"EFFICACY OF COMBINATION THERAPY WITH ORAL IRON CHELATORS ON ENDOCRINE COMPLICATIONS IN CHILDREN WITH TRANSFUSION DEPENDENT B-THALASSEMIA MAJOR - A ONE YEAR HOSPITAL BASED INTERVENTIONAL STUDY"

BY
REG NO. BM0120005

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Dr. TANMAYA METGUD M.D.
Professor & Head,
Department of Pediatrics,
J. N. Medical College,
Nehru Nagar,
Belagavi-590010

Date: 3/1/2023
Place: Belagavi.

KAHER BELAGAVI J.N. MEDICAL COLLEGE

Dr.N.S.MAHANTASHETTI M.D.
Principal
J.N.Medical College,
Nehru Nagar,
Belagavi-590010.

PRINCIPAL
J.N. Medical College,
BELAGAVI- 590 010

Date: 3/1/2023
Place: Belagavi.

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JAWAHARLAL NEHRU MEDICAL COLLEGE
(Recognized by Medical Council of India, New Delhi)
Accredited 'A+' Grade by NAAC (3rd Cycle) Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA
0831 - 2471350 0831 - 2470759 www.jnmc.edu principal@jnmc.edu

Ref No: MDC/PG/ Date: 21-12-2022.

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Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BM0120005,
Postgraduate Student,
2020-21 Batch,
Department of Paediatrics,
J. N. Medical College, Belagavi.

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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 (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ \66 **Date: 25/01/2021**

To,

REG NO: BM120005
PG student in Paediatrics,
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 ON ENDOCRINE COMPLICATIONS IN CHILDREN WITH TRANSFUSION DEPENDENT B-THALASSEMIA MAJOR – A ONE YEAR HOSPITAL BASED INTERVENTIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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

(Dr. Harsha Hegde)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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LIST OF ABBREVIATIONS

B	-	Beta
/cumm	-	Per cubic millimeter
SC	-	Sickle thalassemia
AR	-	Autosomal recessive
BT	-	Blood Transfusion
CBC	-	Complete Blood Count
DFO	-	Deferoxamine
DFP	-	Deferiprone
DFX	-	Deferasirox
fl	-	Femto litre
gm/dl	-	Grams per decilitre
g%	-	gram percent
Hb	-	Haemoglobin
HbA	-	Haemoglobin A
HbA2	-	Haemoglobin A2
HbC/β	-	Haemoglobin C/ beta
HbD	-	Haemoglobin
HbE	-	Haemoglobin E
HbF	-	Fetal Haemoglobin
HbS/ β	-	Haemoglobin S/beta
HBsAg	-	Surface antigen for Hepatitis B virus
HCV	-	Hepatitis C virus
HIV	-	Human Immunodeficiency Virus
HPLC	-	High Performance Liquid Chromatography

HSCT	-	Hematopoietic Stem-Cell Transplant
LIC	-	Liver Iron Concentration
LPI	-	Labile Plasma Iron
mg/kg/d	-	milligram per kilogram per day
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
NTDT	-	Non Transfusion Dependant Thalassemia
pg	-	picogram
RBC	-	Red Blood Cell
RDW	-	Red Cell Distribution Width
SD	-	Standard Deviation
SF	-	Serum Ferritin
USG	-	Ultrasound
WBC	-	White Blood Cell
WHO	-	World Health Organisation
α2	-	Alpha 2

ABSTRACT

Background and objectives

The thalassemias are hereditary anaemias characterized by reduced synthesis of one or more of the globins that form the haemoglobin tetramer. Iron overload in β-thalassemia major usually results in iron-induced cardiomyopathy, liver disease, and endocrine complications. Various authors have reported a high incidence of growth retardation, delayed puberty and endocrine dysfunction in poly transfused thalassemic patients. Iron chelation therapy effectively acts to prevent long-term complications of iron overload,- in transfusion-dependent thalassemia major patients. This study intends to see the incidence of endocrine disorders (hypothyroidism, diabetes, hypogonadism, GH deficiency), growth and pubertal delay and also to assess the efficacy of combined oral chelation therapy on these endocrine complications.

Materials and methods

This interventional study was conducted from January 2021 to December 2021 in the Department of Pediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. 40 children of age 12 to 18 years with transfusion dependent beta thalassemia on single oral iron chelator were enrolled. All the children enrolled in the study was examined for pubertal development by Tanners staging, their height and weight were also be recorded for monitoring their growth. All children were tested for their baseline Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), Growth hormone (GH), Thyroid Stimulating Hormone (TSH), Free Thyroxine (FT4), Fasting blood sugar (FBS) ,2 hr post prandial blood sugar (PPBS), in boys serum Testosterone and in girls serum Oestradiol were tested. All the children who showed

an abnormal endocrine profile received combination of oral iron chelators, Deferasirox at the dose 30 mg/kg/day Q24hourly and Deferiprone at the dose 75 mg/kg/day Q8hrly for 6 months. At the end of the study the children who received oral chelation therapy were again followed up with the investigations and growth parameters to check for any improvement. The data was analysed using R version 4.2.0 statistical software. Chi-square test, Welch's t- test and Mann –Whitney tests were used.

Results

Out of the 40 children enrolled 21 (52.5%) were males and 19 (47.5%) were females. Mean age of children in the study were 14.10 ± 1.85 years. Out of the 40 children studied, 38 children had at least one endocrinopathy. In this study majority of patients had their height, weight and BMI below expected for their age. 19 (47.5%) children out of the 40 children examined, had a Tanner's stage of 1, while 10 (25%) children fell into Tanner's stage 2, 9 (22.5%) children had Tanner's stage of 3 and 2 (5%) of children had Tanner's stage of 4. There was a significant difference in tanners staging at baseline and 6 months with a p value of < 0.05. After 6 months of post intervention with combined oral chelation there was significant difference in the mean value of LH (p-value 0.0017), FSH (p-value <0.001), GH (p-value < 0.001), Testosterone (p value 0.0019), Oestradiol (p value-0.0141) and FBS (p-value <0.001). There were no significant adverse effects observed after combined oral chelation.

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 endocrine functions, growth and sexual maturation and significant decrease in ferritin levels in children with transfusion dependant thalassemia major.

Keywords:

Oral iron chelators, Beta thalassemia, Endocrine complications

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INTRODUCTION

Thalassemias are a group of anemias, characterized by reduced synthesis of one or more of globin chains that form the hemoglobin tetramer. β-thalassemia is caused by mutations in the β-gene with an autosomal recessive inheritance. The reduced or absent β-globin synthesis and the excess of unpaired α-globin chains cause premature death of the erythroid precursors in bone marrow i.e. ineffective erythropoiesis and peripheral hemolysis, leading to anemia, bone marrow expansion, skeletal deformity and increased gastrointestinal iron absorption [1].Beta thalassemias are usually caused by point mutations but at times, deletions in the beta globin gene are seen on chromosome number 11, which leads to reduced in beta+ or absent in beta0 synthesis of the beta chains of hemoglobin [2].

Each year more than 40,000 babies are born with β-thalassemia according to a report from World Health Organization in 2008, out of whom about 25,500 have transfusion-dependent β-thalassemia. The annual number of new-borns expected to be born with β-thalassemia are 20,420 in the Southeast Asia region, 9914 in Eastern Mediterranean region, 1019 in European countries, and 341 in America [3].

Currently, prevalence of Thalassemia carriers in India is found to be 3.7% of the total Indian population. Every year there is an increase in Thalassemia carriers of 50,000. In India around 2-3% of the population is found to be affected by β-thalassemia major. It is estimated that each year about 20,000 children in India are born with β-thalassemia [2]. Rate of carrier state for beta-thalassemia gene in South India is found to be about 1-3%, while the incidence of beta thalassemia trait was shown to be 2.16% [4].

Treatment of thalassemia major consists of conventional modalities which include regular blood transfusions, adding an iron chelator , hydroxyurea , splenectomy in selected cases ,supportive treatment of complications and providing a good psychosocial support . More recent and non conventional treatment includes a hematopoietic stem cell transplant, which is the only treatment modality that offers a complete cure. Other newer methods include drugs like Activin receptor ligand traps- Sotatercept & Luspatercept, JAK2 Inhibitors- Ruxolitinib, Ferroportin Inhibitors- VIT-276, Transmembrane Protease Serine 6 (TMPRSS6)- IONIS TMPRSS6-LRx, a targeted gene therapy and fetal hemoglobin modulation. These treatment options have shown to improve the quality of life drastically and the life expectancy of children with thalassemia major. But they still experience a wide variety of issues especially related to their development, growth, psychological effects, transfusion related infections and various effects of iron toxicity in the different systems of body like the endocrine, liver and the heart being the most commonest [5] .

Endocrine complications have been reported in a large population of children receiving regular blood transfusion therapy due to the iron overload in the different organs. Delayed onset of puberty and hypogonadism is the commonest endocrine complications seen in these children. The incidence of hypogonadism and delayed puberty was found to be approximately 24%. Primary hypothyroidism (4%-24.4%) and diabetes which has an estimated incidence of 9.4% were among the other endocrine complications. Growth faltering can be seen which is mainly due to a low growth hormone production which is seen in a large number of children i.e. about 20-30% [6] .

The mainstay of treatment for removing iron accumulation associated with regular blood transfusion is iron chelation. The different chelators available are deferoxamine, deferasirox and deferiprone. Deferoxamine was the first used iron chelator but is not absorbed well from the GI tract and hence has to be administered through parenteral route. Deferiprone is an oral iron chelator which is given in a dose of 75mg/kg/day and this dose has been known to substantially reduce or stabilize the serum ferritin levels. Deferasirox was the first oral iron chelator available and used in patients above 2 years of age. Deferasirox has a good oral bioavailability, long half life and has high affinity and specificity to iron [7].

Combined chelation results in a constant presence of chelating agent in the patient's circulation, preventing entry of free Labile Plasma Iron (LPI) into the cells and this helps to protect these cells from reactive oxygen species which are the main cause for organ damage, hence resulting in better & improved chelation and which in turn results in a decrease in total iron in the body which is estimated by S. Ferritin levels at timed intervals [8].

Combination therapy of deferasirox with deferiprone is desirable as it avoids the use of parenteral route and can be taken orally [9]. Combined therapy helps to reduce the iron overload more effectively than monotherapy, also reducing the need for high doses of individual therapy. There was found to be a significant decrease in incidence and even reversal of the endocrine complications seen due to the iron overload with oral chelation therapy (deferiprone and deferasirox). Combination therapy with deferasirox and deferiprone was found to be tolerated well and generally safe and resulted in only a few adverse effects like gastrointestinal upset [48].

Even though a lot of literature is available on the effect of combination therapy on cardiac complications, very few studies are carried out in India which comments about its effect on the endocrine complications. This study intends to see the incidence of endocrine disorders like hypothyroidism, diabetes, hypogonadism, growth and delay in puberty and also to assess the efficacy of combined oral chelation therapy on these endocrine complications.

OBJECTIVES

PRIMARY

To assess the efficacy of combined oral chelation therapy (deferiprone and deferasirox) on endocrine complications in children with transfusion dependent beta thalassemia major.

SECONDARY

1)To study the incidence of endocrine disorders (hypothyroidism, diabetes, hypogonadism) changes in children with transfusion dependent beta thalassemia major.

2)To assess the growth and pubertal changes in children with transfusion dependent beta thalassemia major.

REVIEW OF LITERATURE

Thalassemias form a group of anemias due to inherited disorders of globin chains in which there is an imbalance between the α-globin and the β-globin chain production which leads to defects in hemoglobin production [10]. The thalassemias are a heterogeneous group of single-gene disorders which are more common in some parts of the world, like the Mediterranean, Indian Sub-continent, Southeast Asia and Africa[11].

β-Thalassemia:

β-thalassemia is an autosomal recessive disorder of β- globin gene and around 7% of the world's population are the carriers of this genetic trait. 95 % of the thalassemia births worldwide were from Middle Eastern, Asian and Indian regions [12] .In the Indian subcontinent, 2-3% of the general population and a high percentage of 17% of certain high-risk communities like Sindhis, Punjabis ,Khatris are affected by β-thalassemia [2].

HISTORICAL:

By 20th century's beginning, European clinicians became aware of a syndrome with anemia in infancy which was associated along with the enlargement of spleen. The first description of thalassemia clinically was explained by two paediatricians from Detroit, Thomas B. Cooley and Pearl Lee. But the actual term of thalassemia was coined by a scientist named George Whipple [13]. They studied children from Italy with severe anemia (low levels of red blood cells), delayed and poor growth, large sized abdominal organs, and early childhood death. RBCs of these children had shown increased resistance to hypotonic solutions and features of reasonable leukocytosis with nucleated erythrocytes were seen when examined by microscopy of

peripheral blood. These children had a hemolytic facies which consisted of prominent facial skeletal features [14].

Cooley presented his findings to the American Pediatric Society in 1925, naming this disorder erythroblastic anemia, now known as Cooley's anemia. The name Thalassemia is derived from Greek language with "thalassa" which means "the sea" and emia meaning "blood", because the condition was first described in people who were living near the Mediterranean Sea; even though the disease is also prevalent in Africa, the Middle East, and Asia by George Hoyt Whipple, a Nobel prize winner, and W. L. Bradford, a professor of pediatrics at the University of Rochester in 1936 [15].

In 1946, it was found out that the cause of thalassemia was attributed to abnormal hemoglobin structure [16]. Wolman was the first to propose that chronic blood transfusion may be the answer to prevent many problems and resolved most of the childhood symptoms and had led to a major improvement in survival.

Deferiprone, which is an oral iron chelator was discovered in 1981. Dr E Donald Thomas in 1982, was the first to perform a bone marrow transplantation on a thalassemic patient. The first bone marrow transplantation in India was successfully done in 1986 by Dr M. Chandy at Christian Medical College, Vellore.

DEFINITION:

Thalassemia syndromes are a type of hereditary blood disorders which are "characterised by decreased or absent globin chain synthesis, which results in decreased haemoglobin content in the red blood cells, also decreased production of RBC, and hence anaemia.". The inheritance of thalassemia major is in an autosomal recessive pattern [17].

Table 1: Major hemoglobin disorders (18)

α- globin chain disorders	**β – globin chain disorders**
HbH disease α-thalassemia (hydrops fetalis) α-thalassemia	Thalassemia carrier Thalassemia intermedia Thalassemia major
	Compound thalassemia HbS-β -thalassemia HbE-β -thalassemia HbD-β -thalassemia Other rare thalassemia

β -THALASSEMIA:

β-thalassemia is a group of hereditary blood disorders which is characterized by anomalous or reduced synthesis of the β chains of hemoglobin.

CLASSIFICATION OF β -THALASSEMIA:

Thalassemia major	Homozygous state
Thalassemia intermedia (NTDT)	Homozygous/heterozygous state
Thalassemia minor	Heterozygous state
β thalassemia with associated Hemoglobin anomalies HbC/ β thalassemia HbE/ β thalassemia HbS/ β thalassemia	
Hereditary persistence of fetal hemoglobin and β thalassemia	
Autosomal dominant β thalassemia	
β thalassemia associated with other manifestations:- β thalassemia-trichothiodystrophy X-linked thrombocytopenia with thalassemia.	

Children with thalassemia major develops symptoms like severe anemia, become blood transfusion dependent and develop obvious hepatosplenomegaly before they reach the age of 2 years. Thalassemia intermedia shows moderate anemia but may not necessarily be on blood transfusions, shows moderate hepatosplenomegaly, bone abnormalities and growth retardation. While children with thalassemia minor is usually a silent carrier.

Sickle Thalassemia

Sickle thalassemia occurs from the inheritance of one β S gene and a thalassemia defect on the other β gene in a child . The clinical phenotype will be depending on the type of β thalassemia gene inherited. Hence in S β thalassemia (β^O), the thalassemic globin gene will produce no protein hence only the β globin comes from the Hb S chromosome. While in the case of a child with $S\beta^+$ thalassemia, the thalassemic β globin (β^+) is the gene which produces protein in variable amount i.e. low to near normal levels, so that there is some Hb A present. The clinical features of sickle β thalassemia are very variable .$S\beta^O$ thalassemia has features similar to sickle cell anemia in the aspect of hematology, disease onset, course, and prognosis . $S\beta^+$ thalassemia patients show minimal to moderate anemia, and its onset, course, and prognosis similar to hemoglobinopathy SC [19] .

EPIDEMIOLOGY:

The total incidence of thalassemia is predicted to be about 1 in 100,000 population the world annually. Thalassemia is a global disease but showing more predominance in a few regions of the world.

Both alpha- and beta-thalassemia are found to be more prevalent in tropical and subtropical regions of the world, particularly where malaria has been endemic. Even

though the reason for this is not clearly defined, this association is told to be due to carriers of the genetic mutation found to have a higher degree of protection against malarial antigen.

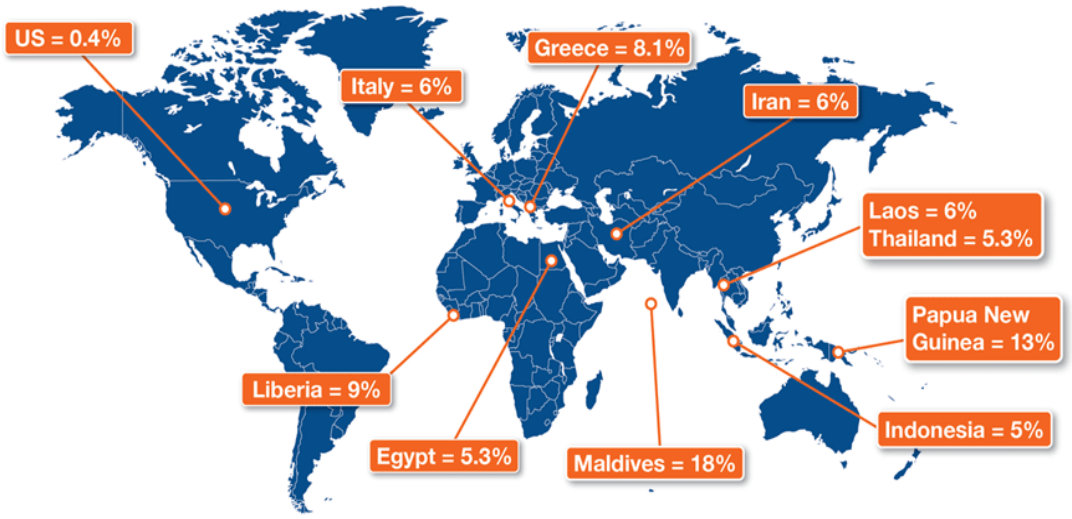


Figure 1: The approximate distribution of the β-thalassemia worldwide

Nearly 4% of the world's population are carriers of alpha and beta thalassemia. The prevalence thalassemia is between 1-17%, mean being about 3.5%. Beta-thalassemia is more common in Mediterranean countries, India, Central Asia, Middle East, Southern China, and countries along the north coast of Africa and South America [17].

Thalassemia is present now in almost all the nations around the world due to intermarriage between varied ethnic groups and the migration of people across countries. 1.5% of the global population has a carrier state for β-thalassemia. Secondary to migration of population between different places, marriage between different ethnic groups or races, is a reason that thalassemia is prevalent in now in almost every country.

INDIA :

India was found to have the largest number of children with Thalassemia major in the world which accounts to about 1 to 1.5 lakhs and almost 42 million carriers of β- thalassemia trait. The prevalence of β thalassemia trait has been reported to be as high as 10.3 8% in the rural parts of West Bengal and in central India, it was found to be 9·59% [20].

In India, the overall carrier rate is measured to be about 4.05, with an incidence of 11,316 births with thalassemia each year. Presently, the prevalence of carrier state of Thalassemia is 3.7% of its total population in India. The rate of annual increment of Thalassemia carrier is around 50,000. Approximately 20,000 children are born each year in India with β-thalassemia major.

India being a country with high numbers of consanguineous marriage accounts for an estimated 10.4% of world population, as they follow their specific caste. Due to the more number of consanguineous marriages and the lack of premarital check up in India, it leads to a many number of disorders. One such disease as a result is Thalassemia which is seen almost in every community of India today. Hence beta-thalassemia has been seen in different castes and communities like Gujaratis, Sindhis, Marathas, Khojas, Bori Muslims, Jains, Baniyas and Punjabis [21].

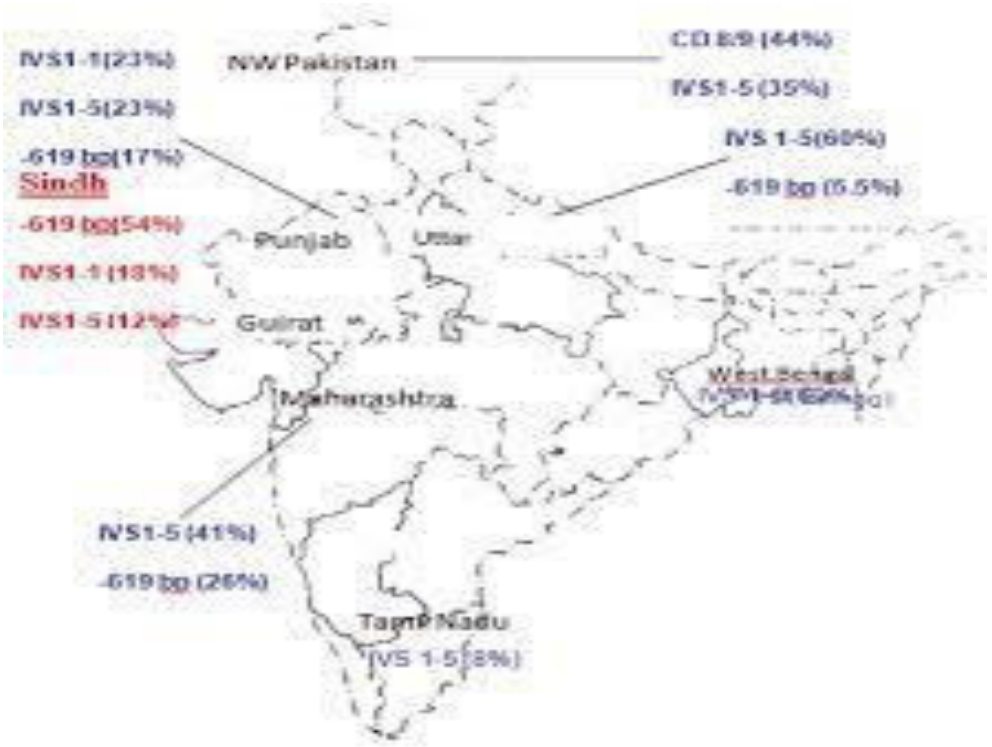


Figure 2: Map showing distribution of beta thalassemia in India

TRANSMISSION:

Beta-thalassemia major and beta-thalassemia intermedia are inherited in an autosomal recessive (AR) manner, which indicates that both copies of the *HBB* gene in each cell have the mutations. The parents of a child with an autosomal recessive condition will each carry one copy of the mutated gene and are referred to as carriers. When two carriers reproduce, each child will have a 25% i.e.1 in 4, chance to be affected, while a 50% i.e. 1 in 2 chance to be a carrier like each parent, and a 25% i.e. 1 in 4 chance to be unaffected *but* not a carrier.

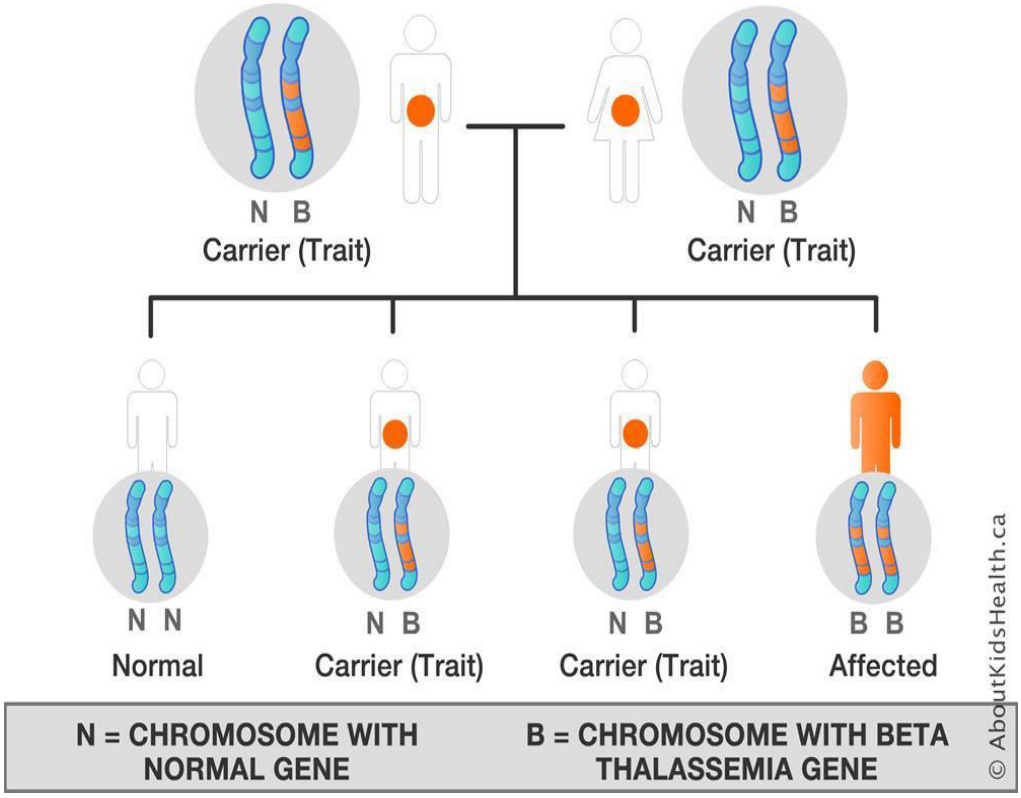


Figure 3: Inheritance of thalassemia

Sometimes, people with only one *HBB* gene mutation in each cell (carriers) do have mild anemia. These people are known to have 'beta-thalassemia minor' or 'beta-thalassemia trait'. In a small group, the disease can also be autosomal dominant, here only one mutated copy of the gene in each cell is enough to cause the disease.

Thalassemia syndromes can be classified based on the requirement of blood transfusion, clinical severity into:-

A) Transfusion Dependent Thalassemia's (TDTs)

B) Non-Transfusion Dependent Thalassemia's (NTDTs)

Patients with TDT's require regular blood transfusions, iron chelating agents to overcome the effects of iron overload. They will present at an early age with complaints of increasing pallor, abdominal distension and respiratory distress [18].

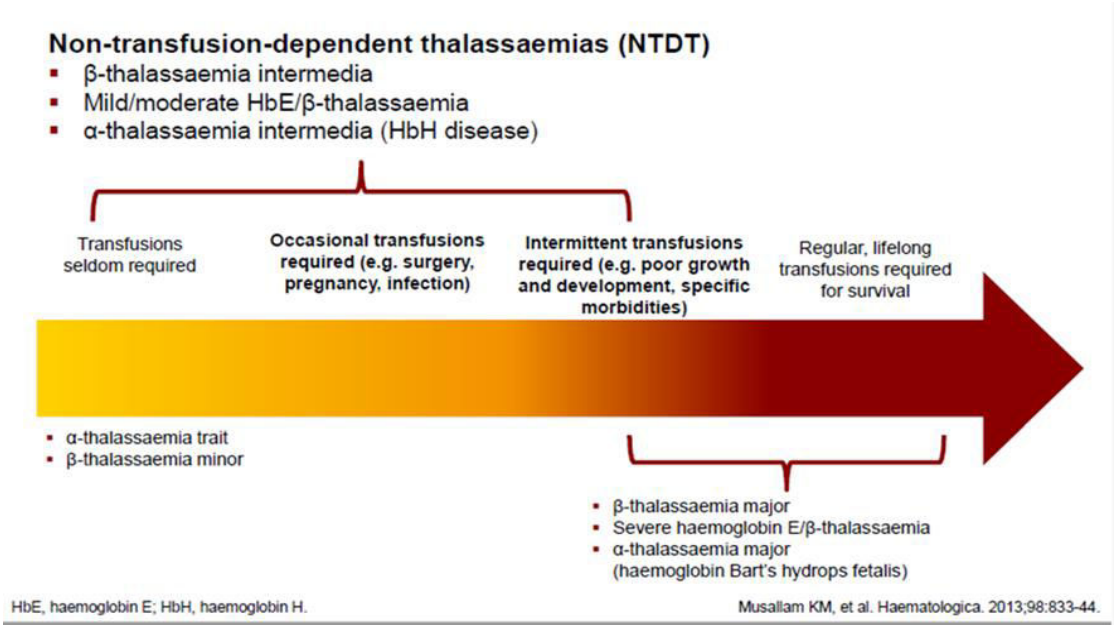


Figure 4: Phenotypic classification of thalassemic syndromes based on clinical severity and transfusion requirement

PATHOPHYSIOLOGY

The primary defect present in β-thalassemia is decreased or lack of β-globin chains production with relative excessive in synthesis of α-chains, hence these α chains in excess, precipitate and form inclusions in erythroid precursors and lead to its destruction in the bone marrow and hence leading to ineffective erythropoiesis.

The free α globin chains and the inclusion bodies are very unstable and hence they precipitate in the red blood cells which lead to the damage of the membranes of RBCs and in turn shorten their survival which causes anemia and in turn also leads to increased production of erythroid precursors. This ineffective erythropoiesis and the compensatory expansion of the marrow with erythroid hyperactivity characterizes thalassemia [22] .

As a response to the anaemia, body produces more erythropoietin, hence patients with high level of erythropoietin can be seen to have an increase in the

production of red cell precursors. This increased erythropoiesis will result in in metabolic activity, growth retardation, increased cardiac overload and congestive cardiac failure. The reason behind an enlarged spleen in thalassemia is due to extramedullary haematopoiesis and increased entrapment of blood in the organ.

In thalassemias, erythrokinetic and morphologic studies showed anemia was a resultant of the death of erythroid precursors in marrow and most probably other sites of extramedullary erythropoiesis. It is known that the erythroid precursors, and not the myeloid precursors, undergoes accelerated apoptosis as detected by an increase in the DNA laddering, which is a sign of enhanced nucleosomal DNA cleavage. It was also found that erythroid apoptosis in thalassemia major was approximately fourfold above the normal as shown by quantitative studies.

Haemoglobin can be oxidatively altered at the 93-cysteine site, and also the band 3 would have undergone oxidative clustering. These changes can lead to a defective or impaired nitric oxide release and thus leads to the deficiency of vasodilator activity. This lack of vasodilatory effect may contribute to the pulmonary hypertension which is now being increasingly seen in cases of thalassemia intermedia[23].

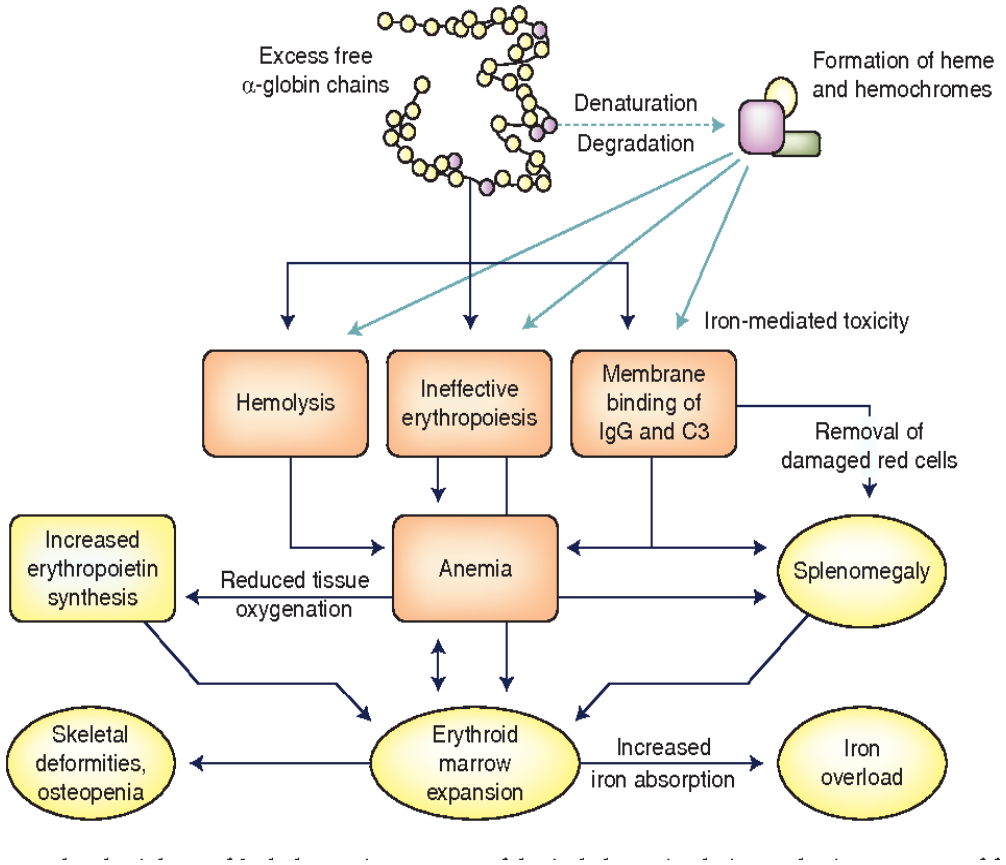


Figure 5: Pathophysiology in Beta Thalassemia Major

Iron overload which is one of the main issues in thalassemia is primarily due to two causes:

(a) Increased absorption of iron from the gut secondary to the increased erythropoiesis

(b) Iron overload from the transfusions that the patients receive over the time

Factors contributing to bony deformities, dental anomalies and other skeletal changes include increase in the erythropoietic activity which causes marrow expansion, also endocrine disorders including primary hypogonadism, growth hormone deficiency ,low vitamin deficiency and oral chelation therapy.

After phagocytosis of red blood cells which are transfused by the reticuloendothelial system, labile cellular iron (LCI), is released this in turn will bind

to plasma transferrin which is circulating. One cellular iron will bind to two Fe3+ molecules. The non-transferrin-bound iron (NTBI) will start to appear in the plasma and will accumulate in different group of cells like the hepatocytes, cardiomyocytes, pituitary and pancreatic cells when the ability of transferrin iron-binding becomes exceeded i.e. the transferrin saturation will be at 60–80%.

Features like oxidant damage, apoptosis, fibrosis, cellular dysfunction and necrosis which is seen in target organs, like heart, liver and the endocrine glands is contributed by reactive oxygen species which is produced as a cause of labile cellular iron . Iron transport is known to be different for different organs and hence this may be the reason behind the different loading rates seen in MRI. The probable reason behind fact that iron overload is seen first in the liver may be that the rate of unloading of iron in the liver is much faster than in heart and other endocrine organs.

Each unit of transfused packed RBCs (PRBCs) usually will contain 200–250 mg of iron; therefore, the amount of iron per year is estimated to be 4800–12,000 mg of with an average of 2–4 PRBCs per month while in comparison only about 400–700 mg of iron absorbed from the diet per year, which also may be lost through cell sloughing and bleeding. Iron in mg/ml of blood can be estimated from the formula - 1.16 times the haematocrit of the blood product which is transfused. The main mechanism of iron accumulation in transfusion dependent thalassemias is due to transfusion therapy, while other reasons can be attributed to secondary hepcidin suppression and increased intestinal absorption. In cases of non transfusion dependent thalassemia, iron accumulation preferentially occurs more in liver than in the heart [13] .

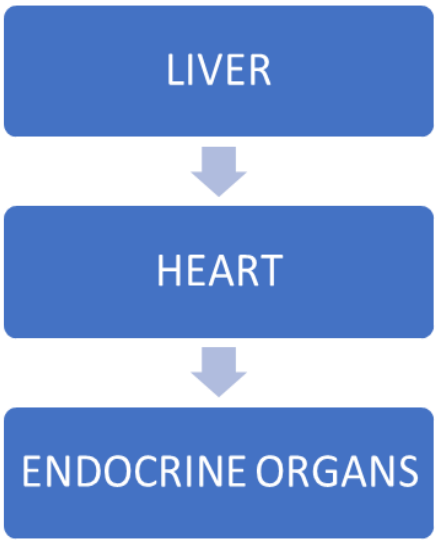


Figure 6: Organ systems involved during iron toxicity

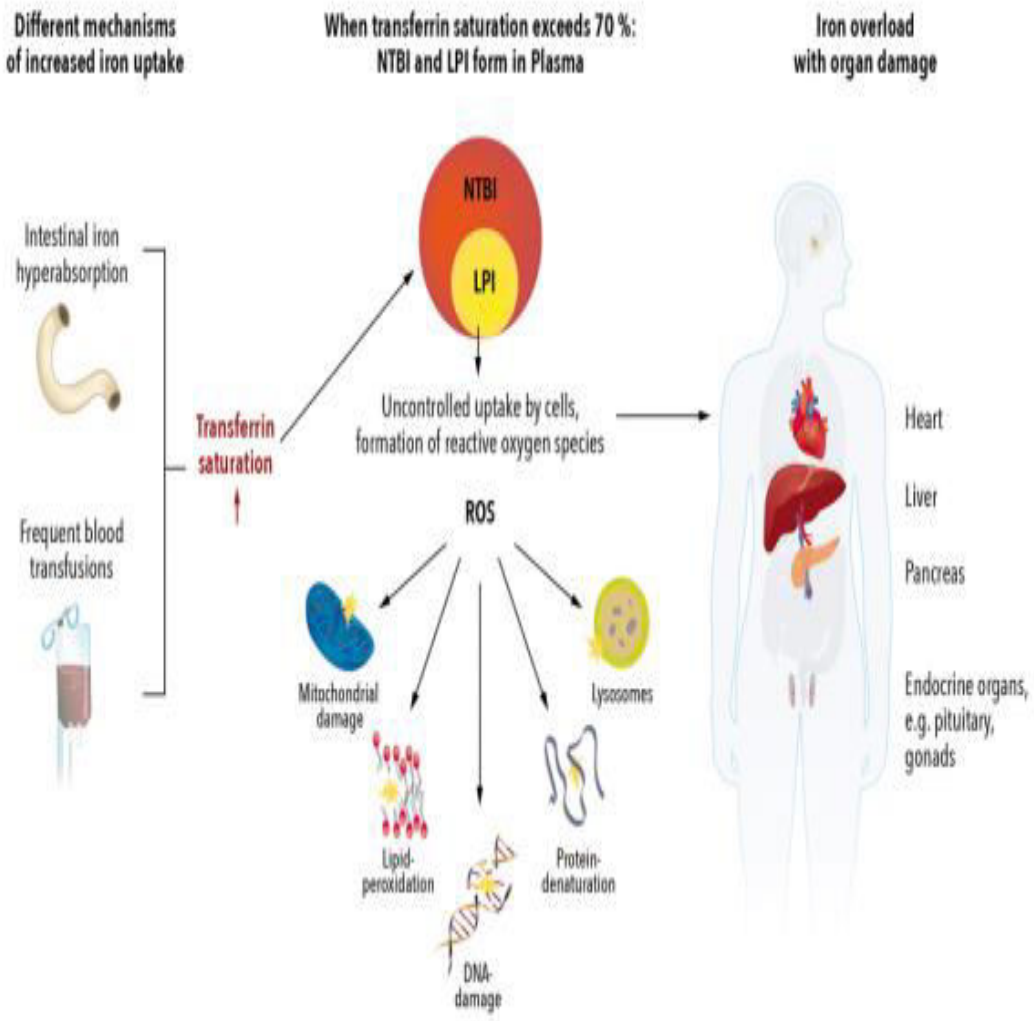


Figure 7: Pathophysiology of iron overload

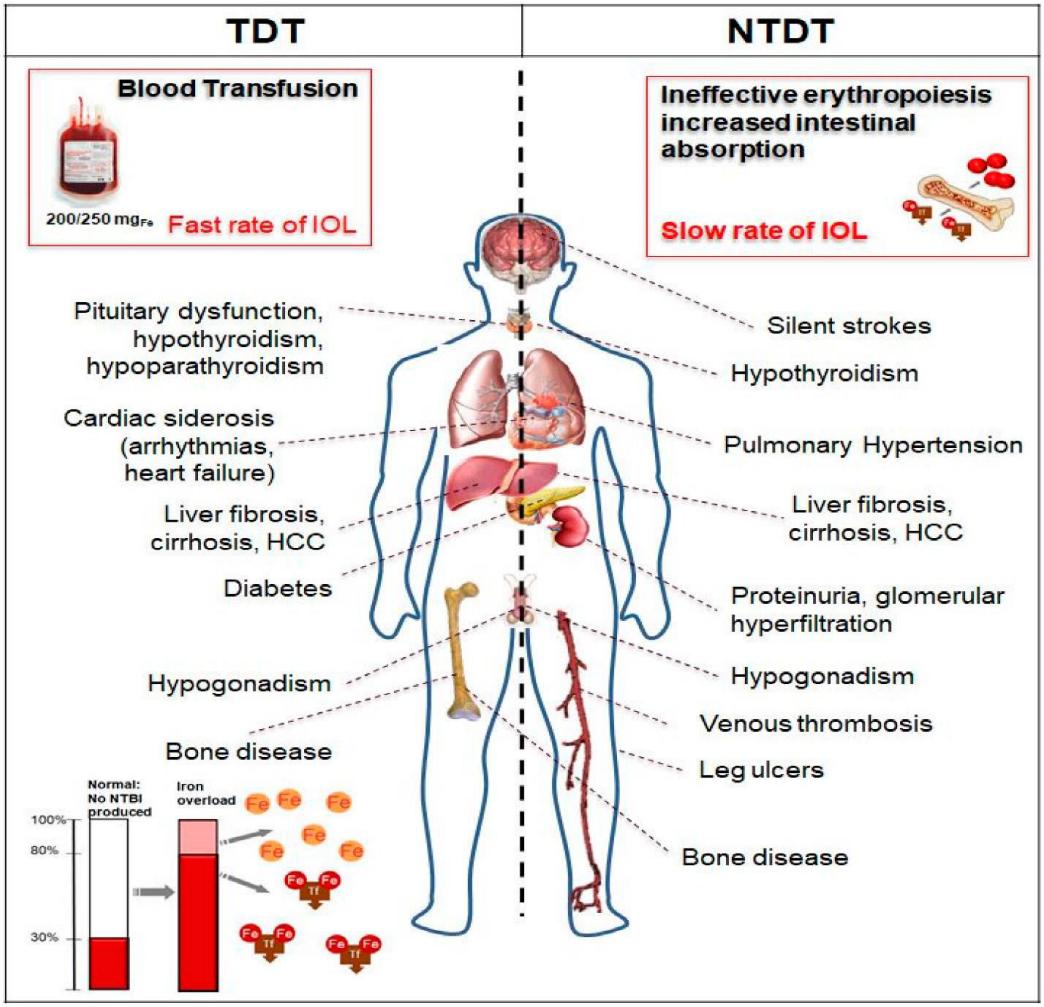


Figure 8: Clinical manifestations in TDT and NTDT

CLINICAL FEATURES

The most common age of presentation of β-thalassemia major is between 6-24 months. The main symptoms are characterized by:

(a) Severe microcytic anaemia

(b) Hepatosplenomegaly

(c) Jaundice

The clinical manifestations of thalassemia major can be divided to the following:

a) General features :

- Progressive pallor
- Fatigue
- Dyspnoea on exertion
- Poor appetite
- Palpitations
- Poor growth
- Failure to thrive
- Congestive cardiac failure
- Hyperpigmented dry skin
- Osteoporosis leading to easy fractures

b) Features due to hemolysis:

- Jaundice and hepatosplenomegaly
- Hyperuricemia(gout)
- Gallstones

c) Features due to ineffective erythropoiesis:

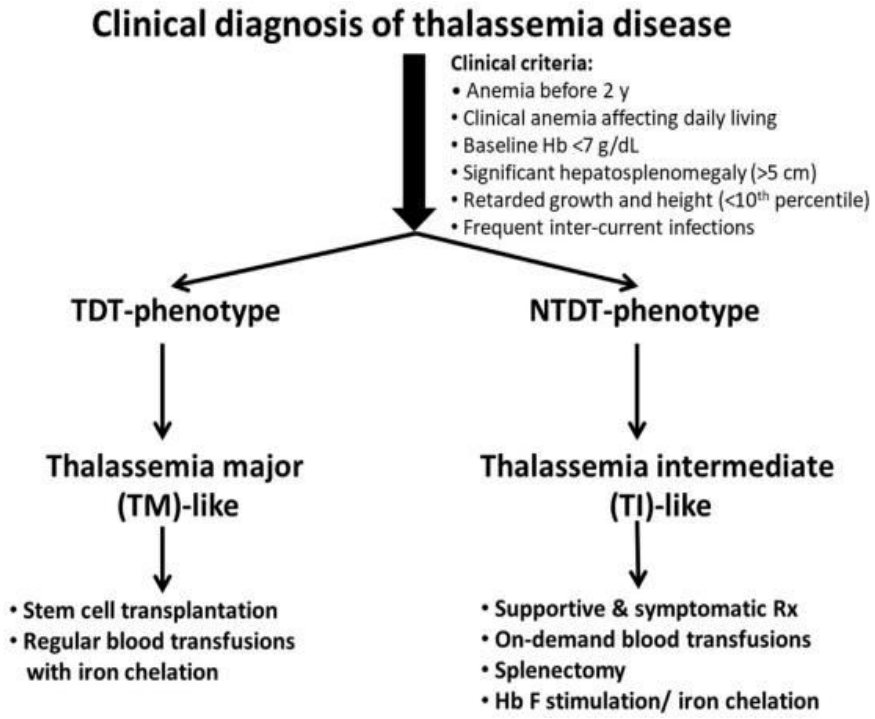
- Maxillary hyperplasia (chipmunk facies)
- Increased space ,overbite and malocclusion of teeth
- Frontal bossing
- Chronic sinusitis
- Impaired hearing

Children present with varying degrees of pallor, failure to thrive or gain weight, excessive crying, irritability and hepatosplenomegaly. Children who are poorly or untreated usually develop bossing of the frontoparietal bone, depressed nasal bridge, prominence of the malar bones, protrusion and malocclusion of the teeth – these features together called thalassemic facies. Repeated episodes of fever secondary to infection, and progressive spleen & liver enlargement causes abdominal distension which is one of the presenting features in children with thalassemia major.

The clinical manifestations of iron overload usually dominate the clinical phenotype of individuals with severe b-thalassemia. Cardiac dysfunction is the main clinical issue that can even lead to early death. Endocrine abnormalities seen are hypogonadism, low levels of growth hormone, hypothyroidism, and diabetes mellitus, are also important problems faced due to effects of iron overload.

Osteoporosis is often seen in children with thalassemia, which may be a reflection of marrow expansion, endocrinal hormone deficits, iron overload, and the potential side effects of chelators. Thinning of the cortex and subclinical and clinical fractures can occur with minimal trauma. Hypercoagulable state, which increases the risk for thromboembolism, is seen in children with thalassemia which is attributed to platelet activation, red cell membrane damage, and activation of endothelial cells [25].

CLINICAL DIAGNOSIS:



β-Thalassemia major is characterised by low Hemoglobin parameters (<7 g/dl). RBC indices will show microcytic hypochromic anemia, MCV between > 50- < 70 fL and MCH between > 12-< 20 pg.

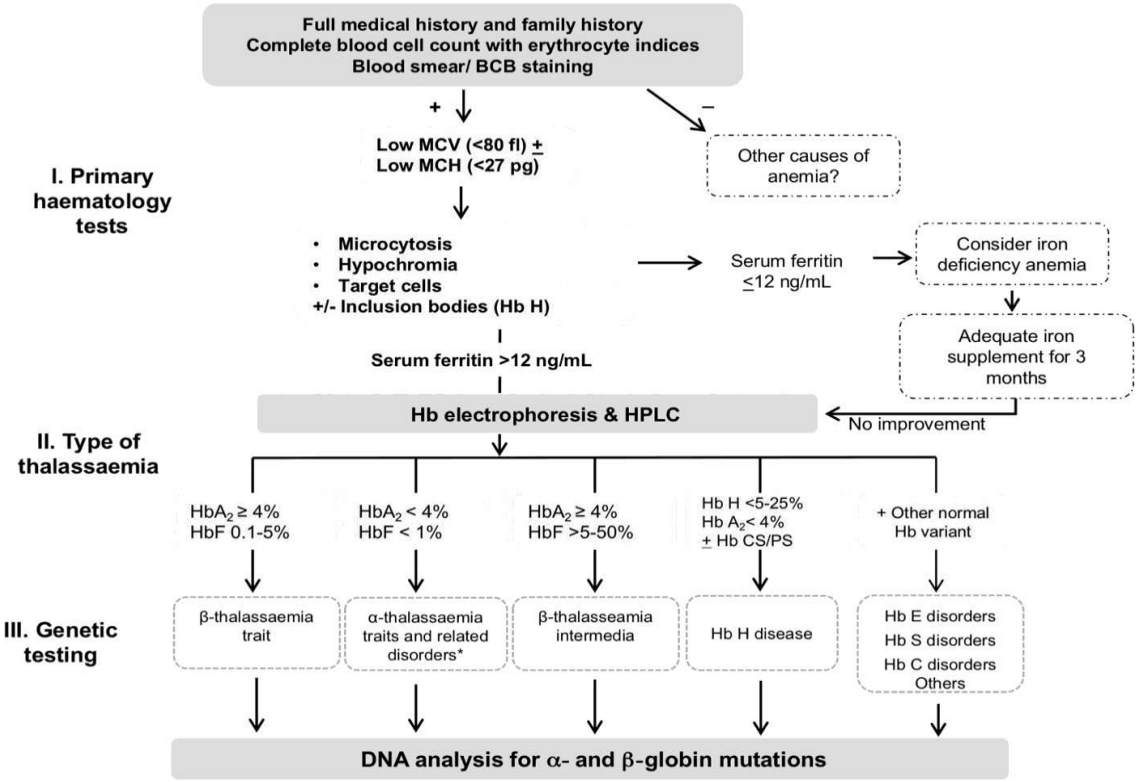
Peripheral blood smear

RBC shows moderate to severe anisocytosis, teardrop cells, microcytic hypochromic and nucleated RBC's. Degree of extent of low haemoglobin levels can be corresponding with the number of erythroblasts and the number will be elevated after post-splenectomy. Few morphologic changes will be seen in RBC's for carriers than affected individuals. Erythroblasts are generally not seen.

Hb Electrophoresis/HbE

HPLC is accurate, reliable and widely used nowadays. Normal level of HbF in an un transfused patient with thalassemia major below age of 1 year varies between 50-100%. The value of HbA_2 is between 2-7% which depends on the genotype, while a child who has received transfusion HbF may be low and non diagnostic.

HbA is absent in ß0 thalassemia homozygotes and HbF will comprise 92-95% of total Hb. HbA levels is found to be between 10 - 30% and HbF in range of 70 - 90% in homozygotes of β-thalassemia and ß+/ ß0 genetic compounds. HbA2 will vary in beta-thalassemia homozygotes and it is usually more in beta-thalassemia minor.



TREATMENT:

Treatment of β thalassemia major is a lifelong entity. Mainstay of therapy includes blood transfusion at regular intervals. If no regular transfusions and chelation therapy is not available, majority of children with thalassemia major will not survive beyond the age of 20- 23 years.

The main pillars 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.
4. Dietary restrictions and supplements: Diet with high caloric intake is recommended along with consumption of supplements like folic acid, calcium, zinc and multivitamins.
5. Hydroxyurea: The ability of hydroxyurea to induce γ-globin is its most important action in β-haemoglobinopathies, this will help to reduce the frequency of blood transfusion in transfusion dependent β-Thalassemia Major. Recommended dose to begin with is 10–15 mg/kg/day, which can be gradually increased to a typical dose of 15–30 mg/kg/day with a maximum dose of 35 mg/kg/day .

Haemoglobin levels should be maintained between at least 9 to 10 g/dL with regular transfusion therapy, which allows improved growth and development and also reduces hepatosplenomegaly, skeletal deformities.

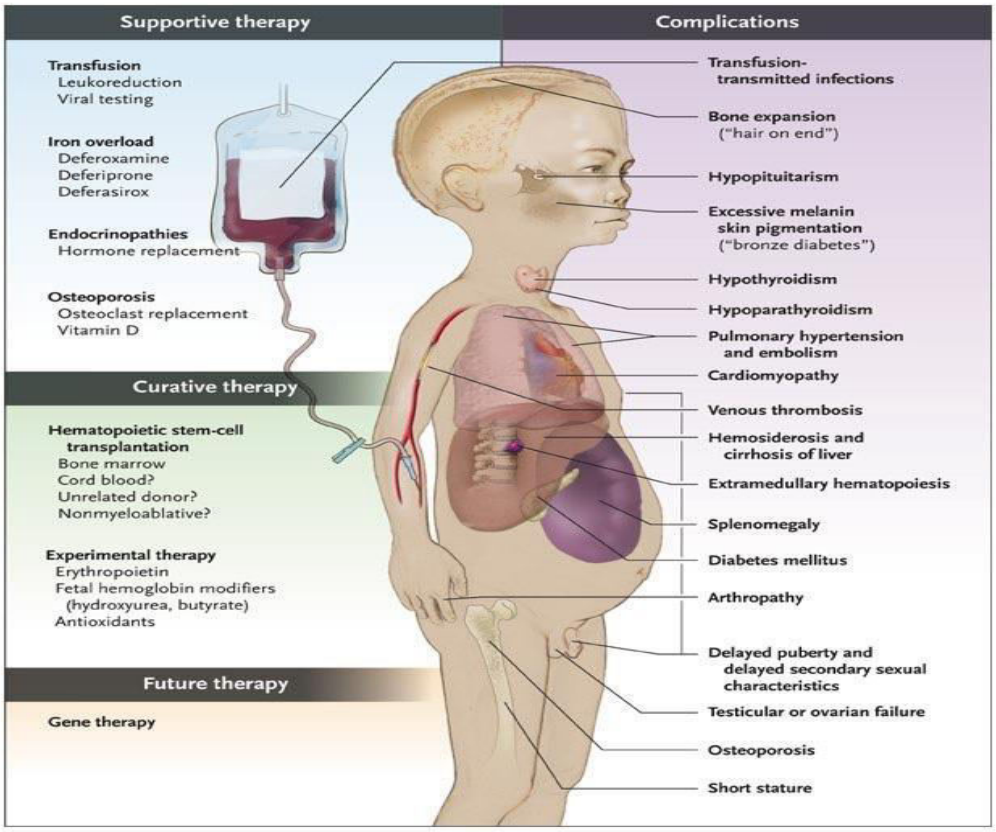


Figure 9 : Complications and treatment of thalassemia

Therapy	Advantages	Disadvantages
Blood transfusion	• Suppresses ineffective erythropoiesis, thus limiting downstream pathophysiological complications • Regular transfusion plus iron chelation therapy is associated with improved long-term survival in TDT • Role in ameliorating certain morbidities in observational studies with NTDT	• Lifelong transfusions required every 2–5 weeks in TDT • Risks of blood-borne infection, alloimmunization, and iron overload
Iron chelation	• Long-term use improves liver and myocardial iron levels and function, and improves endocrine function in TDT • Can reduce systemic and hepatic iron burden in NTDT • Oral formulations now available	• Not effective for all patients • Frequent side effects that require regular monitoring • Demanding regimen of parenteral formulation • Poor adherence among some patients • High cost
Hydroxyurea	• May improve haematological outcomes in specific NTDT populations • Low cost	• Lack of robust evidence of benefit
Splenectomy	• May improve growth, QoL, and haemoglobin concentration, thus avoiding transfusions for some patients	• Risk of sepsis • Increasing awareness of other risks from NTDT studies, including venous thrombosis and other vascular manifestations • May reduce ability to scavenge toxic free iron species, as evident from NTDT studies
HSCT	• Potentially curative for patients with TDT • 90% survival rate in patients; disease-free survival rates > 80% in TDT • Improves HRQoL of children with severe disease • Long-term cost-effectiveness	• Appropriate only for a subset of patients ○ Young age ○ Compatible sibling donor • 5–10% risk of mortality ○ Intensive myeloablative conditioning required, graft-versus-host disease, and graft failure • Potential impairment of fertility • Requires access to technology at major treatment centre • Substantial one-off cost of procedure

HRQoL, health-related quality of life; HSCT, haematopoietic stem cell transplantation; NTDT, non-transfusion-dependent thalassaemia; TDT, transfusion-dependent thalassaemia; QoL, quality of life.

Figure 10: Advantages and disadvantages of treatment modalities of thalassemia

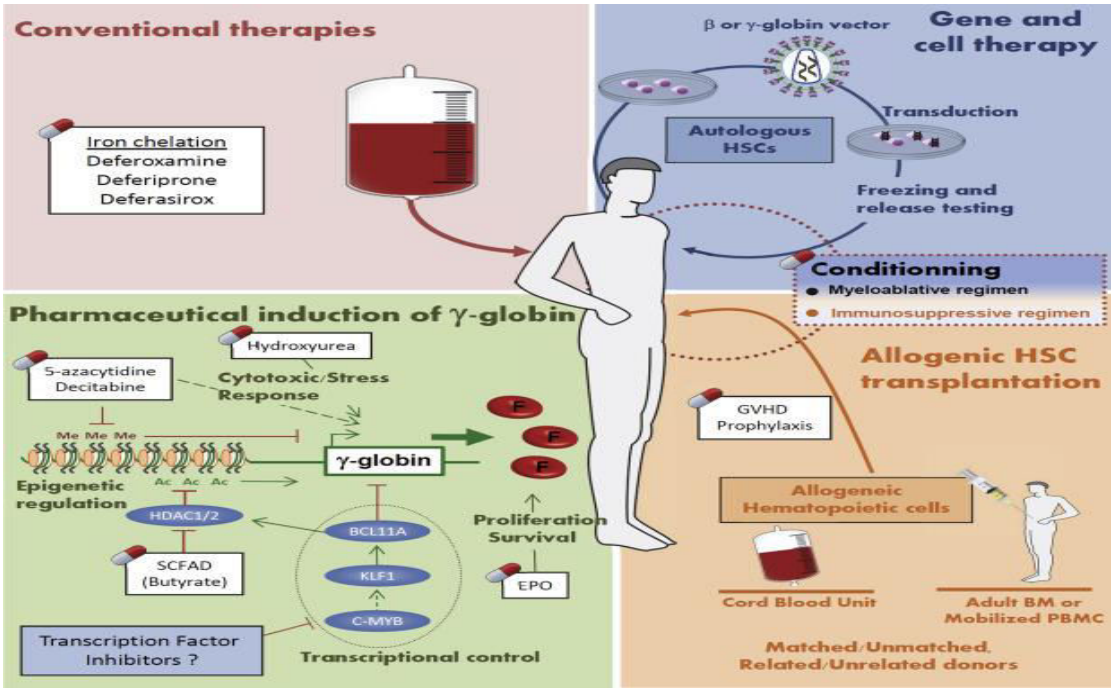


Figure 11: Newer treatment modalities of thalassemia

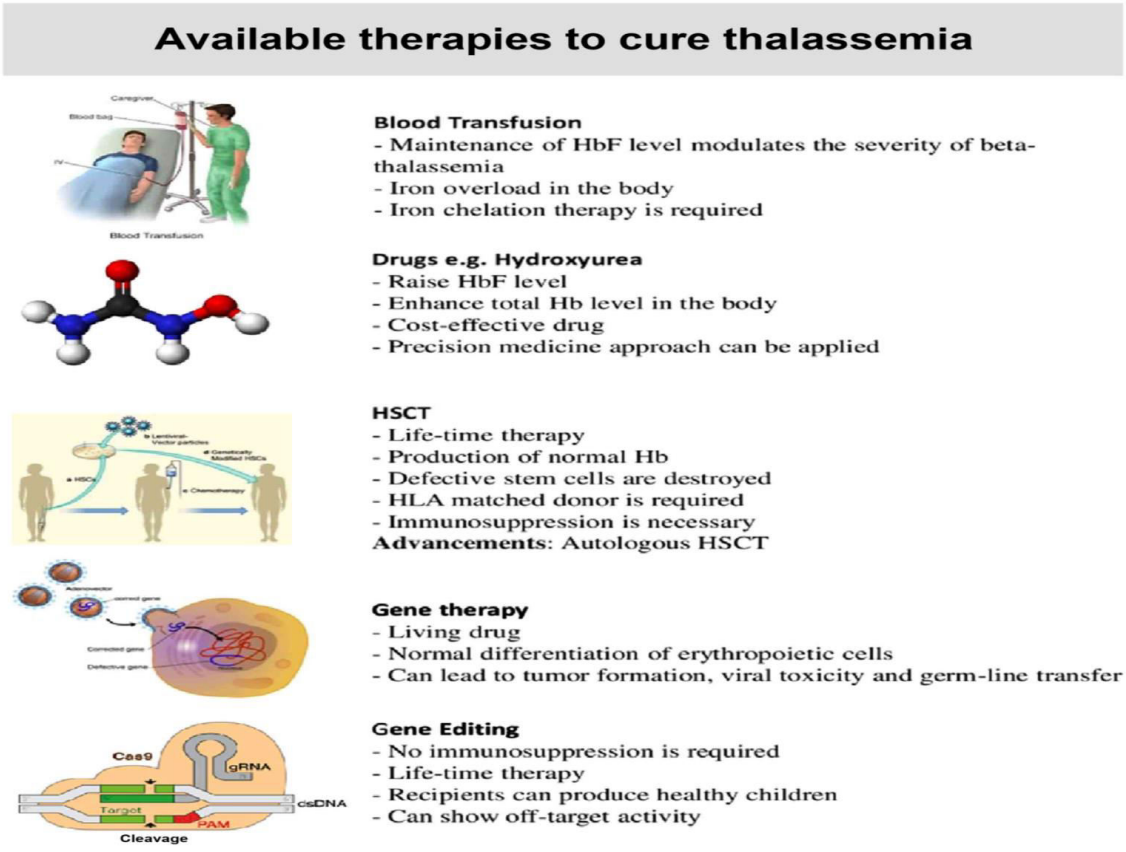


Figure 12: Treatment of thalassemia

ENDOCRINE COMPLICATIONS IN THALASSEMIA MAJOR

INTRODUCTION:

Iron overload in heart and liver is known to be one of the leading cause of morbidity and mortality among children with transfusion-dependent β-thalassemia major who receive regular blood transfusions. Various authors have reported quite a high incidence of growth retardation, delayed puberty and endocrine dysfunction in multiply transfused thalassemic patients.

PREVALANCE:

De Sanctis, et al published a review article" Guidelines for management of endocrinopathies in thalassemia" which showed Incidence/prevalence of endocrinopathies in iron overloaded β-thalassemia major patients in geographical areas as Hypogonadism seen is 35.2% in Cyprus ,42% in Greece ,49% in Italy and 50% in Turkey. The prevalence of Diabetes mellitus/IGT in 27% in Greece, 9.4% in Cyprus ,8.7% in Iran. Short stature or Growth hormone deficiency was seen in 35% patients in Cyprus, 32% in Greece, 39.3% patients in Iran. The incidence of Primary hypothyroidism is found to be 6.2% in Italy, 7.7% in Iran and around 29% in Turkey[26]

Inati et al, did a review article on Endocrine and Bone Complications in β-Thalassemia Intermedia: Current Understanding and Treatment, showed the prevalence of short stature in children and adults with thalassemia is approximately 25% regardless of the type of the thalassemia or the serum ferritin level and 20%–30% of patients with thalassemia have growth hormone (GH) deficiency. Hypogonadic hypogonadism was observed in 24% of patients, affecting girls more than boys. The prevalence of diabetes mellitus and impaired glucose tolerance is 9.4%

and 7.1% and the prevalence of hypothyroidism ranges from 4% to 24.4% in β-thalassemia major [27].

Sharma Et al studied the endocrinopathies in adolescents with thalassemia major receiving oral iron chelation therapy in a prospective cohort study .In 89 patients who were evaluated ,55% were of short stature and 27% had a height Z score of <= 3 . Delayed puberty and /or hypogonadism was present in 54.1 %. It showed that overall 49.4% adolescents had at least one endocrinopathy which included hypothyroidism, hypoparathyroidism, hypogonadism, impaired glucose metabolism or short stature with height for age < 3 SD [28].

ETIOLOGY:

The exact mechanism behind endocrine dysfunction due to iron overload presently remains unclear. However, when iron levels in the body become too high, it can cause saturation of transferrin, and the non-transferrin-bound iron (NTBI) species tend to circulate in the plasma. The amount of unbound iron within cells or in plasma is very labile and can undergo reoxidation cycle between Fe2+ and Fe3+, hence this generates reactive oxygen species (ROS), leading to lipid peroxidation.

Lipid peroxidation when it occurs under the circumstances of iron overload causes generation of both unsaturated and saturated aldehydes. Both these forms of aldehydes have been implicated in cellular dysfunction, cell death and cytotoxicity.

Apart from iron overload, other factors responsible for the organ damage are:

(a) Chronic hypoxia secondary to anemia that will potentiate the toxicity of deposition of iron in endocrine glands

(b) Viral infections

(c) Individual susceptibility have been implicated in causing endocrine dysfunction (26)

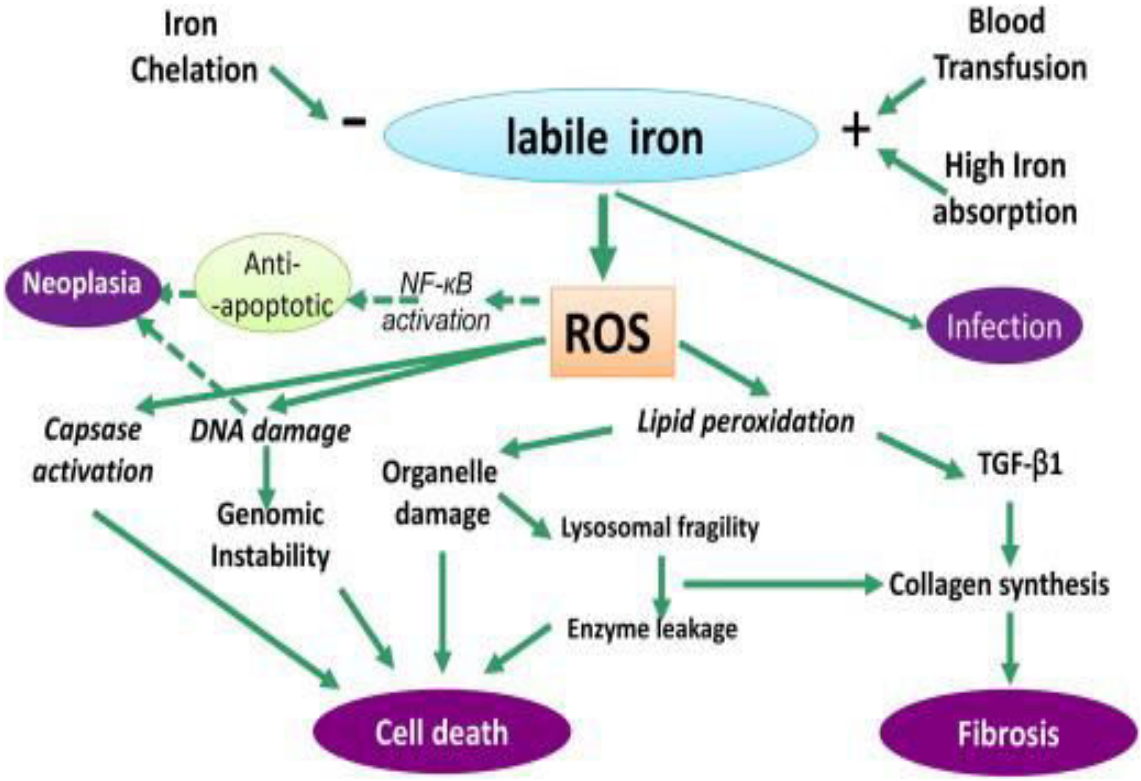


Figure 13: Cellular mechanism of iron toxicity

HYPOGONADISM AND DELAYED PUBERTY:

Delayed puberty and hypogonadism is one of the most common endocrine complications seen in patients with β -thalassemia major. It's prevalence is around 24%. It can be attributed to iron-mediated damage leading to dysfunction of the hypothalamic-pituitary axis. Delayed puberty can be defined as no attainment of puberty in females by 13 years and by age of 14 years in males.

Pathogenesis:

Iron toxicity on the gonadotrope cells is not the only cause of hypogonadotropic hypogonadism in children with thalassemia, but there is toxicity to adipose tissue and leptin. Leptin indirectly stimulates the Kiss1 neurons in the arcuate nucleus by acting on the hypothalamic– pituitary–gonadal (HPG) axis. During

puberty there is an increase in the concentration of the leptin which causes and initial increase in the LH and FSH. Several studies have been carried out on estimating the leptin levels in different age groups of children with thalassemia and level of leptin was found be to low in all of them. Hence, one cause of pubertal delay in these children can be attributed to the low circulating leptin level.

It is found that the ovarian reserve is usually normal in the majority of female thalassemia patients, while in males, there is evidence of interstitial fibrosis in testes with small, heavily pigmented, undifferentiated seminiferous tubules and absence of Leydig cells due to the direct consequence of iron. Deposition of iron in anterior pituitary glands can be present from ten years of life but the clinical manifestations due to this is usually seen only after the onset of puberty. The gonadotropin reserve when significantly diminished, along with a markedly reduced spontaneous pulsatile gonadotropin activity it can lead to irreversible damage of the HPG axis [29].

Definition of hypogonadism can be an absence of testicular development in males and breast development in females by 16 years of age. Hypogonadic hypogonadism is the most frequently seen and very often undertreated endocrine manifestation of β-thalassemia. It is more prevalent among girls than boys. The clinical spectrum is very varied and can range from hypogonadism to a simple delay in starting and developing of puberty .

Associations of Hypogonadic hypogonadism are :

(a) Early onset of blood transfusion

(b) Serum ferritin of approximately 2000 ng/mL

(c) High liver iron concentration (LIC)

(d) Increasing age

(e) Treatment with Hydroxy urea (27)

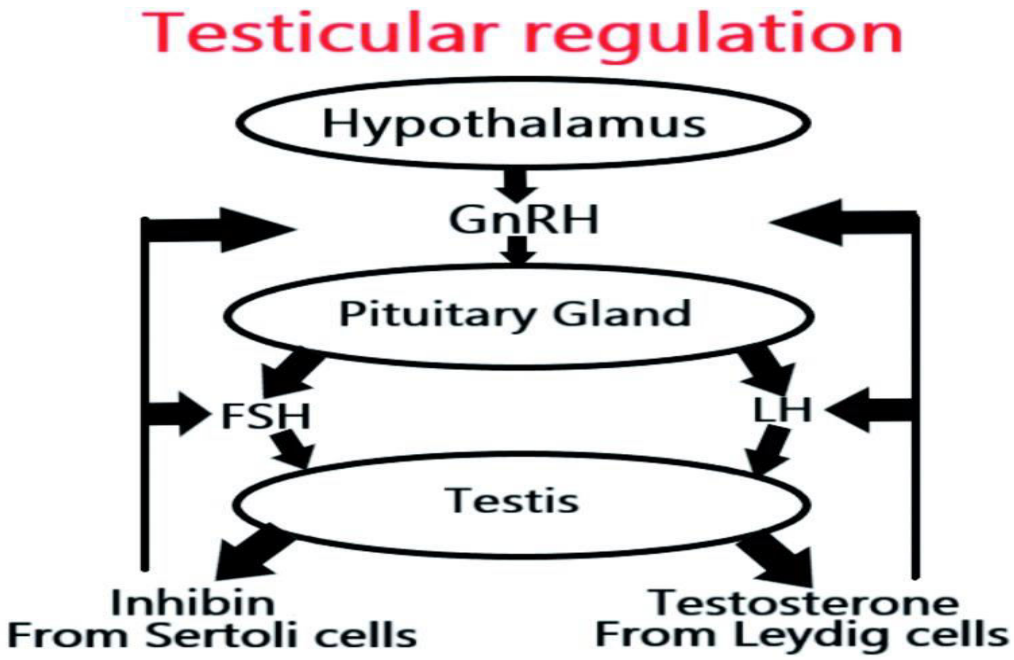


Figure 14: Testicular regulation of gonadotrophins

Investigations:

- Tanner staging should be assessed every 6 months starting from the age of 12 years in children undergoing regular blood transfusions.
- Girls who do not attain puberty by the age of 13 years and boys by the age of 14 years should be screened by checking the levels of LH, FSH, and estradiol in girls, while testosterone in boys.
- Low FSH and LH for that age infers hypogonadotropic hypogonadism which indicates a lesion in the hypothalamic-pituitary pathway.
- Pelvic ultrasound can be helpful in assessing ovarian and uterine maturation in thalassemic patients with hypogonadism.
- MRI scan to know the iron accumulation in pituitary gland.

Treatment:

a) Oral ethinyl estradiol with dose of 2.5–5 μg (can be given upto 10μg) daily for 6 months in female

b) When breakthrough uterine bleeding does not occur low oestrogen-progesterone hormone replacement can also be tried

c) IM injection of testosterone enanthate or cypionate at dose of 30–50 mg every month for 6 months

d) Other preparations of testosterone like topical testosterone gel

Follow up:

Repeat LH, FSH, testosterone and oestradiol every 6 months

STUNTED GROWTH AND SHORT STATURE:

Children with β thalassemia major often present with constitutional delay of growth and a resultant reduction in the final height when compared to the target height for that age. The prevalence of stunted stature is around 30-60% in different studies. Short stature, usually takes place from age of 5-6 years in boys and around the age of 8 years in girls. But nowadays, children who are well transfused and chelated with oral iron chelators usually grow normally till at least the first 10 years of life. Short stature can be defined as a height more than 2 SD below mean height for that age i.e. below 3rd percentile.

Pathogenesis:

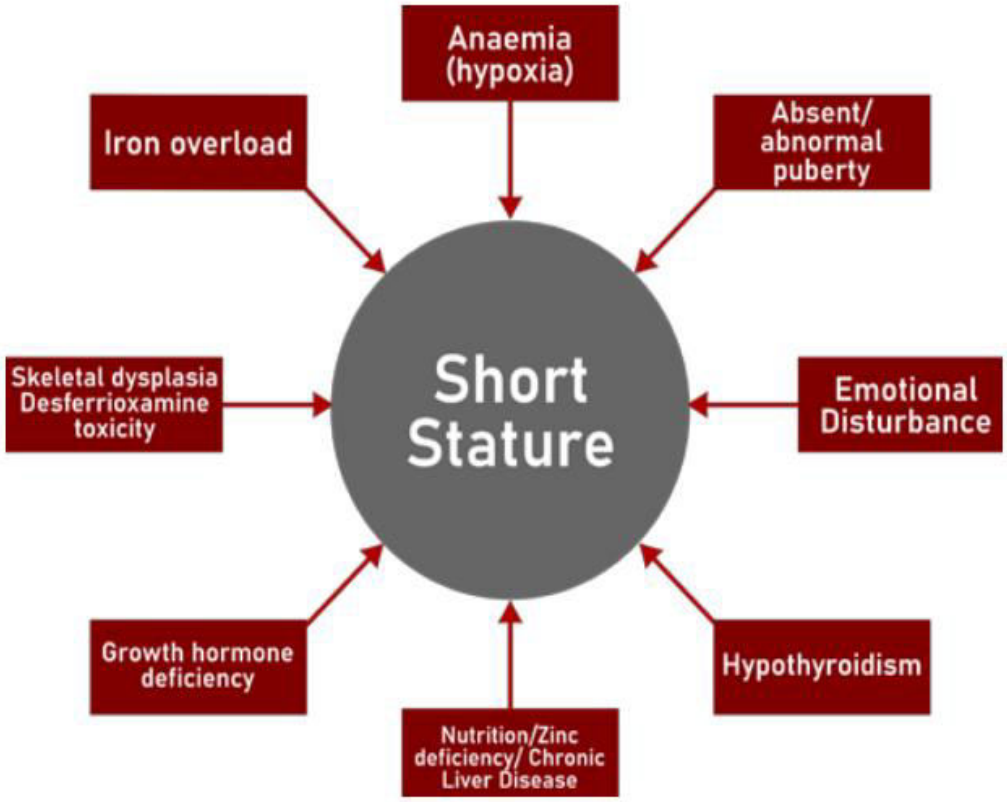


Figure 15: Pathogenesis of short stature in thalassemia

Factors affecting growth in children with thalassemia major:

→ Chronic anemia with chronic tissue hypoxia

→ Bone dysplasia due to chelator toxicity

→ Zinc deficiency and other nutritional deficiencies

→ Hypothyroidism

→ Hypogonadism/delayed puberty

→ Impairment of the GHRH-GH-IGF-I axis

→ Transfusion regimen, age of initiation, and the type of iron chelation therapy

According to age when the child is affected, 3 different phases of growth abnormalities or disturbances are identified. Each of these phases are known to have different etiologies. During the first phase short stature is mainly as a cause of hypoxia, anaemia, ineffective erythropoiesis and nutritional deficiencies. During second phase, which occurs in the late childhood, iron overload affecting the GH-IGF-1 axis and potential endocrine side effects are the main reason behind growth faltering. In the third phase of growth abnormality which usually occurs after the age of 10-11 years, delayed or arrested puberty is an important factor which contributes to growth failure in adolescent children with β thalassemia major.

Investigations:

Evaluation of patients with short stature or growth delay include:

- Dietary assessment
- Assessment of GH secretion: Growth Hormone insufficiency/deficiency may be diagnosed by a reduced levels of GH to provocative tests:

  IV at dose of 0.1 unit/kg. Blood glucose level less than 2.2 mmol/L after the injection indicates a GH deficiency.

→Clonidine stimulation test: Tablet clonidine when given at the dose of 150 μg/m^2 of the body surface area and GH level was estimated at 0 , 30, 45, 60, and 90 min after intake of the tablet. A GH level with <10 ng/mL was considered to have growth hormone deficiency [30].

- Low IGF-1 levels
- MRI of the hypothalamic–pituitary region - to evaluate pituitary iron overload and the size of pituitary gland

Treatment:

a) Adequate blood transfusion to attain pretransfusion hemoglobin level >9g/l.

b) Adequate chelation to maintain a serum ferritin value of < 1,000 ng/ml.

c) Use of new iron-chelators which have lower toxicity on the skeleton.

d) Correction of nutritional deficiencies like protein-calorie, folate, vitamin D, vitamin A, zinc, carnitine

e) Oral zinc sulphate supplementation to patient with zinc deficiency.

f) Management of pubertal delay

- Treatment of any hypothyroidism or impaired glucose tolerance
- Exogenous GH administration -dose of 0.16–0.24 mg/kg/week (22–35 μg/kg/day) [31].

Follow up:

Annual growth screening should be done from the age of 9 years.

- Serum TSH and FT4
- Serum calcium, ionised calcium, inorganic phosphate, magnesium and ALP
- Serum IGF-1
- Serum zinc
- Screening for coeliac disease
- X- ray of wrist and hand, tibia and spine to exclude any presence of platylospondylosis or metaphyseal cartilaginous dysplasia changes.
- GH estimation [32].

DIABETES MELLITUS AND IMPAIRED GLUCOSE TOLERANCE

Glucose intolerance usually occurs at an earlier age during adolescence in β-thalassemia major patients, while diabetes can frequently occur at later stages and is usually secondary to iron overload and subsequent chronic liver disease. Prevalence is found to be 10% to 24% in various studies. Impairment of pancreatic β-cells can be characterised in various forms ranging from insulin resistance with hyperinsulinemia with a normal glucose tolerance, insulin resistance with glucose intolerance, progressive impairment of β-cell function with a reduction of insulin secretion and finally leading to insulin-dependent DM. The progression to the last stage is a relatively slow process and does not occur all at once [33].

Pathogenesis:

The cause behind the eventual development of diabetes in children with β thalassemia major can be attributed to impaired insulin excretory function which occurs due to chronic iron overload in pancreas, activation of selective immune system against pancreatic β-cells which leads to cell damage , or pancreatic cell death due to fat transformation. Initially the impaired glucose tolerance is accredited to iron mediated insulin resistance and not because of decrease in insulin production. β-cell damage and insulin deficiency will develop subsequently due to the iron toxicity. Serum ferritin of 3000 ng/mL has shown to have a higher association with developing diabetes or impaired glucose tolerance .

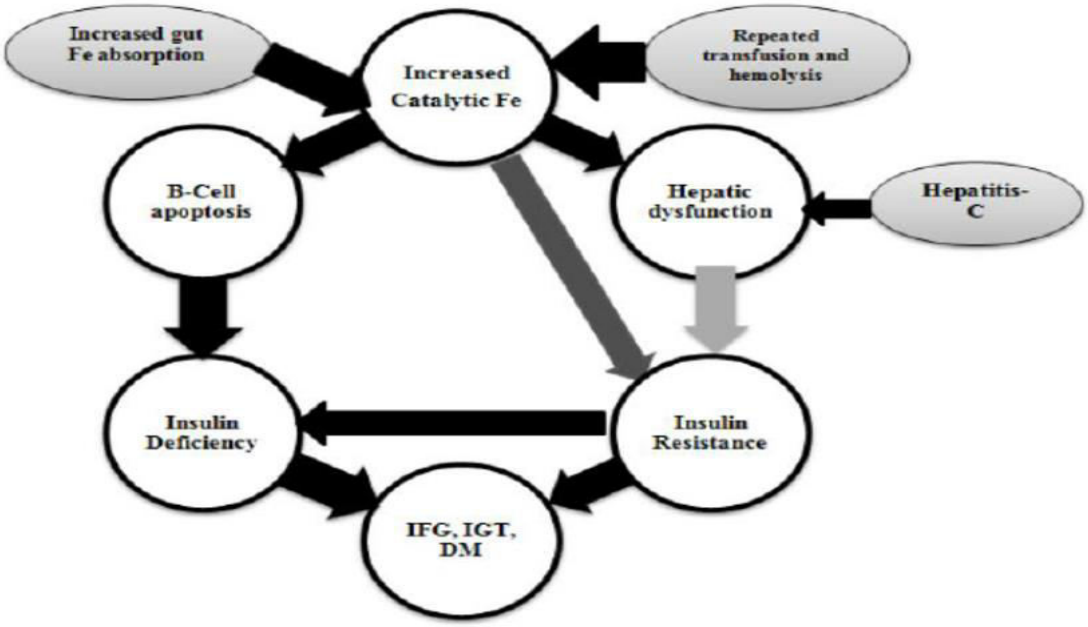


Figure 16: Cellular mechanism of Diabetes mellitus and impaired glucose tolerance

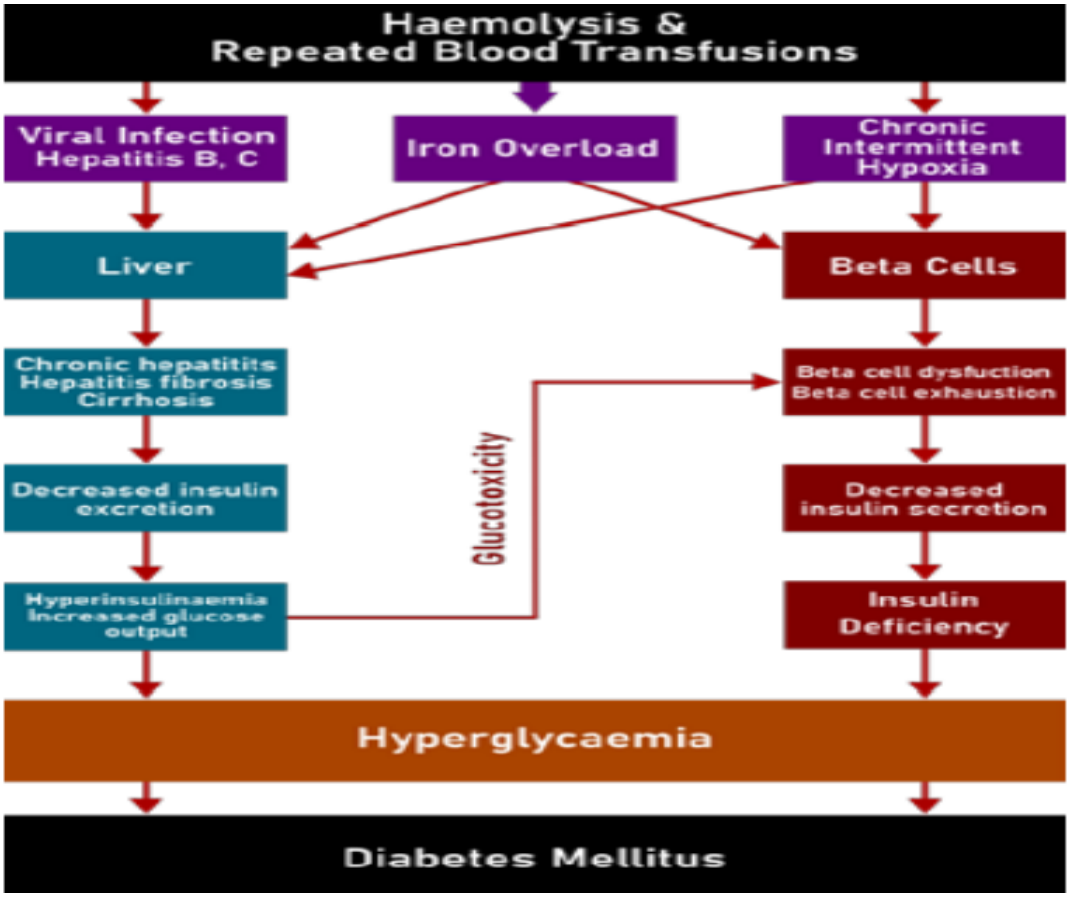


Figure 17: Pathogenesis of Diabetes mellitus in thalassemia

Clinical features:

Clinical features may range from asymptomatic glycosuria to ketosis and ketoacidosis Patients may present with symptoms of increased thirst, frequent urination, extreme hunger, unexplained weight loss, fatigue.

Complications:

- ❖ Cardiovascular disease
- ❖ Cerebrovascular disease
- ❖ Peripheral vascular disease
- ❖ Microvascular complications like diabetic retinopathy, nephropathy and neuropathy

Investigations:

- Impaired fasting glucose was defined as blood glucose level between 100 and 125 mg/dL.
- If the fasting serum glucose is >110 mg/dl, oral glucose tolerance test is indicated
- A 2-hour OGTT -better if combined with insulin secretion determination. Blood glucose between 140 and 199 mg/dL- impaired glucose tolerance (IGT)
- Diabetes mellitus can defined as fasting blood glucose concentration of ≥126 or a blood glucose level ≥200 mg/dL after 2 hours of meal intake.

Treatment:

a) Intensive chelation therapy and prevention and treatment of chronic hepatitis C infection

b) Healthy diabetic diet

c) Regular physical activity

d) Drugs - metformin, glibenclamide, sitagliptin, acarbose.

e) Subcutaneous injections of insulin daily

Follow up :

- Glucose monitoring at home using glucometers
- Urine ketones if blood sugar >250 mg/dl
- Renal function tests and urine and serum albumin test every six monthly to know the kidney function
- Evaluation of retinopathy [34].

HYPOTHYROIDISM:

Hypothyroidism is a late complication of the iron overload in the thyroid gland which can ultimately lead to parenchymal fibrosis of the gland. It's incidence is around 6 to 30 %. Preclinical hypothyroidism is defined by a normal level of thyroxine (T4) and free thyroxine (FT4), a normal basal TSH and TSH values slightly increased after the Thyrotropin releasing Hormone (TRH) test. Subclinical hypothyroidism can be defined as a normal serum level of T4 level and FT4 with a slightly increased TSH level in the blood.

Pathogenesis:

Reactive oxygen species (ROS) which is generated due to the toxicity of the excess unbound iron within the cells, leads to lipid peroxidation and generates saturated and unsaturated aldehydes which have been implied in cytotoxicity and cellular dysfunction and eventual cell death. Splenectomy is a specific risk factor for hypothyroidism in β- thalassemia major. Other contributing factors include chronic

anemia, poor compliance with oral medication like desferrioxamine and an elevated serum ferritin levels reaching 3000 ng/mL . The risk of developing hypothyroidism increases significantly with every 1 mg Fe /kg increase in the liver iron concentration. Primary hypothyroidism can occur from second decade of life, while secondary hypothyroidism is more rare and autoimmune thyroiditis is almost always absent in cases with thalassemia major.

Clinical features:

Patients with subclinical hypothyroidism may be asymptomatic and diagnosed on routine screening, while patients with primary hypothyroidism can present with short stature, delayed puberty, fatigue, cold intolerance, constipation, weight gain and dry skin [35].

Complications:

- Cardiac failure and pericardial effusion
- Osteoporosis
- Infertility

Investigations:

- Measurement of serum TSH and T4 level
  - Preclinical hypothyroidism – Normal T4 level and marginally increased TSH level for the age.
  - Mild hypothyroidism – Marginally low T4 level and high TSH level
  - Overt hypothyroidism -Low T4 level with an elevated TSH level
- Thyroid autoantibodies
-
- Ultrasonography of the neck to identify any abnormalities

Treatment:

a) Central hypothyroidism can be treated with Levothyroxine

b) In subclinical hypothyroidism -intense chelation and careful monitoring of the patient and the patient carefully monitored.

c) Treatment with amiodarone can lead to cardiac dysfunction due to rapid progression from subclinical to severe hypothyroidism

Follow up:

- Free T4 and TSH every 3-4 monthly
- Serum ferritin.
- Electrocardiogram (ECG) and echocardiogram -in severe case every 6-12 monthly
- Hypothalamic-pituitary MRI, in patients with central hypothyroidism.

TREATMENT OF ENDOCRINE COMPLICATIONS

Endocrine dysfunction	Diabetic mellitus Fasting blood glucose, oral glucose tolerance test, blood glucose 2 hours after meal Hypothyroidism TSH, FT4, bone age (X-ray of wrist and hand) Calcium metabolism and Osteopenia/Osteoporosis Calcium, phosphorus, alkaline phosphatase, 25-hydroxy vitamin D, parathyroid hormone osteocalcin, C-terminal telopeptide, bone densitometry Hypogonadism Tanner staging, LH, FSH, and sex steroids (estradiol in females and testosterone in males), bone age (X-ray of wrist and hand), pelvic ultrasound	Management consists of: hormone-replacement therapies, vitamin D, bisphosphonates, and calcium supplementation with follow-up bone densitometry

IRON CHELATORS

Chelating agents are the cornerstone in the management of children with β thalassemia major who are on regular blood transfusions. Early initiation of chelating agents will help to avoid complications which occur due to iron deposition in various tissues especially the endocrine organs.

Criteria for starting chelation therapy are:

1. Child who has received at least a 100ml/kg packed cell transfusions
2. Serum ferritin level exceeding 1000 µg/L
3. When the child is at least above 3 – 5 years old
4. Liver iron concentration which is measured only after 1 year of onset of regular transfusions, should exceed a 7 mg/g dry weight of liver

Properties of an ideal iron chelator:

(a) High affinity for iron in ferric form and low affinity for ferrous form and other metals

(b) High chelating efficiency- should be able to chelate a large quantity of iron and have long half-life.

(c) Attain negative iron balance

(d) Good tissue and cell penetration.

(e) There should be no redistribution of iron

(f) Tolerability should be good

(g) Orally bioavailable

(h) Slow rate of metabolism- effective in once or twice daily doses

(i) Affordable

(j) Easily available [36].

Property	Deferoxamine	Deferasirox	Deferiprone
Stoichiometry (chelator: iron)	Hexadentate (1:1)	Tridentate (2:1)	Bidentate (3:1)
Usual dose	25–60 mg/kg/day over 8–24 hours	20–40 mg/kg/day once daily	75–100 mg/kg/day in three divided doses
Route of administration	Subcutaneous, intravenous	Orally dispersible tablet	Oral tablet or suspension
Half-life	20–30 minutes	7–16 hours	1.5–2.5 hours
Excretion	Urinary, fecal	Fecal	Urinary
Ability to remove liver iron	+++	+++	++[9]
Ability to remove cardiac iron	++[#]	++[99]	+++
Typical adverse events	Local reactions Sensorineural hearing loss Ophthalmic changes Allergic reactions Bone abnormalities Increased risk of *Yersinia* and *Klebsiella* infections Pulmonary at high doses Neurological at high doses	Gastrointestinal Rash Rise in creatinine Proteinuria Elevated hepatic enzymes Gastrointestinal bleeding (rare) Fulminant hepatic failure (rare) Renal insufficiency (rare)	Gastrointestinal Neutropenia/Agranulocytosis Arthralgia Elevated hepatic enzymes
Availability	Licensed	Licensed	Licensed in Europe and Asia as second-line agent; not licensed in North America

Figure 18: Iron Chelators used in thalassemia

DEFEROXAMINE:

Deferoxamine (DFO) is a hexidentate iron chelator, was the first used iron chelator for treating transfusional iron overload in the 1960s. DFO has played a very important role in increasing the life expectancy and quality of life of thalassemic children with iron overload who did not usually live into their teens. It is not well absorbed via the gastrointestinal tract and hence should be administered via parenteral route. Also the drug's plasma half-life is very short being approximately 20 minutes, thus this drug usually has to be administered as a continuous infusion of 25 to 50 mg/kg given over 8 to 12 hours, 5 to 7 days per week either via subcutaneous or intravenous route.

The parenteral route of administration is painful and time consuming, hence it creates issues in treatment adherence especially in children. Average dose regularly used is 20-40 mg/ kg body weight in children. Efficacy of DFO is well established and multiple studies have shown an improvement and reduction in the serum ferritin level with regular administration of DFO. It is efficacious in causing substantial iron excretion and causing a favourable negative iron balance in children undergoing regular transfusions.

Adverse effects:

(a) Local infusion site reactions like induration and erythema

(b) High-frequency hearing loss

(c) Ophthalmologic toxicity

(d) Growth retardation

(e) Skeletal changes -rickets-like lesions and genu valgum

(f) Acute pulmonary toxicity with respiratory distress, hypoxemia

(g) Acute neurotoxicity- with high doses

DEFERASIROX:

Deferasirox (DFX) was the first oral iron chelator approved for use in thalassemia. DFX is a triazole compound which binds iron in a 2:1 ratio and is a tridentate chelator. DFX is a drug with a high specificity for iron and it is supplied as orally dispersible tablets which can be dissolved in water or juice and should be taken empty stomach at least 1/2 hour before a meal. Deferasirox is rapidly absorbed, achieving peak plasma levels within 1–3 hours after administration.

DFX protects the cells from the toxicity of Non Transferrin-Bound Iron (NTBI). . Half-life ranges from 7 to 16 hours, hence be used as a once-daily dosing regimen. The accepted dose used is 20-30mg/kg/day. Overall significant reduction in iron toxicity is seen. Doses of deferasirox should be guided by the goal of maintaining an optimal reduction of the excess body iron stores, keeping in mind about the ongoing transfusional requirements and trends in ferritin and liver iron content during treatment.

Adverse effects:

(a) Nephrotoxicity – ranging from mild elevation in serum creatinine to intermittent proteinuria

(b) Gastrointestinal disturbances- nausea, vomiting and abdominal pain

(c) Hepatotoxicity- Elevation of transaminases above five times the baseline, fulminant hepatic failure and gastrointestinal bleeding

(d) Diffuse, maculopapular skin rashes

(e) Ocular toxicity in form of cataract or lenticular opacities

(f) Audiotoxicity [37].

DEFERIPRONE:

Deferiprone (DFP) was first introduced in the 2000 as a second-line therapy for thalassemic patients with iron overload who had developed adverse effects to deferoxamine or had contraindications to the use of DFO. Deferiprone is a bidentate oral iron chelator which means that each ferric iron binds with three molecules of the drug. DFP has a short plasma half-life lasting for 1.5 to 2.5 hours, due to which the drug should be given in a thrice daily dosage. Iron excretion in deferiprone is almost exclusively via the renal route with very minimal excretion via feces.

The dose which is recommended for DFP is 75mg/kg/day in three divided doses . Deferiprone was found to be most useful in patients with a high serum ferritin and showed improved efficacy in removing iron when the iron burden is high The compliance in patients with deferiprone was found to be more than patients using deferoxamine.

Adverse effects:

(a) Agranulocytosis - absolute neutrophil count $>500 \times 10^9$ /L

Neutropenia is usually reversible after discontinuation of the drug but can recur if the drug is restarted. In clinical practice, it is advised to check the blood counts every week which can be cumbersome.

(b) Gastrointestinal symptoms like nausea, abdominal pain and vomiting

(c) Arthropathy with pain and/or swelling of the large joints including knee joint

Attributed to free radical injury.

(d) Elevation in serum alanine aminotransferase (ALT) – often transient and resolves even if the drug is continued

(e) Anorexia [36].

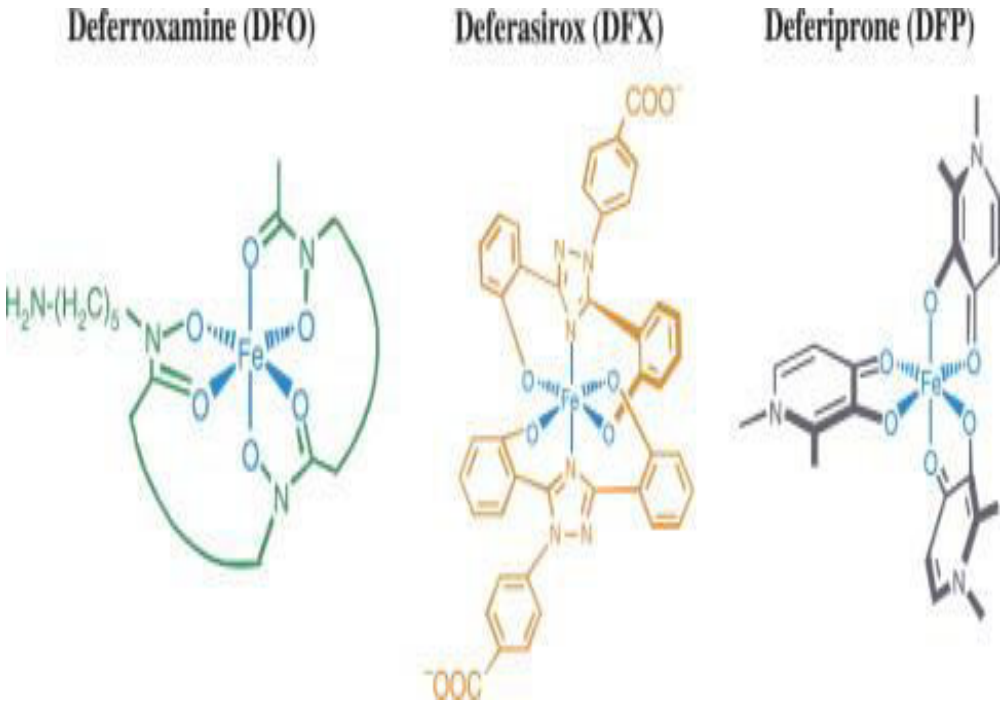


Figure 19: Molecular structure of iron chelators

EFFECT OF COMBINATION THERAPY IN ENDOCRINE COMPLICATIONS IN CHILDREN WITH THALASSEMIA MAJOR

Farmaki K, Tzoumari I, Pappa C conducted an observational study on the effect of oral chelators in reversing or preventing the iron overload in transfusion dependent thalassemia major patients which included 16 patients and conducted in the Thalassemia Unit, of the General Hospital of Greece showed that following 24 months of combined oral chelation, mean 2 h glucose in OGTT decreased significantly (111± 24 vs. 150±87 mg/dl, p= 0.007). All females had shown an improvement in their LH and FSH responses to GnRH test. Among males, who were either hypogonadal or eugonadal, all improved in their LH levels to GnRH test. All males had an increment in their mean testosterone level (9.5± 1.8 vs. 7.6± 2.4 ng/ml) . No new cases of hypogonadism were seen. Regarding thyroid function one out of the two with subclinical hypothyroidism presented with a slight increase in FT4 levels

and reduced his thyroxin dose. New cases of hypothyroidism were not found in the study [38].

Poggi1 M et al conducted a retrospective cohort study on 165 thalassemic patients with mean age 39.9 ± 8.3 years on the longitudinal changes of endocrine and bone disease in patients with β-thalassemia major receiving different iron chelators over 5 years. The study showed that patients on deferasirox had shown a decrease in prevalence of hypogonadism by 3.4 % and diabetes mellitus by 1.8 %, while there was no change in hypothyroidism. Patients on treatment with deferoxamine had shown a decrease in prevalence of hypothyroidism (−2.5 %), while patients on deferiprone had showed an increase in prevalence of diabetes (+5.5 %) and no change in the prevalence of any other endocrinopathies were observed [39.]

Farmaki et al conducted a longitudinal study on normalisation of total body iron load with intensive combined chelation which reversed cardiac and endocrine complications of thalassaemia major which enrolled 52 patients. Following chelation with combined therapy, the study had seen a statistically significant decrease in post-challenge glycaemia ($P < 0.001$), and also significant increase in insulin secretion ($P < 0.005$) and also 14 out of 18 patients who had subclinical or compensated hypothyroidism presented a significant increase in mean FT4 ($P < 0.001$) and mean FT3 ($P < 0.001$) and an additional significant decrease in the mean TSH ($P < 0.001$).7 out of 14 hypogonadal males showed normal mean testosterone levels and normalized their LH–FSH levels as shown by their GnRH response. In previously eugonadal females the mean oestradiol level increased significantly and LH and FSH responses to GnRH improved [40].

MATERIAL AND METHODS

This study was done from January 2021 to December 2021 under the Paediatrics Department, KLES Dr. Prabhakhar Kore Hospital & Medical Research Center, Belagavi.

Study Design:

Interventional study

Study duration:

One year i.e. from January 2021 to December 2021

Place of study:

KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

Source of Data:

Registered patients aged 12 to 18 years diagnosed with Thalassemia Major on blood transfusion and long term oral chelation therapy at KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Selection criteria

Inclusion criteria:

1. Pediatric patients with transfusion-dependent thalassemia aged 12-18 years on single oral iron chelator (Deferasirox)

Exclusion criteria:

1. Patients on more than 1 oral chelator
2. Non transfusion-dependent patients
3. Other haemoglobinopathies like sickle cell disease

4. Chronic infections like TB, HIV, HEP-C
5. Allergy to any of the oral iron chelators
6. Those on other hormonal therapy.

Sample size:

The minimum sample size formula based on prevalence rate is

$$n = \frac{z_\alpha{}^2 P(1-P)}{d^2}$$

where P is the percentage of prevalence and d is the percentage likely difference in the prevalence.

z_α is linked with the level of significance. For 5% level of the significance $z_\alpha = 1.96$.

Ref:

With P = 73.3% and d = 20% of P = 18.33%, the sample size is 35.

To make the study more confirmative, the sample size will be raised to 40.

Informed Consent:

The parents of children who fulfil the eligibility criteria will be briefed about the nature of the study and written informed consent will be taken for enrolment in the study.

Methodology:

After obtaining ethical clearance children between 12-18 years of age who were registered in thalassemia day-care centre of KLE Dr Prabhakar Kore Charitable Hospital & Medical Research Center affiliated to KLE Academy of Higher Education

and Research's J N Medical College, BELAGAVI and who fulfilled the inclusion criteria were chosen. After detailed history, informed consent was obtained from the parents after explaining the purpose of the study. The participant's data was recorded in a structured proforma.

All the 40 children enrolled in the study were examined for pubertal development by Tanners staging. Their weight was recorded using an electronic scale and height using a stadiometer and was plotted on WHO growth charts to identify any short stature or underweight. All the children underwent laboratory tests for Thyroid profile which included TSH, FT4, FSH and LH while female patients were tested for estradiol on the second day of their cycle and in the male patients testosterone hormone was estimated. Oral glucose tolerance test was carried out to know about the glycaemic profile of these children.GH assay after clonidine stimulation at 60 minutes was done in all the participants.

38 children out of the 40 children enrolled, showed an abnormal endocrine profile. These children were to receive combination of oral iron chelators, hence Deferiprone was started at the dose of 75 mg/kg/day Q8hrly, along with Deferasirox at the dose 30mg/kg/day Q24hourly for 6 months. Over the course of the study, children were monitored to look for decreased leukocyte count, neutropenia and anemia using laboratory parameters i.e. CBC every month. Also S. Ferritin, Liver function tests and Renal function tests were monitored every 3rd month for toxicity.

At the end of the study all the children were again followed up with the investigations and anthropometric parameters to check for any improvement.

Children enrolled received blood transfusion every 3 weekly as per their pre transfusion hemoglobin.

Investigations:

Thyroid Stimulating Hormone (baseline and after 6 months)

Free T4 (baseline and after 6 months)

Luteinizing Hormone (baseline and after 6 months)

Follicle Stimulating Hormone (baseline and after 6 months)

Testosterone (males) (baseline and after 6 months)

Estradiol (females) (baseline and after 6 months)

Growth Hormone (baseline and after 6 months)

Oral Glucose Tolerance Test/HbA1c(baseline and after 6 months)

Complete blood count (monthly for 6 months)

Serum ferritin levels (at baseline, 3rd and 6th month)

Renal function tests (at baseline, 3rd and 6th month)

Liver function tests (at baseline, 3rd and 6th month)

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.

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. Growth hormone assay was done after overnight fasting, following administration of oral clonidine at a dose of 150µg/m^2 and drawing sample at 60 minutes. In girls LH, FSH and estradiol were estimated on day 2 of their menstrual cycle. In boys, testosterone was sent along with LH and FSH. Oral glucose tolerance test was done after administering of glucose at a dose of 1.75g/kg(max75g) after overnight fasting of 12 hours. Another plain bulb containing 4ml will be sent for testing urea, creatinine, serum ferritin, liver enzymes (SGOT, SGPT).

- Calibrated DPC IMMULITE 1000 analyzer was used for estimation of FSH, LH, estradiol FT4, TSH and GH

- Estimation of testosterone was using LCMS-8060NX

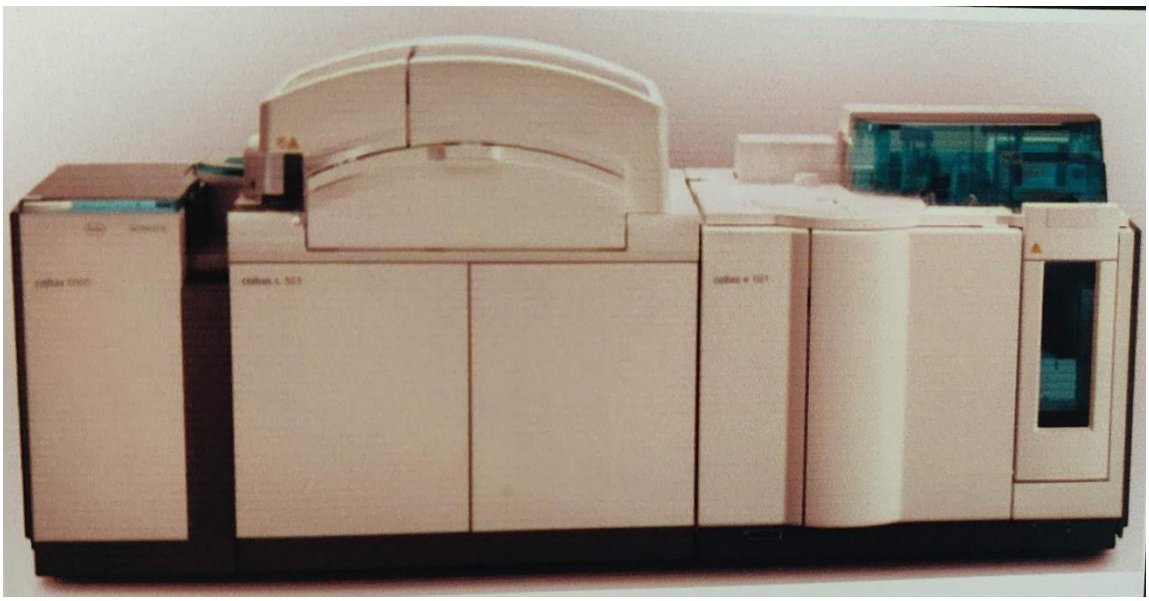
- Estimation of Hemoglobin was done by Cyanomethaemoglobin method on Mindray CAL 80 analyser, before the commencement of the study.

- 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

Beckmann coulter used for analysis of complete blood picture

Calibrated DPC IMMULITE 1000 analyzer



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.

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.

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

Reference values for endocrine function

Growth hormone	<10ng/ml
Estradiol	30-400pg/ml
Testosterone	300ng/dl
LH & FSH	5-20mIU/ml
TSH	0.27-4.2mIU/ml
FT4	0.7-1.9ng/dl

RESULTS

This hospital-based longitudinal study was done from January 2021 to December 2021. Forty children between the ages of 12 and 18 years with transfusion-dependent beta-thalassemia on a single oral iron chelator (Deferasirox) and fulfilling the inclusion criteria 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 .These patients were examined for their pubertal development by Tanner's staging , their weight and height recorded and also underwent laboratory investigations for LH, FSH, GH, TSH , FT4 , FBS and 2hr PPBS .They were also monitored for S. ferritin levels and other blood investigations for the safety of the drug. All patients who were found to have an abnormal endocrine profile were started on Tab. Deferiprone 75mg/kg/day Q8hrly and continued to receive Tab Deferasirox at a dose of 30mg/kg/day Q24hourly for 6 months before being reassessed for the endocrine profile.

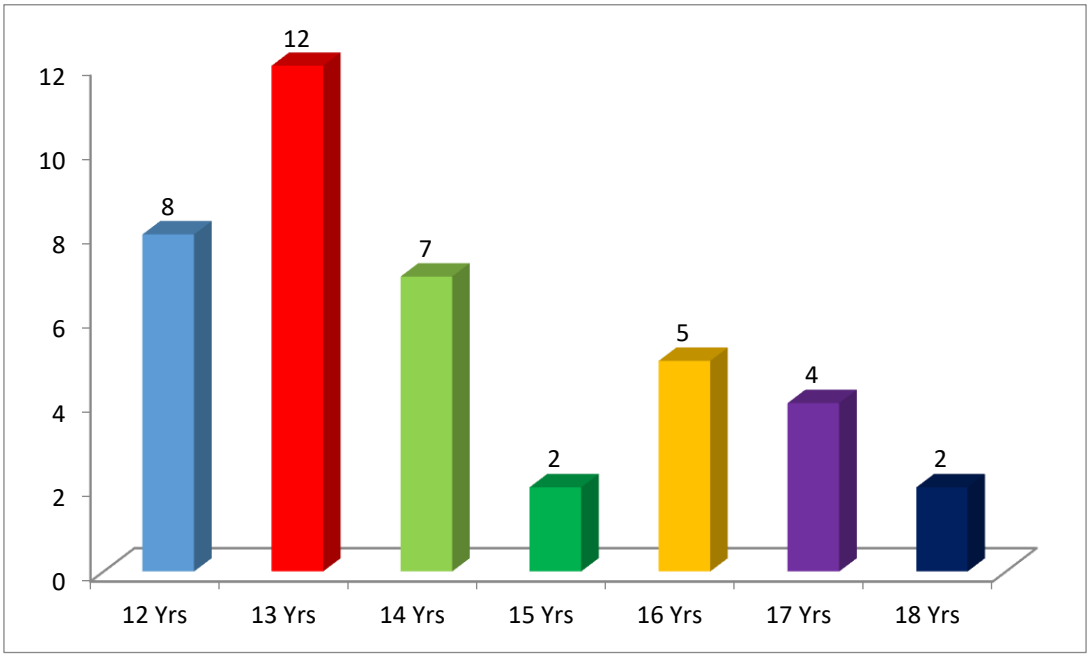
The data was analysed using statistical software R version 4.2.0 and the final results were tabulated and interpreted as below.

Table 1: Distribution of subjects according to age

AGE DISRIBUTION	Number	Percentage
12 Yrs	8	20
13 Yrs	12	30
14 Yrs	7	17.5
15 Yrs	2	5
16 Yrs	5	12.5
17 Yrs	4	10
18 Yrs	2	5

Variables	Sub Category	Number of observations (%)
Age (years)	Mean ± SD Median (Min, Max)	14.10 ± 1.85 13.5 (12, 18)

Figure 1: Distribution of subjects according to age.

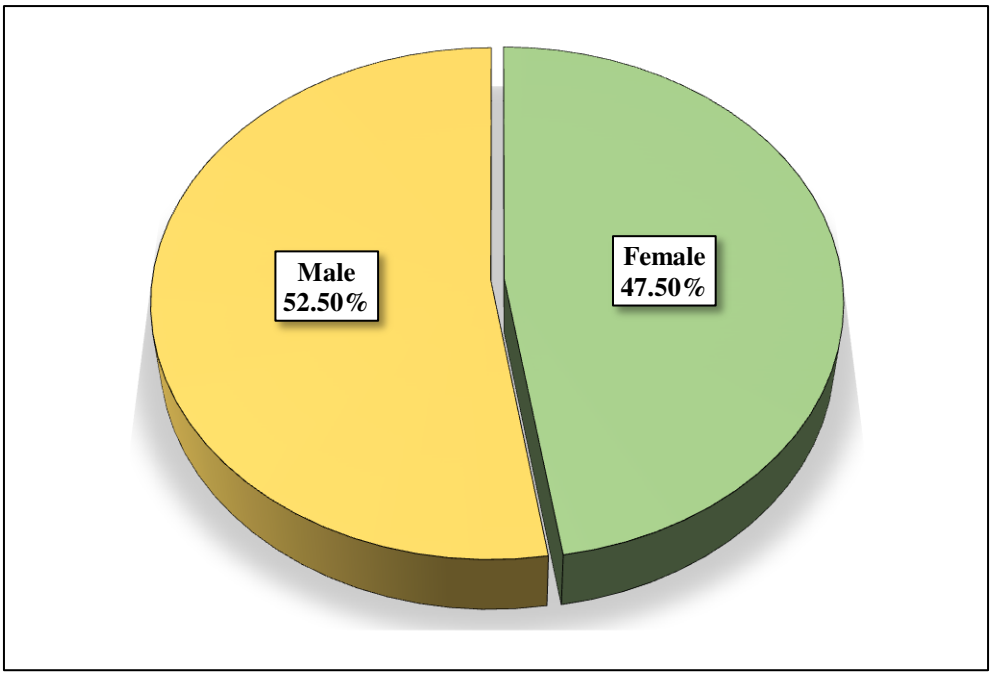


In present study 12 patients were13 years , 8 patients were 12 years and 7 patients were 14 years .

Table 2. Distribution of children according to gender.

Variables	Sub Category	Number of observations (%)
Gender	Female	19 (47.5%)
	Male	21 (52.5%)

Figure 2: Distribution of subjects according to gender.

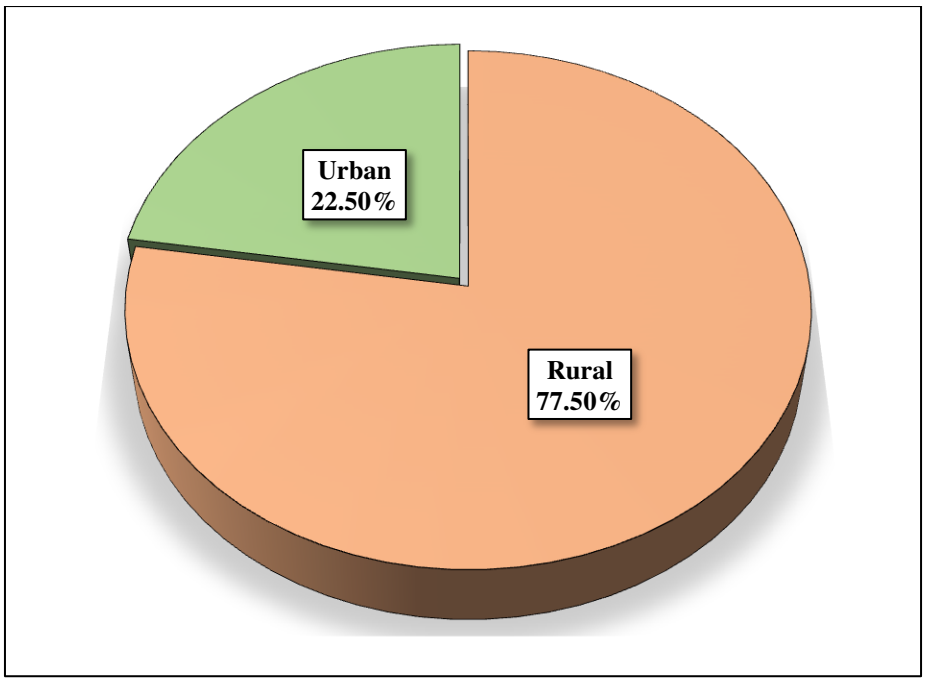


Out of 40 subjects, 21 (52.5%) were males and 19 (47.5%) were females

Table 3. Distribution of children according to residential area

Variables	Sub Category	Number of observations (%)
Residence area	Rural	31 (77.5%)
	Urban	9 (22.5%)

Figure 3: Distribution of subjects according to residential area.



In our study 31 (77.5%) subjects were residing at rural areas and 9 (22.5%) were residing at urban areas.

Table 4. Distribution of children according to their socioeconomic status

Variables	Sub Category	Number of observations (%)
Socio economic status	Lower class	10 (25%)
	Lower middle class	27 (67.5%)
	Middle Class	3 (7.5%)

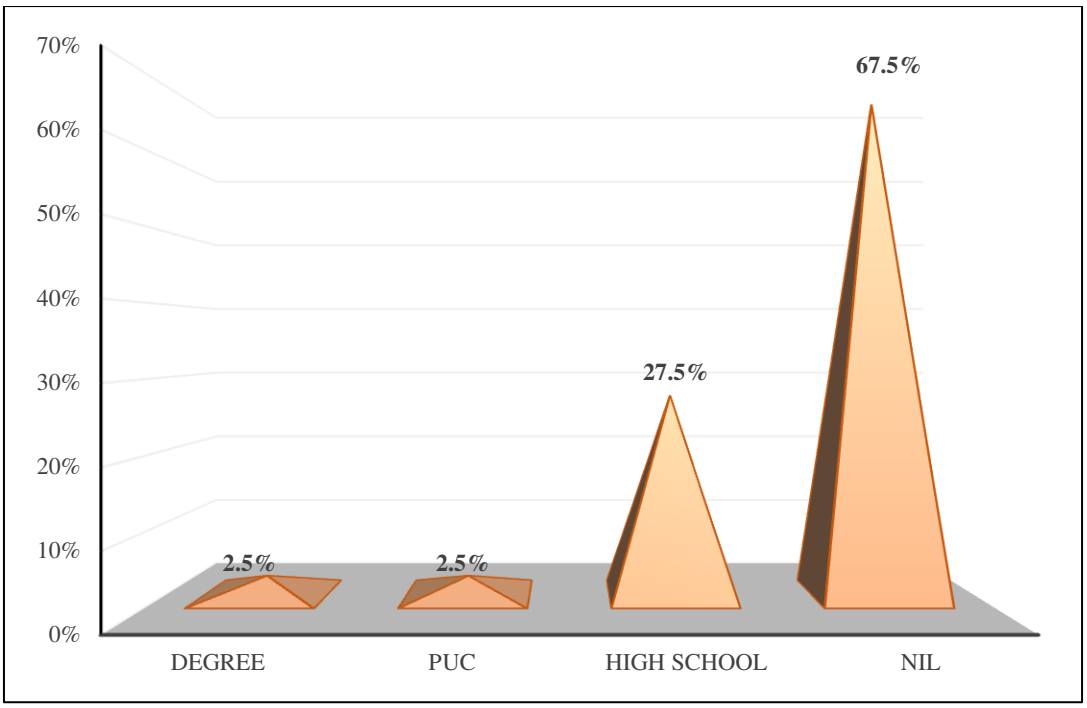
Figure 4: Distribution of subjects according to socioeconomic status.

In the present study 27 (67.5%) subjects belonged to lower middle class, 10 (25%) children belong to lower class and only 3 (7.5%) children belonged to middle class.

Table 5. Distribution of children according to the mother's educational level

Variables	Sub Category	Number of observations (%)
Mother's education level	Degree	1 (2.5%)
	PUC	1 (2.5%)
	High school	11 (27.5%)
	Nil	27 (67.5%)

Figure 5: Distribution of subjects according to mother's education status.

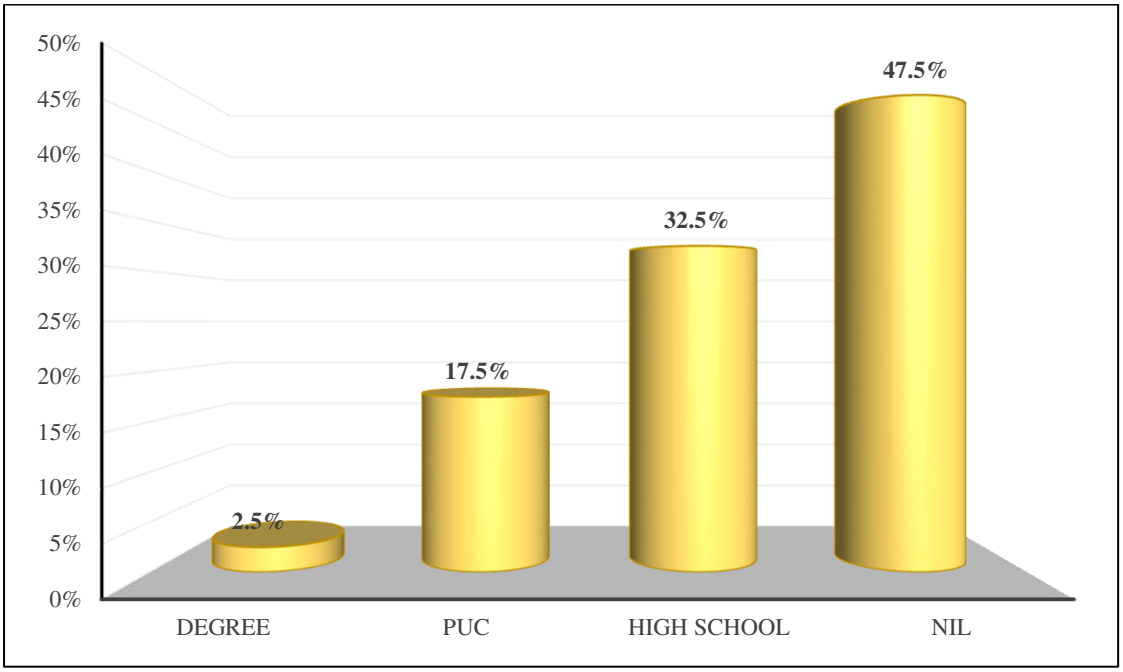


In this study, majority (67.5%) of the mothers have not attended any form of education, while 27.5% have attended High school and 2.5% have attended degree and PUC.

Table 6. Distribution of children according to the father's educational level

Variables	Sub Category	Number of observations (%)
Father's education level	Degree	1 (2.5%)
	PUC	7 (17.5%)
	High school	13 (32.5%)
	Nil	19 (47.5%)

Figure 6: Distribution of subjects according to Fathers education status.



In this study, majority (47.5%) of the father's have not attended any form of education ,while 32.5% have attended High school and 2.5% have attended degree and 17.5% have attended PUC.

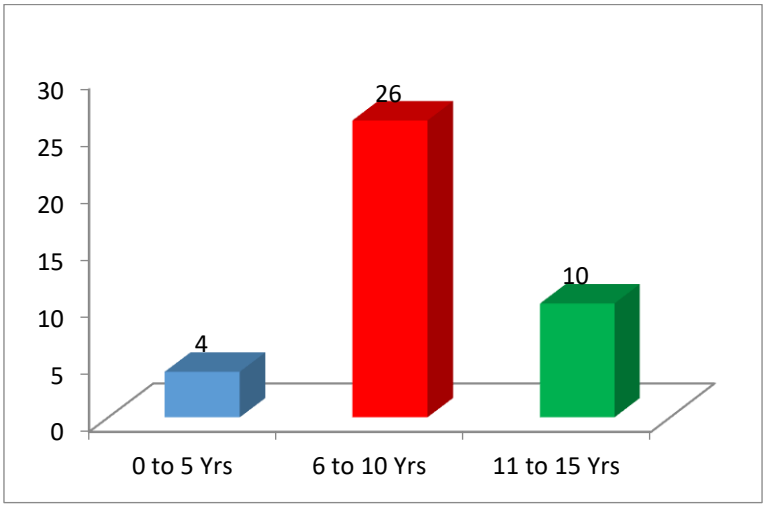
Table 7: Distribution of subjects according to their school grade.

Variables	Sub Category	Number of observations (%)
School grade	6	5 (12.5%)
	7	14 (35%)
	8	10 (25%)
	9	4 (10%)
	10	3 (7.5%)
	12	4 (10%)

Table 8: Distribution of subjects according to duration of treatment in years

Duration of treatment in years	Number	Percentage
0 to 5 Yrs	4	10
6 to 10 Yrs	26	65
11 to 15 Yrs	10	25

Figure 7: Distribution of subjects according to duration of treatment in years.

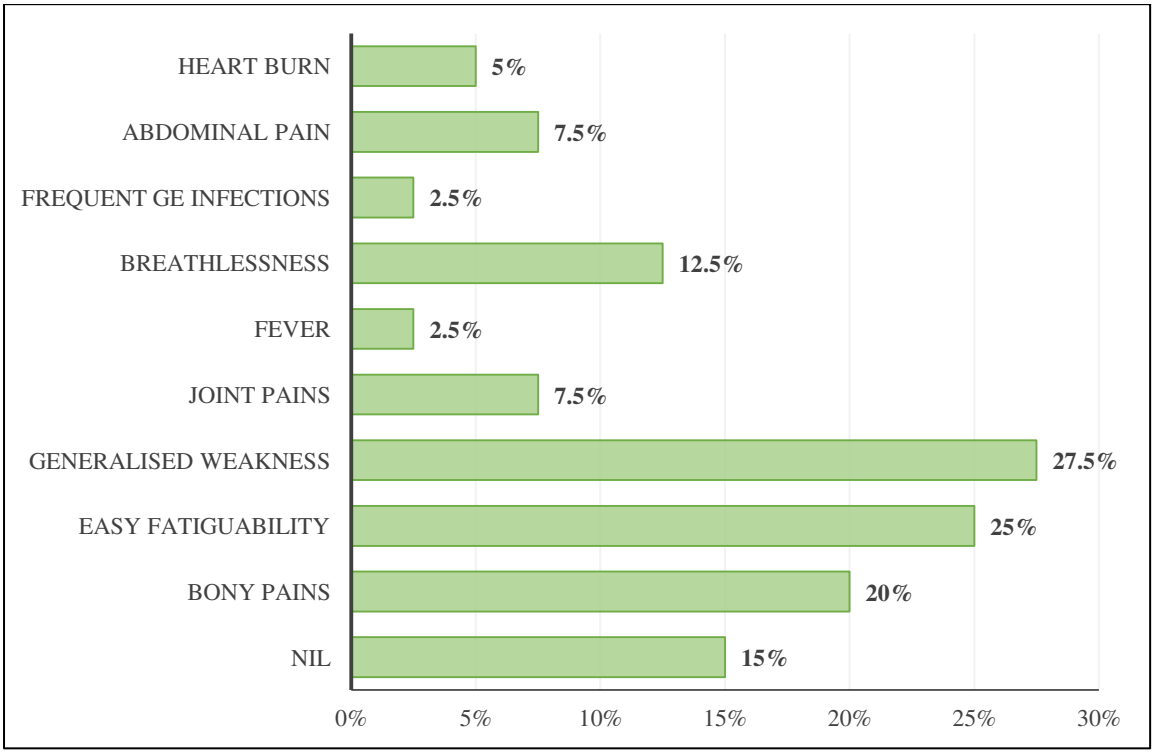


In the present study 26 patients were on Deferasirox for between 6 to 10 years , 10 patients were on treatment for 11 to 15 years and around 4 patients were on Deferasirox for less than 5 years

Table 9: Distribution of subjects according to present complaints on history.

Variables	Sub Category	Number of observations (%)
Present complaints	Heart burn	2 (5%)
	Abdominal pain	3 (7.5%)
	Frequent GE infections	1 (2.5%)
	Breathlessness	5 (12.5%)
	Fever	1 (2.5%)
	Joint pains	3 (7.5%)
	Generalised weakness	11 (27.5%)
	Easy fatiguability	10 (25%)
	Bony pains	8 (20%)
	Nil	6 (15%)

Figure 8: Distribution of subjects according to Present complaints.

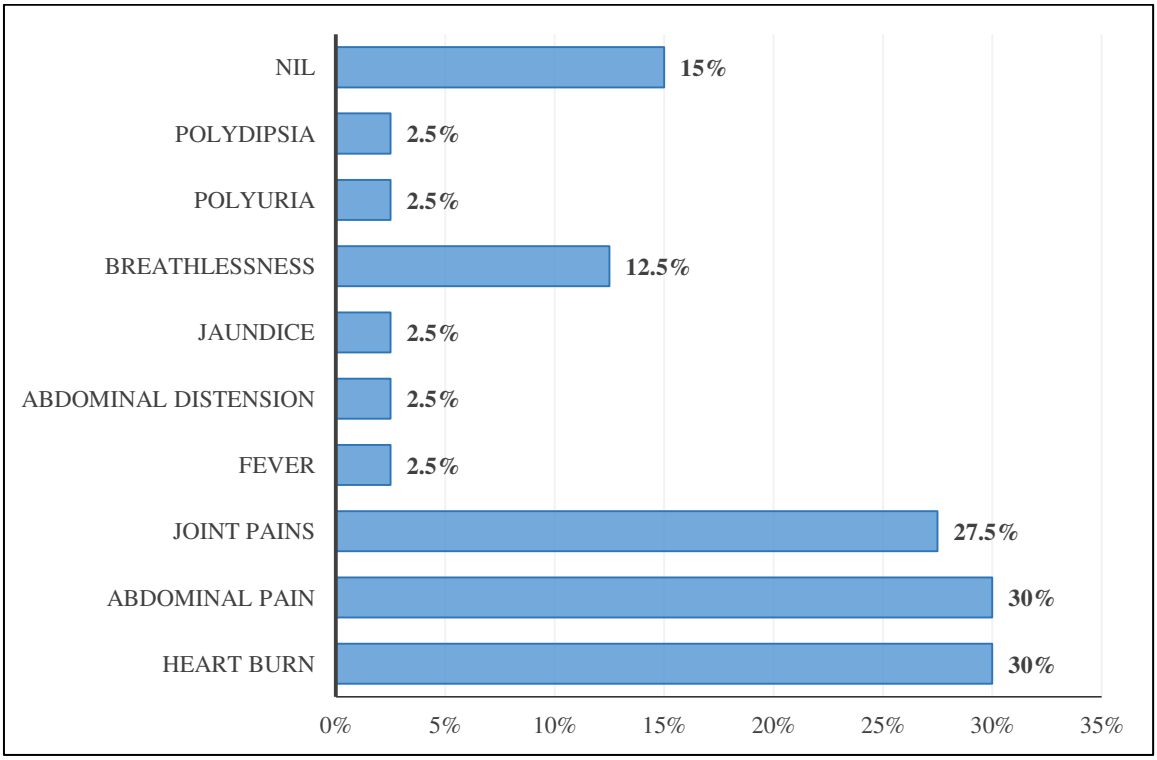


Out of 40 subjects, 6 (15%) had no complaints while 11 (27.5%) had generalised weakness, 10 (25%) had Easy fatiguability and 8 (20%) had bony pains.

Table 10: Distribution of subjects according to any history of present illness

Variables	Sub Category	Number of observations (%)
History of Present Illness	NIL	6 (15%)
	Polydipsia	1 (2.5%)
	Polyuria	1 (2.5%)
	Breathlessness	5 (12.5%)
	Jaundice	1 (2.5%)
	Abdominal distension	1 (2.5%)
	Fever	1 (2.5%)
	Joint pains	11 (27.5%)
	Abdominal pain	12 (30%)
	Heart Burn	12 (30%)

Figure 9: Distribution of subjects according to History of Present Illness.

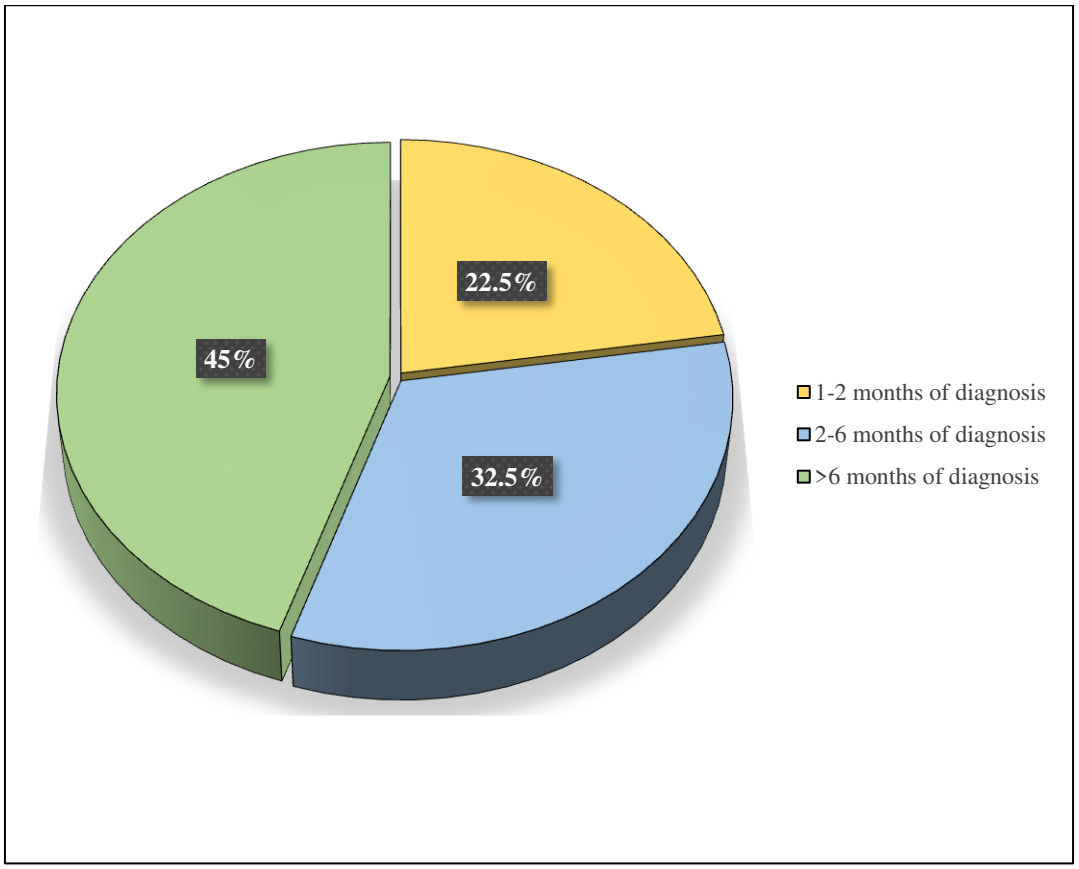


Out of 40 subjects, 6 (15%) had no present history, while 12 (30%) had abdominal pain and heart burn , 11 (27.5%) had joint pain and 5 (12.5%) had breathlessness.

Table 11: Distribution of subjects according to onset of blood transfusion from time of diagnosis

Variables	Sub Category	Number of observations (%)
Onset of Blood Transfusion	1-2 months of diagnosis	9 (22.5%)
	2-6 months of diagnosis	13 (32.5%)
	>6 months of diagnosis	18 (45%)

Figure 10: Distribution of subjects according to Onset of Blood Transfusion.

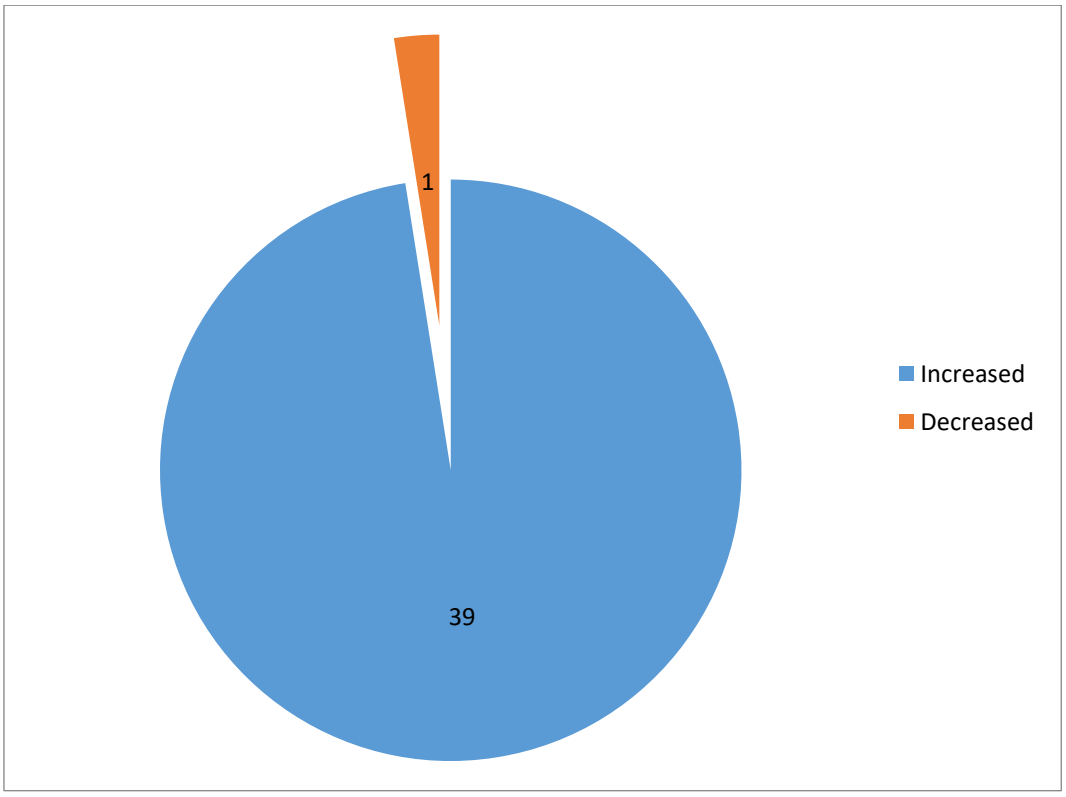


In the present study ,18 (45%) subjects had onset of blood transfusion after more than 6 months of diagnosis , 13 (32.5%) after 2-6 months of diagnosis and 9 (22.5%) children after 1-2 months of diagnosis .

Table 12: Distribution of subjects according to frequency of blood transfusion

Variables	Sub Category	Number of observations (%)
Frequency of BT	Decreased	1 (2.5%)
	Increased	39 (97.5%)

Figure 11: Distribution of subjects according to frequency of blood transfusion

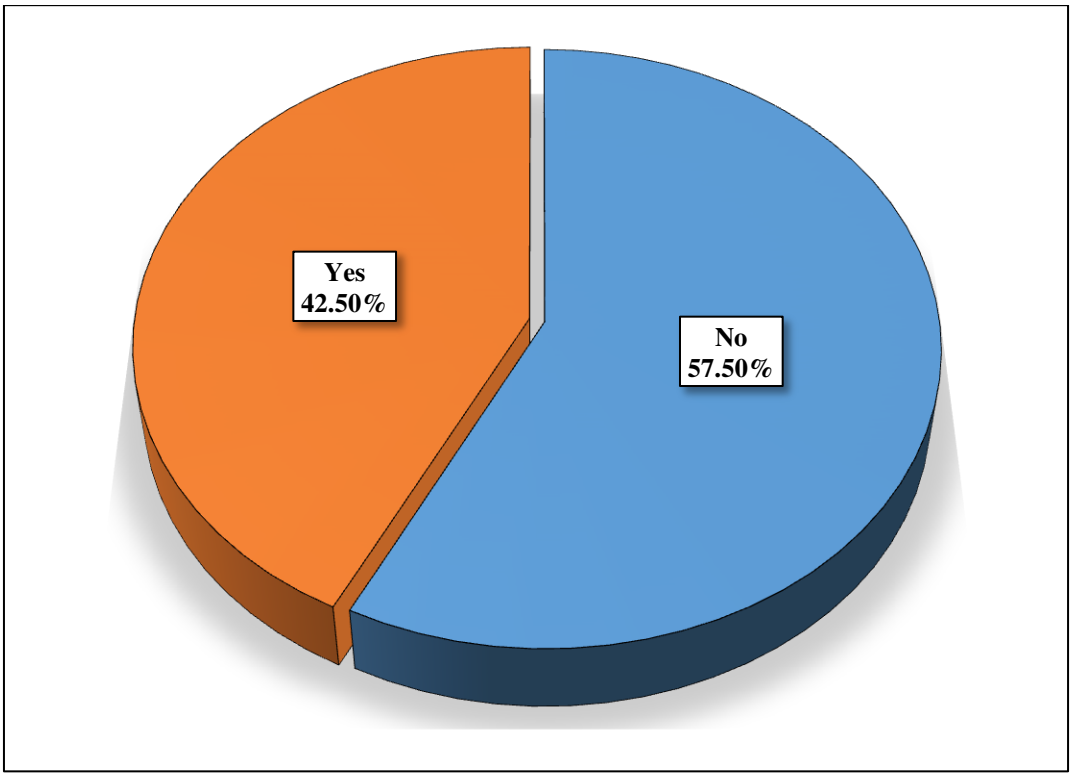


In this study only one child had a decrease in the frequency of blood transfusion, rest of the 39 patients had increased frequency of blood transfusion

Table 13: Distribution of subjects according to growth retardation on history .

Variables	Sub Category	Number of observations (%)
Growth retardation	No	23 (57.5%)
	Yes	17 (42.5%)

Figure 12: Distribution of subjects according to growth retardation on history .

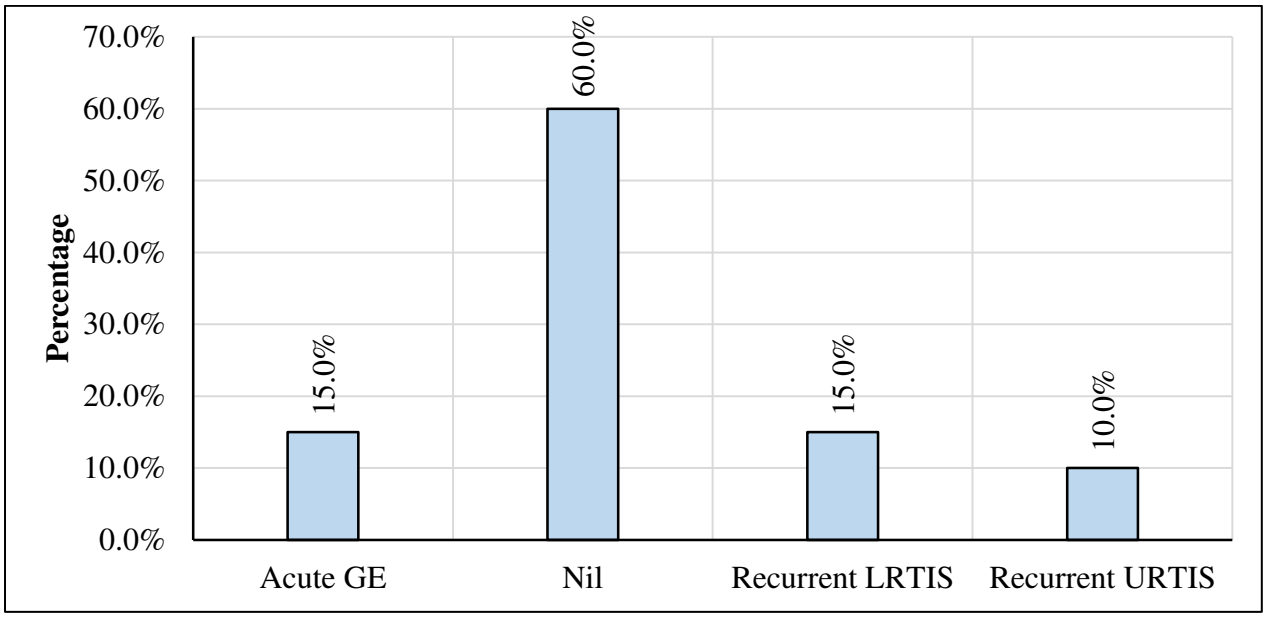


On history around only 17 (42.5%) of the parents had noticed growth retardation in their children

Table 14 : Distribution of subjects according to other co morbidities .

Variables	Sub Category	Number of observations (%)
Other co morbidities	Acute GE	4 (15%)
	Nil	24 (60%)
	Recurrent LRTIs	3 (15%)
	Recurrent URTIs	3 (10%)

Figure 13: Distribution of subjects by other comorbidities.



Out of 40 subjects most of the patients i.e 60% had no other comorbidities, while 15% had acute GE and recurrent LRTIs .

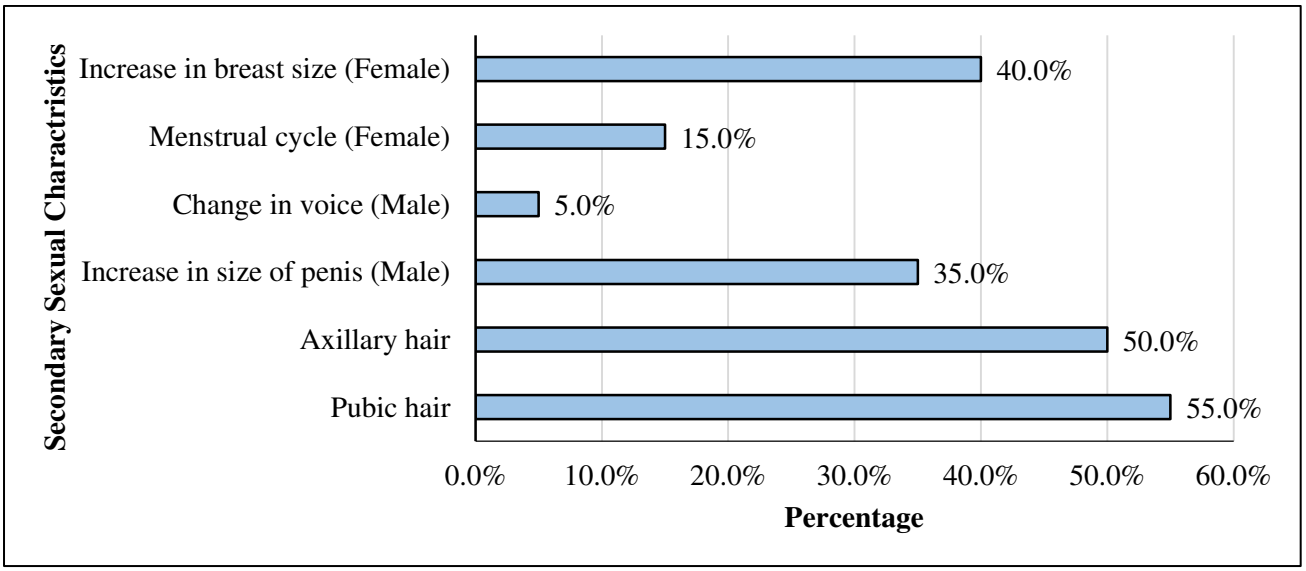
Table 15: Distribution of subjects according to history of secondary sexual characteristics

Variables	Sub Category	Number of observations (%)
History of secondary sexual characteristics	No	20 (50%)
	Yes	20 (50%)

Table 16: Distribution of subjects according to sexual characteristics on history.

Variables	Sub Category	Number of observations (%)
Secondary sexual characteristics (n=20)	Increase in breast size (Female)	8 (40%)
	Menstrual cycle (Female)	3 (15%)
	Change in voice (Male)	1 (5%)
	Increase in size of penis (Male)	7 (35%)
	Axillary hair	10 (50%)
	Pubic hair	11 (55%)

Figure 14: Distribution of subjects by secondary sexual characteristics



In females, increase in breast size was seen in 8 (40%), menstrual cycle was attained in 3 (15%). In male children increase in size of penis was seen in 7 (35%) and change in voice was noted in only one (5%) child. Axillary hair growth was seen in 10 and pubic hair growth was seen in 11 of the patients.

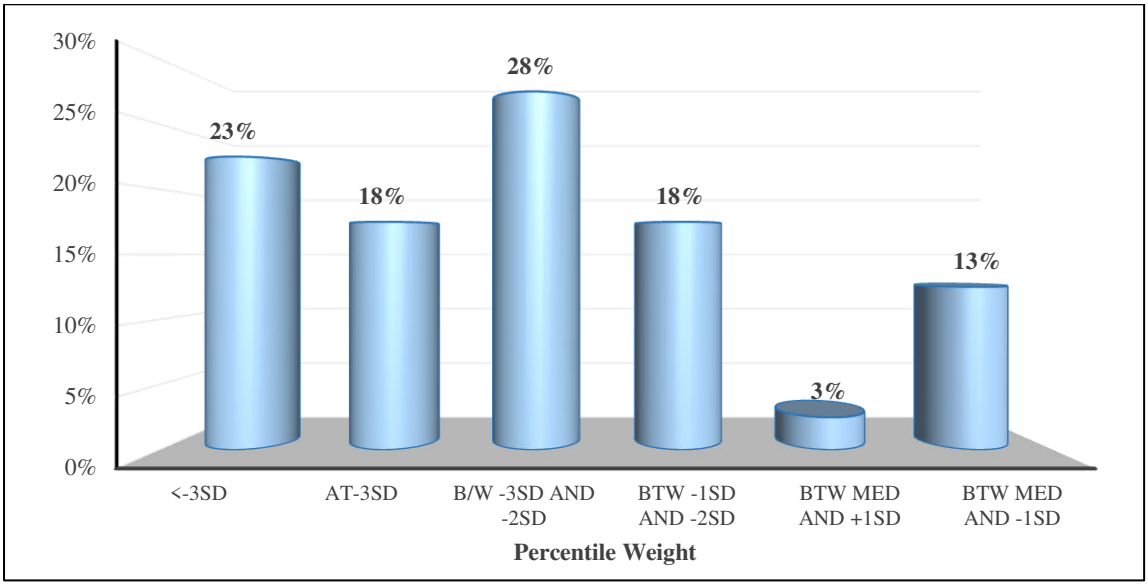
Table 17: Distribution of subjects according to transfusion related complications

Variables	Sub Category	Number of observations (%)
Number of reactions	Nil	40 (100%)
H/O infection	Nil	39 (97.5%)
	Yes, HIV positive	1 (2.5%)
Sibling status	NO	34 (85%)
	YES	6 (15%)
Death of Sibling	No	38 (95%)
	Yes	2 (5%)
Antenatal Testing	No	38 (95%)
	Yes	2 (5%)
Immunisation History	Fully immunised and Hepatitis B vaccine taken	33 (82.5%)
	Not fully immunised	7 (17.5%)

Table 18: Distribution of variables according to percentile weight.

Variables	Sub Category	Number of subjects (%)
Percentile weight	<-3SD	9 (22.5%)
	AT-3SD	7 (17.5%)
	B/W -3SD AND -2SD	11 (27.5%)
	BTW -1SD AND -2SD	7 (17.5%)
	BTW MED AND +1SD	1 (2.5%)
	BTW MED AND -1SD	5 (12.5%)

Figure 15: Distribution of subjects according to percentile weight.

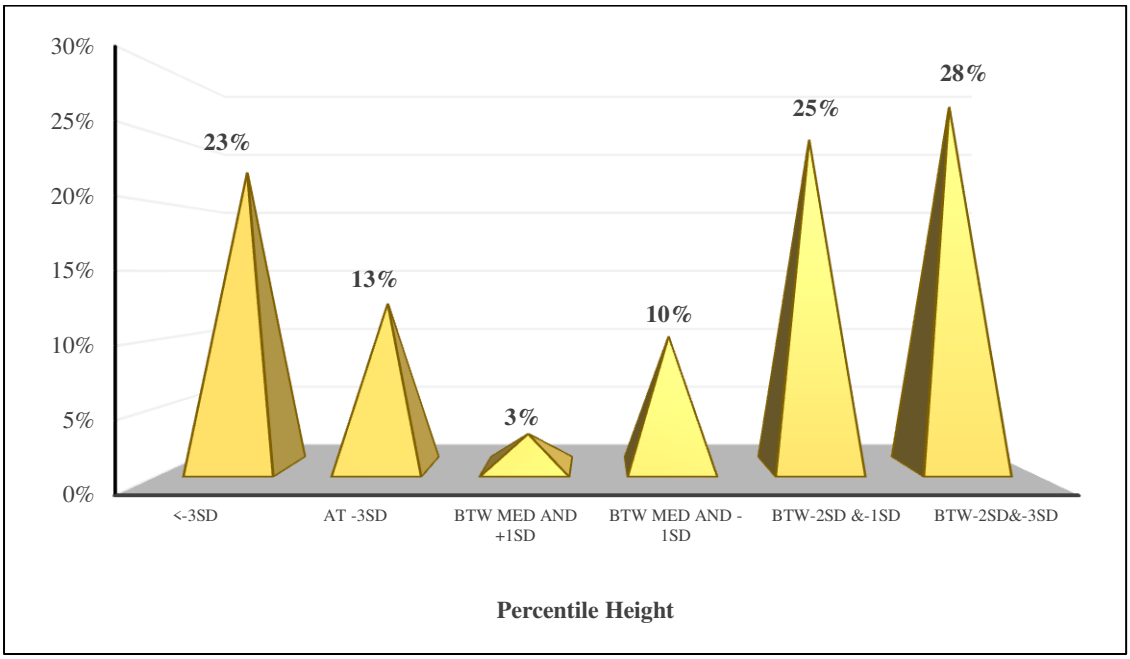


We observe that, 27.5% children had percentile weight between -3SD and -2SD , 23% children had percentile weight <-3SD.

Table 19: Distribution of variables according to percentile height.

Variables	Sub Category	Number of subjects (%)
Percentile Height	<-3SD	9 (22.5%)
	AT -3SD	5 (12.5%)
	BTW MED AND +1SD	1 (2.5%)
	BTW MED AND -1SD	4 (10%)
	BTW-2SD &-1SD	10 (25%)
	BTW-2SD&-3SD	11 (27.5%)

Figure 16: Distribution of subjects according to percentile height.

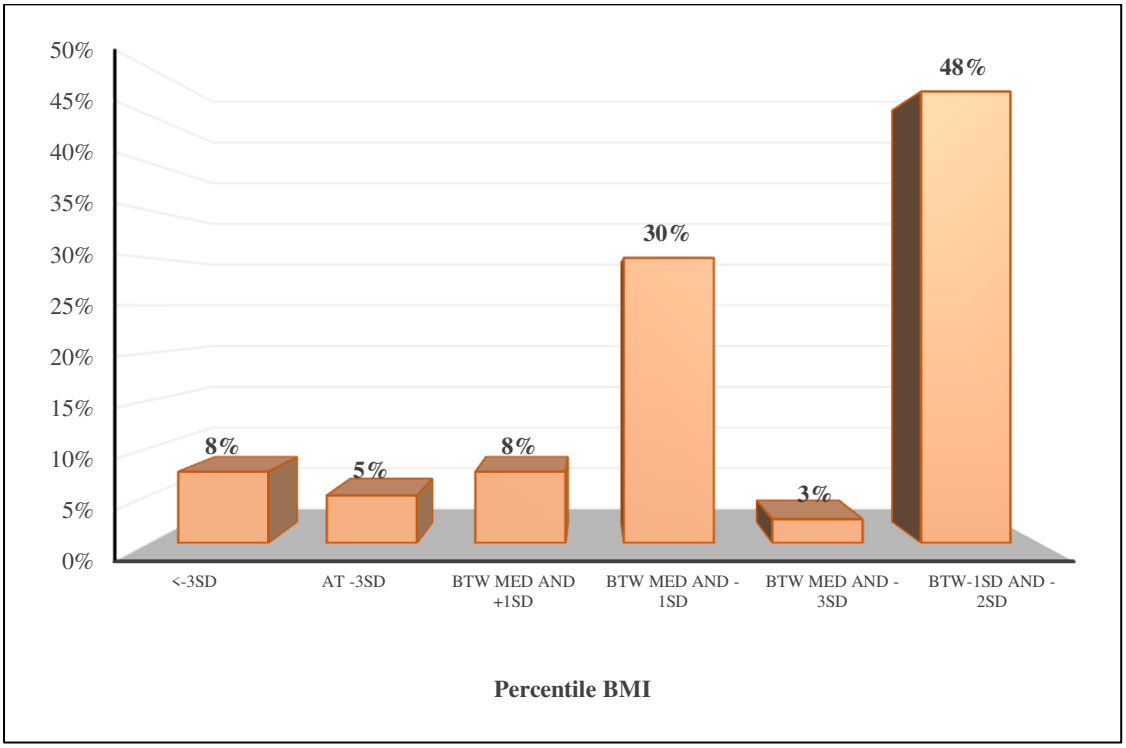


We observe that 27.5% had percentile height between -3SD and -2 SD, 25% had height between -2SD and-1SD and 23% had percentile height <-3SD

Table 20: Distribution of variables according to percentile BMI.

Variables	Sub Category	Number of subjects (%)
Percentile BMI	<-3SD	3 (7.5%)
	AT -3SD	2 (5%)
	BTW MED AND +1SD	3 (7.5%)
	BTW MED AND -1SD	12 (30%)
	BTW MED AND -3SD	1 (2.5%)
	BTW-1SD AND -2SD	19 (47.5%)

Figure 17: Distribution of subjects according to percentile BMI.

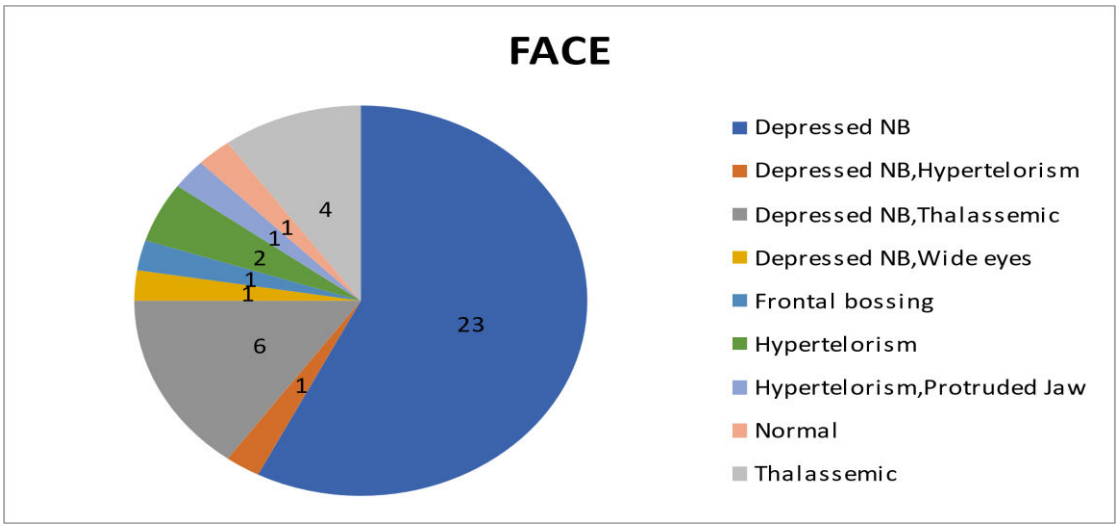


We observe that 47.5% of children had percentile BMI between -1SD and -2SD ,30 % children had a percentile BMI between median and +1SD

Table 21 : Distribution of variables according to facial features.

Variables	Sub Category	Number of observations (%)
Face	Depressed nasal bridge	23(57.5%)
	Depressed nasal bridge ,Thalassaemic facies	6 (15%)
	Depressed nasal bridge ,Hypertelorism	1 (2.5%)
	Frontal bossing	1 (2.5%)
	Depressed nasal bridge ,Wide eyes	1 (2.5%)
	Hypertelorism	2 (5%)
	Thalassemic facies	4(10%)
	Normal	1(2.5%)
	Hypertelorism,Protruded Jaw	1 (2.5%)

Figure 18: Distribution of subjects according to facial features.

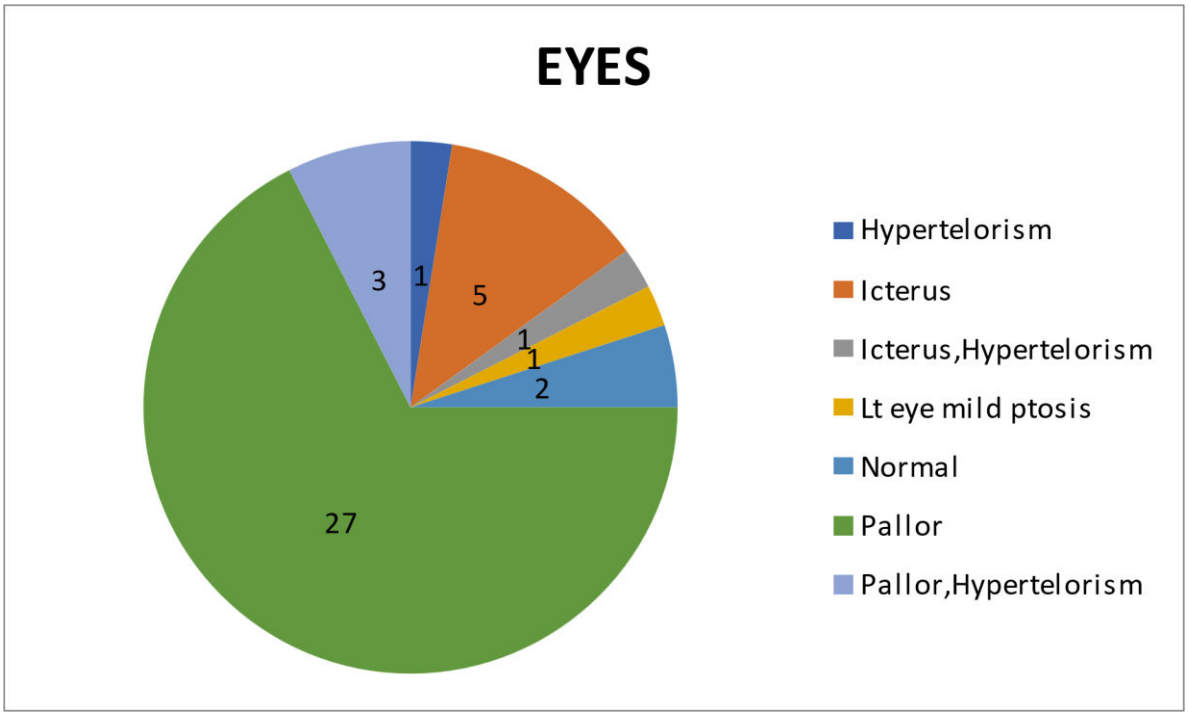


In the present study we observe that 23 patients had depressed nasal bridge, 4 children had thalasemic facies and only one child had normal facial features.

Table 22 : Distribution of variables according to features in eyes

Variables	Sub Category	Number of observations (%)
Eyes	Pallor	27 (67.5%)
	Icterus	5(12.5%)
	Pallor, Hypertelorism	3(7.5%)
	Icterus,Hypertelorism	1 (2.5%)
	Left eye mild Ptosis	1 (2.5%)
	Hypertelorism	1 (2.5%)
	Normal	2 (5%)

Figure 19: Distribution of subjects according to features in eyes.

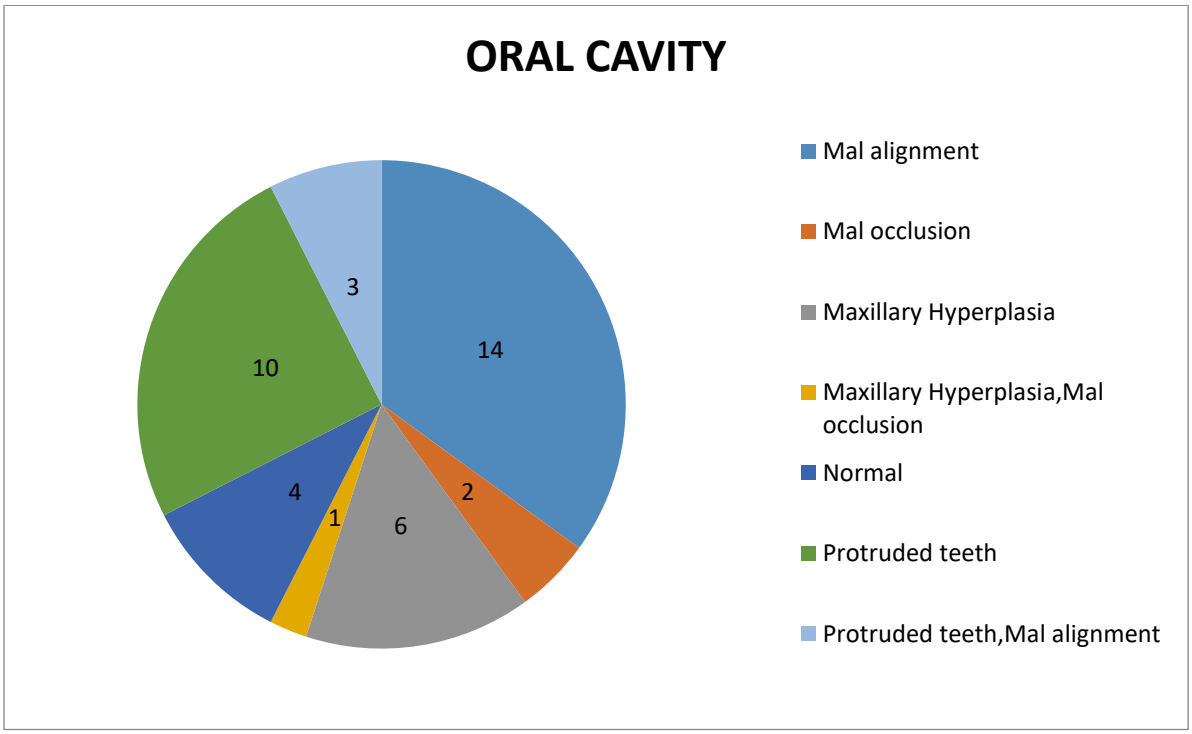


In our study 27 children had pallor, 5 children had icterus ,one child had ptosis and 2 children had normal eyes.

Table 23 : Distribution of variables according to features in oral cavity.

Variables	Sub Category	Number of observations (%)
Oral cavity	Hyperplasia of maxillary bone	6 (15%)
	Protruded teeth	10 (25%)
	Dental malalignment	14 (35%)
	Hyperplasia of maxillary bone, malocclusion	1 (2.5%)
	Dental malocclusion	2 (5%)
	Protruded teeth, dental malalignment	3 (7.5%)
	Normal	4 (10%)

Figure 20: Distribution of subjects according to findings in oral cavity.

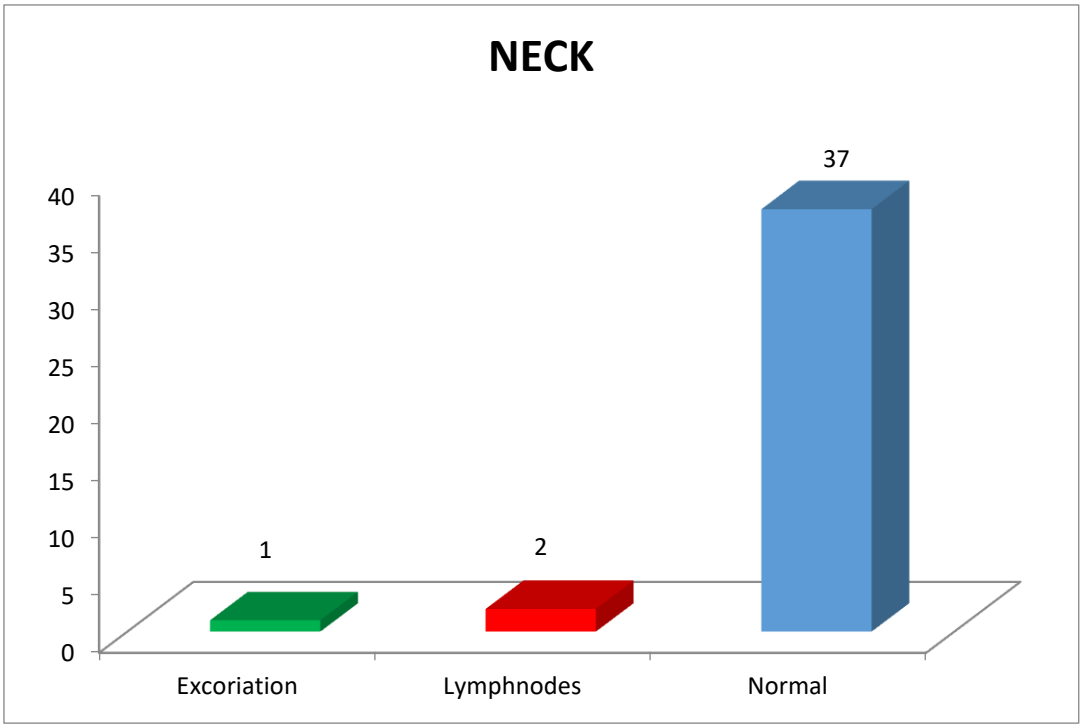


In our study 14 children out of 40 had dental malalignment ,10 children had protruded teeth and 6 children in the study had maxillary hyperplasia.

Table 24 : Distribution of variables according to features in neck.

Variables	Sub Category	Number of observations (%)
Neck	Excoriation marks	1 (2.5%)
	Lymph nodes palpable	2 (5%)
	Normal	37 (92.5%)

Figure 21: Distribution of subjects according to features in neck.

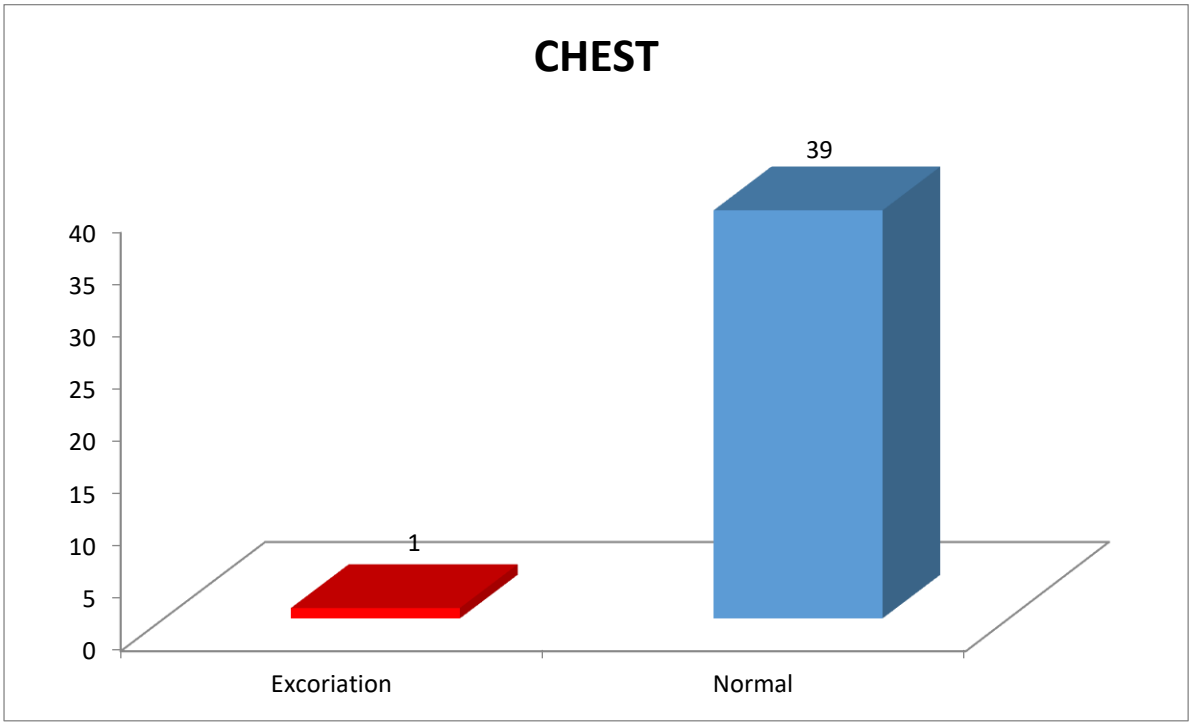


In the present study 37 children had normal findings in the neck, in 2 children lymph nodes were palpable and 1 child had excoriations in the neck.

Table 25 : Distribution of variables according to findings in chest.

Variables	Sub Category	Number of observations (%)
Chest	Excoriations present	1 (2.5%)
	Normal	39 (97.5%)

Figure 22: Distribution of subjects according to findings in chest.

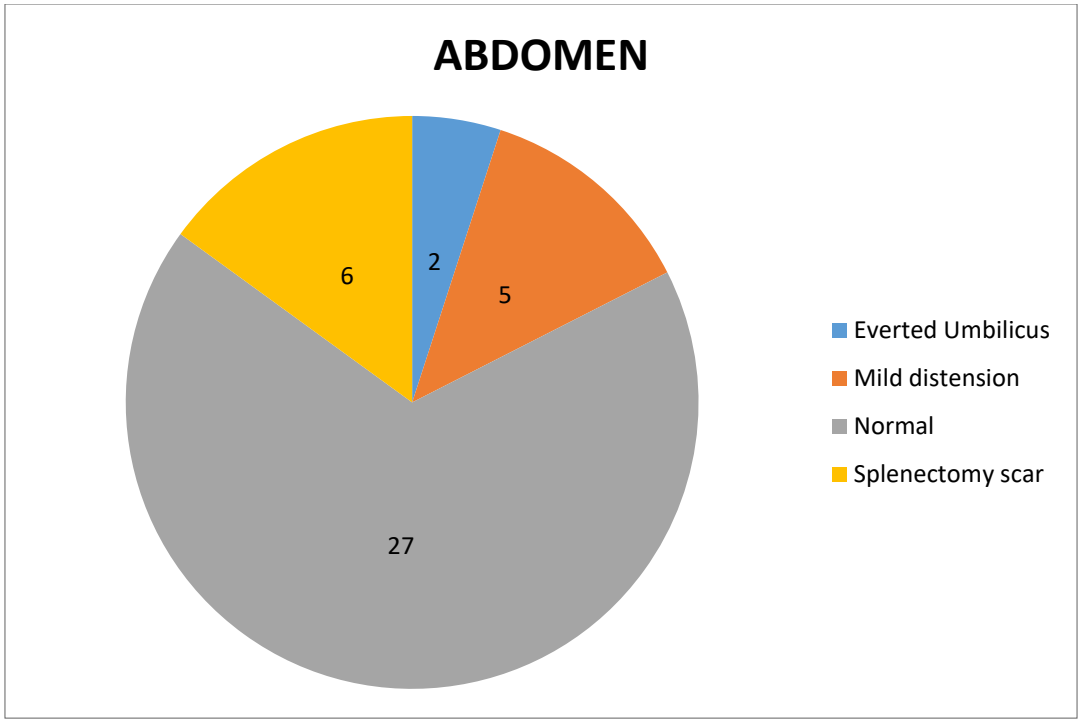


In this study only one child had excoriations in chest, all the other 39 children had normal chest findings.

Table 26 : Distribution of variables according to findings in abdomen.

Variables	Sub Category	Number of observations (%)
Abdomen	Everted Umbilicus	2 (5%)
	Mild distension	5 (12.5%)
	Splenectomy scar	6 (15%)
	Normal	27 (67.5%)

Figure 23: Distribution of subjects according to findings in abdomen

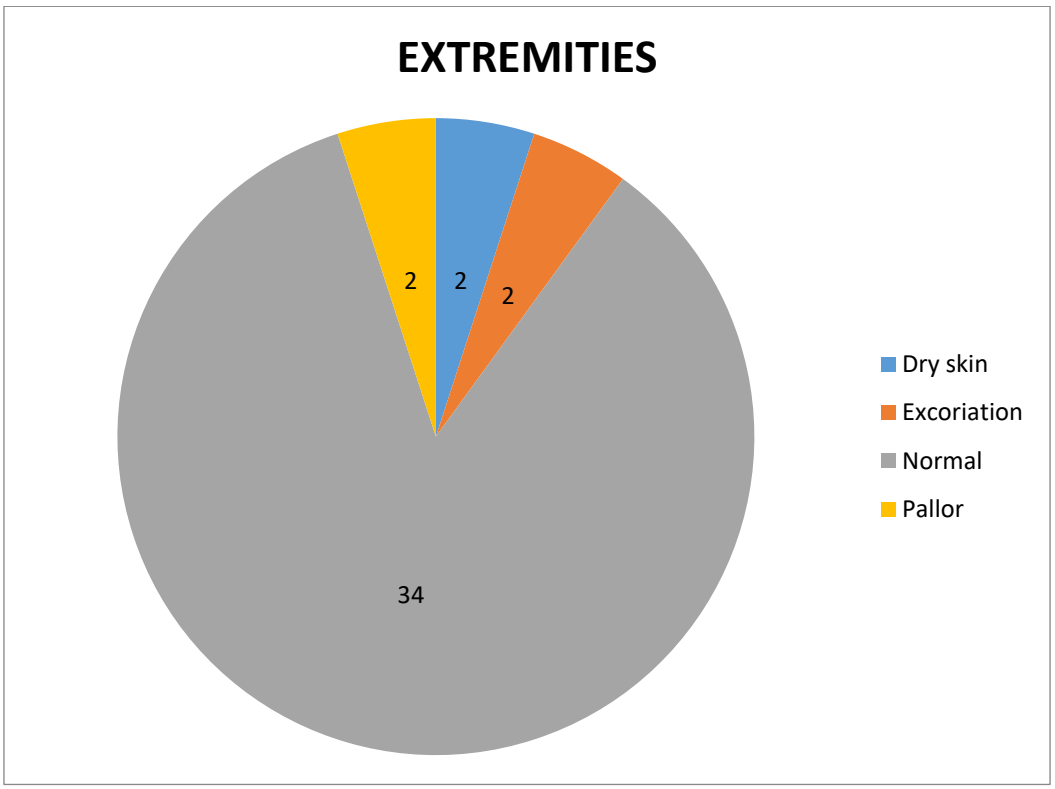


In our study 27 patients out of 40 had normal findings in the abdomen ,6 children had a splenectomy scar and 5 children had mild abdominal distenstion

Table 27 : Distribution of variables according to findings in extremities.

Variables	Sub Category	Number of observations (%)
Extremities	Dry skin	2 (5%)
	Excoriation marks	2 (5%)
	Pallor	2 (5%)
	Normal	34 (85%)

Figure 24: Distribution of subjects according to findings in extremities

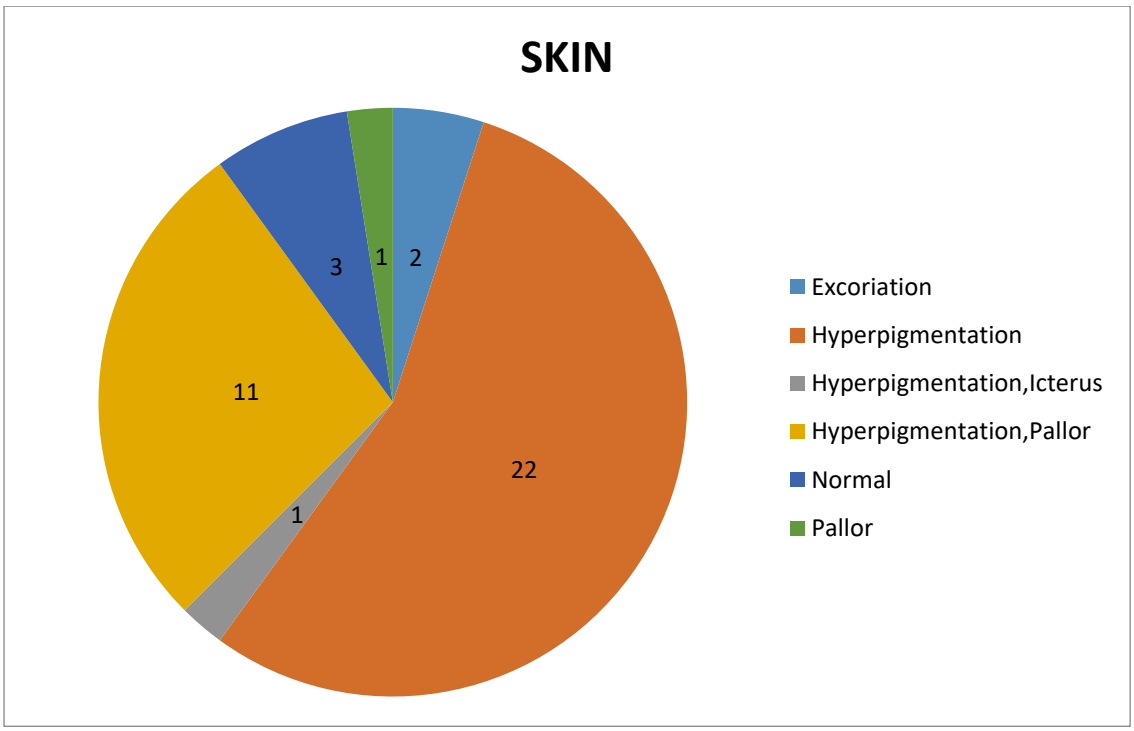


In our study 34 patients had normal findings , 2 children had dry skin ,2 children had pallor in their extremities and 2 other children had excoriation in their extremities.

Table 28 : Distribution of variables according to findings in skin.

Variables	Sub Category	Number of observations (%)
Skin	Normal	3 (7.5%)
	Hyperpigmentation	22 (55%)
	Hyperpigmentation, Icterus	1 (2.5%)
	Hyperpigmentation,Pallor	11 (27.5%)
	Pallor	1 (2.5%)
	Excoriation marks	2 (5%)

Figure 25: Distribution of subjects according to findings in skin



In this study 22 subjects out of 40 had hyperpigmentation of the skin, 11 subjects had hyperpigmentation and pallor of the skin ,2 subjects had excoriation of the skin and 3 subjects had normal skin.

Table 29 : Overall status of Tanner's staging at the baseline of the study

Total number	Abnormal	Normal	Percentage of abnormal
40	39	1	97%

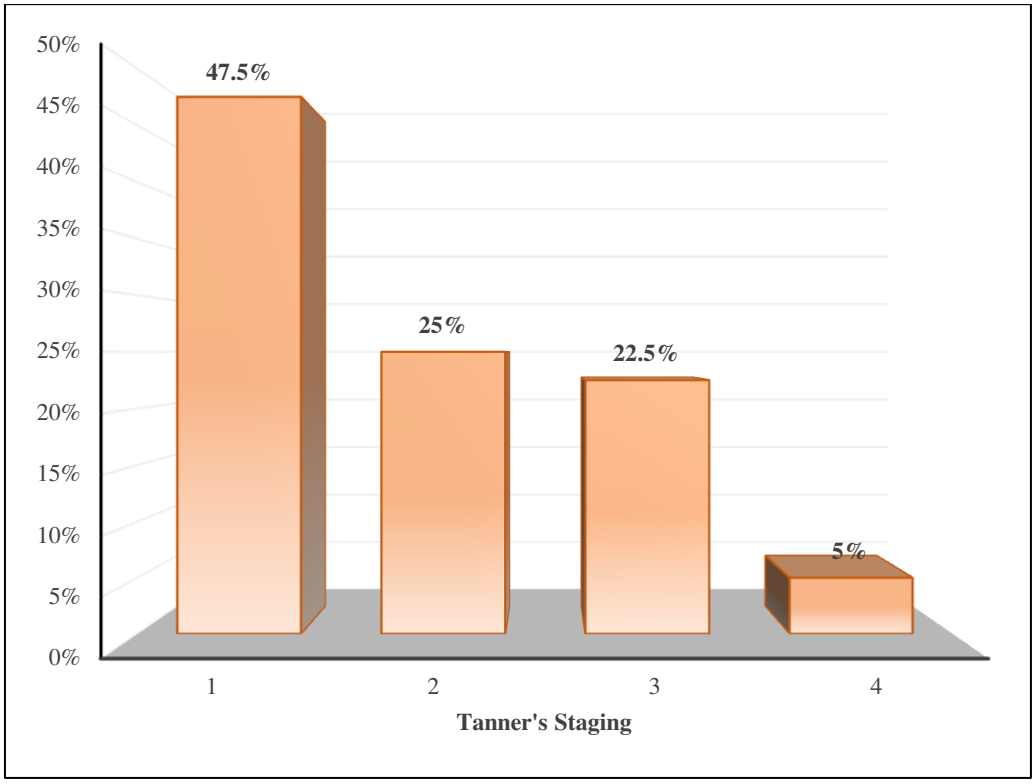
Table 30: Tanner's staging after 6 months of post intervention

Total number	Abnormal	Normal	Improved	Percentage of improved
40	37	3	33	89%

Table 31: Distribution of subjects according to Tanner's staging at baseline.

Variables	**Sub Category**	**Number of observations (%)**
Tanner's Staging At baseline	1	19 (47.5%)
	2	10 (25%)
	3	9 (22.5%)
	4	2 (5%)

Figure 26: Distribution of subjects according to Tanner's staging at baseline.

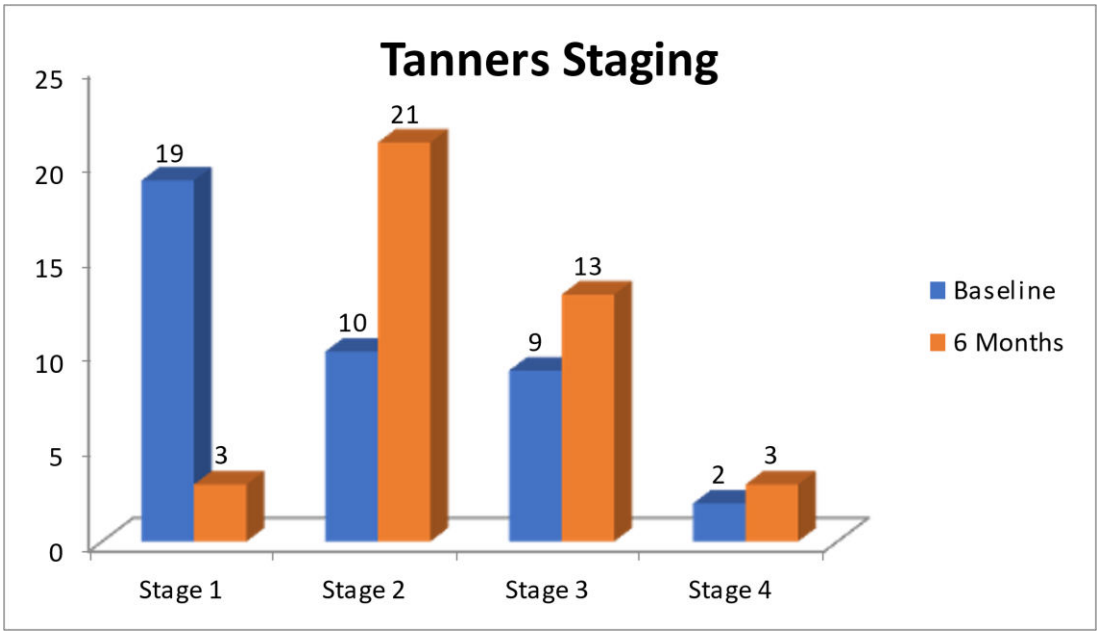


19 (47.5%) children out of the 40 children examined, had a Tanner's stage of 1 , while 10 (25%) children fell into Tanner's stage 2 , 9 (22.5%) children had Tanner's stage of 3 and 2 (5%) of children had Tanner's stage of 4

Table 32: Comparison of Tanner staging

Tanners Staging	At Baseline	At 6 Months
Stage 1	19 (47.5%)	3 (7.5%)
Stage 2	10 (25%)	21 (52.5%)
Stage 3	9 (22.5%)	13(32.5%)
Stage 4	2 (5%)	3 (7.5%)

Figure 27: Comparison of Tanner's staging post intervention.



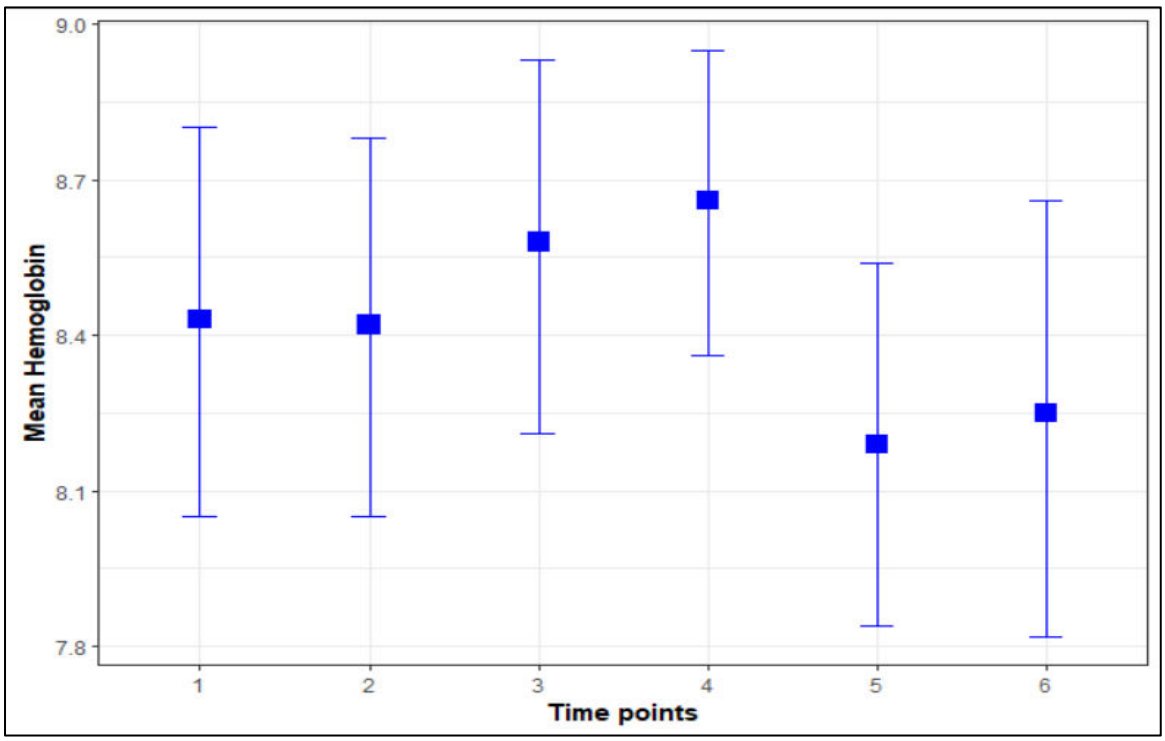
On applying Chi square test there is significant difference in tanners staging at baseline and 6 months with a p value of < 0.05.

Table 33: Comparison of haemoglobin over time.

Timepoints	Haemoglobin		p-value
	Mean ± SD	Median (Min, Max)	
1	8.43 ± 1.22	8.40 (4.60, 12.10)	0.5584[F]
2	8.42 ± 1.22	8.50 (3.80, 10.80)	
3	8.58 ± 1.17	8.70 (5.60, 10.60)	
4	8.66 ± 0.98	8.70 (6.30, 10.60)	
5	8.19 ± 1.15	8.40 (5.30, 10.60)	
6	8.25 ± 1.36	8.45 (4, 10.50)	

Abbreviation: F – Friedman's test.

Figure 28: Mean plot of Haemoglobin over time.



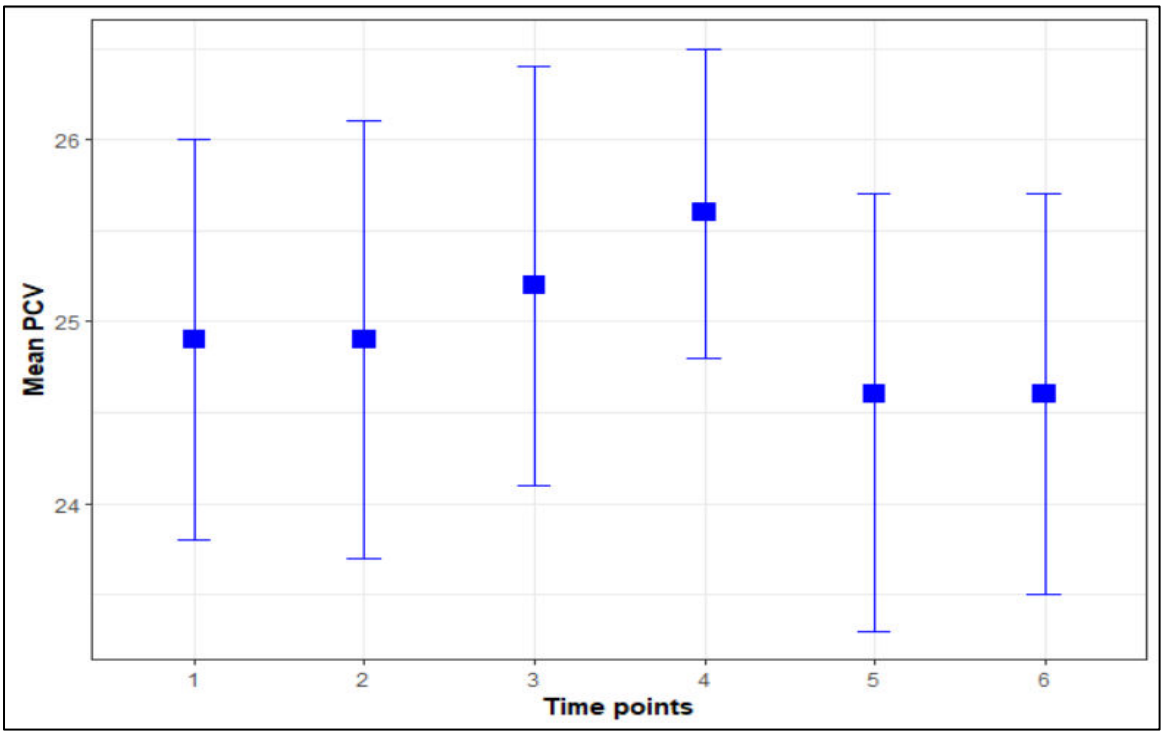
From Friedman's test, we observe that, there is no significant difference in the distribution of haemoglobin over time.

Table 34: Comparison of PCV over time.

Timepoints	PCV		p-value
	Mean ± SD	Median (Min, Max)	
1	24.93 ± 3.75	25.05 (11.80, 36.40)	0.9528[F]
2	24.91 ± 3.87	24.95 (10.50, 33.30)	
3	25.25 ± 3.73	25.70 (15.90, 31.60)	
4	25.62 ± 2.72	26.10 (17.20, 30.50)	
5	24.55 ± 3.98	25.15 (14.30, 32.10)	
6	24.64 ± 3.53	24.80 (14.40, 30.20)	

Abbreviation: F – Friedman's test.

Figure 29: Mean plot of PCV over time.



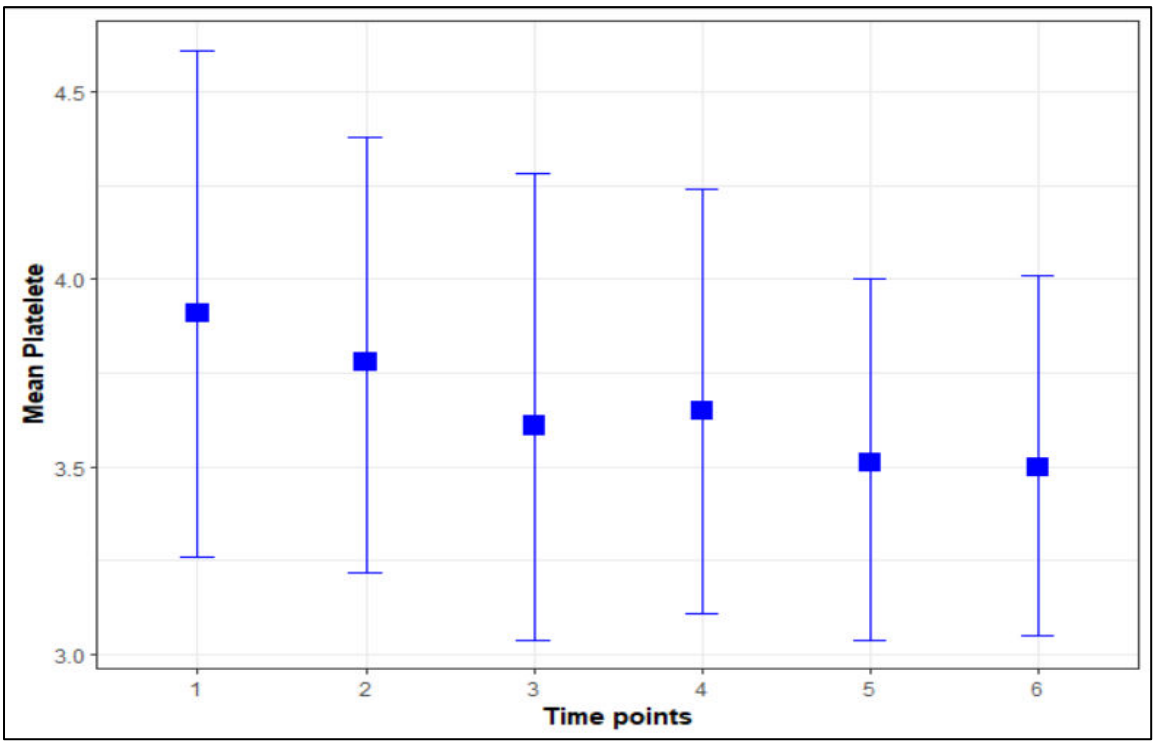
From Friedman's test, we observe that, there is no significant difference in the distribution of PCV over time.

Table 35: Comparison of platelet over time.

Timepoints	Platelet		p-value
	Mean ± SD	Median (Min, Max)	
1	3.91 ± 2.19	3.30 (0.68, 9.97)	0.2012[F]
2	3.78 ± 1.89	3.55 (0.93, 8.95)	
3	3.61 ± 2.04	2.87 (1.05, 9.72)	
4	3.65 ± 1.83	3.33 (0.80, 8.97)	
5	3.51 ± 1.54	3.34 (1.04, 6.88)	
6	3.50 ± 1.58	3.24 (0.93, 7.94)	

Abbreviation: F – Friedman's test.

Figure 30: Mean plot of Platelet over time.



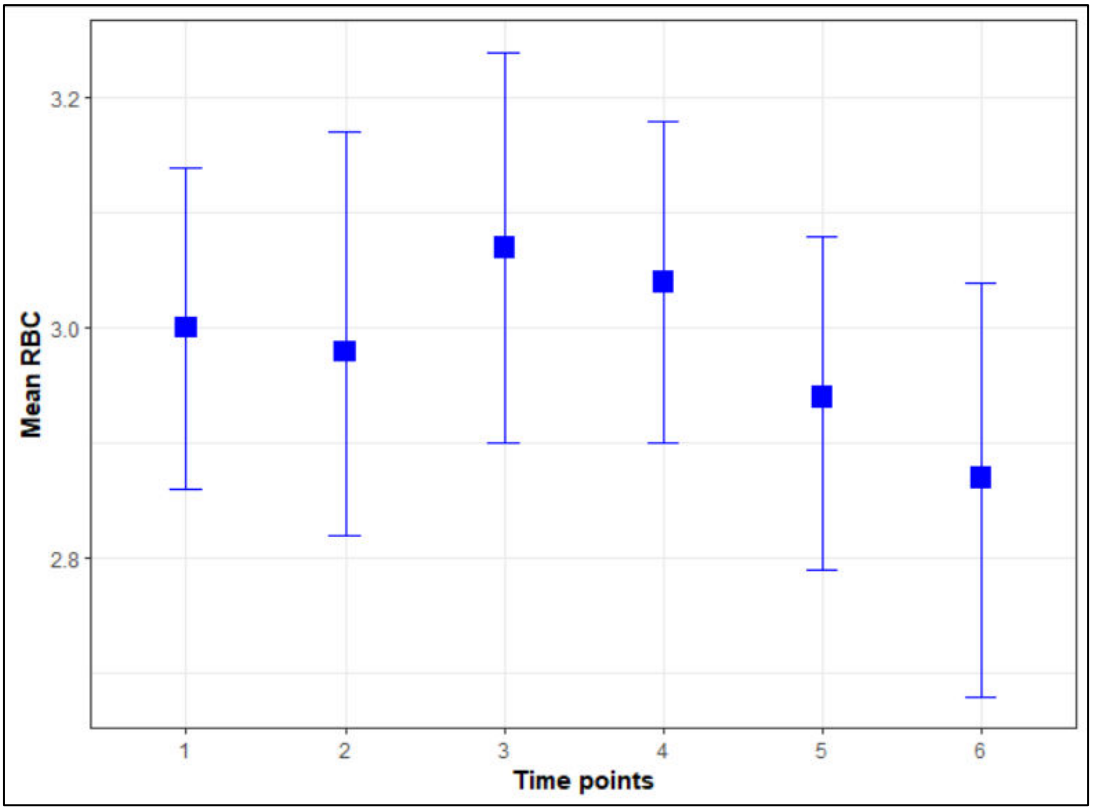
From Friedman's test, we observe that, there is no significant difference in the distribution of Platelet over time.

Table 36: Comparison of RBC over time.

Timepoints	RBC		p-value
	Mean ± SD	Median (Min, Max)	
1	3 ± 0.45	3.01 (1.38, 4.12)	0.43[F]
2	2.98 ± 0.57	3 (1.51, 5.34)	
3	3.07 ± 0.55	3.10 (1.76, 4.03)	
4	3.04 ± 0.44	3.07 (1.93, 4.20)	
5	2.94 ± 0.47	3 (1.57, 4.04)	
6	2.87 ± 0.58	2.93 (1.22, 4.20)	

Abbreviation: F – Friedman's test.

Figure 31: Mean plot of RBC over time.



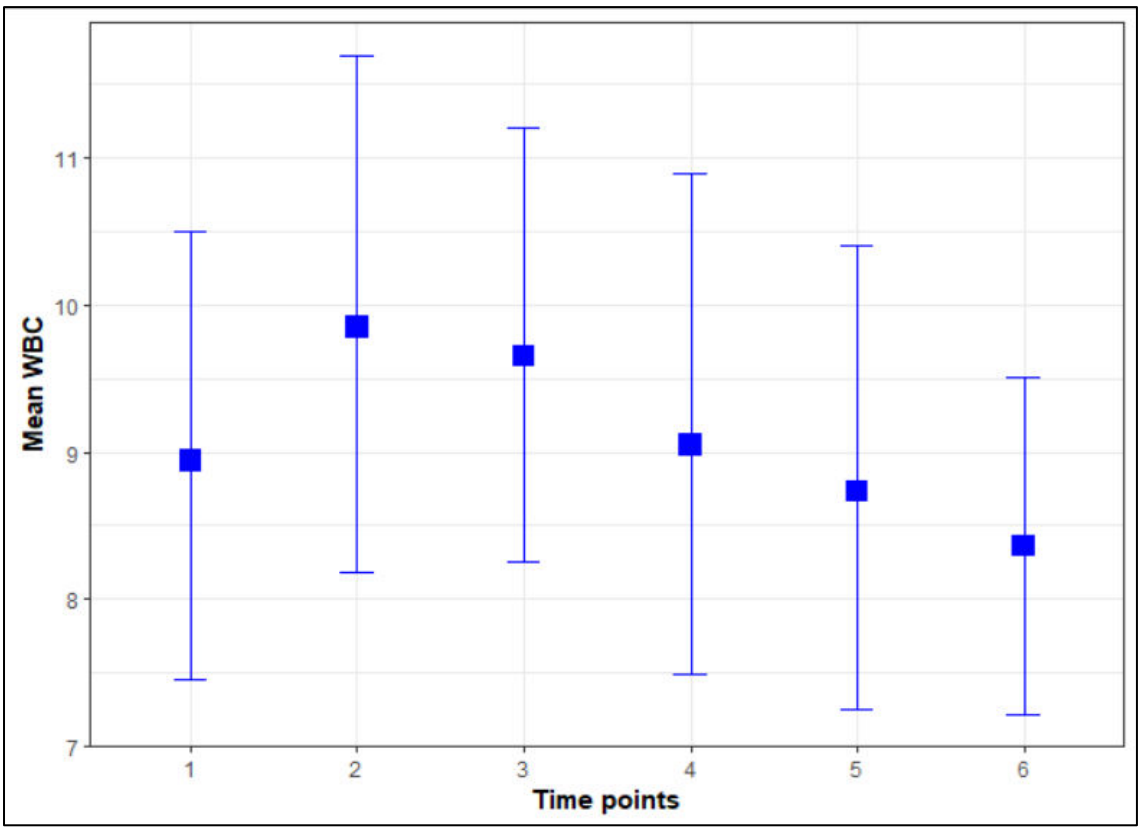
From Friedman's test, we observe that, there is no significant difference in the distribution of RBC over time.

Table 37: Comparison of WBC over time.

Timepoints	WBC		p-value
	Mean ± SD	Median (Min, Max)	
1	8.94 ± 5.10	7.50 (2.30, 23.56)	0.08198[F]
2	9.85 ± 5.83	8.15 (2.79, 29.10)	
3	9.65 ± 4.80	9.59 (2.13, 25.48)	
4	9.05 ± 5.50	8.34 (2.07, 31.85)	
5	8.73 ± 5.14	7.52 (1.81, 26.60)	
6	8.36 ± 3.70	7.78 (2.62, 16.20)	

Abbreviation: F – Friedman's test.

Figure 32:Mean plot of WBC over time.



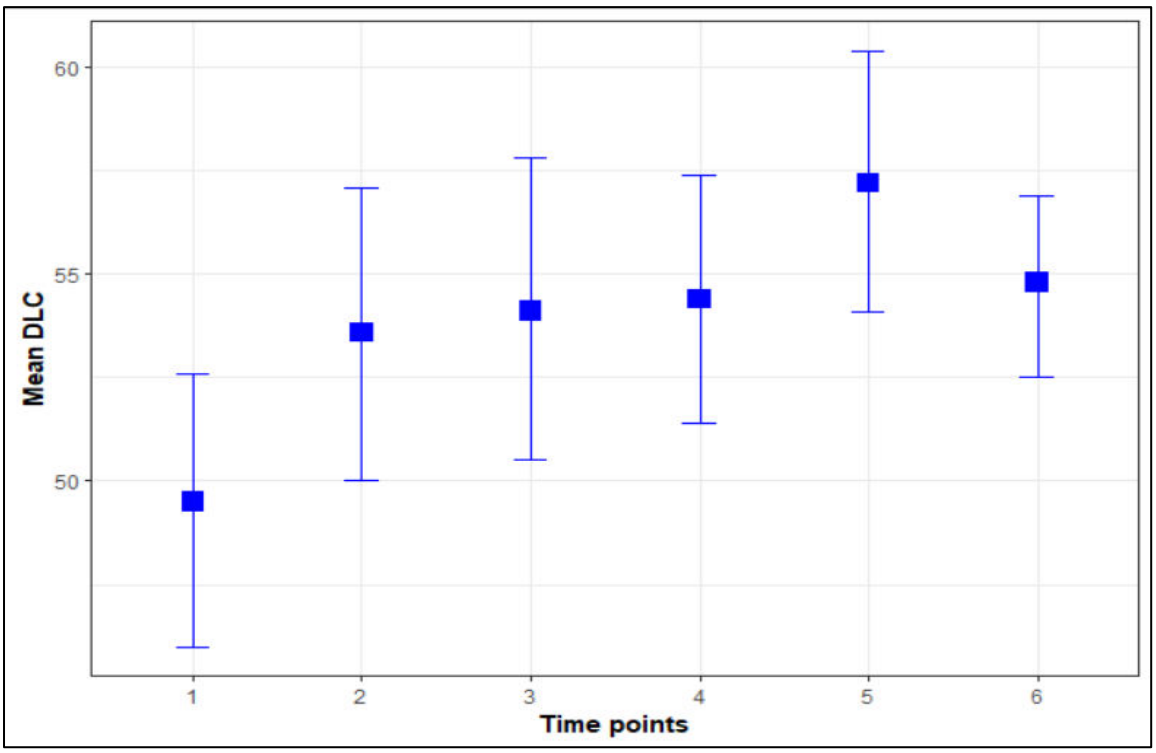
From Friedman's test, we observe that, there is no significant difference in the distribution of WBC over time.

Table 38: Comparison of DLC over time.

Timepoints	DLC		p-value
	Mean ± SD	Median (Min, Max)	
1	49.53 ± 10.83	50 (8.07, 71)	**0.0183[F]***
2	53.65 ± 11.46	54.50 (16, 82)	
3	54.12 ± 11.73	55.50 (26, 81)	
4	54.38 ± 9.66	54 (30, 81)	
5	57.17 ± 10.07	56 (38, 85)	
6	54.85 ± 6.46	55 (39, 68)	

*Abbreviation: F – Friedman's test, * indicates statistical significance.*

Figure 33: Mean plot of DLC over time.



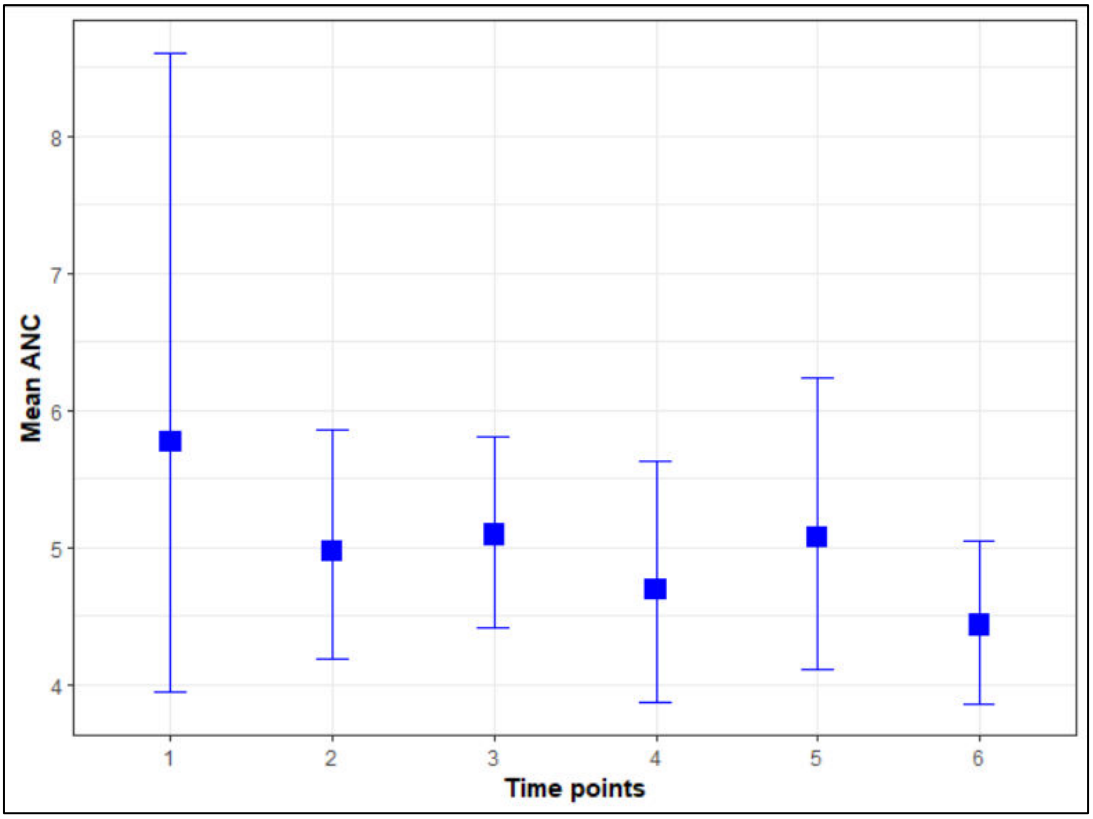
From Friedman's test, we observe that, there is significant difference in the distribution of DLC over time. Further, from pairwise Wilcoxon test, we observe that, there is significant difference in the distribution of DLC between 1st and 5th timepoints (p-value = 0.02).

Table 39: Comparison of ANC over time.

Timepoints	ANC		p-value
	Mean ± SD	Median (Min, Max)	
1	5.77 ± 8.22	4.04 (0.40, 54)	0.4059[F]
2	4.97 ± 2.74	4.28 (1.40, 13.32)	
3	5.09 ± 2.25	4.73 (2, 11.20)	
4	4.69 ± 2.82	4.40 (1.02, 14.31)	
5	5.07 ± 3.50	4.04 (0.89, 17.36)	
6	4.43 ± 1.96	4.26 (1.26, 8.43)	

Abbreviation: F – Friedman's test.

Figure 34: Mean plot of ANC over time.



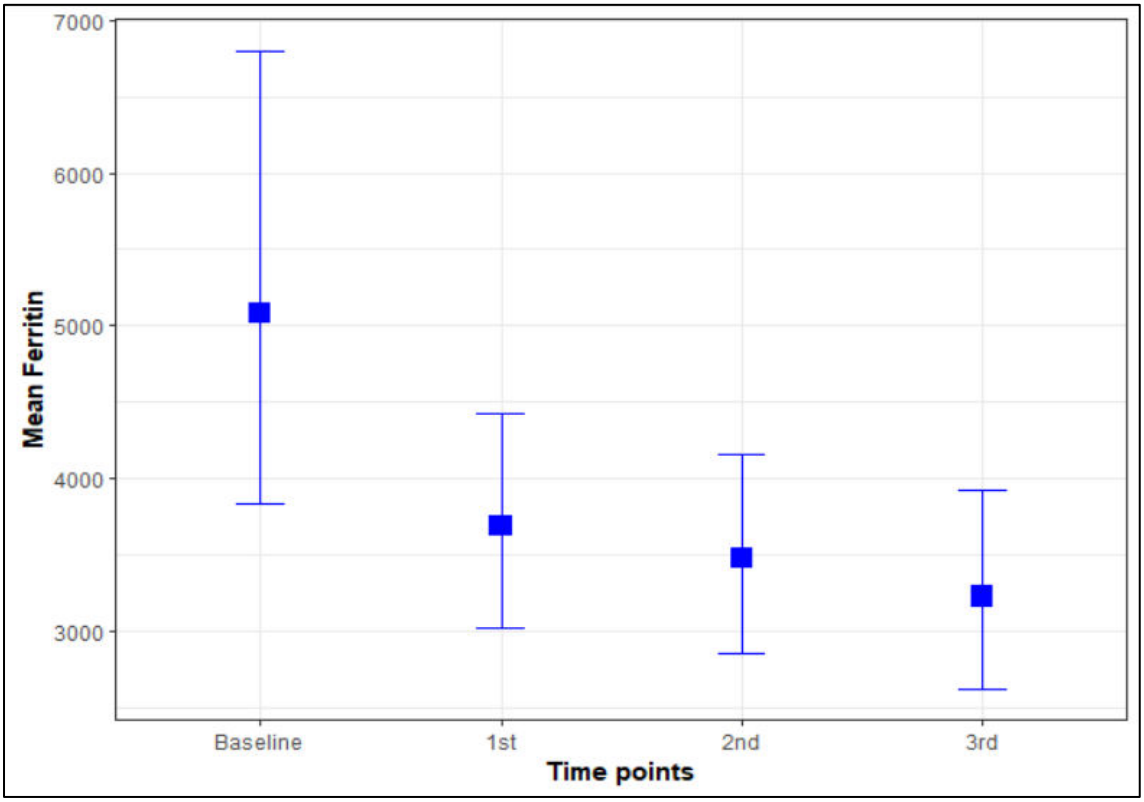
From Friedman's test, we observe that, there is no significant difference in the distribution of ANC over time.

Table 40: Comparison of Ferritin over time.

Timepoints	Ferritin		p-value
	Mean ± SD	Median (Min, Max)	
Baseline	5082.82 ± 4910.40	3869.50 (1066, 31088)	**< 0.001[F]***
1st	3687.34 ± 2256.14	3049 (727.70, 10221)	
2nd	3480.25 ± 2173.15	2985 (834, 9882)	
3rd	3230.78 ± 2167.28	2944.50 (635, 10295)	

*Abbreviation: F – Friedman's test, * indicates statistical significance.*

Figure 35: Mean plot of Ferritin over time.



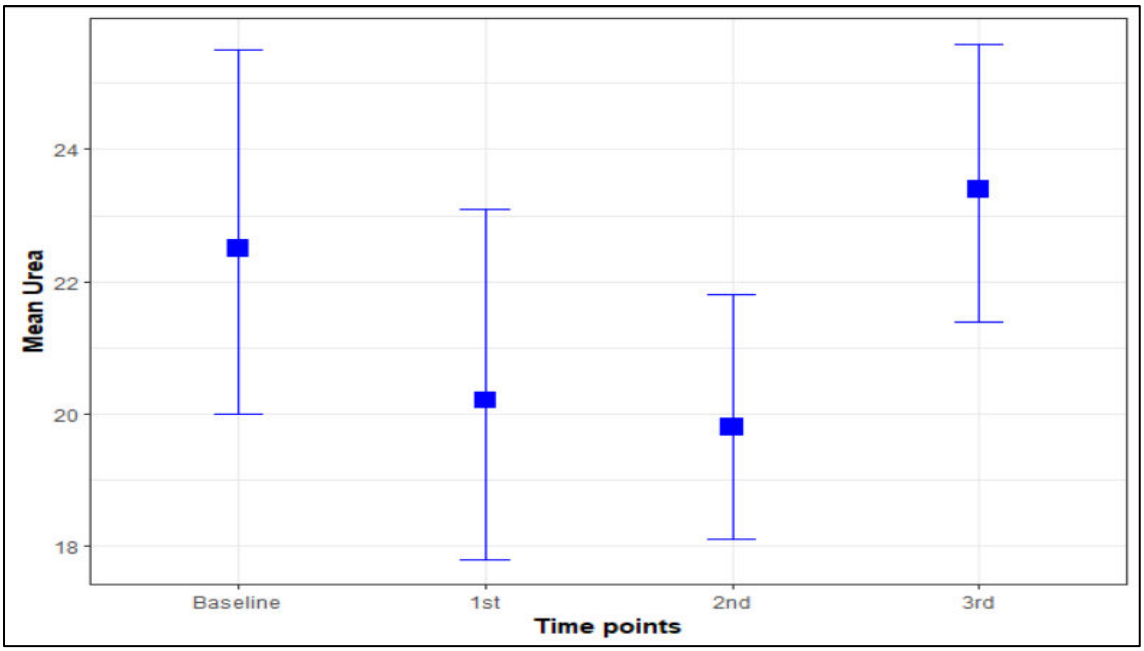
From Friedman's test, we observe that, there is significant difference in the distribution of Ferritin over time. Further, from pairwise Wilcoxon test, we observe that, there is significant difference in the distribution of Ferritin between baseline and 1st time point (p-value = 0.00038), baseline and 2nd time point (p-value = 0.00012) and baseline and 3rd time point (p-value = 0.0001).

Table 41: Comparison of urea over time.

Timepoints	Urea		p-value
	Mean ± SD	Median (Min, Max)	
Baseline	22.52 ± 8.98	19.50 (9, 54)	**0.0049[F]***
1st	20.23 ± 8.65	18.50 (10, 58)	
2nd	19.85 ± 6.01	19 (10, 35)	
3rd	23.45 ± 6.85	21.50 (14, 39)	

*Abbreviation: F – Friedman's test, * indicates statistical significance.*

Figure 36: Mean plot of urea over time.



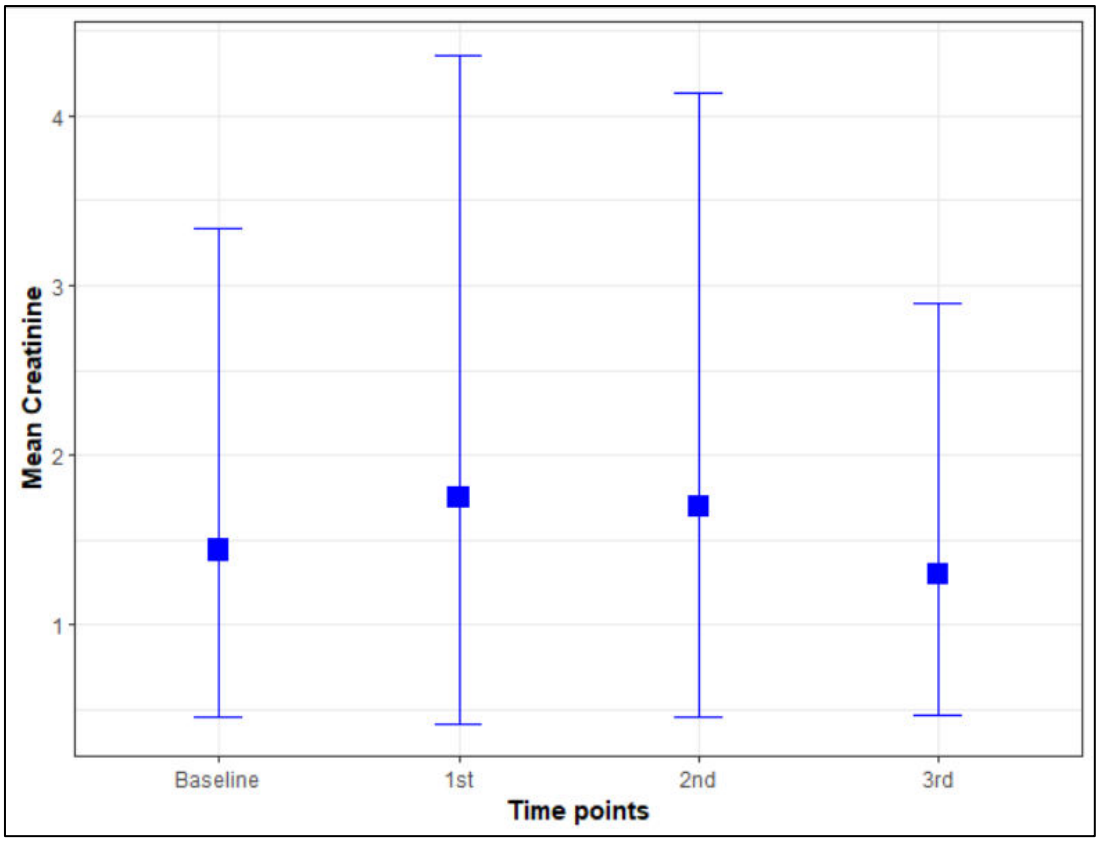
From Friedman's test, we observe that, there is significant difference in the distribution of urea over time. Further, from pairwise Wilcoxon test, we observe that, there is significant difference in the distribution of urea between 1st and 3rd time point (p-value = 0.025) and between 2nd and 3rd time point (p-value = 0.024).

Table 42: Comparison of creatinine over time.

Timepoints	Creatinine		p-value
	Mean ± SD	Median (Min, Max)	
Baseline	1.44 ± 5.93	0.50 (0.20, 38)	0.5042[F]
1st	1.75 ± 8.15	0.40 (0.20, 52)	
2nd	1.70 ± 7.67	0.50 (0.20, 49)	
3rd	1.29 ± 4.98	0.50 (0.20, 32)	

Abbreviation: F – Friedman's test.

Figure 37: Mean plot of Creatinine over time.



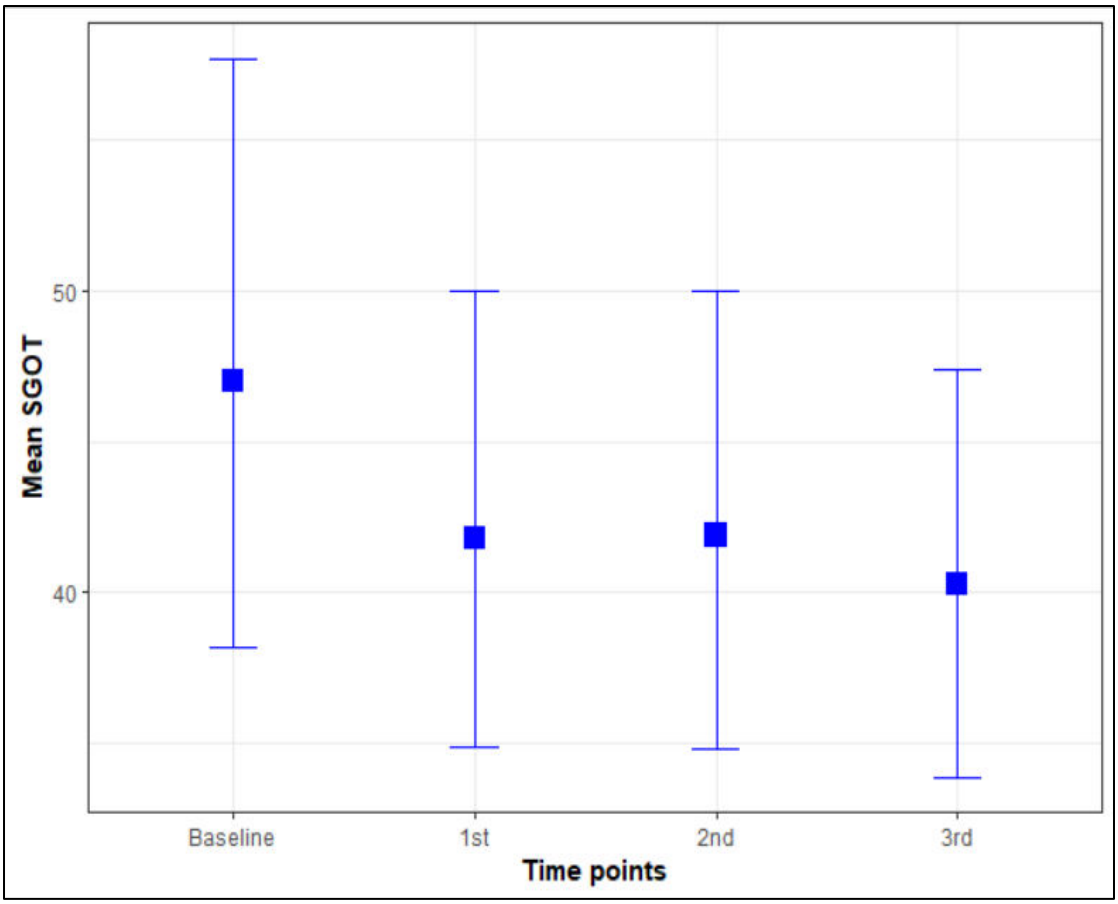
From Friedman's test, we observe that, there is no significant difference in the distribution of creatinine over time.

Table 43: Comparison of SGOT over time.

Timepoints	SGOT		p-value
	Mean ± SD	Median (Min, Max)	
1	46.98 ± 32.36	35 (13, 194)	0.621[F]
2	41.85 ± 24.54	34.50 (12, 143)	
3	41.92 ± 25.44	34 (10, 140)	
4	40.30 ± 22.33	33.50 (15, 132)	

Abbreviation: F – Friedman's test.

Figure 38: Mean plot of SGOT over time.



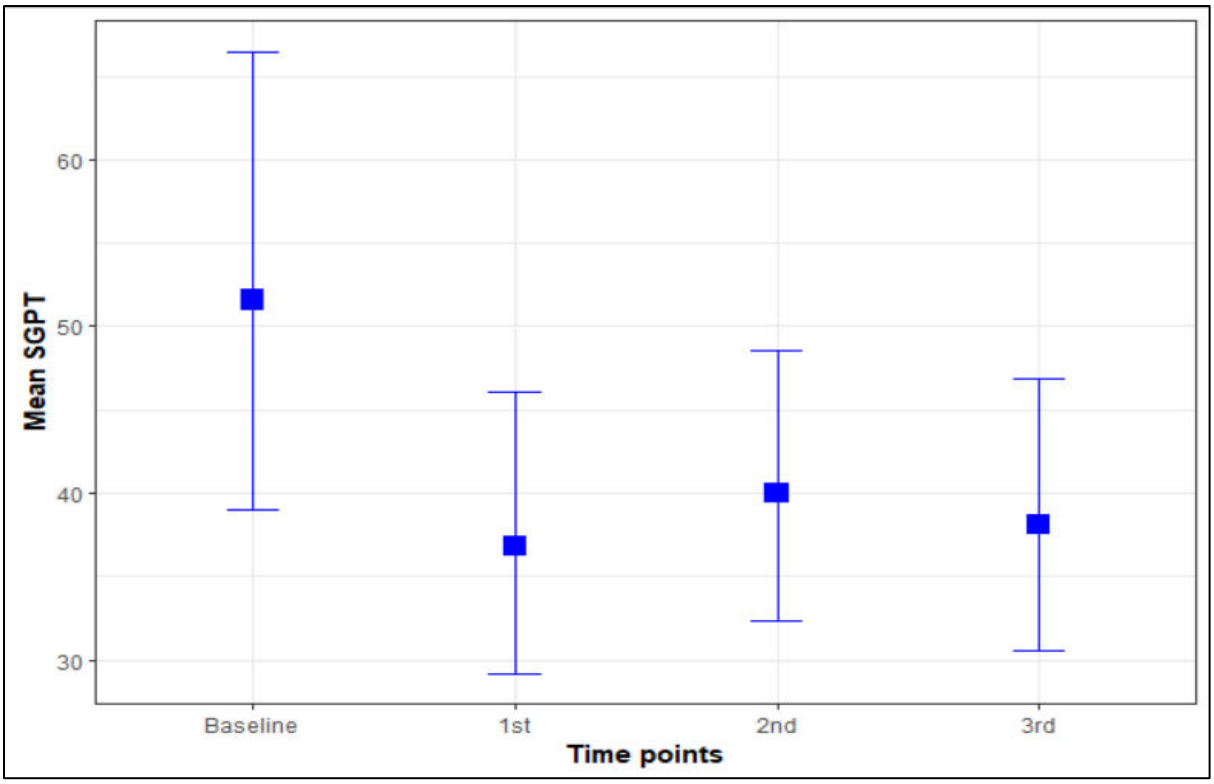
From Friedman's test, we observe that, there is no significant difference in the distribution of SGOT over time.

Table 44: Comparison of SGPT over time.

Timepoints	SGPT		p-value
	Mean ± SD	Median (Min, Max)	
Baseline	51.65 ± 44.53	37 (12, 210)	**0.0117[F]***
1st	36.77 ± 26.95	36 (8, 161)	
2nd	39.95 ± 6.99	32 (12, 143)	
3rd	38.10 ± 26.46	32.50 (6, 140)	

*Abbreviation: F – Friedman's test, * indicates statistical significance.*

Figure 39: Mean plot of SGPT over time.



From Friedman's test, we observe that, there is significant difference in the distribution of SGPT over time. Further, from pairwise Wilcoxon test, we observe that, there is significant difference in the distribution of SGPT at baseline and 1st time point (p-value = 0.011)

Table 45: Incidence of various endocrinopathies

Variable	Normal	Abnormal
LH	7 (17.5%)	33 (82.5%)
FSH	4 (10%)	36 (90%)
GH	2 (5%)	38 (95%)
Testosterone	2 (9.5%)	19 (90.5%)
Estradiol	7 (36.8%)	12 (63.2%)
TSH	38 (95%)	2 (5%)
FT4	39 (97.5%)	1 (2.5%)
FBS	37 (92.5%)	3 (7.5%)
2 hr PPBS	35 (87.5%)	5 (12.5%)

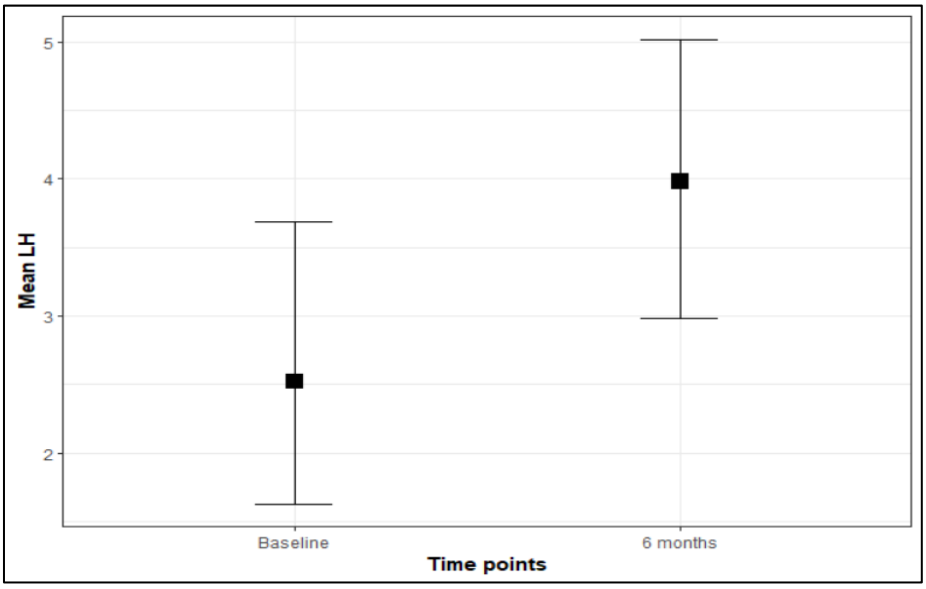
Table 46: Comparison of mean value endocrine profile post intervention

Variables	Time points		p-value
	Baseline	At 6 months	
LH (mIU/ml)	2.52 ± 3.35 1.46 (0.21, 17.94)	3.98 ± 3.34 3.44 (0.10, 11.06)	**0.0017^{W*}**
FSH (mIU/ml)	2.76 ± 1.85 2.50 (0.10, 7.87)	4.19 ± 2.74 3.59 (0.37, 11.27)	**< 0.001^{W*}**
GH (ng/ml)	3.08 ± 3.34 2.06 (0.09, 13.84)	8.87 ± 7 10.59 (0.09, 29.91)	**< 0.001^{W*}**
TSH (µIU/ml)	2.35 ± 0.98 2.24 (0.92, 5)	2.58 ± 1.37 2.39 (0.32, 5.88)	0.3498^{W}
FT4 (ng/dl)	1.28 ± 0.29 1.23 (0.85, 2.65)	1.33 ± 0.55 1.19 (0.78, 3.62)	0.9946^{W}
Testosterone (ng/dl)	5.53 ± 8.57 0.44 (0.09, 22.77)	11.82 ± 12.04 9.65 (0.09, 35.34)	**0.0019^{W*}**
Estradiol (pg/ml)	34.64 ± 36.29 18.22 (4.75, 115.70)	73.49 ± 84.98 40.64 (7.44, 340.22)	**0.0141^{W*}**
FBS (mg/dl)	89.95 ± 14.22 88 (68, 126)	97.42 ± 17.26 92.50 (68, 168)	**< 0.001^{W*}**
2 HR PPBS (mg/dl)	152.82 ± 36.29 137 (94, 262)	157.75 ± 21.54 153.50 (110, 194)	0.0890^{W}

*Abbreviation: W – Wilcoxon test, * indicates statistical significance.*

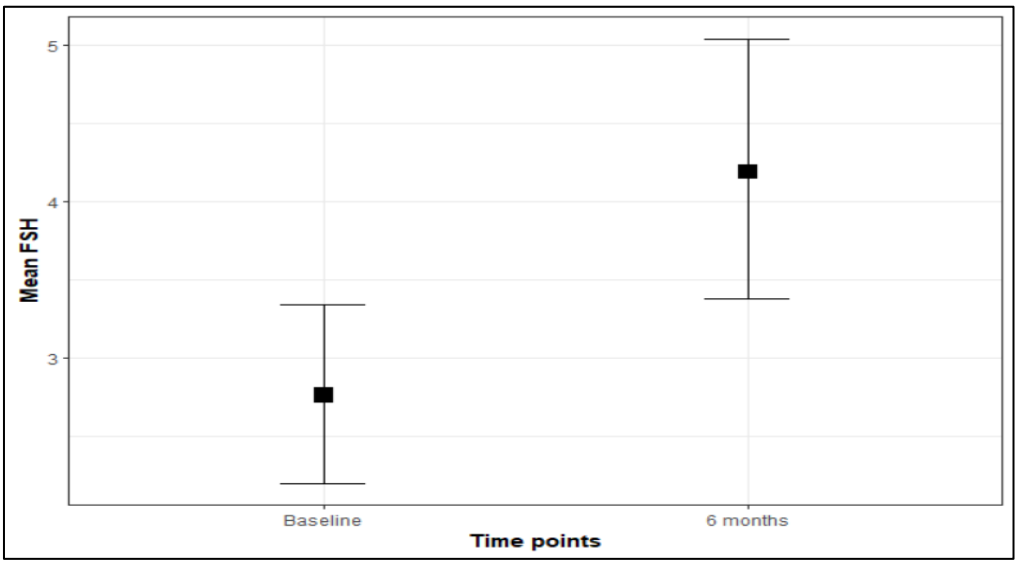
From Wilcoxon test, we observe that, there is significant difference in the distribution of LH, FSH, GH, Testosterone, Estradiol and FBS over time. There is no significant difference in the distribution of TSH, FT4 and 2 HR PPBS over time.

Figure 40: Mean plot of LH over time.



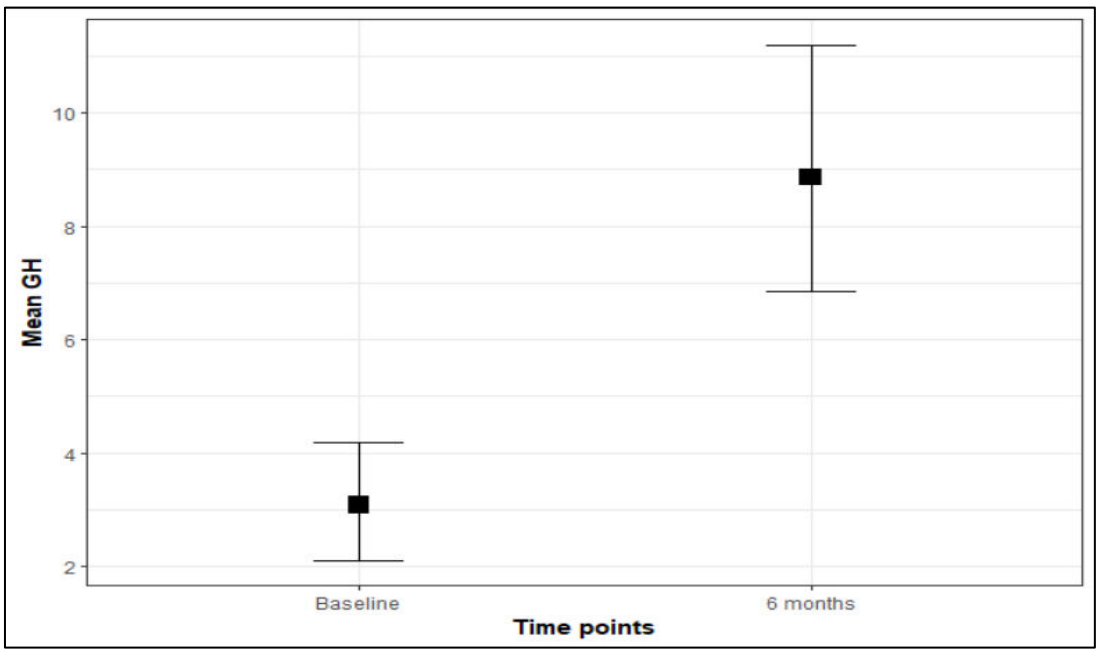
From Wilcoxon test, we observe that, there is significant difference in the distribution of LH

Figure 41: Mean plot of FSH over time.



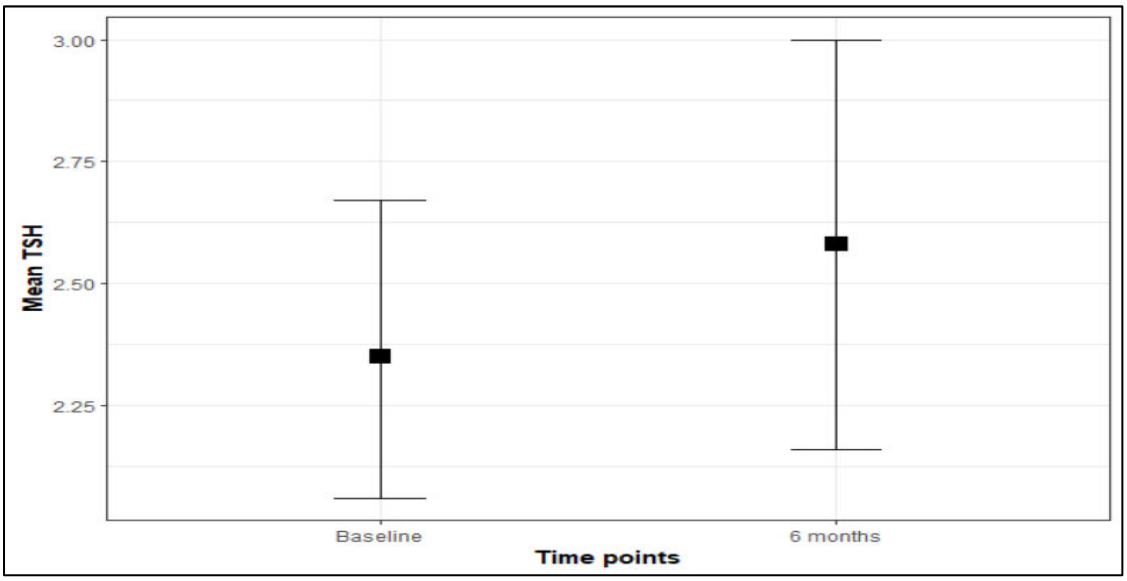
From Wilcoxon test, we observe that, there is significant difference in the distribution of FSH

Figure 42: Mean plot of GH over time.



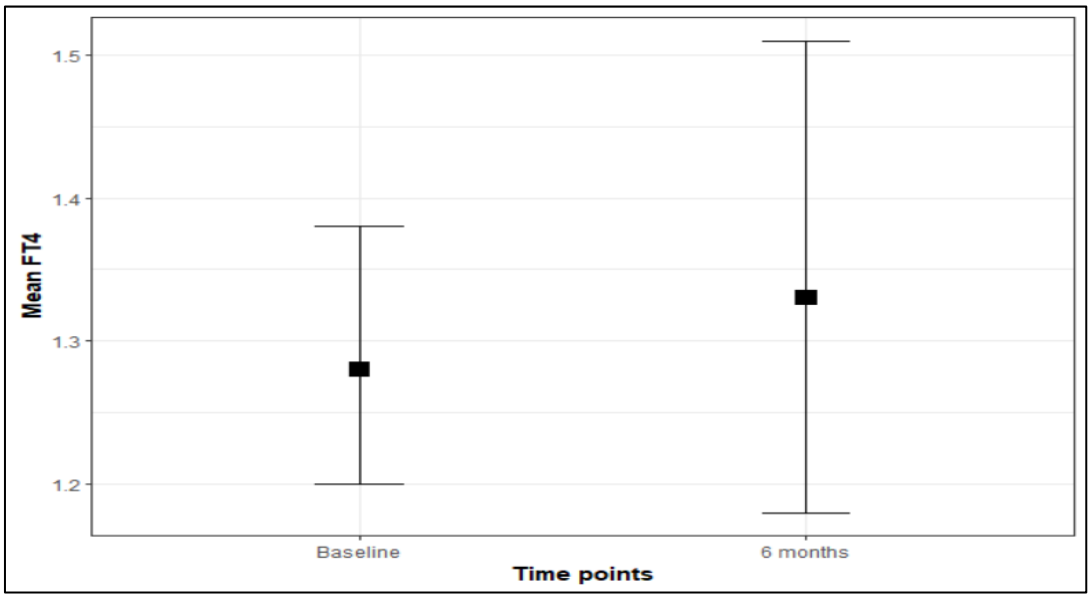
From Wilcoxon test, we observe that, there is significant difference in the distribution of GH

Figure 43: Mean plot of TSH over time.



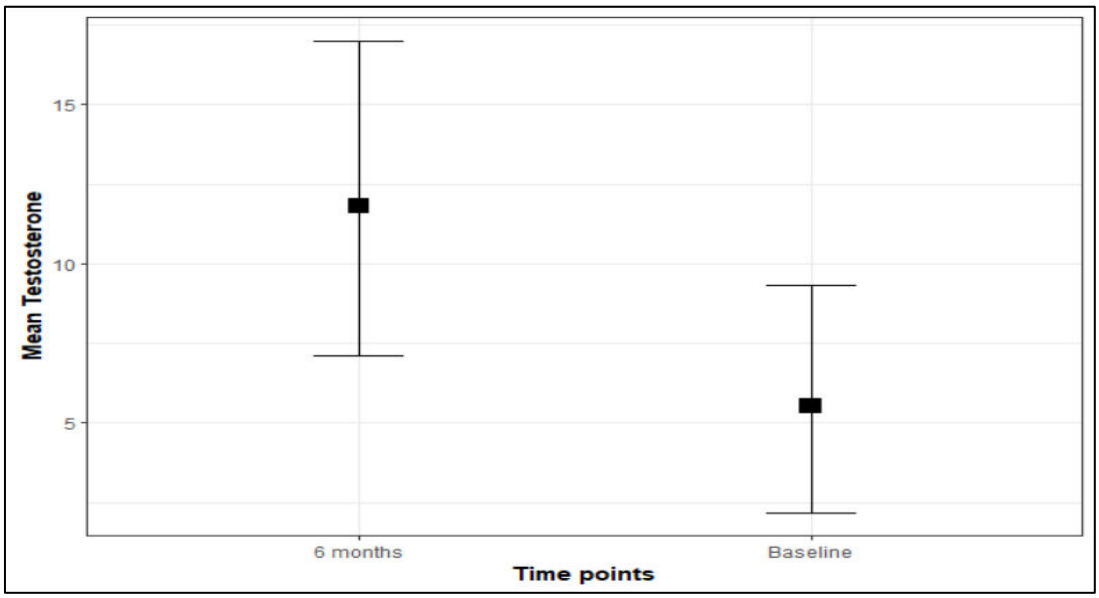
From Wilcoxon test, we observe that, there is no significant difference in the distribution of TSH

Figure 44: Mean plot of FT4 over time.



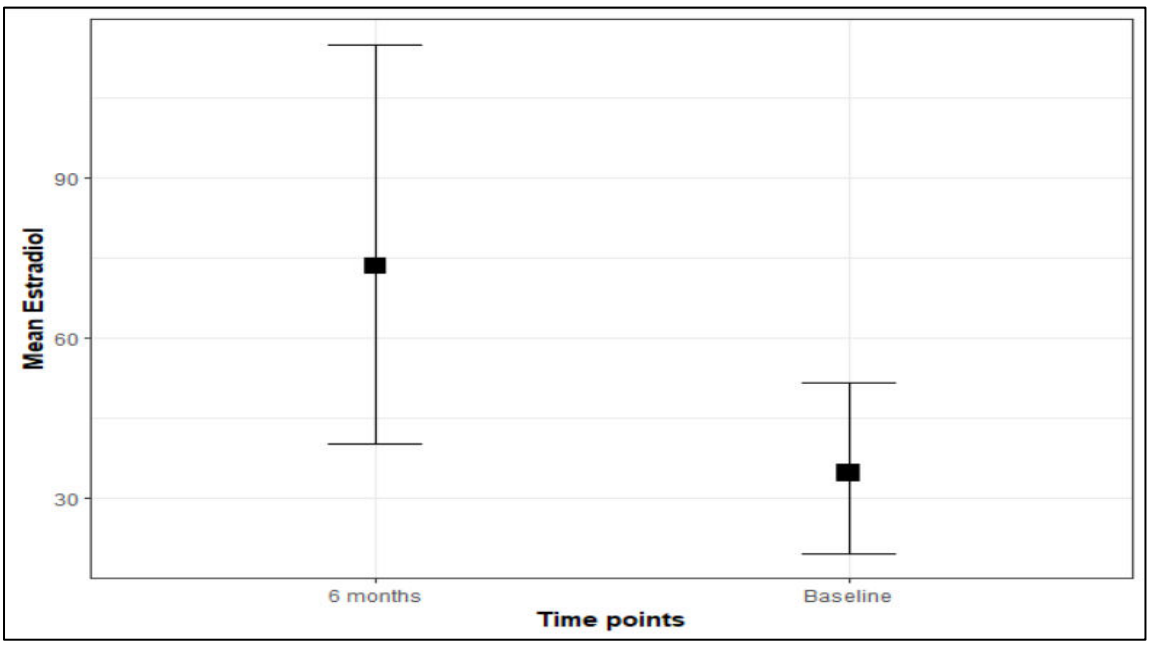
From Wilcoxon test, we observe that, there is no significant difference in the distribution of FT4

Figure 45: Mean plot of Testosterone over time.



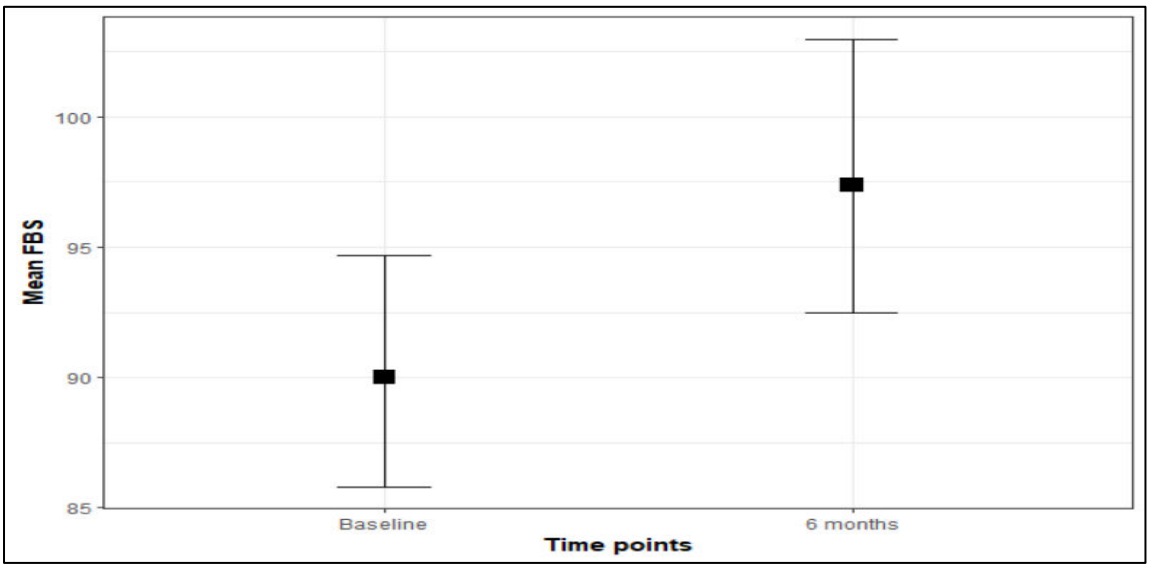
From Wilcoxon test, we observe that, there is significant difference in the distribution of Testosterone

Figure 46: Mean plot of Estradiol over time.



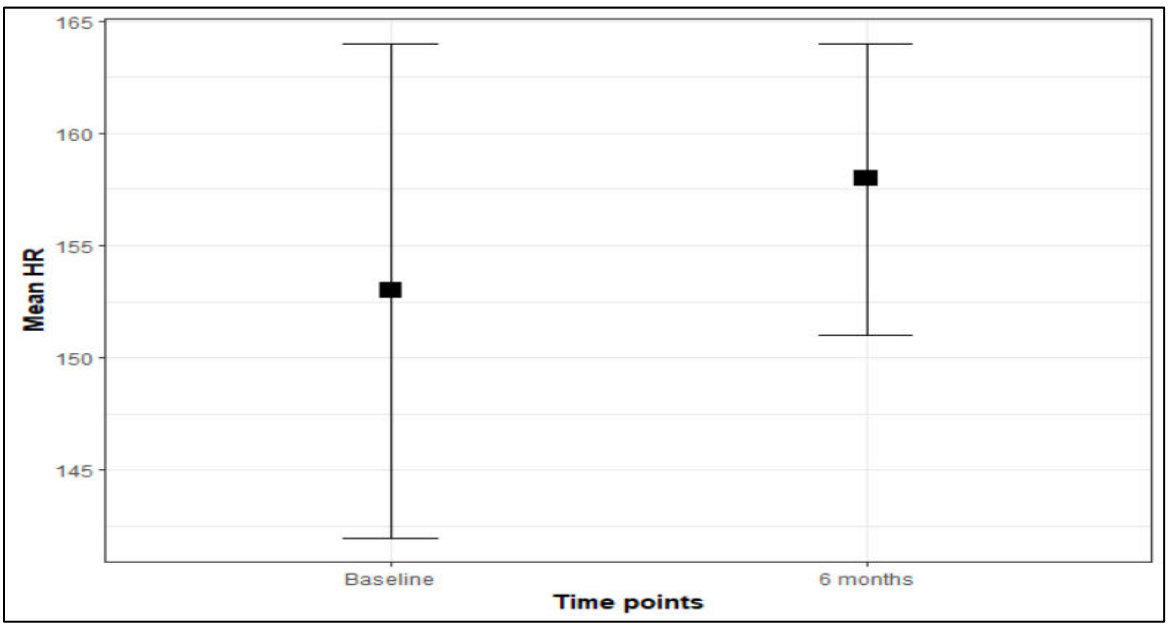
From Wilcoxon test, we observe that, there is significant difference in the distribution of Estradiol

Figure 47: Mean plot of FBS over time.



From Wilcoxon test, we observe that, there is no significant difference in the distribution of FBS

Figure 48: Mean plot of 2HR PPBS over time.



From Wilcoxon test, we observe that, there is no significant difference in the distribution of 2 HR PPBS

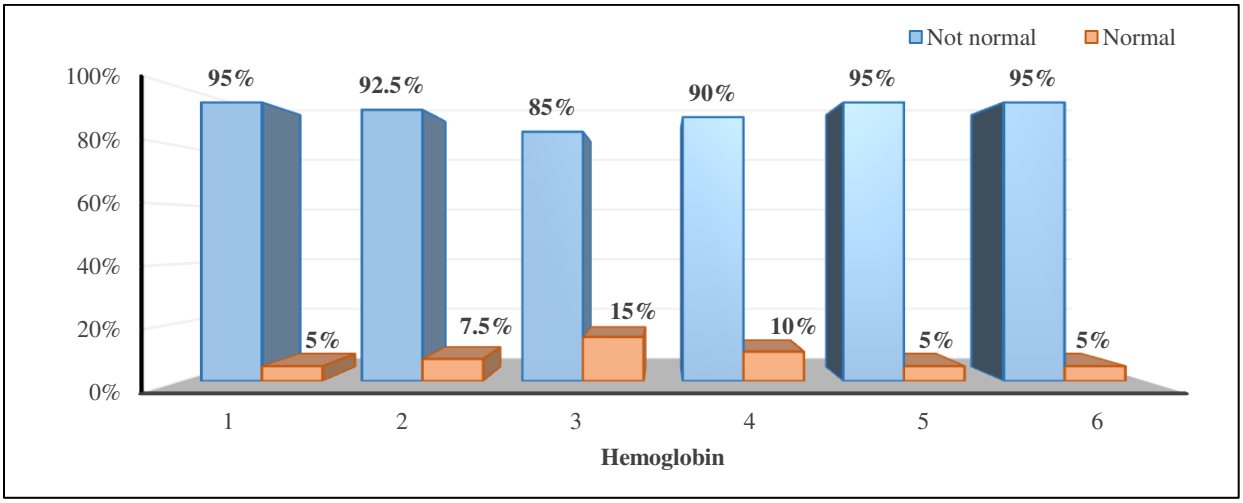
Table 47: Comparison of variables between time points.

Variables	Sub Category	Time point 6		p-value
		Not normal	Normal	
Haemoglobin	Not normal	36	2	1^{MN}
	Normal	2	0	
PCV	Normal	1	0	$< 0.001^{MN*}$
	Not normal	39	0	
Platelet	Normal	13	3	0.2278^{MN}
	Not normal	8	16	
RBC	Normal	0	1	1^{MN}
	Not normal	1	38	
WBC	Normal	23	3	0.2278^{MN}
	Not normal	8	6	
DLC-Neutrophil	Not normal	12	7	0.0455^{MN*}
	Normal	18	3	
ANC	Normal	30	2	0.4497^{MN}
	Not normal	5	3	

*Abbreviation: MN – Mcnemar test, * indicates statistical significance.*

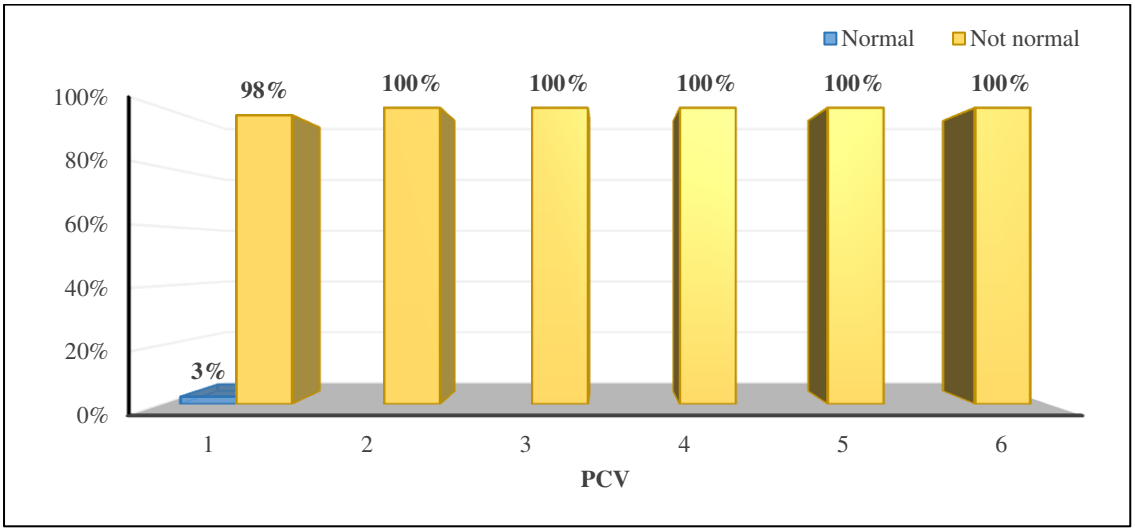
From McNemar test, we observe that, there is significant difference in the distribution of PCV and DLC-Neutrophil between 1st and 6th timepoints.

Figure 49: Distribution of subjects according to haemoglobin over time.



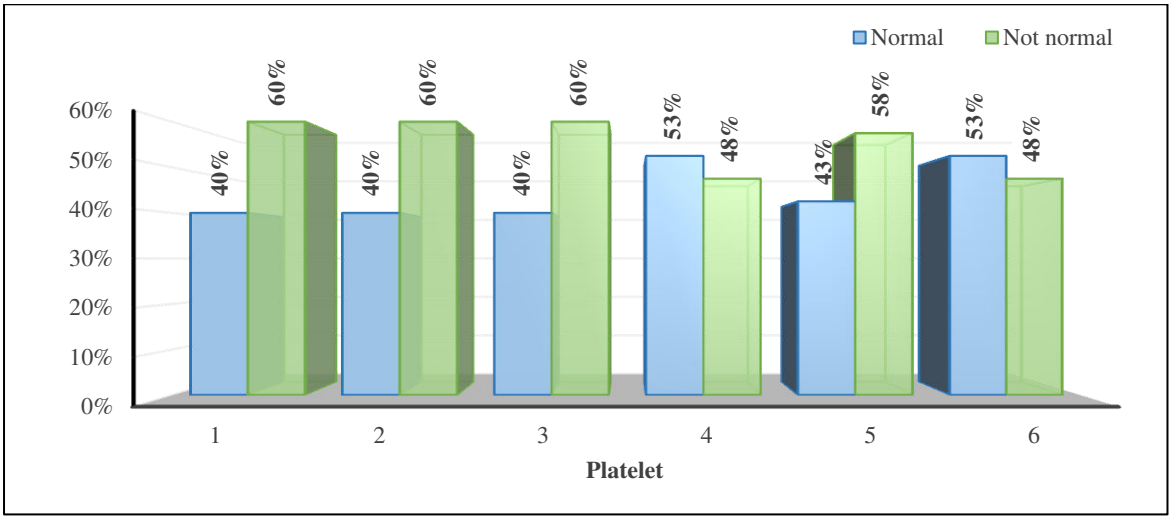
From McNemar test, we observe that, there is no significant difference in the distribution of hemoglobin between baseline and 6 months post intervention .

Figure 50: Distribution of subjects according to PCV over time.



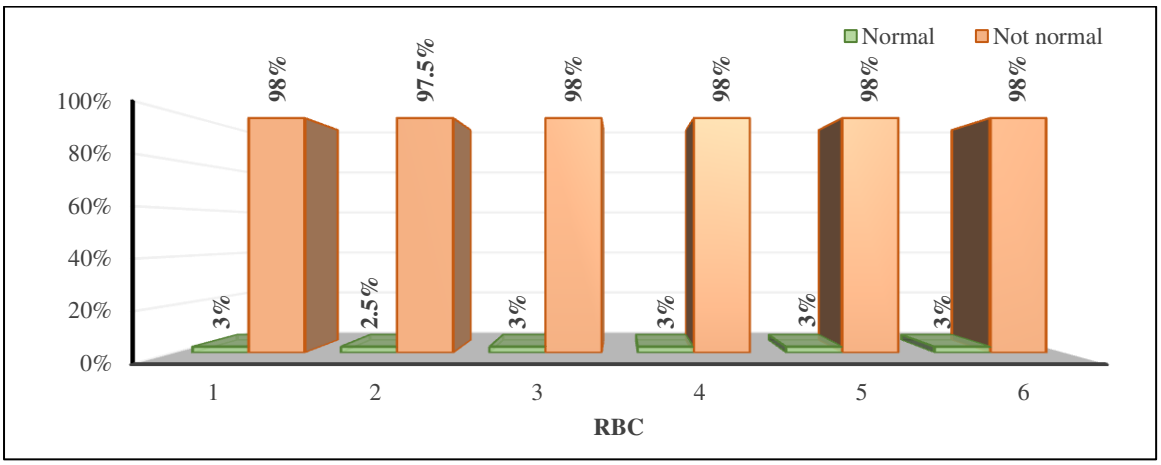
From McNemar test, we observe that, there is significant difference in the distribution of PCV between baseline and 6 months post intervention.

Figure 51: Distribution of subjects according to platelet over time.



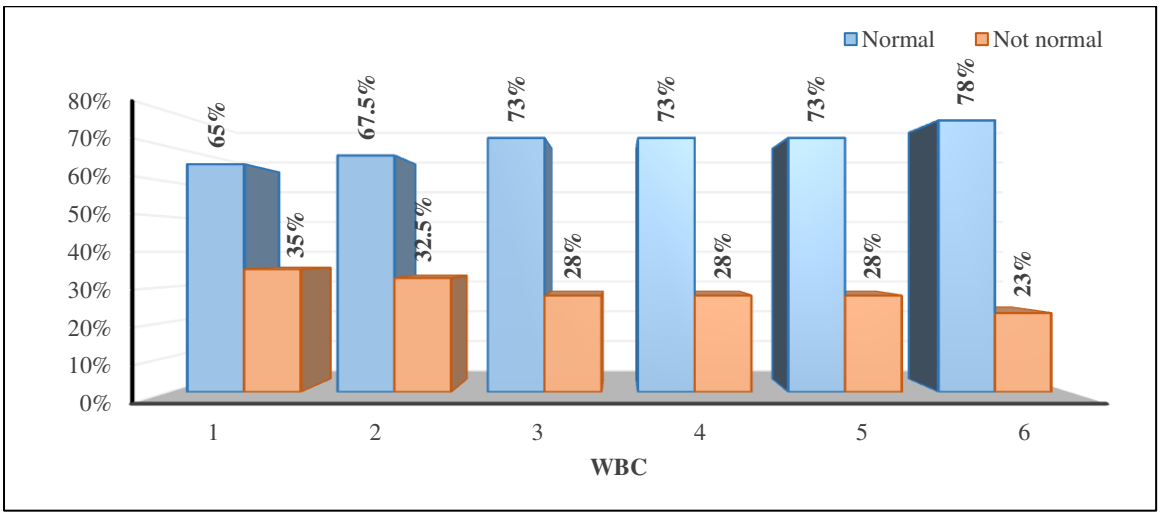
From McNemar test, we observe that, there is no significant difference in the distribution of platelet between baseline and 6 months post intervention.

Figure 52: Distribution of subjects according to RBC over time.



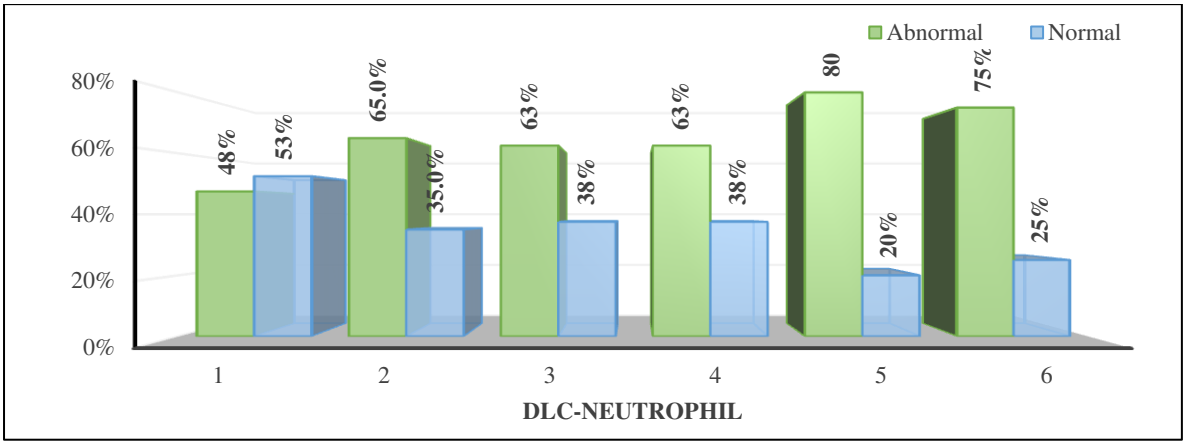
From McNemar test, we observe that, there is no significant difference in the distribution of RBC between baseline and 6 months post intervention.

Figure 53: Distribution of subjects according to WBC over time.



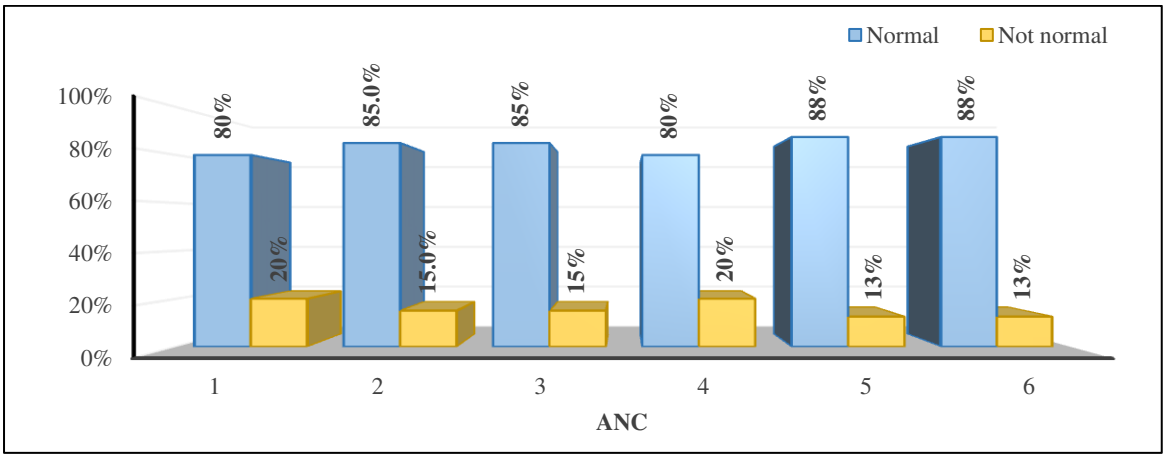
From McNemar test, we observe that, there is no significant difference in the distribution of WBC between baseline and 6 months post intervention.

Figure 54: Distribution of subjects according to DLC-Neutrophil over time.



From McNemar test, we observe that, there is significant difference in the distribution of DLC-Neutrophil between baseline and 6 months post intervention.

Figure 55: Distribution of subjects according to ANC over time.



From McNemar test, we observe that, there is no significant difference in the distribution of ANC between baseline and 6 months post intervention.

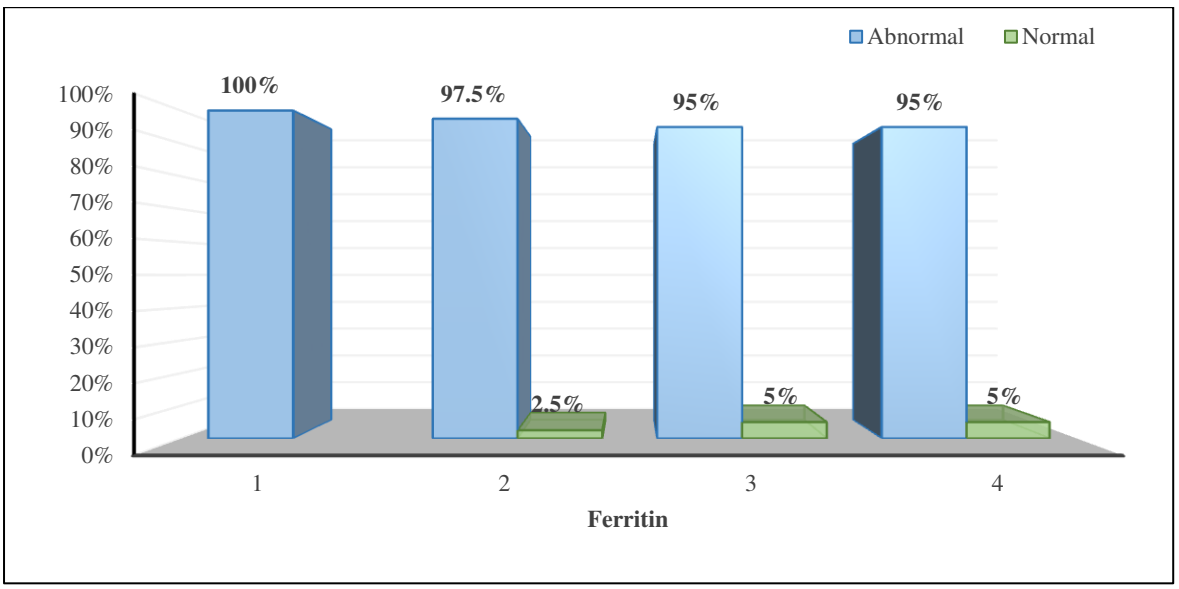
Table 48: Comparison of variables between time points.

Variables	Sub Category	3rd		p-value
		Not normal	Normal	
Ferritin	Not normal	38	2	0.4975MN
	Normal	0	0	
Urea	Normal	33	1	0.1306MN
	Not normal	6		
Creatinine	Normal	37	1	1MN
	Not normal	1	1	
SGOT	Normal	18	4	0.7518MN
	Not normal	6	12	
SGPT	Normal	24	5	0.7728MN
	Not normal	7	4	

*Abbreviation: MN – Mcnemar test, * indicates statistical significance.*

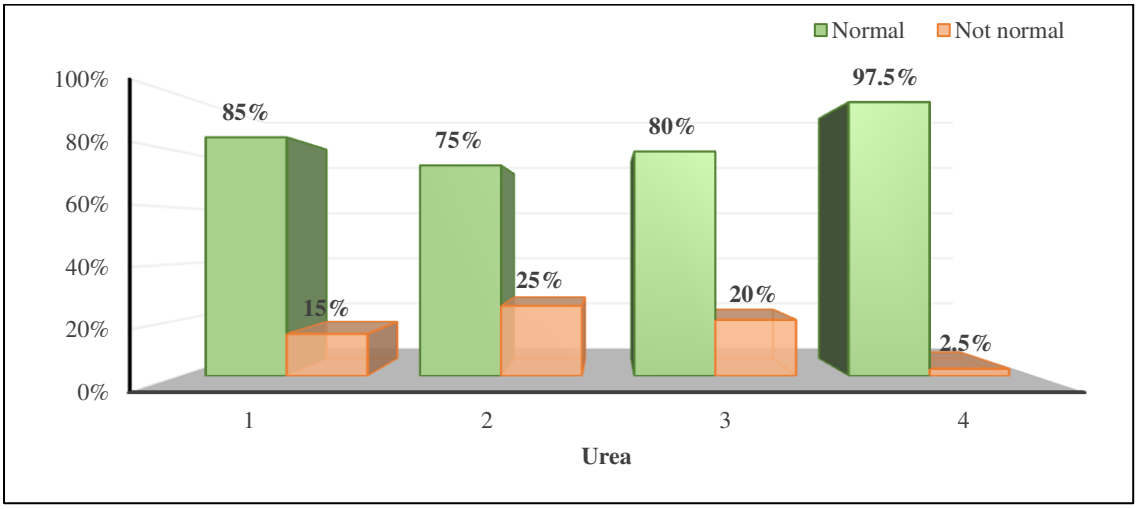
From McNemar test, we observe that, there is no significant difference in the distribution of variable between baseline and 3rd timepoints.

Figure 56: Distribution of subjects according to Ferritin over time



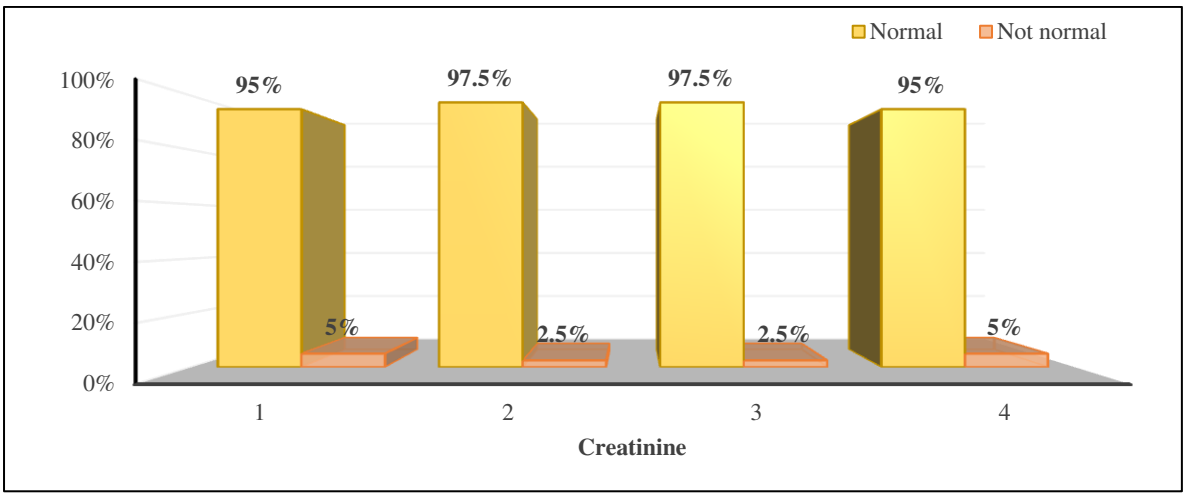
From McNemar test, we observe that, there is no significant difference in the distribution of ferritin between baseline and 6 months post intervention

Figure 57: Distribution of subjects according to Urea over time.



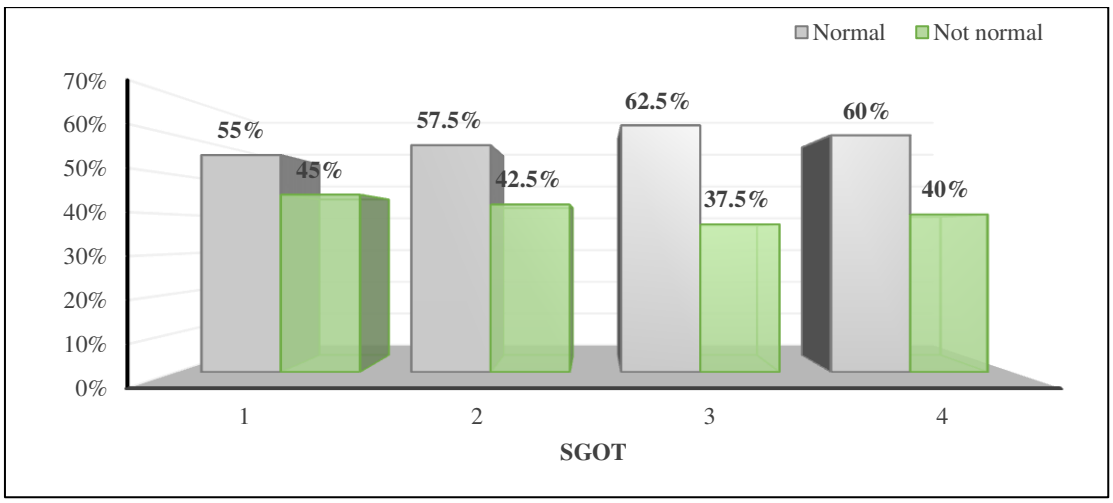
From McNemar test, we observe that, there is no significant difference in the distribution of urea between baseline and 6 months post intervention

Figure 58: Distribution of subjects according to Creatinine over time.



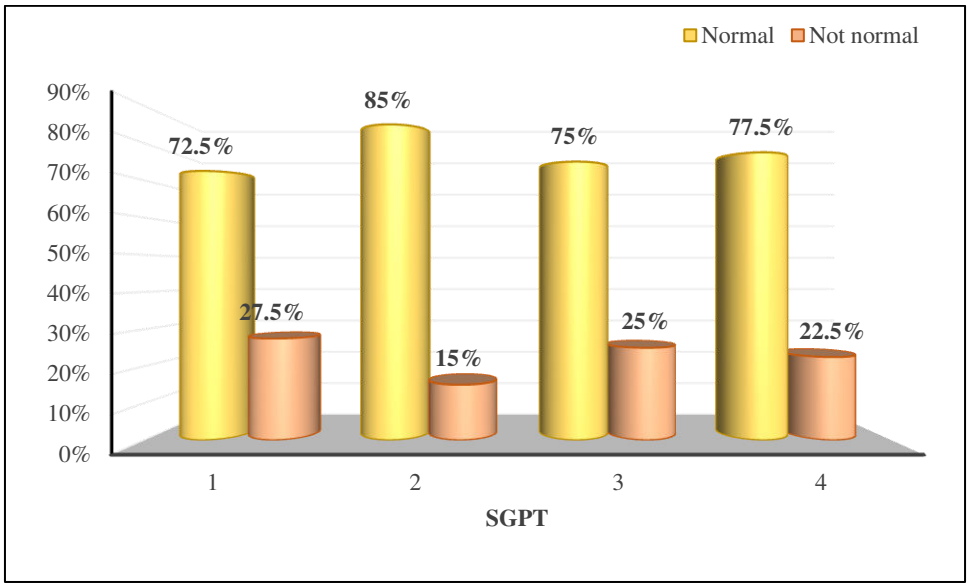
From McNemar test, we observe that, there is no significant difference in the distribution of creatinine between baseline and 6 months post intervention

Figure 59: Distribution of subjects according to SGOT over time.



From McNemar test, we observe that, there is no significant difference in the distribution of SGOT between baseline and 6 months post intervention

Figure 60: Distribution of subjects according to SGPT over time.



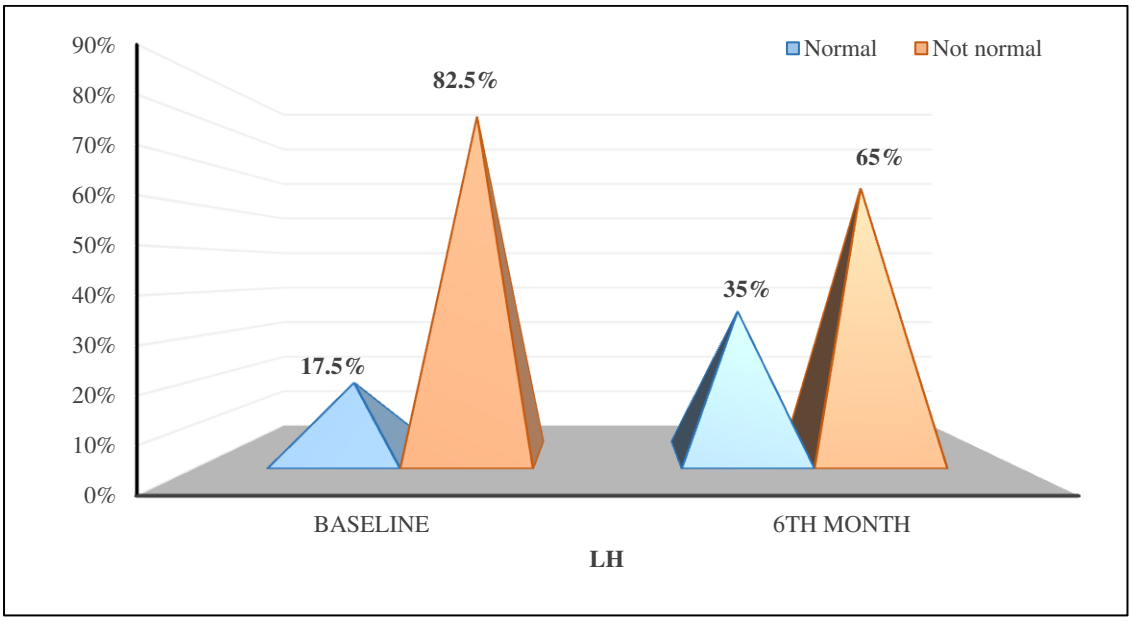
From McNemar test, we observe that, there is no significant difference in the distribution of SGPT between baseline and 6 months post intervention

Table 49: Distribution of improvement of endocrine profile over 6 months

VARIABLE	NORMAL	ABNORMAL	IMPROVEMENT	P-VALUE
LH	7	33	25	<0.0001*
FSH	4	36	31	<0.0001*
TESTOSTERONE	2	19	17	<0.0001*
ESTRADIOL	7	12	11	<0.0001*
GH	2	38	35	<0.0001*
TSH	38	2	0	-
FT4	39	1	0	-
FBS	37	3	2	1
2HR PPBS	35	5	0	-

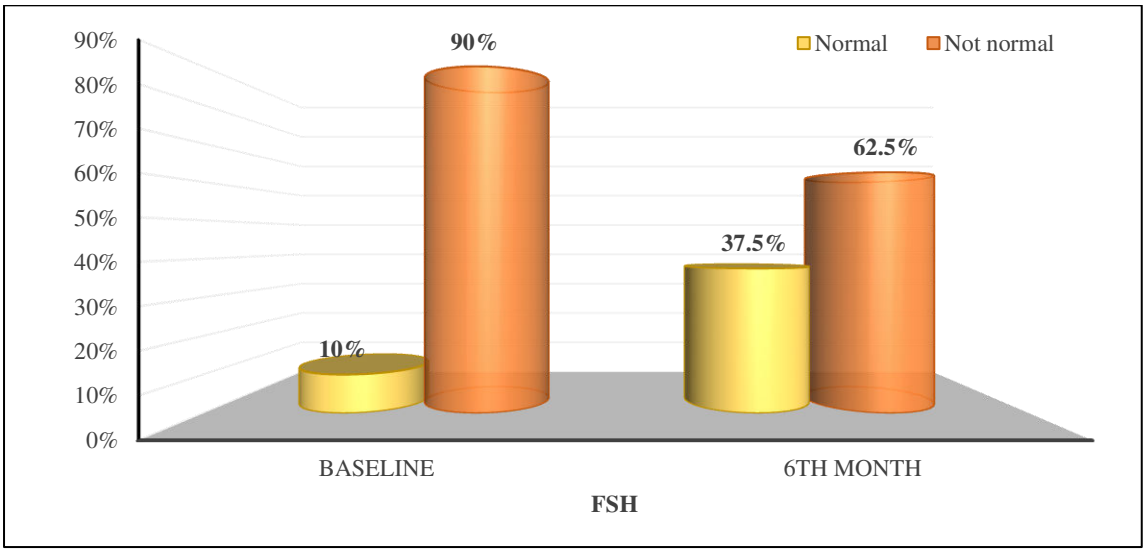
By proportion test, proportion of improved levels at 6th month was significantly more in patients with abnormal level of LH, FSH,Testosterone ,Estradiol and GH.

Figure 61: Distribution of subjects according to LH over time.



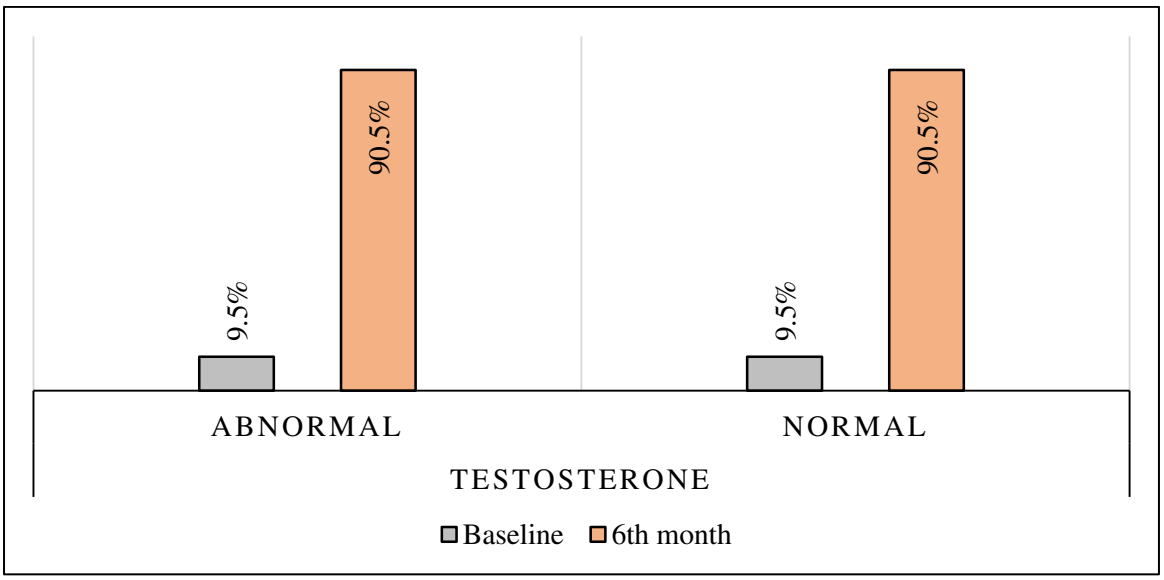
By proportion test, proportion of improved levels at 6th month was significantly more in patients with abnormal level of LH

Figure 62: Distribution of subjects according to FSH over time.



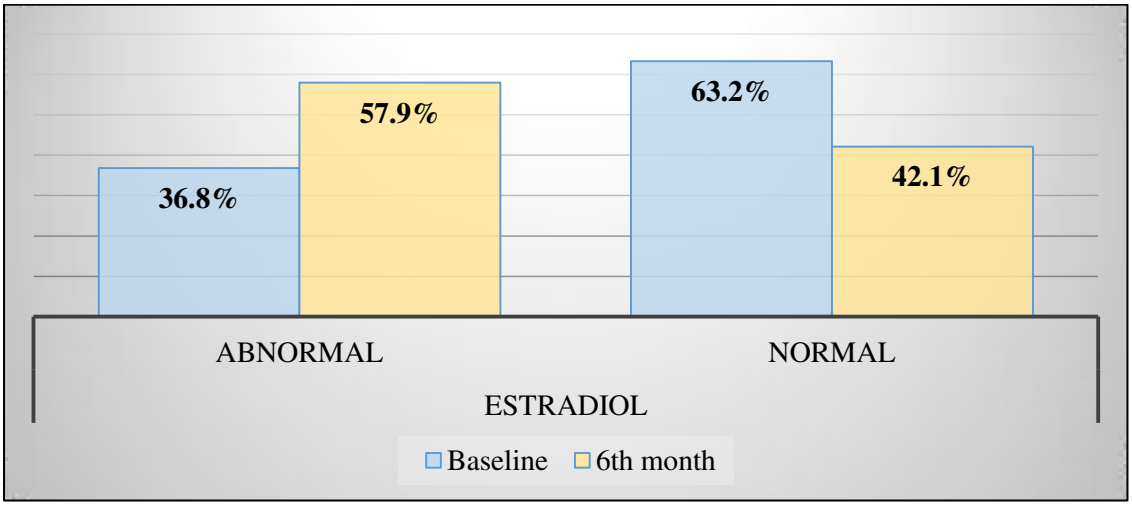
By proportion test, proportion of improved levels at 6th month was significantly more in patients with abnormal level of FSH

Figure 63: Distribution of subjects according to Testosterone over time



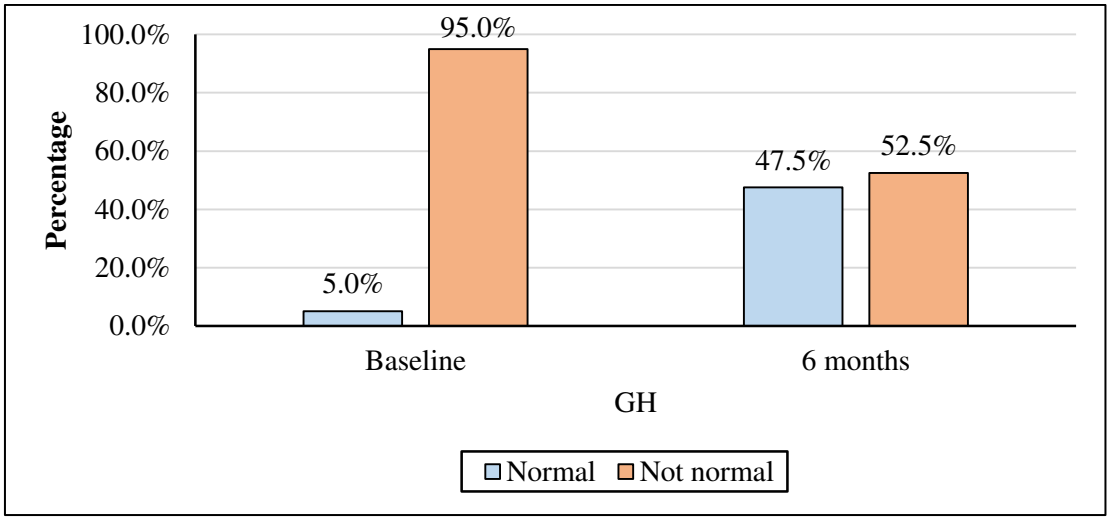
By proportion test, proportion of improved levels at 6th month was significantly more in patients with abnormal level of FSH

Figure 64: Distribution of subjects according to Estradiol over time



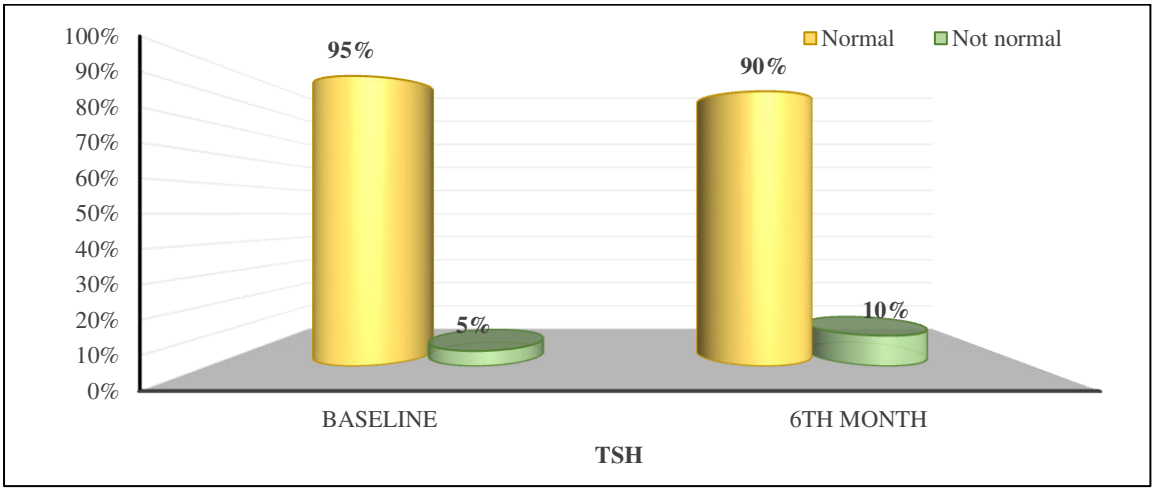
By proportion test, proportion of improved levels at 6th month was significantly more in patients with abnormal level of Estradiol

Figure 65: Distribution of subjects according to GH over time.



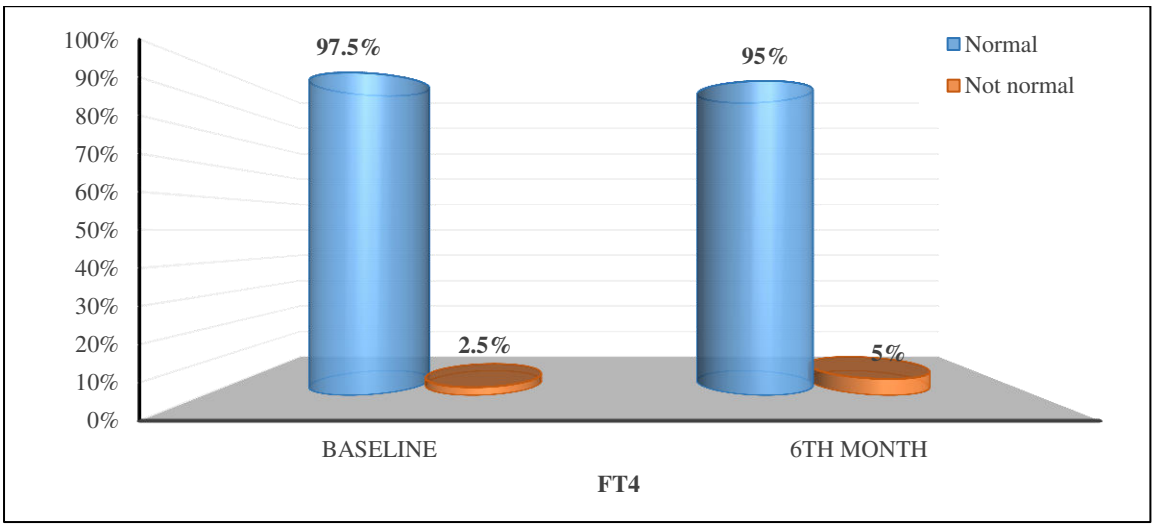
By proportion test, proportion of improved levels at 6th month was significantly more in patients with abnormal level of GH.

Figure 66: Distribution of subjects according to TSH over time.



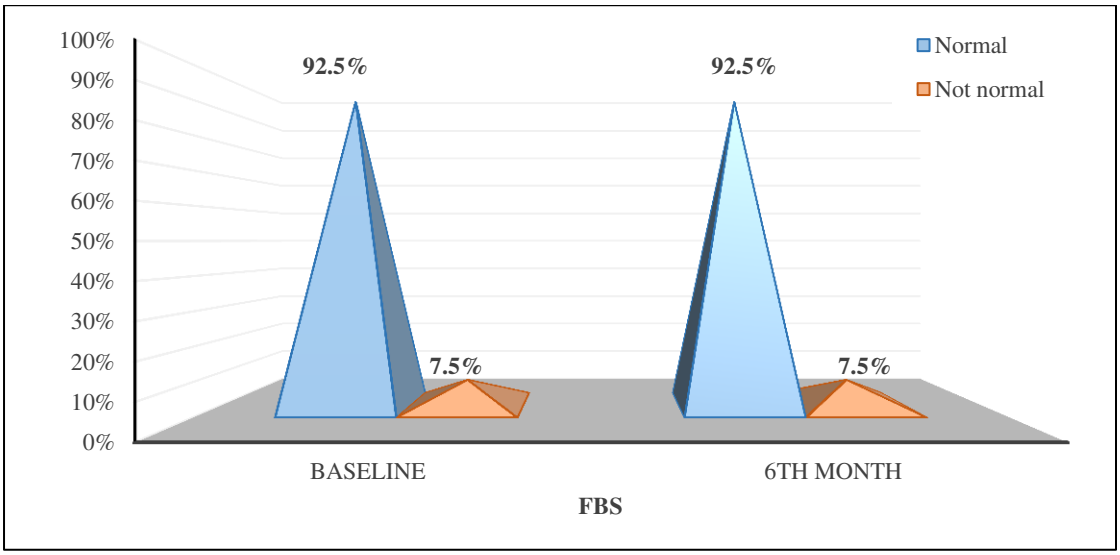
By proportion test, proportion of improved levels at 6th month was not significantly more in patients with abnormal level of TSH.

Figure 67: Distribution of subjects according to FT4 over time



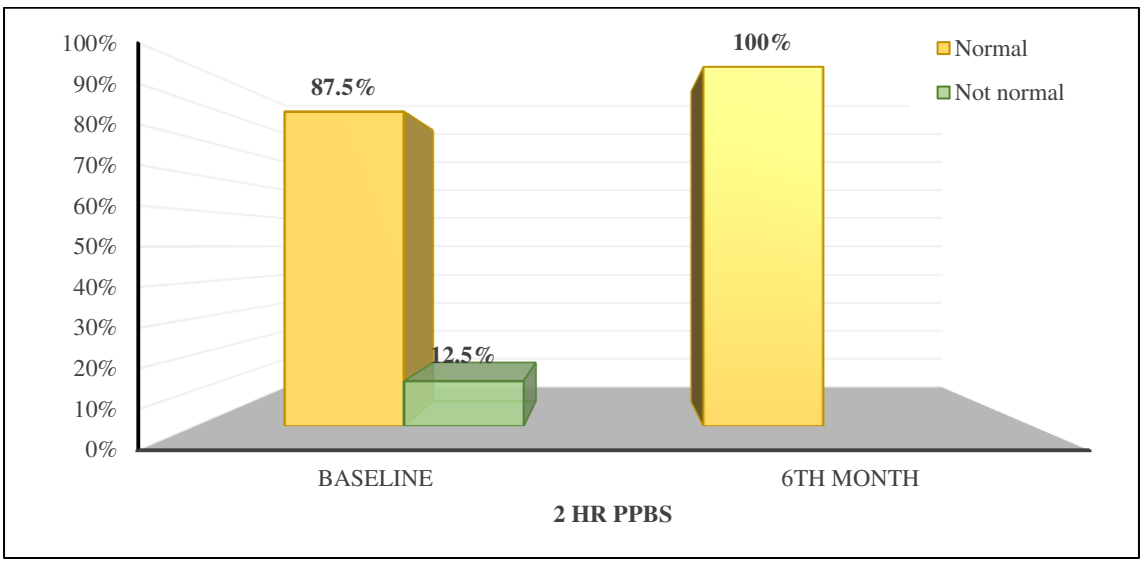
By proportion test, proportion of improved levels at 6th month was not significantly more in patients with abnormal level of FT4.

Figure 68: Distribution of subjects according to FBS over time.



By proportion test, proportion of improved levels at 6th month was not significantly more in patients with abnormal level of FBS.

Figure 69: Distribution of subjects according to 2 HR PPBS over time.



By proportion test, proportion of improved levels at 6^{th} month was not significantly more in patients with abnormal level of 2 HR PPBS.

DISCUSSION

Beta thalassemia is a common genetic disorder and also an important public health problem in many parts of the world. Due to chronic blood transfusions, Beta thalassemia is associated with multi-organ involvement like endocrinological, cardiac, skeletal, hypogonadal problems. Although survival of children with β thalassemia major has improved, endocrine complications continue to compromise growth and have a detrimental effect on the quality of life.

Overload of iron in different endocrine organs due to the multiple blood transfusions received in these children is known to be one of the leading causes of morbidity among children with transfusion-dependent β-thalassemia major. Apart from iron overload other factors which may be held responsible for the endocrine disorders may be chronic hypoxia, viral infections and individual susceptibility. It is very important to identify endocrinopathies, growth retardation and pubertal delay as soon as possible, in order to possibly reverse or retard its progression. With the use of iron chelators it has shown to delay the development of iron induced damage, hence improving the survival of these patients.

Management of different endocrine complications are based on the respective hormonal replacements. Combined oral chelation has showed an improvement in the endocrine profile and also increased compliance among children. DFP is a bi-dentate chelator, together with Deferasirox, it provides long plasma half-life of chelation for LPI binding. Its use has been linked to lower iron-related 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 endocrine function like hypogonadism, hypothyroidism, growth hormone deficiency diabetes mellitus and impaired glucose tolerance in children.

This hospital-based longitudinal study was done from January 2021 to December 2021. Forty children between the ages of 12 and 18 years with transfusion-dependent beta-thalassemia on a single oral iron chelator(Deferasirox) and fulfilling the inclusion criteria 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 .These patients were examined for their pubertal development by Tanner's staging , their weight and height recorded and also underwent laboratory investigations for LH, FSH, GH, TSH , FT4 , FBS and 2hr PPBS .They were also monitored for S. ferritin levels and other blood investigations for the safety of the drug. All patients who were found to have an abnormal endocrine profile were started on Tab. Deferiprone 75mg/kg/day Q8hrly and continued to receive Tab Deferasirox at a dose of 30mg/kg/day Q24hourly for 6 months before being reassessed for the endocrine profile.

In the present study, number of boys were 21 (52.5%) and number of girls were 19 (47.5%) suggesting a male preponderance. The male predominance observed in the present study was in agreement with a similar study done by Flynn et al on hormonal changes in thalassemia major where out of 31 patients studied number of males were 16 and females were 15 [41]. Study done by Padma et.al about demographics of thalassemia in India on 180 children, shown 111 males (61.7%) and 69 females (38.3%) also shown male predominance agreement to our present study [42]. Ideally, β-thalassemia major which is having an autosomal recessive inheritance,

males and females should be in equal proportion but a slightly higher proportion was seen in males in the present study. The 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.

In this study, age ranged from 12 to 18 years. The mean age was 14.10 ± 1.85 years and the median age was 13.5 years. More than half, 27 (68%) were aged between 12 to 14 years, nearly 7 (18%) were between 15 to 16 years and 5 (14%) were between 17 to 18 years. Pubertal development and endocrine abnormalities can be assessed usually after the age of 12 years. The age group is in agreement to a similar study done by Sharma et al. (2014) to determine the growth and endocrine complications in children with TM receiving oral iron chelation with deferiprone with a mean age of 13.6 (2.5) years [6].

Regarding the demographic characteristics, in the present study, the majority of the children 31 (77.5%) belong to the rural area. According to modified Kuppuswamy's scale majority i.e. 27 (67.5%) were in lower middle class, 10 (25%) in the lower class and only 3 (7.5%) in the middle class. Furthermore, the history of consanguineous marriage was reported by 70 % of the children's parents. Thalassemia status of the siblings in the family was positive in 6 children (15%) and the death of the sibling was seen in 2 families (5%). Study done by Padma 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, thalassemia status is seen in 23.3% of the patients, which is in

agreement with our present study [42]. The higher incidence of consanguinity in our present study is probably due to marriage between close relatives in North Karnataka.

Concerning history, in this study Out of 40 subjects, 6 (15%) had no complaints while 11 (27.5%) had generalised weakness, 10 (25%) had Easy fatiguability and 8 (20%) had bony pains. Abdominal pain, fever, heart burn, breathlessness and frequent gastrointestinal upset were reported in in the rest of the 30% patients . None of the patients in this study had developed any reactions to blood transfusion. The frequency of blood transfusion had increased with increasing age in almost all children except one i.e. 97.5%.

On history around only 17 (42.5%) of the parents had noticed growth retardation in their children. Half of the patients (n=20) had given a history of attaining secondary sexual characteristics and majority of these children were above the age of 16 years. Out of the secondary sexual characteristics attained in females, increase in breast size was seen in 8 (40%), Menstrual cycle was attained in 3 (15%). In male children increase in size of penis was seen in 7 (35%) and change in voice was noted in only one (5%) child. Axillary hair growth was seen in 10 and pubic hair growth was seen in 11 of the patients.

In the present study, 8/40 (20%) 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. 12/40 (30%) 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 [42].

ANTHROPOMETRY (WEIGHT, HEIGHT AND BMI) :-

In this study 9 (22.5%) children out of 40 had a percentile weight of < -3SD for their age ,7 (17.5%) children were at -3SD for their age ,11 (27.5%) children were between -2SD and -3SD for their age ,1 (2.5%) child was between median and +1SD of their age and 5 (12.5%) children were between median and -1SD for their age. Percentile height of the children showed that 9 (22.5%) were less than -3SD for their age , 5 (12.5%) children fell at -3SD for their age ,11 (27.5%) children were between -2SD and -3SD for their age , 10 (25%) children feel between -1SD and -2SD for their age , 4 (10%) children were between median and -1SD for their age and 1 (2.5%) child had their height between median and +1SD for their age .A study by Moiz et al on anthropometric measurements in children having transfusion-dependent beta thalassemia , showed that 40% children were underweight 42% of the patients studied were malnourished and 65% were stunted [43] . Badiger and Baruah did a study of growth pattern in regularly transfused thalassaemic children of age group of 2 years to 12 years in Assam, India which included 38 thalassemic children and found that around 34.21% transfusion dependent children had under-nutrition and stunting was seen in 50% children who more irregularly transfused (81.25%) when compared to regularly transfused children which was highly significant ($p<0.001$). Under-nutrition was more among irregularly transfused children when compared to children who were regularly transfused. Also a delay in pubertal spurt was seen in 66% children in the study [44]. A study by Karamifar et al, found that in 43.8% of the patients the weight was below the third percentile for age and 65.7% of patients had short stature (height below the 3rd percentile for age) [45] .

PUBERTAL CHANGES :-

In the present study, on examination of the pubertal development by Tanner's staging 19 (47.5%) children out of the 40 children examined, had a Tanner's stage of 1 , while 10 (25%) children fell into Tanner's stage 2 , 9 (22.5%) children had Tanner's stage of 3 and 2 (5%) of children had Tanner's stage of 4. None of the children in the study had Tanner's stage of 5. There was a significant difference in tanners staging at baseline and 6 months with a p value of < 0.05. This delayed pubertal changes observed in this study was consistent with a study done by Faraj et al. on physical growth patterns in children with transfusion-dependent thalassemia at Wasit city where all the 50 patients included in the study had showed delayed puberty according to the SMR staging [46].

RELATION OF SERUM FERRITIN WITH COMBINATION THERAPY :-

In this study, mean S. Ferritin levels recorded at baseline was 5082.82 ± 4910.40. At 6 months post-intervention, S.Ferritin values showed a statistical significance with mean value of 3230.78 ± 2167.28 and a p -value of < 0.001. 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 showed a decrease 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 a reduction in serum ferritin levels compared to DFP and DFX monotherapy (P=0.035 and 0.040 respectively) [47]. 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) [8]. Farmaki et al., showed that with DFP-DFX combination there was reduction in serum ferritin values [38]. 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 [48]. 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 [49].

COMBINATION THERAPY AND RENAL FUNCTION:-

The mean urea level recorded in our study in the intervention group was 22.52 ± 8.98 and at 6 months was 23.45 ± 6.85. From Friedman's test, it was seen that there was significant difference in the distribution of urea over time.

Mean creatinine level was 1.44 ± 5.93 at the baseline and 1.29 ± 4.98 at 6 months post intervention. This improvement in creatinine was in contrary to a study conducted by MS Elalfy which showed that 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%) [8].

COMBINATION THERAPY AND LIVER FUNCTION:-

In the present study, mean SGOT at the baseline was 46.98 ± 32.36 and at the end of 6 months was 40.30 ± 22.33. Mean SGPT levels at baseline was 51.65 ± 44.53 with a decline of mean SGPT levels by 38.10 ± 26.46 at 6 months was significant (p= 0.0117*). Hence in our study, no rise in S. transaminases was observed, 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 [8].

INCIDENCE OF DIFFERENT ENDOCRINE DISORDERS :-

HYPOGONADISM:-

In the present study, 33 (82.5%) out of the 40 patients had a low LH level for their age . 36 (90%) children had low FSH level, 19 (90.5%) out of 21 boys in this study showed a low S.Testosterone value for their age and 12 (63.2%) out 19 girls had low S.Estradiol level for their age. Hence the incidence of hypogonadism was 63-90 % in our study . Vogiatz et al conducted a study on the differences in the prevalence of growth, endocrine and vitamin D abnormalities among the various children with thalassemia syndromes in North America in 2009, which showed that hypogonadism was the most frequent endocrinopathy and affected both genders and was present in 14.3% of females and 25.5% of males younger than 20 years [50] A study done by Shalitin et al, found out that sixteen (59%) out of 27 patients were found to have hypogonadotrophic hypogonadism in the study [51] . De Sanctis, et al published a review article" Guidelines for management of endocrinopathies in thalassemia" which showed the prevalence of endocrinopathies in iron overloaded β-thalassemia major patients in different parts of the world where hypogonadism was seen is 35.2% in Cyprus ,42% in Greece ,49% in Italy and 50% in Turkey [26].

GROWTH HORMONE DEFICIENCY :-

In our study 38 (95%) children had low levels of GH value for their age. GH deficiency was the most common endocrine abnormality found in this present study. Theodoridis et al conducted a study on growth and management of short stature in

children with thalassaemia major where approximately 20% - 30% of patients have growth hormone (GH) deficiency [52] . GH insufficiency/deficiency was observed in 72% of thalassaemic patients with skeletal dysplasia, while only 41% of patients who did not have skeletal dysplasia when a study on growth hormone secretion and bone histomorphometric study in thalassaemic children with acquired skeletal dysplasia due to desferrioxamine was done by Sanctis et al [53]. Short stature or Growth hormone deficiency was seen in 35% patients in Cyprus, 32% in Greece, 39.3% patients in Iran in another study by De Sanctis [26] .

HYPOTHYROIDISM: -

Around 38 children i.e 95% of the children had normal levels of TSH and 39 (97.5%) children had normal Free T4 levels. Hence the incidence of hypothyroidism was found to be only 1-2 % in our study. Casale et al conducted a study on endocrine function and bone disease during long-term therapy with deferasirox in patients with beta-thalassemia major and found that 19.8% patients had hypothyroidism and there were no incident cases of hypothyroidism in the study [54]. In a study conducted in Iran only 9 (6%) out of 150 children had primary hypothyroidism [55]. The incidence of Primary hypothyroidism is found to be 6.2% in Italy, 7.7% in Iran and around 29% in Turkey according to a study by De Sanctis [26].

IMPAIRED GLUCOSE TOLERANCE AND DIABETES MELLITUS:-

In respect to the blood glucose profile, only 3 (7.5%) children had an abnormal fasting blood sugar and 5 (12.5%) children had an abnormal 2 hr post prandial blood glucose in our study, therefore the incidence of impaired glucose tolerance and diabetes mellitus was about 8-13%. In a study conducted by Mehrvar et al in Iran out

of 437 patients 28 (5.4%) had diabetes [56]. Gulati et al conducted a study on Early Onset of Endocrine Abnormalities in ß-Thalassemia Major in a Developing Country which showed that only three out of 38 patients tested (7.9%) had abnormalities of glucose tolerance which is similar to our study [57]. It was found that eight (11.9%) children had impaired fasting glucose, 7 (10.4%) had impaired glucose tolerance, and 1 (1.4%) subject had diabetes at baseline in a study conducted on glucose homeostasis and effect of iron chelation on β Cell Function in children with β-Thalassemia Major by Gomber et al in 2018 [58].

EFFECT OF COMBINATION THERAPY IN ENDOCRINE PROFILE :-

In our study 38 (95%) children had low levels of GH value for their age at the start of the study . The mean GH value at the baseline was 3.08 ± 3.34 ng/ml and the mean value of GH after 6 months of intervention was found to be 8.87 ± 7 ng/ml. From Wilcoxon test, we observed that, there was a significant statistical difference in the difference in the mean value of GH after 6 months of combined oral chelators, with a p-value of < 0.001. We found out that 35 children out of 38 with an initial low GH had shown an improvement in their GH values with a statistical significance of p-value <0.001.

Regarding the gonadotrophins, the mean value of LH at the start of the study was 2.52 ± 3.35 mIU/ml while the mean value at the end of the study was 3.98 ± 3.34 mIU/ml .Hence there was a significant difference in the mean value post intervention with a p-value of 0.0017. At the baseline, out of 40 children included in the study 33 children had an abnormal LH while only 7 children had normal levels of LH. Out of the abnormal 33 ,25 children had shown an improvement in their LH level hence showing a statistical significance with p-value <0.001. The mean FSH level at the

start of the study was found to be 2.76 ± 1.85 mIU/ml and post intervention for 6 months was 4.19 ± 2.74 mIU/ml, hence showing a significant difference in the improvement of the mean value with a p-value of <0.001. Also 36 children in the study had a low level of FSH for their age in which 31 children showed an improvement in the levels post intervention with combined oral iron chelators with a p-value of <0.001.

In the present study 19 subjects out of the 21 males had a low testosterone for their age , in which 17 of them had shown an improvement in their testosterone value after the study with a p-value of <0.001.The mean testosterone level at the start of the study was 5.53 ± 8.5 ng/dl versus 11.82 ± 12.04 ng/dl at the end of the study . From Wilcoxon test, there was a significant difference in the distribution of testosterone with p-value of 0.0019. Out of the 19 females ,12 females had an abnormal estradiol at the baseline which showed an improvement in 11 females at the end of the study with a statistical significance. The mean estradiol at the start of the study was 34.64 ± 36.29 pg/ml ,while post intervention the mean value showed an improvement to 73.49 ± 84.98 pg/ml which was significant with a p-vale of 0.0141.

In regard to the thyroid profile , the mean value of TSH at the start of the study was 2.35 ± 0.98 µIU/ml and at the end of 6 months post intervention was 2.58 ± 1.37 µIU/ml , which showed no statistical significance with a p-value of 0.3498. Mean value of Free T4 at the baseline was 1.28 ± 0.29 ng/dl while at the end of the study it was 1.33 ± 0.55 ng/dl ,which also did not show any significant difference with p-value of 0.9946. Majority of the children i.e 38 and 39 patients had a normal TSH and Free T4 respectively .None of subjects had any improvement in their thyroid profile .

In the present study with respect to glucose homeostasis , only 3 subjects had an abnormal fasting blood sugar in which 2 subjects had shown an improvement but this was not a significant improvement . At the start of the study only 5 children had an abnormal 2hr post prandial blood sugar and none of them showed any significant improvement at the end of the study . The mean value of FBS at the baseline was 89.95 ± 14.22 mg/dl and at the end of 6 months of intervention the mean FBS value was 97.42 ± 17.26 mg/dl which was statistically significant with a p-value of <0.001 . Mean value of 2hr PPBS at the start of the study was 152.82 ± 36.29 mg/dl while at the end of the study was 157.75 ± 21.54 mg/dl which did not show a statistical significance with p-value of 0.0890 .

These findings are similar to a study conducted on the effect of oral chelators in reversing or preventing the iron overload in transfusion dependent thalassemia major patients by Farmaki et al which showed that after 24 months of treatment with combined oral chelation, mean 2 h glucose in OGTT decreased significantly (111± 24 vs. 150±87 mg/dl, p= 0.007). All females in the study had shown an improvement in their LH and FSH. Among male patients, all improved their LH values to GnRH test. All males increased their mean testosterone level (9.5± 1.8 vs. 7.6± 2.4 ng/ml) . No new cases of hypogonadism were observed and regarding thyroid function one out of the two with subclinical hypothyroidism presented with slight improvement in FT4 levels and reduced his thyroxin dose while no new cases of hypothyroidism were observed [38].

There was also a retrospective cohort study on 165 thalassemic patients by Poggi M et al on the longitudinal changes of endocrine and bone disease in adults with β-thalassemia major receiving different iron chelators over 5 years which showed that

patients on deferasirox had a decrease in the prevalence of hypogonadism (−3.4 %) and diabetes (−1.8 %), while no change was noted for hypothyroidism [39].

Following intensive combined chelation, a study conducted by Farmaki et al on normalisation of total body iron load with chelation with intensive combined therapy which reverses cardiac and endocrine complications of thalassaemia major, observed a statistically significant decrease in post-challenge glycaemia ($P < 0.001$), and a significant increase in insulin secretion ($P < 0.005$) and also 14/18 who had subclinical or compensated hypothyroidism presented a significant increase in mean FT4 ($P < 0.001$) and mean FT3 ($P < 0.001$) and an additional significant decrease in the mean TSH ($P < 0.001$).7 out of 14 hypogonadal males achieved normal mean testosterone levels and normalized their LH–FSH response as shown by their GnRH response. In previously eugonadal females the mean oestradiol level increased significantly and LH and FSH responses to GnRH improved [40].

LIMITATION AND SCOPE OF THE STUDY

The limitations of our study is that it is a single centre study, small sample size and the absence of a control group. So, we recommend to conduct the study including multiple centres, with a larger sample size and along with a control group for comparison.

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 endocrine functions, growth and sexual maturation and significant decrease in ferritin levels in children with transfusion dependent thalassemia major.

Annual laboratory investigations and routine monitoring of growth and sexual maturation will help in early intervention and prevention of endocrine abnormalities. Hence it should be routinely done in all children with transfusion dependent thalassemia major as it is a treatable and even potentially a reversible complication.

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 endocrine dysfunction in India. As the endocrine complications in children with thalassemia is possibly a reversible condition early diagnosis and prompt intervention is of utmost importance 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 endocrine complications.

This hospital-based longitudinal study was done from January 2021 to December 2021. Forty children between the ages of 12 and 18 years with transfusion-dependent beta-thalassemia on a single oral iron chelator(Deferasirox) and fulfilling the inclusion criteria 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 .These patients were examined for their pubertal development by Tanner's staging , their weight and height recorded and also underwent laboratory investigations for LH, FSH, GH, TSH , FT4 , FBS and 2hr PPBS .They were also monitored for S. ferritin levels and other blood investigations for the safety of the drug. All patients who were found to have an abnormal endocrine profile were started on Tab. Deferiprone 75mg/kg/day Q8hrly and continued to receive Tab Deferasirox at a dose of 30mg/kg/day Q24hourly for 6 months before being reassessed for the endocrine profile.

1. In this study, mean age of children were 14.10 ± 1.85 years . 12 patients were 13 years , 8 patients were 12 years and 7 patients were 14 years .
2. Out of 40 subjects, 21 (52.5%) were males and 19 (47.5%) were females and the male to female ratio was 1.1:1 .
3. In the study 31 (77.5%) subjects were residing at rural areas and 9 (22.5%) were residing at urban areas.
4. Majority of the children i.e 27 (67.5%) subjects belonged to lower middle class , 10 (25%) children belong to lower class and only 3 (7.5%) children belonged to middle class.
5. In this study ,majority (67.5%) of the mothers have not attended any form of education ,while 27.5% have attended High school and 2.5% have attended degree and PUC.
6. Majority (47.5%) of the father's have not attended any form of education ,while 32.5% have attended High school and 2.5% have attended degree and 17.5% have attended PUC.
7. History of consanguineous marriage was noted in 70 % of the children's parents.
8. In the present study 26 patients were on Deferasirox for between 6 to 10 years , 10 patients were on treatment for 11 to 15 years and around 4 patients were on Deferasirox for less than 5 years
9. Out of 40 subjects, 6 (15%) had no complaints while 11 (27.5%) had generalised weakness, 10 (25%) had Easy fatiguability and 8 (20%) had bony pains.
10. Out of 40 subjects, 6 (15%) had no present history ,while 12 (30%) had abdominal pain and heart burn , 11 (27.5%) had joint pain and 5 (12.5%) had breathlessness.

11. In the present study ,18 (45%) subjects had onset of blood transfusion after more than 6 months of diagnosis , 13 (32.5%) after 2-6 months of diagnosis and 9 (22.5%) children after 1-2 months of diagnosis .
12. In this study only one child had a decrease in the frequency of blood transfusion, rest of the 39 patients had increased frequency of blood transfusion
13. On history around only 17 (42.5%) of the parents had noticed growth retardation in their children .
14. Out of 40 subjects most of the patients i.e 60% had no other comorbidities, while 15% had acute GE and recurrent LRTIs.
15. In females, increase in breast size was seen in 8 (40%), menstrual cycle was attained in 3 (15%). In male children increase in size of penis was seen in 7 (35%) and change in voice was noted in only one (5%) child. Axillary hair growth was seen in 10 and pubic hair growth was seen in 11 of the patients.
16. 27.5% children had percentile weight between -3SD and -2SD , 23% children had percentile weight <-3SD , 17% had percentile weight at -3SD and between -1SD and -2SD , 12% had percentile weight between median and -1SD and 2% children had percentile weight between median and +1SD.
17. 27.5% had percentile height between -3SD and -2 SD, 25% had height between -2SD and-1SD and 23% had percentile height <-3SD ,13% children had percentile height at -3SD , 10% had percentile height between median and -1SD and 3% had height between median and +1SD .
18. In this study 47.5% of children had percentile BMI between -1SD and -2SD ,30 % children had a percentile BMI between median and +1SD ,8% children had percentile BMI below -3SD and also between median and +1SD , 5% children had a percentile BMI at -3SD and 3% children .

19. In the present study it was observed that 23 patients had depressed nasal bridge, 4 children had thalasemic facies and only one child had normal facial features.
20. In our study 27 children had pallor , 5 children had icterus ,one child had ptosis and 2 children had normal eyes while 14 children out of 40 had dental malalignment ,10 children had protruded teeth and 6 children in the study had maxillary hyperplasia .
21. In the present study 37 children had normal findings in the neck, in 2 children lymph nodes were palpable and 1 child had excoriations in the neck and also in this study only one child had excoriations in chest , all the other 39 children had normal chest findings.
22. In our study 27 patients out of 40 had normal findings in the abdomen ,6 children had a splenectomy scar and 5 children had mild abdominal distention. In this study 22 subjects out of 40 had hyperpigmentation of the skin, 11 subjects had hyperpigmentation and pallor of the skin ,2 subjects had excoriation of the skin and 3 subjects had normal skin.
23. At the start of the study ,19 (47.5%) children out of the 40 children examined, had a Tanner's stage of 1, while 10 (25%) children fell into Tanner's stage 2 , 9 (22.5%) children had Tanner's stage of 3 and 2 (5%) of children had Tanner's stage of 4 .After the study 89% children showed an improvement in their Tanner's stage and this change was statistically significant.
24. The mean hemoglobin at the start of the study was 8.43 ± 1.22 g/dl and at the end of the study was 8.25 ± 1.36. This change in hemoglobin after the study was not statistically significant with p-value of 0.5584.

25. Mean White Blood Count (WBC) at the at first follow up was 8940 ± 5100 while at the end of the study was 8360 ± 3700 and there was no significant difference in the distribution of WBC over time (p-0.08).
26. Mean S. Ferritin levels recorded at the baseline of the study was 5082.82 ± 4910.40 and at the end of post intervention was 3230.78 ± 2167.28. There exists a significant difference in the mean S. Ferritin levels from baseline to 6 months (p-value<0.001).
27. It was observed that, there was significant difference in the distribution of urea over time with mean urea level at the start of the study being 22.52 ± 8.98 while at the end of the study being 23.45 ± 6.85 (p value-0.0049)
28. Mean creatinine value was found to be 1.44 ± 5.93 at the start of the study and no significant change was observed after intervention.
29. Mean SGPT level at the start of the study was 51.65 ± 44.53 while at the end of 6 months post intervention there was a significant reduction to 38.10 ± 26.46 (p-0.011).
30. Out of the 40 children studied 38 children had at least one endocrinopathy.
31. The incidence of hypogonadism was found to be 63-90 %, GH deficiency was 95%, hypothyroidism was 2% while 7.5% children had an abnormal fasting blood sugar and 12.5% children had an abnormal 2 hr post prandial blood glucose.
32. After 6 months of post intervention with combined oral chelation there was significant difference in the mean value of LH (p-value 0.0017), FSH (p-value <0.001), GH (p-value < 0.001), Testosterone (p value 0.0019), Estradiol (p value-0.0141) and FBS (p-value <0.001).

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ANNEXURE I – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

"EFFICACY OF COMBINATION THERAPY WITH ORAL IRON CHELATORS ON ENDOCRINE COMPLICATIONS IN CHILDREN WITH TRANSFUSION DEPENDENT B-THALASSEMIA MAJOR -A ONE YEAR HOSPITAL BASED INTERVENTIONAL STUDY"

Principal Investigator : REG NO. BM120005

Guide: Dr. _____

Name of the participant :

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 2021 to May 2022 by me.

Introduction

Patients with severe thalassemia major suffer endocrine and other abnormalities before their eventual death from iron overload due to repeated blood transfusions. Iron overload is particularly harmful in children, as the accumulation of excess iron in the anterior pituitary gland can interfere with normal endocrine function, leading to growth failure and adverse effects on sexual maturation. Intensive combined chelation normalized patients' iron load and thereby prevented and reversed cardiac and multiple endocrine complications associated with transfusion iron overload. This study is aimed at studying the efficacy of combination therapy with oral iron chelators(deferiprone and deferasirox) on endocrine complications in children with transfusion dependent b-thalassemia major

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 an interventional study with oral combination therapy of deferiprone and deferasirox which appears to have a beneficial effect on the endocrine profile The risks involved are nausea, vomiting, abdominal pain, joint pains, fever etc., for which appropriate medical treatment will be provided has been explained.

Compensation

In the event that you become injured as a result of taking part in this study, treatment will be offered to you or you will be given information about where to receive medical care. But you/your insurance company will be responsible for the costs. However no reimbursements, compensation or free medical care will be given

Privacy and Confidentiality

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

Storage of sample

Samples are immediately being sent for processing to the laboratory

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

REG NO. BM120005

Department of Pediatrics

JNMC, Belagavi-590010

Dr. _____

MBBS, MD (Pediatrics)

Professor, Department of Pediatrics

JNMC, Belagavi-590010

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

Dr. Harsha Hegde

Chairperson , JNMC,IEC

& Scientist D,

ICMR,National Institute of Transfusion Medicine

Belgavi

Phone No.9480422500

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 participant: _____

Date: _____

Name: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator: _____

Date: _____

Name: _____

ANNEXURE II – PROFORMA

"EFFICACY OF COMBINATION THERAPY WITH ORAL IRON CHELATORS ON ENDOCRINE COMPLICATIONS IN CHILDREN WITH TRANSFUSION DEPENDENT B-THALASSEMIA MAJOR - A ONE YEAR HOSPITAL BASED INTERVENTIONAL STUDY."

INFORMATION OF CHILD:

Name/ID No.:

Age

Sex:

Date of birth

Address:

Rural/Urban

Phone no:

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

Std:

DETAILS OF THALASSEMIA HISTORY:

- Age of onset of thalassemia :
- Any present complaints :
- Management history:

(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 (Months/Days)

Quantity of blood transfusion-

No of reactions : 1/2/3/4

Type - fever / chills / others

- H/o infections in the past – HIV/ HBsAg/STD/Malaria
- Others
- **Drugs** : chelating agents :-

Desferoxamine

Dose /Duration :-

Deferiprone-

Dose/Duration :-

Deferasirox-

Dose/Duration:-

Any other : Pantoprazole/ Ca / FA / Zinc / B Complex

Treatment History in past:

IMMUNISATION HISTORY:

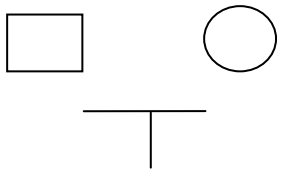
VACCINES	PRIMARY	BOOSTER
BCG		
DPT		
OPV		
HEPATITIS		

MEASLES		
MMR		
TYPHOID		

FAMILY HISTORY:

INFORMATION OF PARENTS-

Consanguinous / non consanguinous:



Siblings of the child & their thalassemia status:

Deaths of the siblings: Yes / No

Any antenatal testing done Yes / No

History of Present Illness:-

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

- Any growth retardation(if yes since when)
- Jaundice
- Menstrual history (in case of girls)
- Breathlessness
- Polyuria /Polydipsia
- H/o secondary sexual characteristics-No/Yes If yes, since when

  Pubic hair/Axillary hair/Increase in size of penis(male)/Change in voice(male)/menstrual cycle(female)/Increase in breast size(female)

EXAMINATION:

General Physical Examination-

Vitals:

HR-

RR-

CFT-

Temperature-

BP-

ANTHROPOMETRY:

	Measured	Expected	Percentile
Weight			
Height			
BMI			

Inference -

Head to Toe-

Face-

Eyes-

Ears-

Oral cavity-

Neck-

Chest-

Abdomen-

Extremities-

Congenital markers-

Skin-

Other Systems:

CVS:

RS:

PA:

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

Other-

CNS:

Tanners staging :

Female

	Pubic hair	Breast
1	Pre adolescent	Pre adolescent
2	Sparse ,lightly pigmented ,straight in medial border of labia	Breast and papilla elevated as small mould ,diameter of areola increases
3	Darker ,beginning to curl , increased amount	Breast and areola increase in size , no contour separation
4	Coarse,abundantly ,curly but less than in adult	Areola and papilla form secondary mould
5	Adult feminine triangular ,spread to medial surface of thigh	Mature , nipple projects ,areola part of general breast contour

Male

	Pubic hair	Penis	Testes
1	None	Preadolescent	Preadolescent
2	Scant, long , slightly pigmented	Minimal change / enlargement	Increased size of scrotum ,pink ,texture altered
3	Darker ,starting to curl , small amount	Lengthens	Larger
4	Resembles adult type but smaller in quantity,curly,coarse	Larger , glans penis and breadth increases in size	Larger , darker scrotum
5	Adult distribution , spread to medial side of thigh	Adult size	Adult size

Investigations:-

	1	2	3	4	5	6
Hb						
PCV						
PLT						
RBC						
WBC						
DLC						
ANC						

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

	Baseline	**At 6 months**
LH		
FSH		
GH		
TSH		
FT4		
TESTOSTERONE(MALES)		
ESTRADIOL(FEMALES)		
OGTT		

SL NO	AGE	SEX	DOB	ADDRESS	RURAL/ URBAN	PH NO	SOCIOECONOMIC STATUS	MOTHER'S EDUCATIONAL STATUS	FATHER'S EDUCATIONAL STATUS	CLASS	AGE OF ONSET	PRESENT COMPLAINTS	ONSET OF BLOOD TRANSFUSION	FREQUENCY OF BT	IF FREQUENCY OF BT	QTY OF BT
1	12	M	06.10.2009	CHIKKODI	1	9108929958	4	2	2	6	11 MONTHS	2,4	2	2	1	10750
2	13	F	22.07.2007	GADAG	1	819766590	4	1	2	8	1 YEAR 6 MONTHS	2,4	3	1	1	21500
3	13	F	09.03.2008	KHANGAV	1	8722748045	4	2	2	6	6 MONTHS	1	2	2	1	10200
4	12	F	14.02.2009	ANGOL	1	8971709717	3	3	4	7	11 MONTHS	5,7	1	2	1	15000
5	16	M	25.01.2005	ATHANI	1	6363230031	4	1	2	9	1 YEAR 4 MONTHS	3,4	2	2	1	18200
6	13	F	01.01.2008	RAIBAG	1	9535371274	5	1	1	7	8 MONTHS	3	1	2	1	19200
7	17	M	27.07.2003	BAGALKOT	2	9449260689	4	1	2	12	1 YEAR	3,4	3	1	1	27500
8	17	M	16.12.2003	BAILHONGAL	1	9964043205	3	2	2	12	6MONTHS	4	1	2	1	45500
9	13	F	15.02.2008	BELAGAVI	1	9844860039	4	1	1	8	6 MONTHS	8	1	2	1	14750
10	16	M	20.03.2005	BAGALKOT	1	8277713899	5	1	1	8	6 MONTHS	3	2	1	1	17760
11	14	F	26.09.2006	BAGALKOT	1	8277713899	5	1	1	7	6 MONTHS	1	2	1	1	13200
12	14	F	20.07.2006	CHIKKODI	1	6366601046	4	1	1	7	1 YEAR	2	2	1	1	19200
13	16	M	24.08.2004	RAMDURG	1	7349627457	4	1	2	10	10 MONTHS	3,4	3	2	2	28800
14	17	M	27.05.2003	RAMDURG	1	9740526941	4	1	1	9	1 YEAR 3 MONTHS	2	3	1	1	500
15	12	F	11.08.2009	ATHANI	1	9686157296	4	2	3	7	6 MONTHS	3	1	2	1	16750
16	14	F	08.08.2007	RAMDURG	1	9648901258	5	1	1	7	11 MONTHS	2	3	1	1	10230
17	12	M	22.02.2009	HUKKERI	2	9972693588	4	1	3	6	2 YEARS	2	2	1	1	10180
18	13	F	30.11.2007	HUKKERI	1	8970705580	4	2	2	7	2 YEARS	7	3	1	1	28750
19	12	M	05.12.2007	YAMAKANMANDI	1	9980013997	4	2	3	7	1 MONTH	3,4	3	1	1	36000
20	14	M	30.06.2006	BAGALKOT	2	9008328629	4	2	3	8	9 MONTHS	1	3	2	1	7500
21	13	F	20.06.2007	GOKAK	2	7829522365	4	1	1	7	10 MONTHS	9	3	1	1	221
22	17	M	22.08.2004	HULLKANI	1	7649356676	5	1	1	10	9 MONTHS	4	3	1	1	8960
23	13	F	01.01.2008	ATHANI	1	9972139997	5	1	1	6	6 MONTHS	3,7	3	1	1	9750
24	16	F	08.03.2005	KADOLLI	1	8050451576	4	1	2	10	1 YEAR 5 MONTHS	2,4	3	1	1	24500
25	15	F	12.07.2005	RAIBAG	1	9945999348	4	2	3	9	9 MONTHS	3	3	1	1	31500
26	13	M	02.06.2008	BAGALKOT	2	7022242959	4	1	2	8	6 MONTHS	6	2	2	1	20250
27	14	M	01.01.2007	BAGALKOT	2	9902749067	4	4	1	8	1 YEAR	7	3	2	1	13500
28	14	M	27.01.2007	RAMDURG	1	9686518695	4	1	1	8	5 MONTHS	2	2	2	1	21200
29	12	M	13.06.2009	MUGALKAD	1	9945999348	4	2	3	7	8 MONTHS	3	3	2	1	7000
30	15	M	27.06.2006	KHANAPUR	2	9770887432	3	2	3	9	7 MONTHS	4	1	2	1	22500
31	13	F	01.01.2009	BAGALKOT	1	8546869471	5	1	1	8	1 YEAR	10	3	2	1	9600
32	18	M	30.09.2003	KITTUR	1	9980706246	4	1	1	12	1 YEAR	7	1	2	1	48000
33	13	M	03.03.2009	RAJBAG	1	9164935427	5	1	1	6	8 MONTH	9	2	2	1	8750
34	12	F	18.03.2010	CHIKKODI	1	9844331515	4	2	2	7	1 YEAR	1	3	1	1	16750
35	13	M	21.10.2009	HUKKERI	1	7795583239	5	1	1	7	2 YEARS	5,10	1	2	1	11750
36	13	M	04.02.2009	HUKKERI	1	9164296437	4	1	1	8	7 MONTHS	9	2	2	1	11750
37	14	F	06.10.2007	BIJAPUR	2	8277890379	4	1	1	7	1 YEAR 4 MONTHS	1	2	2	1	11300
38	18	F	02.01.2003	DHARWAD	2	9620010035	4	1	2	12	2 YEARS	1	2	2	1	16800
39	12	F	14.12.2011	SAUDATTI	1	9591294549	4	1	2	7	1 YEAR 6 MONTHS	5	1	2	1	8250
40	16	M	17.09.2005	HUKKERI	1	9113262983	5	1	1	8	1 YEAR	4	3	2	1	32000

NO: OF REACTIONS	H/O INFECTIONS IN PAST	OTHERS	DESFEROXAMINE	DEFERIPRONE	DEFERASIROX		ANY OTHER	TREATMENT HISTORY IN PAST	INFO OF PARENTS	SIBLING STATUS	DEATH OF SIBLING	ANTENATAL TESTING	ANTENATAL TESTING
	1		DOSE,DURATION	DOSE,DURATION	DOSE	DURATION				NO			
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	1,2,3,4	NIL	1	NO	2	2	2
NIL	1	ACUTE GE	NIL	NIL	1000MG/DAY	11 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	RECURRENT LRTIS	NIL	NIL	1000MG/DAY	10 YEARS	2,3,4,5	TRIVIAL MR ,TR,HYPERSPLENISM	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	1250MG/DAY	10 YEARS	1,2,3,5	NIL	1	NO	1	2	2
NIL	1	ACUTE GE	NIL	NIL	1750MG/DAY	14 YEARS	1,2,3,4,5	SPLENECTOMY DONE	1	NO	2	2	2
NIL	1	RECURRENT LRTIS	NIL	NIL	1000MG/DAY	11 YEARS	1,2,3,5	SPLENECTOMY DONE	1	NO	2	2	2
NIL	1	RECURRENT URTI	NIL	NIL	1000MG/DAY	8 YEARS	1,2,3,5	LVEF 53% ON 2D ECHO	1	NO	2	2	2
NIL	1	ACUTE GE	NIL	NIL	2250MG/DAY	12 YEARS	1,2,3,4,5	SPLENECTOMY DONE	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	1,2,3,5	TRIVIAL TR,SPLENECTOMY DONE	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	7 YEARS	1,2,3,5	SPLENECTOMY DONE	1	YES	2	2	2
NIL	1	RECURRENT URTI	NIL	NIL	1250MG/DAY	9 YEARS	1,2,3,5	TRIVIAL MR,SPLENECTOMY DONE	1	YES	2	2	2
NIL	1	RECURRENT LRTIS	NIL	NIL	1750MG/DAY	9 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	RECURRENT URTI	NIL	NIL	1250MG/DAY	6 YEARS	1,2,3,5	TRIVIAL MR,TR,AR	1	NO	1	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	15 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	750MG/DAY	7 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	750MG/DAY	11 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	RECURRENT LRTIS	NIL	NIL	1500MG/DAY	12 YEARS	2,3,4,5	NIL	1	YES	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	2,3,4	NIL	2	YES	2	2	2
NIL	1	RECURRENT LRTIS	NIL	NIL	1500MG/DAY	12 YEARS	2,3,4,5	NIL	1	YES	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	2,3,4	NIL	2	YES	2	2	2
NIL	1	ACUTE GE	NIL	NIL	1250MG/DAY	8 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	2 YEARS	2,3,5	NIL	2	NO	2	2	2
NIL	1	ACUTE GE	NIL	NIL	500MG/DAY	6 YEARS	2,3,5	NIL	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	1,2,3,5	NIL	1	NO	2	2	2
NIL	1	RECURRENT URTIS	NIL	NIL	1250MG/DAY	3 YEARS	2,3,4,5	NIL	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	1250MG/DAY	6 YEARS	2,3,4,5	SPLENECTOMY DONE	2	YES	2	1	1
NIL	YES,HIV POSITIVE	RECURRENT LRTIS	NIL	NIL	1000MG/DAY	10 YEARS	2,3,5	HIV POSITIVE ON TREATMENT	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	750MG/DAY	10 YEARS	2,3,5	NIL	1	NO	2	2	2
NIL	1	ACUTE GE	NIL	NIL	1250MG/DAY	9 YEARS	1,2,3,5	SPLENECTOMY DONE	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	1750MG/DAY	4 YEARS	1,2,3,5	NIL	2	YES	2	1	1
NIL	1	RECURRENT LRTIS	NIL	NIL	1500MG/DAY	11 YEARS	1,2,3,5	NIL	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	750MG/DAY	9 YEARS	2,3,4	NIL	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	2000MG/DAY	15 YEARS	2,3,4	SPLENECTOMY DONE ,TRIVIAL TR,MR	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	6 YEARS	1,2,3,4,5	TRIVIAL TR ,MR	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	2,3,4	NIL	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	1250MG/DAY	9 YEARS	2,3,5	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	8 YEARS	2,3,4	TRIVIAL TR ,MR	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	5 YEARS	1,2,4,5	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1750MG/DAY	10 YEARS	2,3,4	NIL	1	NO	2	2	2
NIL	1	NIL	NIL	NIL	1000MG/DAY	10 YEARS	1,2,4,5	NIL	2	NO	2	2	2
NIL	1	NIL	NIL	NIL	750MG/DAY	12 YEARS	1,2,5	TRIVIAL MR	1	NO	2	2	2

IMMUNISATION HISTORY	HISTORY OF PRESENT ILLNESS	GROWTH RETARDATION	HISTORY OF SECONDARY SEXUAL CHARACTERISTICS	SECONDARY SEXUAL CHARACTERISTICS	VITALS					WEIGHT			HEIGHT			BMI				
					HR	RR	CFT	TEMP	BP	MEASURED	EXPECTED	PERCENTILE	MEASURED	EXPECTED	PERCENTILE	MEASURED	EXPECTED	PERCENTILE	FACE	EYES
1,3	4	1,6 YEARS	2	6	74/MIN	22/MIN	< 3SEC	98F	98/66MMHG	26KG	39KG	B/W -3SD AND -2SD	134CM	149CM	BTW-2SD &-1SD	15.29	17.5	BTW-1SD AND -2SD	1	1
2,3	1,3	2	1	6	68/MIN	24/MIN	< 3SEC	98F	96/68MMHG	23KG	43KG	<-3SD	130CM	155CM	<-3SD	13.6	18.8	BTW-2SD AND -3SD	1	2
1,3	3	2	2		88/MIN	24/MIN	< 3SEC	99.1F	102/68MMHG	28KG	39KG	AT-3SD	140CM	156.4CM	BTW-2SD&-3SD	14.2	18.8	BTW-2SD AND -3SD	1,2	1
1,3	4,8	2	2		102/MIN	26/MIN	< 3SEC	98F	108/70MMHG	30KG	39.5KG	B/W -3SD AND -2SD	139CM	151.2CM	BTW-1SD&-2SD	15.7	18	BTW-1SD&MED	3	1
1,3	3	1,9 YEARS	1	1,2	68/MIN	24/MIN	< 3SEC	96.8F	120/70MMHG	40KG	58KG	B/W -3SD AND -2SD	150CM	172CM	BTW -2SD AND -3SD	18.2	20.5	BTW-1SD AND -2SD	1	1,3
1,3	4	1,9 YEARS	2		91/MIN	26/MIN	< 3SEC	98F	86/62MMHG	27KG	43KG	B/W -3SD AND -2SD	136CM	155CM	<-3SD	14.6	18.8	BTW-1SD AND -2SD	4	1
1,3	NIL	1,8 YEARS	1	2,3	68/MIN	24/MIN	< 3SEC	96.9F	120/70MMHG	47KG	65KG	<-3SD	147CM	175CM	<-3SD	17.4	21.1	BTW-1SD&-2SD	1	1,3
1,3	7	2	1,16 YEARS	1,2	64/MIN	22/MIN	< 3SEC	97.6F	120/74MMHG	54KG	65KG	BTW -1SD AND -2SD	174CM	175CM	BTW MED AND -1SD	19.2	21.1	BTW MED AND -1SD	1	2
1,3	9,10	2	1	6	108/MIN	23/MIN	< 3SEC	98F	106/68MMHG	25KG	43KG	<-3SD	135CM	155CM	BTW -2SD AND -3SD	13.8	18.8	BTW -2SD AND -3SD	2	1
1,3	NIL	2	1,16 YEARS	3	102/MIN	22/MIN	< 3SEC	98.7F	98/66MMHG	30KG	53.5KG	AT -3SD	138CM	172CM	AT -3SD	15.8	20.5	BTW -2SD AND -3SD	1	4
1,3	NIL	2	1, 14 YEARS	6	86/MIN	21/MIN	< 3SEC	98F	94/70MMHG	29KG	46.5KG	AT -3SD	136CM	159CM	AT -3SD	15.7	19.6	BTW -1SD AND -2SD	1,2	3
1,3	3,4	2	2		88/MIN	24/MIN	< 3SEC	98.6F	94/68MMHG	42KG	46.5KG	BTW MED AND -1SD	142 CM	159CM	BTW -1SD AND -2SD	29	19.6	BTW MED AND +1SD	1	1
2,3	3	1,8 YEARS	1,16 YEARS	1	72/MIN	24/MIN	< 3SEC	98F	96/74MMHG	31.7KG	53.5KG	AT -3SD	143CM	172CM	AT -3SD	15.5	20.5	BTW-2SD AND -3SD	1	1
1,3	3	1,8 YEARS	1,16 YEARS	1,2,3	94/MIN	20/MIN	< 3SEC	98.2F	106/78MMHG	25KG	57KG	AT -3SD	142CM	175.2CM	AT -3SD	12.4	21.2	AT -3SD	1,2	2
1,3	3	2	2		68/MIN	22/MIN	< 3SEC	98F	102/68MMHG	32.7KG	39.5KG	BTW -1SD AND -2SD	139CM	151.2CM	BTW -1SD AND -2SD	17.2	18	BTW MED &-1SD	1,5	5
1,3	3	2	2		88/MIN	17/MIN	< 3SEC	98F	98/64MMHG	22KG	43KG	AT -3SD	132CM	156CM	AT -3SD	12.6	18.8	AT -3SD	1	2
1,3	4	2	2		84/MIN	24/MIN	< 3SEC	98F	88/62MMHG	25KG	39KG	BTW-3SD AND -2SD	129CM	149CM	BTW -1SD AND -2SD	15	17.5	BTW -1SD AND -2SD	1	1
1,3	8	2	1, 13 YEARS	1,6	90/MIN	20/MIN	< 3SEC	99F	96/68MMHG	37KG	43KG	BTW MED AND -1SD	149CM	156CM	BTW MED AND -1SD	16.6	18.8	BTW MED AND -3SD	6	5
1,3	1	1,9 YEARS	2		96/MIN	20/MIN	< 3SEC	98F	100/60MMHG	22KG	39KG	<-3SD	127CM	149CM	<-3SD	13.7	17.5	BTW -2SD AND -3SD	3,7	1
1,3	NIL	1,10 YEARS	1	1,2,3	76/MIN	24/MIN	< 3SEC	98F	94/66MMHG	39KG	46KG	BTW -1SD AND -2SD	157CM	163CM	BTW-1SD&-2SD	14.4	19	BTW-1SD AND -2SD	1	1
1,3	1,3	2	2		76/MIN	22/MIN	< 3SEC	97.6F	98/70MMHG	27KG	39KG	AT -3SD	140CM	156.4CM	BTW -2SD AND -3SD	14.1	18.8	BTW -2SD AND -3SD	2	1
1,3	1	2	1,6 YEARS		68/MIN	24/MIN	< 3SEC	98F	98/64 MMHG	28KG	53.5KG	<-3SD	139CM	173CM	<-3SD	14	20.5	<-3SD	1	2
1,3	1,3,4,8	2	1,13 YEARS	6	82/MIN	20/MIN	< 3SEC	99F	100/70MMHG	34KG	39KG	BTW MED AND -1SD	150CM	156.4CM	BTW MED AND -1SD	15.1	18.8	BTW -1SD AND -2SD	1	1
1,3	4	2	1,15 YEARS	5,6	80/MIN	20/MIN	< 3SEC	98F	100/70MMHG	35KG	50KG	BTW-3SD AND -2SD	144CM	162CM	BTW -2SD AND -3SD	17.8	20.7	BTW -2SD AND -3SD	1	1
1,3	NIL	1,8YEARS	1,15 YEARS	1,5,6	81/MIN	18/MIN	< 3SEC	98F	98/66MMHG	45KG	50KG	BTW -1SD AND -2SD	142CM	161CM	BTW -2SD AND -3SD	22.3	20.2	BTW +1SD AND MED	1	1
1,3	5	2	1,13 YEARS	3	78/MIN	22/MIN	< 3SEC	98F	99/68MMHG	29KG	44KG	BTW -2SD AND -3SD	137CM	156CM	BTW -2SD AND -3SD	15.3	18.9	BTW MED AND -1SD	1	1
2,3	8	2	2	2	64/MIN	20/MIN	< 3SEC	98F	100/70MMHG	22KG	46KG	<-3SD	125CM	163CM	<-3SD	14.6	19	BTW -2SD AND -3SD	2	1,3
1,3	NIL	2	1,13 YEARS	1,2,3	78/MIN	24/MIN	< 3SEC	98F	100/66MMHG	33KG	46KG	BTW MED AND -1SD	143CM	163CM	BTW -1SD AND -2SD	16.3	19	BTW MED &-1SD	1,2	2,3
1,3	1	1,8 YEARS	2		102/MIN	24/MIN	< 3SEC	98F	96/70MMHG	42KG	40KG	BTW MED AND +1SD	150CM	149CM	BTW MED AND +1SD	18.6	17.5	BTW MED AND +1SD	3	1
1,3	1	1,11 YEARS	1,13 YEARS	1,2	82/MIN	22/MIN	< 3SEC	98F	102/76MMHG	40KG	51KG	BTW -2SD AND -3SD	156CM	162CM	BTW -2SD AND -3SD	16.6	20.4	BTW-1SD AND -2SD	1,3	1
1,3	1	1,8 YEARS	2		76/MIN	22/MIN	< 3SEC	98F	94/66MMHG	27KG	39KG	< -3SD	138CM	156.4CM	< -3SD	14.1	18.8	BTW-2SD AND -3SD	2	1
2,3	8	1,12 YEARS	1,15 YEARS	1,2,3	78/MIN	28/MIN	< 3SEC	98.6F	102/80MMHG	48KG	68KG	BTW -2SD AND -3SD	154CM	179CM	BTW -2SD AND -3SD	20.8	22	BTW MED AND -1SD	1,2	1
1,3	1,3	1,8 YEARS	2		78/MIN	22/MIN	< 3SEC	98F	100/66MMHG	28KG	44KG	BTW -2SD AND -3SD	135CM	156CM	BTW -2SD AND -3SD	15.5	18.9	BTW MED AND -1SD	1	1
2,3	4	1,8 YEARS	2		85/MIN	20/MIN	< 3SEC	98F	98/60MMHG	30.7KG	39.5KG	BTW -1SD AND -2SD	136CM	151.2CM	BTW -1SD AND -2SD	17	18	BTW MED AND -1SD	1	1
1,3	1,4	1,10 YEARS	2		86/MIN	23/MIN	< 3SEC	98.6F	86/66MMHG	31KG	39KG	BTW MED AND -1SD	140CM	149CM	BTW MED AND -1SD	15.8	17.5	BTW MED AND -1SD	1	1
1,3	3	2	2		72/MIN	20/MIN	< 3SEC	98.6F	92/60MMHG	28KG	44KG	BTW-2SD AND -3SD	135.5CM	156CM	BTW -2SD AND -3SD	15	18.9	BTW MED AND -1SD	1	1
1,3	1,6	2	2		72/MIN	24/MIN	< 3SEC	98.2F	96/60MMHG	25KG	46.5KG	< -3SD	137CM	159CM	< -3SD	13.8	19.6	<-3SD	1	1
2,3	1	2	1,12 YEARS	1,2,4,5	76/MIN	21/MIN	< 3SEC	98.6F	98/66MMHG	50KG	64KG	BTW -1SD AND -2SD	164CM	175CM	BTW -1SD AND -2SD	18	21.1	BTW MED AND -1SD	1	1
2,3	4	2	2		66/MIN	20/MIN	< 3SEC	98F	86/60MMHG	33KG	39.5KG	BTW -1SD AND -2SD	138CM	151.2CM	BTW-1SD&-2SD	16.8	18	BTW MED AND -1SD	1	1
1,3	4	1,14 YEARS	2		76/MIN	20/MIN	< 3SEC	98.6F	116/68MMHG	28.9KG	56KG	< -3SD	144CM	172CM	< -3SD	14.45	20.5	<-3SD	1,2	1

HEAD TO TOE								CVS	RS	P/A
EARS	ORAL CAVITY	NECK	CHEST	ABDOMEN	EXTREMITIES	CONGENITAL MARKERS	SKIN			
NORMAL	1	1	NORMAL	1	1	NIL	2,4	S1,S2+,SHORT SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,MILD DISTENSION
NORMAL	2,3	1	NORMAL	2	1	NIL	2	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,MILD DISTENSION,TENDERNESS =IN EPIGASTRIC REGION
NORMAL	2	1	NORMAL	2	1	NIL	2	S1,S2+,PANSYSTOLIC MURMUR+	AIR ENTRY B/L+	SOFT ,NON TNEDER
NORMAL	3	1	NORMAL	3	1	NIL	2,4	S1,S2+,SHORT SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	2	1	NORMAL	4	2	NIL	2	S1,S2+,SOFT SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NONTENDER,SPLENECTOMY SCAR
NORMAL	4	1	NORMAL	3	1	NIL	2,4	S1,S2+,SOFT SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NONTENDER
NORMAL	1,4	1	NORMAL	3	2	NIL	2,4	S1,S2+,EJECTION SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	3	1	NORMAL	3	3	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER ,SPELNECTOMY SCAR+
NORMAL	2	1	NORMAL	1	1	NIL	4	S1,S2 +,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	3	1	NORMAL	4	1	NIL	2	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,SPLENECTOMY SCAR+HEPATOMEGALY
NORMAL	3	1	NORMAL	4	1	NIL	2	S1,S1+PANSYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT ,SPLENECTOMY SCAR+,HEPATOMEGALY
NORMAL	5	1	NORMAL	3	1	NIL	1	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NONTENDER
NORMAL	3	1	NORMAL	3	1	NIL	2,4	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	3	1	NORMAL	2	1	NIL	2,3	S1,S2 +,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER,HEPATOSPLENOMEGALY
NORMAL	1	2	NORMAL	3	1	NIL	5	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	2,3	1	NORMAL	3	1	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON THENDER
NORMAL	2	1	NORMAL	3	1	NIL	1	S1,S2+NO MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER ,BS+
NORMAL	3	1	NORMAL	3	1	NIL	1	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TNEDER
NORMAL	5	1	NORMAL	3	1	NIL	2,4	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	3	1	NORMAL	3	1	NIL	1	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TNEDER
NORMAL	5	1	NORMAL	3	1	NIL	2,4	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	3	1	NORMAL	3	1	NIL	2	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NONTENDER
NORMAL	2	1	NORMAL	3	1	NIL	2	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,MNOSOFT,NON TENDER,BS
NORMAL	3	1	NORMAL	3	1	NIL	2	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	2	1	NORMAL	3	1	NIL	2,4	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	3	1	NORMAL	3	1	NIL	2,4	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	5	1	NORMAL	4	1	NIL	2,4	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,SPLENECTOMY SCAR+HEPATOMEGALY
NORMAL	3	3	EXCORIATIONS PRESENT	3	1	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER
NORMAL	5	1	NORMAL	3	1	NIL	2,4	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER ,BS+
NORMAL	1	1	NORMAL	4	1	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER ,SPELNECTOMY SCAR+
NORMAL	4	1	NORMAL	3	1	NIL	2,4	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT , NON TENDER
NORMAL	1	1	NORMAL	3	5	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,NON TENDER ,BS+
NORMAL	2	1	NORMAL	3	1	NIL	5	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	2	3	NORMAL	4	1	NIL	2	S1,S2+, SYSTOLIC MURMUR +	AIR ENTRY B/L +,OCCASIONAL CREPS	SOFT, NON TENDER ,SCAR +
NORMAL	3	1	NORMAL	2	1	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT,EPIGASTRIC TENDERNESS +
NORMAL	1	1	NORMAL	3	5	NIL	2	S1,S2+, NO MURMUR	AIR ENTRY B/L+	SOFT,HEPATOSPLENOMEGALY +
NORMAL	1	1	NORMAL	3	1	NIL	2	S1,S2+, NO MURMUR	AIR ENTRY B/L+	SOFT , EPIGASTRIC TENDERNESS+
NORMAL	2	1	NORMAL	3	1	NIL	2	S1,S2+, NO MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	2,4	1	NORMAL	3	1	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	2	1	NORMAL	3	3	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	3	1	NORMAL	2	1	NIL	2	S1,S2+,SYSTOLIC MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER
NORMAL	3	1	NORMAL	3	1	NIL	2	S1,S2+,NO MURMUR	AIR ENTRY B/L+	SOFT ,NON TENDER

LIVER SPAN			SPLEEN SPAN			CNS	TANNERS STAGING		HEMOGLOBIN(g/dL)						PCV(%)						PLATELET(10^4 /µl)						RBC (10^(		
1ST	2ND	3RD	1ST	2ND	3RD		BASELINE	AT 6MONTHS	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3
13CM	13CM	13CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	1	2	9.1	8.7	9.2	8.4	8	9.8	26.9	27.9	26.8	24	24.5	27.8	3.89	3.7	3.99	3.82	3.67	4.06	3.35	3.22	3.31
12CM	12CM	12CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	2	2	8.9	8	8.9	8.9	8.9	8.9	25.4	25	26.3	25.9	28.1	26.8	3.6	5.34	4.26	5.4	4.33	3.81	2.81	2.93	2.82
12CM	12CM	12CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	2	8.6	7.4	5.9	6.3	5.3	4	26.9	20	15.9	17.2	14.3	14.4	1.49	1.5	1.64	1.01	1.04	0.93	2.93	2.23	1.76
10CM	10CM	10CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	1	2	7.9	10.5	9.4	9.4	9.2	9.4	24.6	29.9	29.2	27.5	27.9	28	3.99	4.19	3.69	3.73	3.15	3.66	3.1	3.45	3.77
12CM	12CM	12CM	NIL	NIL	NIL	NAD	3	3	8.3	8.02	6.88	7.8	8.3	8.4	25.8	24	20.2	22.8	23.8	25.9	9.2	7.02	5.4	5.48	4.88	4.42	2.94	2.72	2.02
11CM	11CM	11CM	NIL	NIL	NIL	NAD	1	2	6.6	10.1	8.9	8.2	8.8	10.3	21.2	31.3	27.5	26.2	25.4	29.3	6.2	7.1	8.16	6.69	6.52	7.56	2.5	3.71	3.24
12 CM	12 CM	12 CM	1CM BELOWLCM	1CM BELOWLCM	1CM BELOWLCM	NAD	3	3	9	10	10.1	9.1	7.7	6.9	26.1	28.3	30.6	27.1	23.9	21.6	4.24	3.62	4.14	3.36	3.4	4.67	3.03	3.32	3.74
14CM	14CM	14CM	NIL	NIL	NIL	NAD	4	4	9	9.1	8.7	8.9	9.9	9.3	26.4	25.5	26.9	27.4	28.2	28.9	6.82	6.5	5.93	6.12	4.93	5.28	2.82	2.8	3.16
13CM	13CM	13CM	NIL	NIL	NIL	NAD	2	3	4.6	8.8	8.6	8.3	7.5	6.9	11.8	23.7	26.9	26	25.8	19.4	5.33	3.75	3.23	4.36	4.07	2.51	1.38	2.46	2.69
12CM	12CM	12CM	NIL	NIL	NIL	NAD	2	3	8.8	8.2	8.5	8.4	8.6	9.8	27	25.6	26.3	23.5	26.3	30.2	6.78	6.54	2.82	6.65	5.59	6.07	3.13	2.9	2.93
11CM	11CM	11CM	NIL	NIL	NIL	NAD	2	2	8.3	8.7	8.5	10	10.6	8.2	25	25.9	24.4	28.8	31.6	24.6	3.88	5.52	5.56	4.13	3.24	4.44	3.2	3.07	2.74
12 CM	12 CM	12 CM	1CM BELOWLCM	1CM BELOWLCM	1CM BELOWLCM	NAD	1	2	9	9	8.4	7.9	8.4	10	26.1	26.8	24.6	22	24.9	28.4	2.51	2.45	1.96	2.03	2.63	2.5	2.99	3.2	3.12
10CM	10CM	10CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	2	3	7	8.7	7	7.2	8.1	6.3	20.9	24.5	21.3	21.7	24.7	19.4	2.91	2.86	2.3	2.5	2.41	3.51	2.67	3.06	2.72
13CM	13CM	13CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	3	3	8.1	3.8	9.4	7.9	6.8	7.8	22.9	10.5	27.3	23.4	19.8	24.7	1.5	1.75	2.85	2.83	1.81	2.75	2.7	1.51	3.24
12CM	12CM	12CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	2	9.7	9.7	10.6	8.7	8.4	9.3	29.3	33.3	31.6	26.4	25.5	28.6	5.6	3.63	5.04	4.5	5.07	5.35	3.55	5.34	4.03
11CM	11CM	11CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	2	2	8.1	8.6	10.1	10	5.9	9.4	23.3	25.7	30.6	28.5	17.8	27.5	2.27	3.1	2.84	2.25	1.87	3.36	2.27	3.1	2.84
12CM	12CM	12CM	1CM BELOWLCM	1CM BELOWLCM	1CM BELOWLCM	NAD	1	2	7.8	7	7.3	10.4	8.6	8.7	22.3	20	21	30.5	24.7	24.3	1.9	1.71	1.78	2.95	2.63	1.93	2.93	2.43	2.35
13CM	13CM	13CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	3	3	10.5	9	10.6	10	9	9.1	30.7	26.8	31.6	29.1	27.3	28.1	5.53	4.93	4.39	3.91	4.87	5.18	3.79	3.27	3.74
14CM	14CM	14CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	2	9.1	9.5	8.2	8.7	6.9	8	26.4	27.5	24.1	24.5	20.5	23.5	2.59	3.12	2.23	2.6	2.41	2.76	3.04	3.11	2.74
13CM	13CM	13CM	3CM BELOW LCM	3CM BELOW LCM	3CM BELOW LCM	NAD	3	4	8.4	8.9	8.4	10.2	9.2	8.5	25	25.2	25	29.3	27.6	24.2	5.7	6.02	6.19	5.9	4.63	4.19	3.2	2.95	2.95
10CM	10CM	10CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	1	2	9.1	8.2	5.6	7.7	6.3	7.3	26.2	23.6	17.3	23.5	19.1	20.5	2.95	1.99	1.83	3.43	3.1	3.5	3.32	3.16	2.46
13CM	13CM	13CM	1CM BELOWLCM	1CM BELOWLCM	1CM BELOWLCM	NAD	3	3	8.6	7.1	9.1	7.6	7.2	6.6	26.1	21.8	27.5	28.6	21.9	19	2.26	1.91	2.35	1.64	1.34	1.36	3.51	3.09	3.99
12 CM	12 CM	12 CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	2	3	7.8	7.3	8.6	8.4	9.4	9.3	21.7	25.9	25.2	27.7	28.3	25.3	2.64	3.25	3.27	3.16	3.34	3.38	3.4	2.33	3.22
14CM	14CM	14CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	3	3	9.5	8.4	8.8	7.6	7.5	8.3	27.4	25.1	27.6	23.5	24.4	25.8	2.13	2.29	1.91	1.88	1.73	1.74	3.5	3.17	3.72
11CM	11CM	11CM	NIL	NIL	NIL	NAD	3	3	12.1	8.5	8.5	8.9	8.5	7.5	36.4	24.8	24.2	27.5	26.5	22.6	5.24	4.04	1.55	2.84	3.59	2.93	4.12	2.74	2.71
13 CM	13 CM	13 CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	2	2	8.2	8.8	9	10.6	8	7.6	24.3	24.2	25.2	26.2	23.8	22.1	2.9	1.88	2.66	2.34	2.36	1.94	2.7	3	3.8
12CM	12CM	12CM	1CM BELOW LCM	1CM BELOW LCM	1CM BELOW LCM	NAD	2	2	7.7	8.4	8.5	8.8	7.6	7.2	22.8	24.6	25.5	26.8	21.9	21.7	1.96	2.26	2.43	1.81	4.84	1.91	2.8	2.91	3.18
14CM	14CM	14CM	NIL	NIL	NIL	NAD	2	3	9.3	9.7	10.1	9.6	6.6	8.6	27.4	26.7	19.7	28	22.3	24.3	6.48	6.03	5.6	6.11	6.05	5.08	3.15	2.9	3.16
10CM	10CM	10CM	NOT PALPABLE	NOT PALPABLE	NOT PALPABLE	NAD	1	2	8	7.1	7.3	7.7	7.8	8.6	22.5	19.9	22.2	23.7	23.7	26.5	2.23	1.72	1.86	1.24	1.13	3.12	2.74	2.29	2.6
12CM	12CM	12CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	3	3	8.5	8.4	8.8	8.3	8.9	8	25.1	24.9	24.7	24.1	26.8	24.2	3.17	4.33	3.3	3.72	3.89	3.73	3.16	3.22	2.93
13CCM	13CM	13CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	2	6.1	9.4	8.7	8.9	8.5	7.6	18.7	27.9	25.9	24.1	25.5	21.8	2.46	3.7	4.12	3.61	3.33	2.24	2.57	3.47	3.26
14CM	14CM	14CM	NIL	NIL	NIL	NAD	4	4	8.2	7.1	8.9	8.8	10.1	10.5	25	21.6	27.8	27.6	32.1	30.2	8.34	6.19	8.95	6.95	6.51	4.81	2.73	2.31	2.99
12CM	12CM	12CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	2	8.7	8.7	8.2	7.5	9.6	8.4	25.7	24.2	23.7	22.7	27	24.6	2.79	2.5	2.33	2.7	3.11	3.12	3.18	3	3.08
12CM	12CM	12CM	1CM BELOW LCM	1CM BELOW LCM	1CM BELOW LCM	NAD	1	2	8.3	8.3	10.2	10.1	8.9	9	23.8	23.4	29.7	28.9	28.4	25.8	3.44	3.36	3.71	3.18	3.34	2.24	2.97	2.96	3.81
11CM	11CM	11CM	JUST PALPABLE	JUST PALPABLE	JUST PALPABLE	NAD	1	1	7.3	6.5	7.3	8.5	6.3	6.4	21	19.7	22.2	25.7	16.9	19	3.72	3.84	2.89	3.38	3.76	2.75	2.56	2.37	2.65
12CM	12CM	12CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	1	8.3	7.8	7.6	8.2	8.8	7.9	24.9	24	21.2	24.6	25.7	25	9.97	8.95	9.72	8.97	6.88	7.94	3.09	2.87	2.31
13CM	13CM	13CM	1CM BELOW LCM	1CM BELOW LCM	1CM BELOW LCM	NAD	1	2	9.4	7.8	9.4	9.32	8.2	5.2	28.2	23.3	27.6	23.8	15.4	22.1	1.71	2.22	2.02	3.02	2.28	1.25	3.18	2.85	3.24
14CM	14CM	14CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	2	7	8.5	8.9	9	8.8	9.2	22.4	26.9	28	27	28.3	28	0.68	0.93	1.05	0.8	1.29	3.08	3	3.53	3.83
12CM	12CM	12CM	1CM BELOW LCM	1CM BELOW LCM	1CM BELOW LCM	NAD	1	2	9.9	10.8	9.6	9	8.44	9.2	29.2	32.3	27.3	27.4	27.1	26.4	3.44	3.48	2.72	3.3	3.45	3.1	2.77	3.38	3.63
14CM	14CM	14CM	2CM BELOW LCM	2CM BELOW LCM	2CM BELOW LCM	NAD	1	1	8.4	8.4	6.7	7.1	8.2	8.5	24.5	24.4	19.4	21.5	24.4	24.9	2.12	1.94	1.77	1.72	2.07	1.92	3.24	3.01	2.45

5 /µl)			WBC (10^3/µL)						DLC-NEUTROPHIL (%)						ANC (10^3/µl)						FERRITIN (µg/L)				UREA (mg/dL)				CREATININE (mg/dL)				SGOT (U/L)				SGPT (U/L)				LH
4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	BASELINE	1ST	2ND	3RD	BASELINE	1ST	2ND	3RD	BASELINE	1ST	2ND	3RD	BASELINE	1ST	2ND	3RD	BASELINE	1ST	2ND	3RD	BASELINE
3.06	2.94	3.31	6.55	6.17	5.47	5.33	6.33	9.24	47	49	52	56	60	60	3.55	3.16	2.88	3.96	3.75	5.41	2667	2366	1910	1777	22	16	25	28	0.4	0.7	0.5	0.6	28	19	21	31	13	11	12	57	0.51
2.85	3.36	3.04	16.81	20.11	16.1	12.29	11.06	13.08	54	55	26	57	65	55	8.5	10.27	5.5	6.33	6.97	7.2	2099	1808	1372	1351	13	14	22	15	0.5	0.3	0.4	0.4	68	44	47	31	72	45	42	17	0.28
1.93	1.57	1.22	3.4	2.79	3.24	2.07	4.02	5.02	52	54	59	50	55	55	1.8	1.4	2	1.02	2.1	2.52	3056	2461	1470	3241	18	17	15	20	0.8	0.6	0.5	0.7	91	41	16	33	88	43	31	33	0.87
3.29	3.4	3.11	10.44	11.64	14.18	9.3	11.5	12.93	44	60	57	51	57	55	4.66	5.38	8.31	4.88	5.4	6.11	3851	3390	2930	4549	20	13	21	27	0.7	0.7	0.5	0.4	32	31	16	18	23	26	22	21	4.05
2.4	2.9	3.1	23.56	11.28	11.2	10.33	6.8	16.2	47	50	50	62	54	44	11.2	6.2	6.1	6.2	3.8	8.4	3682	3024	1889	1740	30	18	32	30	0.6	0.4	0.4	0.4	33	32	30	30	34	18	35	32	1.44
3.1	2.87	3.21	19	16.4	16.65	31.85	10.6	15.66	58	40	78	49	56	55	10.1	5.33	8.41	14.31	5.83	7.8	3888	2627	4676	4485	16	15	12	16	1.02	0.4	0.4	0.5	23	50	53	49	40	36	71	31	1.55
3.36	3.06	2.73	9.69	10.55	10.32	8.36	9.78	7.63	50	65	47	46	47	60	4.8	6.52	5.02	4.77	4.62	4.32	1491	1136	1126	1114	33	27	22	22	0.5	0.4	0.6	0.5	22	24	29	15	12	12	13	6	5.21
3.12	3	3.23	11.4	18.4	12.95	11.71	8.92	13.14	56	70	40	40	60	47	5.92	13.26	5.87	4.88	5.46	6.11	5603	4653	5025	4010	19	20	13	22	0.4	0.5	0.3	0.5	43	45	39	46	34	38	48	58	5.02
2.48	2.27	1.67	11.31	10.22	9.48	10.48	11.33	10.77	51	72	56	60	50	60	5.33	7.24	4.67	6.77	6.42	6.18	4000	3800	3388	2360	31	12	12	29	0.3	0.2	0.5	0.4	60	35	80	55	45	41	46	59	0.36
2.63	2.81	3.59	19.59	19.29	11	15.4	20.32	14.93	59	82	70	79	83	68	11.06	13.32	9.33	11.06	17.36	8.43	3732	5122	2986	2967	20	18	16	18	0.7	0.8	0.6	0.5	31	32	36	34	19	18	19	17	1.72
3.05	3.3	2.94	10.7	10.2	14.72	9.45	13.03	13.74	42	57	39	60	38	58	4.81	5.81	4.12	5.11	3.48	7.88	6548	4377	7535	6050	14	10	10	19	0.4	0.5	0.4	0.4	44	49	41	64	34	40	41	51	1.74
2.58	3.01	3.25	7.79	8.59	6.69	5.5	4.98	6.98	50	57	58	60	54	59	3.82	4.76	4.08	2.92	3.06	4.2	10050	10173	9216	10295	15	20	19	39	0.4	0.7	0.5	0.4	109	58	140	84	145	80	143	101	10.31
2.88	3.21	2.6	5.53	6.42	6.71	6.13	5.83	5.34	52	47	50	47	44	54	2.56	3.12	3.2	3.24	2.53	2.46	8297	8290	2300	4758	19	18	13	18	0.3	0.3	0.5	0.6	32	26	49	25	38	17	32	12	2.51
2.44	2.34	3.03	4.9	4.88	10.3	7.45	4.46	7.38	44	60	62	55	60	63	2.01	2.91	6.72	3.85	2.94	4.13	3597	3933	2633	4110	27	15	18	18	0.5	0.4	0.8	0.5	89	34	73	20	69	38	93	16	1.09
3.24	3.12	3.44	10.58	5.84	9.7	8.83	10.18	9.36	64	54	42	50	52	59	7.22	3.46	4.12	4.03	6.11	5	1615	2268	1769	1836	26	14	25	19	0.3	0.3	0.7	0.4	28	18	24	23	14	11	14	14	0.55
3.44	3.22	2.19	7.2	8.6	8.39	7.47	8.35	9.35	59	56	60	54	63	65	3.97	5.32	5.64	3.62	5.28	5.03	6266	4369	3906	2316	18	38	21	32	0.5	0.8	0.3	0.4	71	36	32	60	101	46	32	140	1.61
3.61	3.14	2.83	3.3	4.14	3.62	3.22	4.45	2.62	50	56	60	51	60	53	1.58	2.8	2.08	1.5	2.92	1.26	3963	4226	4003	3755	23	58	19	16	0.5	0.3	0.3	0,4	66	45	43	53	50	16	32	40	0.21
3.83	3.49	3.77	10.45	9.15	8.34	8.96	8.92	11.05	55	61	55	58	55	65	5.86	5.88	4.61	5.02	4.81	6.22	3644	3268	1710	1050	42	32	16	29	0.4	0.7	0.4	1	19	20	22	15	17	11	13	11	2
2.79	2.56	2.89	6.75	7.49	5.93	6.65	3.06	5.71	46	50	54	60	52	65	3.3	3.54	2.88	3.27	3.03	2.88	5794	2871	4200	2922	23	22	18	25	0.4	0.4	0.4	0.6	25	21	27	22	14	8	14	15	0.43
3.3	3.03	2.64	10.69	14.48	11.9	10.65	22.43	9.69	43	45	57	54	80	56	1.2	8.6	6.3	5.62	17.2	4.6	31088	2122	2188	1376	18	21	24	36	0.9	0.3	0.8	0.6	31	22	26	20	210	17	18	20	1.48
3.3	2.8	4.2	9.32	6.18	10.84	10.89	6.82	7.94	52	53	40	60	50	50	4.52	3.2	4.8	6.21	3.4	5.1	3266	2849	2984	2202	18	19	21	20	38	52	49	32	39	61	37	30	44	26	30	22	0.85
3.32	3.01	2.5	5.19	4.33	4.3	3.83	2.58	2.75	55	53	60	52	44	45	2.58	2.4	2.94	1.6	1.36	1.3	6185	4645	4260	3557	20	16	14	14	0.4	0.4	0.4	0.6	194	143	59	132	189	161	84	86	3.11
2.6	2.66	3.2	5.2	7.45	7.22	5.53	6.59	4.68	55	39	50	50	60	55	2.52	2.72	3.52	2.58	3.94	2.74	3072	1854	2803	1224	9	18	18	23	0.3	0.5	0.4	0.5	32	30	35	20	24	15	16	18	6.66
3.36	3.33	3.57	6.2	7.7	6.73	5.87	6.35	6.23	46	62	57	39	60	63	3.66	5.12	6.11	3.14	3.91	4.18	4360	4895	4522	4992	16	21	24	25	0.6	0.4	0.3	0.4	43	58	47	43	50	60	58	46	5.94
3.14	2.87	2.44	9.6	29.1	12.93	14.25	16	8.05	71	16	52	30	42	60	6.81	3.18	6.96	5.14	7.26	4.96	1898	1220	937.1	635	46	26	15	22	0.6	0.4	0.6	0.4	46	39	10	21	101	50	26	34	17.94
4.2	2.6	2.4	2.3	3.2	3.8	2.4	4.7	3.3	16	45	42	50	60	53	0.4	1.92	2.02	1.3	3	2.01	12499	10221	9882	9801	16	17	14	17	0.8	0.6	0.6	0.4	13	14	22	32	31	36	37	34	0.29
3.41	2.75	2.64	4.77	5.07	6.22	5.44	1.81	4.84	53	60	66	60	56	50	2.83	2.63	4.31	2.91	0.89	2.62	2624	2284	3902	3201	19	33	24	34	0.4	0.5	0.4	0.6	35	12	29	42	35	22	27	30	0.46
2.47	2.02	2.05	13.87	11.47	10.36	12.6	10.55	12.7	49	60	50	62	55	55	6.77	6.89	5.67	7.88	5.66	6.72	6127	5588	5062	4052	54	12	26	21	0.5	0.2	0.5	0.6	54	68	87	55	33	45	61	53	1.61
2.58	2.5	2.92	14.7	11.43	10.48	8.32	8.15	11.9	40	24	30	58	60	55	6.8	5.21	4.88	4.92	5.38	4.2	2823	2488	1930	1794	30	23	15	18	0.6	0.4	0.6	0.5	42	47	26	50	64	57	46	50	4.74
2.9	3.35	3.35	3.09	7.2	13.34	6.97	6.58	5.11	8.07	56	81	50	56	44	54	4.13	10.11	3.88	3.44	2.58	4065	3299	3006	3861	26	10	14	18	0.5	0.5	0.4	0.6	27	24	24	37	19	24	33	26	3.38
2.76	3.14	2.68	8.65	9.11	12.64	12.33	8.56	6.85	56	50	63	64	85	52	3.6	4.36	6.2	6.1	6.8	3.46	2348	1889	1660	1642	17	22	16	21	0.5	0.4	0.2	0.2	31	21	31	37	17	16	16	16	0.72
3	3.46	3.34	17.34	20.52	25.48	19.3	26.6	9.8	50	49	41	40	52	56	2.3	4.2	3.8	2.8	13.12	4.52	6818	3074	3712	1610	18	15	31	20	0.5	0.4	0.9	0.5	52	56	69	35	35	40	61	35	5.33
2.88	3.3	3.13	5.4	6.62	7.14	2.94	6.91	7.06	55	50	63	42	57	50	2.92	3.26	3.94	2.1	3.53	3.5	1394	1951	2123	1870	24	21	28	24	0.2	0.4	0.6	0.4	31	31	42	31	35	24	19	30	0.7
3.55	3.28	2.95	6.02	7	6.53	8.97	8.13	8.32	47	52	60	53	55	52	4.1	4.9	3.66	4.88	4.5	4.38	1585	1601	1313	1585	18	20	30	34	0.2	0.5	0.5	0.4	19	32	23	25	13	37	16	18	0.42
3.15	1.89	2.28	6.6	7.72	6.58	6.16	5.83	6.27	50	56	50	65	61	55	4.6	3.8	4.8	5	3.06	3.58	4542	3532	4094	3233	24	21	22	34	0.5	0.3	0.5	0.6	67	97	40	45	49	40	49	43	0.64
2.88	2.89	2.78	12.46	20.3	20.6	19.4	9.47	9.22	48	45	62	81	65	55	5.6	8.8	11.2	12.33	5.3	5.68	5204	2796	4167	3566	23	27	35	18	0.5	0.3	0.6	0.4	35	61	86	76	44	41	61	57	0.34
3.16	2.66	1.79	6.58	5.81	6.03	6.12	6.81	2.92	57	65	60	50	60	45	3.12	3.01	4.06	3.32	4.13	1.41	9532	8451	8450	6917	18	13	18	20	0.3	0.4	0.4	0.7	78	75	78	65	75	69	75	63	2.5
3.75	4.04	3	3.25	3.87	5.06	5.63	6.39	5.96	40	58	55	60	56	54	2.8	4	2.4	3.06	3.92	2.88	2646	2345	2455	1894	14	14	15	15	0.8	0.8	0.4	0.9	26	30	23	27	36	26	18	25	0.4
3.08	3.45	2.8	3.42	8.58	10.72	9.63	9.82	6.72	50	53	72	60	69	50	4.88	4.66	8.1	5.26	5.03	3.38	6328	5500	4882	4613	27	21	22	24	0.4	0.4	0.5	0.4	43	61	32	33	62	74	64	36	0.48
2.74	2.89	2.81	4.05	4.74	2.13	4.18	4.13	3.78	60	60	39	50	39	39	2.7	2.12	2.31	1.02	2.16	2.03	1066	727.7	834	920	17	22	19	38	0.3	0.4	0.4	0.5	27	32	33	48	38	36	30	51	1.34

(mIU/ml)	FSH (mIU/ml)		GH (ng/ml)		TSH (µIU/ml)		FT4 (ng/dl)		TESTOSTERONE(MALE) (ng/dl)		ESTRADIOL(FEMALE)(pg/ml)		FBS (mg/dl)		2 HR PPBS (mg/dl)	
AT 6 MONTHS	BASELINE	AT 6 MONTHS	BASELINE	AT 6 MOTNHS	BASELINE	AT 6 MOTNHS	BASELINE	AT 6 MONTHS	BASELINE	AT 6 MONTHS	BASELINE	AT 6 MONTHS	BASELINE	AT 6 MONTHS	BASELINE	AT 6 MONTHS
0.1	0.64	0.72	2.28	1.08	2.25	1.08	1.07	1.44	0.087	0.087			89	90	200	181
2.57	1.35	2.93	0.58	20.99	1.76	2.75	1.27	1.03			18.22	7.44	84	103	124	137
0.55	2.43	1.92	0.99	3.98	1.76	5.88	1.32	1.15			5	13.25	126	107	168	194
6.31	4.43	5.88	6.09	13.63	1.41	1.28	1.02	1.11			48.84	67.26	72	88	176	132
5.77	1.76	10.47	1.24	2.1	2.31	2.54	1.3	0.78	302.1	350.54			96	109	160	192
10.32	4.72	6.82	0.62	2.13	1.22	1.36	1.14	1.18			8.88	20.77	72	88	210	138
6.67	1.62	2.74	6.2	12.17	2.6	5.36	1.2	1.46	20.1	9.77			95	107	156	173
4.93	3.11	3.09	0.15	0.09	2.24	3.06	0.85	0.93	22.29	34.8			88	93	170	184
3.72	2.75	2.75	2.75	11.33	2.87	1.76	1.53	1.04			8.1	10.34	78	86	262	188
8.07	2.89	5.76	0.24	0.31	3.69	4.81	1.39	1.46	1.46	14.29			96	102	136	138
2.2	4.4	3.37	0.28	0.53	5	3.99	1.08	1.61			48.07	118.7	76	90	108	133
10.42	2.47	6.23	0.63	1.11	4.79	1.18	1.18	0.9			115.7	340.22	126	130	184	172
5.08	2.88	2.46	2.06	5.73	1.93	1.21	1.07	1.01	3.77	14.09			111	116	163	183
3.04	1.27	3.17	3.01	10.93	3.34	5.66	1.47	1.15	0.087	0.159			97	102	138	162
0.1	1.94	2.38	8.63	1.07	3.31	3.65	1.51	1.34			5	15	94	106	174	188
1.85	6.22	5.33	0.96	2.33	2.39	2.53	1.32	1.07			29.7	40.64	109	110	128	156
2.1	1.8	1.96	13.84	29.91	2.1	3.79	1.41	1.37	0.087	0.451			122	124	182	174
11.06	6.9	11.27	5.89	13.42	1.38	3.49	1.16	3.41			4.75	182.1	86	88	128	143
0.93	2.7	2.89	6.24	10.82	1.31	2.08	1.41	1.11	0.087	0.087			87	103	122	177
8.64	2.09	4.39	0.09	4.09	1.42	1.13	1.2	1.13	1.42	15.43			104	108	211	194
0.44	3.1	4.15	3.27	12.4	2.19	3.24	1.19	1.31			5	23.45	98	102	123	144
4.15	3.57	6.82	0.55	1.28	1.5	1.74	1.37	0.78	1.63	10.59			76	82	132	150
0.44	7.87	0.37	3.03	12.3	2.24	0.32	1.1	1.28			13.05	156.3	94	102	146	138
6.45	6.38	5.57	4.27	10.76	3.3	3.73	0.98	1.39			89.17	108.3	89	88	129	145
4.61	4.89	4.11	2.07	12.1	4.1	2.54	1.23	1.38			94.92	53.88	72	86	102	110
0.44	0.1	4.15	8.89	12.4	2.28	3.24	1.48	1.31	0.443	23.24			68	70	188	123
3.72	2.78	6.84	1.65	23.72	3.59	2.42	1.48	1.52	0.092	2.36			84	88	122	133
8.44	0.93	11.23	1.61	10.77	1.72	1.08	1.38	1.44	0.27	8.28			81	76	172	160
4.74	3.68	6.01	12.28	20.64	1.27	2.07	1.06	1.12	22.77	30.59			90	86	208	153
1.26	1.01	1.38	0.75	13.67	1.22	1.22	0.88	1.07	316	355.4			84	168	94	170
1.42	2.36	2.47	1.2	9.49	1.78	4.1	1.38	0.9			8	34.22	92	102	126	152
7.92	3.69	3.81	0.14	4.97	1.36	1.79	2.65	3.62	5.88	6.68			74	86	113	148
0.1	0.63	0.74	2.7	3.13	2.13	1.68	1.13	1.18	0.087	0.087			78	92	122	154
0.43	2.17	2.5	0.35	1.96	3.09	2.36	1.55	1.35			32.32	15.53	78	94	128	163
0.1	1.43	1.5	3.78	10.66	1.5	1.64	1.01	1.08	0.087	0.087			93	88	186	146
0.1	0.59	0.67	7.27	12.49	0.92	1	1.24	1.22	0.087	3.49			88	90	135	152
8.31	3.16	5.24	0.84	9.33	3.11	3.1	1.08	1.12			90.24	138.44	100	89	190	183
6.4	0.1	5.09	0.96	10.53	2.49	1.49	1.6	1.48			9.59	48.99	83	68	136	166
3.16	2.54	7.12	2.59	11.88	2.5	2.1	1.29	1.6			11.74	13.34	80	98	133	147
2.16	0.95	1.34	2.37	2.59	2.76	3.65	1.22	1.2	0.092	9.65			88	92	128	134