Role of Zinc Supplementation in Improving Bone Density in Late Childhood Patients with Beta Thalassemia Major

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Abstract

Background: Beta thalassemia is a category of recessively inherited hemoglobin abnormalities that were initially identified by Cooley and Lee and are defined by faulty synthesis of the -globin chain. Severe anemia brought on by the homozygous condition need frequent blood transfusions. A range of problems, including endocrine, metabolic, skeletal, and growth abnormalities are being reported as a result of increased iron storage in the body, despite the fact that such therapies extend the patient's life.

Aim: To improve the bone mass in patients with thalassemia through assessing the effect of zinc supplementation on bone density.

Patients and Methods: Eighty children with beta thalassemia who also had low bone mineral density (BMD) and low serum zinc were included in a randomized controlled experiment. The patients were then randomly divided into 2 groups, one of which got zinc supplements for 12 months while the other group received no supplements. At the conclusion of the trial, BMD and serum zinc levels were once again evaluated. Dual-energy X-ray absorptiometry (DXA) scan was used to assess BMD.

Result: There was no statistically significant difference in the supplement group and non-supplement group regarding gender, age, serum ferritin, hemoglobin level, serum zinc (69.3±10/ 73.1±8) [*p*-value 0.07] and BMD-z score (-2.02±-0.5/ -2.1±-0.6) [*p*-value 0.4]. After 12 months; the zinc supplemented group showed a statistically significant elevation of serum zinc level compared to the non-supplemented group (86.8±11.3/78.7±11.5) [*p*-value 0.001] respectively. Also the zinc supplemented group showed a significant increase in BMD - Z score (-1.6±-0.5) compared to the control group (-2.1±-0.6) [*p* -value 0.001].

Conclusion: In children with Beta-thalassemia, a zinc supplement is crucial for increasing BMD and avoiding osteoporosis.

Key words: ***Thalassemia, zinc, bone mineral density***.

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INTRODUCTION

The most prevalent hereditary hemoglobinopathy in the world, affecting 4.4 out of every 10,000 live births, is thalassemia. Thalassemia disorders impact around 200 million individuals worldwide [1].

Thalassemia is the most prevalent genetically inherited hemoglobin disease and the most common hemoglobinopathy in Egypt. The disease's carrier rate ranges from 5.3% to 9%, while the gene frequency is 0.03%. Thalassemia was thought to affect 1000–1.5 million live births annually. According to multicenter studies conducted in 2014, the carrier rate in Egypt is between 9% and 10% [2].

Hemolysis, poor oxygenation, and reduced erythropoiesis are caused by reduced or absent beta globin chain synthesis. According to Fung et al. [3] and Inati et al.[4], many patients need repeated blood transfusions to maintain life, promote development, and prevent severe bone abnormalities.

While many adults with thalassemia have low bone density, hypogonadism, growth hormone deficiency, diabetes, and suppressed immunological function, children and adolescents with thalassemia usually have poor growth and delayed pubertal development [5].

More than 300 metalloenzymes are affected structurally by zinc, which performs a number of fundamentally significant biological roles. In addition to growth retardation, hypogonadism, diabetes, red blood cell fragility, poor immunological function, and poor bone mineralization, zinc deficiency is also associated with these symptoms [6].

According to several research, people with beta-thalassemia major often have zinc deficits [7]. Thalassemia patients have been shown to have suboptimal zinc status, which is indicated by low zinc levels in the plasma and leukocytes, low alkaline phosphatase activity, and high urine zinc excretion. Children with thalassemia have demonstrated improved growth with zinc supplementation, suggesting that certain individuals may have growth restriction due to zinc deficiency [8].

Thalassemia patients are at risk for marginal zinc status due to a variety of causes. These include increased hemolysis, inefficient erythropoiesis, which raises zinc needs, and routine chelator usage, which can raise zinc levels in the urine and feces. Additionally, a lot of people who have thalassemia and iron overload also have diabetes, which causes more zinc to be lost [9].

In order to measure bone density, dual-energy X-ray absorptiometry (DXA) is employed. The lumbar spine and proximal femur are the optimal areas for DXA scans. Calcium and bone mineral may be measured using a DXA approach that is based on transmission measurements taken at two photon energies [10].

Aim of the Study

The aim of this study is improving health and care of thalassemic children throughout improving the bone health in those with β thalassemia major.

PATIENT AND METHODS

This Randomized Controlled Trial (RCT) was conducted at pediatric haematology clinic and diagnostic radiology departments in Suez Canal University Hospital, Ismailia, Egypt. This study enrolled eighty beta-thalassemia major patients attending to Suez Canal University hospital hematology outpatient clinic. The study included children 6 to 12 years of age with Beta thalassemia major. Bone marrow transplant recipient, or currently prescribed treatment for low bone mass other than calcium or vitamin D, or currently participating in another trial with a medication known to affect bone mineral density, and with chronic use of systemic corticosteroids were excluded from the study. The included study population were randomly allocated into one of two groups: Intervention group: - forty children with β. Thalassemia major with low bone mineral density who received oral zinc supplementation (25 mg/d). Non-intervention group: - forty age and gender matched thalassemia children as a control group who didn't not receive zinc supplementation.

METHODS OF THE STUDY:

All children included in the study were subjected to: full history taking, Physical Examinations, laboratory assessment in the followings (haemoglobin Level, Serum zinc level, Serum ferritin level and liver & kidney function).

Determination of serum levels of zinc was measured using spectrophotometric method using bio diagnostic colorimetric zinc kit (CAT.NO.Zn 21 20)

Kit content:

Table (1) Bio-diagnostic zinc reagent and kit components:

Component	Concentration
Standard (R1)	**200 µg/dl**
Buffer (R2) Carbonate buffer (PH 9.5)	**50 Mm/L**
Chromogen(R3) (zincon)	**0.05 Mm/L**

PROCEDURE:

1- Three test tubes were prepared included blank, standard and samples tubes in a zinc free tubes with reaction volumes indicated in table(2).

Table (2) reaction volumes and component:

	Blank ml	**standard ml**	**Sample ml**
D.water	0.5	**-**	**-**
Standard(R1)	**-**	0.5	**-**
Sample	**-**	**-**	**0.5**
Buffer (R2)	0.5	0.5	**0.5**
Chromogen(R3)	0.5	0.5	**0.5**

2- Reaction component was mixed and incubated for 10 minutes at room temperature.
3-Absorbance of the standard ($A_{Stanard}$) and sample (A_{Sample}) was measured against blank at wavelength 610nm.
4- Serum zinc was calculated according to the following equation:
Zinc in sample(µg/dl) =($A_{sample}/A_{standard}$)× Standard concentration
Reference range: 64-124 µg/dl

IV-Bone scan for mass density

Evaluation of bone density and mineralization status was assessed by doing bone density by dual-energy X-ray absorptiometry (DXA) at baseline, and 12 month of zinc supplementation, using Lunar prodigy Primo DXA system (analysis version:14.10) at anteroposterior lumbar spine (L1-L4).

The results of a bone density test are presented as a Z score. It shows how much higher or lower the bone density, the lower score means weaker bone.

V- Duration of therapy and follow up.

The intervention group: the participants received oral zinc supplementation (25 mg) daily for 1 year with weekly regular follow up (started from December 2021-2022). The dosage for the intervention is 2-3 times the Recommended Dietary Allowance (RDA) for most age and gender groups (8-11 mg/d)**.The non-intervention group**: didn't received zinc supplementation. Both groups were subjected to serum zinc measurement and bone mass density evaluation by DXA scan at the start and at the end of the study.

STATISTICAL ANALYSIS

Data entry and statistical analyses were conducted using IBM SPSS STATISTICS version 22. Summary statistics were then computed, including means, SDs, and 95% CIs for all the variables in each group. Data throughout the thesis are reported as means& SDs unless stated otherwise. Zinc supplemented group and non-treated group were compared, and data were analyzed at baseline for continuous outcomes including anthropometric measures and biochemical profiles measure and BMD-z and by using Student's t tests for normally distributed data and a Mann-Whitney U test for highly skewed data. Zinc supplemented group continuous outcomes including anthropometric measures, serum zinc and BMD-z and were compared before and after zinc supplementation by using paired sample t-tests for normally distributed data and a Wilcoxon signed rank test for highly skewed data. Pearson's chi-square or Fisher's exact tests were used to assess differences in categorical variables (e.g., gender) at baseline, p value < 0.05 was considered as statistically significant. Correlations were done for parametric variables using Pearson correlation coefficient and for non - parametric variables using spearman rank correlation.

RESULTS

A total of eighty β thalassemia major patients 41 (51.3%) male and 39(48.7%) female within the age range between 6-12years old with mean age 8.21±2.09 years contribute to the study patients were divided randomly into 2 group the first group received zinc supplementation **(intervention group)** while the other group not received zinc supplementation **(non-intervention group)**. Table (3) shows that there was no statistically significant difference in distribution regarding gender, age, residence between the intervention group and the non-intervention group.

Table (3): Frequency of residence, gender and age in the intervention and non-intervention groups:

	Intervention group		**Non-intervention group**		**Chi-square**	**p-value**
	N	%	N	%		
Urban	16	40%	15	37.5%	.818	1(ns)
Rural	24	60%	25	62.5%		
Male	20	50%	21	50%	0.823	1(ns)
Female	20	50%	19	50%		
Serum zinc ≤70(µg/dl)	23	57.5%	15	37.5		
Age(year)					**t-test**	0.71(ns)

(mean ±SD)	8.3±2.11	8.13±2.09	0.372	

ns: non-significant the t-test is non-significant at 95% level of confidence.

Table (4) illustrates initial analysis that showed no significant differences in baseline anthropometric measures, plasma zinc, and bone mineral density- Z score within the intervention group and the non-intervention group**.**

Table (4) Baseline data for patient characteristics including anthropometric measures and biochemical profiles measures in the intervention group and non-intervention group.

Variable	Intervention group	Non-intervention group	T/Z	p-value
Hight	124.13±12.36	123.45±11.98	-0.005	0.99
Weight	26.8±7.01	26.2±6.79	-.35	0.73
BMI	17.01±1.51	16.87±1.73		0.80
Hb(gm/dl)	7.32±0.47	7.45±0.48	-1.26	0.21
Serum ferritin (ng/ml)	1753±278.08	1688±279.59	t-test 1.043	0.98
Duration of chelation therapy /years	5.1±1.9	4.44±1.82	1.61	0.11
Plasma zinc (µg/dl)	69.35±10.24	73.17±8.14	**t-test** -1.839	0.07
BMD-z score	-2.02± -0.57	-2.10± -0.60	-0.77	0.44

Data presented as mean ± SD, statistically significant difference, **p < 0.01& *P <0.05 (*) compared to the non-intervention group, BMI: Body mass index; BMD: bone mineral density.

Table (5) shows follow up of both intervention group and non -intervention group After 12 months of oral zinc supplementation although weight, height and BMI increased in the intervention group but still no significant difference between the two groups. On the other hand, serum zinc in the intervention group showed statistically significant elevation compared to the non-intervention group (p=0.001), and the same for BMD-z score of the anteroposterior spine that showed statistically significant elevation of bone mineral density in the intervention group compared to the non-intervention group(p=0.001).

Table (5) Comparison of anthropometric measure, serum zinc and BMD-z score between intervention group and non-intervention group after zinc supplementation:

Variable	intervention group	Non-intervention group	Z	p-value
Hight	**127.68±12.60**	**125.86±12.11**	**-0.636**	**0.23**
Weight	**30.57±6.90**	**29.23±6.80**	**-1.204**	**0.52**
BMI	**18.49±1.32**	**18.20±1.65**	**-0.953**	**0.34**
Serum zinc (µg/dl)	**86.80±11.39****	**78.70±11.59**	**-3.757**	**0.001**
BMD-z score	**-1.66± -0.55****	**-2.1± -0.6**	**-3.434**	**0.001**

Data presented as mean ± SD, statistically significant difference, **p < 0.01& *P <0.05 (*) compared to the non-intervention group, BMI: Body mass index; BMD: bone mineral density.

Table (6) shows comparison of anthropometric measures serum zinc and bone mineral density Z score in the intervention group before and after 12 month of zinc supplementation which revealed that statistically significant increase in all variables after zinc supplementation (p=0.001)**.**

Table (6) Comparison of anthropometric measures, serum zinc and BMD-z score of patients in intervention group before and after zinc supplementation:

	Base line	Post -intervention	z	P - value
Weight (kg) **Min-max** **Mean ±SD.** **Median (IQR)**	 **16-40** **26.80±7.02** **19.63(29)33**	 **21.5-45** **30.57±6.94** **23.85(31)35.85**	**-5.516**	**0.001**
Hight (cm) **Min-max** **Mean ±SD.** **Median (IQR)**	 **102-140** **124.12±12.36** **126(111-136)**	 **107-145** **127.67±12.10** **129(114.75-138.50)**	**-5.576**	**0.001**
BMI **Min-max** **Mean ±SD.** **Median (IQR)**	 **13.72-20.41** **17.01±1.51** **17.25(15.71-18.11)**	 **15.36-21.40** **18.49±1.32** **18.50(17.47-19.27)**	**-5.471**	**0.001**
Serum zinc **Min-max** **Mean ±SD.** **Median (IQR)**	 **50-90** **69.38±10.22** **66.5(61.25-79)**	 **70-110** **87.05±11.35** **86(77.25-95)**	**-5.518**	**0.001**
BMD-z score **Min-max** **Mean ±SD.** **Median (IQR)**	 **-1.20 - -3.40** **-2.02±-0.57** **-2.05(-1.6 - -2.30)**	 **-0.9-3** **-1.66± -0.55** **-1.6(-1.2 - -1.97)**	**-5.551**	**0.001**

IQR: Inter quartile range SD: Standard deviation Z: Wilcoxon signed ranks test statistically significant difference, **p < 0.01& *P <0.05 (*) post supplementation compared to pre-supplementation, BMI: Body mass index; BMD: bone mineral density.

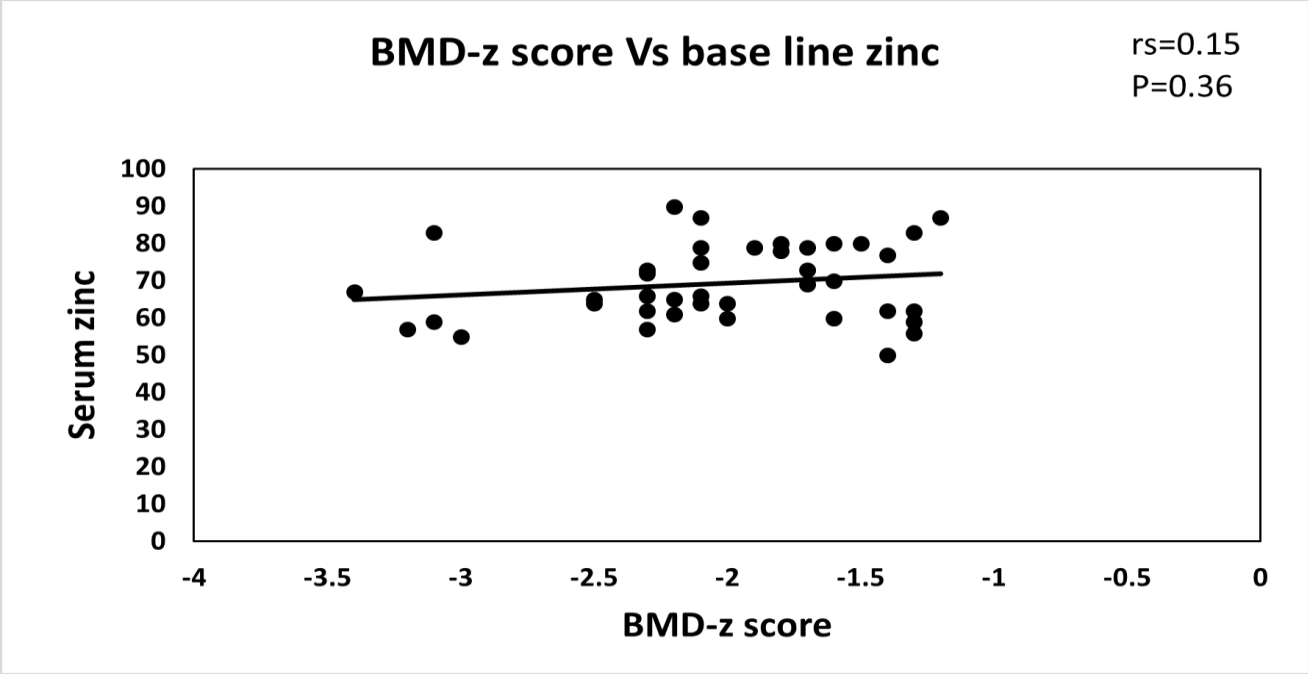

Figure (1) Correlation between BMD-z score, base line serum zinc in the intervention group(n=40). Correlation is significant at 0.05 level (2 tailed)

rs: Spearman coefficient **BMD**: bone mineral density

Figure 1 illustrate spearman rank correlation between BMD-z score and base line serum zinc in the intervention group which reveal a non-significant correlation (p=0.36).

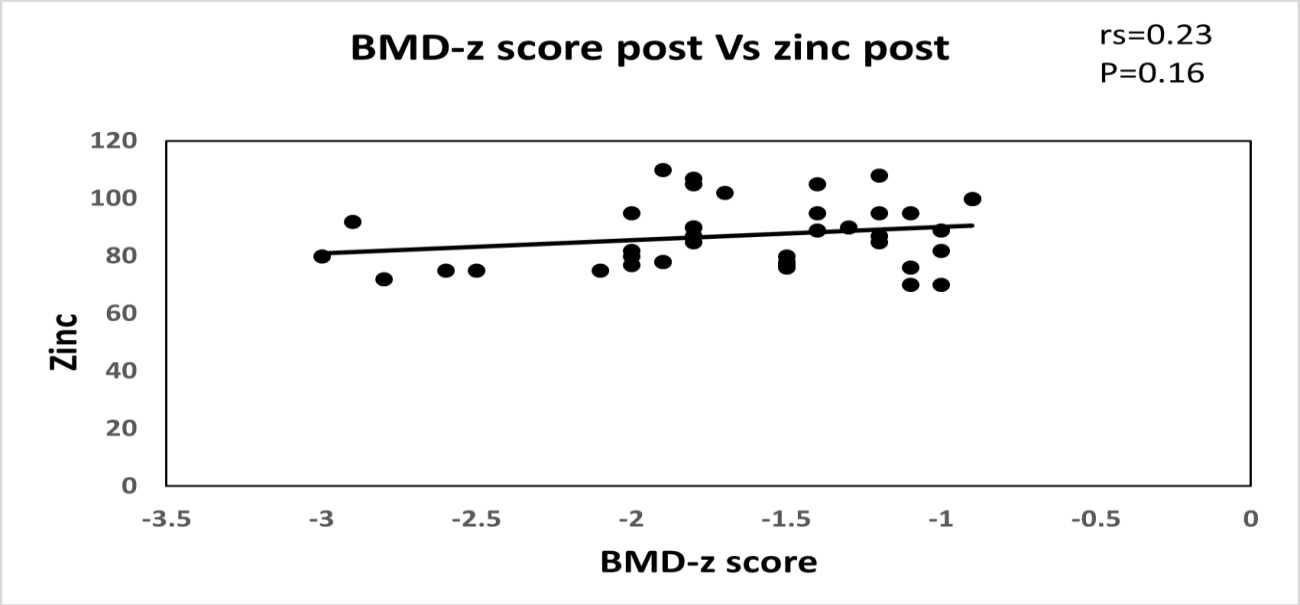

Figure (2) Correlation between BMD-z score, serum zinc post intervention in the intervention group(n=40). Correlation is significant at 0.05 level (2 tailed)

rs: Spearman coefficient **BMD**: bone mineral density

Figure 2 illustrates spearman rank correlation between BMD-z score and serum zinc post supplementation in the intervention group which reveal a non-significant correlation (p=0.16).

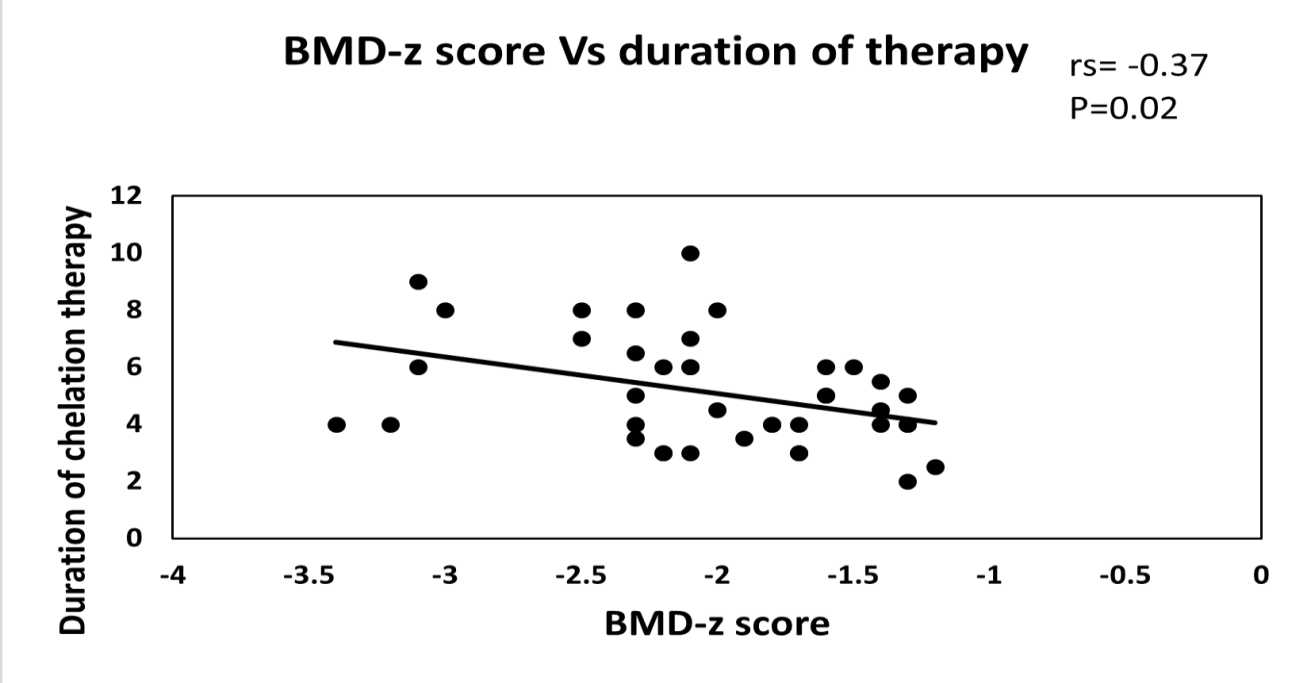

Figure (3) Correlation between BMD-z score, duration of chelation therapy in the intervention group (n=40). Correlation is significant at 0.05 level (2 tailed)

rs: Spearman coefficient BMD: bone mineral density

Figure 3 illustrates the spearman rank correlation between BMD-z score and duration of chelation therapy in the intervention group which reveals a significant negative correlation (p=0.02).

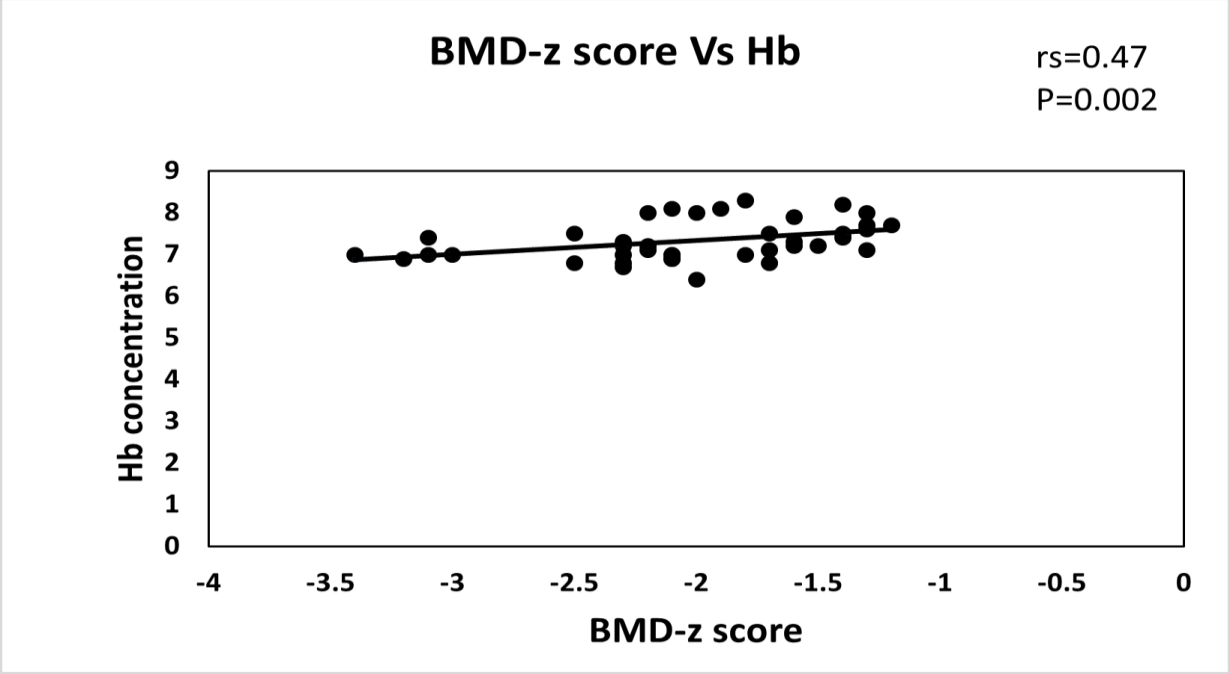

Figure (4) Correlation between BMD-z score and Hb(d) in the intervention group(n=40). Correlation is significant at 0.05 level (2 tailed)
rs: Spearman coefficient **BMD**: bone mineral density **Hb**: hemoglobin

Figure 4 illustrates spearman rank correlation between BMD-z score and hemoglobin concentration in the intervention group which reveal a significant positive correlation (p=0.002).

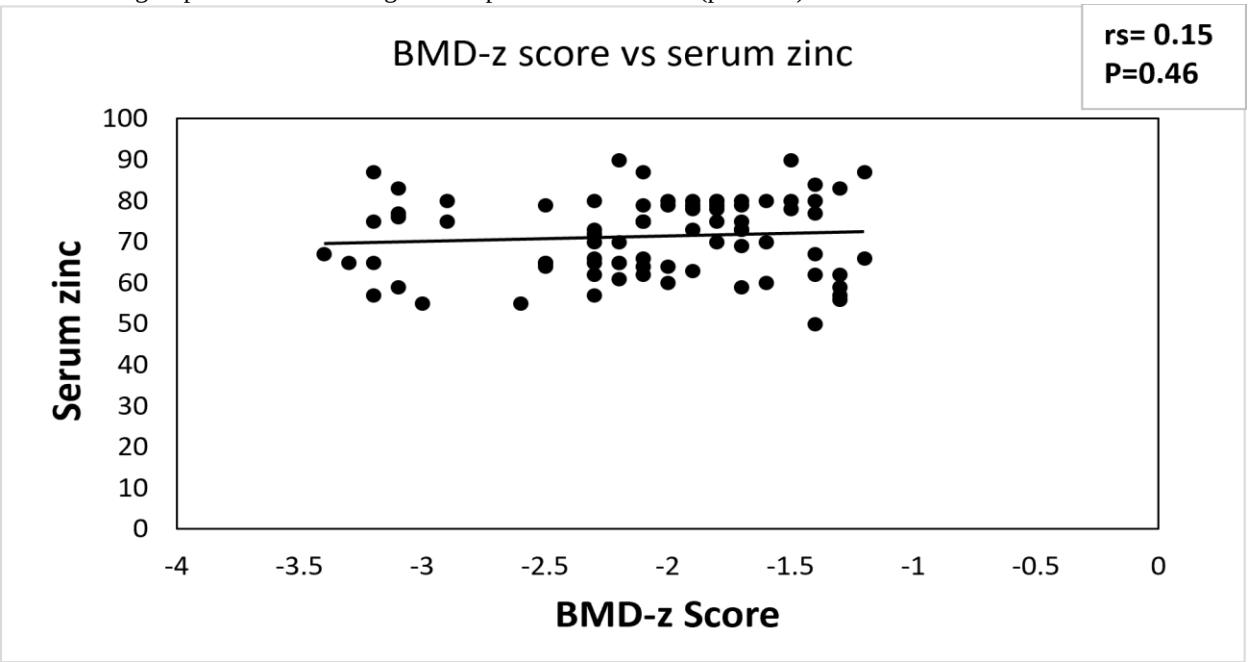

Figure (5) Correlation between BMD-z score and serum zinc in the whole study population(n=80). Correlation is significant at 0.05 level (2 tailed)
rs: Spearman coefficient **BMD**: bone mineral density

Figure 5 illustrates the spearman rank correlation between BMD-z score and serum zinc in the whole study population which reveals a non-significant positive correlation (p=0.46).

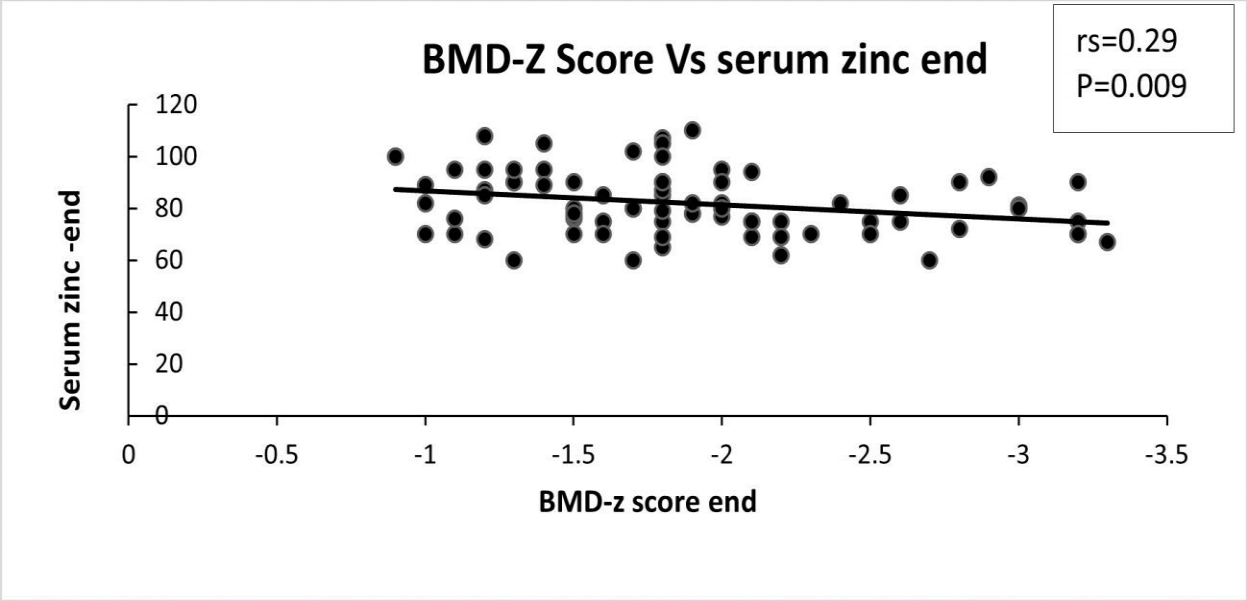

Figure (6) Correlation between BMD-z score and serum zinc post intervention in the whole study population(n=80). Correlation is significant at 0.05 level (2 tailed)
rs: Spearman coefficient **BMD**: bone mineral density

Figure 6 illustrates the spearman rank correlation between BMD-z score end and serum zinc end in the whole study population which reveals a significant positive correlation (p=0.009).

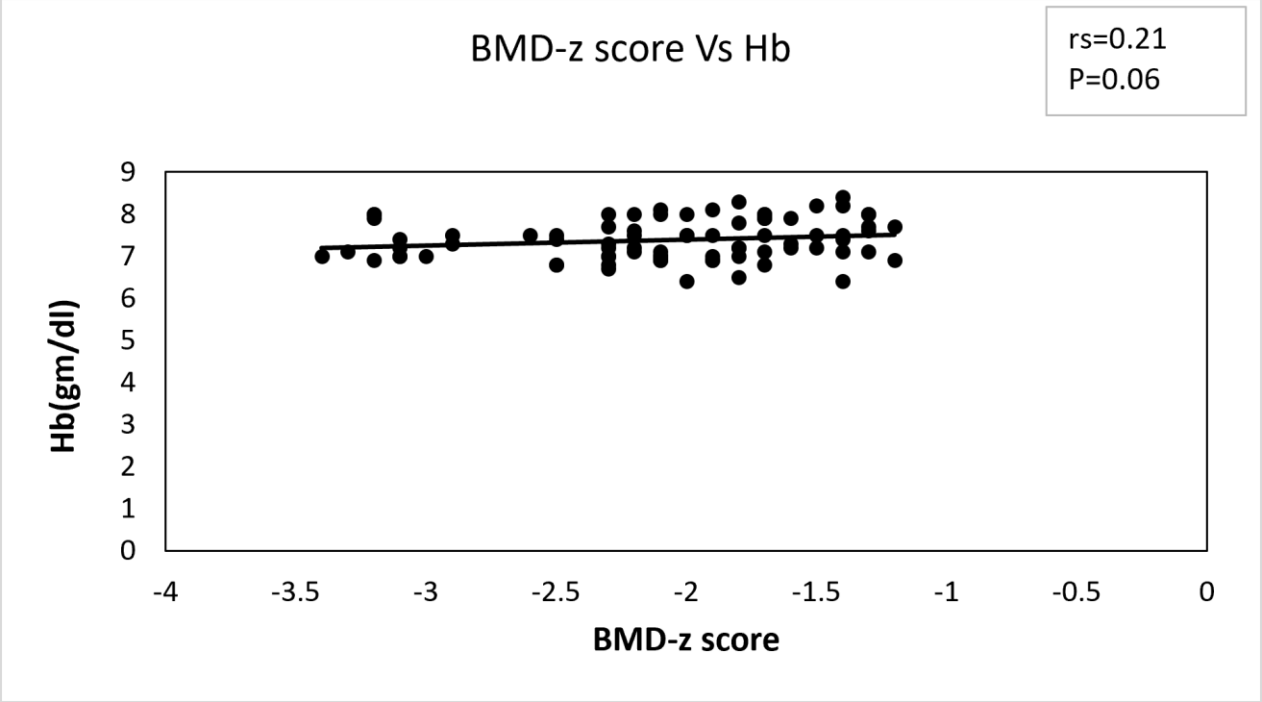

Figure (7) Correlation between BMD-z score and Hb in the whole study population (n=80). Correlation is significant at 0.05 level (2 tailed)
rs: Spearman coefficient **BMD**: bone mineral density

Figure 7 illustrates spearman rank correlation between BMD-z score and hemoglobin concentration in the whole study population which reveal a non-significant correlation (p=0.06).

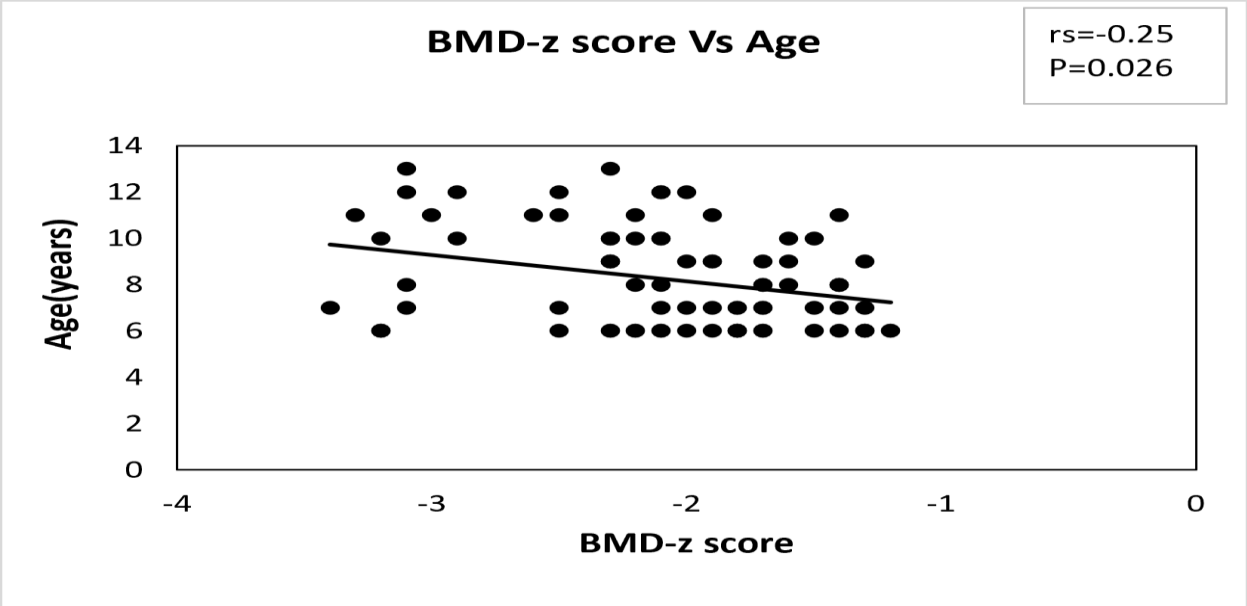

Figure (8) Correlation between BMD-z score and age in the whole study population (n=80). Correlation is significant at 0.05 level (2 tailed)
rs: Spearman coefficient **BMD**: bone mineral density

Figure 8 illustrate spearman rank correlation between BMD-z score and age in the whole study population which reveal a significant negative correlation (p=0.02).

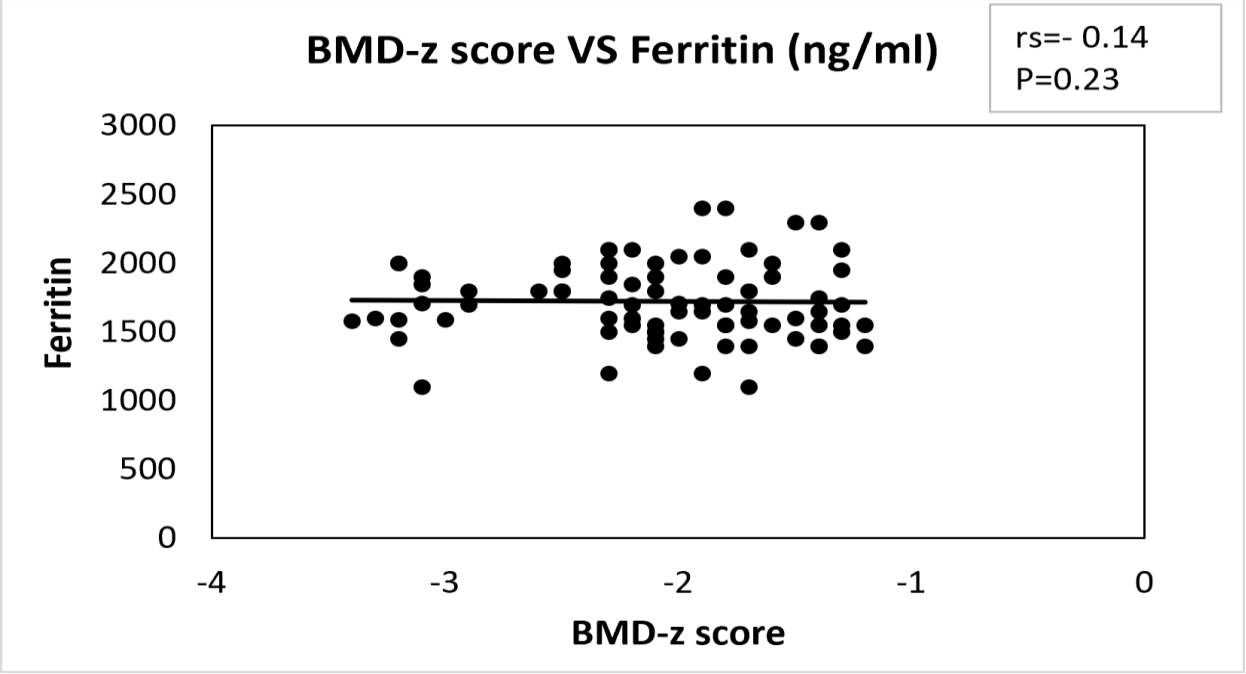

Figure (9) Correlation between BMD-z score and serum ferritin in the study population (n=80). Correlation is significant at 0.05 level (2 tailed)
rs: Spearman coefficient **BMD**: bone mineral density

Figure 9 illustrates spearman rank correlation between BMD-z score and serum ferritin in the whole study population which reveal a non-significant negative correlation (p=0.23).

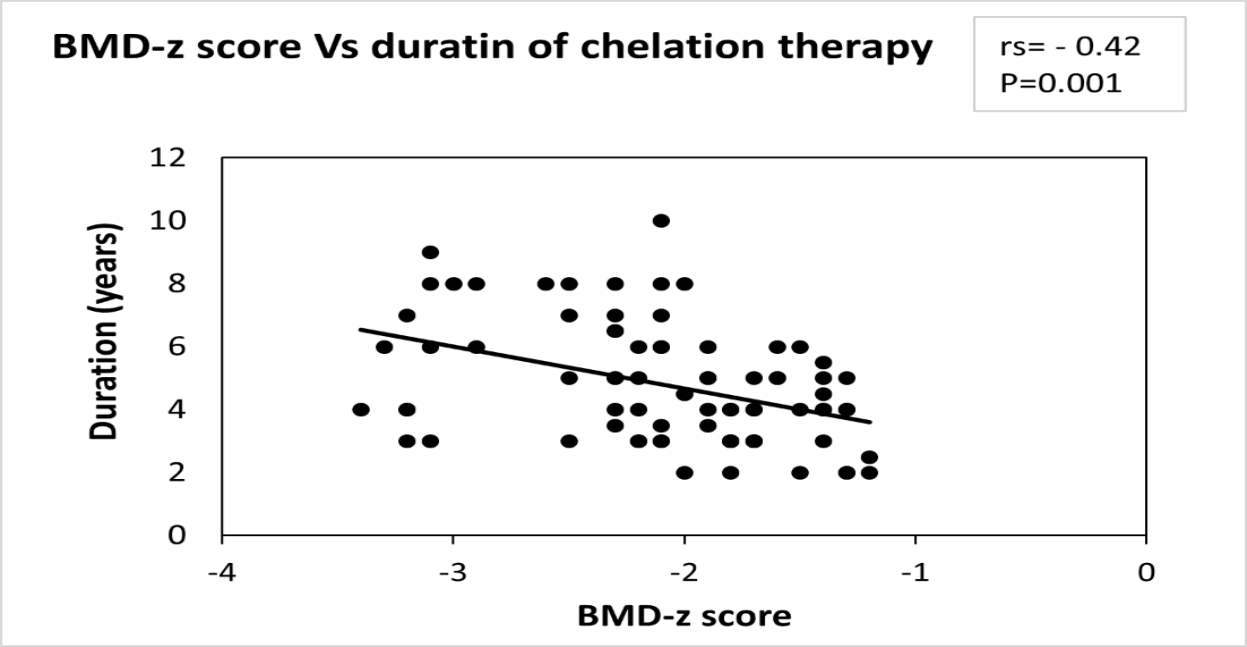

Figure (10) Correlation between BMD-z score and duration of chelation therapying the whole study population (n=80).

Figure 10 illustrates spearman rank correlation between BMD-z score and duration of chelation therapy in the whole study population which reveal a significant negative correlation (p=0.001).

Table 7 illustrates linear regression analysis of variables that were independently associated with BMD-z score to evaluate the predictivity of these variables linear regression analysis illustrates that serum zinc and duration of chelation therapy could be a predictor for low BMD in thalassemic children.

Table (7) Linear regression analysis for BMD post intervention(dependent variable)and (explantory varables)including serum zinc, ferritin , Hb, age, duration of chelation therapy:

Model	Variables	B	S.E	Beta	Sig	t	95%CI for B	
							Lower bound	Upper bound
1	Hb	0.03	0.15	0.021	0.8	0.19	-0.26	0.32
	Ferritin	$9.6*10^{-5}$	0.001	0.04	0.69	-0.39	-0.01	0.001
	Duration of chelation therapy	-0.2	0.09	-0.59	**0.03***	-2.19	-0.38	-.02
	Age	0.11	0.008	0.37	**0.2**	1.37	-0.05	0.27
	Serum zinc end	0.01	0.006	0.26	**0.02***	4.64	0.003	0.025
Constant		-3.04	1.33	- 0.02	-2.28		-5.69	-0.38

***Statistical significance at 0.05 level**

DISCUSSION

The current study's objective was to assess how zinc supplementation affected children with thalassemia major's bone density in order to improve bone health in thalassemia major patients.

Previous cross-sectional investigations found substantial BMD deficiency (osteoporosis) in 62% of Chinese thalassemia major children at the spine and 35% at the left hip [11,12]. Leung et al. [12] highlighted the significance of routine DXA monitoring in these patients by reporting a significant frequency of severe BMD loss in adolescents and young adults with thalassemia. The lumbar spine and femoral region of thalassemic patients have a

significant prevalence of poor bone mineral density, according to several research [13,14].

In a research including 72 thalassemic children between the ages of 6 and 39, Biswas et al. found that 20.8% of patients had poor bone mineral density, but only in the left femoral neck, while 79.2% of patients were found to have normal bone mineral density. Low bone mass was substantially correlated with the total number of blood transfusions and serum ferritin level in transfusion-dependent thalassemic patients [15]. Additionally, Nakavachara et al. [16] show that most children with thalassemia, whether they were transfusion-dependent or not, had normal BMD. They also noted that patients with TD thalassemia (TD: 4%–14%) experienced more lumbar spine damage from low bone mass than patients with NTD thalassemia (NTD: 1.7%–10.2%).

In a research by Bordbar and colleagues that included 713 thalassemia patients ranging in age from 10 to 30 years, it was shown that LBM was more commonly detected in the lumbar spine in around two-thirds of the patients [17].

The causes of low BMD, osteoporosis, skeletal morbidity, and a higher risk of fracture in thalassemia are complex and have been linked to a variety of hereditary and acquired factors, including dietary and metabolic factors [18–20].

According to De Sanctis et al. [19], these endocrine dysfunctions represent significant pathogenic processes connecting TM, poor BMD, and a greater risk of fracture. Androgen and estrogen insufficiency, which play significant roles in the formation and maintenance of the skeleton, are examples of endocrine dysfunction associated with iron. It has been demonstrated that estrogen lessens the activation of the nuclear factor kappa-B signaling receptor in osteoclasts and their progenitor cells. By boosting cytokine production or sensitivity to cytokines such interleukin (IL)-1, IL-6, tumor necrosis factor-, and prostaglandin, androgen and estrogen deprivation promotes bone resorption.

According to De Sanctis et al.[19], thyroid hormones modulate chondrogenesis, bone mineralization, bone turnover, BMD, and have anabolic effects to promote peak bone mass accumulation throughout growth. In children, delayed bone aging, growth arrest, and short stature have all been linked to hypothyroidism, which has been proven to reduce bone turnover and increase fracture risk.

In spite of taking vitamin D supplements, 92.5% of the Egyptian youngsters with thalassemy were found to have osteopenia and osteoporosis in a prior study [21].

In accordance with several studies on thalassemic children, which found decreased serum zinc in thalassemic children compared to control [22,23], the current study found that 57.5% of patients with thalassemia in the zinc supplementation group show a plasma zinc concentration 70 g/dl, which is used as a clinical marker of suboptimal zinc status, while 37.5% in Matter et al. [24] discovered that the zinc level in thalassemia patients was lower than the control value.

According to the current study, zinc supplementation considerably raised the plasma zinc level in the intervention group, which was also accompanied by an increase in BMD. Fung et al. [3] found that functional zinc deficiency existed in thalassemia patients despite apparently adequate dietary zinc intakes (122% of RDA) and plasma zinc levels. They also found that supplementing with zinc increased plasma zinc levels, improved appetite, and increased bone mineral density (BMD), with whole-body bone mineral increasing by 2-4% over the control group. These improvements are comparable to those previously seen with bisphosphonate therapy for thalassemia. In addition, zinc supplementation increases zinc levels in thalassemia patients with diabetes mellitus and also improves glycemic control, increases insulin secretion, and decreases iron burden, which may improve BMD in those patients [24].

According to Wu et al.'s [25] research, zinc is positively correlated with BMD in children under the age of three. They found that children with normal BMD have higher serum levels of zinc than children with low BMD and that the incidence of normal BMD increases as the serum level of zinc increases.

According to the research, a low intake of zinc (less than 3 mg/day) may increase the risk of fractures as well as the onset of osteopenia and osteoporosis. In terms of preserving bone mineral density and promoting quicker fracture healing, zinc supplementation (40–50 g/day) may be advantageous for bone health [26].

Our study had limitations. First, the sample size was relatively small which may lead to biased results and representations in the study. Second, history taking was simple and not illustrative enough for the lifestyle and nutritional habits of the children. Third, poor compliance of patient parents and adherence to the prescribed zinc supplementation at home.

CONCLUSION

We draw the following conclusions from the current study: bone mineral density deficit is common in children with thalassemia, routine DXA monitoring is advised for beta thalassemia patients for early detection, and zinc supplementation in beta

thalassemia children resulted in significant improvements in BMD, illustrating the importance of zinc for bone health. We recommend a nutrition education program for thalassemia children parents to improve knowledge about zinc supplementation for bone health. Further thoughtful studies investigate the effect of zinc supplementation on BMD in early childhood thalassemia children.

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