"EFFICACY OF BISPHOSPHONATES, VITAMIN D AND CALCIUM SUPPLEMENTS IN TRANSFUSION DEPENDENT THALASSEMIA CHILDREN WITH LOW BONE MINERAL DENSITY – A ONE YEAR HOSPITAL BASED INTERVENTIONAL STUDY"

By

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LIST OF ABBREVIATIONS USED

β	-	Beta
/cumm	-	Per cubic millimetre
ACE	-	Angiotensin-converting enzyme
AEs	-	Adverse events
ANOVA	-	Analysis of variance
ARMS	-	Amplification refractory mutation system
BT	-	Blood transfusion
BMD	-	Bone mineral density
BMT	-	Bone marrow transplant
BMI	-	Body Mass Index
BTM	-	B thalassemia major
CBC	-	Complete blood count
CI	-	Confidence interval
CNS	-	Central nervous system
CVS	-	Cardiovascular system
DC	-	Differential count
DEXA	-	Dual Energy X-ray Absorptiometry
DFX	-	Deferasirox
DNA	-	Deoxyribonucleic acid
EPO	-	Erythropoietin
FDA	-	Food and drugs administration
fl	-	Femto litre
g/dl	-	Grams per decilitre
gm%	-	gram percent
Hb	-	Haemoglobin

HbA	-	Haemoglobin A
HbA2	-	Haemoglobin A2
HbC/ β	-	Haemoglobin C/beta
HbD	-	Haemoglobin D
HbE	-	Haemoglobin E
HbE/ β	-	Haemoglobin E /beta
HbF	-	Fetal hemoglobin
HbS/ β	-	Haemoglobin S/beta
HBsAg	-	Surface antigen for hepatitis B virus
HCV	-	Hepatitis C virus
HIV	-	Human immunodeficiency virus
HLA	-	Human leukocyte antigen
HPLC	-	High performance liquid chromatography
hr	-	Hour
HSCT	-	Hematopoietic stem-cell transplantation
Kg/m^2	-	Kilograms per square meter
LCR	-	Locus control region
LDH	-	Lactate dehydrogenase
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration
MCV	-	Mean corpuscular volume
mg/kg	-	Milligrams per kilogram
min	-	Minute
ml/kg	-	Milli litre per Kilogram
ml/kg/day	-	Milli litre per kilogram per day
mm	-	Milli meters
mo	-	Month

mRNA	-	Messenger ribonucleic acid
n	-	Total number
ng/ml	-	Nanograms per milli litre
NIH	-	National institute of health
NTDT	-	Non transfusion dependent thalassemia
p	-	Probability value
PC	-	Platelet count
PCR	-	Polymerase chain reaction
pg.	-	Pico gram
PRC	-	Packed red blood cell
RBC	-	Red blood cells
RCT	-	Randomized clinical trial
RCTs	-	Randomized controlled trials
RDW	-	Red cell distribution width
RS	-	Respiratory system
SD	-	Standard deviation
SEM	-	Standard error mean
TM	-	Thalassemia major
TI	-	Thalassemia intermedia
vs.	-	Versus
WBC	-	White blood cell
α2	-	Alpha 2

ABSTRACT

Efficacy of Bisphosphonates in the treatment of Thalassemia induced Low Bone Mineral Density - A One Year Hospital Based Interventional Study.

Objectives

To assess the efficacy of Tab Alendronate in improving bone mineral density in transfusion dependent β-thalassemia(TDT) children and the prevalence of low BMD in relation with duration of disease, height and serum ferritin.

Methods

This hospital based prospective interventional study done between Jan 2018 to Dec 2018.Total 42 cases of TDT between 8-18 years were selected after randomization . Demographic data including duration of disease, anthropometry, serum ferritin and calcium were recorded. Dual Energy X-ray Absorptiometry (DEXA) scan using Lunar prodigy paediatric software was done at lumbar spine (L1-L4) in 42 children, Of which 26 children had low BMD and treated with Tablet Alendronate 10 mg/ day , vitamin D, calcium for 6 months. DEXA scan was repeated after 6 months.

Results

Mean Z-scores of 42 patients was -1.15 + 1.49 S.D. The prevalence of low BMD was seen in 61.9% children. Mean Z-score significantly improved after 6 months of treatment (-2.18 + 0.83 S.D to -1.72 + 0.6, p value <0.0001). Low BMD was statistically significant with longer duration of disease(r = -0.5843, p value < 0.0001), short stature, low BMI and high ferritin levels.

Conclusion

Prevalence of low BMD was seen in 61.9% children. Treatment with Tab Alendronate is highly effective and safe in low BMD thalassemia children. Low BMD was associated with longer duration of disease, high ferritin levels and short stature. Annual BMD measurement is advised to prevent skeletal morbidity.

Keywords Beta thalassemia children ºDEXA º Alendronate º low bone mineral density

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INTRODUCTION

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

Thalassemia is a heterogeneous disorder with varied phenotypes, ethnicities account for the global public health problem(2). It is estimated that around 3,00,000 - 4,00,000 children are born in the world with some haemoglobin disorder every year, out of which around 1,00,000 children with thalassemia are contributed by India(3). Worldwide 56,000 conceptions are having thalassemia disorder, out of which 30,000 have thalassemia major(4). Northern India is having high Carrier rate of about 3-15% when compared to Southern India which is 1-3% (3).

Thalassemia is more commonly seen in low and middle-income countries. It is estimated that the cost of travel expenses, supportive care management of a thalassemia major child to be Rupees 100000-. 250,000 / year in India(5).

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

The age of presentation in beta-thalassemia major will be usually between 6-24months with Hepato-splenomegaly, mild jaundice, and severe microcytic anaemia. Regular blood transfusions and chelating agents were the mainstay of treatment in thalassemia major, which will increase the life expectancy of the patient.

In recent years, where the blood transfusion facility is increased to many areas, newer treatment modalities are available, their life expectancy is increased. Along with increased life expectancy, age-related conditions like delayed puberty, hypothyroidism, bony deformities, cardiac complications are more prevalent and requires lifelong management plans. Progressive iron deposition in tissues secondary to repeated blood transfusions and enhanced dietary absorption of iron due to ineffective erythropoiesis leads to involvement of multiple organs like liver, heart, endocrine system, bones. Osteoporosis and osteopenia remains still the major morbidity in transfusion-dependent thalassemia patients, in spite of regular blood transfusions and iron-chelating therapy(6). Pathogenesis of osteoporosis in thalassemia major is complex and different from that of bone deformities in thalassemia intermedia, where distortion of bone is secondary to ineffective erythropoiesis & bone marrow expansion(7).

Osteoporosis is defined as “A disease characterized by low bone mass and micro architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk” (W.H.O). Even in well-treated thalassemia major patients, prevalence of osteoporosis or osteopenia was 40-50 %(8). Etiology of osteoporosis and osteopenia in thalassemia major is multifactorial involving genetic and acquired factors, like bone marrow expansion, delayed puberty, altered growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis, excessive iron deposition in bone, desferrioxamine bone toxicity and vitamin D deficiency.

Osteoporosis can be diagnosed by X-ray, DEXA(dual-energy x-ray absorptiometry), Quantitative computed tomography(QCT)(9). DEXA is the gold standard method for diagnosing bone mineral density (BMD). DEXA is a simple, non-invasive procedure, which will be used to check bone mineral density at the lumbar spine, femur neck, distal radius and whole body minus head(10).

Mainstay of management of osteoporosis in thalassemia major is prevention, which can be achieved by regular transfusions preventing anaemia, management of iron overload with chelating agents, proper nutrition, physical activity, cessation of smoking and hormonal replacement therapy (HRT) in case of hypogonadism patients. Patients should be supplemented with calcium and vitamin – D in adequate dosages. Calcitonin, a potent osteoclasts inhibitor used in treating osteoporosis(11).

Bisphosphonates belong to the group of pyrophosphates, used in osteoporosis prevention and treatment as anti-resorption agents. Bisphosphonates usage in the treatment of pediatric patients has been increased in recent years(12). Bisphosphonates have been proved to be effective in postmenopausal osteoporosis, glucocorticoid-induced osteoporosis and Paget's disease. Fracture rate of osteoporosis will be decreased with bisphosphonates usage by improving the acquisition of bone mass. Most commonly used agents are Zolendronate, Pamidronate, Alendronate, Clodronate. Bisphosphonates should be supplemented with calcium and vitamin –D3. Before initiating bisphosphonate treatment, calcium levels should be checked, because of the risk of hypocalcemia(13).

Alendronate is a new orally available bisphosphonate, belongs to nitrogen-containing bisphosphonate. Alendronate decreases the incidence of vertebral, hip and forearm fractures by increasing the bone mineral density (BMD) of the spine, hip and

total body(14). Alendronate is safe and well-tolerated in the pediatric age group without any major side effects. Alendronate is available in oral tablet form, easy way of administering the drug(15).

There are very few studies about bisphosphonates usage and prevalence of osteoporosis in transfusion-dependent thalassemia children in India. Hence this study was undertaken to assess the efficacy of bisphosphonate in reducing osteoporosis or improving bone mineral density. This is probably the first study about the efficacy of alendronate on thalassemia induced osteoporotic children in India.

OBJECTIVES

The objectives of this study were;

PRIMARY:

Bone density & improvement in bone mineral density after Calcium, Vitamin-D supplementation and bisphosphonates.

SECONDARY:

Prevalence of low bone mineral density among thalassemia patients using DEXA scan.

REVIEW OF LITERATURE

The thalassemias are a group of hemolytic anaemias due to inherited defects in the haemoglobin production. The thalassemias are a heterogeneous group of single-gene disorders more common in some parts of the world, more prevalent in the Mediterranean, Indian Sub-continent, Southeast Asia and Africa.[16]

B-Thalassemia:

β thalassemia is a global health problem and is the commonest autosomal recessive disorders worldwide. 95 % of the thalassemia births worldwide were from Middle Eastern, Asian and Indian regions.[5] Thalassemia is commonly seen in people of African descent. The highest incidences are reported in Cyprus (14%), Sardinia (12%) and South-East Asia.[4]

HISTORICAL

In the 20th century beginning, European physicians noticed an anaemia syndrome with enlargement of spleen in infancy. The first clinical description of thalassemia is given by Detroit paediatricians Thomas B.Cooley and Pearl Lee[41]. Cooley and Lee had described four Italian children with anaemia, discolouration of the skin, sclera enlargement of spleen and liver. Red blood cells of these children have increased resistance to hypotonic solutions and the peripheral blood smear showed moderate leukocytosis with nucleated erythrocytes. They used to have hemolytic facies with prominent cranial and facial bones.

Previously chronic childhood anaemias were named under a group called as Von Jaksch's anaemia, later it was separated by Cooley and Lee and named it as "Erythroblastic anaemia or Cooley's anaemia.[41]

The term 'thalassemia' was coined by Whipple and Bradford in 1932, which was taken from the Greek word 'Thalassa' meaning 'black sea', and 'mia' meaning 'blood'.[42] Based on the severity, Valentine and Neel classified the milder forms of Cooley's anaemia as 'Thalassemia minor' and the more severe entity as 'Thalassemia major'. In 1925, it was described that the disease was running in the families by Rietti and told to be familial condition.[43] In 1936, Lehndorff first proposed that the condition is also inherited.

Mukerjee reported the first case of beta-thalassemia from India in 1938 from Calcutta.[44] Wolman in 1964 was the first to suggest that chronic blood transfusion may be able to prevent many of the problems of the disease. Lesky, et al and Model, et al. in 1974 were able to initiate clinical trials with desferrioxamine, an iron chelator, in an attempt to promote the excretion of large percentage of transfusional iron overload. Deferiprone an oral iron chelator was discovered in 1981. In 1982 Dr E Donald Thomas performed the first bone marrow transplantation on a thalassemic patient.[45] The first bone marrow transplantation in India was successfully done by Dr M. Chandy at Christian Medical College, Vellore.[46]

Table.1 Historical landmarks in the field of hemoglobin and thalassemia.[47]

Year	Landmark	Author
1628	Circulation of the blood	Harvey
1862	Oxygen binding pigment is named 'hemoglobin'	Hoppe – Seyler
1866	Fetal blood is alkali-resistant	Korber
1913	Structure of haem	Kuster
1925-1927	Molecular weight of hemoglobin	Adair, Svedberg
1925	Description of 'thalassemia'	Cooley, Lee
1932	Term 'thalassemia' first used	Whipple & Bradford
1937-1944	Inheritance of thalassemia	Caminopetros, Neel, Valentine, Silvestroni
1944-1946	Sickle-cell thalassemia	Silvestroni, Bianco
1948	Alkali-resistant hemoglobin in thalassemia	Vecchio
1949	Malaria hypothesis	Haldane
1955	HbA2 raised in some thalassemia	Kunkel & Wallenuis
1957	Ineffective erythropoiesis in thalassemia	Sturgeon & Finch
1958	Adult hemoglobin controlled by two gene Loci	Smith & Torbert
1959	Three – dimensional structure of hemoglobin	Perutz
1959	α andβ thalassemia hypothesis	Ingram and Stretton
1960-1963	Structure of α, β, γ and δ chains	Konigsberg et al, Schroeder et al., Jones et al, Braunitzer et al., Hill et al
1962	Chelation therapy – Desferrioxamine	Sephton – Smith
1964	High level transfusion for thalassemia	Waldman
1965	Imbalanced globin synthesis in α andβ Thalassemia	Weatherall, Clegg
1966	Consequences of globin imbalance	Nathan, Gunn
1970	Genetics of α thalassemia	Na-Nakron and Wasi
1973	Dominantly inherited β thalassemia	Weatherall et al

1974	Liver iron level controlled by Desferrioxamine	Barry et al
1979	Restriction – fragment length polymorphism for prenatal diagnosis	Kan and Dozy
1979	Stop – codon mutation in β globin mRNA	Chang and Kan
1979	β Thalassemia due to gene deletion	Orkin et al
1980-1981	Globin genes sequenced	Lawn et al., Spritz et al., Barralle et al
1981	Mutations in β thalassemia cloned in DNA	Spritz et al., Westaway and Williamson.

DEFINITION:

"β thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent β globin chain synthesis, resulting in reduced haemoglobin in red blood cells, decreased RBC production and anaemia." They are inherited in the autosomal recessive pattern.[2]

Annual births with major haemoglobin disorders.[1]

b-thalassemia major	22,989
HbE b thalassemia	19,128
HbH disease	9568
Hb Bart's hydrops (a0/a0)	5183
SS disease	217,331
SC disease	54,736
S b thalassemia	11,074

β thalassemia can be classified into:

B THALASSEMIA
Thalassemia major
Thalassemia intermedia
Thalassemia minor
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
o β thalassemia-trichothiodystrophy
o X-linked thrombocytopenia with thalassemia.

The phenotypes of homozygous or genetic heterozygous compound β-thalassemia include Thalassemia major and Thalassemia intermedia. Patients with thalassemia major manifest by 2 years of life and needs blood transfusions regularly for their survival. Thalassemia intermedia manifests in late ages and does not require regular blood transfusions. Heterozygous β-thalassemia manifests in clinically silent carrier state, except in the rare dominant forms. HbC/ β-thalassemia and HbE/β thalassemia manifests in a wide range of spectrum and phenotypes of severity.[16]

Epidemiology

β-thalassemia is a global health problem and the commonest hemoglobinopathies. Mutations, base substitutions or insertion of nucleotides in β-globin genes will cause β-thalassemia. β-thalassemia is more common in certain geographical areas/populations because mutations are relatively population specific.[11]

Figure 1: World Wide Distribution Of Thalassemia

The incidence of symptomatic β thalassemia major individuals throughout the world is estimated as 1 in 100,000. As per Thalassemia International Federation (TIF,) throughout the world, only 2,00,000 β-thalassemia patients are present and taking treatment on registration regularly.[55] HbE/β thalassemia is the most common combination of β thalassemia with abnormal haemoglobin or structural haemoglobin variant and has a high prevalence in Southeast Asia, with a carrier frequency of 50%.[16]

Due to the migration of people, Thalassemia is now present in almost all the nations of the world and intermarriage between varied ethnic groups. 1.5% of the global population has a carrier state for β-thalassemia.[4] Secondary to migration of population between different regions, marriage between different ethnic groups, races, thalassemia is prevalent in almost every country. In India, the overall beta-thalassemia carrier rate is estimated to be 4.05% and incidence of β-thalassemia homozygotes births to be 11,316 per year.[49]

INDIA

β-thalassemia is the commonest single-gene haemoglobinopathy in Indian population. India comprises ten per cent of the total world thalassemia births every year. Grow K et al. The β – thalassemia carrier frequency varies from 3.0 to 17.0% In India.[48]

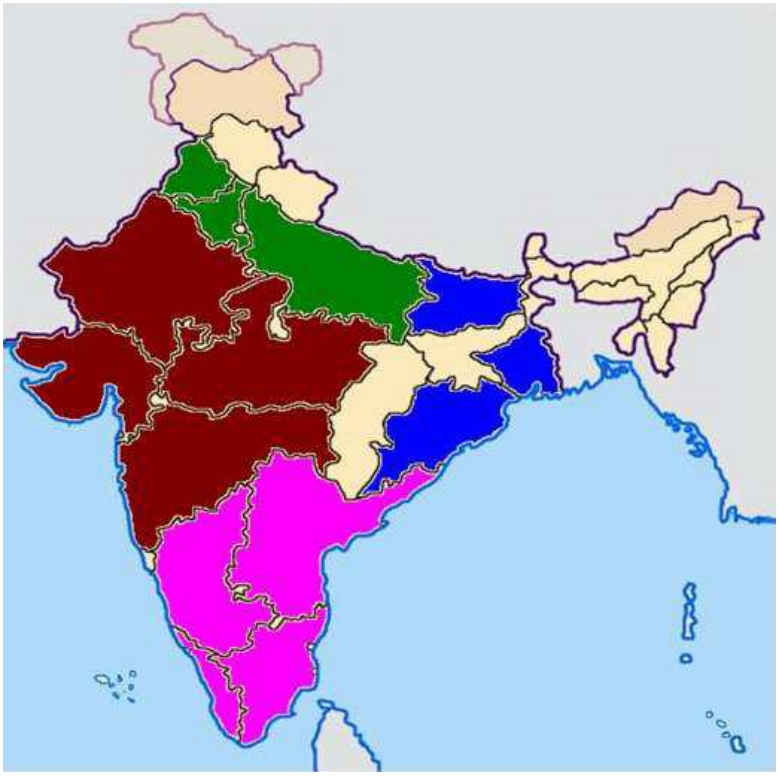


Figure 2. Map showing distribution of beta thalassemia in India[50]

In India, as per WHO update on β-thalassemia, an overall carrier frequency of 3–4% was seen, which would render the carrier rate between 35.6 and 47.5 million nationwide. Prenatal diagnosis and detection of carrier status are required to decrease the mutant load in the gene pool.[50]

In a non-Mediterranean region, the first case of Thalassemia was reported from India. Chouhan DM [1983] reported that beta-thalassaemia has been observed in different castes and communities like Gujaratis, Sindhis, Marathas, Khojas, Bori Muslims, Jains, Baniyas and Punjabis.[51]

India is a multilingual country with different religions, which are further subdivided into different castes. Consanguineous marriage is commonly seen and accounts for an estimated 10.4% of world population, as they follow their specific caste. Due to more consanguineous marriages and the lack of premarital checkup in India, it leads to many disorders. Thalassemia is one of them. In almost every community, thalassemia will be seen.[17]

Hereditary Transmission

Beta thalassemia's show autosomal recessive inheritance pattern. Affected child parents will be heterozygotes and contain a single copy of pathogenic gene mutation in the beta-globin chain. 25 % chances of children getting affected if parents are heterozygotes, 50% chances of asymptomatic carrier, & 25% chances of not getting affected & carrier.[52]

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

A) Transfusion Dependent Thalassemia's (TDTs)

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

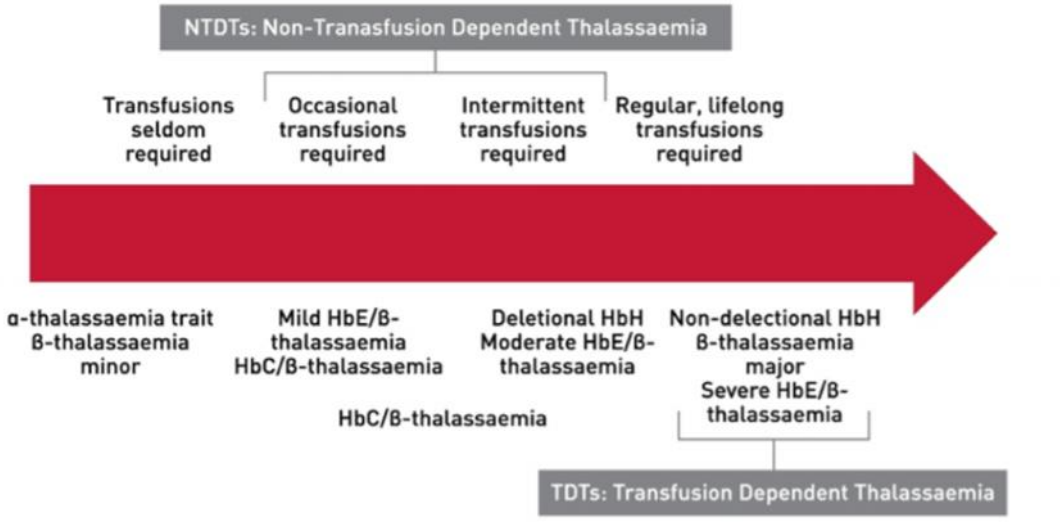


Figure 3: Phenotypic nclassification of thalassaemia syndromes based on clinical severity and transfusion requirment.[8]

TDT's would be requiring regular blood transfusions, iron chelating agents to avoid iron overload. They will be presenting at an early age with increasing pallor, abdominal distension and respiratory distress.[53]

Phenotypic heterogeneity

β-thalassemia is having three forms

1. Thalassaemia Major also called "Cooley's Anaemia" or "Mediterranean Anaemia"
2. "β-thalassaemia carrier - Thalassaemia Intermedia & Thalassaemia Minor"
3. β-thalassaemia trait / heterozygous β-thalassaemia

PATHOPHYSIOLOGY:

Basic defect present in β-thalassemia is decreased or lacking of β-globin chains production with relative excessive synthesis of α-chains.[54]

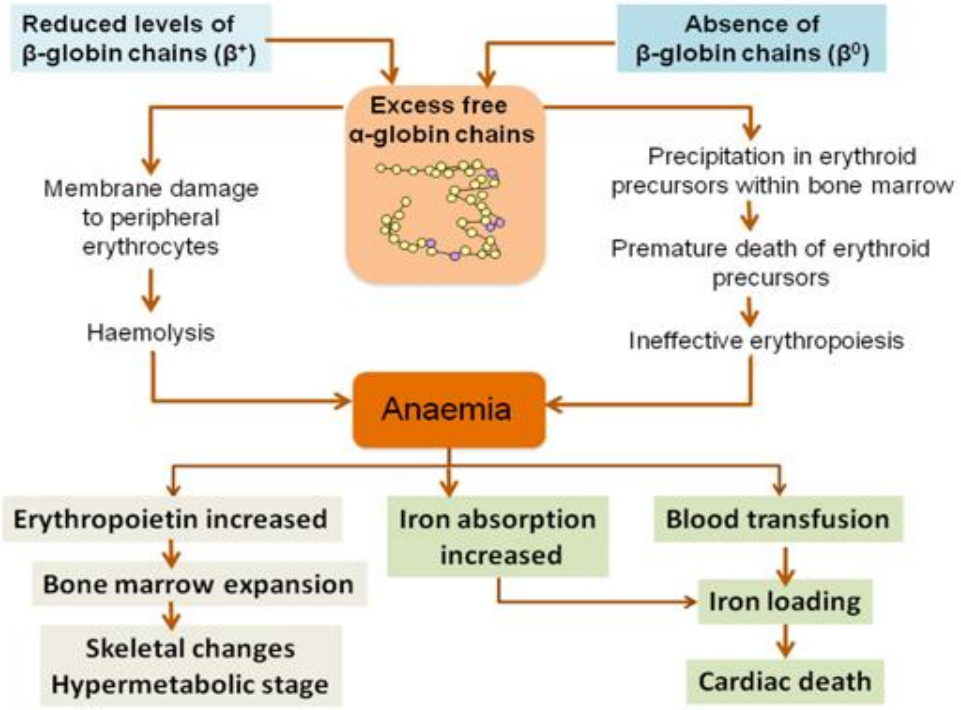


Figure 4: Pathophysiology of thalassemia

Direct significant changes are

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

Decreased haemoglobin production causing reduction of mean cell haemoglobin and mean cell volume has a minor clinical significance.

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

CLINICAL DIAGNOSIS

Usual age of presentation of β-thalassemia major will be between 6-24 months[5] characterized by

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

Failure to thrive, increasing pallor will be seen. Repeated fever episodes secondary to infection, and progressive spleen & liver enlargement causing abdominal distension. In cases of improper treatment or unavailability of transfusion facilities, the clinical picture will be short stature, pallor, jaundice, muscle weakness, genu valgum, hepatosplenomegaly, ulcers over the leg, and skeletal deformities secondary to bone marrow expansion.[8]

Skeletal complications like bone deformities of legs and craniofacial variations characterized by thalassemic facies (skull bossing, prominent malar eminence, depressed nasal bridge and maxillary hypertrophy, which causes exposure of the upper teeth).[56]

Hematologic Diagnosis

β-Thalassemia major is considered by decreased Hemoglobin parameters (<7 g/dl). RBC indices have microcytic hypochromic anemia, MCV between > 50 < 70 Femto litre and MCH between > 12< 20 pg.[57]

Peripheral blood smear

RBC will show moderate to severe anisocytosis, teardrop cells, microcytic hypochromic and nucleated RBC's. Degree of low haemoglobin levels will be correlated with the number of erythroblasts and will be more in number post-splenectomy. Few morphologic changes will be seen in RBC's for carriers than affected individuals. Erythroblasts are generally not seen.[8]

HPLC/electrophoresis

HPLC electrophoresis is diagnostic and advised both for the child and parents to look for transmission of the genes. β-thalassemia type will vary depending on the Hb pattern. HbA will be absent in ß0 thalassemia homozygotes and HbF institutes 92-95% of total Hb. HbA levels will be in between 10 - 30% and HbF in range of 70 - 90% In homozygotes of β-thalassemia and ß+/ ß0 genetic compounds. HmA2 varies in beta-thalassemia homozygotes and it is higher in beta-thalassemia minor.[2,52]

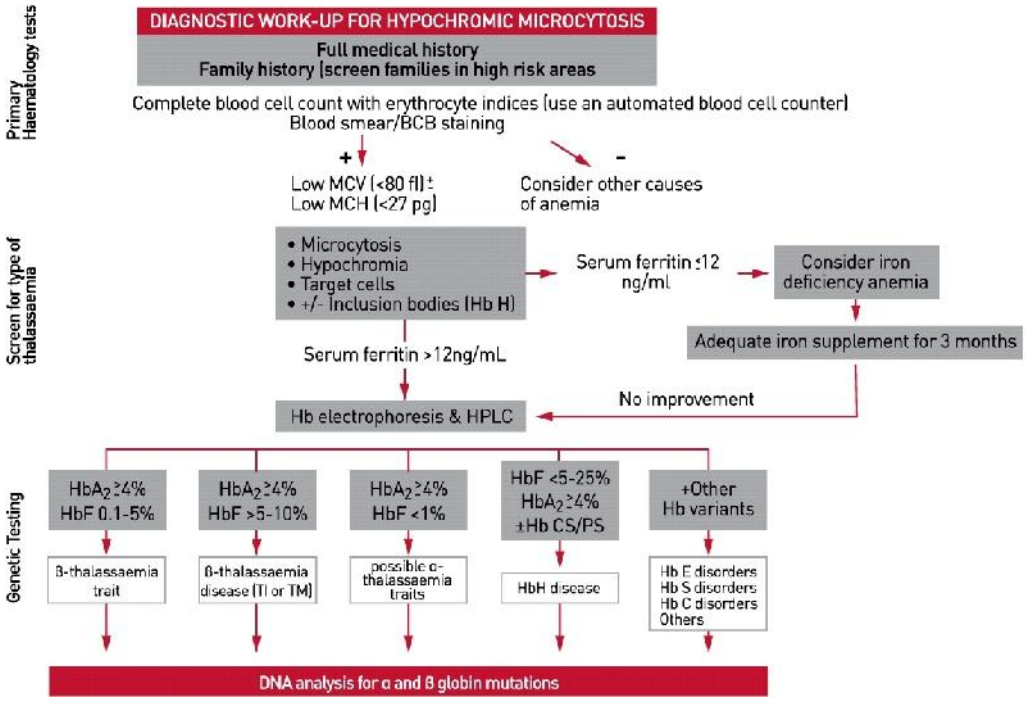


Figure 5: Diagnostic algorithum for individuals with hypochromic microcytosis[8]

TREATMENT:

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

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.

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

CURRENT TREATMENT OPTIONS FOR THALASSEMIA

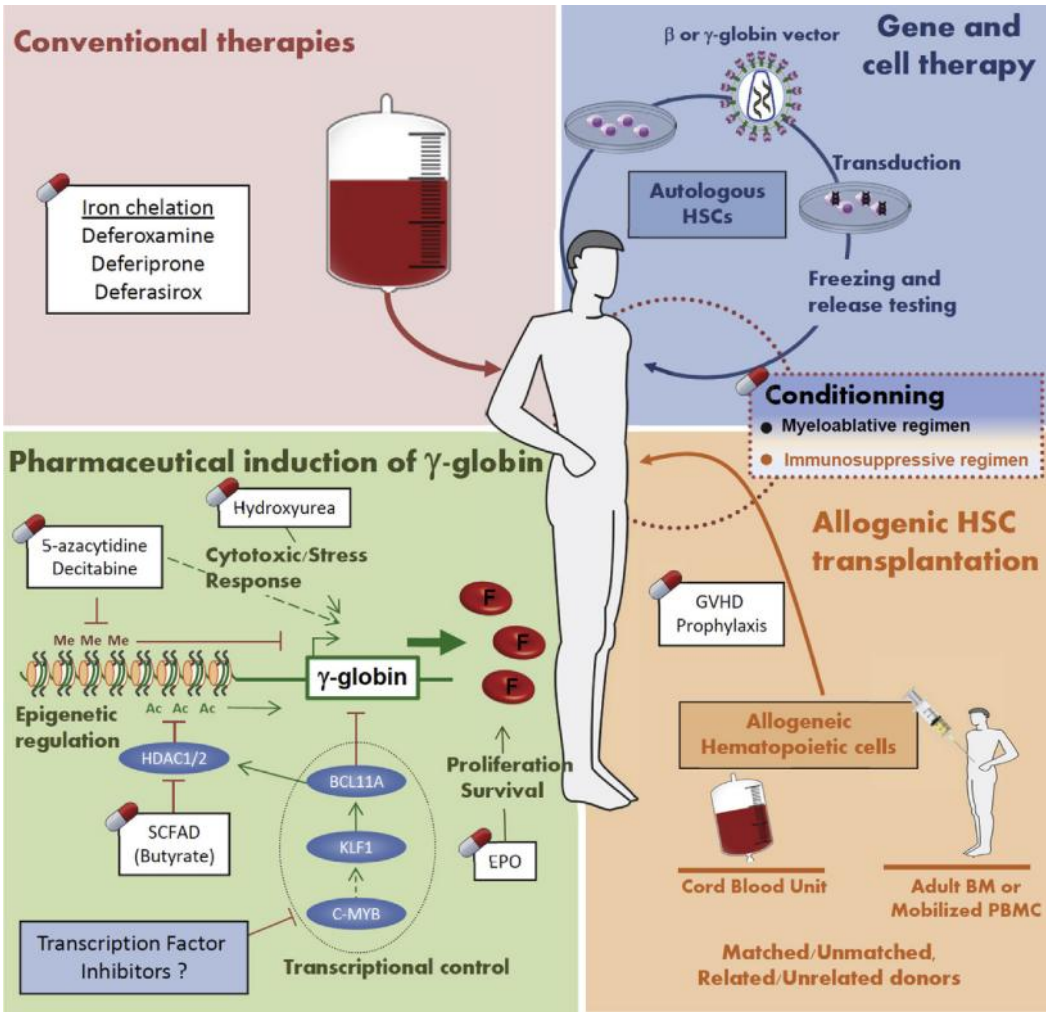


Figure 6: Current treatment option for thalassemia

OSTEOPOROSIS IN THALASSEMIA:

INTRODUCTION:

Osteoporosis is one of the most common morbidity associated with thalassemia major patients. As the age of thalassemia patients increases and repeated blood transfusions, inspite of regular chelating agents osteoporosis is becoming a prominent problem.[58] Early diagnosis of low bone mineral density is essential to prevent skeletal morbidity. So regular bone mineral density assessment is required.

DEFINITION:

As per World Health Organization (WHO), "Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures."[102]

INCIDENCE:

Incidence of osteoporosis is increasing nowadays because of improvement in treatment modalities, availability of blood transfusion facilities along with chelating agents and management of complications at different levels of care. Based on different studies, Z-score cut off, age the prevalence of osteopenia or osteoporosis in well-treated Thalassemia major patients varies with a frequency of 40–50% in the studied population.[33,34,37]

PREVALENCE OF OSTEOPOROSIS IN DIFFERENT STUDIES.

According to Voskaridou E, et al, osteopenia or osteoporosis frequency in well treated Thalassemia major patients is approximately 40 - 50 %.[7]

Study	Subjects (n)	Gender	Mean age (year)	Osteoporosis (%)	Osteopenia (%)	Criteria for diagnosis of osteoporosis
Jensen et al (8)	82	38 males 44 females	25 (male) 27 (female)	51.2%	45.1%	Osteopenia: Z-score between -1 and -2.5 SD Osteoporosis: Z-score below -2.5 SD.
Vogiatzi et al (25)	31	14 males 17 females	15.3	61.3%	22.6%	Osteopenia: Z-score between -1 and -2 SD Osteoporosis: Z-score below -2 SD
Vogiatzi et al (26)	236	116 males 120 females	24.4	49.1%	30.5%	Osteopenia: Z-score between -1 and -2 SD Osteoporosis: Z-score below - 2 SD
Pirinççioğlu et al (27)	47	23 males 22 females	7.42	62%	NA	Osteopenia: Z-score between -1 and -2.5 SD Osteoporosis: Z-score below -2.5 SD
Aslan et al (28)	47	25 males 22 females	NA	53.1%	44.6%	Osteopenia: Z-score between -1 and -2.5 SD Osteoporosis: Z-score below -2.5 SD
Izadyar et al (29)	40	21 males 19 females	23.0	12.5% (femoral level) 37.5% (lumbar level)	37.5% (femoral level) 47.5% (lumbar level)	Osteopenia: Z-score between -1 and -2.5 SD Osteoporosis: Z-score below -2.5 SD
Tzoulis et al (30)	99	49 males 50 females	36	55.5%	NA	Osteoporosis: Z-score below - 2 SD

NA: not available, SD: standard deviation

Figure 7 : Prevalence of osteoporosis/osteopenia in different studies

Study done by Vogiatzi et al in 31 children with mean age of 15.3 years showing prevalence of osteopenia 22.6% and osteoporosis as 61.3% with Z-scores of -1 to -2 S.D and below – 2 S.D respectively.[59] In an study conducted by Rashid et.al on 42 patients with mean age of 17.1 $\pm$ 4.2 years in India showing prevalence of osteoporosis in 50% and osteopenia in 31% children.[61] Study conducted by Kritanjali Singh et.al(2014) about the low bone mineral density in thalassemia major in India showing a prevalence of lumbar osteoporosis and osteopenia was 42.5% and 37.5% respectively.[34]

AETIOLOGY:

Bone disease in thalassemia is multifactorial and complex. Broadly divided into genetic and acquired factors.

GENETIC FACTORS:

In the development of osteoporosis/osteopenia in thalassemia, genetic factors play an important role. Collagen type Ia1 (COLIA 1) Sp1 site gene polymorphism is seen more commonly in females (female: male ratio was 2:1)32,33. Collagen type-1 was the major protein in bone matrix and so affecting this gene will cause severe osteoporosis.

Table.2 Genetic Variants In Pathogenesis Of Osteoporosis In Thalassaemia.[93,94]

Genes & polymorphism	Underlying mechanism
COLIA-1[a] (Sp1 polymorphism)	procollagen type-I structure down-regulation & synthesis
TGF-b[c]and COLIA 1	Reduced osteoblast function and proliferation
VDR[b] (FokI and BsmI polymorphisms)	Down-regulation of gender-related growth factors

a-Collagen type Ia1 gene.

b-Vitamin D receptor.

c-Transforming growth factor-beta.

Table.3 Acquired Factors Causing Reduced Bmd In Beta-Thalassaemia.[18,21,62,63]

1- Bone marrow expansion	
2- Iron overload	
3- Desferrioxamine	
4- Endocrine disorders	Hypothyroidism Secondary hypogonadism Hypo-parathyroidism Diabetes Mellitus GH/IGF-1 axis disorder
5- Nutritional deficiencies	Calcium, Zinc deficiency Vitamin C & D deficiency
6- Decreased physical activity	

OVERVIEW OF BONE FUNCTION AND REMODELLING:

Bone is a reservoir of mineral metabolism like calcium, phosphorus, magnesium and an active tissue which will be remodelled and metabolically changing constantly. Balanced between both osteoclasts and osteoblasts is essential for the turnover of the bone structure. Bone metabolism at a microscopic level occurs at focused sites on the surface of the bone, called as bone metabolism unit (BMU).[64] Osteoclasts and osteoblasts play a crucial role in bone metabolism at the fundamental BMU site. Turnover of bone is started by osteoclasts corroding the mineralized bone surface. Osteoclasts will be attracted to the new BMU site by different factors like growth factors, mechanical load, and cytokines, where they form lacunae by eroding the bone matrix,. Resorption will be ceased by the osteoblasts recruitment by secreting fresh matrix and gradually filling the resorbed cavity. Hydroxyapatite will mineralize the newly formed matrix, giving its BMU tensile strength.[65]

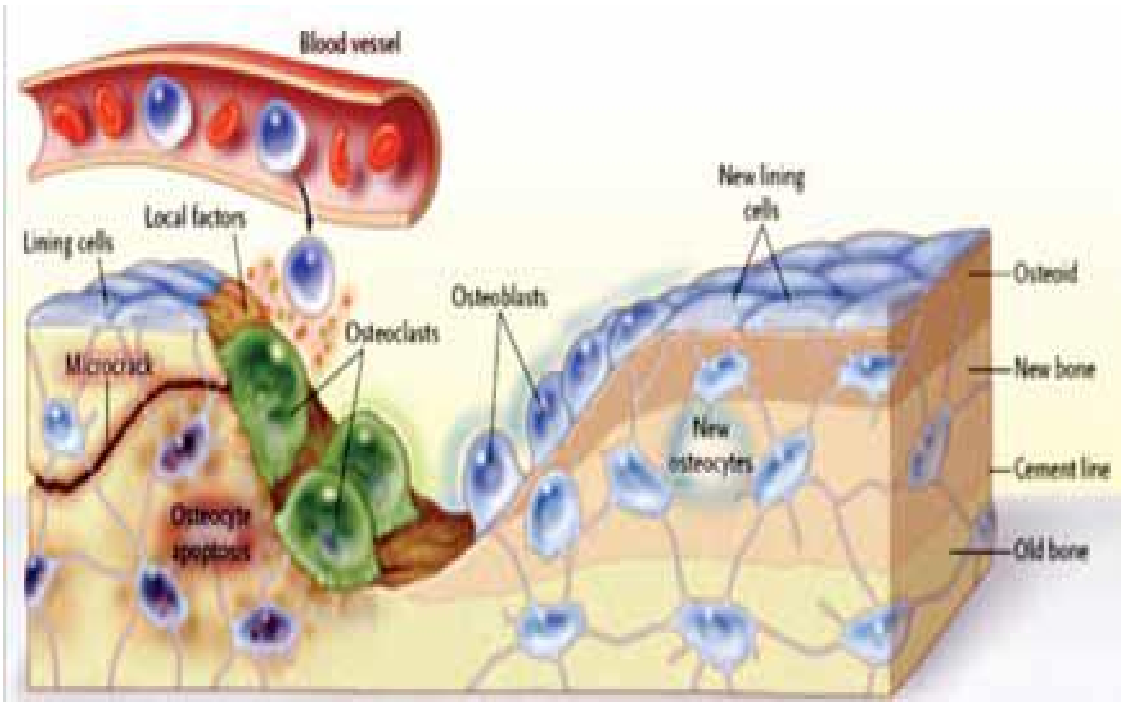


Figure 8 : Structure of bone

PATHOGENESIS:

Osteoporosis in TM is complex and due to multifactorial causes which include bone marrow extension, genetic factors, endocrine disorders and iron toxicity. Marrow expansion itself will cause disruption of bone formation by mechanical forces, causing cortical thinning, and the main reason for the distortion of bones and fragility in patients of thalassaemia major.[21] The pathophysiology of low bone mineral density in TM varies from bone deformities pathogenesis found in non-transfused patient.[101]

Several factors like genetic and acquired will play a role in the pathogenesis of osteoporosis in Thalassemia-major.

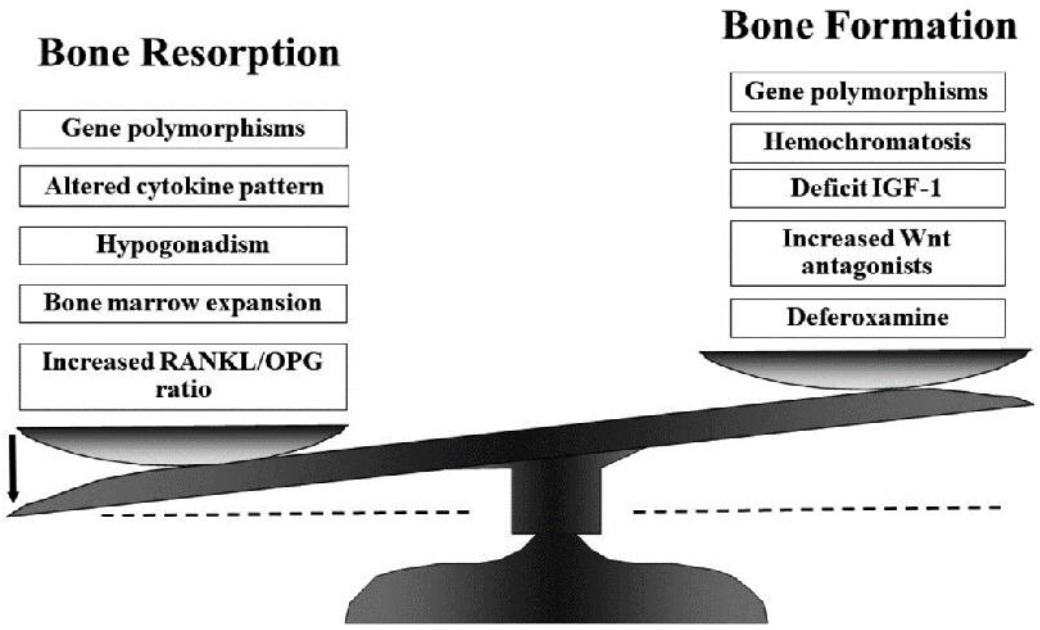


Figure 9 : possible causes for uncoupling bone turnover in thalassemia patients[19]

MARKERS [100]

BONE RESORPTION	BONE FORMATION
1. NTX*	1. b-ALP*
2. CTX*	2. OC*
3. RANK-LIGAND	3. PINP*
4. ICTP*	
5. Dickkopf-1 protein	
6. Sclerostin	

"NTX, N-terminal cross-linking telopeptide of collagen type-I; CTX, C-terminal cross-linking telopeptideof collagen type-I; ICTP, carboxyterminal cross-linking telopeptide of collagen type-I;RANKL, receptor–activator of nuclear factor-kappa B ligand; bALP, bone-specific alkaline phosphatase;OC, osteocalcin; PINP, Procollagen I Intact N-terminal."

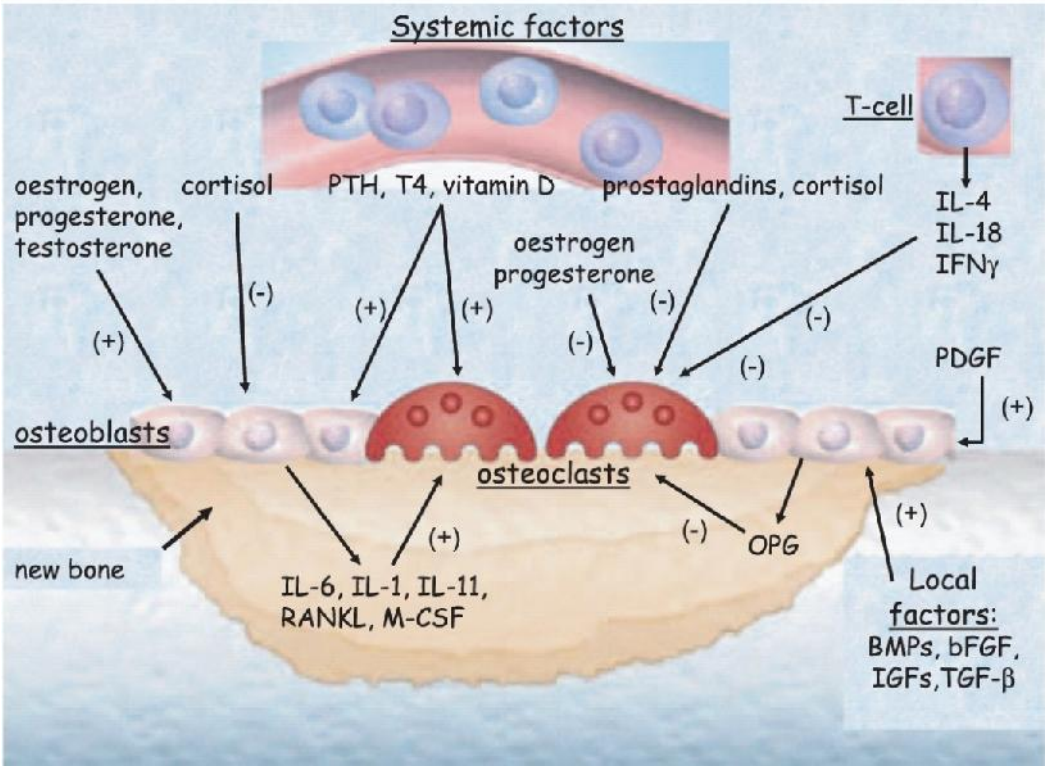


Figure 10: Regulation of bone remodelling[6]

BONE MARROW EXPANSION:

- Bone marrow expansion is a typical finding in patients with TM, due to ineffective erythropoiesis.[18]
- It causes interruption of bone formation due to mechanical forces, leading to thinning of the cortex, bone alteration & increased brittleness of bones.
- Ineffective erythropoiesis in thalassemia major is not completely suppressed even with regular blood transfusions.[19]

IRON OVERLOAD IN ENDOCRINE GLANDS & BONE:

- Regular blood transfusion is required in thalassemia, but effects in iron overload.
- Excessive iron deposition will be seen in almost all the tissues, but primarily affects the liver, endocrine glands and heart.
- Chelating agent should be used early to avoid excessive iron deposition in vulnerable organs that improves life expectancy.[66]
- Impaired puberty was the most common endocrine abnormality noticed. Iron deposition in bone cause damage to osteoid maturation and inhibits its mineralization leading to focal osteomalacia.[21]

HYPOGONADISM:[67]

Gonadal impairment is seen in thalassemia major, due to pituitary gonadotrophic haemosiderosis and deposition of iron in testes and ovaries.[68] Hypogonadism leads to osteoporosis and osteopenia in the general population also.[69] Hormonal therapy will be helpful for the management of osteoporosis, but not for longer duration.

Hypogonadism is due to a malfunction of the gonadotropic cells of the pituitary gland and/or the cells of the gonads (testes-ovaries). In related trials, it is reported that the complication is observed in 30-55% of patients of both sexes and often begins at a relatively young age.GF-1 axis disorder. This is due to a disorder of the somatotropic cells of the pituitary gland as well as possible concomitant hepatopathy. The incidence of the complication has not been firmly established, as

most trials indicate that it usually develops in 7.9-15% of all patients, although higher rates of up to 32% have also been reported.

DESFEROXAMINE :[21,70]

Chelating agents are a cornerstone in the treatment of thalassemia major who are on regular blood transfusions. Early initiation of chelating agents will avoid complications secondary to iron deposition in various tissues. Desferrioxamine is the treatment of choice for iron overload in TM long time. Chelating action of desferrioxamine is not only specific for iron, which also inhibits DNA, collagen synthesis, osteoblast precursor differentiation and augments apoptosis of osteoblasts. [71]

VITAMIN -D DEFICIENCY [62]

Vitamin D deficiency is commonly seen in TM patients who are suffering from osteoporosis. Vitamin-D helps in absorption of calcium and phosphorous through the gut into the blood which will enhance the formation of bones. In comparison to healthy controls, decreased 25-hydroxyvitamin D levels, are a common finding and are correlated with ferritin levels and age inversely. Decreased sun exposure because of reduced physical activity and defective skin synthesis due to jaundice causes vitamin-D deficiency.[20]

Hypoparathyroidism affects 13.5-14.6% of patients and can lead to severe hypocalcemia, hypercalciuria and inadequate production of active vitamin-D

IRON DEPOSITION IN BONE

Deposition of excessive iron in bone affects osteoid maturation and inhibits mineralization, causing focal osteomalacia. Incorporation of iron into calcium hydroxyapatite crystals subsequently affects the growth of bone and reduces basic multicellular unit(BMU) tensile strength. Increased thickness of osteoid, maturation time and lag in the mineralization time in TM patients were observed by Mahachoklertwattana et al.[21]

DIABETES MELLITUS: [72]

Diabetes mellitus in β-Thalassemia usually begins as a kind of type-2 diabetes in the second decade of life or later. This occurs at a rate of 7-11%, while prediabetes is seen with much greater frequency.

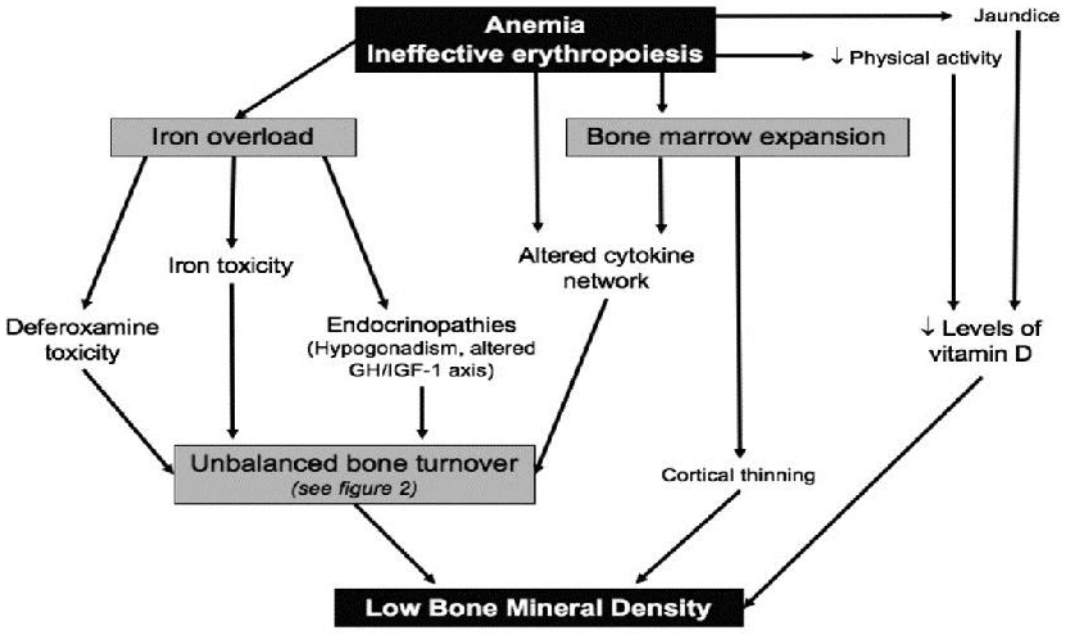


Figure 11 : pathogenesis of low bone mineral density in thalassemia patients.[19]

CLINICAL FEATURES:

Increased risk of fractures.

Low back pain

Bone pains[99]

Bowlegs

Scoliosis

Weakness and fatigue secondary to hypocalcemia

Short stature

Haemolytic facies.

Table.4 Fracture Prevalence In Thalassemia Major Patients

Study	Subjects (n)	Gender	Mean age (year)	Fracture prevalence (%)
Exarchou et al (32)	62	36 males 26 females	16.7	32.2%
Finsterbush et al (33)	61	30 males 31 females	16.0	49.1%
Ruggiero and De Sanctis (3)	977	472 males 505 females	NA	19.7%
Voglatzi et al (34)	379	177 males 202 females	20.2	16.6%
Fung et al (31)	152	80 males 72 females	25.5	38.8%
Sutipornpalangkul et al (35)	136	48 males 88 females	30.8	44.1%

NA: not available

Fracture prevalence is seen in 19 to 49 % of thalassemia children based on their age and other factors like nutrition, chelating agents.[59,60] Low back pain at the lumbar area and generalized body pains will be seen in thalassemia patients. Short stature will be commonly associated with transfusion dependent thalassemia children.[21]

DIAGNOSIS OF OSTEOPOROSIS IN CHILDREN:

Detection of decreased bone mineral density changes early in the children to prevent late skeletal complications like scoliosis, vertebral fractures, short stature, bony deformities. There are different methods to detect osteoporosis in children like X-ray, DEXA, QCT (quantitative ultrasound),p-QCT (peripheral computed tomography). DEXA has been considered as gold standard means for diagnosis of low bone mineral density.[22]

X-ray is usually done in clinical practice to evaluate bone status, which looks for the morphology of bones but doesn't give quantitative data regarding bone density. It requires >25% changes in bone as compared to osteoporosis evident in BMD to detect radiographically.[23]

- For quantifying bone mineral content and bone mineral density, three methods are present.[24]

1. Dual Energy X-ray Absorptiometry(DEXA)
2. Peripheral computed tomography (p-QCT)
3. High-resolution p- QCT (HR-pQCT)
4. Quantitative ultrasound(QUS)

DEXA is the most commonly used and preferred technique, because of easy feasibility, availability, low cost, safety and accuracy.[25]

DEXA scan can be performed at different sites of the body, most commonly at

- Lumbar spine
- Total Body except head
- Femur
- Wrist

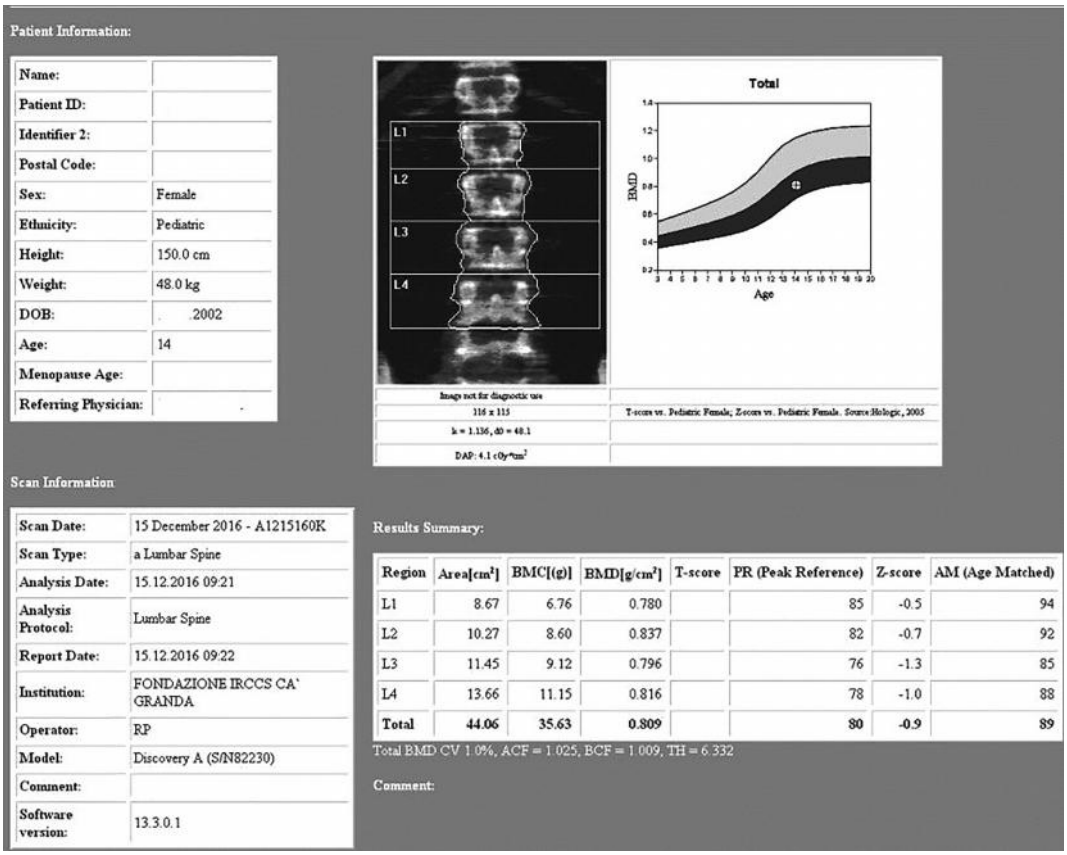
DEXA at lumbar spine is the preferred site in children, because of more trabecular pattern and have more precision & speed of measurement. Recommended site in children is posterior-anterior lumbar spine between L1-L4.[22,26]

"The BMD is the amount of mineral present in the projected area of the bone (g/cm2), which will contribute for 60–80% of bone mechanical resistance. BMD is the simplest parameter for quantifying bone strength in clinical practice.".[9]

What to include in a DEXA report[73]

DEXA report will be accurate and complete when it contains details like age,sex, height for age, body mass index, BMD value. DEXA manufacturer, model and software version must be seen.

For patients, with age less than 30 years, Bone Mineral Density values should be given in Z-scores. "Z-scores is the number of standard deviations (SD) above or below the mean for the patient's age, sex and ethnicity".[74] Usage of T-score is inappropriate and to be avoided while reporting in a pediatric DEXA scan report. BMD Z-scores are diagnostically significant in children and adolescents when below -1 SD, that means a low bone mass/bone mineral density for age and called as osteoporosis when Z-score is below -2 S.D.[59,60]



Patient Information:

Name:	
Patient ID:	
Identifier 2:	
Postal Code:	
Sex:	Female
Ethnicity:	Pediatric
Height:	150.0 cm
Weight:	48.0 kg
DOB:	.2002
Age:	14
Menopause Age:	
Referring Physician:	

Image not for diagnostic use
116 x 115
T-score vs. Pediatric Female; Z-score vs. Pediatric Female. Source Hologic, 2005
k = 1.136, d0 = 48.1
DAP: 4.1 cGy*cm²

Scan Information

Scan Date:	15 December 2016 - A1215160K
Scan Type:	a Lumbar Spine
Analysis Date:	15.12.2016 09:21
Analysis Protocol:	Lumbar Spine
Report Date:	15.12.2016 09:22
Institution:	FONDAZIONE IRCCS CA' GRANDA
Operator:	RP
Model:	Discovery A (S/N82230)
Comment:	
Software version:	13.3.0.1

Results Summary:

Region	Area[cm²]	BMC[(g)]	BMD[g/cm²]	T-score	PR (Peak Reference)	Z-score	AM (Age Matched)
L1	8.67	6.76	0.780		85	-0.5	94
L2	10.27	8.60	0.837		82	-0.7	92
L3	11.45	9.12	0.796		76	-1.3	85
L4	13.66	11.15	0.816		78	-1.0	88
Total	**44.06**	**35.63**	**0.809**		**80**	**-0.9**	**89**

Total BMD CV 1.0%, ACF = 1.025, BCF = 1.009, TH = 6.332

Comment:

Figure 12 : DEXA Scan report

INDICATIONS OF DEXA IN CHILDREN:

1. Osteogenesis imperfecta
2. Cerebral palsy epidermolysis bullosa
3. Endocrine Diseases : Type I- D.M,Hypogonadism, Growth hormone deficiency
4. Genetic Diseases: Turner syndrome, Cystic fibrosis, Marfan syndrome
5. Hematologic Diseases : Thalassemia, Sickle cell disease.
6. Glucocorticoid therapy as in nephrotic syndrome.
7. Anorexia nervosa

TREATMENT:

MANAGEMENT OF THALASSEMIA INDUCED LOW BMD:

Management of low BMD in thalassemia starts with prevention and treatment of early bone loss. So annual BMD check is essential. Regular physical activity should be encouraged along with other treatment modalities like

1. Nutrition and physical activity
2. Regular blood transfusion with Hb level above 9-10.5 g/dl.[75]
3. Calcium and vitamin D supplementation.[76]
4. Bisphoshonates.[98]
5. Calcitonin[95,97]
6. Hormonal replacement therapy[96]
7. Denosumab [77,78]

BISPHOSPHONATES IN PEDIATRICS:

Bisphosphonates are analogues of inorganic pyrophosphate, used in the management of calcium and bone metabolic disorders.[27] Use of bisphosphonates increasing in children for the treatment many diseases associated with bone metabolism in recent years.

Most commonly used bisphosphonates in children are Zolendronate, Alendronate, Pamidronate, Neridronate.

Name	R1	R2	Oral	Parenteral	Dose	Relative potency
Etidronate	OH	CH_3	+	+	5–40 mg/kg per day 400 mg per day per 2 weeks, every 3 months	1
Clodronate	C	Cl	+	+	1,200 mg per day (subdivided into 3 doses) 2 mg/kg per day (200–250 ml ISS 2–3 h, every 3–6 months)	10
Pamidronate	OH	$CH_2CH_2NH_2$		+	0.5–1.5 mg/kg per day for 3 days (200–250 ml ISS 3 h, every 2–6 months)	100
Neridronate	OH	$(CH_2)_5NH_2$		+	1–2 mg/kg per day (250 ml ISS/25 mg 3 h, every 3–6 months)	100
Alendronate	OH	$(CH_2)_3NH_2$	+		1–2 mg/kg per week 5 (<20 kg) to 10 (>20 kg) mg per day 70 mg per week	100–1,000
Ibandronate	OH	$CH_2CH_2N(CH_3)$(pentyl)		+	2 mg (250 ml ISS 2 h, every 3 months)	1,000–10,000
Risedronate	OH	CH_2-3-pyridine	+		15 mg per week (<40 kg) 30 mg per week (>40 kg) 2 mg/kg per week	1,000–10,000
Zoledronate	OH	CH_2-(imidazole)		+	0.015–0.05 mg/kg (50 ml ISS 30–45 min, every 3–6 months)	>10,000

R1: when an OH group binding to hydroxyapatite is enhanced. R2: it exerts a pharmacological effect (potency) on osteoclasts [1]. ISS = Isotonic saline solution. Note: the most widely used bisphosphonates in pediatric patients are alendronate, pamidronate and zoledronate.

FIGURE 13- Bisphosphonates Used In Pediatric Age Group And Doses.[79]

STRUCTURE:

They differ in structure from pyrophosphates, with carbon atom attached to phosphate in place of oxygen. Strong binding capacity of bisphosphonate to bone tissue is attributed to P-C-P motif.[13] Anti –resorptive efficiency of bisphosphonate depends on the R2 side chain.

Bisphosphonates are subdivided into two main groups based on the R2 chain.

1. Nitrogen-containing moiety.
2. Non-nitrogen containing moiety.

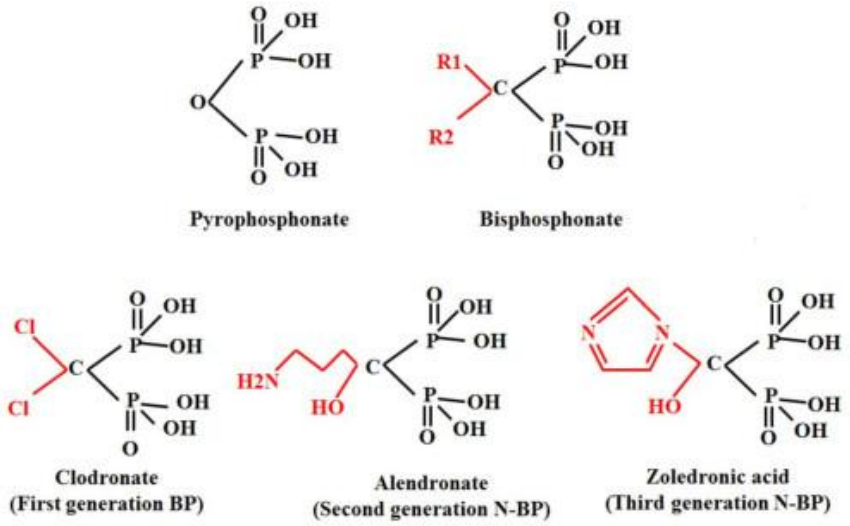


Figure 14: Structure of Bisphosphonate

Zolendronic acid is the most commonly used bisphosphonate in treating osteoporosis in adults which is available only in IV form but not oral. Alendronate is available in form and safe in children in view of less chances of hypocalcemia.

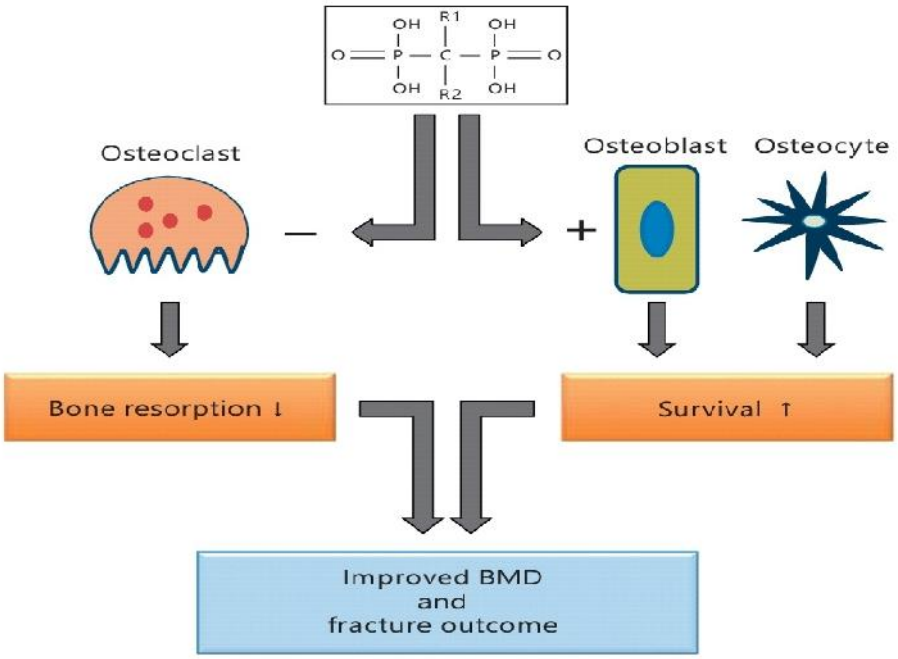


Figure 15 : Mechanism of action of bisphosphonates[92]

ALENDRONATE:

STRUCTURE & PROPERTIES:

Alendronate sodium trihydrate is a bisphosphonate which belongs to nitrogen-containing moiety, used commonly for the prevention or treatment of osteoporosis as a bone resorption inhibitor. It has a high affinity for calcium ions, so binds to bone.

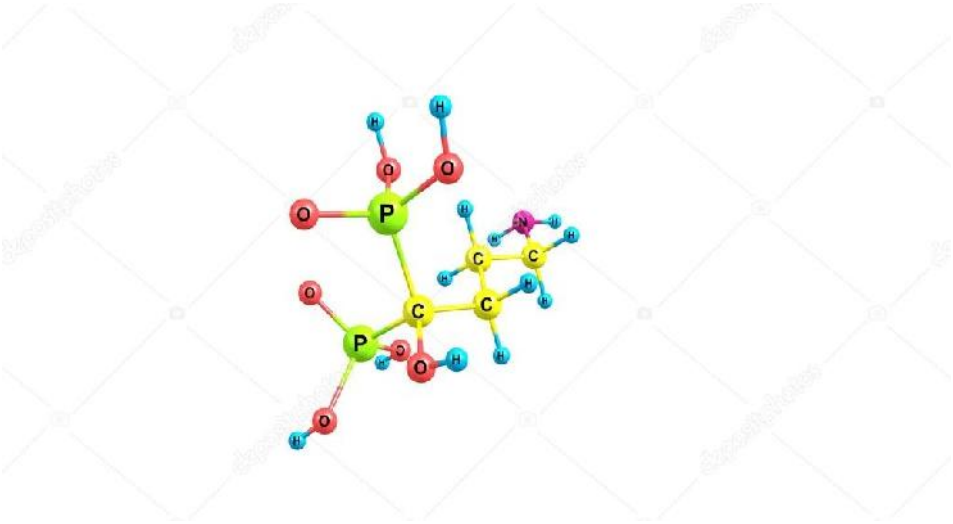


Figure 16 : Structure of alendronate.

PHARMACOKINETIC PROPERTIES: [81,83]

- Oral bioavailability was very low, about 0.64%. [82]
- Predominantly binds to albumin.[89]
- Only eliminated through the renal route.[82]
- Terminal elimination half-life is 10years in postmenopausal women.[81]
- Bioavailability will be reduced if taken along with beverages, calcium, antacids, food.
- Increase in pH increases its bioavailability.

MECHANISM OF ACTION: [84]

It mainly acts by inhibition of osteoclast function and stimulation of osteoblast & osteocyte activity OSTEOCLAST MEDIATED :

1. Induces apoptosis of osteoclasts and reduces its number.[80]
2. Disruption of ruffled border or cytoskeleton of osteoclasts
3. Effects on osteoclast precursors.

Metabolic effects of alendronate are

1. Inhibition of protein tyrosine phosphatase
2. Inhibition of steps in mevalonate pathway

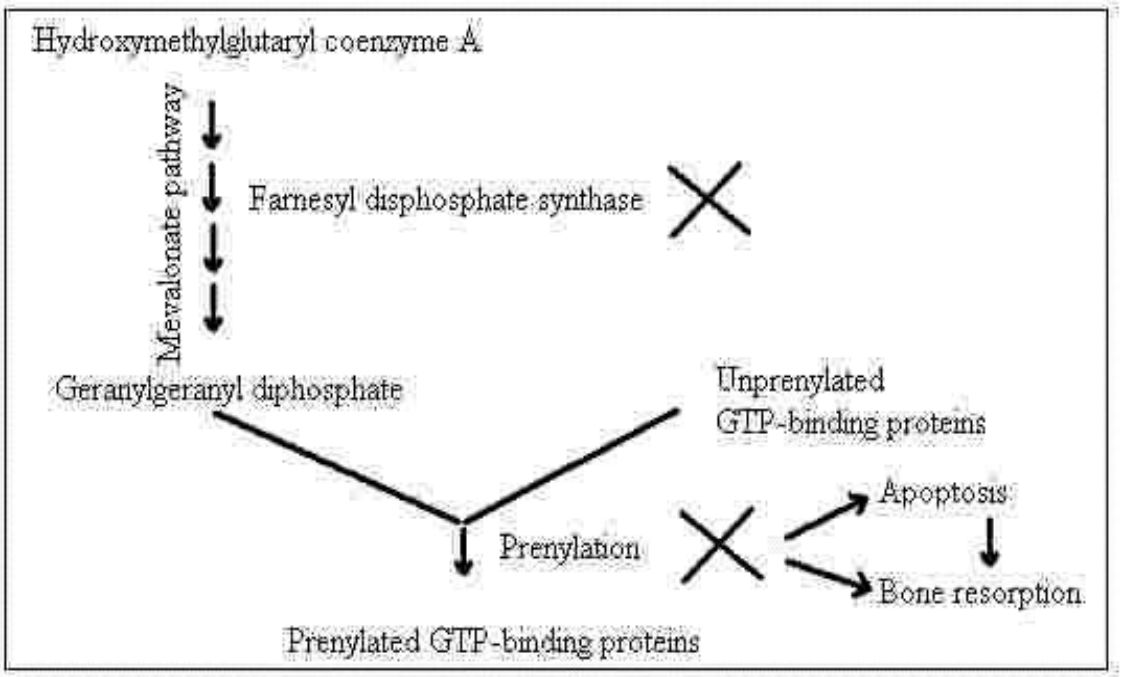


Figure 17: MOAof alendronate [84]

THERAPEUTIC USES:

Alendronate is being used in prevention and treatment of disorders related to calcium and bone metabolism.

USES:
Osteogenesis imperfecta
Glucocorticoid-induced osteoporosis
Vitamin D intoxication
Paget's disease.
Primary hyperparathyroidism.
Malignancy-induced hypercalcemia
Osteoporosis in thalassemia.

DOSAGE:

Alendronate is given in two schedules, one is 70mg once in a week for adults and other is 5mg for <20kg, 10 mg for >20kg once daily in children(Baroncelli & Bertelloni, 2014). Tablet should be taken on an empty stomach, along with plenty of water and should not lie down or sleep for 30minutes after taking the tablet to avoid esophagitis, reflux disease. Alendronate should not be taken along with anta cids, calcium supplements, food or with beverages which will affect the absorption through the gut.

ADVERSE EFFECTS:[85]
Esophagitis
Oesophageal ulcer
Erosive esophagitis[86]
Dysphagia[87]
Abdominal pain, nausea
Constipation /diarrhea.
Hypocalcemia
Delay in bone healing[91]

Alendronate has an acute mucosal damage rate comparable with nonsteroidal antiinflammatory drugs. Abdominal pain, nausea, dyspepsia, constipation and diarrhea were reported in 3 to 7% of patients receiving alendronate in two large three-year studies.[88]

A cross-sectional observational study was conducted by Mahesh et.al in a tertiary care centre in Delhi from November 2011 to January 2013, aged 2–18 years comparing total of 32 patients and 32 age and sex-matched healthy controls. The mean height (133.84 ± 13.40 cm), weight (32.28 ± 10.25 kg), and BMI (17.70 ± 3.86 kg/m2) of cases were lower in comparison to controls but only weight ($P = 0.023$) and BMI ($P = 0.030$) were statistically significant. (Delhi). This study shows children with transfusion-dependent thalassemia are more prone to low bone mineral densities than normal children.

A randomized, placebo-controlled study done by Morabito et al (2002) to evaluate oral alendronate or clodronate intramuscular administration on BMD for 2 years, safety & tolerability in 25 osteoporotic thalassemia patients. DEXA scan (Hologic QDR 4500 W, Waltham, MA) was done to assess BMD at lumbar spine and femoral neck. Patients were randomized into three groups, each group takes a placebo (8 patients) or clodronate 100 mg, i.m., once in 10 days (8 patients) or 10 mg alendronate oral every day (9 patients) along with 500 mg/d calcium & 400 IU cholecalciferol/day. Alendronate treatment daily has normalized bone turnover rate, and lead to a rise in BMD at the spine and the hip.[28] Alendronate caused few side effects like esophagitis, but there is no discontinuation of any patient.

Skordis N et.al conducted a study is to evaluate the effect of Alendronate and Pamidronate on bone mass in thalassemia major patients, treated in St George's University London Medical School at the University of Nicosia on 53 patients (22 males, 31 females). Thalassaemic patients arbitrarily divided into 2 groups. Group A contains 29 patients with a mean age of 33, 32 years were given alendronate and group B contains 24 patients with a mean age of 34, 36 years taken pamidronate for 2 years duration. With the improvement in bone mineral density (BMD) values, the effectiveness of both drugs will be compared. In Pamidronate group, mean BMD after treatment improved from -2.813 to -2.174 ($p<0.001$) at the lumbar spine and the mean BMD at the hip from -2.138 to -2.078 ($p=0.018$). After alendronate treatment, BMD at the lumbar spine was from -2.720 to -2.602 ($p=0.059$) and the changes in BMD at the femoral neck from -2.035 to -2.007 ($p=0.829$).

Omid Reza Zekavat et al. studied the relative efficacy of Alendronate & Zoledronic acid in 734 thalassemia major patients from thalassemia centre in Iran,

Dastgheib hospital, Shiraz University of Medical Sciences. Mean age of the study group is 29.1 ± 8 years. Patients with age more than 9 years having Low Bone Mineral density with a Z score of − 2.0 in the femoral neck or lumbar (L1-L4) spines. Patients are divided into two groups for treatment. One group received Alendronate (Fosamax, Merck & Co., Inc) 70 mg weekly for 1-year duration. Other groups were treated with Zolendronate (Zometa, Novartis®) 4 mg (0.05 mg/kg in children) Iv infusion over 30 min once in 6months for 2years. calcium 500 mg/day and vitamin D 400 IU supplements were given daily. BMD was assessed by using the Hologic system DXA (Discovery QDR, USA). Z-score has improved from − 3.06 ± 0.99 S.D to − 3.04 ± 0.83 in the alendronate group.[29]

Fatemeh Shirani et.al conducted a study on the evaluation of Alendronate efficacy on bone mineral density in Iran on thalassemia patients between 20 to 50 years old. osteoporotic patients were diagnosed based on DEXA scan after which placed on treatment of oral regimen of alendronate 10mg daily. After a year, their densitometries were repeated and compared to the changes in BMD(g/cm) and T-score. serum calcium, phosphorus and alkaline phosphatase levels were measured at the beginning and the end of the year and the results were compared.[30]

Rashid Merchant et al. conducted a study to evaluate osteopathy in thalassemia patients by bone mineral densitometry (BMD) and biochemical indices on 42 children with age group of 10-25 years using the similar software in our present study LUNAR PRODIGY G.E, showing prevalence of osteoporosis(Z-score <-1 S.D) as 50% and osteopenia (Z-score <-2 S.D) as 31%.[6] In this study, there is statistically significant increase in osteoporosis with advancing chronological age(P value = 0.046).

METHODOLOGY

This study was done from January 2018 to December 2018 under the Paediatrics Department, KLES Dr. Prabhakhar Kore Hospital & Medical Research Center, Belagavi.

Study design

The study design was a hospital-based one-year longitudinal study.

Study duration and period

This one year study was carried out from January 2018 to December 2018.

Place

This study was conducted in the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi a tertiary care teaching hospital attached to J.N Medical College, Belagavi.

Source of data

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

Sample size

A total of 42 registered cases of transfusion-dependent beta-thalassemia were studied.

Sampling procedure

The minimum sample size formula based on prevalence is

n =

n = sample size

where P is the percentage of prevalence 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 α = 1.96. With P = 65% and d = 25% of P, the sample size is 33 For a better result, the sample size would be taken around 42. According to this formula, the minimum effect size to estimate the effect of alendronate was 33. However, 42 children fulfilled the selection criteria during the study period hence were enrolled.

SELECTION CRITERIA

Inclusion Criteria

Patients between 8-18 years old, who are diagnosed as thalassemia major on regular blood transfusions.

Exclusion criteria

1) Skeletal dysplasias

2) Other haemoglobinopathies like sickle cell disease

3) Congenital heart diseases

4) Chronic infections like TB, HIV, HEP-C

Already on drugs like calcium supplements

Ethical clearance

In the beginning, the study was approved by the Ethical and Research Committee, J.N Medical College, Belagavi.

Informed Consent

The parents of children who fulfilled the inclusion criteria were briefed about the nature of the study and written informed consent was taken from parents before enrolment in the study. (Annexure I).

Method of collection of data

Children between the age of 8-18 years of age who are enrolled in thalassemia daycare centre of KLE university's JN medical college hospital, BELAGAVI and

who fulfil inclusion criteria were chosen. Detailed history was taken and informed consent was obtained from the parents after explaining the purpose of the study. Patients data were recorded in a structured proforma.

Then the child was assessed for bone mineral density by DEXA scan of the lumbar spine (L1-L4) which is the gold standard for measuring bone mineral density, along with serum calcium, phosphate, serum albumin at the time of enrolment. Children who are with any skeletal dysplasia, congenital heart diseases, chronic infections like HIV, TB, HEP-C, already on drugs like calcium supplements and other haemoglobinopathies like sickle cell disease were excluded.

Based on the Z –Scores obtained in the DEXA scan, children whose Z-scores less than -1 gm/cm2 were selected as low bone mineral density. Then the patient was given calcium supplements, vitamin-D and oral bisphosphonates (alendronate at a dose of 10mg daily) for 6 months, followed by which reassessment of bone mineral density was done with DEXA scan of the lumbar spine. The selected children were treated with alendronate 10 mg/kg/day orally for 6 months. Calcium was supplemented at 500mg/day and vitamin-D3 400 units/day.

TAB ALENDRONATE (BRAND NAME - OSTEOFOS 35MG) was divided into 10mg sachets by drug division and dispensed to parents. Tab CALCIMEN, containing calcium 500 mg, vitamin –D3 400 IU was given. The parents and children were counselled to take alendronate tablet on empty stomach before 30minutes of breakfast and were advised not to lie down for 1 hour to prevent oesophagitis . Side effects associated with alendronate like gastritis, oesophageal burn, vomiting, GERD, giddiness, constipation, abdominal pain were explained.

If the above symptoms were noted, the child was prescribed Tab RANTAC (ranitidine 150mg) to take orally, and drink plenty of water. The patients were asked to continue their chelating agents and folic acid.

INVESTIGATIONS:

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

· Human immunodeficiency virus (HIV) – at the time of enrolment

· Hepatitis C virus (HCV) - at the time of enrolment

· Surface antigen for hepatitis B virus (HBs-AG) - at the time of enrolment

· Hemoglobin - at the time of enrolment

· Renal function tests - at the time of enrolment

· Liver function tests - at the time of enrolment

· Serum ferritin levels - at the time of enrolment

· Calcium, phosphorous - at the time of enrolment

· Alkaline phosphatase - at the time of enrolment

· Ultrasonography for liver and spleen - at the time of enrolment.

· ECG - at the time of enrolment.

HIV, HCV, HBsAG was done at the time of enrolment, as it was a part of the exclusion criteria. Haemoglobin estimation was done at the time of every admission before blood transfusion as a part of the guidelines. Renal function tests were done at the time of enrolment to look for any renal dysfunction because alendronate is known to have renal excretion and may cause renal toxicity. Liver function tests and ferritin levels are done to look for correlation between their levels and severity of low bone mineral density. Calcium was done at the time of enrolment to look for any

hypocalcemia because calcium levels should be normal before starting bisphosphonates which are known to cause hypocalcemia. ECG was done at the time of enrolment to rule out arrhythmias

ECHOCARDIOGRAM - PHILIPS CX 50 REVISION 3.1.1 SOFTWARE

DEXA SCAN: at the time of enrolment and after 6months of treatment with alendronate and calcium, vitamin – D3 supplementation.

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 haemoglobin. Another plain bulb containing 4ml will be sent for urea, creatinine, serum ferritin, thyroid profile, calcium, phosphorous, liver function test.

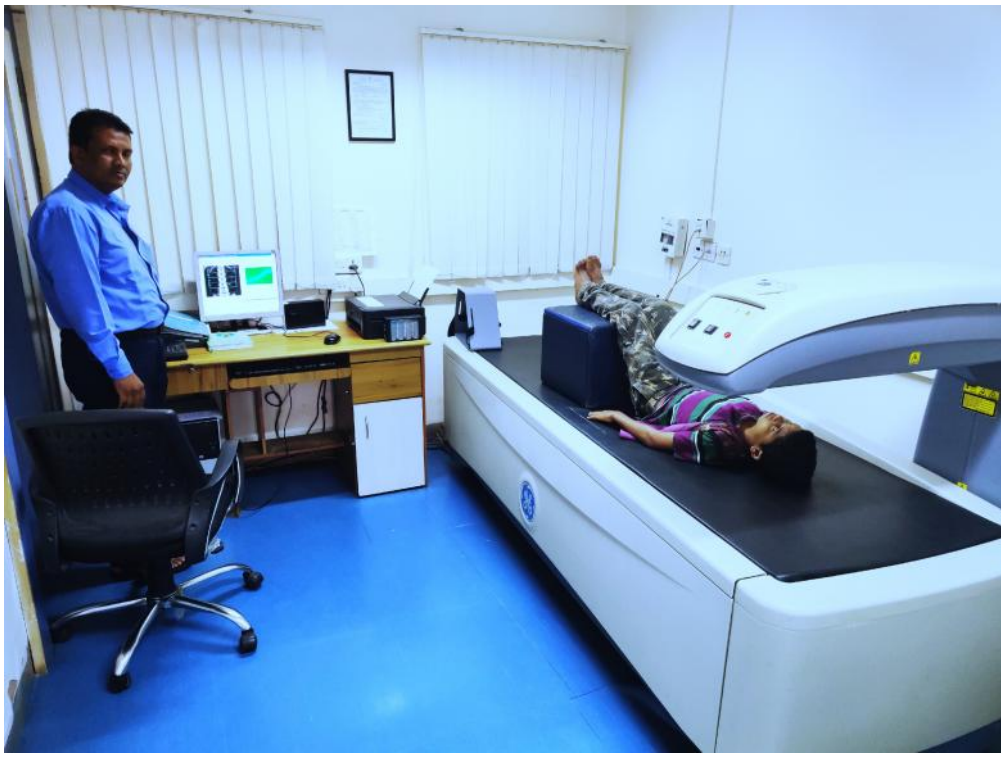
Estimation of Hemoglobin was done by Cyanomethaemoglobin method on Mindray CAL 80 analyser, before the commencement of the study.

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

Estimation done by COBAS E 501 analyser.

• Urea – urease UV method
• Creatinine – enzymatic method(IFCC-IDMS)
• Bilirubin – diazotization
• Total Protein – biuret method
• Albumin – BCP method
• SGPT,SGOT – U.V without P5P method
• Alkaline phosphatase – PNPP,AMP Buffer method

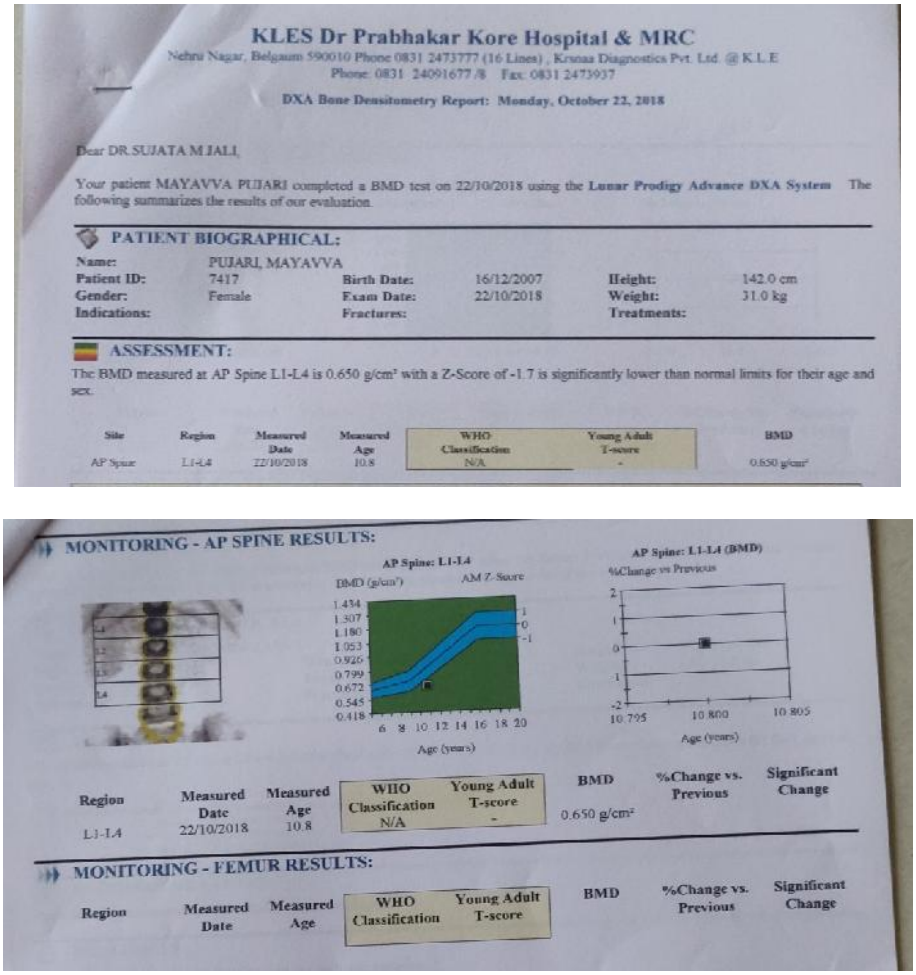
Photograph 1- Beckmann Coulter - For Complete Blood Picture Analysis



Photograph – 2 DEXA Machine – Lunar Prodigy Pediatric Software

DEXA SCAN:

In the present study, Lunar Prodigy Advance Pediatric Software System was used for assessment of bone mineral density. “Z-scores is the number of standard deviations (SD) above or below the mean for the patient’s age, sex and ethnicity”(26). Usage of T-score is inappropriate and to be avoided while reporting in a pediatric DEXA scan report. It is a simple test, which is non-invasive, without any radiation effects taken in supine position with 90 degree flexion at hip and knees.


KLES Dr Prabhakar Kore Hospital & MRC

Nehru Nagar, Belgaum 590010 Phone 0831 2473777 (16 Lines) , Krsnaa Diagnostics Pvt. Ltd. @ K.L.E

Phone: 0831 24091677 /8 Fax: 0831 2473937

DXA Bone Densitometry Report: Monday, October 22, 2018

Dear DR.SUJATA M JALI,

Your patient MAYAVVA PUJARI completed a BMD test on 22/10/2018 using the Lunar Prodigy Advance DXA System The following summarizes the results of our evaluation.

PATIENT BIOGRAPHICAL:

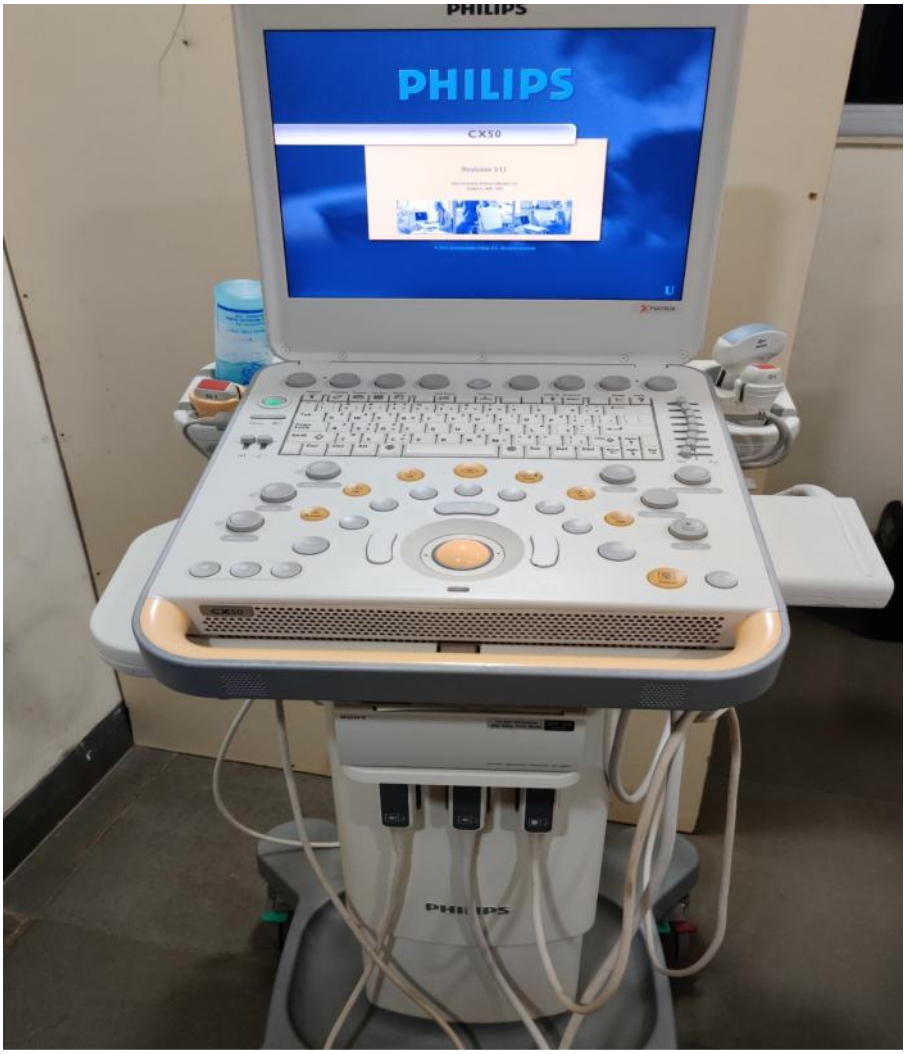
Name:	PUJARI, MAYAVVA				
Patient ID:	7417	Birth Date:	16/12/2007	Height:	142.0 cm
Gender:	Female	Exam Date:	22/10/2018	Weight:	31.0 kg
Indications:		Fractures:		Treatments:	

ASSESSMENT:

The BMD measured at AP Spine L1-L4 is 0.650 g/cm² with a Z-Score of -1.7 is significantly lower than normal limits for their age and sex.

Site	Region	Measured Date	Measured Age	WHO Classification	Young Adult T-score	BMD
AP Spine	L1-L4	22/10/2018	10.8	N/A	-	0.650 g/cm²

Photograph – 3 DEXA Scan Report



Photograph - 4: Echocardiogram - Philips CX 50 Revision 3.1.1 Software

FOLLOW UP

The children were treated and followed up to 6months of treatment to evaluate the compliance and side effects/complications of alendronate. The child was examined per abdomen, checked for heart rhythm, and evaluated clinically when the child used to get admission for regular blood transfusions.

Outcome variables

The children were monitored for the following parameters.

PREVALENCE OF LOW BONE MINERAL DENSITY

BONE DENSITY AND IMPROVEMENT IN BONE MINERAL DENSITY

Prevalence of Low Bone Mineral Density in transfusion-dependent thalassemia children will be checked.

Z-scores will be compared before and after receiving 6 months of bisphosphonates (alendronate). Improvement in bone mineral density was assessed concerning Z-scores after receiving 6 months of alendronate, vitamin-D3 and calcium.

SEVERITY OF LOW BONE MINERAL DENSITY:

Based on Z-scores it was categorized as

> -1 gm/cm^2	NORMAL
-1 to -2 gm/cm^2	OSTEOPENIA
< - 2 gm/cm^2	OSTEOPOROSIS

Statistical analysis

The data was coded and tabulated on excel spreadsheet and master chart was prepared (Annexure IV). The data was analyzed using SPSS version 20.0 statistical software. The categorical data was expressed in terms of rates, ratios and percentages and the continuous data was expressed as mean ± standard deviation. Student 't' test/ paired t test was used to find the improvement in Z-scores before and after 6months of the treatment. A probability value (p value) of less than or equal to 0.05 at 95% confidence interval was considered as statistically significant.

This hospital based prospective interventional study was done from January 2018 to December 2018. A total of 42 registered cases of transfusion dependent beta thalassemia under the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi were enrolled. All the 42 children underwent DEXA scan of which 26 children had low bone mineral density. These 26 children were treated with tablet alendronate 10mg/day once daily for 6months since the dose for children above 20kg is 10mg/day. They were supplemented with tablet calcium 500mg/day and tablet vitamin D 400 IU daily orally for 6 months and evaluated for osteoporotic recovery in terms of improvement in Z-scores in DEXA scan.

The data was analyzed and the final results were tabulated and interpreted as below.

RESULTS

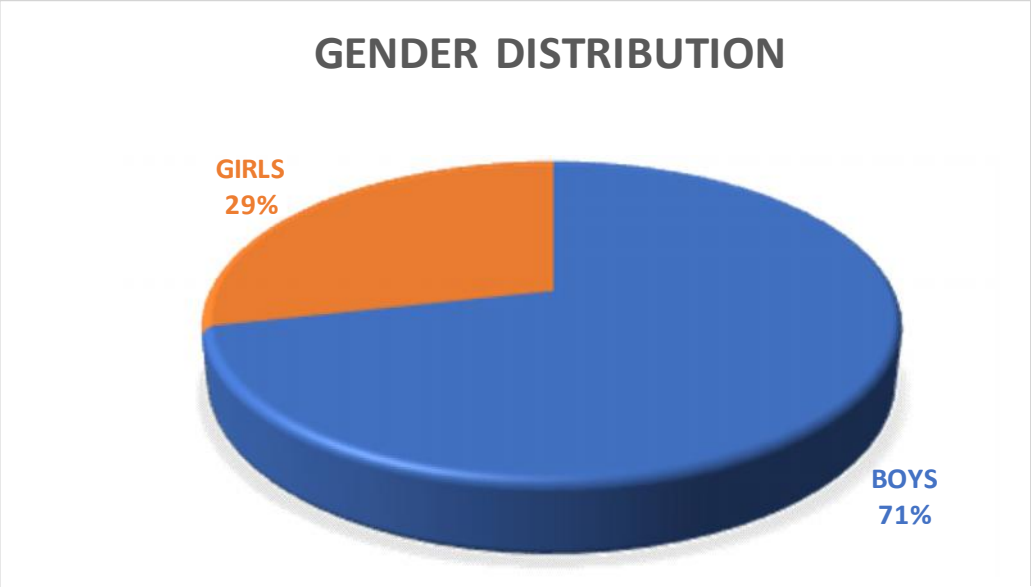
This hospital-based prospective interventional study was done from January 2018 to December 2018. A total of 42 registered cases of transfusion-dependent beta-thalassemia under the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi were enrolled. These children were treated with alendronate 10 mg/day once daily orally along with calcium and vitamin-D supplementation for 6 months and evaluated for improvement in bone mineral density and prevalence of osteoporosis.

The data was analyzed and the final results were tabulated and interpreted as below.

Table 5. Distribution of children according to gender.

GENDER	Distribution (n= 42)	
	Number	Percentage
Boys	30	71.42
Girls	12	28.58
Total	42	100

GRAPH 1.

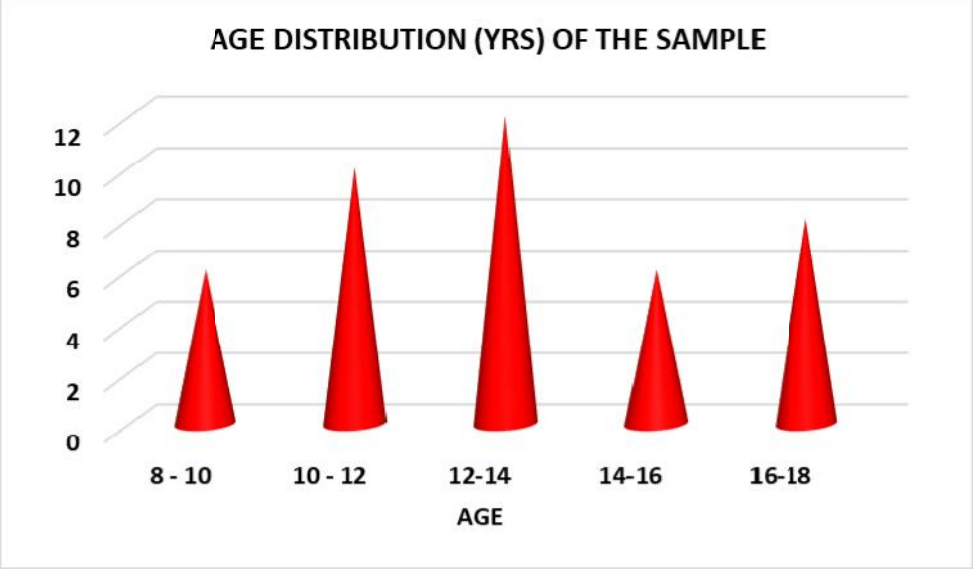


In the present study, most of the children were boys (71%) and 29 % of the children were girls and the boy to girl ratio was 2.5:1.

Table 6. Distribution of children according to the age

Age group (YEARS)	Distribution (n=42)	
	NUMBER	PERCENTAGE
8 – 10	6	14.29
10 – 12	10	23.81
12-14	12	28.57
14-16	6	14.29
16-18	8	19.05
TOTAL	42	100.00

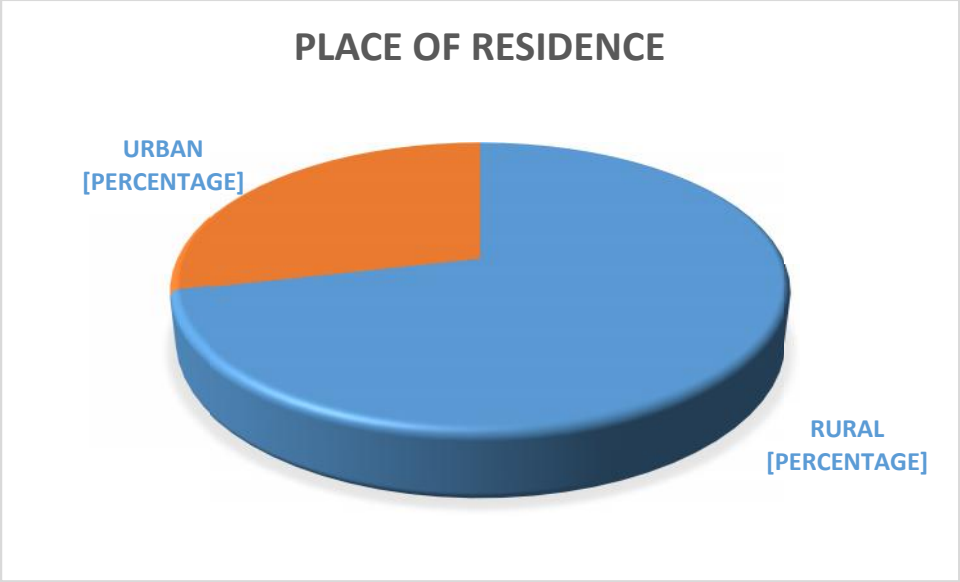
GRAPH 2.



In this study most of the children 22 (52%) were aged between 8 to 12 years and nearly 11 (26%) were between 13 to 15 years and 8 (19%) were between 16 to 18 years .The mean age was 12.64 $\pm$ 2.77 years. The median age was 12 years and ranged between 8 years to 18 years.

Table 7. Distribution of children according to the place of residence

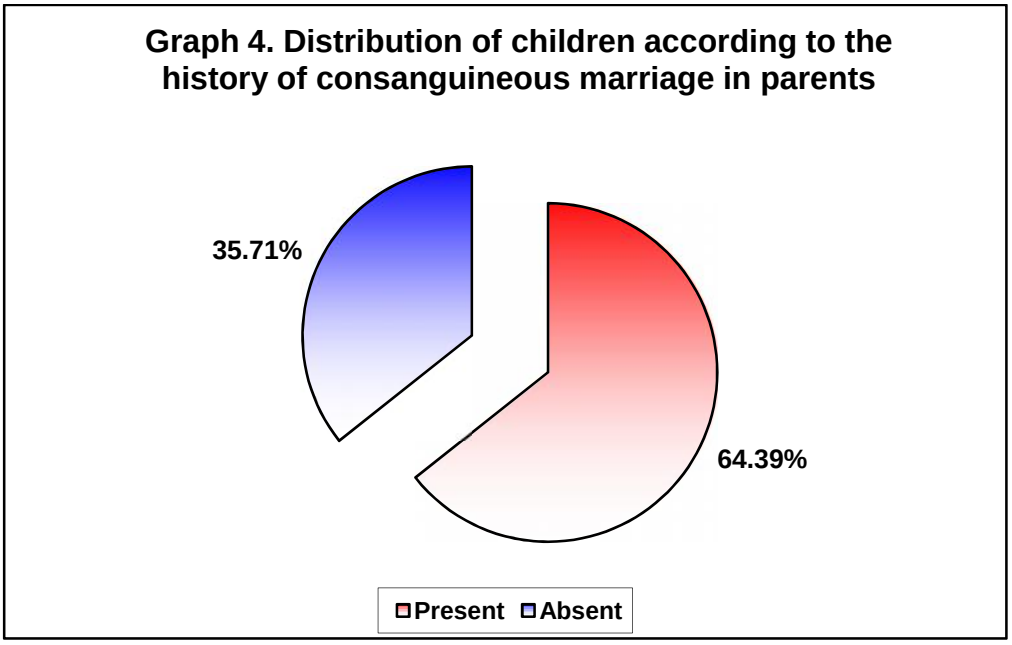
Plate of residence	Distribution (n=42)	
	Number	**Percentage**
Urban	12	29
Rural	30	71
Total	**42**	**100.00**



In the present study, majority of children 30 (71%) belonged to rural area, 12(29%) belongs to urban area.

Table 8. Distribution of the children according to the history of consanguineous marriage in parents

History	Distribution (n=42)	
	Number	**Percentage**
Present	27	64.28
Absent	15	35.71
Total	**42**	**100.00**

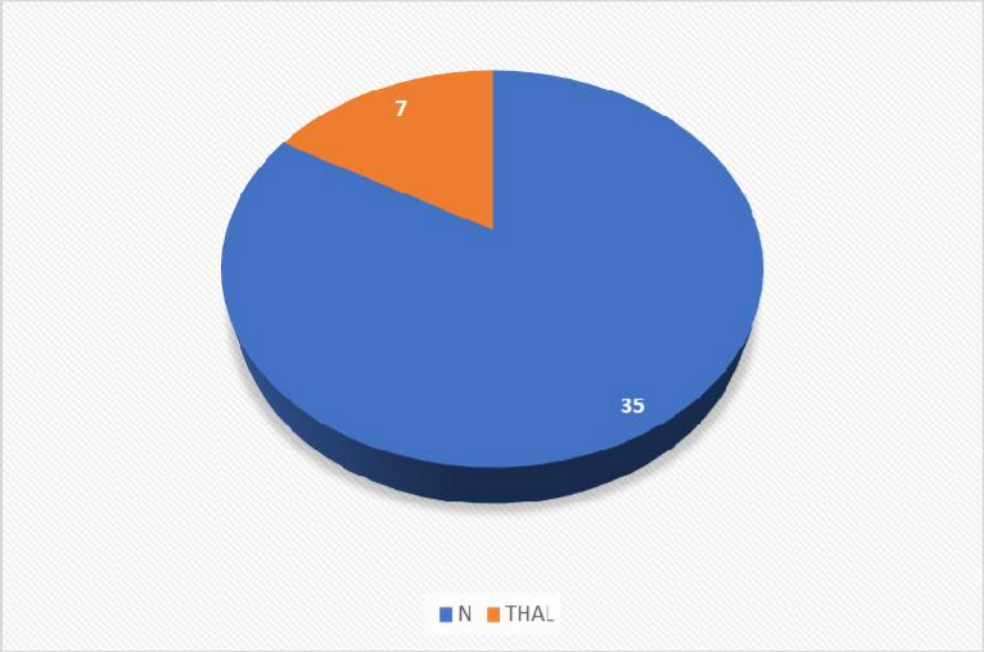


In the present study 64.39 % of the children's parents reported history of consanguineous marriage.

Table 9. Thalassemia status of the siblings in the family

SIBLING STATUS	NUMBER	PERCENTAGE
Normal	35	83.33
Thalassemia	7	16.67
TOTAL	42	100.00

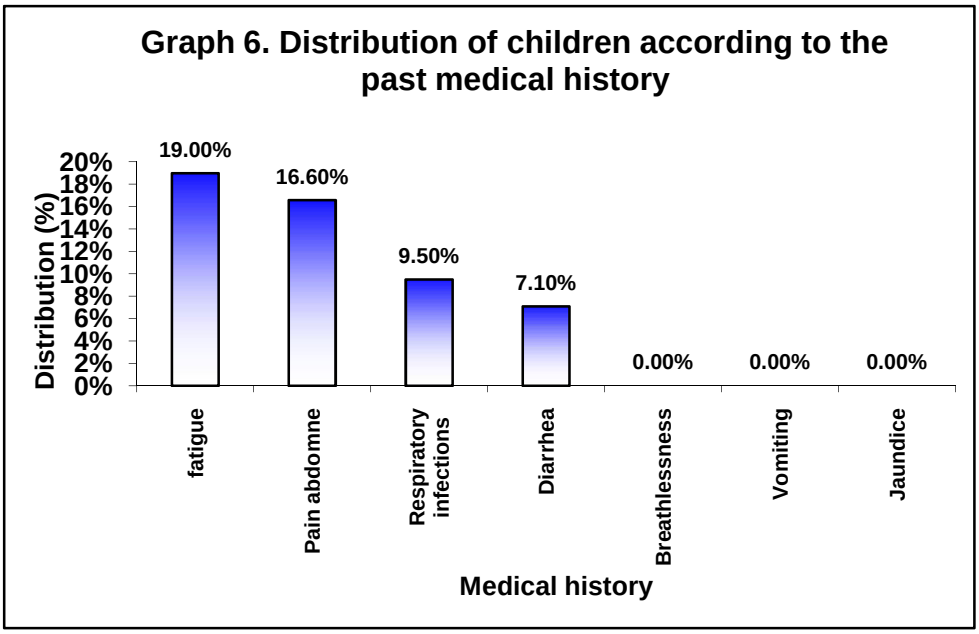
Graph 5.Thalassemia status of the siblings in the family



In the present study, Thalassemia status of the siblings in the family was noted in 7 children (16%).Death was noted in 3 siblings.

Table 10. Distribution of children according to medical history.

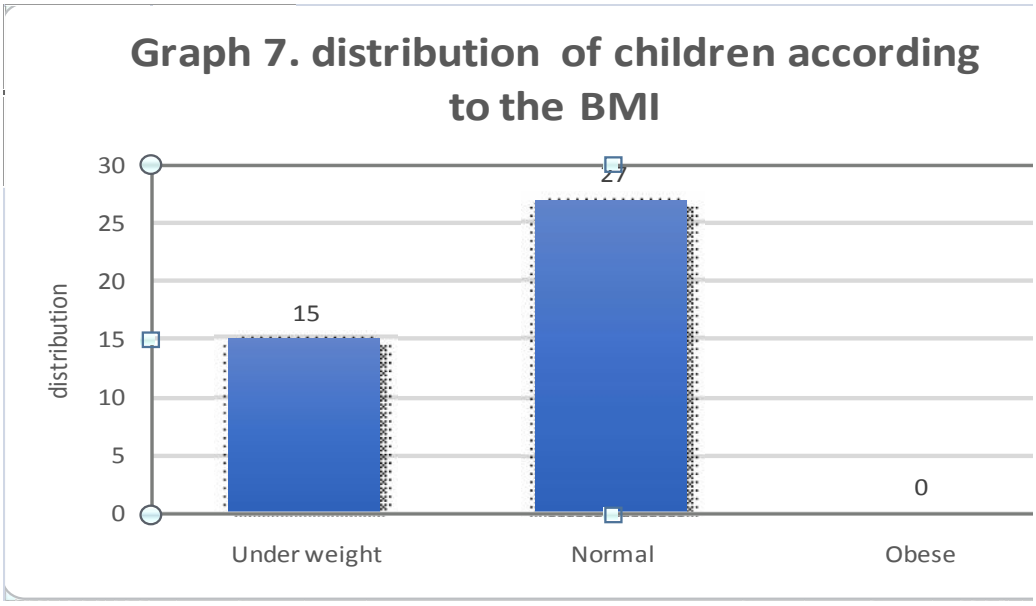
Medical history	Distribution (n=42)	
	Number	Percentage
Fatigue	8	19.00
Pain abdomen	7	16.60
Respiratory infections	4	9.50
Diarrhea	3	7.10
Breathlessness	0	0.00
Vomiting	0	0.00
Jaundice	0	0.00



In this study most of the children had history of fatigue (19%) followed by pain abdomen (16.6%), respiratory infections (9.5%) and diarrhoea (7.5%).

Table 11. Distribution of children according to the BMI (WHO)

Body Mass Index	Distribution (n=42)	
	Number	**Percentage**
Under weight	15	35.71
Normal	27	64.29
Obese	0	0.00
Total	42	100

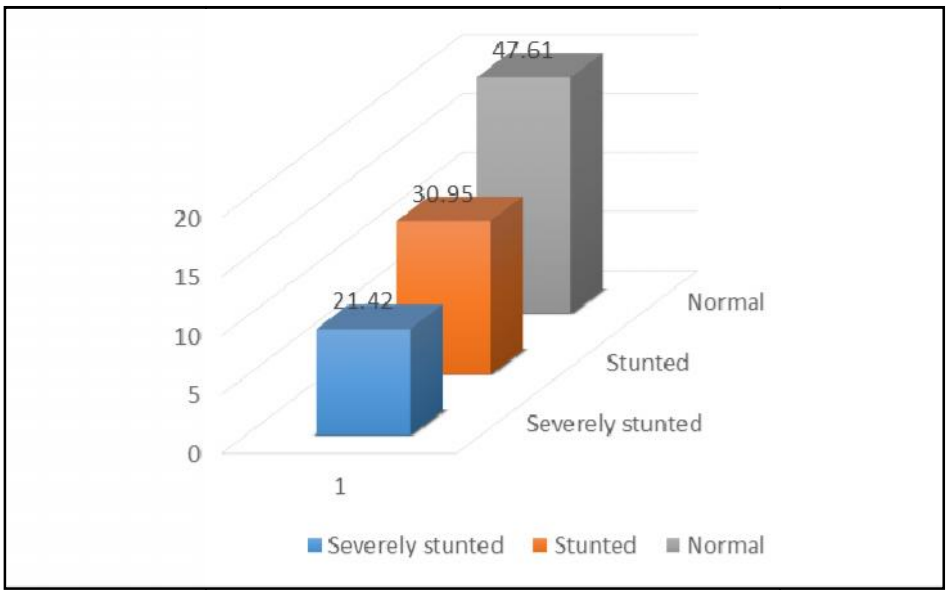


In this study 15 children (35.71%) are Under Weight and most of the children 27(64.29%) are normal.

Table12. Distribution of children according to the height for age (WHO)

Height for age	Distribution (n=42)	
	Number	**Percentage**
Severely stunted	9	21.42
Stunted	13	30.95
Normal	20	47.61
Total	42	100

Graph 8. Distribution of children according to the height for age

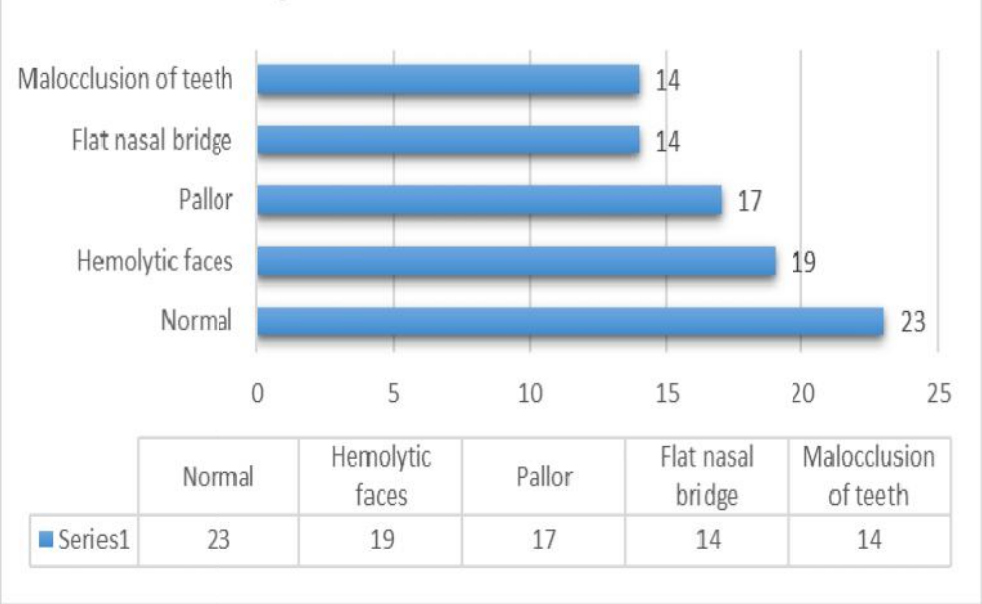


In the present study most of the children had short stature 22(52.38%), of which 13(30.95%) children had stunting and 9 (21.42%) children were severely stunted. 20 children (47.62%) had normal height for age.

Table 13. Distribution of the children according to head to toe examination

Findings	Distribution (n=42)	
	Number	Percentage
Normal	23	54.76
Hemolytic faces	19	45.23
Pallor	17	42.50
Flat nasal bridge	14	35.00
Malocclusion of teeth	14	35.00

Graph 9. Distribution of the children according to the head to toe examination

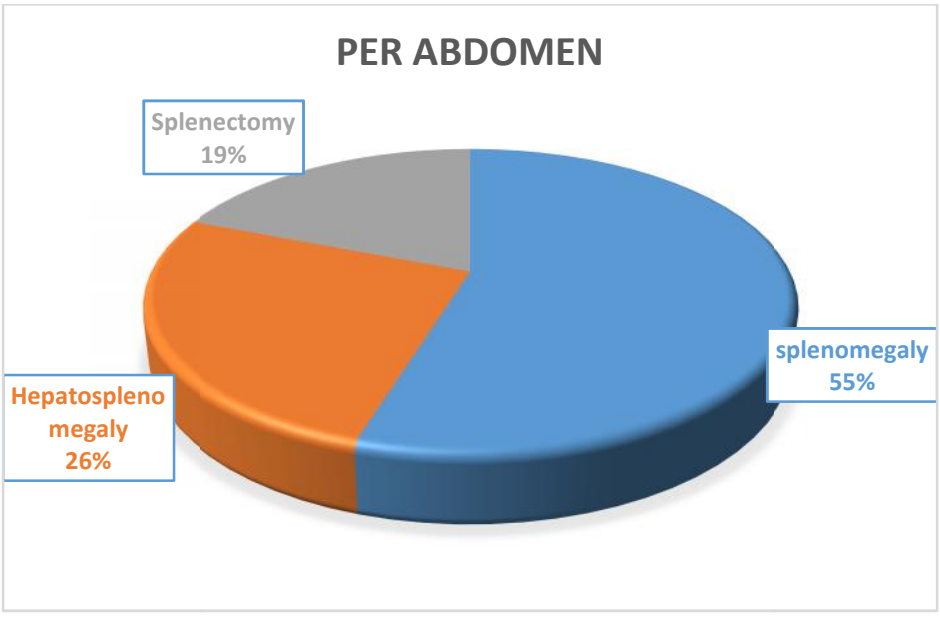


In the present study head to toe examination revealed hemolytic faces in 45.23%, pallor in 42.5% of the children and flat nasal bridge, malocclusion of teeth in 35% of the children.

Table 14. Distribution of the children according to the systemic examination - Per abdomen

Findings	Distribution (n=42)	
	Number	**Percentage**
Splenomegaly	23	54.76
Hepatosplenomegaly	11	26.19
Splenectomy	8	19.00
Total	**42**	**100.00**

Graph 10. Distribution of the children according to the systemic examination - Per abdomen

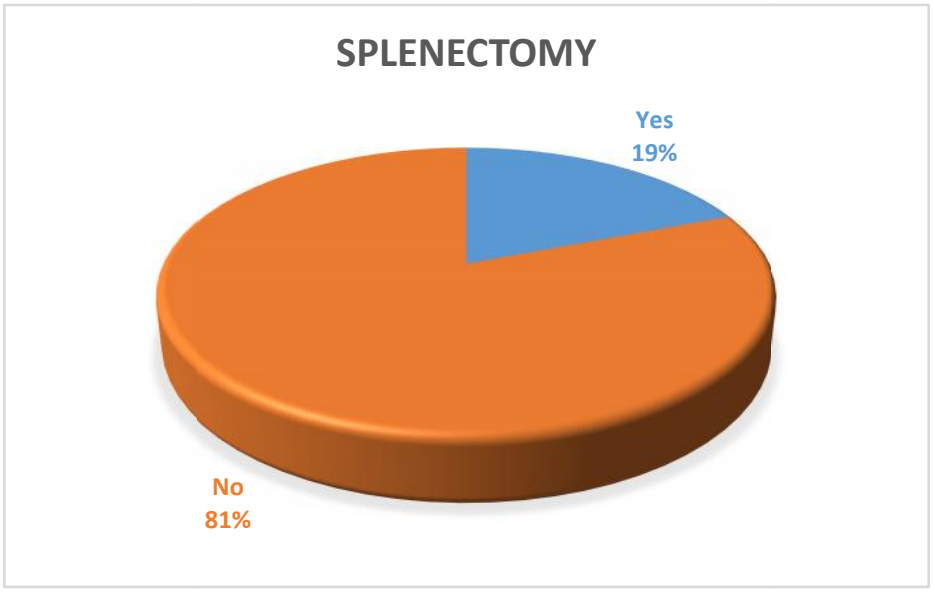


In the present study hepatosplenomegaly was noted in 26%, splenectomy in 19% and splenomegaly in 55%.

Table 15. Distribution of the children according to the splenectomy

Splenectomy	Distribution (n=42)	
	Number	**Percentage**
Yes	8	19.00
No	34	81.00
Total	**42**	**100.00**

Graph 11. Distribution of the children according to the splenectomy

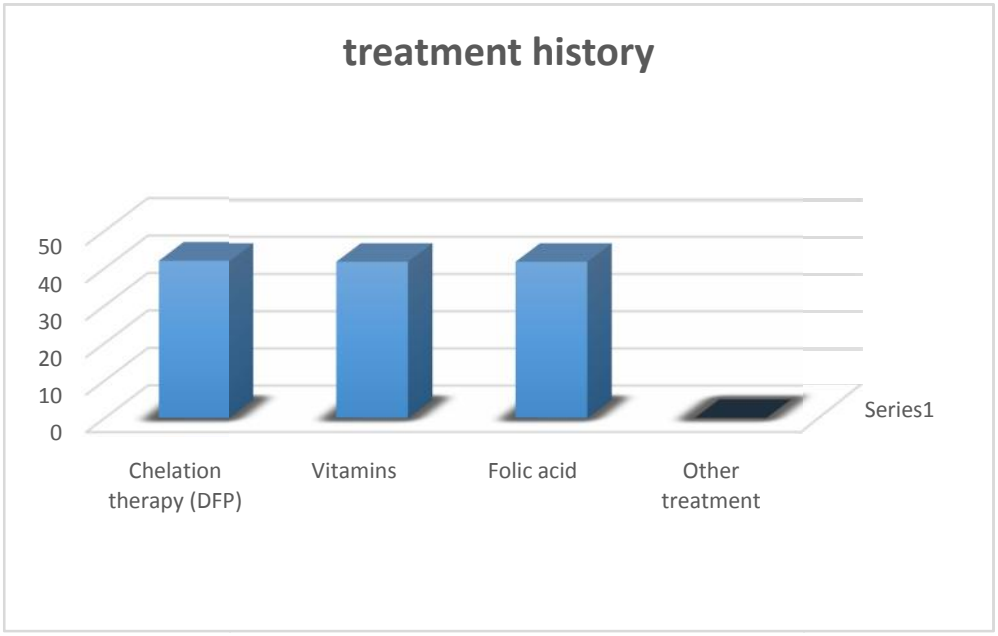


In the present study, Out of 42 children, 8 (19%) children underwent splenectomy.

Table.16: Distribution of the children according to the treatment history

History	Findings	Distribution (n=42)	
		Number	**Percentage**
Treatment history	Chelation therapy (deferasirox)	42	100.00
	Vitamins	42	100.00
	Folic acid	42	100.00
	Other treatment	0	0.00
	Total	**42**	**100.00**

Graph 12. Distribution of the children according to the treatment history



In the present study, 42 children are on iron chelation therapy (Deferasirox), Vitamins, Folic acid supplements.

Table : 17 Blood Parameters

	MEAN	S.D.	MINIMUM	MAXIMUM
HEMOGLOBIN	8.99	0.87	6.8	10.5
BLOOD UREA	25.34	6.25	12	43
CREATININE	0.61	0.17	0.1	0.9

TABLE 18: CALCIUM, PHOSPHOROUS, FERRITIN, ALBUMIN

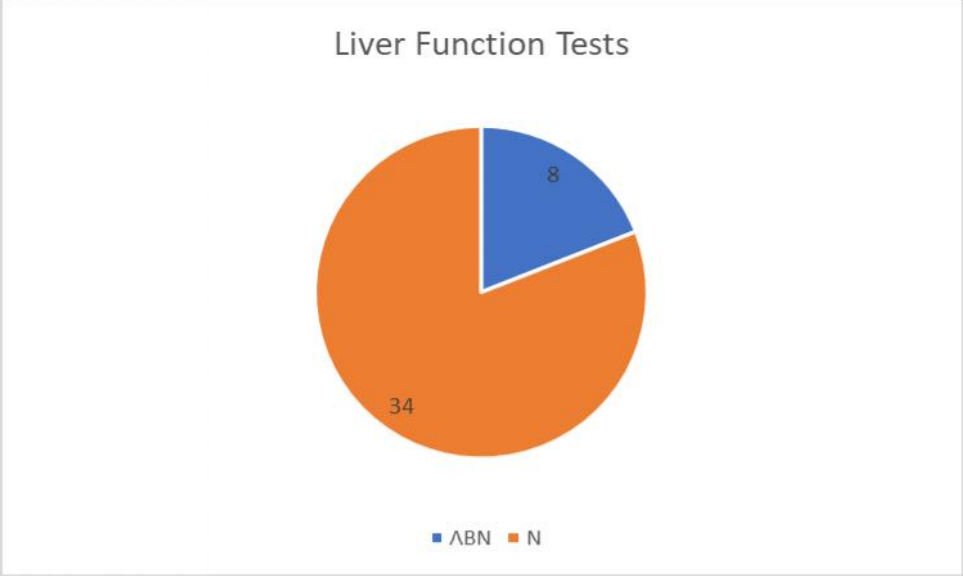
	MEAN	S.D.
FERRITIN	3352.10	2075.59
CALCIUM	8.97	0.33
PHOSPHOROUS	3.90	0.83
ALBUMIN	4.08	0.40
ALKALINE PHOSPHATASE	358.10	165.41

In the present study, Mean haemoglobin was noted as 8.99 $\pm$ 0.87 gm/dl. Mean Blood Urea was noted as 25 $\pm$ 6.25 mg/dl, mean creatinine as 0.61 $\pm$ 0.17.Mean ferritin was noted as 3352.10 $\pm$ 2075.59 mg/dl and calcium was noted as 8.97 $\pm$ 0.33 mg/dl. Mean phosphorous and albumin levels were noted as 3.90 $\pm$ 0.83 gm/dl and 4.08 $\pm$ 0.40 gm/dl.

Table .19-Liver Function Tests:

Liver Function Tests	NUMBER	PERCENTAGE
Abnormal	8.00	19.05
Normal	34.00	80.95
TOTAL	42.00	100.00

Graph 13. Liver Function Tests



In the present study, Liver function tests were abnormal in 19.05% of the children and normal in 80.95% of the children.

Table 20. DEXA scan in all patients- Z-SCORES.

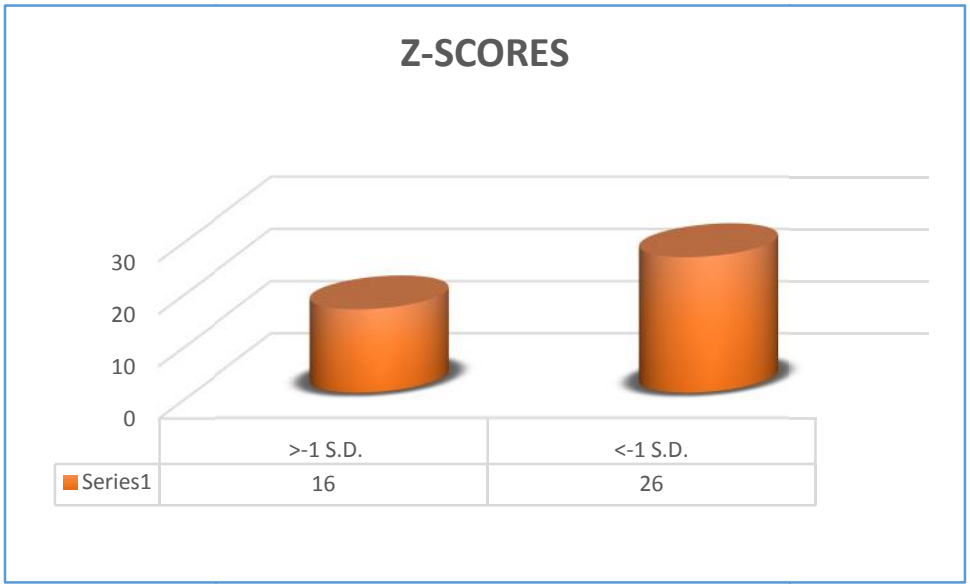
GROUP	MEAN	S.D.	MINIMUM	MAXIMUM	Number
All patients	-1.15	1.49	-4.7	1.2	42
	MEAN	**S.D.**	**MINIMUM**	**MAXIMUM**	**Number**
Low bone mineral density	-2.18	0.83	-4.7	-1.2	26

In the present study, mean Z-scores of all 42 patients in the DEXA scan notes as -1.15 $\pm$ 1.49 S.D and in low bone mineral density group as -2.18 $\pm$ 0.83 S.D.

Table 21: Prevalence of low bone mineral density:

B.M.D	Z-SCORES	Distribution (n=42)	
		Number	**Percentage**
NORMAL	>-1 S.D.	16	29.1
LOW	<-1 S.D.	26	61.9
Total		**42**	**100.00**

Graph 14. Prevalence of low bone mineral density

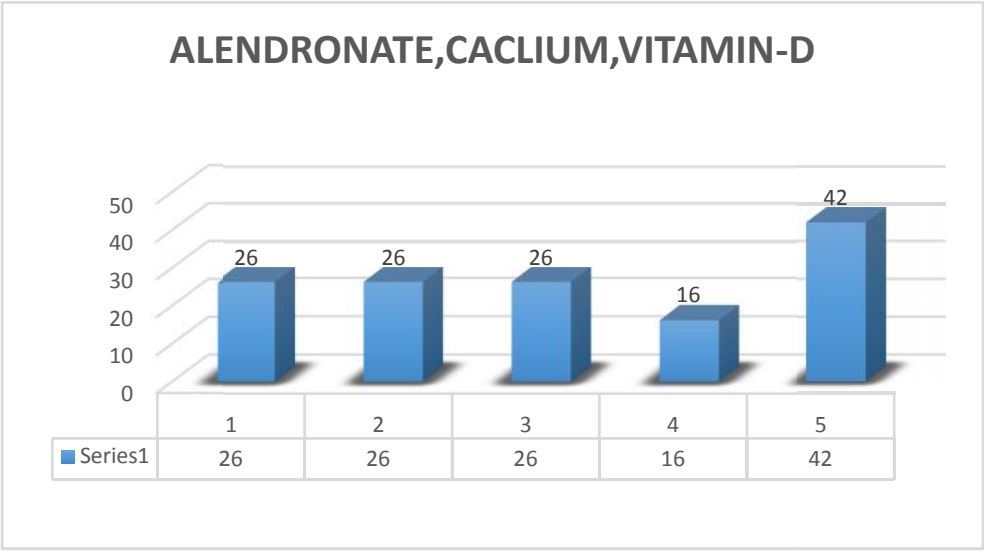


In this study, based on Z-scores, there were total 26 children (61.9%) with low bone mineral density (Z- SCORE <-1 S.D.) and 16 children with normal bone mineral density.

Table 22: No. Of Children On Oral Alendronate, Calcium, Vitamin-D

History	treatment	Distribution (n=42)	
		Number	**Percentage**
Treatment history	ALENDRONATE	26	61.90
	CALCIUM	26	61.90
	VITAMIN – D3	26	61.90
	NOT ON ALENDRONATE	16	28.10
	Total	**42**	**100.00**

Graph 15. No. Of Children on Oral Alendronate, Calcium, Vitamin-D

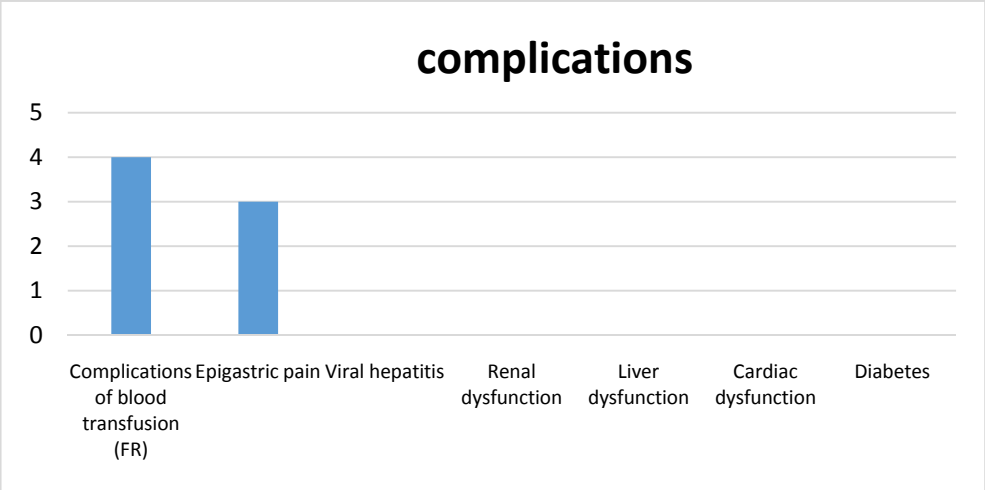


Out of 42 children, 26 (61.9 %) of the children are on Alendronate, calcium and vitamin –D3 supplementation.

Table 23.Distribution of the children according to the treatment complications.

Complications	Distribution (n=40)	
	Number	Percentage
Complications of blood transfusion (FR)	4	9.50
Epigastric pain	3	7.14
Viral hepatitis	0	0.00
Renal dysfunction	0	0.00
Liver dysfunction	0	0.00
Cardiac dysfunction	0	0.00

Graph 16. Distribution of the children according to the complications



In the present study, 4 (9.5%) children had developed minor blood transfusion reactions in the form of rash, fever and chills. 3(7.14%) children had complications of Alendronate tablet in the form of epigastric pain and gastritis. No other complications were present.

Table 24: Relation between Gender and Low Bone Mineral Density:

	OSTEOPOROSIS	**OSTEOPENIA**	**NORMAL**	**TOTAL**
FEMALES	3 (7.14)	4 (9.52)	5 (11.90)	12
MALES	11 (26.19)	8 (19.05)	11 (26.19)	30
TOTAL	14	12	16	42
P -value = 0.7636				

In the present study, osteoporosis is seen in 3 female children, 11 male children. Osteopenia in 4 female and 8 male children. There is no correlation between gender and prevalence of low bone mineral density in the present study.

Table 25: Residence and Low Bone Mineral Density:

	RURAL	**URBAN**	***Row Totals***
Normal	14	2	16
Osteopenia	8	4	12
Osteoporosis	8	6	14
	30	12	**42 (Grand Total)**

Chi-square statistic is 3.5583.The *p*-value is .168779.

There is no correlation between residence and prevalence of low bone mineral density in the present study.

Table 26: Socioeconomic Status And Low Bone Mineral Density

SOCIO-ECONOMIC STATUS	OSTEOPOROSIS	OSTEOPENIA	p VALUE	INFERENCE
Class 3	4	2	0.4726	NS
Class 4	10	10		

There is no correlation between socioeconomic status and prevalence of low bone mineral density in the present study.

Table 27: Short Stature & Low Bone Mineral Density

	Osteopenia	Osteoporosis	Normal	***Totals***
short stature	6 (6.29)	13 (7.33)	3 (8.38)	22
normal	6 (5.71)	1 (6.67)	13 (7.62)	20
N	12	14	16	**42 (Grand Total)**
P value is 0.000264 and statistically significant.				

Short stature is commonly seen in low bone mineral density and it is highly prevalent in children with osteoporosis. Relation between short stature and prevalence of low bone mineral density is highly significant.

Table 28: Low BMI& Bone Mineral Density

BMD	B.M.I		
	LOW	NORMAL	***Row Totals***
Normal	1 (5.71)	15 (10.29)	16
osteopenia	2 (4.29)	10 (7.71)	12
osteoporosis	12 (5.00)	2 (9.00)	14
Column Totals	15	27	**42 (Grand Total)**

P value is <0.00001 and statistically significant.

Relation between BMI and prevalence of low bone mineral density is highly significant.

Table 29 : Ferritin Levels And Z-Scores

	MILD<2000	MODERATE 2000-4000	SEVERE>4000	TOTAL
Normal	10	2	1	13
Osteopenia	6	4	3	13
Osteoporosis	3	5	8	16
	19	11	12	**42**

The chi-square statistic is 10.9013. The *p*-value is .027696. The result is significant at $p < .05$.

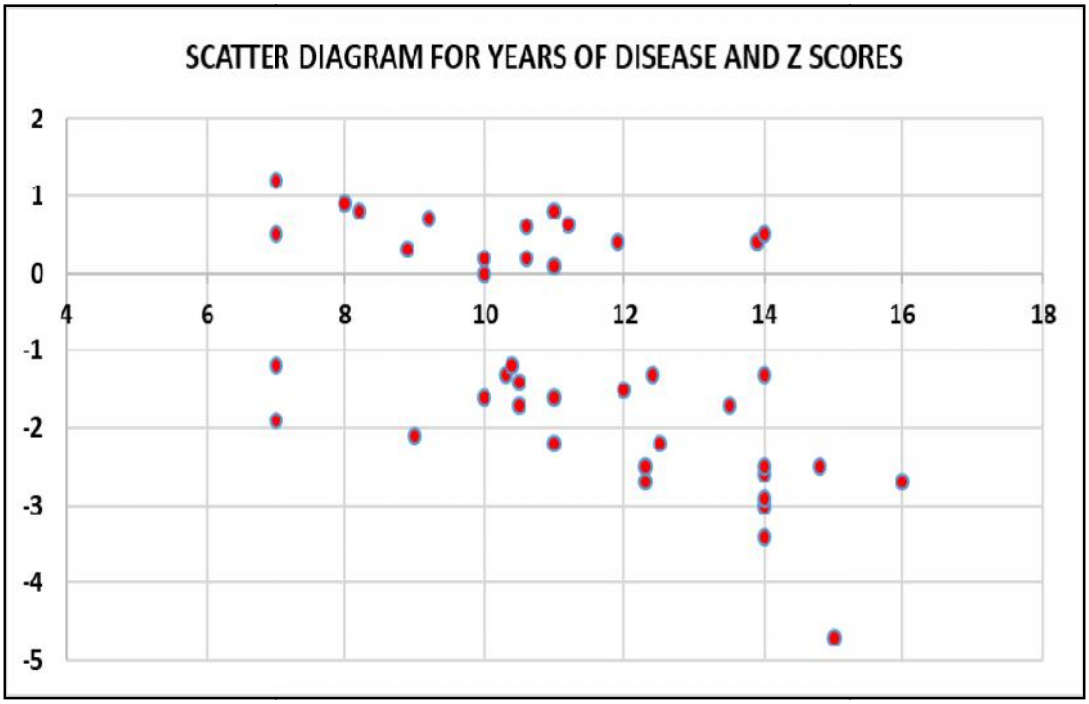
Relation between BMI and prevalence of low bone mineral density is highly significant.

Table 30: Pearson's Correlation Coefficient

BETWEEN YEARS OF THE DISEASE AND Z SCORES :

r	p VALUE	INFERENCE
-0.5843	< 0.0001	HS

Graph 17. BETWEEN YEARS OF THE DISEASE AND Z SCORES:



- There is significant correlation between longer the duration of the disease and low bone mineral density in the present study with r value -0.5843.
- P value was also very significant (P value < 0.0001).

Table 31: calcium levels and low bone mineral density:

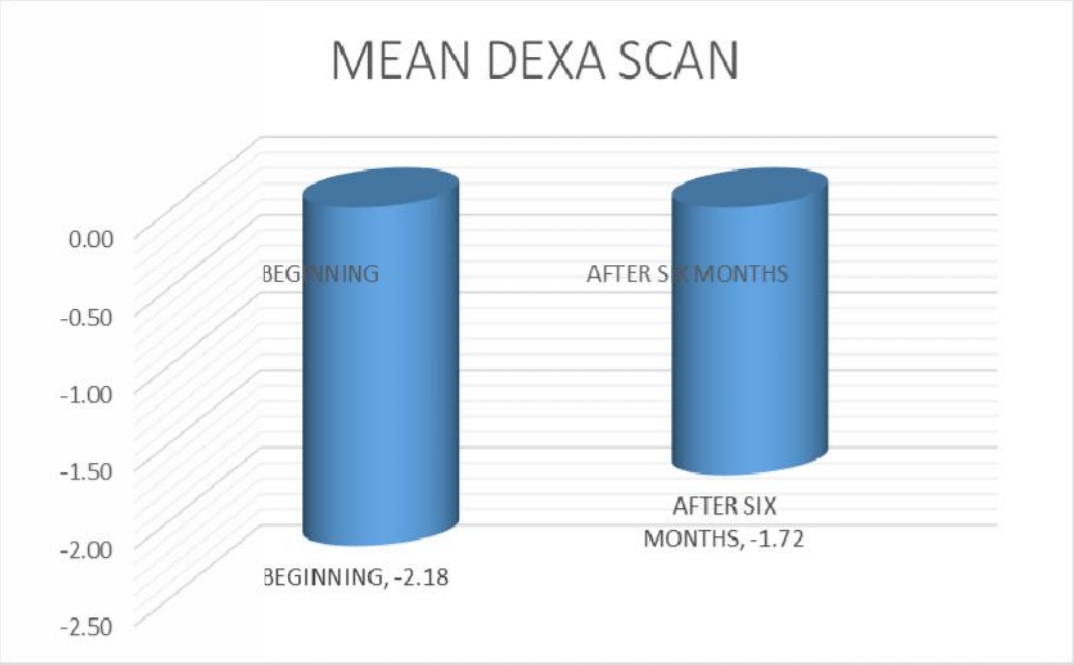
	OSTEOPOROSIS		OSTEOPENIA			
	MEAN	**S.D.**	**MEAN**	**S.D.**	**p VALUE**	**INFERENCE**
CALCIUM LEVELS	8.86	0.38	9.00	0.35	0.3310	NS

There is no correlation calcium levels and prevalence of low bone mineral density in the present study (P value = 0.3310).

Table 32 : Z-Scores Before And After 6months Of Treatment:

BEGINNING				AFTER SIX MONTHS					
MEAN	S.D.	MINIMUM	MAXIMUM	MEAN	S.D.	MINIMUM	MAXIMUM	p VALUE	INFERENCE
-2.18	0.83	-4.7	-1.2	-1.72	0.66	-3.6	-1	< 0.0001	HS

Graph 18. Z-scores before and after 6months of treatment

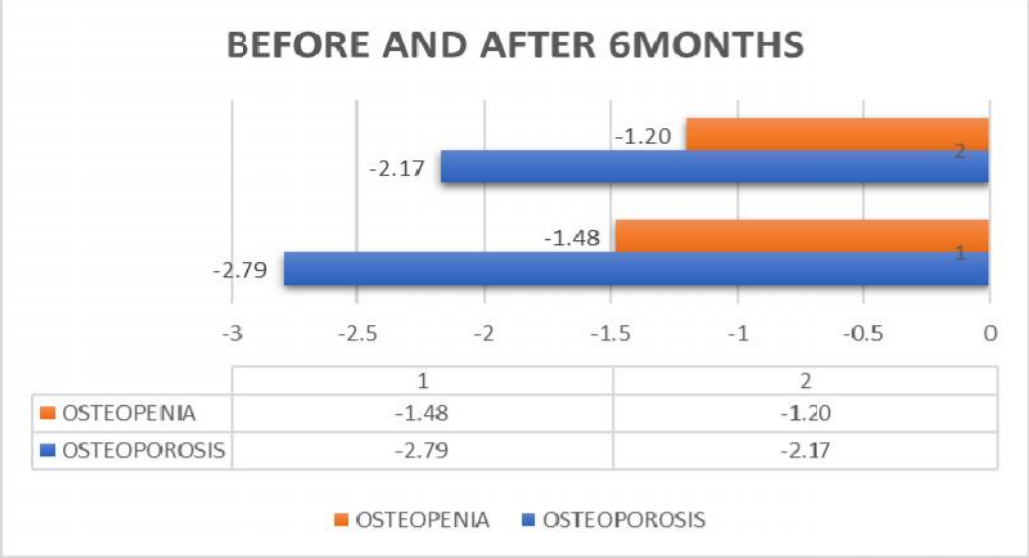


- The mean Z-score has significantly decreased after 6 months of alendronate intake (-2.18 ± 0.83 S.D to -1.72 ± 0.6, p value <0.0001) which is highly significant.

Table 33: Comparison Between Osteoporosis And Osteopenia Group

GROUP	DEXA BEGINNING			DEXA AFTER 6 MONTHS			
	NUMBER OF PATIENTS	MEAN	S.D.	MEAN	S.D.	p VALUE	INFERENCE
OSTEOPOROSIS	14	-2.79	0.66	-2.17	0.57	< 0.0001	HS
OSTEOPENIA	12	-1.48	0.23	-1.20	0.20	0.0046	VS

Graph 19 Comparisonsbetween Osteoporosis and Osteopenia Group



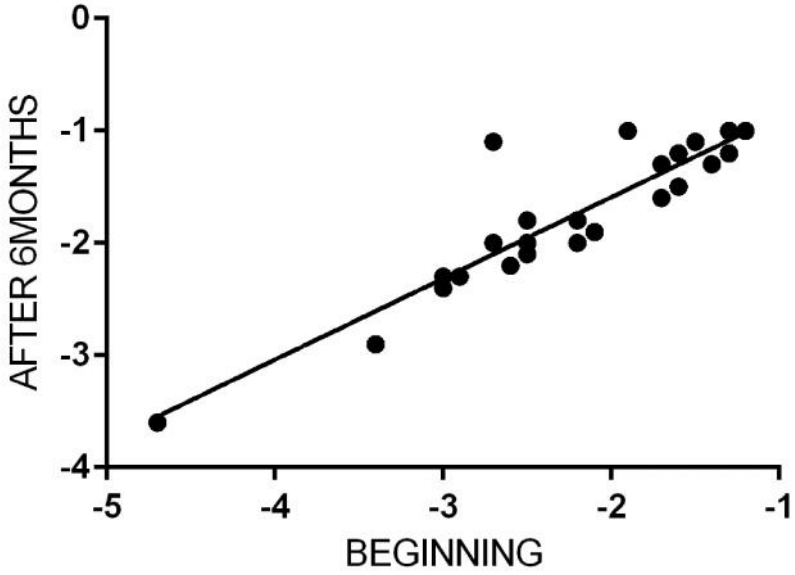
- The mean Z-score decreased after 6 months of alendronate intake from -2.79 ± 0.66 to -2.17 ± 0.57 S.D. in osteoporosis group which is highly significant.(p value= < 0.0001)

- The mean Z-score decreased after 6 months of alendronate intake from -1.48 to -1.20 S.D in osteopenia group, which is very significant (p value = 0.0046).

Table 34. Pearson Coefficient Between Z-Scores Before And After 6months Of Alendronate Treatment:

r	p VALUE	INFERENCE
+ **0.8368**	< 0.0001	HS

GRAPH 20. CORRELATION BETWEEN Z-SCORES BEFORE AND AFTER 6MONTHS OF ALENDRONATE TREATMENT:



There is significant correlation between Z-scores before and after 6 months of treatment with Alendronate. (r value = +0.8368, P value <0.0001).

Table 35.Relation Between Age And Prevalence Of Low Bone Mineral Density.

GROUP	8-11 years	11-14 years	15-18 years	***Row Totals***
normal	10 (62.5%)	5 (31.25%)	1 (6.2%)	16
osteopenia	5 (35.7%)	4 (28.57%)	5 (35.7%)	14
osteoporosis	1 (8.33%)	2 (16.66%)	9 (75%)	12
	16	11	15	**n = 42**
χ^2= 14.9898. The *p*-value is significant with 0.004722				

There is increase in prevalence of low bone mineral density with more age group. Children in the age group of 15-18 years had high prevalence with 9 out of 12 (75%) of osteoporosis. There is significant correlation between age and prevalence of low bone mineral density with P value of 0.004722.

SCOPE AND LIMITATIONS OF THE STUDY

1. Overall , the findings of the present study was that ,treatment with Alendronate 10mg/day along with calcium and vitamin D supplementation is highly effective in improving the bone mineral density in transfusion-dependent thalassemia patients with low bone mineral density, by its anti-resorptive action.
2. Alendronate is safe and well-tolerated in pediatric patients with minor side effects like abdominal pain, esophagitis and without any major side effects like atypical fracture/osteonecrosis of the jaw in the present study.
3. Prevalence of osteoporosis in the present study is 33.3% and osteopenia as 28.57% which is high. Severity of osteoporosis is more with increasing chronological age and duration of disease in years.
4. DEXA is a highly precise and gives accurate measurement of BMD in children. So annual check-up of BMD assessment will be helpful for early detection and prevention of skeletal morbidity.
5. Alendronate is useful in the prevention and treatment of low bone BMD in patients with transfusion-dependent thalassemia.
6. While the findings of the present study need further validation due to limitations like single center study, small sample.
7. Hence further multicentric studies involving large sample size with longer follow up may be helpful in giving precise role of Alendronate in the treatment of transfusion dependent thalassemia children with low BMD.

DISCUSSION

Beta thalassemia is a common genetic disorder and also an important public health problem in many countries. With chronic blood transfusions, Beta thalassemia is associated with multi-organ involvement like endocrinological, cardiac, skeletal, hypogonadal problems. Osteoporosis is the common morbidity seen in transfusion-dependent thalassemia patients, its incidence will be seen increasing with age. Chiefly, it is associated with multiple blood transfusions leading to iron overload & toxicity, delayed puberty, hypogonadism, hypothyroidism, marrow expansion, even chelation therapy with desferrioxamine. It is very important to identify the osteopenia or osteoporotic changes as early as possible to avoid complications like bony deformities, scoliosis, fractures, nerve compression and growth failure. DEXA scan is the gold standard for diagnosing osteoporosis in thalassemia children, which was a non-invasive and simple method.

Treatment of Osteoporosis in thalassemia major includes physical exercise, vitamin –D, calcium supplementation, hormone replacement therapy, bisphosphonates & calcitonin. Most commonly used bisphosphonates in children are alendronate, Zolendronate, pamidronate, clodronate, out of which alendronate was available in oral form. Alendronate is an orally available bisphosphonate which is safe and effective in treating osteoporosis in children, without any major side effects. Alendronate acts by inhibiting osteoclastic activity and increasing osteoblastic activity. Hence the present study was an attempt to assess the efficacy of alendronate in osteoporotic recovery in patients with transfusion-dependent beta-thalassemia major by evaluating bone density with DEXA scan.

This hospital-based longitudinal study was done from January 2018 to December 2018.

The minimum effective sample size to estimate the efficacy of alendronate treatment was 33. There are 205 registered cases of thalassemia in thalassemia day care unit, under the Department of Pediatrics, KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi. Out of 205 cases, 84 cases are transfusion-dependent thalassemia major aged between 8 to 18 years which fulfilled the inclusion criteria. However, based on computerized randomization, 42 children were enrolled. All these 42 children were screened with DEXA scan (LUNAR PRODIGO, G.E. pediatric software) to look for bone mineral density. Out of 42 children, 26 children have low bone mineral density with Z-score <-1 S.D which is low for the appropriate matched age and sex. All the 26 children were treated with alendronate 10mg/day once daily as all the children were above 20kgs,[31] along with calcium 500mg/day and vitamin – D 400 IU daily orally for 6 months and evaluated for osteoporotic recovery in terms of improvement in Z-scores in DEXA scan.

In the present study, most of the children were boys 30 (71.42%) and 12(28.58 %) of the children were girls and the boy to girl ratio was 2.5:1 suggesting male preponderance. The male predominance observed in the present study was in agreement with a similar study done by Mahesh M et al. (2013) to compare bone mineral density in thalassemia major patients with healthy controls, where out of total 32 children, 21 (65.6) males and 11(34.4) females(32). 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.[17] Ideally, β-thalassemia major which is having an autosomal

recessive inheritance, males and females should be in equal proportion but the 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 8 to 18 years. The mean age was 12.64 $\pm$ 2.77 years and the median age was 12 years. More than half 22 (52%) were aged between 8 to 12 years, nearly 11 (26%) were between 13 to 15 years and 8 (19%) were between 16 to 18 years. DEXA scan will be helpful to detect osteopenia/osteoporosis after 8years of age and is more common in the adolescent age group.[33] The age group is in agreement to a similar study done by Khalaf Hussein about bone mineral density in patients with β-thalassemia major in Duhok city with a mean age of 12.8 $\pm$ 1.68 years.[33]

Concerning demographic characteristics, in the present study, the majority of the children 30 (71.5%) belong to the rural area. Furthermore, the history of consanguineous marriage was reported by 61 % of the children parents. Thalassemia status of the siblings in the family was positive in 7 children (16%) and the death of the sibling was seen in 3 families (7.14%). 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.[17] 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 4(9%) children had a history of heartburn, followed by pain abdomen in 2 (4.5%)children. Abdominal pain, nausea, dyspepsia, constipation and diarrhoea were reported in 3 to 7% of patients receiving alendronate in two large three-year studies.[15] The mild reactions related to blood transfusion were noted in 10% of the children like febrile reactions, rash. No other complications like anaphylactic shock seen. All the children (100%) were under chelation therapy (deferasirox), multivitamins and folic acid.

PREVALENCE OF OSTEOPOROSIS :

Out of 42 children, 26 (61.9%) are having low bone mineral density. Osteopenia and osteoporosis is seen in 12children(28.57%) with Z-scores of <-1 S.D. and 14 children(33.3%) with Z-scores of <- 2 S.D. Study conducted by Kritanjali Singh et.al(2014) about the low bone mineral density in thalassemia major in India showing a prevalence of lumbar osteoporosis and osteopenia was 42.5% and 37.5% respectively.[34] Rashid Merchant et al. conducted a study to evaluate osteopathy in thalassemia by bone mineral densitometry (BMD) and biochemical indices on 42 children with age group of 10-25 years using the similar software in our present study LUNAR PRODIGO G.E, showing prevalence of osteoporosis(Z-score <-1 S.D) as 50% and osteopenia (Z-score <-2 S.D) as 31%.[61] Study done by Vogiatzi et al on 236 patients (116 males,120 females with a mean age of 24.4yrs) showed a prevalence of osteoporosis and osteopenia was 49.1% and 30.5% respectively.[35] A study done by Fouzia et al in Lahore has shown the prevalence of osteoporosis as 47.33%. Prevalence of osteoporosis /osteopenia will vary depending on the type of the study, age of the study group, Z-scores cut off. Our present study is correlating with the prevalence of osteopenia, osteoporosis with previous studies in India.

Relation between age and Prevalence of Low Bone Mineral Density :

There is increase in prevalence of low bone mineral density with more age group. Children in the age group of 15-18 years had high prevalence with 9 out of 12 (75%) of osteoporosis. There is significant correlation between age and prevalence of low bone mineral density with P value of 0.004722. Study conducted by Rashid Merchant et al. Showing statistically significant increase in osteoporosis with advancing chronological age (P value = 0.046). [61]

Relation between gender and low bone mineral density:

In the present study, association between gender and prevalence of low bone mineral density is not significant (P-value >0.05, 0.7636), which is in agreement with previous studies done by Chapelon et.al and Shamshieshaz. Study conducted by Hashemieh et al shown no correlation between gender and prevalence of osteoporosis(P-value = 0.376).[36]

Years of onset of thalassemia and low bone mineral density :

Significant correlation was found between years of onset of thalassemia and severity of osteoporosis in children (r -0.5843, p-value < 0.0001, highly significant). Study conducted by Kritanjali Singh et.al(2014) has shown a high prevalence of low bone mineral density with increasing years of transfusion.[34] A study conducted by Hashemieh et al on the prevalence of osteoporosis in Iran showed a correlation between duration of disease and the incidence of osteoporosis(p-value = 0.007).[36] Prevalence of increasing osteoporosis with duration of disease can be due to repeated blood transfusion and increased iron overload, deposition of iron, free radicals, bone

marrow expansion.[6] DEXA scan advised annually to look for low BMD and early treatment for osteopenia/osteoporosis should be started.[37]

Short stature and low bone mineral density:

Out of 42 children, short stature is seen in 22 children(52.3%). In the present study, there is a significant correlation found between short stature and low bone mineral density (p-value <0.001). Study conducted by Kritanjali Singh et.al (2014) in India has shown a significant association of osteoporosis with short stature(P=0.002). Short stature is more prevalent in thalassemia due to the effect of iron overload on the Growth hormone deficiency or insulin-like growth factor I (IGF-I).[38]

B.M.I and LOW BONE MINERAL DENSITY:

In the present study, out of 42 children, low B.M.I was seen in 15 children (35.71%) and 27 children (64.29%) have normal B.M.I. There is a significant association between B.M.I and prevalence of low bone mineral density in the present study (P-value <0.001). This is in agreement with study done by Khalaf Hussein about bone mineral density in patients with β-thalassemia major in Duhok city .[33]

FERRITIN AND Z-SCORES:

There is a statistically significant correlation between the prevalence of low bone mineral density with high ferritin levels in the present study (P<0.02). Results in the present study are in agreement with a similar study done by Ayfer Gozu et.al on bone mineral density in children with beta-thalassemia major in Diyarbikar, with a strong correlation between serum ferritin levels and BMD (r= - 0.856, p=<0.01)(39). Low Z-scores are seen at high ferritin levels, because of chronic blood transfusions leading to excess iron in bones, may affect osteoblast number and activity.[40]

Z-SCORES BEFORE AND AFTER 6 MONTHS OF TREATMENT WITH TAB ALENDRONATE:

The mean Z-score has significantly decreased after 6 months of alendronate, vitamin-D, calcium supplements intake from -2.18 ± 0.83 S.D to -1.72 ± 0.6 (p-value <0.0001) . The mean Z-score in the osteoporosis group has decreased from -2.79 to -2.17 S.D. which is highly significant.(p value= < 0.0001) . The mean Z-score has decreased after 6 months of alendronate intake from -1.48 to -1.20 S.D in osteopenia group, which is very significant (p-value = 0.0046). Significant correlation between Z-scores before and after 6 months of treatment with Alendronate by pearson correlation coefficient (r value = +0.8368, P value <0.0001).

Fatemeh Shirani et.al conducted a study on the evaluation of Alendronate efficacy on bone mineraldensity in Iran on thalassemia patients between 20 to 50 years old. osteoporotic patients were diagnosed based on DEXA scan after which placed on treatment of oral regimen of alendronate 10mg daily. After a year, their densitometries were repeated and compared to the changes in BMD(g/cm^3) and T-score. serum calcium, phosphorus and alkaline phosphatase levels were measured at the beginning and the end of the year and the results were compared. Z-scores improved from -2.611± 0.18 to -2.38 ± 0.13 after 1year treatment of alendronate (P <0.001).[30]

A randomized, placebo-controlled study done by Morabito et al (2002) to evaluate the efficacy of oral alendronate or clodronate intramuscular administration on BMD for 2 years, safety & tolerability in 25 osteoporotic thalassemia patients. DEXA scan (Hologic QDR 4500 W, Waltham, MA) was done to assess BMD at lumbar spine and femoral neck. Patients were randomized into three groups ,each group takes placebo (8 patients) or clodronate 100 mg, i.m., once in 10 days (8 patients) or 10 mg alendronate oral everyday (9 patients,2male + 7female) along with 500 mg/d calcium & 400 IU cholecalciferol/day. Z-score has improved from -2.75 $\pm$ 0.12 to 2.62 $\pm$ 0.10.

CALCIUM & Z-SCORES:

Prevalence of low BMD with calcium levels in the present study, is not statistically significant (P=0.3310). It is correlating with the Study done by Mahesh M et al. (2013) in thalassemia patients, without any significance between calcium and BMD levels (r=0.116, P=0.526).

Overall, the findings of the present study was that ,treatment with Alendronate 10mg/day along with calcium and vitamin D supplementation is highly effective in improving the bone mineral density in transfusion-dependent thalassemia patients with low bone mineral density, by its anti-resorptive action. Alendronate is

safe and well-tolerated in the present study with minor side effects like abdominal pain, epigastric pain which were treated conservatively. There is good compliance with the tablet Alendronate in all 26 children without any dropout. Prevalence of osteoporosis in the present study is 33.3% and osteopenia as 28.57% which is high. Severity of osteoporosis is more with increasing chronological age and duration of disease in years. Prevalence of low BMD in the present study is significantly correlated with short stature, low body mass index, high serum ferritin levels. There is no significant correlation between prevalence of low BMD and calcium levels, rural/urban area, gender and socioeconomic status. DEXA is a highly precise and gives accurate measurement of BMD in children. So annual check-up of BMD assessment will be helpful for early detection and prevention of skeletal morbidity. Alendronate is useful in the prevention and treatment of low bone BMD in patients with transfusion-dependent thalassemia. While the findings of the present study need further validation due to limitations like single center study, small sample. Hence further multicentric studies involving large sample size with longer follow up may be helpful in giving precise role of Alendronate in the treatment of transfusion dependent thalassemia children with low BMD.

CONCLUSION

Based on the findings of the present study it may be concluded that, treatment with alendronate 10mg/day along with calcium and vitamin D supplementation is highly effective in improving the bone mineral density in transfusion-dependent thalassemia patients with low bone mineral density, by its anti-resorptive action. Alendronate is safe and well-tolerated in pediatric patients with minor side effects like abdominal pain, esophagitis and without any major side effects like atypical fracture/osteonecrosis of the jaw in the present study. Prevalence of osteoporosis in the present study is 33.3% and osteopenia as 28.57% and is more with increasing age and duration of disease. So annual check-up of BMD assessment will be helpful for early detection and prevention of skeletal morbidity. Alendronate is useful in the prevention and treatment of low bone BMD in patients with transfusion-dependent thalassemia.

SUMMARY

Alendronate is known to decrease the resorption of bone and is helpful in the treatment of osteopenia/osteoporosis. However, there are very few studies about the alendronate efficacy on transfusion-dependent thalassemia children with osteoporosis & osteopenia in India. This study was aimed to know the prevalence of osteopenia, osteoporosis in transfusion-dependent thalassemia children and assess the efficacy of Alendronate along with vitamin-D, calcium supplements in improving the bone mineral density in patients with transfusion-dependent beta-thalassemia.

This hospital-based longitudinal study was done from January 2018 to December 2018.

The minimum effective sample size to estimate the efficacy of alendronate treatment was 33. There are 205 registered cases of thalassemia in thalassemia day care unit, under the Department of Pediatrics, KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi. Out of 205 cases, 84 cases are transfusion-dependent thalassemia major aged between 8 to 18 years which fulfilled the inclusion criteria. However, based on computerized randomization, 42 children were enrolled. These patients were already receiving chelating agents, multivitamins & folic acid. All the 42 children underwent DEXA scan of which 26 children had low bone mineral density. These 26 children were treated with tablet alendronate 10mg/day once daily for 6months since the dose for children above 20kg is 10mg/day. They were supplemented with tablet calcium 500mg/day and tablet vitamin D 400 IU daily orally for 6 months and evaluated for osteoporotic recovery in terms of improvement in Z-scores in DEXA scan.

The important findings of the present study are summarized as below.

1. Most of the children were boys 30(71%) and 12(29 %) of the children were girls. Boy to girl ratio was 2.5:1.
2. 22 (52%) were aged between 8 to 12 years, 11 (26%) were between 13 to 15 years and 8 (19%) were between 16 to 18 years. The mean age was 12.64 + 2.77 years. The median age was 12 years and ranged between 8 years to 18 years.
3. Majority of the children 30 (71.5%) belong to the rural area, 12(29%) belong to urban area.
4. History of consanguineous marriage was noted in 61 % of the children's parents.
5. Thalassemia status of the siblings in the patients family was noted in 7 children (16%) and the death of the sibling was seen in 3 families (7.14%).
6. In this study most of the children had history of fatigue (19%) followed by pain abdomen (16.6%), respiratory infections (9.5%) and diarrhoea (7.5%).
7. In this study 15 children (35.71%) are Under Weight and most of the children 27(64.29%) are normal.
8. In the present study 22(52.38%) had short stature, of which 13(30.95%) children had stunting and 9 (21.42%) children were severely stunted. 20 children (47.62%) had normal height for age.
9. In the present study head to toe examination revealed hemolytic faces in 45.23%, pallor in 42.5% of the children and flat nasal bridge, malocclusion of teeth in 35% of the children.
10. In the present study hepatosplenomegaly was noted in 26%, splenectomy in 19% and splenomegaly in 55%.
11. Splenectomy was done in 8 patients.

12. All the 42 children (100%) were under chelation therapy (deferasirox), vitamins and folic acid.
13. In the present study, Mean haemoglobin was noted as 8.99 + 0.87 gm/dl. Mean Blood Urea was noted as 25 + 6.25 mg/dl, mean creatinine as 0.61 + 0.17.
14. Mean ferritin was noted as 3352.10 + 2075.59 mg/dl and calcium was noted as 8.97 + 0.33 mg/dl.
15. In the present study, Liver function tests were abnormal in 19.05% of the children and normal in 80.95% of the children.
16. In the present study, mean Z-scores of all 42 patients in the DEXA scan notes as -1.15 + 1.49 S.D.
17. Out of 42 children, 26 (61.9%) are having low bone mineral density (osteopenia-28.57%, osteoporosis-33.3%). The mean BMD in this 26 children was -2.18 + 0.83 S.D
18. Out of 42 children, 26 (61.9 %) of the children are on Alendronate, calcium and vitamin –D3 supplementation.
19. In the present study, 4 (9.5%) children had developed minor blood transfusion reactions in the form of rash, fever and chills .4(9%) children had a history of heartburn, followed by pain abdomen in 2 (4.5%) children, and were treated for the same.
20. In the present study, osteoporosis is seen in 3 female children, 11 male children with Z-scores <-2 S.D. Osteopenia in 4 female and 8 male children with Z-scores <-1 S.D. There is no correlation between gender and prevalence of low bone mineral density in the present study (P value =0.7636).

21. There is no correlation between rural/urban and prevalence of low bone mineral density in the present study. (P value = 0.1687).
22. There is no correlation between socioeconomic status and prevalence of low bone mineral density in the present study (P value = 0.4726).
23. Short stature is commonly seen in low bone mineral density and it is highly prevalent in children with osteoporosis. Relation between short stature and prevalence of low bone mineral density is highly significant.(P value <0.0001).
24. Relation between low BMI and prevalence of low bone mineral density is highly significant (P value <0.0001)
25. Relation between high serum ferritin levels and prevalence of low bone mineral density is statistically significant. (P value = 0.027).
26. There is significant correlation between longer duration of disease duration and low bone mineral density in the present study. The P value is also significant (r value = -0.5843, P value < 0.0001).
27. There is no correlation between calcium levels and prevalence of low bone mineral density in the present study (P value = 0.3310).
28. The mean Z-score has significantly decreased after 6 months of alendronate intake which is statistically significant (-2.18 + 0.83 S.D to -1.72 + 0.6, P value <0.0001).
29. The mean Z-score decreased after 6 months of Alendronate intake from -2.79 + 0.66 to -2.17 + 0.57 S.D. in osteoporosis group which is highly significant. (P value= < 0.0001)
30. The mean Z-score decreased after 6 months of alendronate intake from -1.48 to -1.20 S.D in osteopenia group, which is very significant (P value = 0.0046).

31. There is significant correlation between Z-scores before and after 6 months of treatment with Alendronate. (r value = +0.8368, P value <0.0001).

32. There is increase in prevalence of low bone mineral density with more age group. Children in the age group of 15-18 years had high prevalence with 9 out of 12 (75%) of osteoporosis. There is significant correlation between age and prevalence of low bone mineral density with P value of 0.004722.

Treatment with Alendronate in children with transfusion-dependent beta-thalassemia is highly effective in improving the bone mineral density.

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ANNEXURE I – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

"EFFICACY OF BISPHOSPHONATES, VITAMIN D AND CALCIUM SUPPLEMENTS IN TRANSFUSION DEPENDENT THALASSEMIA CHILDREN WITH LOW BONE MINERAL DENSITY – A ONE YEAR HOSPITAL BASED INTERVENTIONAL STUDY"

Principal Investigator: DR._____

Co – investigator: Dr._____

You have been asked to involve your child in above said research to be conducted at thalassemia day care centre of KAHER's JN medical college hospital, Belgaum by Dr. _____, PG student in the Department of Paediatrics at Jawaharlal Nehru Medical College, Belgaum.

Introduction

PURPOSE OF THE STUDY:

Participation of your child will help us to know the prevalence of osteoporosis in thalassemia patients and intervention with bisphosphonates, vitamin D and calcium supplements to improve the bone mineral density. 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. Hence involving your child in the study is your voluntary decision.

Voluntary participation

Your child's participation in this study is your voluntary decision, whether or not to participate will not affect your current or future relationship with KLEs Dr. Prabhakar Kore Hospital & MRC, Belgaum.

Risk and benefits

There are no major risks involved, other than discomfort and pain caused during collection of biological sample and mild gastric irritation due to bisphosphonates which are preventable.

Due to DEXA scan, early detection of osteoporotic changes is possible, and bone mineral density will be increased with bisphosphonates, vitamin D and calcium supplements.

Privacy and Confidentiality

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

Queries

If you have any queries you may contact

Dr. _____

Department of Pediatrics

JNMC, Belagavi-590010

Dr. _____

Professor, Department of Pediatrics

JNMC, Belagavi-590010

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

DR. ROOPA. M.BELLAD MD DCH

PROFESSOR

DEPARTMENT OF PAEDIATRICS,

KAHER'S

JAWAHARLAL NEHRU MEDICAL COLLEGE,

BELGAUM-590010

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

Signature of the authorized representative/ parent: _____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator: _____

Date: _____

Name: _____

ANNEXURES II –ETHICAL CLEARANCE

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC).

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

Phone: (+ 91-(0)831 Office : 2471350
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 24 **Date: 22/11/2017**

To.

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

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EFFICACY OF BISPHOSPHONATES, VITAMIN-D & CALCIUM SUPPLEMENTS IN TRANFUSION DEPENDENT THALASSEMIA CHILDREN WITH OSTEOPOROSIS, BASEN ON DEXN SCAN – A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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

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

5

ANNEXURE-III- PROFORMA

DATA COLLECTION INSTRUMENT

IP NO:

INFORMATION OF CHILD:

Name:

Age

Sex:

Address:

Socioeconomic status: Class I , II ,III, IV

Parents educational status:

Mother - High school / PUC / degree / University

Father - High school / PUC / degree / University

Phone no -

Std:

DETAILS OF THALASSEMIA HISTORY:

- **Age of onset of thalassemia :**
- **Management history:**

- **Drugs : chelating agents :-**

  Desferoxamine

  Deferiprone

  Deferasirox

Duration :

Any other : FA / Zinc / BC

BLOOD TRANSFUSION HISTORY :

Onset of transfusion -

1-2 months of diagnosis

2-6 months of diagnosis

>6months

No of reactions : 1/2/3/4

Type - fever / chills / others

- **H/o infections in the past - RS / GIT / HEP / HIV**
- **Others**

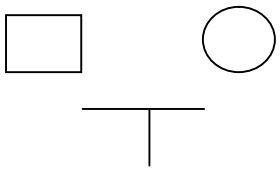
Treatment History in past:

IMMUNISATION HISTORY:

FAMILY HISTORY:

INFORMATION OF PARENTS-

Consanguinous / non consanguinous:



Siblings of the child & their thalassemia status:

Deaths of the siblings: **Yes / No**

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-

SYSTEMIC EXAMINATION:

PA:

Liver span-

Spleen-

Other Systems:

CVS:

RS:

CNS:

PRESENT INVESTIGATIONS-

CBC

FE

Ferritin-

Hb-

RFT-

LFT-

USG-

DEXA AT BEGINNING

ECHO- N/ABN

ECG-

Serum Calcium-

Serum Phosphorous-

Serum Albumin-

REPEAT DEXA SCAN AFTER 6MONTHS OF ALENDRONATE:

ANNEXURE IV–PHOTOGRAPHS

Photograph -5:Thalassemia Ward

Photograph- 6:Taking Demographic Data & Counselling

ANNEXURE-V

KEY TO MASTER CHART

BT	-	Blood transfusions
Cms	-	centimeters
DEXA	-	Dual energy X-ray absorptiometry
DFX	-	deferasirox
CG	-	electrocardiograph
ECHO	–	echo cardiogram
F	-	Female
gm%	-	Gram percent
H	-	Hepatomegaly
HBSAG	-	Surface antigen of the hepatitis B virus
HCV	-	Hepatitis C virus
HF	-	Haemolytic faces
HIV	-	Human immunodeficiency virus
HS	-	Hepatosplenomegaly
Kg/m	-	Kilograms per square meter
M	-	Male
mg/kg/day	-	Milligrams pre kilograms per day
mm	-	Millimeter
ng/mL	-	Nano grams per milliliter
N	-	Normal
NR	-	Non reactive
R	-	Rural
U	-	Urban
USG	-	ultrasonogram
WBC	-	White blood cell
WNL	-	Within normal limits
Y	-	Yes

					History – Brief history	COMPLAINTS								Treatment history						Past history						FAMILY HISTORY			
Serial number	AGE (YRS)	SEX	PLACE OF RESIDENCE	SOCIOECONOMIC STATUS	AGE OF ONSET	Breathlessness	Diarrhea	Heartburn	constipation	Vomiting	ONSET OF TRANFUSIONS	NO. OF REACTIONS	TYPE OF REACTIONS	CHELATING AGENTS	DURATION	Folic acid	zinc	vitamins	immunization	Viral Hepatitis	Renal dysfunction	Liver dysfunction	Cardiac dysfunction	Diabetes	Splenectomy	consanguinous	parents status	siblings status	death of siblings
1	8	M	U	3	1year	N	N	N	N	N	1	N	N	DFR	6YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
2	11	F	R	3	8 months	N	N	N	N	N	1	N	N	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N
3	9	M	R	3	2 years	N	N	N	N	N	1	1	Fever	DFR	5YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N
4	11	F	U	2	9months	N	N	N	N	N	2	N	N	DFR	7YRS	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
5	12	M	U	3	18 months	N	N	Y	N	N	1	2	chills	DFR	6YRS	Y	N	Y	Y	N	N	N	N	N	N	N	N	N	N
6	12	M	R	3	2years	N	N	N	N	N	1	1	rash	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
7	11	F	R	3	6months	N	N	N	N	N	1	N	N	DFR	7YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	thal	Y
8	12	F	R	3	3 years	N	N	N	N	N	1	N	N	DFR	7YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
9	11	M	U	3	6months	N	N	N	N	N	1	N	N	DFR	7YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
10	13	M	R	3	1 year	N	N	N	N	N	1	N	N	DFR	3YRS	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Mother-carrier	thal	N
11	15	M	R	3	18months	N	N	N	N	N	1	N	N	DFR	4YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	both carriers	thal	Y
12	13	M	U	3	9months	N	N	N	N	N	1	N	N	DFR	5YRS	Y	Y	Y	Y	N	N	N	N	N	Y	Y	NK	N	N
13	13	M	R	3	2 years	N	N	N	N	N	1	N	N	DFR	7YRS	Y	N	Y	Y	N	N	N	N	N	N	N	NK	N	N
14	13	M	U	2	9months	N	N	N	N	N	1	1	N	DFR	8YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
15	13	M	R	3	8months	N	N	N	N	N	1	N	N	DFR	8YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
16	15	M	U	2	1year	N	N	N	N	N	1	1	Fever	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
17	14	M	R	3	18months	N	N	N	N	N	2	N	N	DFR	8YRS	Y	Y	Y	Y	N	N	N	Y	N	N	Y	NK	N	N
18	15	F	R	3	1year	N	N	N	N	N	1	N	N	DFR	3YRS	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Mother-carrier	thal	N
19	16	M	U	2	2years	N	N	N	N	N	1	N	N	DFR	6YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
20	16	M	R	3	14months	N	N	N	N	N	1	N	N	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
21	17	M	R	3	3years	N	N	N	N	N	1	N	N	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	both carriers	thal	N
22	17	M	R	3	2years	N	N	N	N	N	1	N	N	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N		Y	NK	N	N
23	17	F	R	3	3years	N	N	N	N	N	1	N	N	DFR	8YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
24	17	M	U	2	3years	N	N	N	N	N	1	2	N	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
25	17	F	U	3	3years	N	N	N	N	N	3	1	N	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
26	18	M	R	3	2years	N	N	N	N	N	1	N	N	DFR	5YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N
27	14	M	U	3	4year	N	N	N	N	N	1	N	N	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
28	10	M	R	3	20months	N	N	N	N	N	1	N	N	DFR	5YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N
29	8	M	R	3	1year	N	N	N	N	N	1	N	N	DFR	4YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N

	General physical examination					Head to toe examination					Systemic examination – PER ABDOMEN						INVESTIGATIONS															DEXA SCAN		TREATMENT		
Serial number	Height (cms)	Inference (height/age)	Weight (kgs)	Body mass index (Kg/m2)	INFERENCE-BMI(WHO)	Face	Eyes	Ears	Chest	Extremities	Liver span	spleen size	others	Cardiovascular system	Central nervous system	Respiratory system	HIV	HCV	HBsAG	hemoglobin	urea	creat	LFT	ferritin	calcium	phosphorous	albumin	alkaline phosphatase	USG	ECG	ECHO	BEGINNING	AFTER 6MONTHS	ALENDRONATE(10MG/KG/DAY)	CALCIUM	Vitamin D3
1	116	-1.99	21	14.38	-1.93	N	N	N	N	N	7	9	HS	N	N	N	N	N	N	9.1	22	0.6	N	1076	9.4	3.7	4.4	420	HS+	N	N	-1.2	-1	Y	Y	Y
2	123	-1.55	21	13.9	-1.44	N	pallor	N	N	N	4	2	N	N	N	N	N	N	N	8.2	21	0.8	N	2100	8.8	3.2	3.4	289	N	N	N	-1.2	-1	Y	Y	Y
3	122	-1.72	22	14.3	-1.23	HF	pallor	N	N	N	10	6	HS	N	N	N	N	N	N	6.8	22	0.4	N	2645	9	3.6	4.7	202	spl+	N	N	-1.9	-1	Y	Y	Y
4	129	-1.38	23	14.11	-2.51	HF	pallor	N	N	N	11	8	HS	N	N	N	N	N	N	10.4	12	0.6	N	3437	8.9	4.3	3.8	332	HS+	N	N	-1.3	-1.2	Y	Y	Y
5	130	-2.69	30	17.2	-0.88	HF	pallor	N	N	N	8	6	N	N	N	N	N	N	N	8.3	24	0.8	N	1702	8.8	3.1	3.9	185	spl+	N	N	-1.6	-1.5	Y	Y	Y
6	138	-1.56	28	16.8	-1.79	HF	pallor	N	N	N	6	9	HS	N	N	N	N	N	N	9.2	28	0.8	N	2688	8.9	4.1	4.5	288	HS+	N	N	-1.6	-1.2	Y	Y	Y
7	142	-0.45	31	14.9	-0.98	N	icterus	N	N	N	7	8	HS	N	N	N	N	N	N	10	33	0.6	abn	3556	8.9	4.7	3.6	673	HS+	N	N	-1.7	-1.3	Y	Y	Y
8	135	-1.99	26	12.9	-3.73	HF	pallor	N	N	N	5	7	N	N	N	N	N	N	N	8.4	30	0.7	abn	2647	9.4	3.6	3.7	458	spl+	N	N	-2.1	-1.9	Y	Y	Y
9	125	-2.69	25	14.6	-0.54	HF	N	N	N	N	5	6	N	N	N	N	N	N	N	8.9	27	0.4	N	3761	9.1	2.9	3.4	312	spl+	N	N	-1.4	-1.3	Y	Y	Y
10	135	-2.83	31	16.5	-1.67	N	N	N	N	N	5	##	splenectomy	N	N	N	N	N	N	9	29	0.6	N	3971	8.2	3.1	4.3	304	#	N	N	-1.5	-1.1	Y	Y	Y
11	134	-4.48	27	14.67	-2.73	N	N	N	N	N	5	8	N	N	N	N	N	N	N	7	25	0.5	N	3224	9.1	3	4.7	244	spl+	N	N	-1.7	-1.6	Y	Y	Y
12	141	-2.02	33	16.66	-0.86	HF	pallor	N	N	N	5	##	splenectomy	N	N	N	N	N	N	9.3	28	0.6	N	3761	9.1	2.7	4.2	540	#	N	N	-2.7	-2	Y	Y	Y
13	130	-3.51	25	12.04	-2.11	HF	N	N	N	N	7	10	N	N	N	N	N	N	N	8.9	20	0.6	abn	5850	8.7	3	4.4	228	spl+	N	N	-2.2	-2	Y	Y	Y
14	138	-2.5	26	13.7	-3.16	HF	pallor	N	N	N	5	6	N	N	N	N	N	N	N	9.1	34	0.8	N	3320	9.2	3.7	3.8	256	spl+	N	N	-2.5	-2	Y	Y	Y
15	122	-3.14	28	15.5	-0.08	N	N	N	N	N	4	7	N	ABN	N	N	N	N	N	9.5	30	0.9	N	2066	9.5	3.7	3.9	179	spl+	ABN	ABN	-1.3	-1.2	Y	Y	Y
16	134	-2.68	29	16.1	-4.13	HF	pallor	N	N	N	4	3	N	N	N	N	N	N	N	7.9	27	0.7	N	4568	8.4	3.2	4.3	284	N	N	N	-3	-2.4	Y	Y	Y
17	155	-2.3	39	16.25	-3.18	HF	pallor	N	N	N	6	7	HS	ABN	N	N	N	N	N	9.4	16	0.8	N	1285	9.1	4.8	3.3	192	HS+	ABN	ABN	-2.2	-1.8	Y	Y	Y
18	138	-3.44	35		0.27	N	pallor	N	N	N	5	##	splenectomy	N	N	N	N	N	N	10.4	43	0.7	N	2622	9.4	3.6	3.9	416	#	N	N	-1.3	-1	Y	Y	Y
19	146	-4.34	34	16	-3.04	HF	pallor	N	N	N	6	6	HS	N	N	N	N	N	N	8.4	30	0.5	N	3265	8.6	3.2	4	226	HS+	N	N	-2.6	-2.2	Y	Y	Y
20	155	-4.49	29	14	-4.8	HF	pallor	N	N	N	5	8	HS	N	N	N	N	N	N	8.4	28	0.4	N	2098	8.5	3.2	4.7	341	HS+	N	N	-2.5	-1.8	Y	Y	Y
21	160	-2.43	43	16.7	-2.02	HF	N	N	N	N	6	10	HS	N	N	N	N	N	N	10.5	19	0.5	abn	3025	8.2	4.1	3.7	368	HS+	N	N	-3.4	-2.9	Y	Y	Y
22	148	-4.44	33	15	-3.64	HF	N	N	N	N	6	9	HS	N	N	N	N	N	N	9.1	22	0.5	abn	7520	8.5	4.5	3.6	330	HS+	N	N	-4.7	-3.6	Y	Y	Y
23	148	-2.43	36	16.4	-1.89	N	N	N	N	N	6	##	splenectomy	N	N	N	N	N	N	10.2	16	0.3	N	6028	9.4	4.8	4.3	134	#	N	N	-3	-2.3	Y	Y	Y
24	160	-2.83	42	16.4	-3.18	HF	N	N	N	N	6	##	splenectomy	N	N	N	N	N	N	9.2	22	0.8	abn	5632	9.1	2.8	3.9	294	#	N	NA	-2.5	-2.1	Y	Y	Y
25	148	-2.88	36	16.43	-2.29	N	N	N	N	N	7	9	HS	N	N	N	N	N	N	9.4	26	0.5	N	6512	8.9	3.6	4.5	410	HS+	N	N	-2.9	-2.3	Y	Y	Y
26	142	-3.45	31	15.6	-2.46	HF	pallor	N	N	N	5	6	spl	N	N	N	N	N	N	7	20	0.4	abn	2658	8.9	3.1	4	247	spl+	N	N	-2.7	-1.1	Y	Y	Y
27	139	-3.73	30	15.5	-1.8	HF	pallor	N	N	N	6	##	splenectomy	N	N	N	N	N	N	8.3	23	0.6	N	1392	8.8	4.7	4.3	723	#	N	N	0.2	##	##	##	##
28	130	-1.95	24.3	14.3	-1.87	N	N	N	N	N	7	9	HS	N	N	N	N	N	N	9.1	16	0.6	N	2340	9	4.4	3.7	256	HS+	N	N	0.8	##	##	##	##
29	133	0.07	25	15.1	-1.41	N	N	N	N	N	4	6	splenomegaly	N	N	N	N	N	N	9.8	32	0.1	N	1295	9.2	4.1	3.8	986	spl+	N	N	1.2	##	##	##	##

31	9	M	R	3	2years	N	N	N	N	N	1	N	N	DFR	5YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N	126	-1.09	27.6	17.5	0.55	N	N	N	N	N	6	9	HS	ABN	N	N	N	N	N	8.5	27	0.8	N	1677	8.9	5.1	4.4	574	HS+	ABN	ABN	0.5	##	##	##	##
32	9	F	R	3	1year	N	N	N	N	N	2	N	N	DFR	6YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N	128	-0.76	23	14	-1.4	N	N	N	N	N	6	8	HS	N	N	N	N	N	N	9.8	23	0.4	N	1124	9.2	4.8	4.5	564	HS+	N	N	0.9	##	##	##	##
33	10	M	R	3	15months	N	N	N	N	N	1	N	N	DFR	5YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N	132	-0.91	28	16.6	-0.22	N	N	N	N	N	5	4	N	N	N	N	N	N	N	9.4	24	0.7	N	1056	9	3.8	4.2	234	N	N	N	0.4	##	##	##	##
34	10	F	R	3	10months	N	N	N	N	N	1	N	N	DFR	6YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N	126	-1.85	26	16.1	-0.04	HF	pallor	N	N	N	7	10	HS	N	N	N	N	N	N	8.1	18	0.5	N	2168	8.6	4.3	4	320	HS+	N	N	0.7	##	##	##	##
35	10	M	R	3	1year	N	N	N	N	N	1	N	N	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N	134	-0.59	28	15.7	-0.53	N	N	N	N	N	5	7	splenomegaly	N	N	N	N	N	N	9.1	24	0.4	N	1652	9.1	4.1	3.5	391	spl+	N	N	0	##	##	##	##
36	11	F	U	3	6months	N	N	N	N	N	1	N	N	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N	147	0.58	28	13	-3.09	HF	N	N	N	N	6	7	splenomegaly	N	N	N	N	N	N	9.4	34	0.8	N	1482	9.6	3.2	4.1	228	spl+	N	N	0.6	##	##	##	##
37	11	F	R	3	6months	N	N	N	N	N	1	N	N	DFR	8YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	THAL	Y	138	-0.76	28	14.7	-1.45	HF	N	N	N	N	9	10	HS	N	N	N	N	N	N	7.9	33	0.7	N	1552	8.9	6	4	509	HS+	N	N	0.2	##	##	##	##
38	13	M	R	3	13months	N	N	N	N	N	1	N	N	DFR	9YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N	136	-1.85	32	16.32	-0.72	N	N	N	N	N	4	3	N	N	N	N	N	N	N	9.6	18	0.5	N	2250	9.1	3.9	4.3	318	N	N	N	0.4	##	##	##	##
39	12	M	R	3	11months	N	N	N	N	N	1	N	N	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N	135	-1.99	27	14.8	-1.7	N	N	N	N	N	7	##	splenectomy	N	N	N	N	N	N	9	21	0.6	N	1458	9.1	4.7	4.5	370	#	N	N	0.1	##	##	##	##
40	12	M	R	3	1year	N	N	N	N	N	1	N	N	DFR	6YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	N	N	142	-1	31	15.6	-1.28	N	N	N	N	N	7	9	HS	N	N	N	N	N	N	9.1	22	0.8	N	2064	9.4	4.2	4	380	HS+	N	N	0.8	##	##	##	##
41	12	M	R	3	10months	N	N	N	N	N	1	N	N	DFR	7YRS	Y	Y	Y	Y	N	N	N	N	N	N	N	NK	N	N	130	-2.69	26	14.8	-1.28	N	N	N	N	N	5	8	SPL	N	N	N	N	N	N	9.2	31	0.6	N	2652	9.1	3.8	4.9	259	spl+	N	N	0.62	##	##	##	##
42	15	M	R	3	1year	N	N	N	N	N	1	N	N	DFR	10YRS	Y	Y	Y	Y	N	N	N	N	N	N	Y	NK	thal	N	168	-0.12	49	17.4	-1.16	N	N	N	N	N	6	##	splenectomy	N	N	N	N	N	N	9.8	30	0.72	N	2204	8.9	3	3.7	328	#	N	N	0.5	##	##	##	##