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Bone Mineral Density (BMD) and Chemical Biomarkers Among Patients with Thalassemia Major and Intermedia in Iran

Ali Reza Ansari-Moghadam [1], Hossein Adineh [2,*], Iraj Zareban [3], Zeinab Almasy [4] and Mahtab Maghsudlu [5]

[1] Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[2] Department of Epidemiology and Biostatistics, Iranshahr University of Medical Sciences, Iranshahr, Iran
[3] Health Education Department, Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[4] Department of Epidemiology and Biostatistics, Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[5] Department of Thalassemia, Transfusion Research Center Tehran, Iran

[*] *Corresponding author*: Epidemiologist and Faculty Member, Department of Epidemiology and Biostatistics, Iranshahr University of Medical Sciences, Iranshahr, Iran. Email: payam.health@yahoo.com

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Abstract

Background: Thalassemia is a common genetic disease in Iran and is most prevalent in northern and southern parts of the country.
Methods: Due to vulnerable metabolic organs of thalassemia patients, the present study was conducted to examine the relationship between Bone Mineral Density (BMD) and thyroid hormones along with some other variables.
Results: A total of 5491 (2647 males and 2634 females) cases were studied in Tehran (the capital of Iran), Sari (in northern Iran), Bandar Abbas, Iranshahr, and Zahedan (in southern Iran). The mean age ± standard deviation of the patients was 24.22 ± 12.7 years (24.4 ± 0.23 in both male and female patients).
Conclusions: Based on the results, 30.6% of the patients had lumbar osteoporosis, 39.0% had lumbar osteopenia, 8.6% had femoral neck osteoporosis, and 40.4% had femoral neck osteopenia. Although thyroid hormones did not correlate with osteoporosis, greater changes were observed in these hormones in patients with thalassemia major than in other patients.

Keywords: Bone Mineral, Thalassemia, Iran

1. Background

Thalassemia is an inherited disorder that occurs due to a defect in hemoglobin chain (1). Thalassemia major (TM) becomes apparent in the first year of life with severe anemia, and Thalassemia intermedia (TI) provides moderate anemia and does not have obvious clinical symptoms (2). These patients are dependent on blood transfusion and at risk of osteopenia and osteoporosis (3). Metabolic-bone disease involves the spinal and femoral neck region and includes the major causes of morbidity in patients with thalassemia major and intermedia (4-8). In addition to progressive process of disease, patients, who do not receive blood transfusions will develop more severe forms complication (6, 9).

Pathogenesis of osteopenia in thalassemia patients is multi-factorial. However, factors, such as diabetes, hypothyroid, toxic effects of sediment iron, and gender were considered as influential factors (10). Gender not only effects the prevalence of disease yet also influences the severity of disorder in bone density. Furthermore, start time and duration of taking Desferal (Deferoxamine) plays a protective role on osteopenia and osteoporosis (11-13). Biological markers, such as levels of TSH, calcium, and alkaline phosphatase, can be used to detect changes in bone metabolism. According to previous studies, there is a relationship between high level of alkaline phosphatase and density of bone (13, 14). Hyper parathyroidism, osteopenia, and osteoporosis are other complications, which usually develop by overload and sediment of iron in glands (15) so that a quarter of thalassemia patients may face worsening of thyroid function (16).

However, regular blood transfusion to maintain hemoglobin levels near normal levels leads to a reduction or prevention of bone deformities (17), yet in general there is high prevalence of osteopenia and osteoporosis in patients with thalassemia (4). In Iran, according to the results of prevalence studies, bone metabolism disease and its complications, such as fractures, is remarkable in the general population, especially in patients with thalassemia (9, 18-20), and imposes a high burden on

the health system (21). Therefore, measurement and determination of bone mineral density in patients with thalassemia major and intermedia at different ages is essential to receive the necessary treatment (3). Also, following thalassemia patients is necessary for better understanding of the severity of bone disease, and diagnosis and management of complications (20, 22, 23).

Assessment of the status of bone mineral density in patients with thalassemia depends on blood transfusion and susceptible risk factors, which demonstrate the extent and severity of problems as well as level of effectiveness of the care and treatment of patients in the country. The aim of this study was to determine the prevalence of low bone mineral density in patients with thalassemia major and intermedia and describe the correlation between bone mineral density and impressive biomarkers in samples from patients with thalassemia in Iran.

2. Methods

This was a cross-sectional study, in which information about the variables under study in a limited time frame were extracted from records of patients. In this study, for each patient, blood tests and X-ray data were collected for last two years. This population study included all patients with thalassemia major (transfusion-dependent) and thalassemia intermedia at a reference center of adult patients in Tehran, Iran. Also, medical records at thalassemia centers in Sari (Mazandaran), Sistan and Baluchestan (Zahedan and Iranshahr), and Bandar-e Abbas were used in this study. Patients, who were inactive or did not had regular visits for more than four years and who had incomplete records, were excluded from study.

In this study, cities with high prevalence of thalassemia, according to the Health Ministry, were selected. Number of thalassemia cases is considerable in south-east cities and border of the Persian Gulf as well as cities located around the Khazar Sea. Therefore, Sari and Tehran from the northern area and Sistan and Baluchestan, and Bandar-e Abbas from south of Iran, were selected. Then, the census method was used to recruit cases, who had a medical record and information about bone mineral density (BMD), ferritin, calcium, alkaline phosphatase, and thyroid hormones. Additionally, demographic characteristics of patients were extracted and examined. Bone mineral density in patients with thalassemia was determined using Dual-DEXA (energy X-ray absorptiometry) at the lumbar spine (L1 - L4) and femoral. According to the World Health Organization definition, Z-score from -1 to -2.5 is considered as osteopenia and less than -2.5 is considered as osteoporosis. This study used population-based Z-scores for the study aims. The reference population for Z-score was considered as defined by the manufacturer of DXA and it was the same for all patients from every city.

Most patients with thalassemia major and intermedia usually underwent regular lab examination for blood biomarkers. The researchers reviewed medical records of patients and extracted information about calcium, ferritin, alkaline phosphates, and thyroid hormones, which had been measured based on a routine and standard method. The survey checklist also included items on age, gender, and age of diagnosis. The process of quality assurance and quality control of data was done by employees familiar to medical records and well-trained data collectors.

2.1. Analysis and Data Description

After determining appropriate distribution tests, descriptive statistics, such as mean and standard deviation (SD) was estimated for numerical data. Analysis of data was performed using Chi-square tests, *t*-test, one-way and two-way analysis of variance (ANOVA) or the equivalent non-parametric tests. All analysis was carried out using the STATA software, version 11.0. The P value was two-sided and $P < 0.05$ was considered significant.

3. Results

Of the total of 5491 cases examined in the study, 2647 (50.1%) were male and 2634 (49.9%) were female. The mean age $\pm$ SD of the patients was 24.22 $\pm$ 12.7 years (24.4 $\pm$ 0.23 in both male and female patients). A total of 999 (19.1%) patients had thalassemia intermedia, 3936 (75.1%) cases had thalassemia major, and 89 (1.7%) had sickle beta thalassemia. Of the 5491 cases examined, 2717 (49.5%) were from Tehran, 739 (13.5%) from Mazandaran, 815 (14.8%) from Hormozgan, 599 (10.9%) from Iranshahr, and 621 (11.3%) from Zahedan. Most of the patients were in the age range of 21 to 30 (n = 1854, 35.1%) years. A total of 797 (15.1%) patients were aged 0 to 10 years, comprising the least frequent age group among the five age groups. The Chi-square test showed significant differences between age groups in terms of the type of thalassemia ($P < 0.001$).

Moreover, 50.1% of cases of thalassemia were male (2647 male versus 2634 female) and there was no statistically significant difference ($P > 0.05$). Table 1 presents the patients' details. Although the age at diagnosis was higher in patients with thalassemia intermedia than in the other patients, the difference was not statistically significant ($P = 0.1$).

The patients' thyroid hormone levels were examined based on their last test results. As shown in Table 1, the mean T3 and T4 levels were higher ($P < 0.05$) and the mean TSH level was lower in patients with thalassemia major (P

Table 1. Characteristics of Patient's Thalassemia[a,b]

Variables	Type of Thalassemia				Total	P Value
	Major	Intermedia	Sickle Beta Thalassemia	Missing Cases		
City						***
Tehran	1695 (43.1)	694 (69.5)	40 (44.9)	288 (61.7)	2717 (49.5)	
Mazandaran	470 (11.9)	125 (12.5)	0	144 (30.8)	739 (13.5)	
Iranshahr	573 (14.6)	0	0	26 (5.6)	599 (10.9)	
Zahedan	613 (15.6)	4 (0.4)	0	4 (0.9)	621 (11.3)	
Bandar-e-Abbas	585 (14.9)	176 (17.6)	49 (55.1)	5 (1.1)	815 (14.8)	
Age, y						***
0 - 10	694 (18.4)	62 (6.5)	6 (7.0)	35 (8.1)	797 (15.2)	
11 - 20	948 (25.1)	114 (11.9)	26 (30.2)	82 (19.1)	1170 (22.3)	
21 - 30	1421 (37.6)	276 (28.8)	30 (34.9)	139 (32.3)	1866 (35.5)	
31 - 40	611 (16.2)	331 (34.6)	13 (15.1)	115 (26.7)	1070 (20.4)	
+40	106 (2.8)	175 (18.3)	11 (12.8)	59 (13.7)	351 (6.7)	
Gender						0.105
Male	1957 (51.1)	445 (47.0)	39 (44.3)	206 (49.8)	2647 (50.1)	
Female	1876 (48.9)	501 (53.0)	49 (55.7)	208 (50.2)	2634 (49.9)	
Age of diagnosis	30.42 ± 56.65	35.49 ± 63.93	32.53 ± 47.05	29.27 ± 52.68	31.20 ± 57.49	0.147
T_3, nmol.L	1.86 ± 1.17	1.78 ± 1.12	1.55 ± 1.21	1.86 ± 0.955	1.83 ± 1.14	0.098
T_4, nmol.L	8.41 ± 2.47	8.14 ± 2.28	8.07 ± 2.82	8.21 ± 2.71	8.33 ± 2.45	*
TSH, micr.dL	3.08 ± 2.07	3.34 ± 2.19	3.47 ± 2.27	3.18 ± 2.36	3.16 ± 2.12	**
Ferritin, micg/dL	2942.5 ± 4587.1	1622.3 ± 3677.6	1675.6 ± 2045.9	2106.1 ± 2329.8	2550.0 ± 4237.1	***
Calcium, mg.dL	8.99 ± 1.105	9.12 ± 1.04	9.05 ± 0.422	9.14 ± 0.83	9.03 ± 1.06	**
Phosphate, mg.dL	4.72 ± 1.14	4.48 ± 1.03	4.28 ± 0.68	4.58 ± 1.09	4.65 ± 1.11	***
HG, g.dL	9.18 ± 1.58	9.03 ± 1.54	9.01 ± 1.67	9.21 ± 1.72	9.15 ± 1.58	0.1
BMD Hip, g/cm^2	0.801 ± 0.255	0.784 ± 0.213	0.74 ± 0.184	0.807 ± 0.170	0.796 ± 0.238	0.28
HIP Z-score	-0.752 ± 1.59	-0.748 ± 1.46	-0.30 ± 1.60	-0.808 ± 1.35	-0.75 ± 1.53	0.74
BMD Spine	0.80 ± 0.25	0.79 ± 0.16	0.81 ± 0.18	0.80 ± 0.16	0.79 ± 0.22	0.90
Spine Z-score	-1.37 ± 2.05	-1.54 ± 1.95	-0.17 ± 2.16	-1.67 ± 1.68	-1.43 ± 2.0	*

[a] Values are expressed as mean ± SD or No. (%).
[b] * P < 0.05, ** P< 0.01, *** P < 0.001.

< 0.01) than in the other thalassemia patients. Overall, the mean T3 level was 1.83 ± 1.1, the mean TSH level 8.33 ± 2.4, and the mean T4 level 3.16 ± 2.12 in the patients.

The mean calcium and phosphate levels differed significantly between different types of thalassemia (P < 0.01) and were lower in patients with sickle beta and thalassemia major than in the other patients. Overall, the mean calcium level was 9.03 ± 1.06 and the mean phosphate level was 4.6 ± 1.1 in the patients. The mean ferritin level differed significantly between different types of thalassemia (P ≤ 0.001). The mean hemoglobin level, however, was the same in all patients with either major, intermedia or sickle beta thalassemia (P = 0.1).

Data on hip BMD was available for 2448 patients and data on spine BMD for 2536 patients (Table 2). Overall, 50.7% of the patients had normal hip Z-score, 40.6% had osteopenia, and 8.6% had osteoporosis. These values differed between the male and female patients. The female patients were significantly more affected by osteoporosis and osteopenia than the male patients (P = 0.002). The highest prevalence of osteoporosis was found in Bandar Abbas, and the number of patients with normal Z-score was significantly more in Zahedan than in all other cities examined (P < 0.001).

Table 2. Bone Mineral Density of the Femoral Neck and Lumbar Spine in Patients with β-Thalassemia and Intermedia[a,b]

Variables	BMD Z-Score of Spine				BMD Z-Score of HIP			
	> -1, Normal	-2.5 to -1, Osteopenia	< -2.5, Osteoporosis	P Value	> -1, Normal	-2.5 to -1, Osteopenia	< -2.5, Osteoporosis	P Value
No. (%)	541 (30.3)	697 (39.0)	547 (30.6)		899 (51.1)	711 (40.4)	151 (8.6)	
City								
Tehran	397 (35.4)	401 (35.7)	325 (28.9)	***	575 (51.5)	438 (39.2)	103 (9.2)	***
Mazandaran	83 (17.1)	238 (49.2)	163 (33.7)		231 (48.8)	209 (44.2)	33 (7.0)	
Iranshahr	-	-	-		-	-	-	
Zahedan	40 (87.0)	3 (6.5)	3 (6.5)		43 (93.5)	2 (4.3)	1 (2.2)	
Bandar-e-Abbas	11 (9.0)	55 (45.1)	56 (45.9)		45 (37.5)	63 (52.5)	12 (10.0)	
Gender								
Male	211 (28.3)	271 (36.3)	264 (35.4)	**	410 (55.3)	266 (35.9)	65 (8.8)	**
Female	301 (31.5)	392 (41.0)	263 (27.5)		443 (47.1)	418 (44.4)	80 (8.5)	
Age, y	24.7 ± 12	23.4 ± 11.3	24.3 ± 11.1	0.38	23.8 ± 11.7	24 ± 11.25	24.5 ± 11.19	0.791
Age of diagnosis	36.3 ± 61.8	32.8 ± 59.1	29.8 ± 54.2	0.2	31.74 ± 57.5	31.66 ± 56.6	43.4 ± 70.8	0.11
T_3, nmol.L	1.9 ± 0.93	1.9 ± 0.84	1.9 ± 1.25	0.3	1.90 ± 0.92	1.95 ± 0.94	1.98 ± 1.5	0.6
T_4, nmol.L	8.48 ± 2.6	8.5 ± 2.3	8.07 ± 2.3	**	8.42 ± 2.4	8.37 ± 2.5	8.36 ± 2.1	0.9
TSH, micr.dL	3.17 ± 2.0	3.18 ± 2.11	3.1 ± 1.96	0.7	3.29 ± 2.0	3.03 ± 1.9	3.08 ± 2.3	0.05
Ferritin	2377 ± 5065	2395 ± 3519	2455 ± 4694	0.9	2277 ± 4061	2398 ± 3329	2703 ± 6249	0.5
Calcium, mg.dL	8.9 ± 1.05	9.05 ± 1.06	9.0 ± 0.99	0.6	9.05 ± 1.06	8.9 ± 1.06	9.08 ± 0.7	0.2
Phosphate, mg.dL	4.69 ± 1.2	4.46 ± 1.0	4.65 ± 1.01	***	4.59 ± 1.05	4.58 ± 1.1	4.68 ± 0.9	0.6
HG, g.dL	9.63 ± 1.4	9.35 ± 1.5	9.25 ± 1.4	***	9.4 ± 1.48	9.3 ± 1.45	9.4 ± 1.40	0.6

[a] Values are expressed as mean ± SD or No. (%).
[b] * P < 0.05, ** P < 0.01, *** P < 0.001.

The mean ± SD hip BMD was obtained based on the patients' place of residence, and its maximum and minimum mean ± SD values were found in Zahedan (1.09 ± 0.84) and Tehran (0.77 ± 0.19; P < 0.001). In terms of age, the maximum mean hip BMD was observed in the age range of 21 to 30. The mean hip BMD differed significantly between the different age groups (P < 0.001). In terms of gender, the mean hip BMD was higher in male than in the female patients. The frequency of osteopenia was significantly higher in patients over 40 and those aged 10 to 19 compared to the other age groups, and the patients aged 21 to 30 had a normal Z-score (P < 0.05).

Pearson's correlation test was used to determine the correlation of hip BMD and lumbar BMD with age, hemoglobin, ferritin, calcium, phosphate, T3, T4, and TSH. The results showed a very poor correlation between these variables (P > 0.05). The correlation between the hip and spine Z-score and the noted variables was not significant (P > 0.05).

In this study, a Z-score of less than -2.5 was taken to indicate osteoporosis, a Z-score between -2.5 and -1 to indicate osteopenia, and a Z-score higher than -1 to indicate a normal BMD. A total of 30.6% of the patients (n = 547) had lumbar osteoporosis, yet this rate was 45.9% in Bandar Abbas. Spine osteoporosis was more common in male patients and osteopenia was more common in female patients (P < 0.01).

The femoral neck hip Z-score revealed femoral neck osteoporosis in 151 (8.6%) of the patients and osteopenia in 711 (40.4%). Femoral osteoporosis was more common in male patients as well as in Bandar Abbas (P < 0.01).

The mean age and the mean age at diagnosis were similar in the patients with osteoporosis and osteopenia and in patients with a normal BMD, and there were no significant differences between them in this regard (P > 0.1).

The present study also examined the correlation of thyroid hormones with osteoporosis and osteopenia. According to the results, thyroid hormone levels were similar in

patients with osteoporosis and osteopenia and in those with a normal BMD ($P > 0.05$), with the exception of T4, which was lower in the patients with osteoporosis than in the others ($P < 0.01$). Phosphate and hemoglobin levels ($P < 0.001$) as well as calcium levels ($P = 0.6$) were lower in patients with spine osteoporosis compared to patients with osteopenia and normal-BMD, although the difference was not statistically significant. Overall, ferritin levels were higher in the patients with hip and spine osteoporosis and osteopenia compared to the normal patients ($P \geq 0.5$).

4. Discussion

The results of this cross-sectional study showed that 30.6% of the patients had lumbar osteoporosis and 39.0% had lumbar osteopenia in contrast to 17% and 35% in the general population, retrospectively (24); 8.6% had femoral neck osteoporosis and 40.4% had femoral neck osteopenia. The hip and spine BMDs were higher in patients with thalassemia major than in those with thalassemia intermedia. Similarly, Kosarian's study showed a lower BMD in patients with thalassemia intermedia than in patients with thalassemia major (25). The results obtained in a study by Origa, however, were inconsistent with these findings (26). The patients with thalassemia major were more affected by hip and spine osteoporosis and osteopenia compared to the other patients and the severity of osteoporosis was similar in the hip and spine. The hip BMD varied with age; it increased until 30 years old and then declined with time. The spine BMD also showed irregular changes; it increased until 30 years old, remained constant from age 30 to 40, and then declined with time. Kosarian reported a reduction of 7% per year in BMD in older adults (25). Vogiatzi et al. reported a decline of -0.38 per year in BMD Z-score in patients with a mean age of six years (27). Although the results of these studies confirm the present findings, it should be noted that the cited studies were conducted in a specific age group while the present study was conducted on a wider group of the population and examined people aged 1 to 59 years and can thus provide more comprehensive results compared to the other studies. In line with the present findings, the results obtained by Pollak et al. reported no reduction in the spine, femoral, and distal BMDs with age (28). Also in line with the present findings, two studies conducted in Tehran (29) showed that spine BMD was worse than femur BMD, although the spine Z-score was lower than the femoral neck or hip Z-scores in both of them.

In this study, thalassemia was nearly the same in male and the female patients (51.1% versus 48.9%); this may be because of the study design. The highest prevalence of thalassemia was observed in the 21- to 30-year-old age group and the prevalence of the disease decreased with age; the highest prevalence of thalassemia major was observed in 21- to 30-year olds, yet its prevalence decreased dramatically after age 30. The present findings are in line with the results of recent studies on the subject and showed that the survival of patients with thalassemia major may increase up to age 30 with appropriate treatments. The frequent blood transfusion in patients with thalassemia major has made them more prone to iron poisoning and thereby anemia (30).

In the present study, the hip Z-score was similar in patients with thalassemia major and intermedia yet lower than in those with sickle beta thalassemia. The spine Z-score was higher in patients with sickle beta thalassemia than in those with thalassemia major and intermedia. The T3, T4, and HG levels were higher in patients with thalassemia major than in the other patients. The TSH level was lower in patients with thalassemia major than in those with thalassemia intermedia, which is inconsistent with the results obtained by Dresner Pollak et al. (28), who found a higher TSH level in patients with thalassemia major than in those with thalassemia intermedia. Vernejoul et al. showed a dramatic reduction in TSH levels and other parathyroid hormones in patients with thalassemia (31). However, information about taking levothyroxine was not extracted and it may have affected the association between Z-score and TSH.

A reduction in BMD is a major predictor of bone fracture. The BMD is determined based on the peak bone mass measured in late youth and the loss in bone density following that peak. Pollak showed that bone density loss continues in patients with thalassemia, even in those, who are receiving proper treatments; Pollak's findings on this subject are thus consistent with the present findings as well as with the results obtained by Vogiatzi et al. (27) and Kosarian et al. (25), which unanimously reported an increased risk of bone density loss with age.

The mean hip BMD was higher in patients with thalassemia major than in those with thalassemia intermedia, yet the mean spine BMD was higher in patients with sickle beta thalassemia than in those with thalassemia major. Most of the patients with bone density loss lacked an obvious marker for this disease, yet there are many factors that can cause the secondary form of the disease, including particular types of pharmacotherapy and certain clinical disorders, such as hyperparathyroidism and hyperthyroidism, increased serum cortisol, and a number of digestive disorders. Furthermore, the majority of the cases with hip and spine osteoporosis were from Tehran, and spine osteoporosis was more common in males while hip osteoporosis was more common in females. The increase in age and the incidence of menopause increase the risk of osteo-

porosis in females, especially in the hip area.

There are numerous risk factors for osteopenia and osteoporosis; Kosarian found that patients with thalassemia intermedia are at a higher risk due to their chronic anemia and the lack of blood transfusion in them, which is inconsistent with the present findings (25). Endocrine disorders in patients with thalassemia are caused by frequent blood transfusions and the deposition of excess iron. In line with the present findings, another study (32) showed a reduction in thyroxine levels and parathyroid hormone levels and an increase in thyrotrophic hormone levels in patients with thalassemia.

4.1. Conclusion

Based on the results, patients with thalassemia major and intermedia are at a higher risk of osteoporosis. Although thyroid hormone disorders are more common in patients with thalassemia major than in other patients, they do not correlate significantly with osteoporosis. Moreover, ferritin and hemoglobin levels are higher in patients with thalassemia major than in other patients, although they do not correlate with osteoporosis.

4.2. Limitation and Strengths

The current authors studied a large sample of thalassemia from around Iran, and presented a general view of complications of thalassemia patients and effective factors. In contrast, lack of consideration of parathyroid hormones, incomplete patient records, not alike and consistent date of blood test were weaknesses of the current study.

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Footnotes

Authors' Contribution: Hossein Adineh participated in data collection and coordination with the managers of the universities. Hossein Adineh and Ali Reza Ansari-Moghadam participated in analysis of data and drafting of the manuscript. All authors helped design the study and critically revise the manuscript.

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