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Evaluation of Biochemical Parameters of Bone Metabolism in Patients with Transfusion Dependent Beta Thalassemia

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Abstract

Hemoglobinopathies are the most common group of autosomal recessive monogenic disorders of hemoglobin production. These include the thalassemia and structural hemoglobin (Hb) variants. Iron overload is the major complication occurring in thalassemia major due to repeated blood transfusion. The consequence of this is that vital organs like liver, heart, kidney are loaded with iron and their function deteriorates progressively which results in osteopenia and osteoporosis. In the present study vitamin D levels along with other biochemical parameters were tested to assess the role of vitamin D deficiency in pathogenesis of thalassemia. This study aimed to assess biochemical parameters of bone metabolism in transfusion dependent beta thalassemia. A of total120 subjects were included in the study, of which 60 were diagnosed cases of beta-thalassemia major and 60 were healthy age and sex matched controls. Subjects were in age group of 6 to 18 years. Proper history was taken along with physical examination for all cases and controls. Blood samples were drawn for Serum ferritin, Calcium and 25-OH-Vitamin D estimation. Analysis was done by applying unpaired T test with P value $p< 0.01$ was considered statistically significant. Out of the 60 cases, 31.67% had short stature (< 3rd percentile) and 25% were underweight (<5th percentile) in the present study. WHO growth charts were used as reference. The Calcium and Ferritin levels in cases ranged from 7.1 to 10.8 mg/dl and 923 to 6929 ng/ml, respectively. They were statistically analyzed by applying unpaired t test and the p values being found to be significant. The Mean ± SD of Vit D in cases was15.5 $\pm$3.9 and that of controls was 22.35 $\pm$ 6.7 by applying unpaired t test p value was statistically significant. This showed that the Vitamin D levels were found to be reduced significantly in cases as compared to the controls. It was found that the Ferritin has a linear negative correlation with Calcium and Vitamin D. To conclude it can be said that there is a positive correlation between bone metabolic dysfunctions and iron overload in patients with transfusion dependent beta thalassemia. Thus, monitoring of ferritin along with Vitamin D and Calcium is required to reduce overall morbidity in these patients.

Keywords: Vitamin D, Calcium, Ferritin, morbidity, Thalassemia.

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1. Introduction

Hemoglobinopathies are the most common group of autosomal recessive monogenic disorders of haemoglobin production. These include the thalassemia and structural haemoglobin (Hb) variants [1]. Iron overload is the major complication occurring in thalassemia major due to repeated blood transfusion. Chelation therapy is done to decrease the rate of iron accumulation due to blood transfusion [2]. Even with the administration of effective iron chelation therapy over 50% of patients die before the age of 35 years, mainly due to poor compliance [3]. Bone marrow transplantation (BMT) is the only cure for thalassemia major and is an accepted therapeutic alternative to lifelong blood transfusion and chelation [4]. However, it is not easily accessible and affordable for all. Iron stores in the body are in the form of ferritin. Small amount of ferritin in the body is secreted into the plasma.

This ferritin concentration is in positive correlation with the size of the total body iron stores in the absence of inflammation [5]. Normal ferritin concentrations differ by age and sex. Concentrations are high at birth, show increase during the first two months of life, and then fall throughout later infancy. Ferritin concentrations begin to rise again at about one year of age and continue to increase into adulthood [6]. In early adolescence males have higher values than females which continues into late adulthood. Packed cell transfusions are repeatedly administered to these patients which results in iron overload. As each unit of packed cells contain approximately 200 mg of iron, a patient who receives 25 units per year, accumulates 5 grams of iron per year in the absence of chelation [7]. This is due to free radical generation which damages membrane lipids and causes cell death eventually leading to organ failure [33]. These patients are also susceptible to increased iron absorption. The consequence of this is that vital organs like liver, heart, kidney are loaded with iron and their function deteriorates progressively [7]. The iron burden on the body can be estimated by means of serum ferritin. The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta thalassemia [8]. A target ferritin of approximately 1000 mg/l is generally recommended standard practice in thalassemia major and other forms of iron overload resulting from blood transfusion. When the serum ferritin level reaches 1000 mg/l (usually after10th to 12th transfusion), it is generally taken as the point to initiate iron chelation therapy [2]. Even though chelators have improved life expectancy of these patients, still there is a risk of osteopenia and osteoporosis if chelation is given in excess [34,35]. Hepatic iron concentration (HIC) is the most useful method for estimating iron load in chronically transfused patients. However, while the method is generally safe, it requires a liver biopsy and an undefinable risk of morbidity (and rarely of mortality) is reported. Patients with thalassemia major are susceptible to osteopenia and osteoporosis. The mechanism of osteoporosis in these patients is multifactorial. Transfusion related iron overload in endocrine organs leads to increased bone resorption, decreased mineralization, impaired growth hormone secretion and vitamin D deficiency that contribute to impairment in achieving an adequate bone mass [9]. Therefore, in the present study vitamin D levels along with other biochemical parameters were tested to assess the role of vitamin D deficiency in beta thalassemia.

2. Material and Methods

Cross sectional, observational study which was conducted at "Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai" comprised of sample size 120 subjects, which were divided into cases and control groups. Group A comprised of 60 diagnosed cases of beta-thalassemia major and group B included 60 non thalassaemic, healthy age and sex matched controls. Patients in age group 6 years to 18 years and on chelation therapy were included. Subjects having any known bone disorder and any other concomitant illness and those taking any medications affecting bone functions other than chelation therapy were excluded. The study was performed after approval from the ethics committee. Then the informed written consent was taken from participants. Sample was collected and was analysed for serum Vitamin D, Calcium and Ferritin. 25-OH-Vitamin D was done on ACCESS 2 Immunoassay system by Beckman Coulter and Calcium and ferritin were done using AU-680 fully automated autoanalyzer by Beckman Coulter. For each parameter, mean and standard deviation was calculated to estimate the significance. The difference between the groups was measured by Students Unpaired 't' test. p value less than 0.05 was considered statistically significant. The relationship between variables was measured by Pearson's correlation coefficient using SPSS software for all analysis.

3. Observations and Results

The anthropometric measurements for height and weight, their Mean, Standard Deviation and results of unpaired t test of Controls and Cases was done. The weight ranged from 16.3 kg to 53.8 kg, the height ranged from 108 cm to 164 cm in cases and BMI ranged from 13 to 20 kg/m^2 in cases. In the controls the respective ranges of weight, height and BMI was 18.7 kg to 76.5 kg, 112 cm to 172 cm and 14.4 to 25.5 kg/m^2. On application of unpaired t test, statistically significant difference was found between the weight, height, BMI of cases and controls. Out of 60 cases, 31.67% had short stature (< 3rd percentile) and 25% were underweight (<5th percentile) in the present study. (WHO growth charts used as reference) (Table 1) [28-29]. Table 2 shows the respective Mean ± SD of Vitamin D, Calcium and ferritin levels in cases and controls. They were statistically analyzed by applying unpaired t test and the p values being found <0.05 were considered significant. The p value was found for all the parameters as mentioned above. Figure 1 shows the distribution of the same data with box and whisker plots comparing the median and distribution of data into individual quartile. The Calcium and Ferritin levels in cases ranged from 7.1 to 10.8 mg/dl and 923 to 6929 ng/ml, respectively. In controls the respective values were 8.1 to 10.8 mg/dl and 11 to 45 ng/ml. Table 3 shows that the vitamin D levels were deficient in 33.3% cases and 11.6% of controls. 53.3% of cases and 43.3% of controls had vitamin D insufficiency whereas 13.3% of cases and 45% of controls had normal levels. Table 4 represents the correlation between individual parameters (Age, Vitamin D, Calcium) and Ferritin. It was found that the Ferritin has a linear negative correlation with Calcium and Vitamin D. Ferritin has a linear positive correlation with Age (Table 4 & Figure 2) [32]. These correlations were all statistically significant.

4. Discussion

It is known that beta thalassemia is an inherited disorder of hemoglobin synthesis due to defective globin chain production leading to ineffective erythropoiesis and anemia. The treatment mainly consists of blood transfusion & prevention of iron overload [17]. Despite giving chelation therapy, the adverse effects due to iron overload are seen which can be attributed to poor compliance and side effects of the chelating drugs [18]. Though the survival of patients with thalassemia major has progressively improved with advances in therapy but bone abnormalities, endocrine derangement and cardiac dysfunction are the most frequent complications, mainly because of iron overload. As Vitamin D is essential for optimal skeletal health like for reducing fracture risk, for calcium homeostasis & mineralization of the bones during infantile and pubertal growth periods, deficiency of vitamin D could be a major cause of impaired

bone health in the patients with transfusion dependent beta thalassemia [19]. Growth retardation in transfusion dependent beta thalassemia patients occurs despite of giving them optimal transfusion and chelation therapy. There is curtailed or delayed preadolescent and adolescent growth spurt. Therefore, these patients do not obtain their full potential stature.

Many patients show poor compliance to chelation therapy and iron overload remains the main cause of growth failure in such patients [5]. In the present study, physical growth was assessed by measurement of height, weight, and BMI, and were compared with that of the controls. Out of 60 cases, 31.67% had short stature and 25% were underweight. The findings of the present study were in concordance with the study conducted by Fahim et al who found that the mean height of the patients was significantly lower than that of controls. 49% of their cases were of short stature and 47% were underweight [15]. Haydeh Hashemizadeh et al., (2013) have compared thalassemic patients with normal individuals and have stated that the patients had short stature and were underweight [30]. Shalitin et al., (2005) conducted a study on 39 patients with thalassemia major. They also noticed short stature of thalassemic patients [21]. Growth failure is common in patients with thalassemia. However, growth failure can be multifactorial in thalassemia. It can be due to chronic hypoxia because of chronic anemia, chelation toxicity, low serum zinc level, hepatic dysfunction due to iron overload in the liver. It can also be due to iron associated endocrinopathies like hypothyroidism, hypoparathyrodism, hypogonadism and growth hormone deficiency. This implies that the current transfusion and chelation therapies, which were introduced approximately 25 years ago, have not significantly improved growth in thalassemia patients. Transfused iron is deposited first within the reticuloendothelial cells, prior to parenchymal iron loading within the heart and liver. However, as in primary iron overload, the majority of complications ultimately result from progressive cardiac and liver failure. For effective management of iron overload frequent evaluation of the body iron stores should be done [23]. The iron status of the body in overload conditions can be assessed by different ways like LIC, endomyocardial biopsy, T_2 MRI which are excellent methods, but these are either not easily available, or invasive, and costly, so these are not routinely used. Serum ferritin measurement, on the other hand is easy to perform frequently. At present, no other serum test is a better predictor for iron overload than serum ferritin level [24]. The findings in the present study were in concordance with the study conducted by Fahim et al, who found that the Ferritin levels were significantly lower in the patients than in controls [15]. In the present study the calcium levels in cases (8.67$\pm$ 0.92) were lower than the controls (9.27$\pm$0.84). The difference in values being statistically significant (p value <0.05). A negative correlation was found between ferritin and calcium levels (r value -0.747, p value <0.001). The findings were in harmony with the study conducted by Meenu Goyal et al., (2010) the calcium levels in their patients (8.42$\pm$0.32) were significantly lower than the controls (9.98$\pm$0.16) [13]. In another study conducted by Amirah Zatil Izzah et al., (2017) have found significantly lower levels of serum calcium in patients as compared with healthy controls (p value = 0.001). There was also a significant negative correlation between calcium and serum ferritin levels (r value = -0.44, p value 0.007) which was in accordance with the present study [25]. In the present study vitamin D levels were found to be low in cases (15.6 $\pm$3.85) as compared to the controls (26.91 $\pm$ 4.19). The difference was statistically significant (p value <0.05). 33.3% of cases and 11.67% of controls were vitamin D deficient stating that vitamin D deficiency is not only present in cases but it is also prevalent in the normal general population, though it was found to be severe in cases. In the cases, 33.3% were vitamin D deficient, 53.3% were found to be insufficient and 13.3% were found to have sufficient levels of vitamin D. There was a strong negative correlation between serum ferritin levels and vitamin D levels in the cases. The vitamin D deficiency had no association with age, as compared among children and adolescents, and gender, which was in harmony with the study conducted by Sultan et al., (2016) [26]. The mean serum level of 25-OH Vitamin D was significantly lower in the thalassemic patients than in controls was seen in the study conducted by Akhouri et al., (2017) 41% had vitamin D deficiency, 46 % had vitamin D insufficiency and only 13% had normal Vit D level. These findings were in concordance with the present study [22]. In the study conducted by Ezzat et al., (2015) found the 43.33% of patients had vitamin D and only 7% had normal levels which were in concordance with the present study. They also found a correlation between LIC and vitamin D levels and stated that vitamin D may prove to be a simpler and convenient indicator over LIC [11]. Rashid merchant et al., (2010) found vitamin D deficiency in 62% Indian thalassemia major children and suggested that vitamin D deficiency was nutritional deficiency and defective hydroxylation of vitamin D in liver due to hemochromatosis as all children had high serum ferritin levels [14]. Vogiatzi et al., (2009) reported in their study that 12% of thalassemic patients were vitamin D deficient and 69.8% had insufficient levels which was in concordance with the present study [12].

Table 1: Showing mean and SD of height, weight and BMI of cases and controls.

	Cases	Controls	P value	Statistical significance
Height	136.37+15.32	143+14.59	0.016	Significant
Weight	30.2+9.27	38.25+12.4	0.0001	Significant
BMI	15.73+1.47	18.19+2.57	0.0001	Significant

Table 2: Mean and SD of serum calcium, vitamin D and ferritin levels of cases and controls.

	Cases	Controls	P value	Statistical significance
Calcium	8.67+0.92	9.27+0.84	0.003	Significant
Vitamin D	15.5 +3.9	22.35 + 6.7	0.0001	Significant
Ferritin	3363.93+1750	32.33+8.05	0.0001	Significant

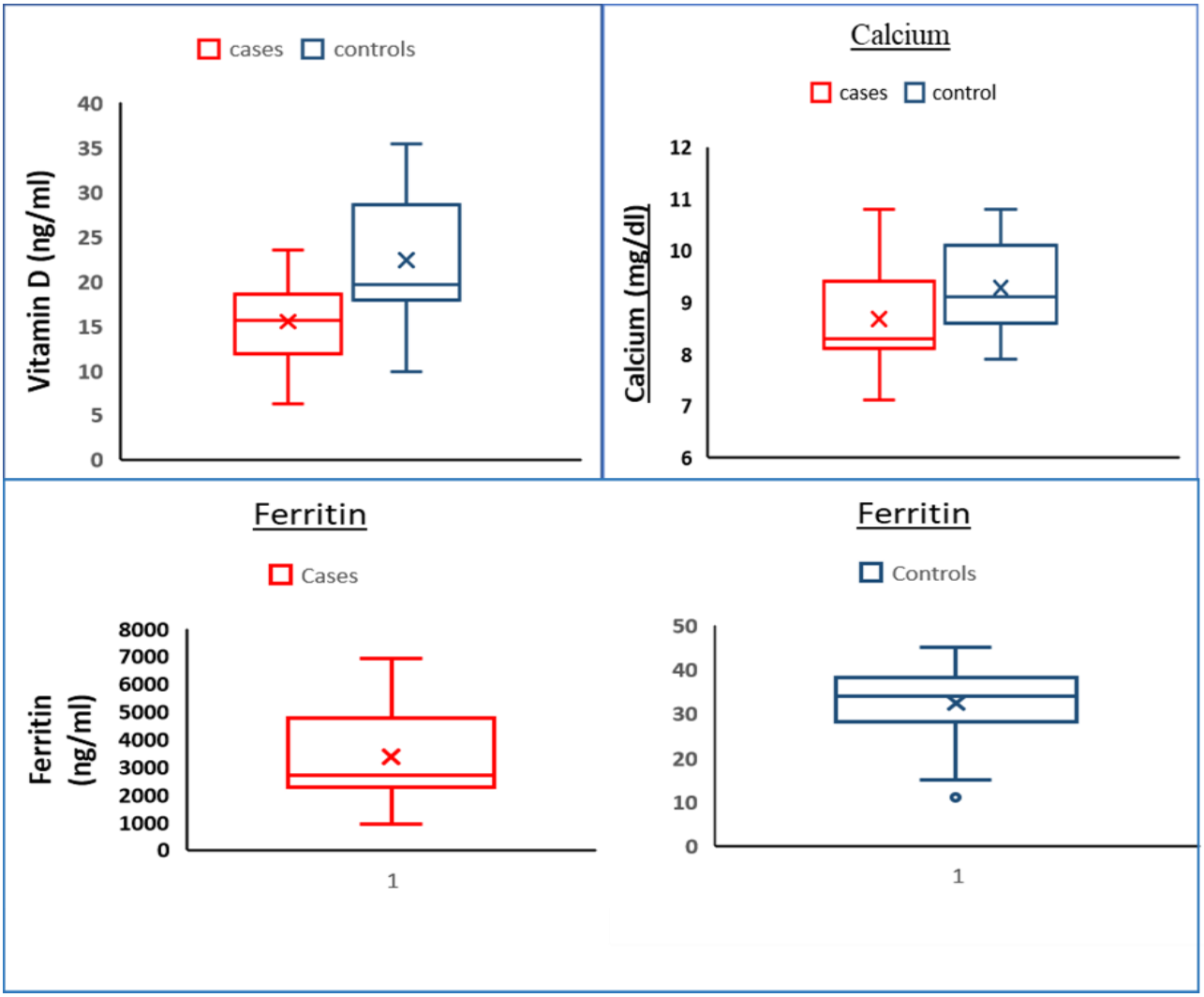

Figure 1: Box and Whisker plots of mean and SD of serum calcium, vitamin D and ferritin levels of cases and controls.

Table 3: Level of vitamin D in cases and controls.

Vitamin D levels	Cases	Controls
Deficiency	20 (33.3%)	7 (11.6%)
Insufficiency	32 (53.3%)	26 (43.3%)

Normal	8 (13.3%)	27 (45%)

Table 4: Pearson correlation: Serum Ferritin with other variables in cases.

Variables	Ferritin vs. Age	Ferritin vs. Vitamin D	Ferritin vs. Calcium
Pearson r	**0.521**	**-0.37**	**-0.747**
P (two-tailed)	**< 0.05**	**0.003**	**< 0.0001**

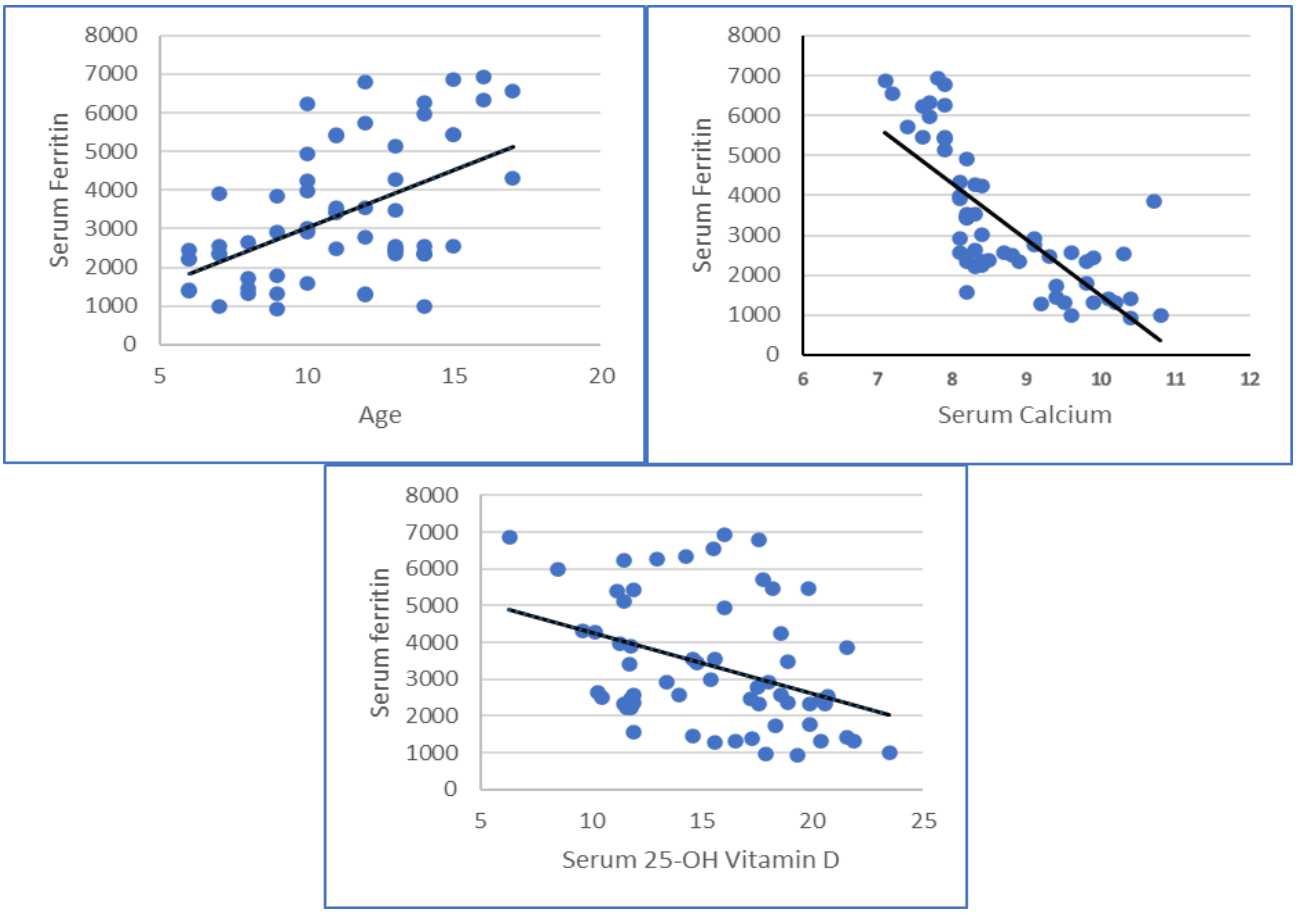

Figure 2: Pearson correlation: Serum Ferritin with other variables in cases.

The present study is in concordance with another study conducted by Fadilah et al. They also found a negative correlation between vitamin D level and ferritin (r value -0.368, p value <0.01). In the study conducted by Agrawal et al., (2016) the mean serum level of 25-OH-vitamin D was significantly lower in thalassemic patients than in healthy controls. Prevalence of vitamin D deficiency among healthy controls was also found in their study, like the findings of the present study [16]. 25-OH Vitamin D deficiency in thalassemia patients is most likely due to hepatic dysfunction due to iron overload in the liver, which led to defective hydroxylation of vitamin D. Though the vitamin D levels are affected by geographic location, cloud cover, application of products like sunscreen but still the findings of high prevalence of vitamin D deficiency and its association with deranged bone metabolism should be a major concern regarding the health of these patients. The individuals with thalassemia are at a greater risk for vitamin D deficiency and related osteopathy like bowing of legs, fractures of long bones and avascular necrosis of hip. Chronic anaemia, undernourishment, hypovitaminosis D and hypoparathyroidism lead to osteopathy at an early age. Therefore, there is a greater need for the regular follow up for assessment of complications and vitamin D supplementation in them [27].

5. Conclusion

This study revealed deranged bone metabolism in patients as evidenced by decrease in calcium and vitamin D, and their negative correlation with ferritin levels. Vitamin D deficiency occurs due to improper hydroxylation due to iron overload in the liver and undernutrition causing alterations in calcium, phosphate homeostasis, reduced osteoblast activity and increased bone resorption rates which ultimately hampers growth in these patients seen in the form of short stature and underweight in many patients. Iron overload impairs osteoid maturation and inhibits local mineralization, resulting in focal osteomalacia. In addition, the incorporation of iron in calcium hydroxyapatite affects the growth of crystals, leading to defective mineralization. Hormonal abnormalities, including thyroid, parathyroid dysfunction, and hypogonadism, are believed to underlie the altered bone turnover observed in β Thalassemia Major. A better understanding of the pathogenetic mechanisms underlying bone abnormalities in β Thalassemia Major is needed to develop targeted treatments. As of now, the early detection of bone demineralization, and the eventual institution of vitamin D and calcium supplementation along with their periodic monitoring are the most effective strategies to reduce the incidence and severity of skeletal complications in these patients.

6. Limitations

Being a cross sectional study patients could not be followed up to evaluate the final height attained and other skeletal complications. Estimation of BMD by DEXA and assessment of parathyroid hormone for better evaluation of bone metabolism could not be done in the present study. Although the present study highlights that bone dysfunction remains an unresolved problem in Beta Thalassemia Major despite transfusion regimens and chelation therapy, there is a need of further studies with larger sample size to accurately evaluate the prevalence and risk factors that lead to bone disease in beta thalassemia.

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