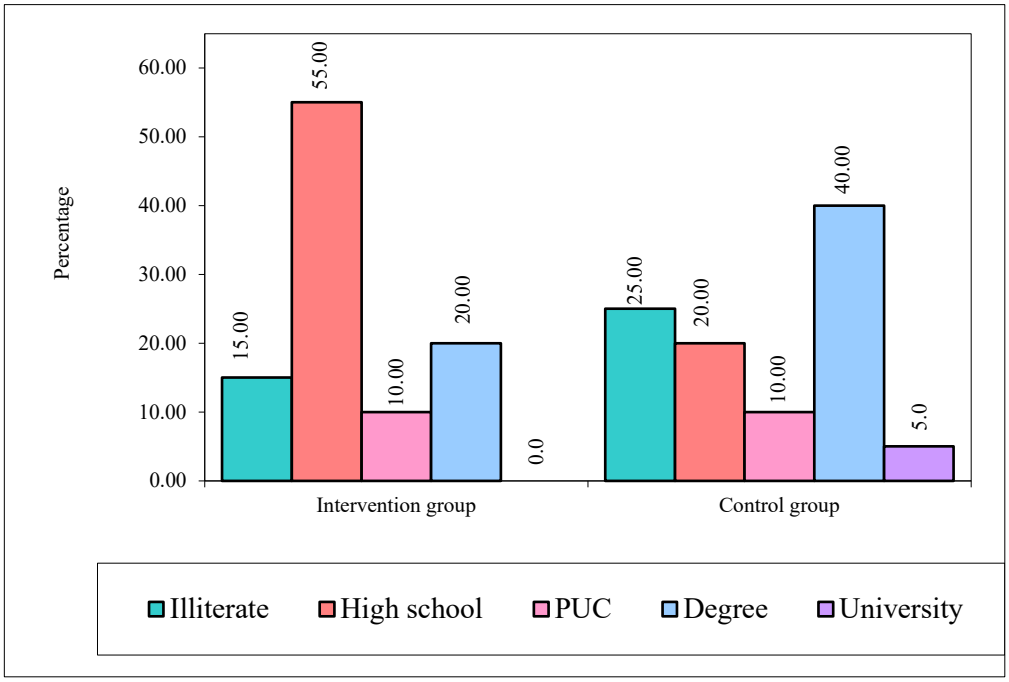
In the current study, majority of the children in both the intervention group (n=13; 65%) and control group (n=12; 60%) were male with a male to female ratio of 1.6. No significant difference was seen in distribution of children according to gender (p= 0.7440).

Graph 3: Comparison of intervention group and control group with Socio-Economic Status



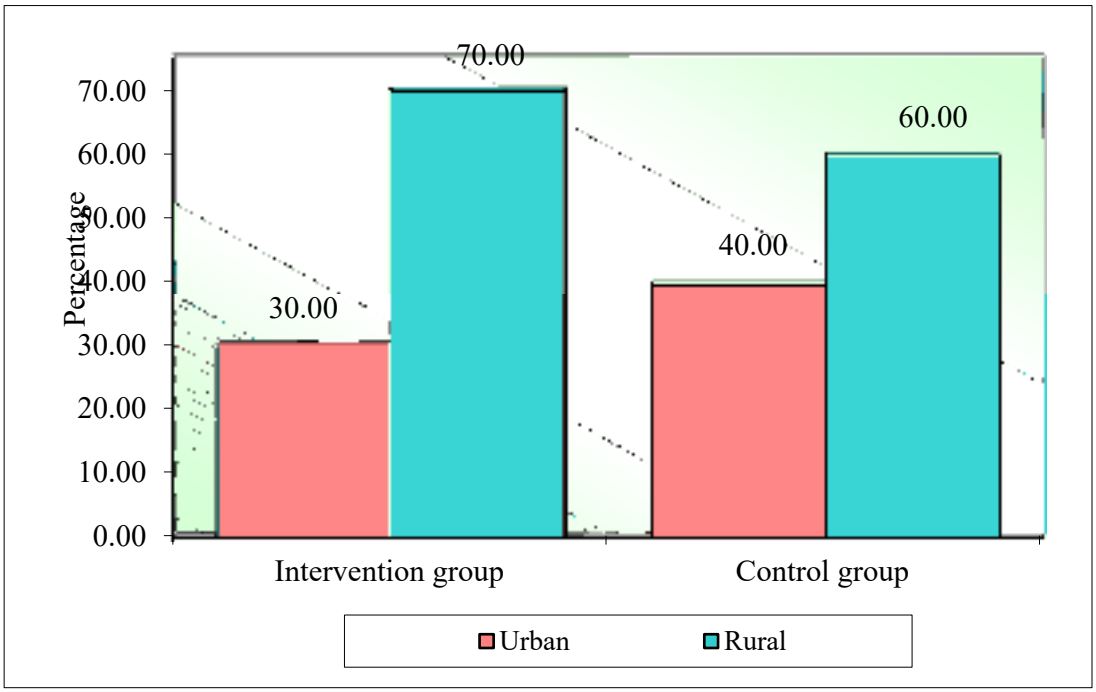
In the current study, 15% children belonged to lower middle class SES in interventional group as compared to 10% in control group, 25% children belonged to middle class SES in interventional group as compared to 15% in control group. 25% children belonged to upper middle class SES in interventional group as compared to 20% in control group. 30% children belonged to Upper class SES in interventional group as compared to 55% in control group. No significant difference was seen in distribution of children according to SES (p=0.5120).

Graph 4: Comparison of intervention group and control group with fathers education.



In the present study, majority of children i.e. 11/20 (55%) had fathers who completed education till high school in interventional group as compared to 4/20 (20%) children in control group. 2/20 (10%) children from both interventional and control group had fathers who completed education till PUC. Only 1 child (5%) in control group had father who had his education till University level compared to none in interventional group. No significant difference was seen in distribution of children according to fathers education (p= 0.1920).

Graph 5: Distribution of children according to the place of residence

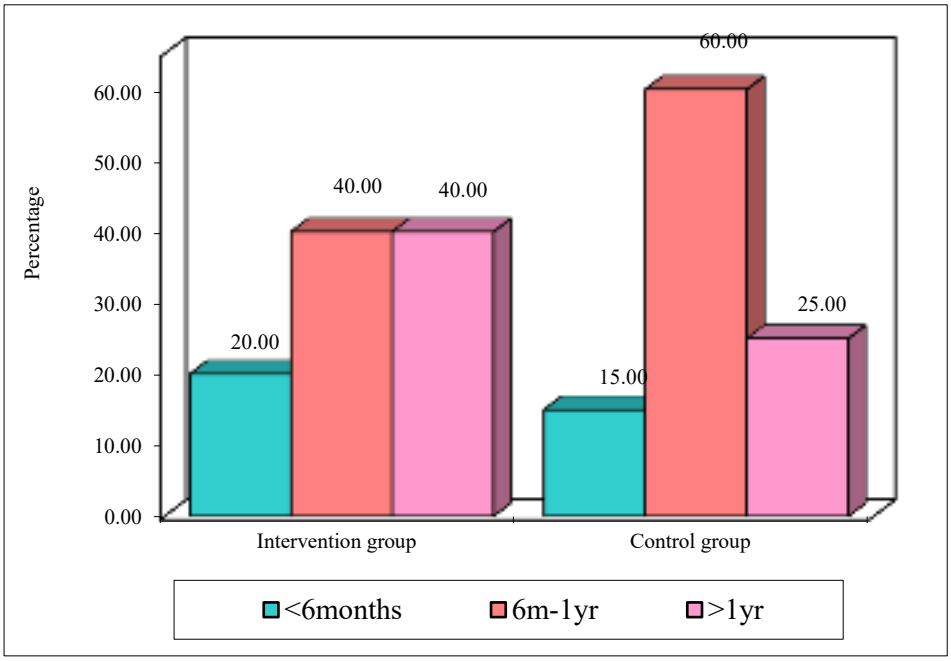


In the present study, majority of children in both the intervention (n=14/20; 70%) and control group (n=12/20; 60%) belonged to rural area. No significant difference was seen in distribution of children according to place of residence (p= 0.5070).

Table 8: Comparison of intervention and control group with years of onset of disease

Years of onset of disease	Intervention group	%	Control group	%	Total	%	χ^2	p-value
<6months	4	20.00	3	15.00	7	17.50	1.6350	0.4410
6m-1yr	8	40.00	12	60.00	20	50.00		
>1yr	8	40.00	5	25.00	13	32.50		
Total	20	100.00	20	100.00	40	100.00		

Graph 6: Comparison of intervention and control group with years of onset of disease



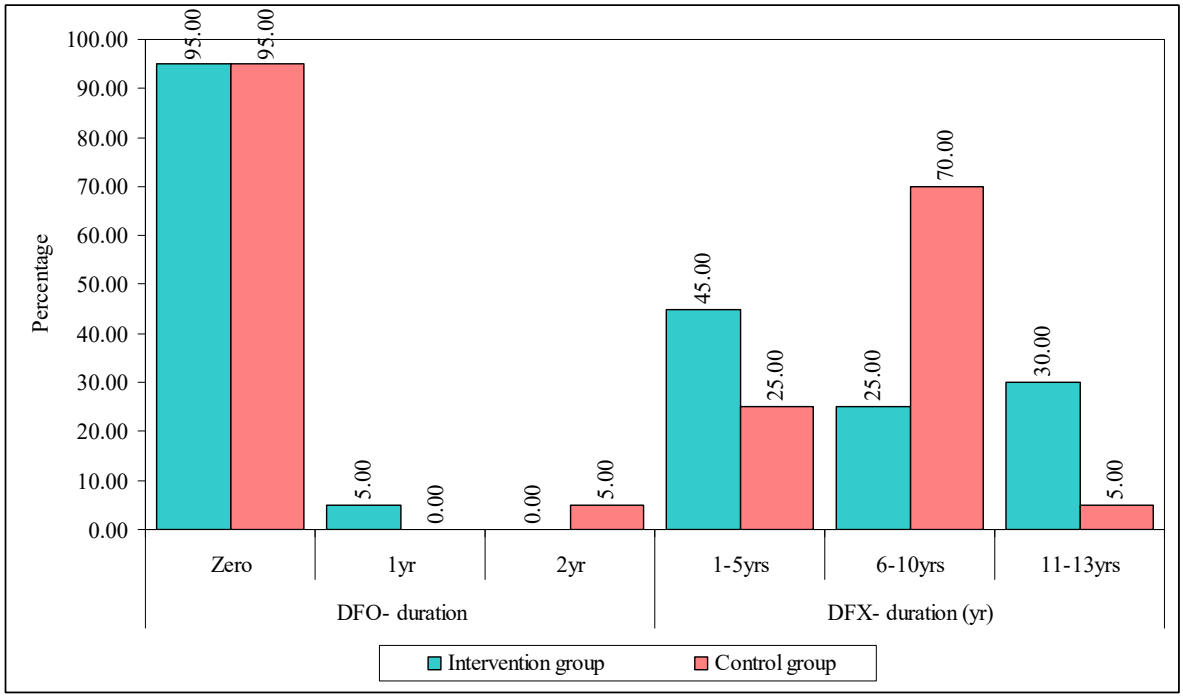
In the present study, 8/20 (40%) children from interventional group and 12/20 (60%) children from control group had the onset of disease at the age of 6 months to 1 year. 8/20 (40%) children from interventional group and 5/20 (25%) children from control group had the onset of disease after 1 year of age**.** However, no significant difference was seen in distribution of children according to years of onset of disease (p= 0.4410).

Table 9: Comparison of intervention and control group with Treatment history in the past.

Treatment history	Intervention group	%	Control group	%	Total	%	χ^2	p-value
Desferrioxamine (DFO)								
No	19	95.00	19	95.00	38	95.00	0.0000	1.0000
Yes	1	5.00	1	5.00	2	5.00		
DFO- duration								
Zero	19	95.00	19	95.00	38	95.00	2.0000	0.3680
1yr	1	5.00	0	0.00	1	2.50		
2yr	0	0.00	1	5.00	1	2.50		
Deferasirox (DFX)								
No	0	0.00	0	0.00	0	0.00	0.0000	1.0000
Yes	20	100.00	20	100.00	40	100.00		
DFX- duration (yr)								
1-5yrs	9	45.00	5	25.00	14	35.00	8.9770	**0.0110***
6-10yrs	5	25.00	14	70.00	19	47.50		
11-13yrs	6	30.00	1	5.00	7	17.50		
Folic acid								
No	2	10.00	0	0.00	2	5.00	2.1050	0.1470
Yes	18	90.00	20	100.00	38	95.00		
Calcium								
No	0	0.00	1	5.00	1	2.50	1.0260	0.3110
Yes	20	100.00	19	95.00	39	97.50		
Zinc								
No	16	80.00	18	90.00	34	85.00	0.7840	0.3760
Yes	4	20.00	2	10.00	6	15.00		
B-complex								
No	3	15.00	3	15.00	6	15.00	0.0000	1.0000
Yes	17	85.00	17	85.00	34	85.00		
Pantoprazole								
No	19	95.00	16	80.00	35	87.50	2.0570	0.1510
Yes	1	5.00	4	20.00	5	12.50		
Total	20	100.00	20	100.00	40	100.00		

*$p<0.05$

Graph 7: Comparison of intervention and control group with DFO- duration and DFX- duration (years).



In the present study, majority of children i.e.19/20 (95%) belonging to both interventional group and control group did not receive DFO chelation therapy.

All 20/20 (100%) children from both groups have been on DFX for iron chelation therapy. 9/20 (45%) children from intervention group received DFX chelation for a period of 1-5 years in comparison to 14/20 (70%) children from control group who received DFX therapy for 6-10 years. This difference in the duration of treatment with DFX between the two groups was statistically significant (p=0. 0110*).

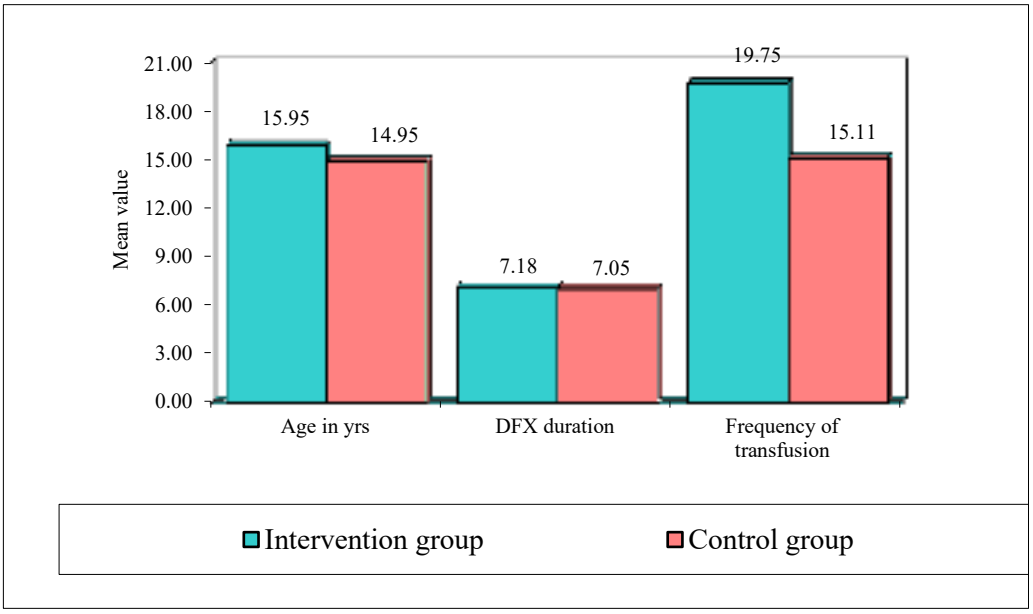
Consumption of calcium, folic acid, zinc, b-complex and pantoprazole showed no difference in both intervention and control groups.

Table 10: Comparison of intervention and control group with mean age, DFX duration in years and Frequency of transfusion (units/year) by t test

Variables	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Age in years	15.95	2.26	14.95	2.63	1.2913	0.2044
DFX duration in years	7.18	3.63	7.05	3.05	0.1178	0.9068
Frequency of transfusion (units/year)	19.75	5.02	15.11	4.90	2.9210	**0.0059***

*p<0.05

Graph 8: Comparison of intervention and control group with mean age, DFX duration in years and Frequency of transfusion (years)

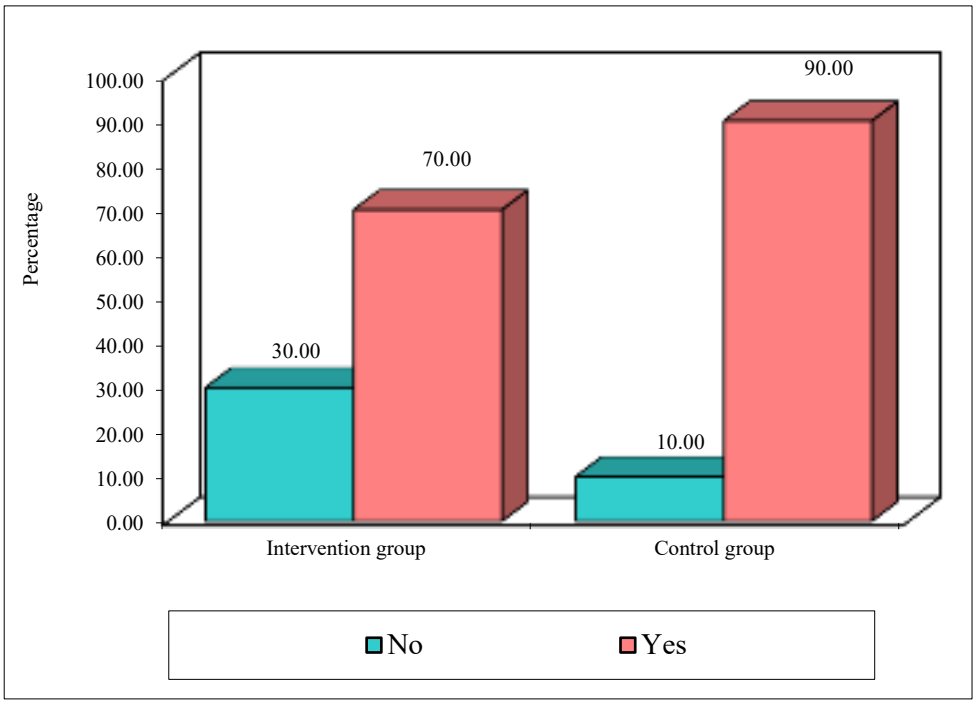


In the present study, mean age of children was 15.95 ± 2.26 years in intervention group and 14.95 ± 2.63 years in control group who received DFX chelation for a mean duration of 7.18 ± 3.63 years and 7.05 ± 3.05 years respectively with higher frequency of blood transfusion in intervention group as compared to control group (19.75 ± 5.02 U/year vs 15.11 ± 4.90 U/year; p= 0.0059*).

Table 11: Comparison of intervention and control group with history of consanguineous marriage

Consanguineous marriage	Intervention group	%	Control group	%	Total	%	p-value
No	6	30.00	2	10.00	8	20.00	0.1140
Yes	14	70.00	18	90.00	32	80.00	
Total	20	100.00	20	100.00	40	100.00	

Graph 9: Comparison of intervention and control group with consanguineous marriage

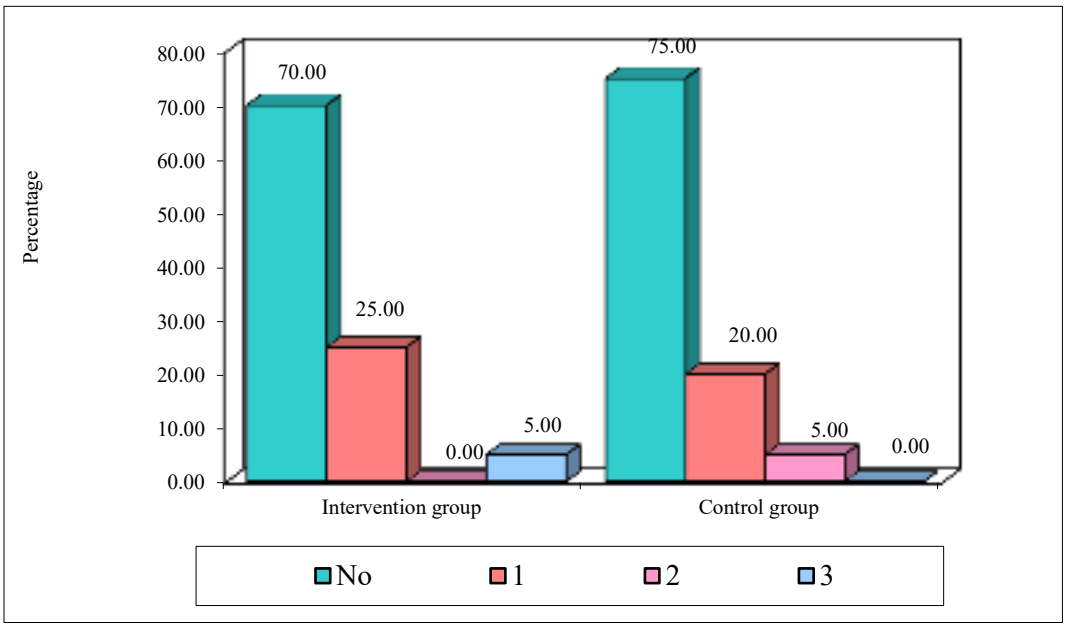


In the current study, 14/20 (70%) children from intervention group and 18/20 (90%) children from control group reported history of consanguineous marriage. The rate of consanguinity is higher in control group as opposed to intervention group but this difference showed no statistical significance (p=0.1140).

Table 12: Comparison of intervention and control group with history of siblings with thalassemia

Siblings with thalassemia	Intervention group	%	Control group	%	Total	%	χ^2	p-value
No	14	70.00	15	75.00	29	72.50	2.1460	0.5430
1	5	25.00	4	20.00	9	22.50		
2	0	0.00	1	5.00	1	2.50		
3	1	5.00	0	0.00	1	2.50		
Total	20	100.00	20	100.00	40	100.00		

Graph 10: Comparison of intervention and control group with Siblings with thalassemia



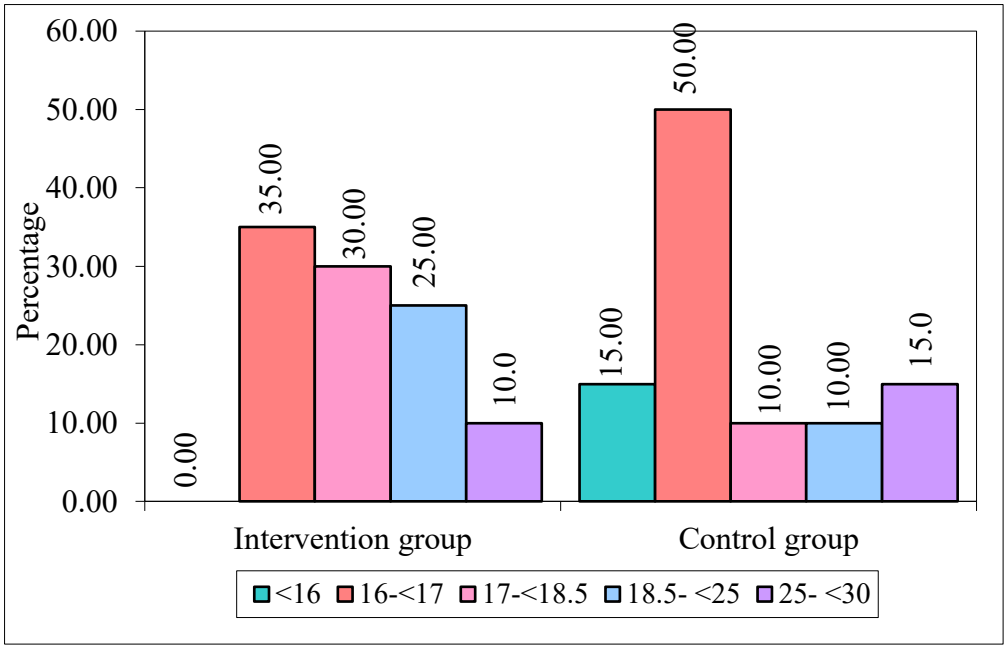
In this study, majority i.e. 14/20 (70%) children in intervention group and 15/20 (75%) children in control group did not have sibling with thalassemia. 5/20 (25%) children in intervention group and 4/20 (20%) children in control group had one sibling with thalassemia. The distribution of children in both the groups according to history of siblings with thalassemia showed no difference and is statistically not significant (p=0.5430)

Table 13: Comparison of intervention and control group with BMI

BMI	Intervention group	%	Control group	%	Total	%	χ^2	p-value
<16	0	0.00	3	15.00	3	7.50	7.0151	0.1350
16-<17	7	35.00	10	50.00	17	42.50		
17-<18.5	6	30.00	2	10.00	8	20.00		
18.5- <25	5	25.00	2	10.00	7	17.50		
25- <30	2	10.00	3	15.00	5	12.50		
Total	20	100.00	20	100.00	40	100.00		

*p<0.05

Graph 11: Comparison of intervention and control group with BMI

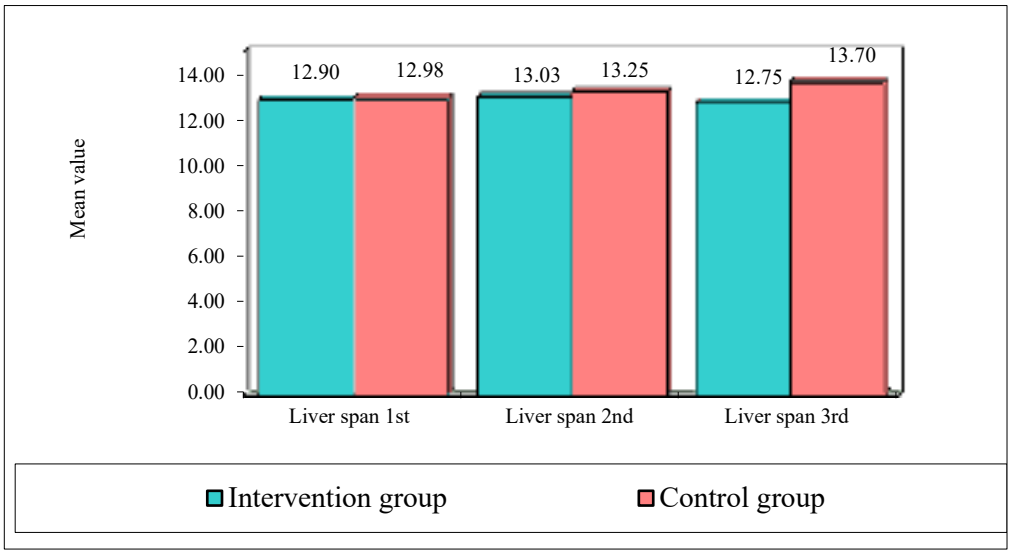


In this study, 3/10 (15%) children in control group were underweight with BMI <16kg/m2 as opposed to none in intervention group. 10/20 (50%) children in control group had BMI between 16-<17kg/m2 as opposed to 7/20 (35%) children in intervention group. Even though the distribution of underweight children was higher in control group than intervention group, the difference was not of statistical significance (p= 0.1350).

Table 14: Comparison of intervention and control group with mean liver span by t test (per abdomen)

Variables	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Liver span 1st	12.90	2.34	12.98	1.98	-0.1096	0.9133
Liver span 2nd	13.03	1.99	13.25	1.82	-0.3600	0.7210
Liver span 3rd	12.75	1.78	13.70	2.15	-1.4732	0.1494

Graph 12: Comparison of intervention and control group with mean liver

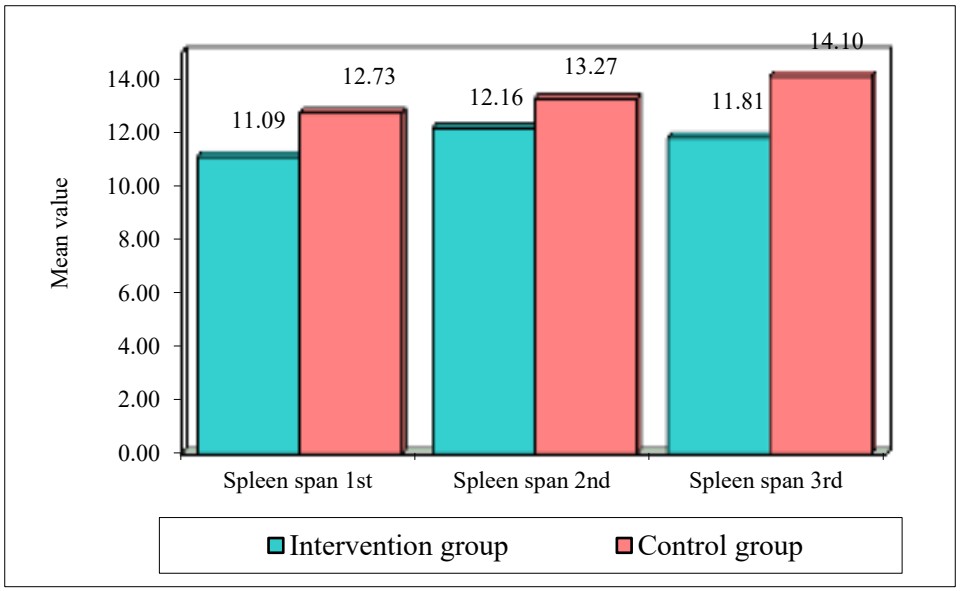


In the present study, the mean liver span measured at baseline of study was 12.90 ± 2.34 in intervention group as compared to 12.98 ± 1.98 in control group (P= 0.9133). The mean liver span measured at 6 months, post-intervention was found to be 12.75 ± 1.78 in intervention group as compared to 13.70 ± 2.15 in control group (p= 0.1494). There was a difference in the mean of liver span measured post-intervention amongst both groups but this difference was not of any statistical significance.

Table 15: Comparison of intervention and control group with mean spleen span by t test (per abdomen)

Variables	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Spleen span 1st	11.09	7.62	12.73	3.41	-0.7699	0.4474
Spleen span 2nd	12.16	3.80	13.27	3.05	-0.8935	0.3790
Spleen span 3rd	11.81	3.67	14.10	3.90	-1.6824	0.1032

Graph 13: Comparison of intervention and control group with mean spleen span



In the present study, the mean spleen span measured at baseline of study was 11.09 ± 7.62 in intervention group as compared to 12.73 ± 3.41 in control group (P= 0.4474). The mean liver span measured at 6 months post-intervention was found to be 11.81 ± 3.67 in intervention group as compared to 14.10 ± 3.90 in control group (p= 0.1032).

The mean spleen size was lower in intervention group as compared to that of control group participants at 6 months, but this difference was not found to be statistically significant.

Table 16: Comparison of intervention and control group with mean pre-transfusion Haemoglobin recorded at baseline and 6 months by independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	8.44	1.09	7.81	1.49	1.5245	0.1357
6 months	9.16	0.81	8.32	1.18	2.6447	**0.0118***

*p<0.05

Graph 14: Comparison of intervention and control group with mean pre-transfusion Hemoglobin recorded at baseline and 6 months

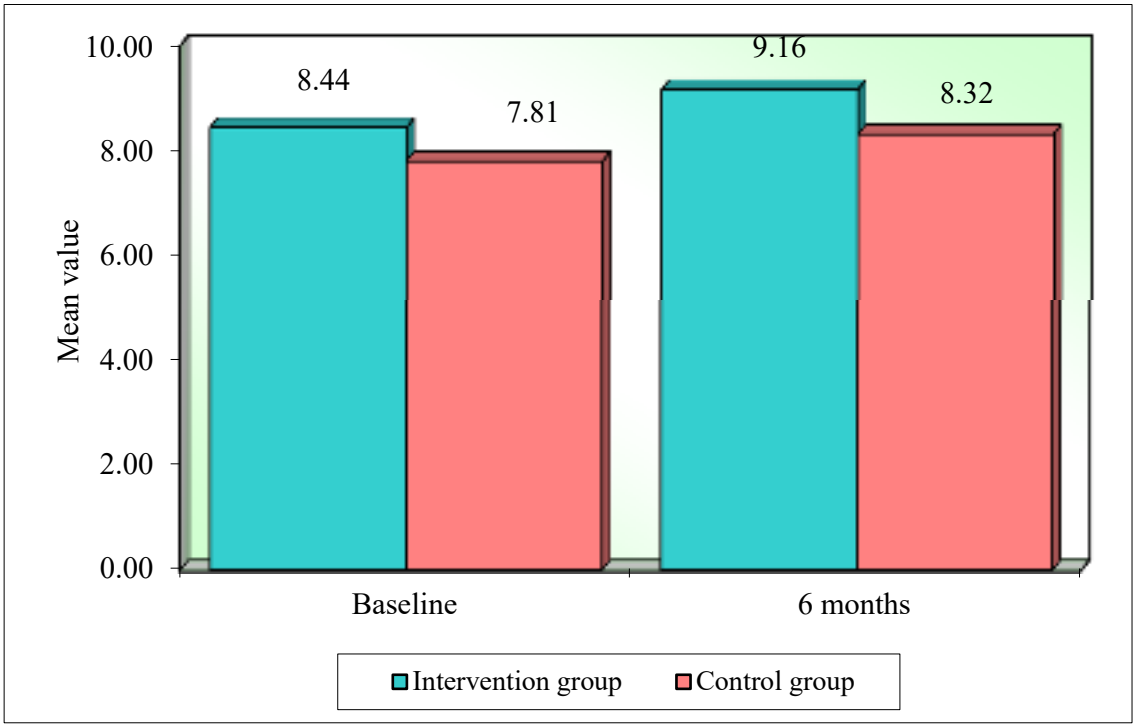


Table 17: Comparison at baseline and 6 months with mean Hb values in intervention and control group by dependent t test

Changes from	Intervention group			Control group		
	Mean Diff.	t-value	p-value	Mean Diff.	t-value	p-value
1st to 12th	-0.73	-2.9020	**0.0091***	-0.51	-1.3651	0.1882

*p<0.05

In the present study, Mean pre-transfusion Hemoglobin measured at baseline did not show any statistical difference between intervention group and control group (8.44 ± 1.09 vs 7.81 ± 1.49; p= 0.1357) but at 6 months, the mean pre-transfusion Hb measured was higher in intervention group as compared to control group (9.16 ± 0.81 vs 8.32 ± 1.18; p= 0.0118*). This difference was found to be of statistical significance.

With comparison to baseline pre-transfusion Hb values, the Hb levels recorded in intervention group at 12th follow-up (6 months) was found to have a mean difference of -0.73 (p= 0.0091*). Whereas, in the control group, the mean difference in pre-transfusion Hb recorded at 6 months as compared to baseline did not show any statistical significance (p= 0.1882).

Graph 15: Comparison of intervention and control group with mean pre-transfusion Hb values at different time points

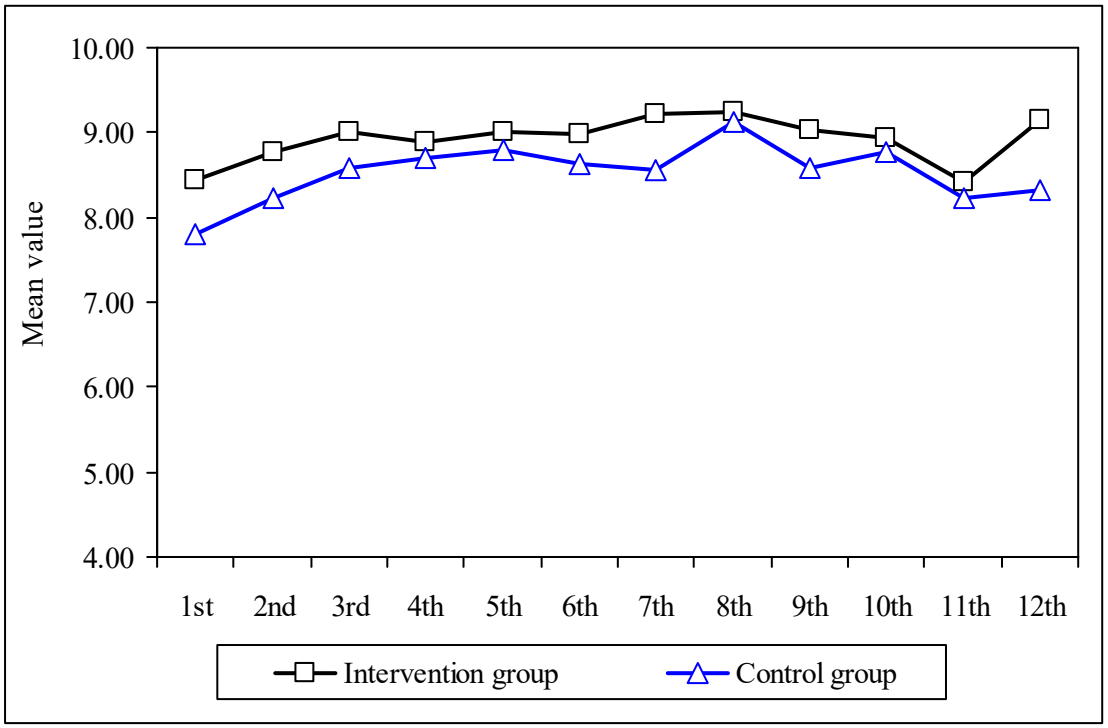
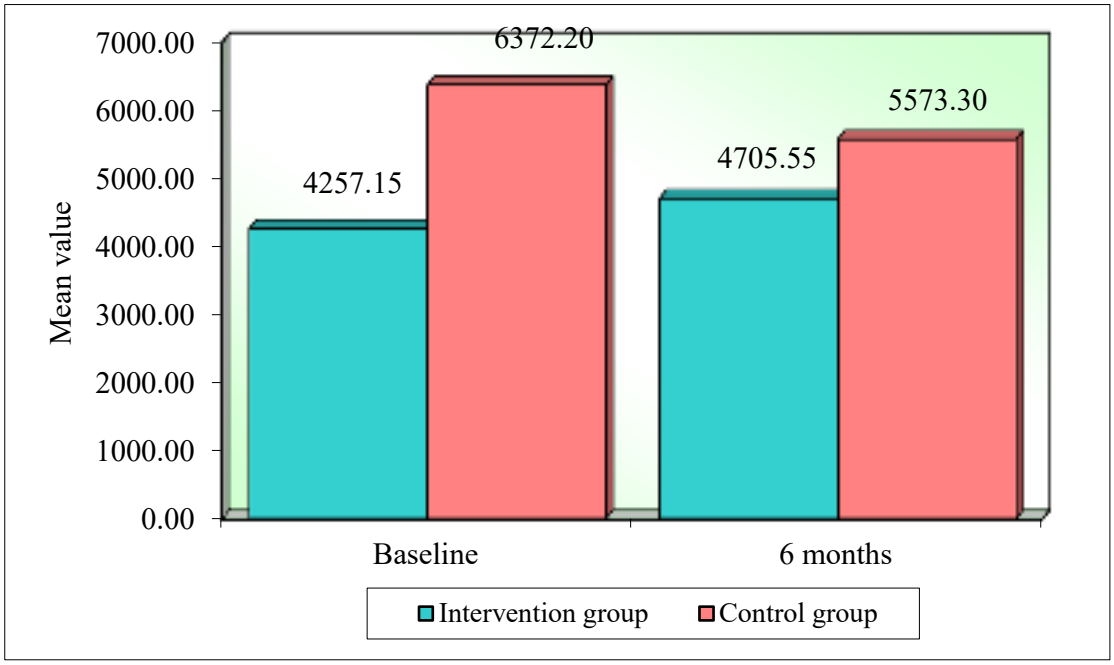


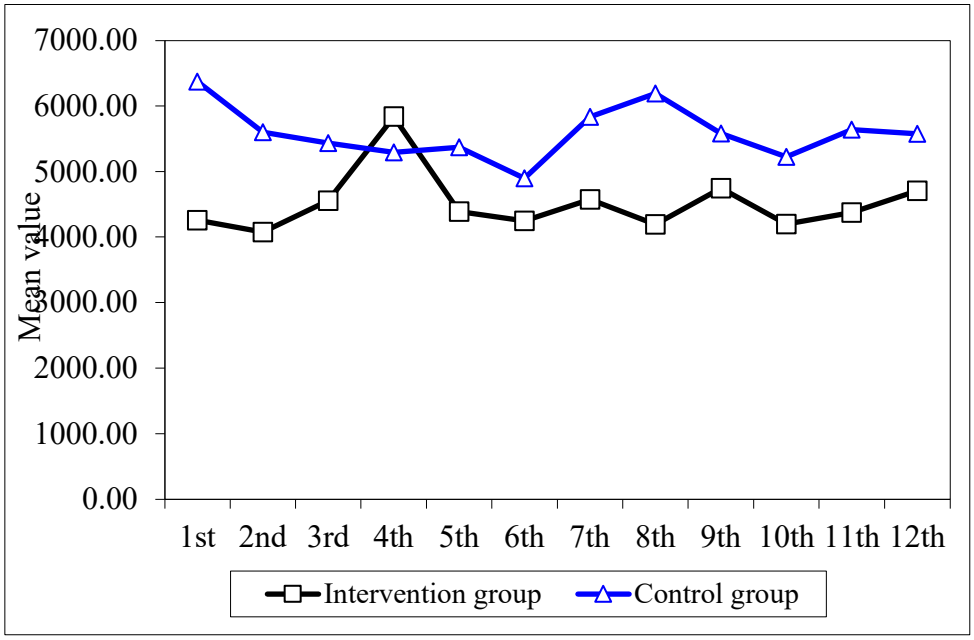
Table 18: Comparison of intervention and control group with mean Absolute Neutrophils Count (ANC) at baseline and 6 months by independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	4257.15	1975.26	6372.20	5553.19	-1.6048	0.1168
6 months	4705.55	1326.82	5573.30	2795.85	-1.2540	0.2175

Graph 16: Comparison of intervention and control group with mean Absolute Neutrophils Count (ANC) at baseline and 6 months



Graph 17: Comparison of intervention and control group with mean Absolute Neutrophil Count (ANC) at different time points



In this study, the mean ANC counts measured both at baseline and at 6 months was lower in intervention group as compared to control group but this difference was not of statistical significance. (4257 ± 1975.26 vs 6372.20 ± 5553.19; p= 0.1168 and 4705.55 ± 1326.82 vs 5573.30 ± 2795.85; p= 0.2175). Hence, none of the groups reported neutropenia.

Table 19: Comparison of intervention and control group with mean Serum Ferritin at baseline and 6 months by independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	4258.35	1676.90	3732.75	3160.81	0.6569	0.5152
6 months	3610.80	1785.21	3030.70	1737.52	1.0414	0.3043

In this study, mean S. Ferritin levels recorded at 6 months post-intervention in intervention group was 3610.80 ± 1785.21 and in control group was 3030.70 ± 1737.52. Thus, mean S. Ferritin levels in both groups were comparable and did not show any difference of statistical significance (p=0.3043).

Table 20: Comparison at baseline and 6 months with mean Serum ferritin in intervention and control group by dependent t test

Changes from		Intervention group			Control group	
	Mean Diff.	t-value	p-value	Mean Diff.	t-value	p-value
BL to 6M	647.55	1.7710	0.0926	702.05	1.1902	0.2486

In this study, there exists a difference in the mean S. Ferritin levels from baseline to 6 months post-intervention, recorded at regular intervals between both the intervention group and control group, but this difference was not of any statistical significance.

Graph 18: Comparison of intervention and control group with mean Serum ferritin at baseline and 6 months

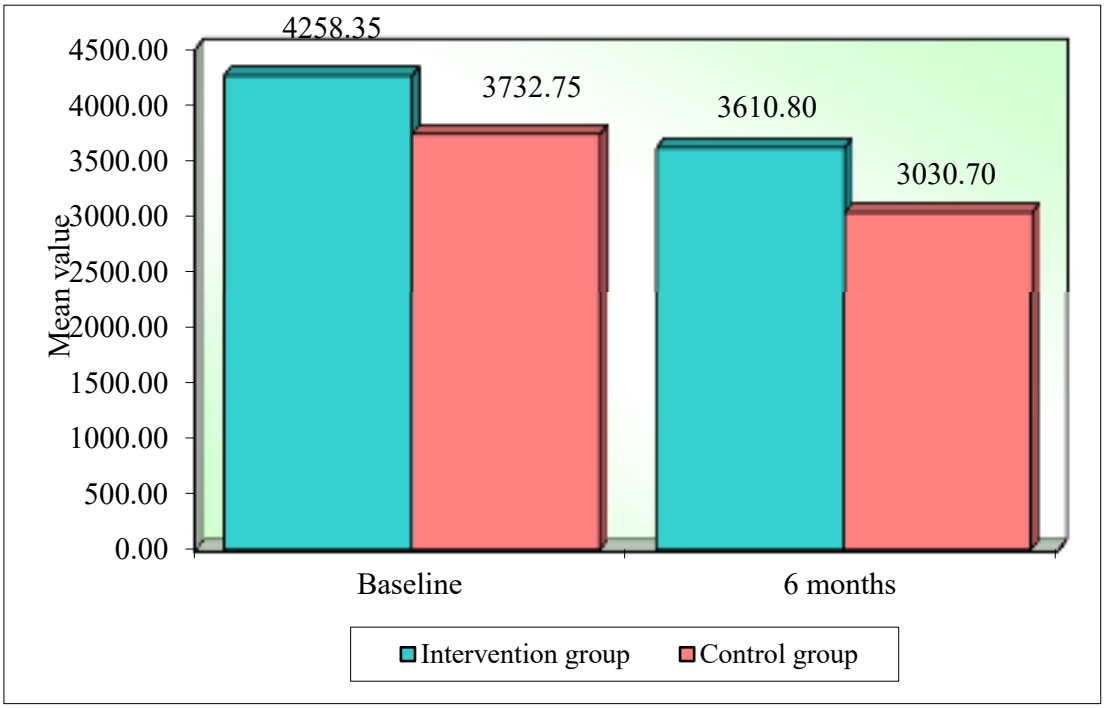
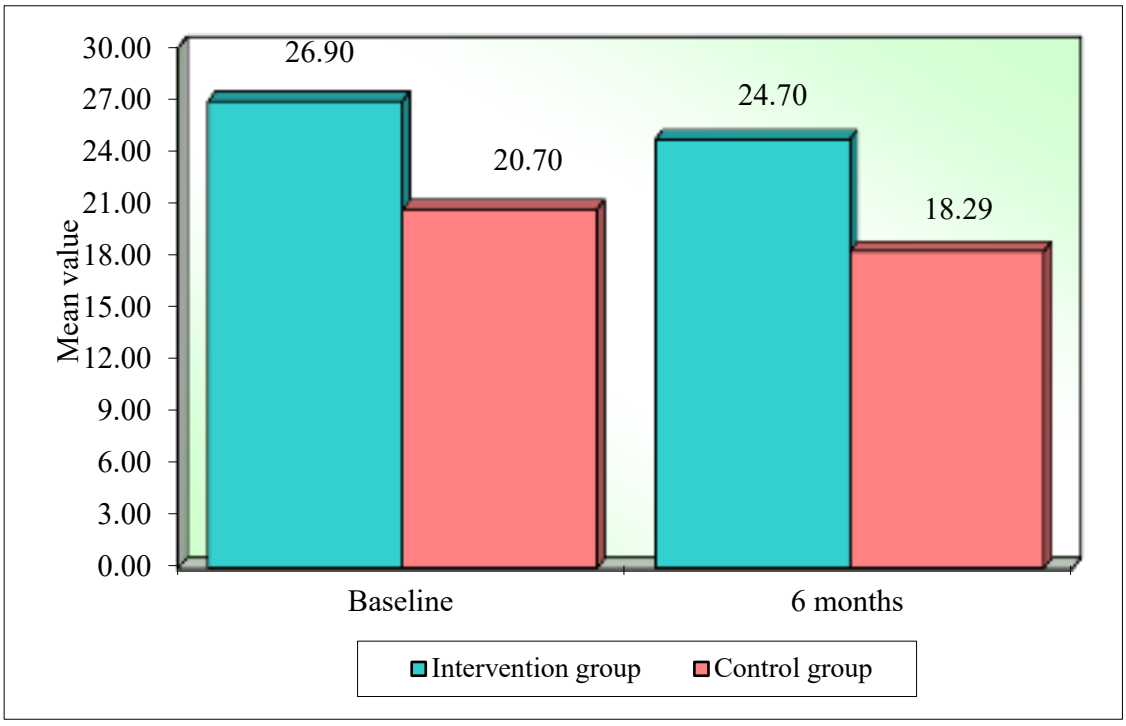


Table 21: Comparison of intervention and control group with mean urea at baseline and 6 months by independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	26.90	10.53	20.70	7.58	2.1365	**0.0391***
6 months	24.70	5.75	18.29	6.90	3.1930	**0.0028***

*p<0.05

Graph 19: Comparison of intervention and control group with mean urea at baseline and 6 months



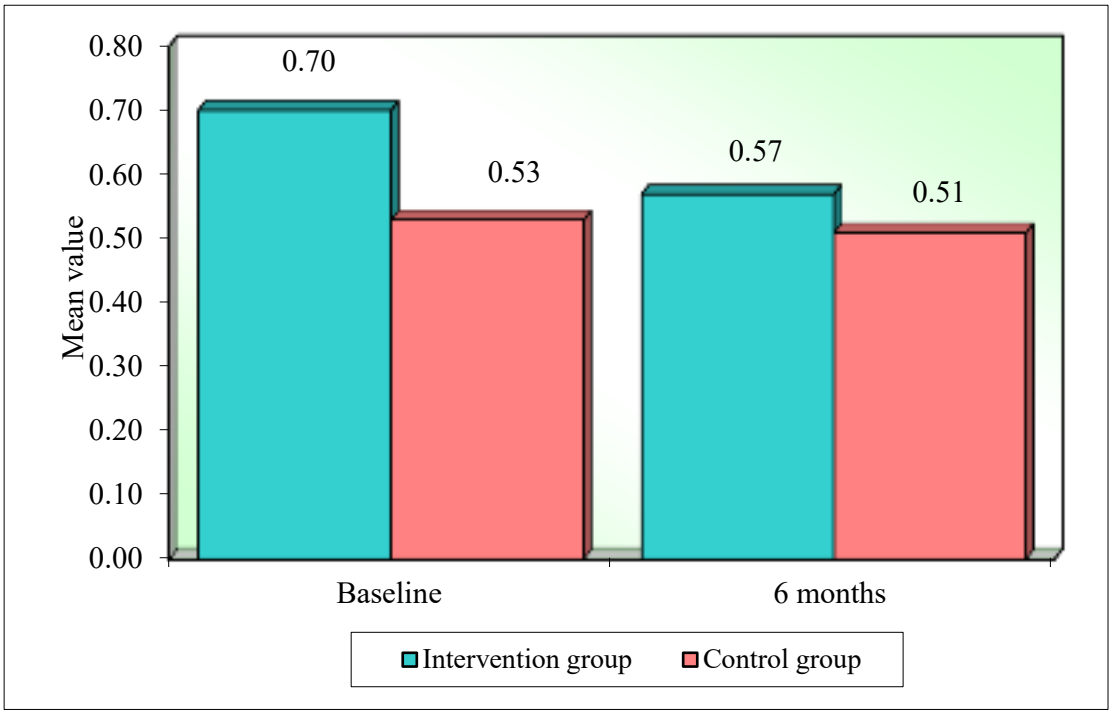
In this study, the mean urea levels recorded at baseline in intervention group was 26.90 ± 10.53 as compared to 20.70 ± 7.58 (p= 0.0391*). There is a decline in mean urea levels from baseline to 6 months in both intervention and control groups. But at 6 months, mean urea levels were higher in intervention group as compared to control group (24.70 ± 5.75 vs 18.29 ± 6.90; p= 0.0028*).

Table 22: Comparison of intervention and control group with mean Creatinine at baseline and 6 months by independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	0.70	0.33	0.53	0.26	1.7517	0.0879
6 months	0.57	0.27	0.51	0.22	0.7614	0.4511

*p<0.05

Graph 20: Comparison of intervention and control group with mean Creatinine at baseline and 6 months.



Mean creatinine levels between the two groups at baseline and 6 months did not show any statistical significance (p= 0.0879, p= 0.4511).

Table 23: Comparison of intervention and control group with mean SGOT at baseline and 6 months independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	50.95	25.78	63.85	52.26	-0.9900	0.3285
6 months	36.82	16.33	40.95	16.95	-0.7847	0.4375

In this study, there exists no difference of any statistical significance in mean SGOT levels from baseline to 6 months post-randomization, recorded at regular intervals between the intervention group and control group.

Table 24: Comparison at baseline and 6 months with mean SGOT in intervention and control group by dependent t test

Changes from	Intervention group			Control group		
	Mean Diff.	t-value	p-value	Mean Diff.	t-value	p-value
BL to 6M	14.13	2.3071	**0.0325***	22.90	2.3085	**0.0324***

*p<0.05

In this study, the difference in mean SGOT levels from baseline to 6 months post randomization was significantly lower in intervention group as compared to control group (14.13 vs 22.90; p= 0.0324*)

Graph 21: Comparison of intervention and control group with mean SGOT at baseline and 6 months

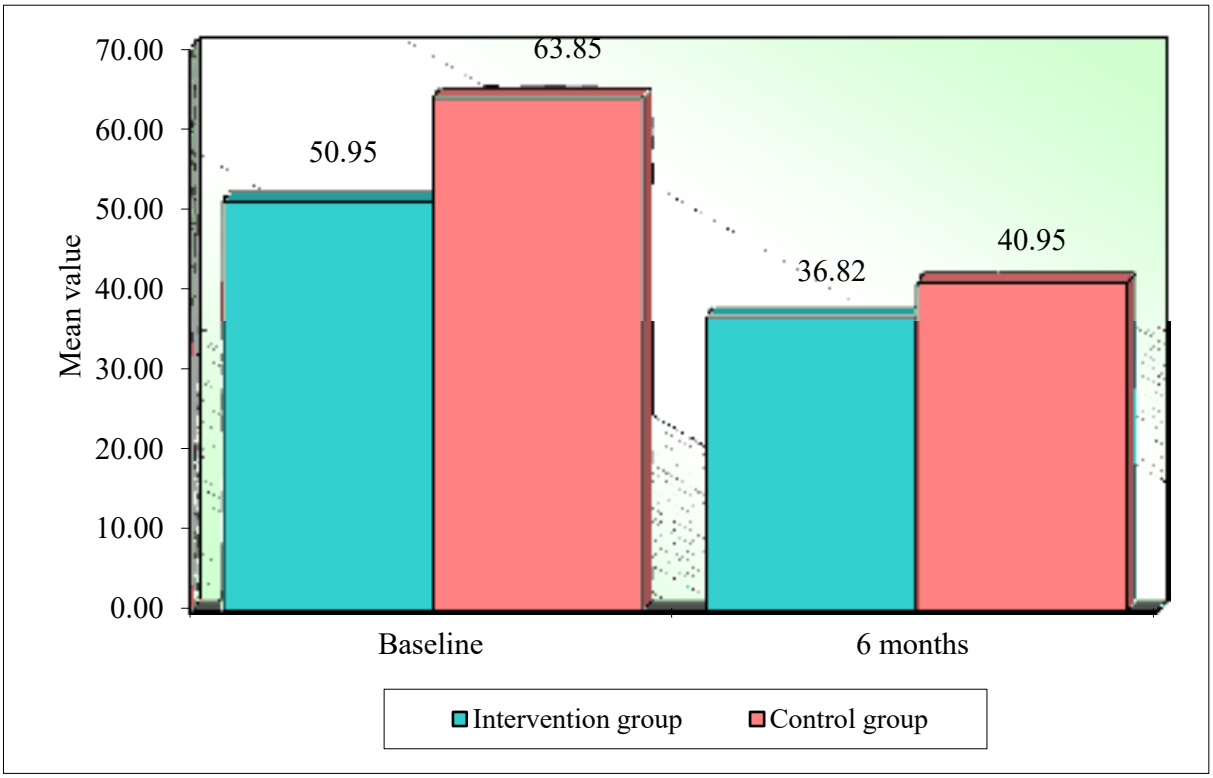


Table 25: Comparison of intervention and control group with mean SGPT at baseline and 6 months by independent t test

Times	Intervention group		Control group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Baseline	46.05	29.82	48.20	44.16	-0.1804	0.8578
6 months	30.85	13.67	33.75	20.52	-0.5260	0.6020

In this study, the difference in mean SGPT levels from baseline to 6 months post-randomization, recorded at regular intervals between the intervention group and control group did not show any statistical significance**.**

Table 26: Comparison at baseline and 6 months with mean SGPT in intervention and control group by dependent t test

Changes from	Intervention group			Control group		
	Mean Diff.	t-value	p-value	Mean Diff.	t-value	p-value
BL to 6M	15.20	2.2815	**0.0342***	14.45	1.5086	0.1479

*p<0.05

In this study, there exists a statistically significant difference in mean SGPT levels at 6 months in the intervention group as compared to baseline values (15.20 vs 14.45; p= 0.0342*). Similarly, difference in mean SGPT levels from baseline to 6months was seen in control group as well but it was not of any statistical significance (p= 0.1479).

Graph 22: Comparison of intervention and control group with mean SGPT at baseline and 6 months

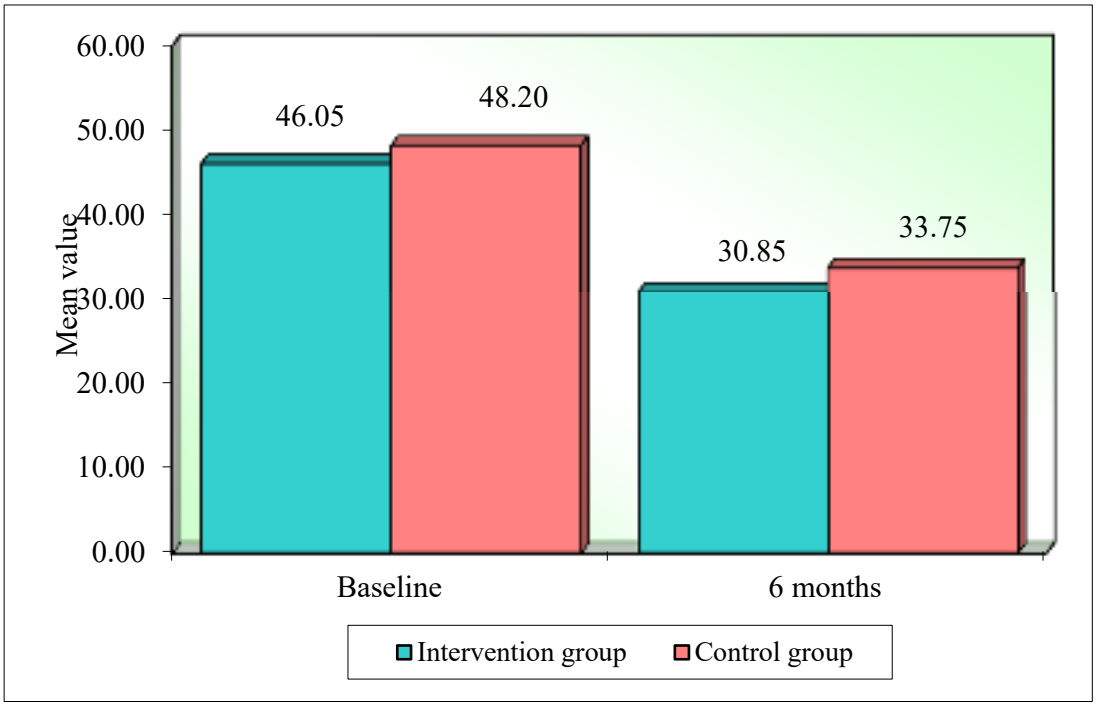


Table 27: Comparison of intervention and control group with mean Liver size and Spleen size obtained by USG at baseline and 6 months by independent t test

Variables	Times	Intervention group		Control group		t-value	p-value
		Mean	Std.Dev.	Mean	Std.Dev.		
Liver size	Baseline	15.64	1.43	14.84	1.53	1.7079	0.0958
	6 months	14.82	1.89	15.72	1.73	-1.5701	0.1247
Spleen size	Baseline	15.54	2.51	15.21	2.52	0.3541	0.7259
	6 months	14.26	1.98	16.09	3.39	-1.8781	0.0705

In this study, at baseline the mean liver size in intervention group was higher than that of control group (15.64 ± 1.43, 14.84 ± 1.53 respectively). But 6 months post-intervention, the mean liver size in intervention group was 14.82 ± 1.89 which has decreased in comparison to baseline values. However, the mean liver size in the control group at 6 months (15.72 ± 1.73) was found to be higher in comparison to baseline values. This difference was not of any statistical significance.

Similarly, at baseline the mean spleen size in both intervention group and control group were found to be comparable (15.54 ± 2.51, 15.21 ± 2.52 respectively). But at 6 months post-intervention, the mean spleen size in intervention group was 14.26 ± 1.98 which has decreased in comparison to baseline values. However, the mean spleen size in the control group at 6 months (16.09 ± 3.39) was found to be higher in comparison to baseline values. This difference was not of any statistical significance.

Table 28: Comparison at baseline and 6 months of Liver size and Spleen size obtained by USG in intervention and control group by dependent t test

Variables	Changes from		Intervention group			Control group	
		Mean Diff.	t-value	p-value	Mean Diff.	t-value	p-value
Liver size	BL to 6M	0.83	2.7084	**0.0139***	-0.88	-2.0993	**0.0494***
Spleen size	BL to 6M	1.28	3.0357	**0.0079***	-0.88	-1.6688	0.1190

*p<0.05

In this study, the difference in mean liver size from baseline to 6 months post-intervention is 0.83 in intervention group which is of statistical significance (p= 0.0139*). The difference in mean liver size in control group is -0.88 which is found to be of statistical significance (p= 0.0494*).

The difference in mean spleen size from baseline to 6 months post-intervention is 1.28 in intervention group which is of statistical significance (p= 0.0079*).

Graph 23: Comparison of intervention and control group with mean Liver size and Spleen size at baseline and 6 months

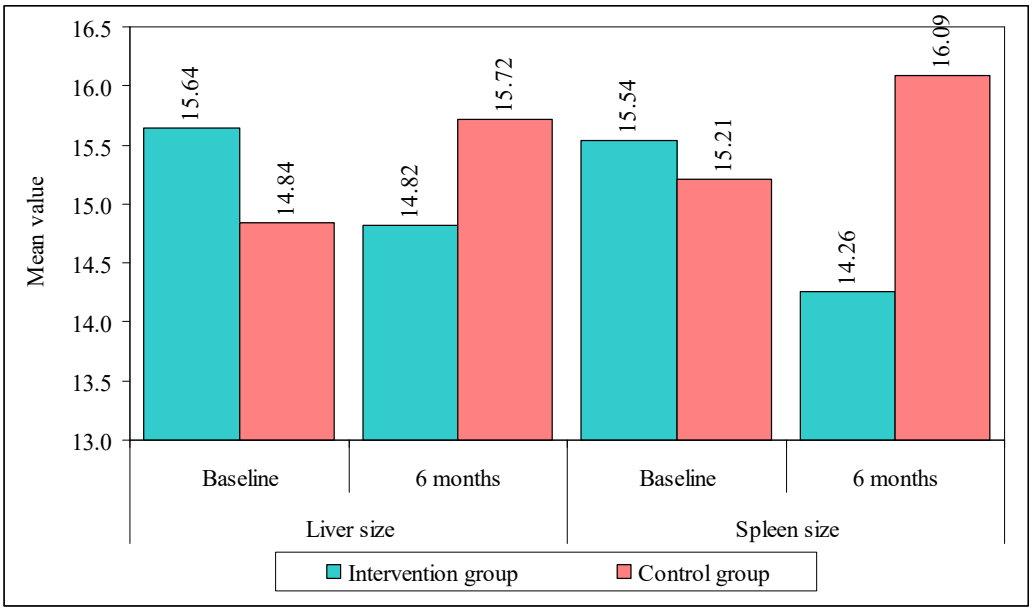


Table 29: Comparison of intervention and control group with mean of all ECHO-TDI parameters at baseline and 6 months by independent t test

ECHO-TDI	Times	Intervention group		Control group		t-value	p-value
		Mean	Std.Dev.	Mean	Std.Dev.		
LVEF	Baseline	57.47	7.20	59.70	4.40	-1.1815	0.2448
	6 months	59.66	4.89	58.07	3.70	1.1591	0.2536
LVEDV	Baseline	72.95	26.73	59.48	17.43	1.8887	0.0666
	6 months	73.15	26.21	65.63	21.17	0.9988	0.3242
LVESV	Baseline	31.70	11.45	29.98	12.17	0.4617	0.6469
	6 months	30.73	12.31	29.22	12.74	0.3811	0.7053
LVIDd	Baseline	4.33	0.48	4.06	0.33	2.0379	**0.0486***
	6 months	4.29	0.49	4.08	0.35	1.5150	0.1380
LVISd	Baseline	3.04	0.56	2.80	0.40	1.5571	0.1277
	6 months	2.82	0.38	2.88	0.38	-0.4958	0.6229
FS	Baseline	31.00	8.74	31.55	7.78	-0.2102	0.8346
	6 months	34.00	6.33	28.40	7.04	2.6439	**0.0118***
MVI-E	Baseline	109.60	29.55	107.50	38.29	0.1942	0.8471
	6 months	119.46	22.81	116.26	20.13	0.4711	0.6403
MVI-A	Baseline	58.82	15.85	58.62	19.69	0.0354	0.9720
	6 months	56.18	30.29	60.78	13.25	-0.6222	0.5375
E/A	Baseline	2.11	0.60	1.93	0.65	0.9349	0.3557
	6 months	1.93	0.55	1.99	0.58	-0.3404	0.7354
MVLI-E	Baseline	17.15	4.83	16.64	3.21	0.3970	0.6936
	6 months	16.98	4.90	18.92	3.55	-1.4327	0.1601
MVLI-A	Baseline	9.16	3.45	7.65	1.78	1.7452	0.0890
	6 months	8.50	2.56	8.24	2.21	0.3439	0.7328
MVLI-S	Baseline	10.84	3.15	10.52	3.97	0.2782	0.7824
	6 months	10.45	2.57	10.28	2.70	0.1926	0.8483
MVSI-E	Baseline	12.55	3.68	12.11	1.96	0.4721	0.6396
	6 months	12.32	3.14	13.38	2.46	-1.1933	0.2402
MVSI-A	Baseline	7.11	2.25	6.78	2.31	0.4503	0.6550
	6 months	6.41	2.43	7.58	2.67	-1.4496	0.1554
MVSI-S	Baseline	8.15	2.20	8.18	3.25	-0.0342	0.9729
	6 months	8.22	1.90	9.24	2.23	-1.5588	0.1273
MVLSAE	Baseline	1.52	0.48	1.60	0.27	-0.6145	0.5426
	6 months	1.73	0.32	1.68	0.22	0.5178	0.6076
MVSSAE	Baseline	1.28	0.29	1.38	0.21	-1.2441	0.2211
	6 months	1.44	0.33	1.33	0.18	1.2579	0.2161
TAPSE	Baseline	2.00	0.36	2.10	0.23	-1.0390	0.3054
	6 months	2.16	0.29	2.14	0.29	0.1630	0.8714

*$p<0.05$

In this study, the ECHO-TDI assessment of the participants was done using the following parameters: LVEF, LVEDV, LVESV, LVIDd, LVISd, FS, Mitral valve inflow velocities E & A, E/A ratio, Mitral valve lateral inflow velocities E, A & S, Mitral valve septal inflow velocities E, A & S, MAPSE of lateral and septal walls, TAPSE. There was no significant difference between the control group and interventional groups at baseline on any of these measured parameters. However, 6months post-intervention, we found a statistically significant difference on 1 of the parameters namely Fractional Shortening (FS). The mean FS after 6 months of treatment in intervention group was i.e 34 ± 6.33 and that of control group was 28.4 ± 7.04. (p= 0.0118*). The decline in FS in control group at 6 months was significant. Also, LVIDd at baseline was 4.33 ± 0.48 in intervention group as compared to 4.06 ± 0.33 in control group. This difference was found to be statistically significant (p= 0.0486*).

Table 30: Comparison of baseline and 6 months with mean of all ECHO-TDI parameters in intervention and control group by dependent t test

ECHO-TDI	Changes from	Intervention group			Control group		
		Mean Diff.	t-value	p-value	Mean Diff.	t-value	p-value
LVEF	BL to 6M	-2.19	-1.3107	0.2056	1.64	1.2909	0.2122
LVEDV	BL to 6M	-0.20	-0.0420	0.9669	-6.15	-1.2208	0.2371
LVESV	BL to 6M	0.98	0.4432	0.6626	0.76	0.2867	0.7774
LVIDd	BL to 6M	0.04	0.3464	0.7329	-0.02	-0.2029	0.8414
LVISd	BL to 6M	0.22	1.9550	0.0655	-0.08	-0.9959	0.3318
FS	BL to 6M	-3.00	-1.9988	0.0601	3.15	1.7978	0.0881
MVI-E	BL to 6M	-9.86	-1.2594	0.2232	-8.76	-0.9424	0.3578
MVI-A	BL to 6M	2.64	0.3557	0.7260	-2.17	-0.6129	0.5472
E/A	BL to 6M	0.18	1.4016	0.1772	-0.06	-0.4206	0.6788
MVLI-E	BL to 6M	0.17	0.1831	0.8567	-2.29	-2.3472	**0.0299***
MVLI-A	BL to 6M	0.67	1.1715	0.2559	-0.59	-1.1565	0.2618
MVLI-S	BL to 6M	0.39	0.5069	0.6180	0.24	0.1950	0.8474
MVSI-E	BL to 6M	0.24	0.3866	0.7033	-1.27	-2.0493	0.0545
MVSI-A	BL to 6M	0.70	1.4326	0.1682	-0.80	-1.0223	0.3195
MVSI-S	BL to 6M	-0.08	-0.1550	0.8785	-1.07	-1.2158	0.2390
MVLSAE	BL to 6M	-0.21	-1.9908	0.0611	-0.09	-1.0553	0.3045
MVSSAE	BL to 6M	-0.16	-2.0990	**0.0494***	0.05	0.6914	0.4976
TAPSE	BL to 6M	-0.16	-1.4043	0.1764	-0.04	-0.4653	0.6470

*$p<0.05$

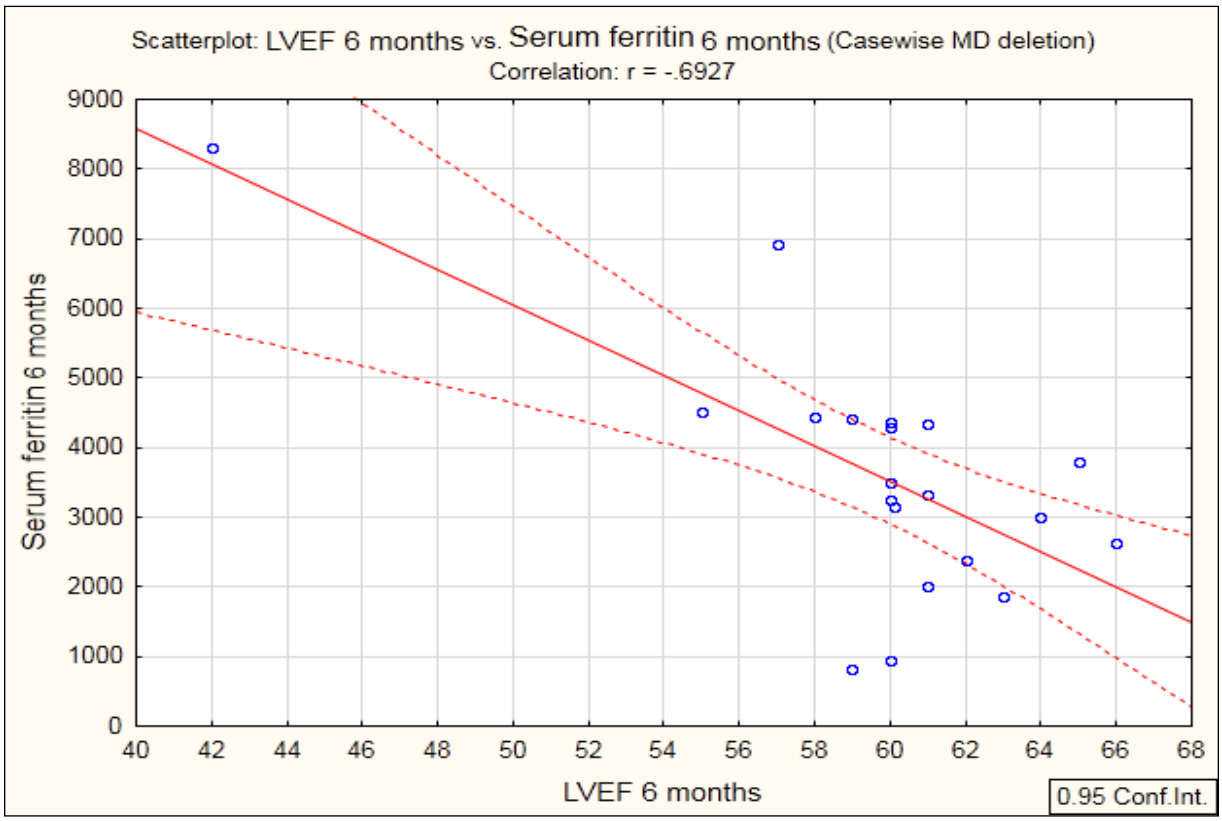
Further, MAPSE of Septal wall (MVSSAE) showed significant improvement from baseline to 6 months (p= 0.0494*) in intervention group and we also observed a statistically significant reduction of MVLI-E velocity from baseline to 6 months post-randomization in the control group however no such significant reduction was found in the intervention group. The difference in mean MVLI-E velocity of -2.29 in control group (t=-2.3472; p= 0.0299*) was found to be statistically significant thus indicating the setting of early diastolic dysfunction in control group.

Table 31: Correlation between Serum ferritin levels at 6 months with ECHO-TDI parameters at 6 months by Karl Pearson's correlation coefficient in intervention group and control group.

ECHO-TDI measurements	Summary	Serum Ferritin (Intervention group)	Serum Ferritin (Control group)
LVEF	r- value	**-0.6927**	0.1306
	p-value	**0.0010***	0.5830
LVEDV	r- value	-0.1373	-0.2154
	p-value	0.5640	0.3620
LVESV	r- value	0.0495	-0.1011
	p-value	0.8360	0.6720
LVIDd	r- value	0.1220	-0.3529
	p-value	0.6080	0.1270
LVISd	r- value	0.3311	-0.2794
	p-value	0.1540	0.2330
FS	r- value	-0.3197	-0.3497
	p-value	0.1690	0.1310
MVI-E	r- value	-0.2152	-0.4059
	p-value	0.3620	0.0760
MVI-A	r- value	0.1838	0.0165
	p-value	0.4380	0.9450
E/A	r- value	-0.3569	-0.2566
	p-value	0.1220	0.2750
MVLI-E	r- value	**-0.5475**	-0.4222
	p-value	**0.0120***	0.0640
MVLI-A	r- value	0.0484	0.3909
	p-value	0.8390	0.0880
MVLI-S	r- value	-0.2136	0.3286
	p-value	0.3660	0.1570
MVSI-E	r- value	-0.3628	0.1733
	p-value	0.1160	0.4650
MVSI-A	r- value	0.1080	0.1890
	p-value	0.6500	0.4250
MVSI-S	r- value	-0.1962	0.1265
	p-value	0.4070	0.5950
MVLSAE	r- value	**-0.6041**	0.1847
	p-value	**0.0050***	0.4360
MVSSAE	r- value	-0.2860	-0.0960
	p-value	0.2210	0.6870
TAPSE	r- value	0.1738	-0.3985
	p-value	0.4640	0.0820

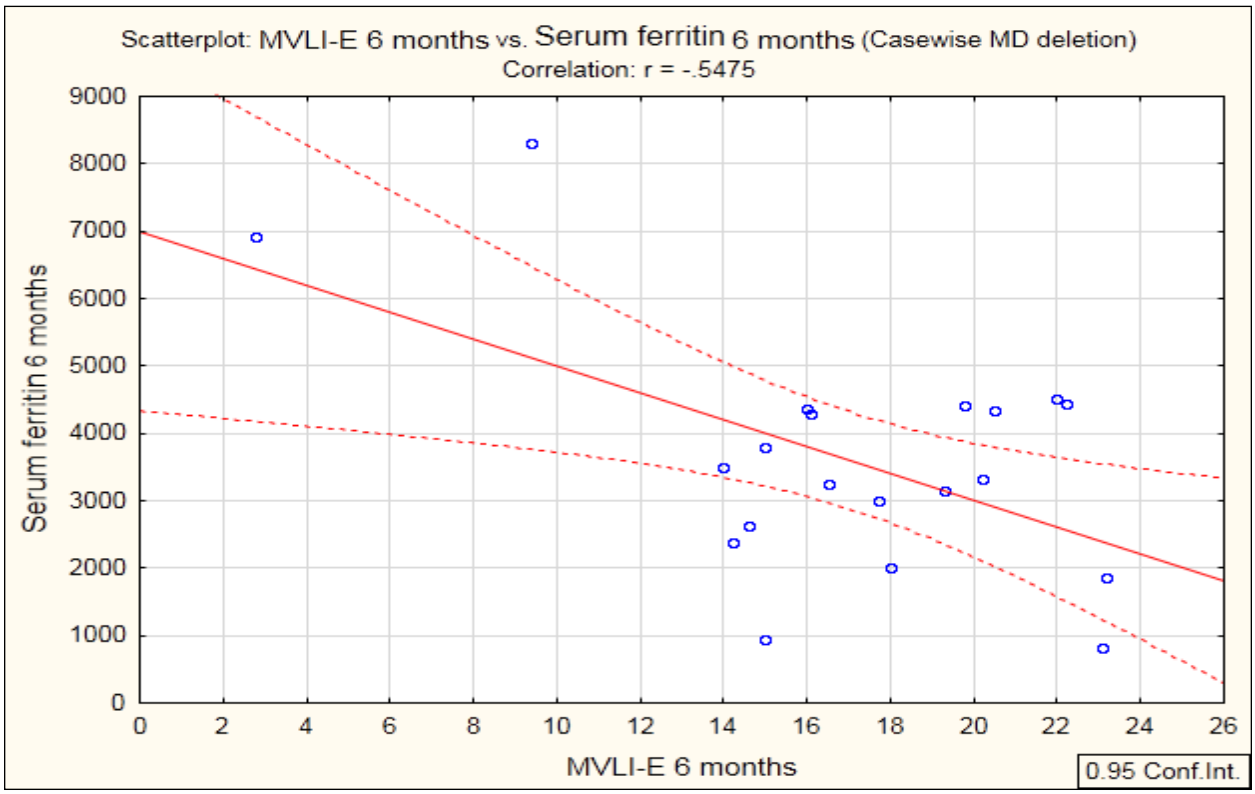
*$p<0.05$

Graph 24: Pearson Correlation between LVEF at 6 months vs S. Ferritin at 6 months in intervention group.



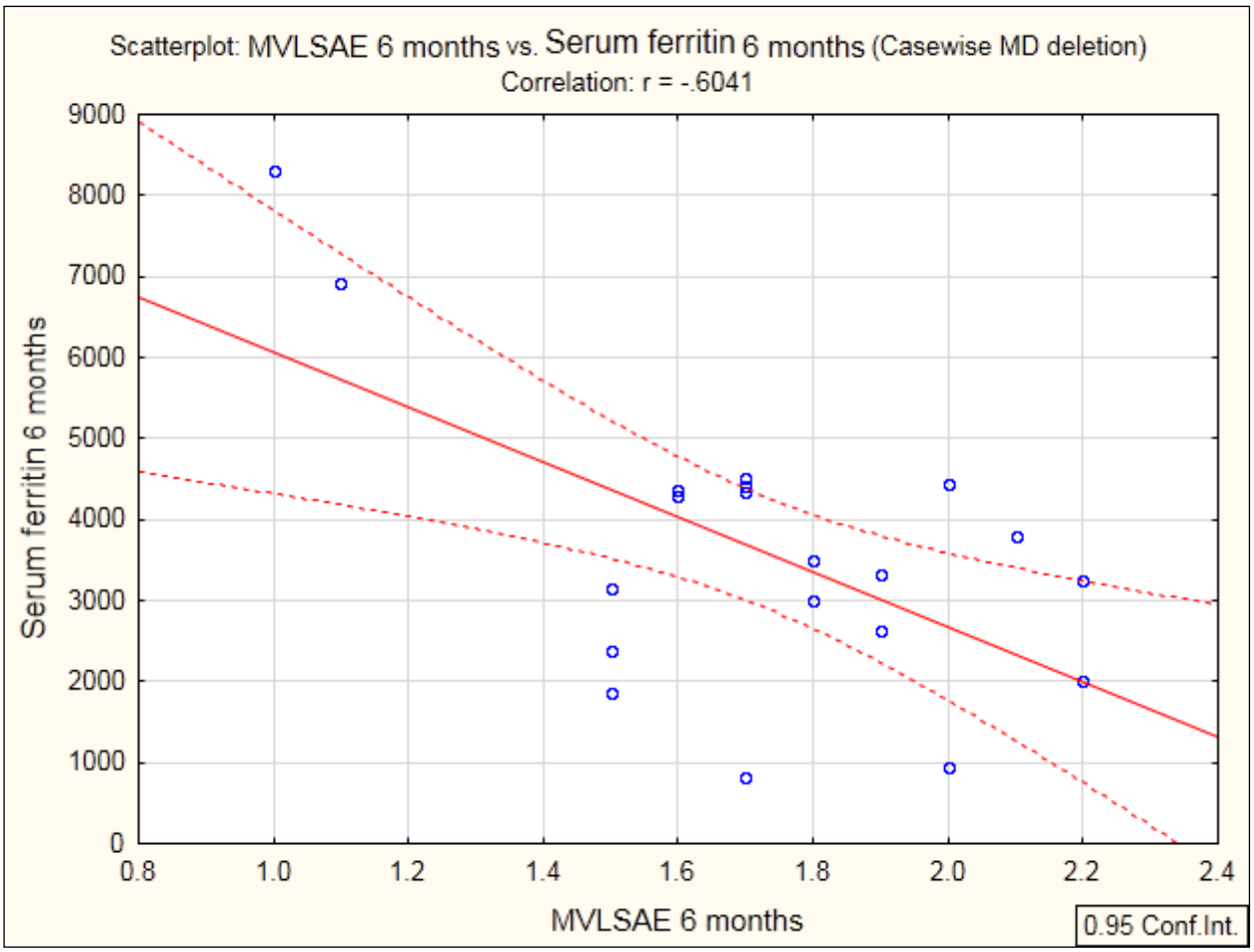
A statistically significant negative linear co-relation exists between LVEF and S. Ferritin levels at 6 months post-intervention (r= -0.6927, p=0.0010*). Hence, from baseline to 6 months, there was a progressive increase in LVEF with decline in S. Ferritin levels recorded in the interventional group.

Graph 25: Pearson Correlation between MVLI-E at 6 months vs S. Ferritin at 6 months in intervention group.



A negative linear co-relation is seen associated with MVLI-E and S. Ferritin levels in intervention group after 6 months of intervention indicating an increase in early diastolic flow velocity (E) measured across Mitral Valve Lateral margin Inflow (MVLI) with progressive decrease in S. Ferritin levels (r= -0.5475, p= 0.0120*).

Graph 26: Pearson Correlation between LVEF at 6 months vs S. Ferritin at 6 months in intervention group.



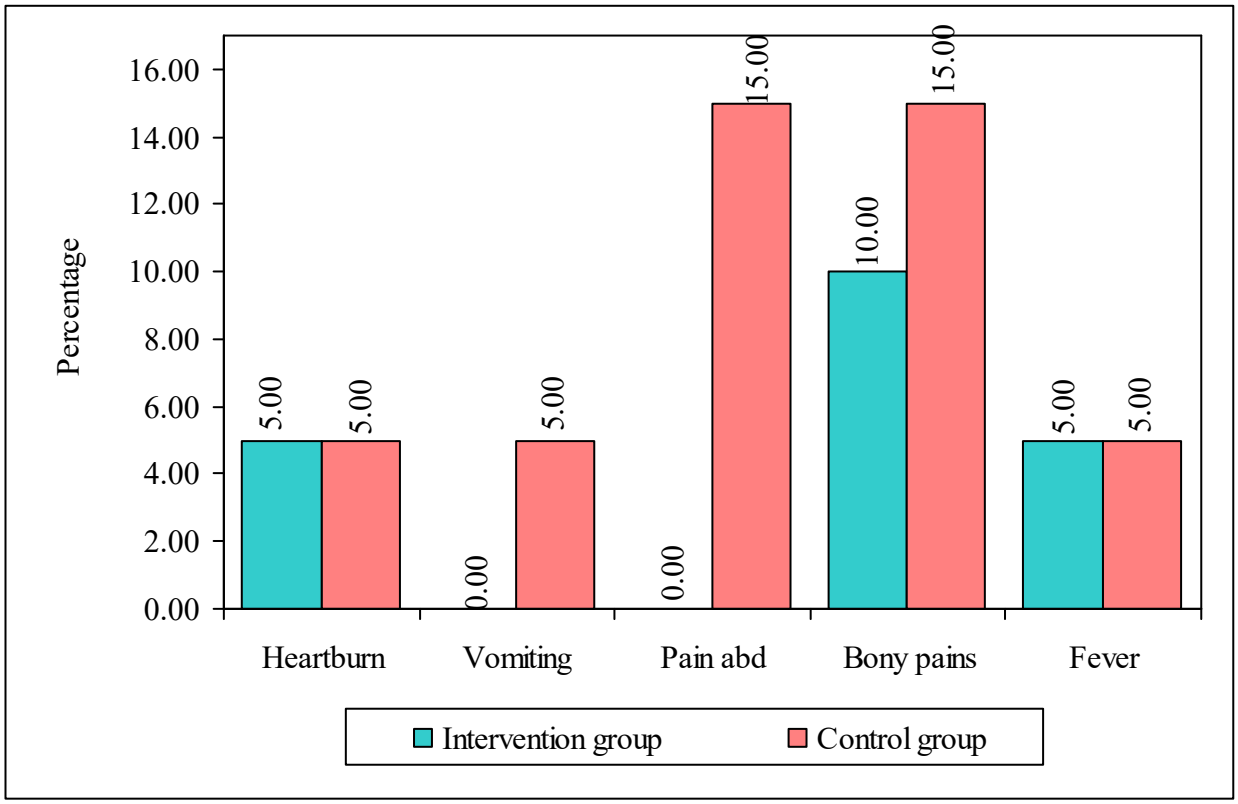
From Baseline to 6 months, there is an improvement in MVLSAE (MAPSE – lateral wall) associated with decline in S. Ferritin levels in the interventional group as recorded by a statistically significant negative linear co-relation between MVLSAE and S. Ferritin levels at 6 months post-intervention (r= -0.6041, p=0.0050*).

Table 32: Comparison of intervention and control group according to the treatment complications

Complaints	Intervention group	%	Control group	%	Total	%	p-value
Heartburn							
No	19	95.00	19	95.00	38	95.00	1.0000
Yes	1	5.00	1	5.00	2	5.00	
Vomiting							
No	20	100.00	19	95.00	39	97.50	0.3110
Yes	0	0.00	1	5.00	1	2.50	
Pain abd							
No	20	100.00	17	85.00	37	92.50	0.0720
Yes	0	0.00	3	15.00	3	7.50	
Bony pains							
No	18	90.00	17	85.00	35	87.50	0.6330
Yes	2	10.00	3	15.00	5	12.50	
Fever							
No	19	95.00	19	95.00	38	95.00	1.0000
Yes	1	5.00	1	5.00	2	5.00	
Total	20	100.00	20	100.00	40	100.00	

In this study, there was no significant adverse event that was reported.

Graph 27: Comparison of intervention and control group with treatment complications



DISCUSSION

Beta thalassemia is a common genetic disorder that also poses a significant public health issue in many countries and India. Beta thalassemia is associated with cardiac dysfunction caused by cardiac iron accumulation especially in beta-thalassemia major patients receiving repeated blood transfusions, manifested as iron overload cardiomyopathy (IOC). One of the major complications is congestive cardiac failure presenting in TM.

Despite good global ventricular function, iron overload in TM causes wall motion abnormalities as an early sign of myocardial involvement. Cardiac T2* MRI is the gold standard investigation because it allows for the estimation of cardiac iron overload, early detection of preclinical changes in EF, therapeutic guidance, and iron chelation therapy follow-up. It is, however, expensive, not widely available in majority of the centres, and requires an expert for reporting. Several studies have shown that Tissue Doppler Imaging when correlated with Cardiac T2* MRI is a sensitive and specific modality for the early detection of myocardial function and accumulation of iron in the heart.[59]

Treatment strategy should aim to reduce the risk of IOC and/or heart failure as well as maintain a pretransfusion Hb of at least 10 g/dl by providing combination chelation therapy and monitoring its compliance. The best option for clearing cardiac iron and stabilizing ventricular function is to use a combination of DFO & DFP or DFP & DFX as both are proven to be equally efficacious.

DFP is a bi-dentate chelator, as well as a small lipophilic molecule capable of entering myocardial cells and facilitating cardiac iron removal by rapidly scavenging

NTBI. Together with Deferasirox, it provides long plasma half-life of chelation for LPI binding. Its use has been linked to lower iron-related cardiac morbidity and mortality. As a result, the current study sought to evaluate the efficacy of combination therapy with oral iron chelators, namely Deferiprone and Deferasirox, in children with transfusion-dependent b-thalassemia, with a focus on cardiac function as measured by echocardiography and tissue Doppler imaging.

This hospital-based single blind randomized controlled trial was done from July 2020 to May 2021. Forty-five children between the ages of 10 and 18 years with transfusion-dependent beta-thalassemia and abnormal conventional 2D-ECHO findings done in last 6 months, were enrolled in the study from thalassemia day care unit under the Department of Pediatrics, KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi. Four splenectomised patients and one patient who died from COVID-19 infection were excluded. A total of 40 patients underwent baseline ECHO-Tissue Doppler Imaging with ECHOCARDIOGRAM - PHILIPS CX 50 REVISION 3.1.1 SOFTWARE, as well as S. ferritin levels, USG assessment of liver and spleen size, and blood investigations, and were then randomly assigned based on a computer generated randomization sequence into the intervention group, with 20 patients receiving Tab. Deferiprone 75mg/kg/day Q8hrly. Both groups continued to receive Tab Deferasirox at a dose of 30mg/kg/day Q24hourly for 6 months before being reassessed for cardiac functions.

In this study, age of the participants ranged from 10 to 18 years. Mean age of children in intervention group was 15.95 ± 2.26 as compared to 14.95 ± 2.63 in control group. The age group is in agreement to a similar study done by MS Elalfy et al., about efficacy and safety of two oral chelators DFX/DFP in severely iron-

overloaded young patients with β-thalassemia major in Egypt with a mean age of 14.05 ± 2.21 years. [9]

Majority of the children in both the intervention group (65%) and control group (60%) were male with a male to female ratio of 1.6:1. The male predominance observed in the present study is similar with the study done by MS Elalfy et al., where group B receiving DFP-DFX combination had 66.6 % male participants as compared to 33.4% female participants showing a male to female ratio of 1.9:1. [9] Study done by Padma Bhatia et.al on demographics of thalassemia in India including 180 children, showed 111 males (61.7%) and 69 females (38.3%) with male predominance agreement to our present study. [22] Ideally in β-thalassemia major with an autosomal recessive inheritance, males and females should be in equal proportion but the higher proportion was seen in males in the present study. This disparity in the present study could be explained due to the gender inequality in our population, where male children are cared better in health-seeking and brought to hospital regularly as it is a chronic illness.

Regarding demographic characteristics, in the present study, majority of the children from intervention group (n=14, 70%) and control group (n= 12, 60%) belonged to rural area.

History of consanguineous marriage was reported was (n=14, 70%) in intervention group and (n= 18, 90%) control group. Thalassemia status of the siblings in the family was positive in 6 children (30%) in intervention group and 5 children (25%) in control group. Study done by Padma Bhatia et al., in thalassemia has shown that out of a total 180 children, 39 (21.6%) children were born to consanguineous parents and remaining 141 (78.3%) to non-consanguineous parents. In the same study [22], thalassemia status in siblings was seen in 23.3% of the patients, which is in

agreement with our present study. The higher incidence of consanguinity in our present study is probably due to the cultural practice of marriage between close relatives in North Karnataka.

In the present study, 7/40 (17.5%) children had the onset of disease at the age of less than 6 months. 20/40 (50%) children had the onset of disease between the age of 6months to 1 year. 13/40 (32.5%) children had the onset of disease after 1 year of age. Study done by Padma Bhatia showed that of the total 180 patients, 102 (56.7%) were diagnosed with thalassemia were between 0 and 6 months, 48 (26.7%) between 6 and 12 months, 24 (13.3%) between 13 and 24 months. [(22)]

COMPARISON BETWEEN MEAN AGE, DURATION OF DEFERASIROX CHELATION AND FREQUENCY OF TRANSFUSION

In the present study, children with mean age of 15.95 ± 2.26 years in intervention group and 14.95 ± 2.63 years in control group received DFX chelation for a mean duration of 7.18 ± 3.63 years and 7.05 ± 3.05 years respectively with frequency of transfusion being 19.75 ± 5.02 transfusions/year in intervention group vs 15.11 ± 4.90 transfusions/year in control group. Although the mean age of the participants and the duration of treatment with DFX is similar between both the intervention and control groups, the frequency of transfusions/year was significantly higher in intervention group as opposed to control group (p= 0.0059*). Cappellini et al., in 2005, conducted an open labelled multicentric RCT with mean age in DFX group 17 ± 9.47 years with blood transfusions of ≥ 8units /year (64). Pennell et al., in 2014 conducted an open labelled RCT where children with thalassemia major on DFX therapy had a mean age 19.8 ± 6.4 years, receiving blood transfusions >10 units/ year. [(65)]

COMPARISON OF PRE-TRANSFUSION HEMOGLOBIN AFTER INTERVENTION:-

The Mean pre-transfusion Hemoglobin measured at 6th month was significantly higher in intervention group as compared to control group (9.16 ± 0.81 vs 8.32 ± 1.18; p= 0.0118*). Study done by Ibrahim M., et al on 100 TM patients below 18 years age treated with DFX for 1 year to evaluate the value of TDI showed that mean Hb of patients was 9.1 ± 2.3 vs 11.5 ± 1.5 of control group. [(7)]

COMPARISON OF ABSOLUTE NEUTROPPHIL COUNTS AFTER INTERVENTION:-

In the present study, the difference in mean ANC and WBC recorded from baseline (1st follow-up) to 6 months post-intervention (12th follow-up) at regular intervals, did not show any difference of statistical significance between the two groups. None of the groups reported neutropenia. However, MS Elalfy showed that with a baseline ANC of 5.1 ± 2.7 in DFP-DFX combination group, at 12 months post-intervention, Neutropenia was reported in 5 patients (10.4%). [(9)]

COMBINATION THERAPY AND SERUM FERRITIN :-

In this study, mean S. Ferritin levels recorded at baseline was 4258.35 ± 1676.90 in intervention group as compared to 3732.75 ± 3160.81 in control group. At 6 months post-intervention, SF values did not show any difference of statistical significance (p=0.3043). Sunil Gomber conducted a study to compare efficacy and safety of oral iron chelators i.e. DFP monotherapy (75 mg/kg/day in 3 divided doses), DFX monotherapy (30 mg/kg/day single dose) and combination of DFP and DFX for 12 months in 49 multi-transfused children with TM. Serum ferritin values decreased

from a mean of 3859.2 ng/mL to 3417.4 ng/mL in DFX alone group and from 3696.5 ng/mL to 2572.1 ng/ mL in the combination group. The combination therapy was more efficacious in causing fall in serum ferritin levels compared to DFP and DFX monotherapy (P=0.035 and 0.040 respectively). [8] Elalfy et al in 2015 compared efficacy of DFP and DFO versus DFP and DFX in 96 β-TM patients aged between 10-18years of age. Mean SF at study end were lower compared to 6 months and to baseline (3219.98±882.25, 3525.57±952.31 and 4289.19±866.21 μg/ L respectively, p=0.001). Farmaki et al., showed that with DFP-DFX combination there was reduction in serum ferritin levels along with improvement in LVEF.[56] Totadri S., showed decrease in mean serum ferritin from 6,769 ng/mL to 3,275 mcg/L in TDT children with DFP-DFX combination therapy.[61] Karami et al., showed significant reduction in ferritin levels to 2800±1900 from 3400±1600 ng/mL before and after treatment, respectively (p <0.6) with DFP-DFX combination chelation therapy. [63]

COMBINATION THERAPY AND RENAL FUNCTION:-

The mean urea level recorded in our study in the intervention group was higher than that of control group at baseline (26.90 ± 10.53 vs 20.70 ± 7.58; p= 0.0391) and at 6 months (24.70 ± 5.75 vs 18.29 ± 6.90; p= 0.0028*). Mean creatinine level were normal both at baseline and at 6 months in both the groups. Whereas, MS Elalfy showed that with a baseline S. creatinine of 0.54 ± 0.21 in DFP-DFX combination group, at 12 months post-intervention, S. creatinine increased by ≥ 33% above baseline on 2 consecutive occasions in 3 patients (6.2%). [9]

COMBINATION THERAPY AND LIVER FUNCTION:-

In the present study, when compared to baseline, the difference in mean SGOT levels at 6 months was significantly lower in intervention group as compared to control group (14.13 vs 22.90; p= 0.0324*). Also, mean SGPT levels at baseline in intervention group was 46.05 ± 29.82 with a decline of mean SGPT levels by 15.20 at 6 months was significant (p= 0.0342*). Hence in our study, no rise in S. transaminases was observed in both groups, whereas, in the study conducted by Elalfy, at baseline the SGPT levels were 38.85 ± 8.01 in group with DFP-DFX combination but at 12 months, 4 patients (8.33%) had ≥ 3 folds increase in SGPT levels with 1 child reported with Acute Cholecystitis. [9]

COMBINATION THERAPY AND LIVER AND SPLEEN SIZE AS ASSESSED BY ULTRASOUND:-

In this study, at baseline the mean liver size in intervention group was higher than that of control group (15.64 ± 1.43 vs 14.84 ± 1.53; p= 0.0958***) by independent t test.*** But at 6 months post-intervention, the mean liver size in intervention group was 14.82 ± 1.89 which has decreased in comparison to baseline values. The mean liver size in the control group at 6 months (15.72 ± 1.73) was found to be higher in comparison to baseline values. This difference was not of any statistical significance (p= 0.1247). The difference in mean liver size from baseline to 6 months post-intervention in intervention group (0.83; p= 0.0139*) and in control group (-0.88; p= 0.0494*) was significantly high ***by dependent t test.***

Similarly, at baseline, the mean spleen size in both intervention group and control group were found to be comparable (15.54 ± 2.51, 15.21 ± 2.52 respectively). But at 6 months post-intervention, the mean spleen size in intervention group was 14.26 ± 1.98 which has decreased in comparison to baseline values. The mean spleen size in the control group at 6 months (16.09 ± 3.39) was found to be higher in comparison to baseline values. This difference was not of any statistical significance (p= 0.0705) ***by independent t test.*** However, the difference in mean spleen size from baseline to 6 months post-intervention was significantly high in intervention group (1.28; p= 0.0079*) ***by dependent t test.***

The difference in mean spleen size from baseline to 6 months post-intervention is 1.28 in intervention group which is of statistical significance (p= 0.0079*).

COMBINATION THERAPY AND ECHO-TISSUE DOPPLER IMAGING: -

In the present study, we found that LVEF, LVESV, LVISd, MVI-E, E/A ratio, MVLI- A & S waves, MAPSE of lateral and septal walls (MVLSAE, MVRSAE), TAPSE in both intervention and control groups were comparable at 6 months. Whereas, LVEDV, LVIDd, MVI-A wave, MVMI- E, A & S wave velocities assessed at 6 months showed difference between the two groups but not of statistical significance. In our study, the mean Fractional Shortening (FS) at 6 months in intervention group was significantly higher than that of control group (34 ± 6.33 vs 28.4 ± 7.04; p= 0.0118*). Ibrahim M., showed FS (%) of 40.6 ± 6.1 as compared to FS (%) of 34 ± 6.33 in this study (7). Further, from baseline to 6 months, we observed that there was significant improvement in MAPSE of Septal wall (MVSSAE) in intervention group (p= 0.0494*). The difference in mean MVLI-E velocity of -2.29 in

control group (t=-2.3472; p= 0.0299*) was found to be statistically significant thus indicating the setting of early diastolic dysfunction in control group receiving DFX monotherapy.

While there are studies that have assessed DFO- DFP combination therapy on cardiac functions with Tissue Doppler Imaging and ECHO (35, 38, 57, 62) or existance of studies that have used T2*MRI to assess improvement in myocardial iron burden in children receiving DFP+DFX combination therapy (9), however, to our understanding from literature review, there are no similar studies that have used Tissue Doppler Imaging to assess efficacy of DFP- DFX combination therapy in improving cardiac functions.

CORRELATION BETWEEN ECHO-TISSUE DOPPLER IMAGING AND SERUM FERRITIN:-

This study demonstrated that in the intervention group, with decline in serum ferritin levels at 6 months, there was a progressive increase in LVEF as shown by statistically significant negative linear correlation between LVEF and S. Ferritin levels (r= -0.6927, p=0.0010*). Similarly, a negative linear correlation is seen associated with MVLI-E and S. Ferritin levels at 6 months indicating an increase in early diastolic flow velocity (E) measured across Mitral Valve Lateral wall Inflow (r= -0.5475, p= 0.0120*). Also, a statistically significant negative linear correlation was found between MVLSAE and S. Ferritin levels at 6 months post-intervention indicating improvement in MAPSE of lateral wall (MVLSAE) associated with decline in S. Ferritin levels at 6 months (r= -0.6041, p=0.0050*).

While studies conducted with iron chelator monotherapy showed no correlation between serum ferritin levels and cardiac dysfunction as assessed by echocardiography, our study with combination oral iron chelator therapy showed positive correlation between the two. [7,60]

Overall, the finding of the present study was that therapy with combination oral iron chelators i.e. Deferiprone & Deferasirox was effective in improving cardiac functions and reducing liver and spleen size in transfusion dependant beta thalassemia major. Tissue Doppler Echocardiography detects early myocardial diastolic and systolic dysfunction rather than just Conventional ECHO. Hence, bi-annual TDE will be both effective and economical in diagnosing cardiac dysfunction in TDT children. Reduction in serum ferritin levels was observed in both groups but not of statistical significance.

While the findings of the study need further validation as it was single center study with a relatively smaller sample size and a short duration of intervention of only 6 months as compared to other larger studies with 1 year intervention. Hence, further multi-centric studies involving larger sample sizes and longer term follow up with larger cohort may focus on the precise role of combination therapy in the prevention and treatment of cardiac dysfunction (IOC) in patients with transfusion-dependent thalassemia major.

CONCLUSION

Based on the findings of this study, it may be concluded that treatment with combination oral iron chelators i.e. Deferiprone and Deferasirox for 6 months was effective in improving cardiac functions, reducing liver and spleen size and significant increase in hemoglobin levels in children with transfusion dependant thalassemia major.

Tissue Doppler Echocardiography can detect early myocardial diastolic and systolic dysfunction than global dysfunction by Conventional ECHO. Hence, bi-annual Tissue Doppler Echocardiography should be done in all children with transfusion dependant thalassemia major, which is economical in diagnosing cardiac dysfunction.

SUMMARY

Deferiprone when used in combination with the other oral iron chelator namely Deferasirox, has proven great efficacy when it comes to reduction in S. ferritin levels and in both prevention and control of iron overload scenario.

However, there are very few studies about the efficacy of this combination therapy on transfusion-dependent thalassemia children with cardiac dysfunction in India. Also with the benefit of detection of early myocardial diastolic and systolic dysfunction by doing Tissue Doppler Imaging rather than just the conventional echocardiography, especially helps in developing countries like ours where T2* MRI is unavailable across many health centres or is not financially economical. This study was aimed to assess the efficacy of combination therapy with oral iron chelators i.e. Deferiprone and Deferasirox in children with transfusion dependant b-thalassemia major on assessing cardiac function by Echocardiography and Tissue Doppler Imaging.

This hospital-based single blind randomized controlled trial was done from July 2020 to May 2021.

Out of 84 patients between the age group of 10-18 years attending thalassemia day care unit under the Department of Pediatrics, KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi and undergoing bi-annual conventional echocardiography, 45 cases of transfusion-dependent beta-thalassemia children with abnormal conventional 2D-ECHO findings were enrolled in the study, amongst which 4 splenectomised patients were excluded and 1 patient death due to COVID-19 infection. Total of 40 patients underwent baseline ECHO-Tissue Doppler Imaging

evaluation performed by Pediatric Cardiologist who was blinded to the treatment allocation in the study along with S. ferritin levels, USG assessment of liver and spleen size amongst other blood investigations and then were randomly assigned based on computer generated randomization sequence into intervention group with 20 patients who received Tab. Deferiprone 75mg/kg/day Q8hrly for 6 months and the other 20 children formed the control group. Both groups continued to receive Tab Deferasirox at 30mg/kg/day Q24hourly dosing for 6 months and were re-assessed for cardiac functions, serum ferritin and liver & spleen size at 6 months.

1. In this study, mean age of children in interventional group was 15.95 ± 2.26 as compared to 14.95 ± 2.63 in control group. No significant difference was seen in distribution of children according to age (p= 0.3420).

1. Majority of the children in both the intervention group (65%) and control group (60%) were male with a male to female ratio of 1.6:1. No significant difference was seen in distribution of children according to gender (p= 0.7440).

2. There were 30% children belonging to Upper class SES in interventional group as compared to 55% children in control group. No significant difference was seen in distribution of children according to Socio-economic status (p=0.5120).

3. Majority of children had fathers who completed education till high school; 55% children in interventional group as compared to 20% children in control group. No significant difference was seen in distribution of children according to fathers education (p= 0.1920).

4. Majority of children in both the intervention (70%) and control group (60%) belonged to rural area.

5. 40% children from interventional group and 60% children from control group had the onset of disease at the age of 6 months to 1 year. However, no significant difference was seen in distribution of children according to years of onset of disease (p= 0.4410).

6. 45% children from intervention group received DFX chelation for a period of 1-5 years in comparison to 70% children from control group who received DFX therapy for 6-10 years. This difference in the duration of treatment with DFX between the two groups was statistically significant (p=0. 0110*).

7. Although the mean age of the participants and the duration of treatment with DFX was similar between both the intervention and control groups, the frequency of transfusions/year was significantly higher in intervention group (19.75 ± 5.02) as opposed to control group (15.11 ± 4.90) (p= 0.0059*).

8. Consanguinity was higher in control group (90%) as opposed to intervention group (70%) but this difference showed no statistical significance (p=0.1140).

9. History of one sibling with thalassemia was recorded in 25% children from intervention group and 20% children from control group while majority i.e. 70% children from intervention group & 75% children from control group did not. The distribution showed no difference and is statistically not significant (p=0.5430)

10. 35% children in intervention group had BMI between 16-<17kg/m2 as opposed to 50% children in control group. Even though the distribution of underweight children was higher in control group than intervention group, the difference was not of statistical significance (p= 0.1350).

11. The Mean pre-transfusion Hemoglobin (g/dl) measured at baseline did not show any statistical significance (p= 0.1357) but after 6 months of intervention, it was higher in intervention group with mean of 9.16 ± 0.81 as compared to 8.32 ± 1.18 in control group (p= 0.0118*). This difference was found to be of statistical significance.

12. With comparison to baseline pre-transfusion Hb values, the Hb levels recorded in intervention group at 12th follow-up (6 months) was found to have a mean difference of -0.73 (p= 0.0091*). Whereas, in the control group, the mean difference in pre-transfusion Hb recorded at 6 months as compared to baseline did not show any statistical significance (p= 0.1882).

13. Mean WBC counts measured at 1st follow-up was 7226.00 ± 3347.60 in intervention group as compared to 13049.00 ± 11410.50 in control group (p= 0.0347) and at 12th follow-up (6 months) was 7614.00 ± 1986.34 in intervention group as compared to 10603.00 ± 5052.31 in control group (p= 0.0185*). These differences in mean WBC counts between both groups were found to be of statistical significance.

14. Mean ANC counts recorded at baseline (p= 0.1168) and 6 months (p= 0.2175) did not show any difference of statistical significance between intervention and control groups.

15. Difference in mean ANC levels recorded from 1st follow-up to 12th follow-up did not show any difference of statistical significance in both the groups. None of the groups reported neutropenia.

16. Mean S. Ferritin levels recorded at 6 months after treatment in intervention group was 3610.80 ± 1785.21 as that of 3030.70 ± 1737.52 in control group. Hence, the results were comparable and did not show any difference of statistical significance (p=0.3043).

17. There exists a difference in the mean S. Ferritin levels from baseline to 6 months post-randomization, recorded at regular intervals between both the intervention group (p= 0.0926) and control group (p= 0.2486), but this difference was not of any statistical significance.

18. At 6 months post randomization, mean urea levels were higher in intervention group as compared to control group (24.70 ± 5.75 vs 18.29 ± 6.90; p= 0.0028*).

19. Mean creatinine levels between the two groups at baseline and 6 months did not show any difference of statistical significance (p= 0.0879, p= 0.4511).

20. Mean SGOT levels at baseline and at 6 months recorded between both the groups did not show any difference of statistical significance. However, difference in mean SGOT levels was significantly lower in both the groups at 6 months post randomization compared to baseline (14.13; p= 0.0325* and 22.90; p= 0.0324*).

21. Difference in mean SGPT levels between the intervention group and control group at baseline and at 6 months did not show any statistical significance.

22. Significant difference in mean SGPT levels of 15.20 at 6 months post-intervention in comparison to baseline values in the intervention group (p= 0.0342*) was found to be significant. Such difference was not recorded in control group.

23. Mean liver size recorded at baseline in intervention group was higher than that of control group (15.64 ± 1.43 vs 14.84 ± 1.53; p= 0.0958). But 6 months after treatment, the mean liver size was lower in intervention group as compared to control group (14.82 ± 1.89 vs 15.72 ± 1.73; p= 0.1247). This difference was not of any statistical significance. However, The difference in mean liver size from baseline to 6 months post-intervention in intervention group (0.83;p= 0.0139*) and in control group (-0.88; p= 0.0494*) was significantly high.

24. Mean spleen size recorded at baseline in intervention group and control group were similar (15.54 ± 2.51 vs 15.21 ± 2.52; p= 0.7259). But 6 months after treatment, the mean spleen size was lower in intervention group as compared to control group (14.26 ± 1.98 vs 16.09 ± 3.39; p= 0.0705). This difference was not of any statistical significance. However, the difference in mean spleen size from baseline to 6 months post-intervention was significantly high in intervention group (1.28; p= 0.0079*).

25. Difference was noted with respect to the ECHO-TDI assessment of the participants using the parameters: LVEF, LVEDV, LVESV, LVIDd, LVISd, FS, Mitral valve inflow velocities E & A, E/A ratio, Mitral valve lateral inflow velocities E, A & S, Mitral valve septal inflow velocities E, A & S, MAPSE of lateral and septal walls and TAPSE, between the interventional group and control group at baseline, but not of statistical significance. However, mean FS after 6 months of treatment in intervention group was higher in comparison to that of control group (34 ± 6.33 vs 28.4 ± 7.04; p= 0.0118*). This decline in FS in control group at 6 months was significant. Like the ejection fraction, this is a measure of the myocardial contractility and is affected by preload and afterload.

If the internal diameter fails to shorten by atleast 25% (<25% FS is abnormal), the efficiency of heart in ejecting blood is impaired. Here systolic function in control group is impaired.

26. Also, LVIDd at baseline was 4.33 ± 0.48 in intervention group as compared to 4.06 ± 0.33 in control group. This difference was found to be statistically significant (p= 0.0486*).

27. In this study, MAPSE of Septal wall (MVSSAE) showed significant improvement from baseline to 6 months (p= 0.0494*) in intervention group and we also observed a statistically significant reduction of MVLI-E velocity from baseline to 6 months post-randomization in the control group however no such significant reduction was found in the intervention group. The difference in mean MVLI-E velocity of -2.29 in control group (t=-2.3472; p= 0.0299*) was found to be statistically significant thus indicating the setting of early diastolic dysfunction in control group.

28. A statistically significant negative linear correlation exists between LVEF and S. Ferritin levels at baseline (r= -0.5189, p= 0.0190*) & at 6 months post-intervention (r= -0.6927, p=0.0010*). Hence, from baseline to 6 months, there was a progressive increase in LVEF with decline in S. Ferritin levels recorded in the interventional group.

29. A negative linear correlation is seen associated with MVLI-E and S. Ferritin levels in intervention group after 6 months of intervention indicating an increase in early diastolic flow velocity (E) measured across Mitral Valve Lateral margin

Inflow (MVLI) with progressive decrease in S. Ferritin levels (r= -0.5475, p= 0.0120*).

30. From Baseline to 6 months, there was an improvement in MVLSAE (MAPSE of lateral wall) associated with decline in S. Ferritin levels in the intervention group as recorded by a statistically significant negative linear correlation between MVLSAE and S. Ferritin levels at 6 months post-intervention (r= -0.6041, p=0.0050*).

31. No significant adverse events were reported in either groups.

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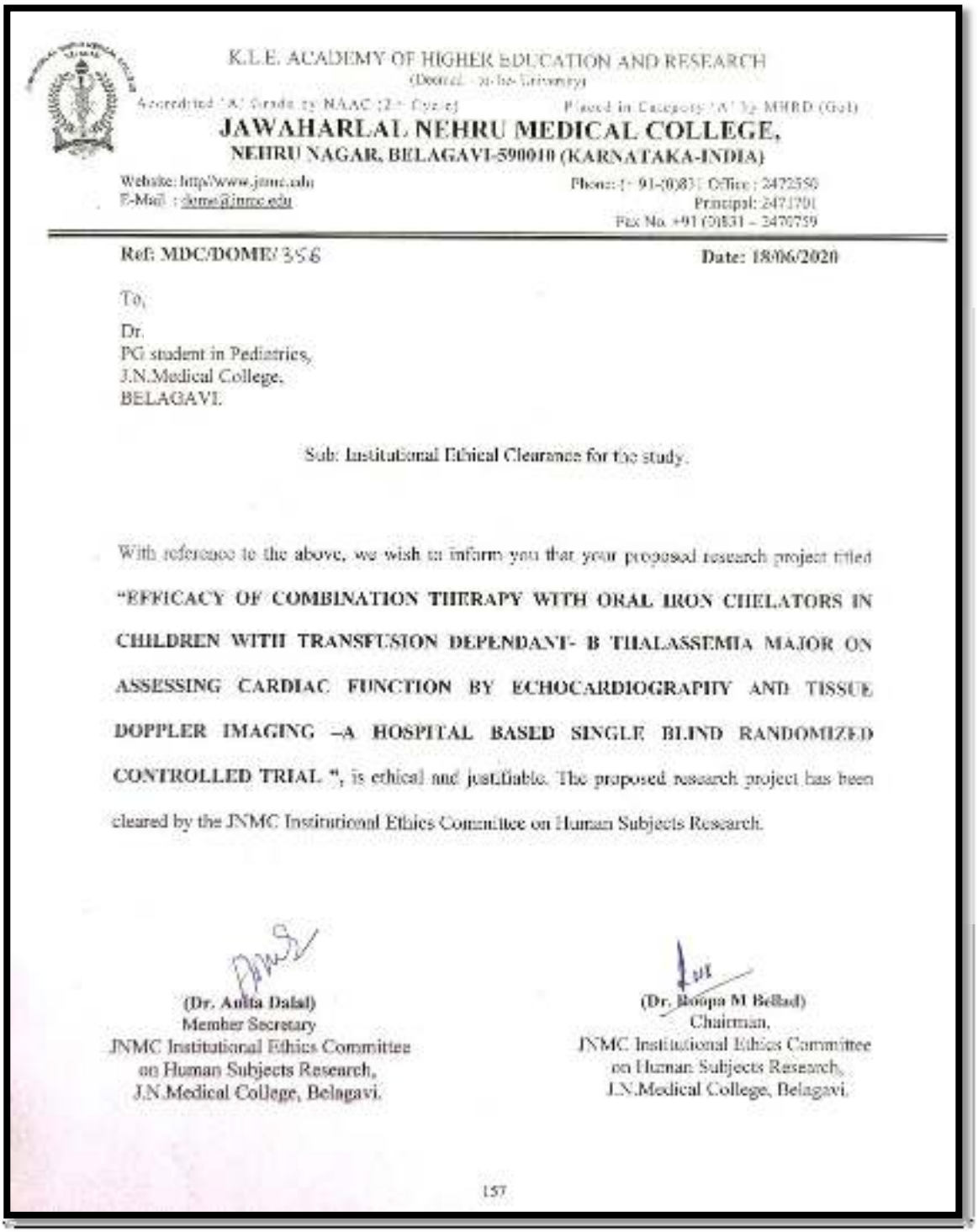
ANNEXURE-I- ETHICAL CLEARANCE LETTER



K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed - to-be University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (Govt)

JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-0831) Office : 2472550
Principal: 2471701
Fax No. +91 (0831 – 2470759

Ref: MDC/DOME/ 356 Date: 18/06/2020

To,

Dr.
PG student in Pediatrics,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EFFICACY OF COMBINATION THERAPY WITH ORAL IRON CHELATORS IN CHILDREN WITH TRANSFUSION DEPENDANT- B THALASSEMIA MAJOR ON ASSESSING CARDIAC FUNCTION BY ECHOCARDIOGRAPHY AND TISSUE DOPPLER IMAGING –A HOSPITAL BASED SINGLE BLIND RANDOMIZED CONTROLLED TRIAL "**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

157

ANNEXURE II – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

"EFFICACY OF COMBINATION THERAPY WITH ORAL IRON CHELATORS IN CHILDREN WITH TRANSFUSION DEPENDANT B-THALASSEMIA MAJOR ON ASSESSING CARDIAC FUNCTION BY ECHOCARDIOGRAPHY AND TISSUE DOPPLER IMAGING- A HOSPITAL BASED SINGLE BLIND RANDOMISED CONTROLLED TRIAL"

Principal Investigator: Dr.

Guide: Dr.

Co-Guide: Dr.

You are hereby requested to involve yourself and your child in the above said research to be conducted at KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from July 2020 to May 2021 by me.

Introduction: In children with Thalassemia Major on regular transfusions, myocardial iron overload eventually leads to congestive heart failure and is the most common cause of mortality in this population. Chelation therapy is used to prevent iron overload as it can reduce iron accumulation in the organs. New echocardiographic techniques such as Tissue Doppler Imaging (TDI) are widely available and can be used to assess cardiac function, as well as to screen and detect subclinical cardiac dysfunction. Hence, the proposed study is aimed at assessing the efficacy of combination therapy with oral iron chelators on assessing cardiac function by Echocardiography and Tissue Doppler Imaging

Voluntary participation: Your and 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.

Risk and benefits: This study is a Randomized Controlled Trial involving intervention with oral combination therapy of deferiprone and deferasirox which appears to be an efficacious modality to reduce cardiac iron overload so as to prevent early cardiac systolic and diastolic dysfunction and in reducing serum ferritin levels in multi-transfused children with thalassemia major monitored by investigations routinely and the outcome assessed by ECHO and TDI. The risks involved like gastric irriatation i.e. nausea, vomiting, abdominal pain, joint pains, fever etc., for which appropriate medical treatment will be provided has been explained.

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.

Financial incentive for participation: You or your child will not receive any financial assistance for participating in this study.

Queries: If you have any queries you may contact

Dr.

Post Graduate Student

Department of Pediatrics

JNMC ,Belagavi-590010

Phone No.

Dr.

MBBS, MD, FRCP, (Pediatrics)

Professor, Department of Pediatrics

JNMC ,Belagavi-590010

Phone No.

Dr.

MBBS, MD, FNB, (Pediatrics)

Pediatric Cardiologist and Professor

JNMC, Belagavi- 590010

Phone No-

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

Dr. Roopa M Bellad

Chairperson of Ethical Committee

JNMC Belgavi-590010

Phone No.9448113403

You will be given a copy of this form for your information and to keep for your records.

STATEMENT OF CONSENT

I hereby voluntarily agree for my and my childs participation in this study. I understand that even if I choose to allow my child 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 authorized representative/ parent: _____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator: _____

Date: _____

Name: _____

ANNEXURE-III - PROFORMA

(GROUP A/B)

INFORMATION OF CHILD:

Name/ID No.:

Age

Sex:

Address:

Socioeconomic status: class I II III IV

SES CLASS	REVISED INCOME CATEGORIES FOR ALL INDIA 2014
1. Upper class	≥ 5357
2. Upper middle class	2652-5356
3. Middle class	1570-2651
4. Lower middle class	812-1569
5. Lower class	≤ 811

Parents educational status:

Mother - High school / PUC / degree / University

Father - High school / PUC / degree / University

Phone no -

Std:

DETAILS OF THALASSEMIA HISTORY:

- Age of onset of thalassemia :
- Management history:
- Drugs : chelating agents :-

a) Desferoxamine-

Dose:-

Duration :

b) Deferasirox-

Dose:-

Duration :

Any other : Pantoprazole/ Ca / FA / Zinc / BC

(YES/NO)

BLOOD TRANSFUSION HISTORY :

Onset of transfusion -	1-2 months of diagnosis	2-6 months of diagnosis	>6months of diagnosis

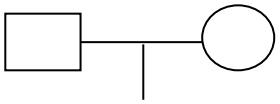
Frequency of blood transfusions- _____ months/year

If frequency of blood transfusions has Increased/ Decreased

FAMILY HISTORY:

INFORMATION OF PARENTS-

Consanguinous / non consanguinous:



Siblings of the child & their thalassemia status:

ANTHROPOMETRY:

	Measured	Expected	Percentile
Weight			
Height			
BMI			

Inference -

	1st (cms)	2nd (cms)	3rd (cms)
Liver span			
Spleen span			

Investigations:-

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Hb												
WBC												
ANC												

	Baseline	**1st**	**2nd**	**3rd**
S. Ferritin				
Urea				
Creat				
SGOT				
SGPT				

	Baseline	**At 6 months**
USG- **Liver size** **Spleen size**		

	Baseline	**At 6 months**
ECHO		
TDI		

ECHO-TDI PARAMETERS

1. Mitral Valve Inflow (cm/s): - E- A- E/A-
2. Mitral Valve Lateral Inflow (cm/s): - E- A- S'-
3. Mitral Valve Septal Inflow (cm/s): - E- A- S'-
4. Mitral Valve Lateral Systolic Annulur Excursion (cm)-
5. Mitral Valve Septal Systolic Annulur Excursion (cm)-
6. TAPSE (cm)-
7. LVEF (%)-
8. FS (%)
9. LVIDd (cm)-
10. LVISd (cm)-

10.LVEDV (ml)-

11. LVESV (ml)-

Complications seen (YES/NO):-

- Heart burn
- Vomiting
- Abdominal pain
- Joint pains
- Fever

ANNEXURE-IV - KEY TO MASTER CHART

1. R. no: Randomization sequence number
2. Group A/B- A-Intervention group, B- control group

3. DEMOGRAPHICS:-

a) Age in years

b) Sex: M- Male, F- Female

c) SES (Socio-economic status) – 0: No, 1: Yes

d) Mothers education – 0: No, 1: Yes

e) Fathers education – 0: No, 1: Yes

f) Residence: Urban/ Rural - 0: No, 1: Yes

4. PAST HISTORY:-

a) Age of onset of disease - 0: No, 1: Yes

b) Treatment with Desferrioxamine (DFO) - 0: No, 1: Yes

c) Desferrioxamine duration in years

d) Treatment with Deferasirox (DFX) - 0: No, 1: Yes

e) Deferasirox duration in years

f) Folic acid, Calcium, Zinc, B-complex, Pantop - 0: No, 1: Yes

g) Blood Transfusion (BT) onset - 0: No, 1: Yes

h) Frequency of transfusion- units/year

i) Consanguinous marriage - 0: No, 1: Yes

j) Siblings with thalassemia - 0: No, 1: Yes

5. ANTHROPOMETRY:-

a) Weight for Age (W/A) in kilograms

b) Height for Age (H/A) in centimeters

c) BMI in kg/m2 - 0: No, 1: Yes

6. GENERAL PHYSICAL EXAMINATION:-

a) Dark skin - 0: No, 1: Yes

b) Hemolytic facies - 0: No, 1: Yes

c) Pallor - 0: No, 1: Yes

d) Flat nasal bridge - 0: No, 1: Yes

e) Dental malocclusion - 0: No, 1: Yes

7. PER ABDOMEN:-

a) Liver span at 1st, 2nd and 3rd follow-up in centimeters

b) Spleen span at 1st, 2nd and 3rd follow-up in centimeters

8. INVESTIGATIONS:-

a) Haemoglobin (Hb) in g/dl

b) White Blood Count (WBC) in cells/cumm

c) Absolute Neutrophil Count (ANC) in cells/cumm

d) Serum Ferritin (SF) at Baseline, 1st , 2nd and 3rd follow-up in ng/ml

e) Urea at Baseline, 1st , 2nd and 3rd follow-up in mg/dl

f) Creatinine at Baseline, 1st , 2nd and 3rd follow-up in mg/dl

g) SGOT at Baseline, 1st , 2nd and 3rd follow-up in IU/L

h) SGPT at Baseline, 1st , 2nd and 3rd follow-up in IU/L

9. USG ABDOMEN:-

a) Liver span at Baseline and 6 months in centimeters

b) Spleen span at Baseline and 6 months in centimeters

10. ECHO- TDI PARAMETERS:-

a) LVEF (Left Ventricular Ejection Fraction) at baseline and 6 months in %

b) LVEDV (Left Ventricular End Diastolic Volume) at baseline and 6 months in millilitre

c) LVESV (Left Ventricular End Systolic Volume) at baseline and 6 months in millilitre

d) LVIDd (Left Ventricular Internal Diameter in diastole) at baseline and 6 months in centimetres

e) LVIDs (Left Ventricular Internal Diameter in systole) at baseline and 6 months in centimetres

f) Fractional Shortening (FS) at baseline and 6 months in percentage

g) MVI (Mitral Valve Inflow) at baseline and 6 months

E- Early diastolic flow velocity in centimetres/second

A- Late diastolic flow velocity in centimetres/second

h) MVLI (Mitral Valve Lateral Inflow) at baseline and 6 months

E- Early diastolic flow velocity in centimetres/second

A- Late diastolic flow velocity in centimetres/second

S- systolic flow velocity in centimetres/second

i) MVSI (Mitral Valve Septal Inflow) at baseline and 6 months

E- Early diastolic flow velocity in centimetres/second

A- Late diastolic flow velocity in centimetres/second

S- systolic flow velocity in centimetres/second

j) MVLSAE (Mitral Valve Lateral Systolic Annular Excursion) at baseline and 6 months in centimetres

k) MVSSAE (Mitral Valve Septal Systolic Annular Excursion) at baseline and 6 months in centimetres

l) TAPSE (Tricuspid valve Annular Plane Systolic Excursion) at baseline and 6 months in centimetres

11. COMPLICATIONS:- Heartburn, vomiting, pain abdomen, joint pains, fever - 0: No, 1: Yes

		DEMOGRAPHIC DATA																		PAST HISTORY																		ANTHROPOMETRY								GENERAL				PER ABDOMEN			
S. No	R. Number	Group A/B	Age	Sex	SES - lower class	SES-lower middle	SES-middle class	SES-upper middle	SES-upper class	mother- high school	mother-Pre-University College	Mother- Degree	Mother- university	Father- High school	Father-PUC	Father- Degree	Father- University	Urban	Rural	age of onset of disease - <6months	age of onset of disease- 6m-1yr	age of onset of disease: >1yr	DFO	DFO- duration	DFX	DFX- duration	Folic acid	calcium	zinc	B-complex	Pantop	BT onset: 1-2m of diagnosis	BT onset: 2-6m	BT onset: >6m	frequency of transfusion- units/yea	Consanguinous marriage	Siblings with thalassemia	W/A	H/A	BMI- <16 kg/m2	BMI- 16-<17 kg/m2	BMI- 17-<18.5 kg/m2	BMI- 18.5 - <25 kg/m2	BMI- 25- <30 kg/m2	BMI: ≥30 kg/m2	dark skin	hemolytic facies	pallor	flat nasal bridge	dental malocclusion	liver span 1st	liver span 2nd	liver span 3rd	spleen span 1st
1	1	B	13	F	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1	0	0	0	1	10	1	1	0	0	0	0	1	0	15	0	0	27	138	1	0	0	0	0	0	0	1	1	0	1	13	13	17	13
2	5	B	15	M	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	10	1	1	0	1	0	1	0	0	2	1	1	36.2.	148	0	1	0	0	0	0	1	1	1	0	1	12	12	11	7
3	7	B	17	M	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	2	1	1	0	1	0	1	0	0	18	1	0	28	130	0	1	0	0	0	0	1	1	1	1	0	12	13	14.5	10
4	9	B	14	F	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	8	1	1	0	1	0	0	0	1	12	1	0	28	144	1	0	0	0	0	0	1	1	1	0	0	14	16	17	14
5	11	B	13	F	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	9	1	1	0	1	0	1	0	0	12	1	0	26	138	1	0	0	0	0	0	1	0	1	0	1	16	16	16	16
6	13	B	12	F	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	7	1	1	0	1	0	0	1	0	16	1	1	24	137	1	0	0	0	0	0	1	1	1	0	0	14	14.5	14.5	15
7	14	B	18	M	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	10	1	1	0	1	0	1	0	0	17	1	2	42	162	0	1	0	0	0	0	0	1	1	1	0	12	12	11	12
8	16	B	23	M	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	1	2Y	1	10	1	1	0	0	0	0	1	0	12	1	0	47	153	0	0	0	1	0	0	0	1	1	0	0	12	14	15	13
9	19	B	10	F	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2	1	0	0	1	0	1	0	0	12	1	0	24	134	1	0	0	0	0	0	1	1	1	0	0	14	14	15	14
10	21	B	18	F	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	6	1	1	1	1	1	0	1	0	16	1	0	47	147	0	0	0	1	0	0	1	1	1	0	0	15	15	15.5	17.5
11	23	B	17	M	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	5	1	1	0	0	0	1	0	0	12	0	1	35	146	0	1	0	0	0	0	1	1	1	0	0	11	12	13	14
12	24	B	14	M	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	8	1	1	0	1	0	0	0	1	24	1	0	22	132	1	0	0	0	0	0	1	1	1	0	1	17	14	13	13
13	25	B	15	M	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	7	1	1	0	1	0	1	0	0	18	1	0	37	146	0	0	1	0	0	0	1	1	1	0	0	10	10	10	10
14	28	B	18	F	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	6	1	1	1	1	1	0	1	0	16	1	0	47	147	0	0	0	1	0	0	1	1	1	0	0	15	15	16	17.5
15	30	B	16	M	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	13	1	1	0	1	1	1	0	0	24	1	0	26.5.	133	1	0	0	0	0	0	1	1	1	1	1	12	14.5	15	10
16	32	B	12	F	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	3	1	1	0	1	0	0	1	0	18	1	0	27	135	1	0	0	0	0	0	1	0	1	0	1	9	10	12	7
17	34	B	13	M	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	10	1	1	0	1	1	0	1	0	12	1	0	25	125	1	0	0	0	0	0	1	1	1	0	1	14.5	14.5	13	12
18	37	B	12	M	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	6	1	1	0	1	0	1	0	0	13	1	0	31	132	0	0	1	0	0	0	1	1	1	1	1	12	12	13	15
19	38	B	15	M	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	3	1	1	0	1	0	0	1	0		1	0	28	139	1	0	0	0	0	0	1	1	1	0	0	12	10.5	10.5	15
20	40	B	17	M	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	6	1	1	0	1	0	0	1	0	18	1	1	30	141	1	0	0	0	0	0	1	1	1	0	1	13	13	12	15

MASTERCHARTS- 2020 (CONTROL GROUP)

		INVESTIGATION																														
spleen span 2nd	spleen span 3rd	Hb-1st	HB2nd	HB-3rd	Hb-4th	Hb-5th	Hb-6th	Hb-7th	hb-8th	hb-9th	hb-10th	hb-11th	hb-12th	WBC-1st	WBC-2ND	WBC-3RD	WBC-4TH	WBC- 5th	WBC-6TH	WBC-7TH	WBC-8TH	WBC-9TH	WBC-10TH	WBC-11TH	WBC-12TH	ANC-1	ANC-2	ANC-3	ANC-4	ANC-5	ANC-6	ANC-7	ANC-8	ANC-9
12.5.	17	7.3	7	10.1	6.1	8.2	7.8	8.9	8.8	8.4	8.6	7.2	7.4	2620	3570	3450	2480	2520	2440	3550	3480	2960	3430	3100	2790	1388	1820	1725	1760	1209	1415	1846	1844	1568
9	9	8.8	9.2	9.2	9.2	8.6	8.4	7.8	9	8.7	8.8	8.9	8.8	6170	7520	8240	7780	8250	8080	8470	9400	6840	8260	8560	10400	2899	4286	4696	4279	4372	4363	5251	4512	3196
10	11	8.4	8.7	9.2	9.2	9.3	10.1	8.3	9.6	10.2	8.8	8.3	7.8	11540	9100	10590	10100	8620	8300	8950	7950	9340	6860	8950	9750	6325	4368	5880	5454	4827	4150	5012	4690	5697
15	15	8.9	7.4	7.4	6.9	9	6.3	7.4	10.3	8.6	9.9	8.7	9	9960	10420	11550	9090	8360	8160	9390	10340	8730	7160	8120	10540	5776	5720	7738	5999	5099	6120	6385	5253	5412
17	17	5.2	4.1	9.4	8	6.9	8.9	9.5	9.5	7.7	10.2	9.5	8.7	15900	15900	9400	15100	12400	11000	15700	20000	19800	12200	11200	10390	8745	8427	2538	6493	4340	3190	5024	7200	4950
15	15	7.6	6.8	4.4	9.8	9.4	8.9	6.7	9.4	8.4	7.8	9.1	6.6	7480	10640	11290	8710	8350	6620	4870	7100	7160	6930	9320	10570	3665	7102	5488	5568	5678	4170	2581	3905	4224
12	10	6.8	9.1	9	9.4	8.5	8.7	8.7	9.1	8.4	9	8.3	9.7	4340	5730	3680	2570	4020	5090	5090	5410	3000	3990	3260	5450	2083	3036	1692	1259	1648	2443	2443	2322	1290
13	14	8.7	8.5	9	9.8	9.4	9.7	10.2	10.7	9.3	9.7	8.9	10.7	41000	26200	24970	21500	21800	14800	19700	18400	18000	20500	21400	23200	12710	14410	12948	10750	9810	7548	9456	9384	8640
14	15	7.5	8	8.3	8.4	9.7	8.8	4.7	6.8	6.6	8.7	4.7	7.5	20000	12370	17740	14000	15440	10100	17900	13500	19020	18300	17900	20000	7600	5781	10799	7980	7546	7575	12790	7830	11590
17	20	9.9	11.1	8.9	10	11	9.9	11.3	7.5	8	8.3	8.3	8.8	7680	8660	7200	7620	9160	8130	8270	7000	8250	5500	5700	8120	5145	5848	4248	4648	6188	5508	5623	4480	5330
14	15	8.8	9.3	8.5	8.4	8.4	8.8	9.5	8.5	8.4	8.4	8.1	8.5	28200	9150	26200	15500	29000	13300	17900	26000	5960	23900	16600	9700	5922	1820	8646	3565	16240	7980	9487	16380	3304
14	14.5	4.2	8.3	8.1	8.6	7.9	8	8.6	8.7	8.8	9.2	7.3	8.7	41300	12000	13000	7700	9600	16100	17400	17900	18500	26150	17900	15100	26019	6000	7150	5698	6336	8211	8178	9845	12395
11	12	9.6	9.1	9.4	8.4	8.6	9.7	9	10.5	11.1	10.5	9.1	9	3160	2460	3230	3300	3770	3610	4030	5490	5890	5620	7800	8920	1488	1549	1937	2079	2412	1728	2400	3239	3654
17	20	9.9	11.1	8.9	10	11	9.9	11.3	7.5	8	8.3	8	9	7680	8680	7200	7620	9160	8130	8270	7000	8250	5500	7680	8200	5092	5902	4248	4626	6228	5528	5623	4480	5362
13.5	15	6.1	6.9	7.9	9.1	8.2	8.5	7.7	8.7	8.3	8.8	7.7	7.8	3650	3870	5570	3870	4260	5760	5530	3540	4570	6100	5470	6230	1861	2476	3355	2322	2726	3628	3318	1734	2513
7.5	6.5	8.5	9.5	9.4	7.9	7.7	8.5	8.6	8.7	9.1	9.2	9.4	8.3	9560	12380	13220	17470	10100	12200	14460	12700	12700	11900	11400	10700	6022	6273	6072	10266	4747	5978	6912	6985	5715
12	13	7.8	7.9	9.5	7.6	9.9	8.8	8.7	10.9	7.1	6.6	8	6	9380	10730	8490	7900	7350	9450	10520	10740	6690	6530	7200	4280	5859	6848	4839	4503	3675	5292	5985	6634	3880
16.5	17	7.9	9.3	8.7	8.9	10	10.1	9.9	10.4	9.7	8.4	9.5	9.4	7130	7540	9880	8950	9780	8780	7920	12600	8140	9760	9080	14300	3279	3543	5292	4385	4205	5092	3484	6552	3888
15	14	7.6	7.1	9.1	11.7	8.3	8.2	8.9	8.8	8.2	8.5	8.3	8.5	15820	15190	9820	14530	13290	10600	19300	19500	19200	11000	13300	16100	11534	13137	5586	12180	8580	6466	13317	14040	16320
14	14	6.6	5.9	7.2	6.6	5.9	4.3	5.5	9.2	8.7	7.6	7.1	6.1	8410	8050	5780	3870	3330	3710	2720	5100	5710	5560	7040	7320	4032	3600	3757	2051	1565	1554	1512	2448	2679

																									USG ABDOMEN																					
ANC-10	ANC-11	ANC-12	SF-BASELINE	SF-1st	SF-2nd	SF-3rd	UREA- BASELINE	UREA-1st	UREA-2nd	UREA-3rd	CREATININE-Baseline	CREATININE-1st	CREATININE-2nd	CREATININE-3rd	SGOT-Baseline	SGOT-1t	SGOT-2nd	SGOT-3rd	SGPT-Baseline	SGPT-1st	SGPT-2nd	SGPT-3rd	LIVER SIZE -Baseline	SPLEEN SIZE-Baseline	LIVER SIZE-6 months	SPLEEN SIZE-6 months	LVEF-Baseline	LVEF-6 months	LVEDV-Baseline	LVEDV-6 months	LVESV-Baseline	LVESV-6 months	LVIDd-Baseline	LVIDd-6 months	LVISd-Baseline	LVISd-6 months	FS-Baseline	FS-6 months	MVI-E (Baseline)	MVI-E 6months	MVI-A (Baseline)
1886	1736	1590	781	2619	4591	5995	23	15	20	18	0.4	0.5	0.7	0.8	45	77	52	41	28	73	60	88	16	16	17	22	60%	60%	52	100	30	55	3.8	4.5	2.9	3.1CM	24	31	100	100	88.2
3882	4194	5304	965	1364	1390	1621	17	28	35	41	0.4	0.6	0.3	0.4	21	18	24	26	15	15	18	13	13.7.	12	14	12	*65%	55	64	98	36	44	4	4.3	2.8	3.1	30	28	129	100	45.4
4321	5251	4387	1633	1577	1491	1136	36	33	27	12	1	0.5	0.4	0.4	122	22	24	25	89	12	12	11	13.5.	10.4.	15.8	11.4	53%	60	67	84	32	30	4.8CM	4.3	3.3	3.3	31	23	100	135	88.2
4009	4709	7035	1627	1564	1668	1890	23	34	13	20	0.9	1.2	0.3	0.2	19	51	30	30	11	135	13	14	14.5	15.1	18	16	*64%	58%	60	95	32	43	3.8CM	4.1	2.2	2.4	42	41	104	130	86.9
5124	5152	4326	12256	5403	7245	4000	23	24	31	20	0.4	0.3	0.3	0.3	188	154	60	62	176	78	74	56	16.9	17	17	18	64%	62%	64	45	36	17	4.1CM	4.2	2.8	2.8	32	33	70	120	44
3811	5498	6195	1976	3084	2984	3266	14	20	15	18	0.4	0.5	0.3	0.8	102	42	83	38	126	58	118	39	15.5.	15.4.	15	15	*63%	57%	39	45	15	19	4.2CM	3.6	2.2	2.9CM	48	19	105	130	65
1560	1825	2616	2222	1284	1641	1637	21	18	30	18	0.4	0.3	0.4	0.6	34	32	27	48	39	33	57	51	16.3.	17.2.	15	15.1.	*60%	49%	52	95.5	32	49	4	4.9	3.1	3.8CM	23	22	140	121	69
4715	9630	11600	4965	7483	3671	4265	25	19	17	10	0.7	0.5	0.5	0.8	75	114	100	63	96	118	84	50	14.3.	15	19.6	14	60%	64.8	56	45	34	16	4.4	3.6	2.8	2.5	36	31	103	110	60
8967	12709	7600	4152	3520	3704	3888	16	18	16	14	0.4	0.4	0.5	0.2	171	90	47	58	58	76	37	27	15.2.	15.1	15.8	15.4	60%	50	52	58	30	24	3.8	3.8	2.6	2.6	32	32	148	110	60
3465	3591	3238	2567	2882	2731	2385	13	22	13	14	0.6	0.5	0.2	0.8	27	27	15	30	17	21	21	26	16	18.7	17.5	21	46%	56%	82	78	46	34	4.4CM	3.9	2.6	2.8	41	28	77	87.6	50
8604	12118	4268	1762	1719	2712	636	18	16	23	25	0.5	0.5	0.3	0.4	36	35	31	27	48	23	30	14	11.3.	15.2	13.2	15.3	64	57%	64	68	36	32	4.1	4.6	2.8	3	32	35	70	171	44
16443	8592	7852	4597	7352	3566	3600	20	21	18	13	0.3	0.1	0.4	0.2	139	90	76	87	62	44	57	62	17	15	14.2	14.8	60%	58	92	72	31	31	4.4CM	4.3	3.5	3.4	20	21	20	93	9
3821	3744	4906	2621	1930	3093	2808	17	18	15	15.7	0.3	0.5	0.6	0.4	30	15	25	27	12	25	19	18	13	12	12.6.	13.2.	55%	60%	87	80	66	50	4.5	4.1	3.5	3.2CM	22	22	190	106	60
3465	4028	4674	2567	2882	2731	2900	13	22	13	15	0.6	0.5	0.2	0.5	27	27	15	22	17	21	21	18	16	18.7	17.5	21	57%	57.30%	37	48	16	20.6.	3.8CM	4.1	2.6	3.2CM	32	22	130	122	50
3538	3446	3239	2872	838	3583	2247	42	29	18	17	1.1	0.4	0.8	0.8	29	29	38	46	33	35	35	50	16	15	16	15.5	63	61%	43	59	16	23	4.2	4.1	2.8	2.7CM	33	34	132	120	55
5712	3534	4815	4786	4068	6548	2585	10	10	10	10	0.2	0.4	0.4	0.5	52	49	44	49	38	38	34	40	14.9.	15.1	15.8	15	60%	61	48	41	23	16	4.1	4.2	2.5	2.6	39	38	80	82.5	50
3787	4176	2525	8139	6089	5325	2679	22	23	19	25	0.8	0.6	0.3	0.6	31	30	60	51	14	20	49	33	15.2.	13.1.	13.8	13.5	60%	62	92	48	34	18	3.8CM	3.7	2.9	2.7	24	27	160	120	90
6499	4320	10439	1140	1274	1676	1870	18	30	21	24	0.4	0.3	0.4	0.4	22	30	25	31	11	15	24	30	12.3	16.4	15.8	17.6	60.00%	58%	54.5.	65	21.5.	25	3.4CM	3.8	2	2.3	41	39	128	141	39.6
8030	10374	10465	2460	3732	5122	2916	25	28	20	18	0.2	0.6	0.7	0.8	42	36	31	32	14	32	19	18	13.8.	16	14.7	15	60%	59	34	33	15	13.7	3.8CM	3.8	2.9	2.8	24	26	84	117	56
2805	4130	4392	10567	5217	8272	8290	18	14	19	18	0.6	0.4	0.3	0.3	65	50	32	26	60	43	38	17	15.4	16	16.0.	14.0.	60	57%	50	55	18	24	3.8	3.7	3.2	2.4CM	25	16	80	109	62

ECHO-TDI																					COMPLICATIONS				
MVI-A 6 months	E/A-Baseline	E/A-6 months	MVLI-E (Baseline)	MVLI-E (6 months)	MVLI-A (Baseline)	MVLI-A (6 months)	MVLI-S (Baseline)	MVLI-S (6 months)	MVSI-E (Baseline)	MVSI-E (6 months)	MVSI-A (Baseline)	MVSI-A (6 months)	MVSI-S (Baseline)	MVSI-S (6 months)	MVLSAE-Baseline	MVLSAE-6 months	MVSSAE-Baseline	MVSSAE-6 months	TAPSE-Baseline	TAPSE-6 months	heartburn	vomiting	pain abdomen	joint pains	fever
60	1.13	1.66	18	11	7	10	7	12	12	21	9	13	7	14	1.40	1.80	1.20	1.40	2.3	2.1	0	0	1	0	0
63	2.8	1.58	18.1	26.1	6.9	7.4	12.3	10.7	11.2	14	5.8	7	7	7.85	1.90	1.60	1.40	1.40	2	2.2	0	0	0	1	0
79	1.13	1.7	17	21.4	7	6.7	10	11.5	12	12.5	6	8.7	9	10	1.60	1.70	1.30	1.50	2.2	2.9	0	0	0	0	0
76.1	1.19	1.7	20.1	17.4	10.6	11.8	8	9.29	15.3	14.7	7.5	8.3	7.6	8.5	1.30	1.60	1.20	1.60	2.8	2.2	0	0	0	0	0
50	1.59	2.4	16.7	17	6	6.6	10.1	10.5	10.9	11.6	5.2	5.4	7.6	7.3	1.50	1.70	1.50	1.20	1.9	2	0	0	0	0	0
82	1.6	1.58	14.2	16.3	5.6	5	8.4	9.3	13.2	11.2	6	6.7	8.6	8.3	1.80	1.30	1.50	1.20	2.1	1.9	0	0	0	1	0
61.3	2	1.97	18.2	16.6	9.5	7.3	8	5.7	13.8	11.3	6	4.6	7.6	6.5	1.20	1.70	1.10	1.20	1.8	2.5	0	0	0	0	0
68.5	1.71	1.6	18.1	17	8	8.9	8	8.6	10.7	11.2	6.5	6.9	8.3	8.2	1.50	1.50	1.30	1.20	2.2	2.1	0	0	0	0	0
40	2.46	2.75	17.8	19.2	6.7	8.6	7	9.3	11.8	15.2	7.5	6.7	9.1	7.6	1.30	1.80	1.20	1.10	2	1.7	0	0	0	0	0
60.6	1.54	1.44	18	15.3	6	7.9	12	9.8	12	14.9	0.4	11.1	2	9.9	1.50	1.50	1.10	1.10	2.2	1.8	0	0	0	0	0
60.8	1.59	2.8	17	23.5	6.6	9.5	10.5	13.6	11.6	14.9	5.4	9.2	7.3	12.6	1.50	2.20	1.50	1.60	2	2	0	0	1	0	0
45	2.22	2	18.1	20	7.8	6	20	9	15.2	13.6	8.2	6	14.6	8	1.10	1.40	1.10	1.60	2.1	2.5	0	0	0	1	0
81.6	3.16	1.29	23	21.5	10.7	9.2	7.5	8.5	11.7	12.5	12.6	11.5	12.9	14.2	1.80	1.60	1.30	1.40	2.1	2	0	0	0	0	0
49.8	2.6	2.44	12.3	19.8	5.5	5.4	20.1	6.4	13.6	16.2	8.4	5.7	14.8	6.8	1.80	1.50	1.50	1.60	2.1	2	0	0	0	0	0
50	2.4	2.4	12	17	8	6	15	7	10	11	8.2	0.7	2	8	1.60	1.70	1.30	1.20	2	2.2	1	1	1	0	0
50.8	1.6	1.62	17	16.8	8.7	9.5	10.5	11.3	11	10.5	8	7.7	7	8	1.40	1.90	1.40	1.30	2.1	2.2	0	0	0	0	0
70	1.77	1.71	12.1	16.9	5.6	7.2	8.1	9.2	10.4	11.6	4.9	7.5	7.2	10.3	2.00	1.60	1.90	1.20	2.4	2	0	0	0	0	0
39	3.23	3.61	18	22.6	10.5	7	8.7	13	16	13	6	7	7.8	8	2.00	1.50	1.80	1.20	1.8	2.6	0	0	0	0	1
56.7	1.5	2	18	24.3	10	11.8	13	14.8	12	14.6	7.8	9.4	10	11.1	1.90	1.90	1.40	1.20	1.8	2	0	0	0	0	0
71.4	1.29	1.52	9	18.7	6.2	12.9	6.2	16.2	7.8	12.1	6.2	8.4	6.1	9.7	1.80	2.10	1.50	1.40	2.1	1.9	0	0	0	0	0

			DEMOGRAPHIC DATA																	PAST HISTORY																				ANTHROPOMETRY							
S. No	R. Number	Group A/B	Age	Sex	SES - lower class	SES -lower middle	SES- middle class	SES- upper middle	SES- upper class	mother- high school	mother-Pre-University College	Mother- Degree	Mother- university	Father- High school	Father-PUC	Father- Degree	Father- University	Urban	Rural	age of onset of disease -<6months	age of onset of disease- 6m-1yr	age of onset of disease: >1yr	DFO Y/N	DFO- duration	DFX Y/N	DFX- duration	Folic acid	calcium	zinc	B-complex	Pantop	BT onset- 1-2m of diagnosis	BT onset: 2-6m	BT onset: >6m	frequency of transfusions- units/yea	Consanguinous marriage	Siblings with thalassemia	infection in past-HIV,HBSAG, HC	W/A	H/A	BMI- <16 kg/m2	BMI- 16-<17kg/m2	BMI- 17-<18.5 kg/m2	BMI- 18.5- <25 kg/m2	BMI- 25- <30 kg/m2	BMI: ≥30 kg/m2	dark skin
1	2	A	18	F	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	8	1	1	1	0	0	0	1	0	15	1	0	0	48	149	0	0	0	1	0	0	1
2	3	A	13	M	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	3.5	1	1	1	1	0	1	0	0	24	0	0	1	33	138	0	0	1	0	0	0	1
3	4	A	15	M	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	8	1	1	0	1	0	1	0	0	24	0	0	0	34	142	0	0	1	0	0	0	0
4	6	A	14	M	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	1	0	0	1	0	24	1	1	0	38	160	1	0	0	0	0	0	0
5	8	A	14	M	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	9	1	1	0	1	0	1	0	0	24	0	0	0	29	129	0	0	1	0	0	0	0
6	10	A	12	F	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	5	1	1	0	1	0	1	0	0	24	0	1	0	35	152	1	0	0	0	0	0	1
7	12	A	18	M	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1yr	1	10	1	1	1	1	1	1	0	0	16	1	0	1	40	143	0	0	0	1	0	0	1
8	15	A	18	M	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	11	1	1	1	1	0	1	0	0	24	0	0	0	42	161	0	1	0	0	0	0	0
9	17	A	14	F	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	11	0	1	0	1	0	1	0	0	14	1	0	0	32	147	1	0	0	0	0	0	0
10	18	A	18	M	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	5	1	1	0	1	0	0	1	0	24	1	1	0	53	173	0	0	1	0	0	0	0
11	20	A	18	F	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	11	1	1	0	1	0	0	0	1	24	1	3	0	38	145	0	0	1	0	0	0	0
12	22	A	13	F	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	12	1	1	0	1	0	1	0	0	18	1	1	0	22.5.	122	0	1	0	0	0	0	0
13	26	A	18	M	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	3	1	1	0	0	0	1	0	0	24	1	0	0	41.7.	173	1	0	0	0	0	0	0
14	27	A	18	M	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	12	1	1	0	1	0	1	0	0	24	1	1	0	51	167	0	0	1	0	0	0	0
15	29	A	18	M	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	5	0	1	0	1	0	1	0	0	12	1	0	0	41	160	0	1	0	0	0	0	1
16	31	A	18	M	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	3	1	1	0	0	0	0	0	1	24	1	0	0	39.8.	154	0	1	0	0	0	0	0
17	33	A	18	M	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	1	5	1	1	0	1	0	1	0	0	12	0	0	0	42	143	0	0	0	1	0	0	1
18	35	A	13	F	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	3	1	1	0	1	0	1	0	0	16	1	0	0	25	133	1	0	0	0	0	0	1
19	36	A	16	F	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1	12	1	1	0	1	0	1	0	0	12	1	0	0	32	135	0	0	1	0	0	0	1
20	39	A	15	M	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1	6	1	1	0	1	0	1	0	0	16	1	0	0	25	132	1	0	0	0	0	0	1

GENERAL PHYSICAL				PER ABDOMEN						INVESTIGATION																									
hemolytic facies	pallor	flat nasal bridge	dental malocclusion	liver span 1st	liver span 2nd	liver span 3rd	spleen span 1st	spleen span 2nd	spleen span 3rd	Hb-1st	HB2nd	HB-3rd	Hb-4th	Hb-5th	Hb-6th	Hb-7th	hb-8th	hb-9th	hb-10th	hb-11th	hb-12th	WBC-1st	WBC-2ND	WBC-3RD	WBC-4TH	WBC- 5th	WBC-6TH	WBC-7TH	WBC-8TH	WBC-9TH	WBC-10TH	WBC-11TH	WBC-12TH	ANC-1	ANC-2	ANC-3	ANC-4	ANC-5	ANC-6
0	1	0	0	15	15	14.5	15	15	15	8.6	10.9	8.4	9.1	10.5	10	8	8.1	8.2	7.9	8.2	8.4	9800	9280	13700	19800	12700	14100	13600	7280	15300	12300	11400	11700	5194	5753	8768	16434	6096	8883
1	1	1	0	12	12	11	15	15	14	8.2	8.1	8.3	9.3	9.1	8.2	8.8	8.8	8	8.3	9.2	8.8	6080	6160	7110	7100	10290	7090	6490	6240	5980	6930	6930	8450	3720	3294	6882	5777	4896	3744
1	1	1	0	15	15	14	13	13	12.5	8.7	9.8	9.2	8.9	9.9	9.1	8.8	8.8	8.2	9.3	9.1	9	6310	6560	11170	9170	7270	7870	8060	7510	6050	7200	10100	9140	3843	3250	6882	4368	3528	4914
1	1	1	1	14	14	14	13	12	12	7.6	6.9	7.9	8	8.8	8.7	9.3	8.2	8.2	8	7.5	8.5	8490	6760	6360	7080	6770	5470	8910	6790	6890	5450	6580	6500	5773	3886	3843	4060	3452	2899
1	1	1	1	13	13	13	13	13	12	9.1	8.1	8.5	7.9	8.4	8.9	8.5	9.2	9.4	8.5	7.9	9.2	3880	7800	6640	8000	8230	8760	9500	6710	8190	5900	6850	7590	1901	4836	3366	4880	4444	4380
0	1	0	0	12	12	12	13	13	12	10.3	8.6	9	9.2	7.9	8.1	9.5	9.2	8.4	7.4	9	9.4	7160	5890	7430	6810	6480	6010	7000	6790	5830	7400	7000	7500	4797	3887	4903	4216	4082	3480
1	1	1	1	16	16	15	15	15	16	8.1	7.4	8.3	8.4	8.6	9.7	8.6	8.3	7.6	7.8	8.9	9	3860	4230	4620	4010	5580	5450	3990	4110	5020	6510	6050	3970	2277	2453	2864	2360	3465	3433
1	1	0	0	12	12	11.5	17	17	16	9.8	10.5	8.8	9.3	8.5	9.8	10.4	9.7	9.5	9.4	8.9	9.7	4430	6490	5290	4040	5100	4780	5740	4420	5190	5420	5590	5780	2772	4478	3121	2280	3366	3489
1	1	0	1	12	12	12	10	9	9	5.6	9.8	9	8.9	8	6.2	10.2	9.3	8.9	9.7	7.8	9.9	5430	5810	4960	4340	6050	3820	5170	4310	4810	5170	5020	6010	3909	3844	2876	2213	4020	2559
1	1	0	1	16	14	14	16	15	14	9.7	9.2	9.9	11.8	9.7	10.4	11.5	10.6	11.3	11.8	10.9	10.5	6340	5810	5570	6230	5500	5020	5100	5330	7910	5420	8920	6240	3930	3654	3174	4049	3245	2400
1	1	1	1	9	9	10	12	12	12.5.	7.3	8.9	10.4	8.3	10.2	8.3	9.7	9.4	8.8	10	9	9.5	4330	4490	5350	3380	4430	4370	5690	4850	4400	3160	4870	6440	2279	2828	3049	2027	3101	2665
1	1	0	0	10	10.5	10.5	9	11.5	11	8.3	7.4	10.7	9	9.5	7.4	9.3	9.1	6.1	6.5	5.4	8.1	8790	7280	10870	12370	10000	9240	12100	8820	6000	6900	6170	7200	5537	4586	6696	9471	6500	6256
1	1	0	0	10	14	14	15	14	14	8.4	8.3	8.6	7.4	8.3	8.6	8.4	8.5	8	8.5	9	9.6	18800	22700	7590	14910	12800	9690	10070	26930	17070	12000	14420	9940	11092	4767	4022	8791	7552	6104
1	1	1	1	10	10	12	10	10	9	9.2	10.7	11.1	9.3	7.8	9.7	8.5	9.9	11.2	9.6	8.5	9.9	8020	9080	6650	8240	6630	5220	4680	6310	10450	6260	5610	6420	5120	5890	4322	5603	4290	3184
1	1	1	0	12	13	13	14	14	12	8.5	8.1	8.7	8	9.3	9.8	9	10	9.3	7.7	8.1	9	5140	6130	4980	5120	5400	6490	6010	5840	7380	6640	7400	6610	3289	3678	3286	3020	3186	3829
1	1	0	1	12	11	11	10	12	14	8.1	9.1	8	8.3	9.4	9.5	10	9.3	10.1	9.6	9.7	8.4	6930	7220	6200	7330	8450	8340	10530	7470	9770	7160	8520	7130	3395	3898	3534	4984	4788	5504
1	1	0	0	18	16	16	15	14	14	7.8	8	7.4	9	9.1	9.6	8.7	11	9.6	8.4	8.4	8	7910	8110	6400	8010	7080	9410	9300	2620	14710	11150	10390	9760	4345	4460	4352	4800	3710	4987
1	1	0	1	14	15	15	15	13	12	6.9	6.8	10.1	10.1	9.4	8.8	8.2	9.6	11.8	11.7	5.5	8.2	4370	3570	4350	5280	5930	5660	5380	5960	6030	7780	3380	6070	2543	2106	2131	3273	3220	3339
1	1	0	1	14	14	12	15	14	14	9.3	9	7.9	7.6	8.2	8.6	9.3	8.9	8.9	8.6	8.2	11.1	10150	11650	11200	20500	12400	9180	8380	14900	9560	9650	15700	11600	4364	5825	7168	17220	6572	3947
1	1	1	1	12	11	11	11.5	10	10	9.2	9.7	9.8	9.9	9.4	10.3	9.5	8.8	9.2	9.9	9	9	8300	7840	9210	10480	7780	8740	8010	8310	11320	8740	10480	8230	5063	4134	5796	6916	4279	4894

																											USG ABDOMEN																			
ANC-7	ANC-8	ANC-9	ANC-10	ANC-11	ANC-12	SF-BASELINE	SF-1st	SF-2nd	SF-3rd	UREA- BASELINE	UREA-1st	UREA-2nd	UREA-3rd	CREATININE- BASELINE	CREATININE- 1st	CREATININE- 2nd	CREATININE- 3rd	SGOT-Baseline	SGOT-1st	SGOT-2nd	SGOT-3rd	SGPT-Baseline	SGPT-1st	SGPT-2nd	SGPT-3rd	LIVER SIZE -Baseline	SPLEEN SIZE-Baseline	LIVER SIZE-6 months	SPLEEN SIZE-6M months	LVEF-Baseline	LVEF-6 months	LVEDV-Baseline	LVEDV-6 months	LVESV-Baseline	LVESV-6 months	LVIDd-Baseline	LVIDd-6 months	LVISd-Baseline	LVISd-6 months	FS-Baseline	FS-6 months	MVI-E (Baseline)
6664	3312	8262	7749	6042	8424	4055	5237	3464	3149	17	27	18	14	0.5	0.5	0.6	0.8	25	57	40	34	25	68	56	22	16.5	16.3	16.6	15.4	61%	60.10%	69	91	27	36	4.5	3.9	3	2.5	33	36%	120
4080	2976	3186	3795	4071	5408	1055	709	677	949	30	30	28	28	0.5	0.4	0.9	0.3	24	19	20	21	18	17	16	12	13	17.7	12	13.6	62%	60%	72	120	26	50	4.4	4.5	3.6	3.2	18	29%	91
4240	3600	3240	3960	5300	6188	4846	7489	4717	4431	44	28	13	29	0.3	0.6	0.3	0.4	113	53	41	38	124	73	14	31	16	14.5.	14.8	13.6	60%	58%	22	24	15	10	4.2	4	2.8	2.5	33	38	142
5785	3819	3536	2916	3705	3835	4113	4287	6383	2000	26	23	15	15	0.8	0.9	0.5	0.4	45	47	34	54	27	38	20	42	18	16.7.	17.4.	15.3.	63%	61%	78	85	36	45	4.7	4.5	3	2.6	36	42	101.4
6365	3819	4176	3127	3604	4500	3987	4284	3969	2382	19	27	20	26	0.4	0.9	0.4	0.3	44	32	26	26	38	22	20	24	16.7.	11.3.	15.8.	11	63%	62.00%	88	74	30	28	4	4.6	2.4	2.8	40	39	128
4270	3734	3439	4884	3360	4350	8429	2967	6114	4337	14	21	21	24	1.1	0.4	0.6	0.6	84	39	37	27	95	35	23	17	17	16.3	16.5.	15.3X7.8.	$ 60%	61%	56	54	20	21	3.9	3.9	2.9	2.5	26	36	115
2457	2542	3650	4680	3780	2262	5706	4894	3092	8305	30	19	12	21	1.4	0.8	1	0.8	48	26	37	30	47	21	34	35	17	15.6	16.5	15.7	*45%	42%	102	84	56	49	5.4	5.5	4.2	4	22	27	87.4
3788	2607	3269	3306	3630	3583	4780	4918	4260	3256	41	49	33	28	0.9	0.7	0.6	0.3	27	23	15	17	17	13	10	10	15.2.	19.1X6.6.	12.1.	16.8	##55%	60.00%	109	84	46	35	4.7	4.3	3.5	2.8	26	35	135
3102	2494	2544	3153	2900	4260	1754	1978	3072	1854	30	29	9	18	0.6	0.5	0.3	0.5	48	42	32	1.4	15	38	24	15	14.8.	14.6	12.4	12	##60%	63	69	66	31	24	4.6	4.3	2.8	3	39	30	142
2907	3357	6004	3240	6675	4492	5291	3962	5348	4406	28	28	28	36	0.8	0.5	0.7	1	21	27	32	39	20	23	40	35	17.7.	18.5X5.	16.3.	15X4.	60%	59	86	115	38	47	3.9	4.8	2.9	3.5	26	27	120
3869	3249	2552	1832	3120	3735	4513	3827	3997	3800	21	29	24	20	0.5	0.5	0.4	0.1	48	42	45	53	53	61	53	43	14.8.	16	15.8.	16.7.	##60%	65%	51	37	26	16	3.8	4.2	2.5	2.8	34	33	104
9075	5544	3660	4347	3763	4464	1826	6266	3906	4360	16	18	38	21	0.4	0.5	0.8	0.3	39	71	36	32	36	101	46	32	13.5.	11.3.	15.3	10.9	60%	60%	52	50	21	20	3.9	3.8	2.3	2.4	41	37	149
5800	12643	14110	6360	7056	5466	3869	2949	4004	3487	25	23	34	22	0.2	0.6	0.9	0.7	37	46	38	46	23	32	28	30	16.2.	15.8	16	14.8	60%	60%	52	55	20	22	3.8	3.9	2.4	2.5	37	36	123
2808	4032	6165	3534	3528	4044	4120	3811	3523	3316	15	28	26	27	0.4	0.5	0.4	0.8	34	75	35	30	25	81	32	23	16.8.	19.7X7.6.	15.1	17.2.	63%	61	140	117	54	46	4.9	4.3	3.4	2.8	31	35	20.2
3180	3422	3690	2655	4144	3828	3069	3693	4244	2628	46	23	19	24	1.3	0.7	0.8	0.7	49	50	48	53	48	62	42	32	14.6.	16.8.	14.7	13.8	##38.4%	66%	37	64	22	22	5	4.5	3.5	2.6	30	42	96
6633	3959	4591	3937	5026	4206	3969	5295	6763	**4285**	20	20	24	25	0.5	0.7	0.5	0.7	104	23	29	30	88	22	30	19	15.8.	14.5	12	15.5.	*58%	60.00%	80	85	36	35	4.2	3.8	3	2.6	29	32	80
4743	1430	4851	5909	5091	6051	6163	3112	4777	4527	45	32	20	24	1.1	1	0.5	0.9	44	30	28	38	40	23	32	38	16.5	13	17	14.9.	*60%	55.00%	75	90	34	42	4.5	5.2	2.9	2.8	36	46	100
3443	3874	3480	4668	1994	3780	5171	5637	8263	6917	26	29	23	34	0.7	0.2	0.5	1	80	75	65	78	65	59	26	64	14.8	16.3	15.3	13.2	##41%	57%	97	52	45	22	4.6	3.4	4.3	2.8	7	18	82
3818	8046	3824	5114	4867	6380	2908	2847	2672	814	13	24	22	32	0.6	0.4	0.8	0.6	39	51	68	39	40	46	78	42	14.4.	16	12.5	15	60%	59	56	52	23	21.5	3.6	4.1	2.5	2.9	31	29	126
4400	5395	6678	4807	5764	4855	5543	4789	4641	3013	32	42	19	26	0.9	0.5	0.6	0.2	66	75	18	50	77	99	55	51	13.5	13.3	12.2	11.9	##60	64%	68	64	28	23	3.9	4.2	2.9	2.8	45	33	130

ECHO-TDI																							COMPLICATIONS				
MVI-E (6 months)	MVI-A (Baseline)	MVI-A (6 months)	E/A-Baseline	E/A-6 months	MVLI-E (Baseline)	MVLI-E (6 months)	MVLI-A (Baseline)	MVLI-A (6 months)	MVLI-S (Baseline)	MVLI-S (6 months)	MVSI-E (Baseline)	MVSI-E (6 months)	MVSI-A (Baseline)	MVSI-A (6 months)	MVSI-S (Baseline)	MVSI-S (6 months)	MVLSAE-Baseline	MVLSAE-6 months	MVSSAE-Baseline	MVSSAE-6 months	TAPSE-Baseline	TAPSE-6 months	heartburn	vomiting	pain abdomen	joint pains	fever
128	60	56.4	2	2.28	16.4	19.3	6	9.3	8.4	9	13.3	13.4	5.6	6.4	7.2	7.4	2	1.5	1.4	1.2	1.7	2	0	0	0	0	0
90	38	5	2.39	1.8	15	15	4	5	8	16	11	13	5	0.9	8	7.6	1.6	2	1.2	1.9	1.8	1.9	0	0	0	0	0
90	60	0.4	2.36	2.25	26.2	22.2	12.6	10.3	13.1	14.1	13.6	14.8	7.2	8.3	9	9.4	1.4	2	1.2	1.5	2	2.1	0	0	0	0	0
130	70.7	80	1.42	1.62	22	18	13	9	8	11	10.2	16	7	8	7.7	13	1.8	2.2	1.5	2.1	2.1	2.5	0	0	0	0	0
140	62	72.7	2	1.92	23	14.2	6	6	17	7.2	14	10.4	8.1	4.9	8.2	6.5	1.3	1.5	1.2	1	1.3	2.2	0	0	0	0	0
128	76	87.6	1.51	1.46	22.1	20.5	13.7	11.4	9.4	10.9	17	13.5	11	7	10	8.9	1.8	1.7	1.4	1.4	1.8	2	0	0	0	0	0
93	31.1	50	2.8	1.8	9.4	9.4	4.7	5.3	7.9	8.5	8.4	8.2	4	5	5.4	6.1	0.8	1	0.7	1	2.2	2.3	1	0	0	1	1
127	53	97.8	2.54	1.29	17.2	16.5	15.3	14.2	9.7	8.7	14.3	12.2	9.7	8.1	8.1	8.9	1.6	2.2	1.1	1.4	1.8	2.4	0	0	0	0	0
143	94.1	50	1.51	2.86	18.7	23.2	10.5	9.5	11.3	12	21.4	20.1	10.4	9.6	10	10.5	2.3	1.5	1.5	1.3	2.7	1.6	0	0	0	0	0
112	70	72	1.71	1.55	18	19.8	9.3	11	16.4	15.8	12.3	15.6	5.2	6.2	8.2	10.3	1	1.7	1.1	1.5	1.7	2.4	0	0	0	0	0
70	72.8	5	1.42	1.4	15.1	15	10.7	10	9.6	8	9.6	11	4	0.9	2	7	2.2	2.1	1.7	1.6	2.2	2.6	0	0	0	0	0
158	60	58	2.48	2.72	15.7	16	10	10.2	8.2	8	10.2	10.4	6.1	5.4	7	7.3	1.6	1.6	1.1	1	2	1.9	0	0	0	0	0
120	61	5	2.01	2.4	12	14	7	7	10.3	9.8	11	9	9	7	8	6	1.9	1.8	1.5	1.4	2.3	2.4	0	0	0	0	0
150	75	61.7	2.66	2.43	20	20.2	11	11.8	13.2	12	14.1	13.6	10	10.1	12.8	11.5	1	1.9	1.1	1.6	2	2.3	0	0	0	0	0
104	49	71.4	1.95	1.45	12	14.6	6.3	5.7	9.5	9.4	7.1	5.9	7	4.1	7.2	6	0.8	1.9	1	1.1	1.6	2.5	0	0	0	0	0
94.2	61	72.6	1.31	1.31	8	16.1	7	8	17.4	10.8	8.1	9.3	7.5	8.5	11.1	7.4	1.7	1.6	1.6	1.7	1.7	1.8	0	0	0	1	0
126	64	86	1.56	1.46	18.2	22	7.6	6	13.3	12	12.6	12	5.7	7	10.2	8	1.5	1.7	1.3	2.1	2.4	2.5	0	0	0	0	0
127	40	87	2.05	1.45	12.4	2.8	6.2	7.7	9.3	7.7	11.2	11	4.2	8	8	8	0.6	1.1	0.7	1.2	2	2	0	0	0	0	0
135	37.1	43.2	3.4	3.1	23	23.1	15	6.1	8.5	8.9	20	13.2	9.8	6.7	7.6	7.4	1.6	1.7	1.7	1.4	2.8	1.9	0	0	0	0	0
124	41.5	61.8	3.13	2	18.6	17.7	7.3	6.4	8.2	9.1	11.6	13.7	5.6	6	7.2	7.2	1.9	1.8	1.5	1.3	1.9	1.8	0	0	0	0	0