

PREDICTORS OF OSTEOPATHY AMONG ADULT PATIENTS WITH THALASSAEMIA MAJOR

Abstract

Background: Beta thalassemia comprises a group of inherited, autosomal, recessive and hematologic disorders characterized by decreased or absent synthesis of β -globin chains. Beta thalassemia patients exhibit an unbalance in bone mineral turnover with increased resorptive rates and suppression of osteoblast activity, resulting in diminished bone mineral density (BMD).

The aim of study was to determine the prevalence of low BMD in patients with β -thalassemia and to find a possible relationship between osteopathy among adult thalassaemic patients and multiple variables as variant endocrinal disorders, iron chelation therapy, calcium and vitamin D supplementation or level, ferritin level and splenectomy.

Subjects and Methods: The current study had been conducted on 80 patients with β -thalassemia major. Thirty seven were males (46.3 %) and 43 patients were females (53.8 %). Their ages ranged from 15 to 33 years with a mean of 20.6 ± 4 years. All patients were subjected to full medical history, full clinical examination, and investigations which included: ALT, AST, bilirubin (total and direct), serum creatinine, calcium, 25(OH) vitamin D, FBG, 2hPPBG, TSH, free T4 and ferritin levels. Assessment of BMD was done by DEXA scan at three sites (AP-spine, left femur and right forearm).

Results: Seventy nine (98.7%) patients had abnormal BMD where 34 (42.5%) patients were osteoporotic, 6 (7.5%) were osteopenic and 39 (48.7%) had both osteopenia and osteoporosis.

Bone mineral density was positively correlated with:

- **Vitamin D** levels ($r = 0.30$, $P = 0.008$ & $r = 0.36$, $p = 0.001$) at left femur and AP spine respectively

- **Calcium** levels ($r= 0.29$, $p= 0.008$, $r= 0.32$, $p=0.004$ and $r= 0.22$, $p=0.05$) at RT forearm, LT femur and AP spine respectively.

We observed a significant **positive** correlation ($r=0.29$, $p=0.008$) between right forearm DEXA scores and calcium levels, and a **negative** correlation with 2 hours post prandial blood glucose and ferritin levels. Regarding DEXA scores at left femur, a significant **positive** correlation was observed with vitamin D ($r= 0.3$, $p= 0.001$) and calcium ($r=0.32$, $p= 0.05$) levels, and a **negative** correlation with the following variables:

- FBG ($r= -0.28$, $p= 0.006$)
- 2h post prandial blood glucose ($r= -0.3$, $p= 0.002$)
- Ferritin levels ($r= -0.46$, $p= 0.001$)

There was a statistically significant difference with p-value of 0.02 between number of sites affected and history of splenectomy, where (40.6%) of these who underwent splenectomy had affection in 3 sites.

There was statistically significant difference ($p= 0.001$) between different DEXA results at left femur regarding **hypogonadism** in males and females while it was statistically significant at right forearm and AP spine only in female patients ($p= 0.04$ and 0.01).

Conclusions: The prevalence of low BMD in patients with β -thalassemia is high. Many factors contribute to the development of such complication. Bone mineral density is a good index of bone status in patients with thalassemia. Most of the patients were not compliant to iron chelating therapy, vitamin D and calcium supplementation. Future studies including large number of patients with different types of hemoglobinopathies are recommended.

Keywords: β -thalassemia major, bone mineral density, osteopathy, 25(OH)vitamin D.

Introduction: Thalassemia is the most common monogenic disorder in the world. Thalassemia major (β -thalassemia) affects a significant segment of the

population in certain areas of the world. Alterations in migration patterns have changed the geographic distribution of this disease and made it a worldwide health problem with a high frequency in Africa, India, Southeast Asia and the Mediterranean area (Singh et al., 2014).

The management of patients with thalassemia has improved markedly over the past few decades with the use of optimized transfusion programs and chelating therapy. With prolongation in the life expectancy, it has been observed that this hemoglobinopathy is associated with a variety of bone disorders like deformities, bony pains, delayed bone age, growth failure, rickets, scoliosis, spinal deformities, nerve compression, pathologic fractures, osteopenia, and osteoporosis (Meena et al., 2015).

Bone mineral density (BMD) measurement by dual X-ray absorptiometry (DEXA) of the lumbar spine, femoral neck and forearm is recommended as one of the most reliable and non-invasive techniques for the assessment of bone mass. DEXA is an excellent non-invasive choice for repeated measurements of any temporal changes of BMD because of its low radiation exposure (Hashemieh et al., 2014).

Subjects and methods:

Patients:

This study included 80 patients with β -thalassemia major who attended the outpatient clinic at Fayoum university hospital.. Thirty seven were males (46.3%) and 43 patients were females (53.8%). Their ages ranged from 15-33 years.

Method:

Full history taking and clinical examination.

Laboratory investigations:

- Complete blood count
- Serum Ca (total), FBS and 2h PPBG
- serum ferritin
- 25(OH) vitamin D

- Dual energy X-ray absorptiometry (DEXA)

Specimen collection:

Blood samples were withdrawn from patients using wide-bore needle slowly from ante cubital vein to avoid hemolysis of RBCs by careful venipuncture. These samples were divided into two aliquots, first aliquot was added to EDTA tube for CBC counts, second aliquot was added to a plain tube, allowed to be clotted for 15 minutes, and then separated by centrifugation at 1000 rpm for 15 minutes for measurements the others. Serum 25(OH) vitamin D was determined by Enzyme linked immunosorbent assay (ELISA). Bone mineral density (BMD) was measurement by dual X-ray absorptiometry (DEXA) of the lumbar spine, femoral neck and forearm

Statistical Analysis

Collected data were computerized and analyzed using Statistical Package for Social Science (SPSS) version 16. Descriptive statistics were used to describe variables; percentproportion for qualitative variables. Mean $\pm$ SD and range for Quantitative variables. Student's t-test was used to compare measures of two independent groups of quantitative data. Chi square test was used to compare two of more than two qualitative groups. P values with significance of less than 5% were considered statistically significant.

Results:

- This study included 80 patients with β -thalassemia major. Thirty seven were males (46.3 %) and 43 patients were females (53.8 %). Their ages ranged from 15 to 33 years with a mean of 20.6 ± 4 years.two (2.5%) patients were diabetic, and25 (31.2%) had symptoms of hypogonadism. Splenectomy was done in 69 (86.3%) patients. None of our patients had abnormal thyroid function.

Table (1): Description of vitamin D, calcium, and ferritin levels among the study group.

Variables	Number (n=80)	%
Vitamin D level		
Severe deficiency < 10 (ng/ml)	48	60%
Deficiency < 20 (ng/ml)	21	26.3%
Insufficiency < 30 (ng/ml)	4	5%
Sufficiency 30:100 (ng/ml)	6	7.5%
Toxicity > 100 (ng/ml)	1	1.3%
Low calcium levels	12	15%
High ferritin levels (>1000 ng/ml)	62	77.5%

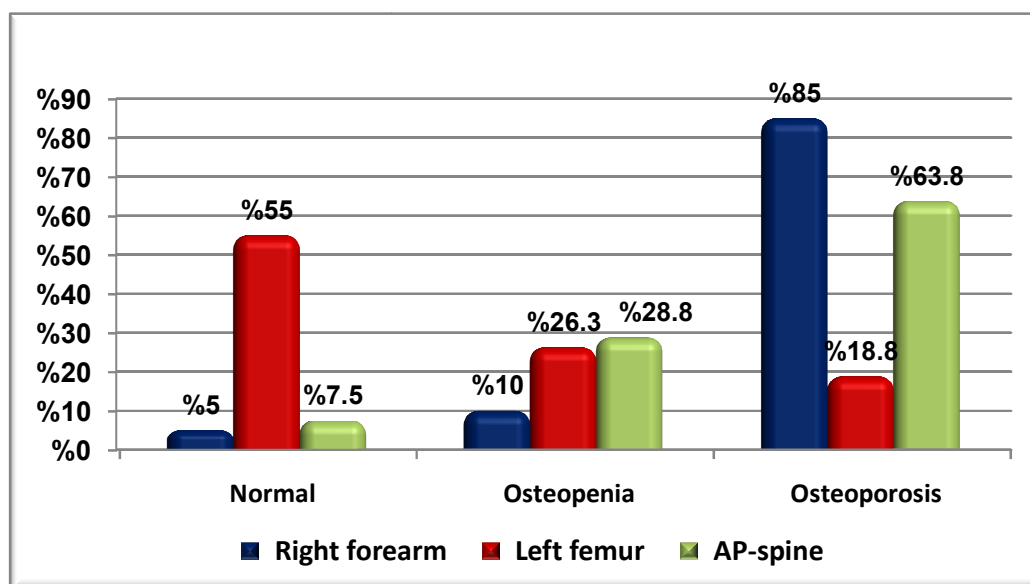


Figure (1): Description DEXA scan results at different sites among the study group.

Table (2): Comparison between different DEXA results at different sites and history of splenectomy

Variables	Splenectomy				p-value	Sig.
	No (n=11)		Yes (n=69)			
	No.	%	No.	%		
Number of sites						
No	1	9.1%	0	0%	0.02	S
One site	1	9.1%	6	8.7%		
Two sites	8	72.7%	35	50.7%		
Three sites	1	9.1%	28	40.6%		

Table(3):Comparison between DEXA scan results in relation to hypogonadismamong study group.

RT-forearm DEXA results			P-value
Normal (n=4)	Osteopenia (n=8)	Osteoporosis (n=68)	
Mean ±SD	Mean ±SD	Mean ±SD	
Males 0/1 (0%)	0/3 (0%)	8/33 (24.2%)	<0.001
Females 0/3 (0%)	0/5 (0%)	17/35(48.6%)	<0.001
LT- femur DEXA results			
Normal (n=44)	Osteopenia (n=21)	Osteoporosis (n=15)	
Mean ±SD	Mean ±SD	Mean ±SD	
Males 1/21(4.8%)	2/10(20%)	5/6(83.3%)	<0.001
Females 3/23(13%)	5/11(45.5%)	9/9(100%)	<0.001
AP-spine DEXA results			
Normal (n=6)	Osteopenia (n=23)	Osteoporosis (n=51)	
Mean ±SD	Mean ±SD	Mean ±SD	
Males 0/3(0%)	0/10(0%)	8/24(33.3%)	0.06
Females 0/3(0%)	2/13(15.4%)	15/27(44.4%)	0.01

Table (4): Comparison between of different DEXA results and calcium levels among study group.

Variables	Hypocalcemic (N=12)		Normocalcemic (N=68)		p-value
	Mean	SD	Mean	SD	
Right forearm	-4.9	2.1	-4.1	1.7	0.1
Left femur	-2.2	1.2	-1.1	0.9	0.002
AP-spine	-4.1	1.7	-2.9	1.4	0.009

Table (5): Comparison between different DEXA results and ferritin levels among study group.

Variables	Ferritin<1000		Ferritin>1000		p-value
	No.	%	No.	%	
Right forearm					
Normal	3	4.8%	1	5.6%	0.1
Osreopenia	4	6.5%	4	22.2%	
Osteoporosis	55	88.7%	13	72.2%	
Left femur					
Normal	31	50%	13	72.2%	0.2
Osreopenia	17	27.4%	4	22.2%	
Osteoporosis	14	22.6%	1	5.6%	
AP-spine					
Normal	4	6.5%	2	11.1%	0.001
Osreopenia	12	19.4%	11	61.1%	
Osteoporosis	46	74.2%	5	27.8%	

Table (6): Comparison between DEXA results and vitamin D among study group.

	RT-forearm DEXA results			P-value
	Normal (n=4)	Osteopenia (n=8)	Osteoporosis (n=68)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Vitamin D	12 $\pm$ 19	14 $\pm$ 13.6	12.1 $\pm$ 15.7	0.9
	LT-femur DEXA results			P-value
	Normal (n=44)	Osteopenia (n=21)	Osteoporosis (n=15)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
	15.1 $\pm$ 20	9.7 $\pm$ 5.6	7.5 $\pm$ 6.1	0.2
	AP-spine DEXA results			P-value
	Normal (n=6)	Osteopenia (n=23)	Osteoporosis (n=51)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
	32 $\pm$ 42.7	14.3 $\pm$ 14.2	9 $\pm$ 7.1	0.002

Table (7): Comparison between DEXA results and FBG 2hPPBG among study group.

	RT-forearm DEXA results			P- value
	Normal (n=4)	Osteopenia (n=8)	Osteoporosis (n=68)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
FBG (mg/dl)	85.5 $\pm$ 6.2	80.1 $\pm$ 5.4	86.4 $\pm$ 35.6	0.9
2 hours PPBG (mg/dl)	106 $\pm$ 11.6	102.5 $\pm$ 9.6	113.6 $\pm$ 52.9	0.8
	LT-femur DEXA results			P-value
	Normal (n=44)	Osteopenia (n=21)	Osteoporosis (n=15)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
FBG (mg/dl)	79.9 $\pm$ 5.9	83.4 $\pm$ 16.4	106.2 $\pm$ 71	0.02
2 hours PPBG (mg/dl)	102.6 $\pm$ 11.7	110 $\pm$ 27	142.8 $\pm$ 104.1	0.02
	AP-spine DEXA results			P-value
	Normal (n=6)	Osteopenia (n=23)	Osteoporosis (n=51)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
FBG (mg/dl)	78 $\pm$ 5.4	80.3 $\pm$ 6.5	89.1 $\pm$ 40.7	0.5
2 hours PPBG (mg/dl)	93 $\pm$ 6.7	102.7 $\pm$ 10.5	118.5 $\pm$ 60.2	0.02

Table (8): Correlation between different DEXA scores with age and laboratory data among study group.

Variables	DEXA score					
	RT-forearm		LT-femur		AP-spine	
	r	p-value	r	p-value	r	p-value
Age (years)	-0.21	0.06	-0.03	0.8	0.03	0.8
HB	0.16	0.1	0.21	0.07	0.14	0.2
FBG	-0.21	0.06	-0.28	0.01	-0.31	0.006
2 hours PPBG	-0.23	0.04	-0.30	0.007	-0.34	0.002
Vitamin D	0.14	0.2	0.30	0.008	0.36	0.001
Calcium	0.29	0.008	0.32	0.004	0.22	0.05
Ferritin	-0.42	<0.001	-0.46	<0.001	-0.37	0.001

DISCUSSION

Thalassemia is the most common monogenic disorder in the world. Thalassemia major (β -thalassemia) affects a significant segment of the population in certain areas of the world. Alterations in migration patterns have changed the geographic distribution of this disease and made it a worldwide health problem with a high frequency in Africa, India, Southeast Asia and the Mediterranean area (Singh et al., 2014).

Many factors contribute to the etiology of low bone mass including: ineffective erythropoiesis which leads to bone marrow hyperplasia, growth hormone and sex steroid deficiencies, hypothyroidism, vitamin D deficiency and severe anemia with presumed, but poorly characterized reduced physical activity. Even well transfused patients with normal gonadal function who are supplemented with calcium have been shown to have low bone mass by dual x-ray absorptiometry (DXA), suggesting other factors are also involved (Fung et al., 2011).

Bone mineral density (BMD) measurement by dual X-ray absorptiometry (DEXA) of the lumbar spine, femoral neck and forearm is recommended as one of the most reliable and non-invasive techniques for the assessment of bone mass. DEXA is an excellent non-invasive choice for repeated measurements of any temporal changes of BMD because of its low radiation exposure (**Hashemieh et al., 2014**).

This study included 80 patients with β -thalassemia major. Thirty seven were males (46.3%) and 43 patients were females (53.8%). Their ages ranged from 15-33 years.

In our study, 79 (98.7%) patients had abnormal DEXA findings in the form of 34 (42.5%) patients were osteoporotic, 6 (7.5%) were osteopenic and 39 (48.7%) had both osteopenia and osteoporosis. These findings were supported by **Hashemieh and colleagues (2014)** who reported that there were abnormal DEXA findings in 274 (84.2%) patient. 214 (65.5%) patients had osteoporosis and 60 (18.7%) patients had osteopenia.

Our study revealed a statistically significant difference ($p = 0.02$) between number of sites affected and splenectomy and 28 (40.6%) of those who underwent splenectomy had affection in the three sites. Up to our knowledge, no other study correlated BMD with history of splenectomy.

Our study showed no statistically significant correlation between age and different DEXA scores at RT forearm, LT femur and AP spine ($r = -0.21$, $p = 0.06$ & $r = -0.03$, $p = 0.8$ and $r = 0.03$, $p = 0.8$ respectively). On the contrary, **Izadyar and colleagues (2012)** reported that there was a significant decrease in the BMD with age ($p = 0.001$) and this study explained this finding based on that, normally peak bone mass is attained shortly after completion of puberty and is stable until the third decade of life. It is just after the age of 30 age-related bone mineral loss starts. However, in thalassaemic patients, this process starts much sooner and progresses more swiftly. Bone mineral density Z score significantly deteriorates with age. It is known that in those patients whose lifespan is getting longer, osteopenia and osteoporosis are among the major causes of morbidity, with a strong negative impact on the quality of life.

Vitamin D deficiency is prevalent in many patients with β -thalassemia major and vitamin D replacement therapy has been shown to increase IGF-I secretion which is vital for skeletal health (**De Sanctis et al., 2015**).

As regards 25(OH) vitamin D levels, our study revealed that 48 (60%) patients had severe deficiency, 21 (26.3%) had deficiency, while 4 (5%) had insufficient and 6 (7.5%) had sufficient levels, and 1 (1.3%) had toxic level.

Similar results were obtained by **Meena and colleagues (2015)**, their study demonstrated that none of the subjects had normal 25(OH) vitamin D levels.

Our study showed a positive correlation between DEXA scores (at AP-spine and left femur) and vitamin D levels ($r = 0.36$ and $r = 0.30$). Supporting our results, **Singh and colleagues (2014)** showed a significant influence of serum vitamin D on Z score of BMD at lumbar spine in the studied group of patients ($r = 0.422$, $P = 0.031$). In addition, **Meena et al., (2015)** observed a positive correlation between BMD and vitamin D levels at the three sites ($r = 0.086$, 0.0236 and 0.0236 at lumbar spine, distal radius and left femur respectively) but it was not statistically significant ($p = 0.641$, 0.193 and 0.194 at lumbar spine, distal radius and left femur respectively). In contrast, **Merchant and colleagues (2010)** found no difference in vitamin D levels between thalassaemic patients with or without evidence of osteopathy.

Moreover, **Tzoulis and colleagues (2014)** found no association between vitamin D status and low bone mass.

In our study, there was a negative correlation between BMD and ferritin levels at RT forearm, LT femur and AP spine ($r = -0.42$, $p < 0.001$ & $r = -0.46$, $p < 0.001$ and $r = -0.37$, $p = 0.001$ respectively). **Morabito and colleagues (2015)** demonstrated that, BMD on lumbar spine and femoral neck was inversely related to serum ferritin levels which could account for direct or indirect bone damage by iron overload status. Meanwhile, **Wong and colleagues (2014)** found that there was no significant relationship between serum ferritin levels and Z-scores at the lumbar spine or femur neck.

We found that, there was a negative correlation between the following variables:

- **2HPP** and DEXA scores at RT forearm, LT femur and AP spine ($r = -0.23$, $p = 0.04$ & $r = -0.30$, $p = 0.007$ and $r = -0.34$, $p = 0.002$ respectively).
- **FBS** and DEXA scores at LT femur and AP spine ($r = -0.28$, $p = 0.01$ and $r = -0.31$, $p = 0.006$ respectively).

The contribution of diabetes mellitus in the pathophysiology of bone complications in thalassemic patients was not well evaluated, and we found few studies in literatures, which correlate diabetes mellitus and BMD. One study showed that thalassemic patients with DM had higher rates of very low BMD compared to those with normal blood glucose levels (**Mylona et al., 2005**). While **Baldini and colleagues (2014)** found that the presence of DM did not distinguish thalassaemic patients without osteopathy from osteopathic ones.

Our study showed statistically significant difference ($p = 0.001$) between different DEXA score at left femur and hypogonadism among males and females and a statistically significant difference at right forearm and AP spine among females only ($p = 0.04$ and 0.01), with higher evidence of osteoporosis among patients with history of hypogonadism.

This is supported by **Izadyar and colleagues (2012)** who found a significant relationship between bone mineral loss and history of gonadal dysfunction ($p = 0.01$) and stated that the anabolic effects of sex steroids in bone are essential, not only for the acquisition of bone mass during adolescence and puberty, but also for the maintenance of peak bone mass during adulthood. In our study, 12 (15%) patients had low calcium level ($<8.5\text{mg/dl}$).

Merchant and colleagues (2010) evaluated the prevalence of osteopathy in β -thalassemia major by bone mineral densitometry and biochemical indices on 42 regularly transfused Indian thalassemic patients, found that 7 (16%) patients had hypocalcemia and 16 (37%) patients had high alkaline phosphatase levels. In contrast, **Modi and colleagues (2012)** reported no alteration in the calcium and phosphate levels in thalassemia patients.

We found a positive correlation between calcium levels and BMD at all three sites, namely, lumbar spine ($r = 0.22$, $P = 0.05$), RT forearm ($r = 0.29$, $P = 0.008$) and LT femur ($r = 0.32$, $P = 0.004$). This was supported by **Meena and colleagues (2015)** who reported a positive correlation between calcium levels and BMD at all three sites, namely, lumbar spine ($r = 0.116$, $P = 0.526$), distal radius ($r = 0.063$, $P = 0.733$) and neck of femur ($r = 0.392$, $P = 0.026$). While **Izadyar and colleagues (2012)** reported that there was no significant correlation between serum concentrations of calcium and BMD of hips and spines (all p values more than 0.1).

CONCLUSIONS

Our study concluded that:

- Seventy nine (98.7%) patients had abnormal DEXA findings (osteopenia and osteoporosis) with high prevalence in the right forearm and lumbar spine.
- Fifteen % of the patients had low calcium level with high mean of DEXA score among patients with normal level of calcium and BMD was positively correlated with calcium on RT forearm, LT femur and AP spine.
- Vitamin D deficiency/insufficiency prevalence was detected in 73 patient (91.2%) and bone mineral density was positively correlated with vitamin D on left femur and AP spine
- Bone mineral density was negatively correlated with **ferritin** on RT forearm, LT femur and AP spine and bone mineral density was negatively correlated with **25HPP** on RT forearm, LT femur and 25 (31.2%) patient had hypogonadism with statistically significant difference between different DEXA results at left femur as regards to hypogonadism among males and females. statistically significant at right forearm and AP spine only among females.

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