Effect of Zinc Supplementation on Linear Growth and Bone Mineral Density in Prepubertal Children with β - Thalassemia Major

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Abstract

Background: Linear growth and bone mineral density are commonly affected in thalassemic children and the aetiology is usually multifactorial.

Objective: To study the effect of zinc supplementation on linear growth and bone mineral density in prepubertal children with beta- thalassemia major.

Methodology: This is an intervention study at hematology clinic, Ain- Shams university, started at Aptil 2012. until April 2014. Forty prepubertal osteoporotic thalassemic children Nineteen males and twenty one females, were supplemented with oral zinc sulfate capsule, once daily for 12 months consecutively. Height percentile (plotted on DEMPU curves), serum zinc level and BMD Z- score (measured by DEXA) were measured both before and after zinc intake.

Results: There was statistically significant improvement when height percentile, serum zinc level andBMD Z- score were measured before and after zinc supplementation (P value <0.001). There was no statistically significant difference between males and females when all these parameters were measured either before or after zinc supplementation (P value >0.05).

Conclusion: Zinc supplementation had a positive impact on linear growth and BMD in prepubertal children with beta- thalassemia major and this effect couldhave been magnified if zinc was provided at an earlier age and for a longer time.

Key words: zinc supplementation- linear growth- prepubertal children- beta thalassemia major

أثر إعطاء الزنك على الكثافة المعدنية للعظام والنمو الخطى فى سن ما قبل البلوغ للأطفال المصابين بأنيميا البحر المتوسط

الخلفية: تعد مشكلة نقص النمو الطولي ونقص كثافة أملاح العظام وما يتبعه من حدوث هشاشة بالعظام من المشكلات الشائعة في الاطفال اللذين يعانون من انيميا البحر المتوسط، خاصة قبل سن البلوغ، وهذا بدوره يؤدي الى ضعف العظام وزيادة قابليتها للكسور مما قد يتعارض مع الامشطة اليومية لهؤلاء الاطفال.

الهدف: تهدف هذه الدراسة الى تقييم نتائج اعطاء عنصر الزنك لهؤلاء الاطفال الذين تم اختيارهم من المترددين على عيادة امراض الدم بقسم الاطفال بمستشفيات جامعة عين شمس على النمو الطولي لهم وعلى كثافة املاح العظام.

المنهج: تم ذلك باعطاء كل طفل ٢٥ جم زنك يوميا بالفم لمدة ١٢ شهر متواصلة ثم بعد ذلك تم قياس الطول ووضعه على منحنى الطول الخاص بالاطفال المصريين، كما تم قياس معامل (Z) كمؤشر للتغيير في كثافة املاح العظام.

النتائج: أظهرت النتائج ان اعطاء الزنك له تأثير ايجابي على زيادة النمو الطولي و زيادة كثافة املاح العظام في هؤلاء الاطفال وأن هناك اختلافا احصائيا واضحا بين الطول وكثافة املاح العظام قبل وبعد اعطاء الزنك. و أظهرت الدراسة والنتائج انه لم يكن هناك اختلافات احصائية واضحة بين الذكور والاناث فيما يتعلق بالزيادة في النمو الطولي او في مقدار كثافة املاح العظام. كما أوصت الدراسة بأنه يتم تتبع النمو الطولي وكثافة املاح العظام في الاطفال المصابين بانيميا البحر المتوسط وان يتم اعطاء هؤلاء الاطفال عنصر الزنك في صورة دواء.

الكلمات الكاشفه: زنك– انيميا البحر المتوسط– عمر ما قبل البلوغ– النمو الطولي– هشاشه العظام

Introduction:

Thalassemia ismonogenetic, hereditary hematologic disorders, inherited as an autosomal recessive blood disorder, characterized by abnormal formation of hemoglobinand presenting as microcytic anemia.[1] Thalassemic patients have unbalanced globin chain synthesis with ineffective erythropoiesis and increased peripheral hemolysis.[2] Expansion of bone marrow cavity, decreased cortical and trabecular bone tissues and osteoporosis are commonly seen in these patients.[3] Symptoms include fatigue, shortness of breath, bone deformities in the face, growth failure and yellow skin.[4] Signs are usually linked toiron overload, infection, bone deformities, enlarged spleen, slowed growth rates, andheart problems.[5]

Pathogenesis of osteoporosis in thalassemic patients is multi- factorial. Bone marrow expansion due to ineffective erythropoiesis results in reduction of trabecular bone tissue.[6] Iron deposition directly in the bone impairs osteoid maturation and inhibits mineralization. Use of iron chelators as desferrioxaminecan lead to lower serum calcium, magnesium and higher phosphorusand parathyroid hormone level levels.[7] Endocrine dysfunction consisting mainly of hypogonadism in addition to hypothyroidism, hypoparathyroidism can impair osteogenesis.[8] Reduced physical activity as a result of the disease complications and parental over- protectiveness can worsen the condition.[9] Growth failure is usually noticed in thalassemics.[10] It could be attributed tochronic anemia, transfusion- related iron overload, and chelation toxicity. Other contributing factors include hypothyroidism, hypogonadism, growth hormone deficiency/ insufficiency, chronic liver disease, undernutrition, and psychosocial stress.[11] Malnutrition, inadequate nutrient intake (zinc, folic acid, vitamin D, carotenoids, and retinol binding proteins) may play a role in growth retardation in these children.[12]

Bone mineral density (BMD) is defined clinically as the amount of bone minerals- mostly calcium and phosphorus- expressed in grams in a given area of bone, expressed in square centimeters.[13] BMD is measured by Dual- energy X- ray absorptiometry (DEXA) which is considered to be the most standardized form and widely used technique due to its ease of use, precision, accessibility and low cost, in comparison to other modalities of BMD measurement. DEXA can measure as little as 2% of bone loss per year. It is fast and uses very low doses of radiation.[14]

Densitometry reporting is done either by using T- score in which BMD is compared to the average score of a healthy 30- year- old person, or Z-score in which BMD value is compared to thata healthy personof the same age, sex, and race as the examined person.[15]

Zinc is trace element and a micronutrient of outstanding and diverse biological and clinical importance.Z inc ions have catalytic, structural, and regulator functions.[16] Zinc is an essential trace element to the structure and function of bone and its metabolism.[17] Itis an essential for all the steps of osteogenesis, including the production of the extracellular organic matrix (osteoid),t he mineralization of the matrix to form bone; and bone remodeling by resorption and deposition.[18] Accordingly, zinc supply can have a positive impact on both bone mineral density and linear growth.[19]

Aim of the study:

To study the effect of zinc supplementation on linear growth and bone mineral density in prepubertal children with beta- thalassemia major.

Subjects And Methods:

- Study Population: This intervention study was conducted on forty prepubertalthalassemic children with beta- thalassemia major, 19 males (47.5%) and 21 females (52.5%).T heir age ranged from 6.2- to 10.8 years (mean age 8.99± 1.32 years). Children were recruited from the regular attendants of Hematology clinic, Department of Pediatrics, Children hospital, Ain Shams University and work was completed in Institute of Postgraduate Childhood Studies, Ain Shams University. The study started at April 2012 and continued until April 2014. All selected children fulfilled the following inclusion criteria: age between 6 and 10 years, diagnostic criteria of β- thalassemia major, history ofrepeated blood transfusion and receiving chelating therapy, osteoporosis as shown by DEXA (Z-score<- 2.5), normal serum calcium and no bisphosphonates therapy.

  An informed consent was obtained from parents after full explanation of the aim of the study and nature of the work. Also approval of the ethics committee of the university was taken.

- Methods: All children were subjected to full history taking, full comprehensive medical examination and pubertal development asssessment. Venous blood sampleswere taken to measure serum zinc level. Blood samples were collected in ice boxes to the Community and Public Health Department laboratory where serum zinc level measurement was carried out in the atomic absorption laboratory using the atomic absorption spectrometer Autosampler Model A 600) Serum zinc values< 80 µg/ dl are considered hypozincemia.[20] The degree of hypozincemia was suggested to be classified according to serum zinc level Degree of hypozincemia was classified according to serum zinc level table (1).

Table (1) Degree of hypozincemia in relation to serum zinc level

Degree of hypozincemia (<80 µg/dl)	Serum zinc level (µg/dl)
Mild	70-< 80 µg/dl
Moderate	60-< 70 µg/dl
Severe	50-< 60 µg/dl

Height and weight were recorded prior to DEXA scanning. Both were plotted on the Egyptian percentile growth charts for stature and body mass index (BMI)[21]. Z score of the spine was recorded as a measure of BMD.

BMD Z-score from 2.5 to- 1 was considered normal, BMD Z-score from< - 1 to -2.5 was considered osteopenia andBMD Z-score< - 2.5 was considered osteoporosis.[22]

All the patients undergoing this study were osteoporotic with Z-score< - 2.5. A new classification of osteoporosis was suggested in order to facilitate the assessment of changes in BMD after zinc supplementation. Osteoporosis was classified as shown in table (2).

Table (2) Degree of osteoporosis according to BMD Z- scoring.

Degree of osteoporosis (<- 2.5 SD)	Z- Scoring
Mild	<- 2.5 to- 3
Moderate	<- 3 to- 3.5
Severe	<- 3.5

Prior to zinc supplementation CBC, serum ferritin, ALT, serum calcium and hemoglobin electrophoresis were obtained by reviewing the patients records. BMI was also recorded.

Zinc was provided to the selected patients in the form of zinc capsules (vita zinc 25 mg once daily).A fter 12 month of zinc supplementationeach patient was subjected to evaluation of serum zinc, DEXA scanning to measure BMD Z-score, putting in consideration to use the same machine used in primary BMD assessment to avoid soft ware differences between machines, which might give inaccurate resultsandheight percentile was measured and plotted in the same way as before zinc supplementation.

Statistical Analysis:

Data obtained from the research will be organized, tabled and analyzed with the help of SPSS/PC. Categorical data were presented as frequencies (n) and percentage (%). Pearson Chi- Square was used to analyze categorical data. P value was calculated and P value less than 0.05 was considered statistically significant. Information and statistical data were provided by IT and, Institute of Postgraduate Childhood Studies.

Results:

Table (3) Frequency distribution of patients regarding their height percentile beforeand after zinc supplementation

	Before Zinc Supplementation		After Zinc Supplementation	
	N	%	N	%
5th-< 10th	12	30.00	6	15.00
10th-< 25th	19	47.50	18	45.00
25th-< 50th	9	22.50	14	35.00
50th-< 75th	0	0.00	2	5.00
Total	40	100.00	40	100.00
Chi- Square (X^2)	5.93			
P- Value	0.115			

Before zinc supplementation 77.5% of patients have less than 25th height percentile. The figure dropped to 60% after zinc was provided. No patients recorded 50th height percentile or more before zinc was given and 5% exceeded 50th percentile after zinc supplementation.

Table (4) Comparison of height, serum zinc level, and BMD Z- scoring, before and after zinc supplementation in the total sample

		Range	Mean± SD	Paired Samples Test	
				t	P- Value
Height (Cm)	Before Zinc Supp.	114.15- 139.35	121.30 ± 12.64	- 1.512	<0.001*
	After Zinc Supp.	118.90 - 150.10	128.80± 13.92		
Serum Zinc (Mg/Dl)	Before Zinc Supp.	52.000- 86.000	67.925± 6.896	- 6.325	<0.001*
	After Zinc Supp.	56.000 - 81.000	70.250± 6.348		
Bmd Z-score (Sd)	Before Zinc Supp.	- 3.900- - 2.600	- 3.008± 0.410	- 5.981	<0.001*
	After Zinc Supp.	- 3.700- - 1.800	- 2.465± 0.496		

There was statistically significant difference when mean serum zinc level, mean height and mean BMD Z-score were measured before, and after zinc supplementation for 12 months (P value< 0.001)

Before zinc was provided, the height ranged between 114.15 and 139.35cm (mean 121.30± 12.64 cm). After zinc was given for one year, the height ranged between 118.90 and 150.10 (mean 128.80± 13.92 cm).

Serum zinc level was measured prior to zinc supplementation. Serum zinc level ranged between 52µg/ dl and 86µg/ dl (mean 67.925± 6.896 µg/dl). Serum zinc was measured after zinc supplementation for one year. Serum zinc level ranged between 56 µg /dl and 81µg/ dl (mean 70.250± 6.348 µg/ dl).

Before zinc supplementation BMD Z-score for the patients in the study sample ranged between- 3.9 and -2.6 (mean- 3.008± 0.410). After zinc was supplemented for one year, Z-score ranged between- 3.7 and -1.8 (mean- 2.465± 0.496).

Table (5) Comparison of height, serum zinc level, and BMD Z- scoring, before and after zinc supplementation in both males and females.

		Gender		T- Test	
		Female	Male		
		Mean± SD	Mean± SD	t	P- Value
Height. (Cm)	Before Zinc Supp.	122.34± 12.22	120.00± 11.543	7.765	0.345
	After Zinc Supp.	129.55± 12.657	127.59± 12.005	7.167	0.269
Serum Zinc	Before Zinc supp. (µg/dl)	66.429± 6.226	69.579± 7.381	- 1.464	0.151
	after zinc supp. (µg/dl)	69.810± 6.577	70.737± 6.226	- 0.457	0.651
BMD Z-score	before zinc supp. (SD)	- 2.971± 0.366	- 3.047± 0.461	0.579	0.566
	After Zinc supp. (SD)	- 2.400± 0.460	- 2.537± 0.537	0.868	0.391

There was no statistically significant difference between males and females when mean height, serum zinc level and BMD Z-score were measured before and after zinc supplementation (P value>0.05).

Table (6) Mean height, serum zinc level and BMD Z-score before and after zinc supplementation in males.

	Before Zinc Supp.	After Zinc Supp.	t	P Value
Mean Height (Cm)	120± 11.543	127.59± 12.005	- 11.231	<0.001*
Mean serum zinc level (µg/dl)	69.579± 7.381	70.737± 6.226	- 6.002	<0.001*
Mean Z score (SD)	- 3.047± 0.461	- 2.537± 0.537	- 12.762	<0.001*

There was statistically significant difference in males when mean serumzinc, height and BMD Z-score were measures before and after zinc supplementation (P value< 0.001).

Table (7) Mean height, serum zinc level and BMD Z-score before and after zinc supplementation in females.

	Before Zinc Supp.	After Zinc Supp.	t	P Value
Mean Height (Cm)	122.34± 12.22	129.55± 12.657	- 11.584	<0.001*
Mean serum zinc level (µg/dl)	66.429± 6.226	69.810± 6.577	- 6.221	<0.001*
Mean Z-score (SD)	- 2.971± 0.366	- 2.400± 0.460	- 12.612	<0.001*

There was statistically significant difference in females when mean serumzinc, height and BMD Z-score were measures before and after zinc supplementation (P value< 0.001).

Table (8) Male and female mean values for serum hemoglobin, WBCs, platelets, serum ferritin, calcium, ALT, and hemoglobin electrophoresis before zinc supplementation.

	Gender	N	Mean	± Sd	t	P Value	Sig.
WBC ($\times10^3$/ml)	M	19	7.4	2.7	1.052	0.282	NS
	F	21	6.9	2.1			
PLT. ($\times10^3$/ml)	M	19	320.2	190.2	1.974	0.061	NS
	F	21	331.4	195.4			
Hb (gm/dl)	M	19	6.2	0.6	2.615	0.595	NS
	F	21	6.8	0.6			
Serum ferritin (ng/ml)	M	19	3755.5	2011.2	0.541	0.601	NS
	F	21	3659.4	2056.4			
ALT (iu/L)	M	19	49.7	22.9	0.079	0.922	NS
	F	21	50.2	24.4			
Serum calcium (mg/dl)	M	19	9.79	0.59	2.822	0.625	NS
	F	21	9.92	0.61			
Hb. Elecroph. Hb. F%	M	19	56.77	26.4	0.082	0.982	NS
	F	21	54.51	23.6			
BMI	M	19	19.4	10.8	0.455	0.422	NS
	F	21	19.6	10.9			

Before zinc supplementation there was nostatistically significant difference between males and femalesregarding the mean values for serum hemoglobin,

WBCs, platelets, serum ferritin, calcium, ALT, and hemoglobin electrophoresis (P value>0.05).

Discussion:

In the present study the impact of zinc supplementation on linear growth and bone mineral density in prepubertalthalassemic children was evaluated. Serum zinc level was measured prior to zinc supplementation. Serum zinc level ranged between 52µg/dl and 86µg/dl (mean 67.925± 6.896 µg/dl). Ninety five percent of the patients undergoing this study showed low serum zinc level (less than 80µg/dl), and only five percent showed normal level (≥ 80µg/dl).T hese findings come near to those obtained by many authors.[23- 25]

With respect to reference cut- off point of serum zinc level (80 µg/dl), 27.5% of patients in this study presented with mild zinc deficiency, with serum zinc level ranging between 70µg/dl and 79µg/dl. Those presented with moderate zinc deficiency (60- 69µg/dl) were%62.5. Severe deficiency (50- 59µg/dl) was reported in only 5% of patients.

Although Keikhaei et.al, 2010 showed hypozincemia in 50% of their patients, 20% of their patients had severe zinc deficiency.[26] This might be because older patients were involved in their study, with mean age (15± 5) years.

Many studies reported decreased serum zinc level in thalassemic patients. Arcasoy et.al. (2001) reported that chronic Zn deficiency occur in the patients with thalassemia major (TM), but they stressed on the importance of measuring serum zinc binding capacity (ZnBC) to evaluate body zinc status. They postulated that Zn deficiency in TM may be due to hyperzincuria, high ferritin levels, hepatic iron load and hepatic dysfunction. The study has been carried out on 30 TM patients. As control group, 13 healthy children were involved. Serum Zn and ZnBC were determined by atomic absorption, then saturation index (SI%: serum Zn/ZnBC x 100) was calculated. Serum Zn levels in all patients were lower than control (p< 0.01). Serum ZnBC was found to be lower in TM patients than control (p< 0.01).

While serum Zn levels decrease and ZnBC increase in nutritional Zn deficiency, serum Zn levels decrease but ZnBC doesn't increase in patients with thalassemia.[23]

Tabatabei et.al. (2003) reported that 84% of ß- thalassemia major patients had zinc deficiency.[27] Fikry et.al. (2003) found apositive correlation between serum level and height/age.[24]

Bekheiia et.al. (2004), reported that low serum zinc was found in 84.8% of their patients. Hypozincemia was severe in 44.7% of them. Patients under this study were aged 10- 20 years. The above mentioned study might suggest that the severity of hypozincemia increases with age.T his probably might pay the attention for early zinc supplementation to avoid the negative impact of zinc deficiency in older age.[28]

Mahyar et.al. (2010) in their study proved low serum zinc level in thalassemic children.[29] Keikhaei et.al. (2010) showed hypozincemia in 50% of their patients in a similar study.[26] Sherief et.al. (2014), showed significantly lower level of zinc in thalassemic group than controls.[25]

Contrary to the results of the former studies, Kosarian et.al. (2000) and Reshadat et.al. (2006) found that serum zinc level in their thalassemic patients and controls was within normal limits.[30, 31]

Mehdizadeh et.al. (2008) found that zinc deficiency in thalassemic patients who are on regular blood transfusion is rare.[32]

Before zinc supplementation BMD Z-score for the patients in the study sample ranged between- 3.9 and- 2.6 (mean- 3.008± 0.410). After zinc was supplemented for one year, BMD Z-score ranged between- 3.7 and- 1.8 (mean- 2.465± 0.496).T he difference between mean BMD Z-score before and after zinc supplementationwas statistically significant (P value< 0.001).

The mean BMDZ-score before zinc was provided, was in the moderate osteoporosis category (- 3.008± 0.410). After zinc supplementation this mean became no longer in the osteoporotic side and moved to the osteopenic side (- 2.465± 0.496).

This in turn can reflect a positive impact of zinc supplementation on these osteoporotic patients. It is remarkable that 67.5% of cases became no longer osteoporotic and had their Z-score more than- 2.5 (osteopenia).A lthough BMD Z- scoring for 67.5% of the patients in this study moved from osteoporosis to osteopenia (63.2% of males and 71.4% of females had their BMD Z- scoring moving from osteoporosis to osteopenia), none of our patients had a normal BMD Z-score at the end of the study. This probably could have been achieved if we start giving zinc earlier and /or if we continue giving zinc for a longer period.

It was also remarkable that zinc intake improved BMD Z- scoring in males and females more or less indifferently.

Leung et.al. (2009) reported that 62% of their thalassemic patients showed low BMD Z-score than normal.[33] Pirinççioğlu. (2011) found significantly lower BMD findings in ß- thalassemic children in comparison to their peers.[34]

Bekheiia et.al. (2004) reported low serum zinc levels in 84.8% of the cases of their study compared to controls. They reported decreased BMD in 68.7% of these patients.[28]

Izadyar et.al. (2012) found that at least 50% of their thalassemic patients suffered from some degree of bone mineral loss, either osteopenia or osteoporosis.[35]

Fung et.al. (2013) came to the conclusion that if zinc supplementation is found to have a clinically important effect, this simple, safe, non- invasive therapy could quickly become a part of the standard care of these young patients and improve overall health in children and adult patients with ß-thalassemia.[36]

Before zinc was provided, theheight ranged between 115.15 and 139.35 cm (Mean 121.30± 12.64 cm). After zinc was given for one year, the height ranged between 118.90 to 150.10 cm (Mean 128.80± 13.92 cm). The difference in mean height before and after zinc was supplemented was statistically significant (P value< 0.001).

Males and females look to react indifferently to zinc supply regarding their mean height change after zinc supplementation.

Arcasoy et.al. (1987) studied Effects of zinc supplementation on linear growth in β- thalassemia. They demonstrated that zinc deficiency is one of the factors responsible for retarded linear growth in beta- thalassemia major. Only the patients who received zinc supplementation showed an acceleration of growth in height.[23]

Faranoush et.al. (2008).I n their studycame to the conclusion that oral zinc sulfate has no significant effect on linear growth of β- thalassemia patients.[37]

Conclusion:

1. Zinc supplementation for one year for prepubertalthalassemic children can have a positive impact on their linear growth as well as their bone mineral density.
2. There is statistically significant difference on measuring mean serum zinc

level, height and BMD Z-score before and after zinc supplementation.

Recommendations:

Zinc supplementation to prepubertalthalassemic children is advised to improve their BMD. This in turn can improve their bone strength and quality leading to a better quality of life. Furthermore, providing zinc to thalassemic children may improve their height velocity, and in turn their final stature.

References:

1. Quirolo K, and Vichinnky E. (2007): Hemoglobin disorders: Behraman RE. **Nelson Textbook of Pediatrics**. 18th ed. Philadephia; Saunders. ; Pp: 2033- 9
2. Lukenn JN. (1999): The thalassemia and related disorders. In: Richard Lee GR, Foeuster J, Lukens J, Parakeras F, Green J, Rodgers GM. **Wintrobes clinical hematology**. 18th ed. Philadelphia; Lippincott, Williams& Wilkins. Pp: 1406- 35.
3. MohamadN, and Jackson N. (1998): Severe thalassemia intermedia: Clinical problems in the absence of hypertransfusion. **Blood Rev**. ; 12: 163- 70
4. DeBaun MR, Frei- Jones M, VichinskyE, Kliegman RM, Behrman RE, Jenson HB, Stanton BF (2011):, Hemoglobinopathies. eds. **Nelson Textbook of Pediatrics**. 19th ed. Philadelphia, PA: Saunders Elsevier; chap 45.
5. Cianciulli P (2008): "Treatment of iron overload in thalassemia". **PediatrEndocrinol Rev** 6 (Suppl 1): 208- 13.
6. Vichinsky EP. (1998): The morbidity of bone disease in thalassemia. Ann N Y **AcadSci** 850: 344- 348
7. Anapliotou L, Kastanias I, Psara P, EvangelouE, Liparaki M, and Dimitriou P. (1995): "The contribution of hypogonadismto the development of osteoporosis in thalassemia major: new therapeutic approaches", **Clinical Endocrinology**, vol. 42, no. 3, pp. 279- 287
8. Wonke B. (1998): Bone disease in beta- thalassemia major. **Br J Haematol** 103: 897- 901.
9. De Sanctis V, Pintor C, andGamberini M. (1995): "Multicentre study on prevalence of endocrine complications in thalassemia major", **Clinical Endocrinology**, vol. 42, no. 6, pp. 581- 586
10. Soliman AT, Khalafallah H, andAshour (2009): Growth and factors affecting it in thalassemia major. **Hemoglobin**; 33:116- 26
11. Skordis N. (2011): The growing child with Thalassaemia. **J Pediatr Endocrinol Metab**; 19: 467- 9
12. Fuchs GJ, Tienboon P, Linpisarn S, Nimsakul S, Leelapat P, and Tovanabutra S. (1996): Nutritional factors and thalassemia major. **Arch Dis Child**; 74: 224- 27
13. Segen's (2012): **Medical Dictionary**. © Farlex.
14. Leib ES, Lewiecki EM, Binkley N, andHamdy RC. (2004):O fficial positions of the International Society for Clinical Densitometry. **J ClinDensitom** 7:1- 6.
15. Kocks J, Ward K, Mughal Z, Moncayo R, Adams J, and Högler W. (2010): Z-score comparability of bone mineral density reference databases for children, **JClinEndocrinolMetab.** ; 95 (10):4 652- 9
16. Szewczyk B. (2013):Z inc homeostasis and neurodegenerative disorders, Front Aging, **Neurosci**, Vol 5, No. 5, Article 33
17. Tapiero H, Tew KD. (2003): Trace elements in human physiology and pathology: zinc and metallothioneins. **Biomed Pharmacother**. ; 57:399- 41
18. Doherty CP, Crofton PM, Sarkar **MA**, Shakur MS, Wade JC, andKelnar CJ. (2002): Malnutrition, zinc supplementation and catch- up growth: Changes in insulin- like growth factor I, its binding proteins, bone formation and collagen turnover. **ClinEndocrinol** (Oxf); 57:391- 399
19. Mamidi RS, Shidhaye P, Radhakrishna KV, Babu JJ, and Reddy PS. (2011): Pattern of Growth Faltering and Recovery in Under- 5 Children in India Using WHO Growth Standards- A Study on First and Third National Family Health Survey. **IndPedvol** 48 (11): 855- 60
20. Perkin Elmer (2011): **Autosampler**, Model A 600 Supplies
21. DEMPU and NRC. (2003): **Diabetes Endocrine and Metabolic Pediatric Unit and the National Research Center**, Cairo University in collaboration with Wright State University, School of Medicine Department of Community Health. Health Research Center. From total sample of 33189 girls and boys.
22. Kanis JA, Black D, Cooper C, Dargent P, Dawson- Hughes B, De Laet C, Delmas P, Eisman J, and Johnell O. (2002):A new approach to the development of assessment guidelines for osteoporosis, **Osteoporos Int**. ; 13 (7):5 27- 36.
23. Arcasoy A, Canata D, Sinav B, Kutlay L, Oguz N, andSen M. . (2001): Serum zinc levels and zinc binding capacity in thalassemia. **J Trace Elem Med Biol**; 15: 85- 87
24. Fikry SI, Saleh SA, Sarkis NN, and Mangoud H. (2003): Study of serum zinc in relation to nutritional status among thalassemia patients in **Damanhour Medical National InstituteJ Egypt Public Health Assoc.** ; 78 (1- 2):7 3- 93.
25. Sherief L, Abd El- Salam S, Kamal N, El safy O, Almalky M, Azab S, H, Amal F. Gharieb A. (2014): Nutritional Biomarkers in Children and Adolescents with Beta- Thalassemia- Major: **An Egyptian Center ExperienceBio**Med **Research International Journal**; Volume 2014 pages1- 7
26. Keikhaei B, Badavi M, Pedram M, Zandian K, Rahim F. (2010):S erum Zinc Level in Thalassemia Major. **Pak J Med Sci**; 26 (4):9 42- 945
27. Tabatabei M, Kamkar M, and Habibzadeh M. (2003): "Metabolic and endocrine complications in beta thalassemia major in a multicenter study", **Tehran University Medical Journal**, vol. 5, no. 1, pp. 72- 73
28. Bekheiia MR, Shahirsaz AA, Kamgar M, Bouzari N, Erfanzadeh G, andPourzahedgilani N. (2004): Serum zinc and its relation to bone mineral density in beta- thalassemic adolescents. **Biol Trace ElemRes**; 97: 215- 24
29. Mahyar A, Ayaz A. PahlevanA,. Mojabi H, Sehhat M, and Javadi A. (2010): "Zinc and copper status in children with beta- thalassemia major", **Iranian Journal of Pediatrics**, vol. 20, no. 3, pp. 297- 302
30. Kosarian M, Valaee N, and MahdyyaneeA. (2000): "Do the desferal receiver thalassemic patients have zinc deficiency?", **Journal of Mazandaran University of Medical Sciences**, vol. 26, no. 10, pp. 1- 6
31. Reshadat S, Kiani A, and Iranfar S. (2006): "Zinc level of major thalassemic patients in Kermanshah", **Behbood**, vol. 2, no. 10, pp. 157- 167
32. Mehdizadeh M, Zamani G, and Tabatabaee S. (2008): "Zinc status in patients with major β- thalassemia", **Pediatric Hematology and Oncology**, vol. 25, no. 1, pp. 49- 54
33. Leung TF, Chu Y, andLee V. (2009): Long- term effects of pamidronate in thalassemic patients with severe bone mineral density deficits.

Hemoglobin. 33: 361- 369.

34. Pirinççioğlu A. (2011):A kpolatV, KöksalO, SökerM Bone mineral density in children with beta- thalassemia major in Diyarbakir, **Bone** vol. 49 819-823
35. Izadyar S, Fazeli M, Izadyar M, Salamati P, and Gholamrezanezhad A. (2012): Bone mineral density in adult patients with major thalassaemia: our experience and a brief review of the literature **Polish Journal of Endocrinology Tom**/Volume 63; 4/ pp264- 70
36. Fung EB, Kwiatkowski JL, Huang JN, Gildengorin G, King JC, and Vichinsky EP. (2013):Z inc supplementation improves bone density in patients with thalassemia: a double- blind, randomized, placebo-controlled trial. **Am J ClinNutr.** Oct; 98 (4):9 60- 71
37. Faranoush M, Rahiminejad MS, Karamizadeh Z, Ghorbani R, andOwji SM. (2008): **Zinc Supplementation Effect on Linear Growth in Transfusion Dependent βThalassemia IJBC**; 1: 29-32