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Original Article

Serum Dickkopf-1 and Correlation with Bone Mineral Density in Patients with Beta Thalassemia Major in North-Eastern of Iran

Zahra Badiee[1]M.D., Mahdieh Sepasi Zangabadi[2]M.Sc., Aref Keshavarzi[2]M.Sc., Mohammad Reza Keramati[2]M.D., Hossein Ayatollahi[2]M.D., Mohammad Hadi Sadeghian[2*]M.D., Seyyede Fatemeh Shams[2]M.Sc., Sepideh Shakeri[2]M.Sc.

[1]Department of Pediatric Hematology, Mashhad University of Medical Sciences, Mashhad, Iran.
[2]Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

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Background and Aims: Iron overload is one of the effects of frequent transfusion in beta-thalassemia major (BTM) patients. Osteopenia and osteoporosis are the secondary complications of frequent transfusion due to toxic effects of iron on osteoblasts. Dickkopf-1 (DKK-1) is a secreted protein which plays an important role in the development of osteoporosis. The aim of this study was to investigate the relationship between DKK-1 protein and iron overload in BTM patients.

Materials and Methods: Fifty BTM patients and fifty healthy individuals participated in this case-control study. Each group were similar in terms of age and gender. We evaluated serum levels of DKK-1 and ferritin by enzyme-linked immunosorbent assay (ELISA) method. Dual-energy X-ray absorptiometry method was used to evaluate patients bone density. Complete blood count and osteoporosis parameters (densitometry test results) were checked in both groups.

Results: The mean age of BMT patients was 25.82 years (24 male/26 female); it was 26.98 years (25 male/25 female) in control group. Significantly, the serum level of DKK-1 was higher in BTM samples ($p<0.001$). Mean of DKK-1 was 34447.1 pg/ml in patients, and 15252.0 pg/ml in controls. Mean of serum ferritin was 3808.6 ng/ml and 73.9 ng/ml in both groups, respectively. A significant association was found between DKK1, ferritin and osteoporosis parameters ($p<0.001$).

Conclusions: Probably, DKK-1 protein plays a significant role in the development and intensification of osteoporosis in BTM patients; it can be used as an applicable factor for early diagnosis of osteoporosis and follow-up in adult BTM patients.

[*]**Corresponding Author:** Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. **Tel:** +985118012584, **Email:** sadeghianmh@mums.ac.ir

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Introduction

Beta-thalassemia syndromes are a family of inherited disorders associated with decreased or absent globin chains production; it leads to hemoglobin reduction in red blood cells and anemia [1]. Osteoporosis is a common metabolic bone disease that is defined by bone mass reduction, bones microstructure weakening and fractures risk increment [2]. Osteoporosis and osteopenia are responsible for substantial fatality in beta thalassemia major (BTM) patients. Osteoporotic fractures have been reported in more than 20% of BTM adults. Usually, iron overload, hepatitis, hypogonadism, diabetes, hypothyroidism, growth hormone deficiency or insulin-like growth factor are the major risk factors of osteoporosis in BTM individuals [3].

Dickkoopof-1 (DKK1) is a secreted protein which belonges to extracellular proteins family. It is coded by DKK1 gene which is located in 10q21.1 situation; it contains 4 exons and 3 introns and 226 amino acids [4, 5]. Generally, there are four types of DKK1 protein the most important one of which is DKK1/2. DKK1 is generated by osteoblasts and bone marrow stromal cells. This protein inhibits Wnt/β-catenin signaling pathway and is a strong inhibitor of both *in vivo* and *in vitro* function of osteoblasts. DKK1 reduction leads to bone anabolic response as a result [5, 6]. Wnt/β-catenin signaling pathway plays an important role in bone mass regulation, maintenance, reconstruction and restoration of bones [5, 7].

Given the importance of osteoporosis, it is vital to identify the factors which can be applied for rapid diagnosis of osteoporosis in BTM patients. Actually rapid diagnosis of osteoporosis elevates BTM life quality.

According to previous studies, DKK1 inhibits the Wnt signaling pathway by binding to LRP5/6. As a result, it prevents osteoblasts maturation and osteogenesis [8]. Therefore, DKK1 may be an effective factor for rapid diagnosis of osteoporosis. The purpose of this study was to evaluate iron overload and serum DKK1 level in BTM patients. Detection of a correlation between iron overload and Dickkopf-1 protein in cases of osteoporosis and bone loss will help achieve a method for early diagnosis, monitoring and follow-up of patients affected with osteoporosis.

Materials and Methods

This case-control study was conducted in Mashhad city during two years. The study concluded fifty BTM patients who had referred to Sarvar polyclinic center, (thalassemia and hemophilia center in Mashhad city) for transfusion. Fifty other healthy individuals matched in terms of age and sex who lacked clinical and laboratory findings of thalassemia disorders as well as bone disease and lesions were included as the control group; they were checked by a physician at the entrance to the study. Minimum age of thalassemic patients was 15 years. After sampling the cases by an expert laboratory technologist, blood samples were kept in sterile tubes for 30 min. at room temperature until the blood turned clot; then

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the serum was separated by centrifuge and stored at -70°C. Variables such as DKK1 protein level, osteoporosis parameters (T.score, Z-score), ferritin, complete blood count (CBC) indices such as red blood corpuscles (RBC), white blood corpuscles (WBC) and platelet count, hematocrit, hemoglobin were examined. CBC indices were evaluated by the cell counter (Sysmex Company) and ferritin was measured by enzyme-linked immunosorbent assay (ELISA) method (Pishtaz Company, Iran) Demographic variables, including age, gender, height and weight were obtained from patients' records. We used kit human ELISA (Abcam Company, America) to determine DKK1 protein content in serum by sandwich ELISA method. In so doing, sera of the studied individuals were collected according to the instructions of the manufacturer. Written consent was also obtained from all the individuals. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (ethics code: 931264).

BMD assay

Dual-energy X-ray absorptiometry (DEXA) method was applied to evaluate the patients' bone density. Bone density was measured typically by DEXA in lumbar spine (Lumbar L1-L4) and femoral neck or Hip. The results of the test included two variables: T-score and Z-score.

T-score: T-score is bone density in comparison with what is normally expected in a healthy young sex match adult. T-score is the number of units -called standard deviations- which bone density may go above or below the average. According to World Health Organization (WHO) criteria, bone density is considered normal when the value is -1 and above. When the value is between -1 and -2.5, the score is a sign of osteopenia (the condition which bone density is below normal and may lead to osteoporosis). Values ≤-2.5 indicate osteoporosis.

Z-score: Z-score is the number of standard deviations above or below what is normally expected for someone with specific age, sex, weight, and ethnic or racial origin. According to WHO criteria, Z-score ≤-2 may suggest the abnormal bone loss being relevent to other factors rather than age.

Statistical analysis

The One-sample Kolmogorov-Smirnov test was used to determine the type of distribution of the quantitative data. Mann-Whitney U and Student T tests were used to describe and analyze data. Data were analyzed by means of a personal computer implemented with dedicated software (SPSS 16.5). Level of significance was determined at < 5%, as usual.

Results

Totally, fifty BTM patients and fifty sex-matched individual were recruited in this study. In the patient group, 52% were female and 48% were male (24 male/26 female). The age range of thalassemia patients was 15-43 years with the mean age of 25.82. In the control group, 50% were female and 50% were male (25 male/25 female). The age range was 15-42 years and the mean age was 26.98. Ferritin level was 3808.6±3418.8 ng/ml and 73.9±47.2 ng/ml in the patient and control group respectively. DKK1 was 34447.1±18632.8 in the thalassemic and 15252.3±4266.1 pg/ml in the

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control groups. A significant difference was obtained between DKK1 protein and ferritin values in both case and control groups (p<0.001). Moreover, a significant difference between RBC indices was evident in two groups under study (p<0.001). No significant differences were observed between WBC count (p=0.2). Pearson correlation test result showed direct and positive correlation between DKKK1 and serum ferritin in the two groups (p<0.001, r=0.40). The relationship between DKK1 protein and hematological parameters are presented in table 1. As is displayed in table 1, DKK1 has the highest positive correlation with platelet and white blood cells count (p<0.001). RBC indices are shown to have indirect and inverse relationship with DKK1 protein. There are no significant correlations between DKK1 and age in both groups (r=-0.05, p=0.5). In addition, the relationship between DKK1 protein and gender was analyzed using independent t-test and no significant correlations was found (p=0.8). The relationship between DKK1 protein and ferritin with osteoporosis indicators has been displyed in table 2. As shown, there is a reverse and negative relationship between serum DKK1 and osteoporosis indicators. According to table 2, there is a reverse and negative correlation between ferritin level and osteoporosis indicators of the lumbar spine and BMD index of the femur. A direct and positive correlation was found between ferritin and femur T and Z-score indices, too.

Table 1. Correlation of DKK1protein and CBC parameters

CBC		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
DKK1	Correlation coefficient(r)	0.55	-0.52	-0.57	- 0.54	-0.23	-0.35	-0.32	0.58
	P-value	<0.001	<0.001	<0.001	<0.001	0.02	<0.001	0.001	<0.001

DKK1=Dickkoopof-1; CBC= Complete blood count; WBC= White blood corpuscles; RBC= Red blood corpuscles; HGB= Hemoglobin; HCT= Hematocrit; MCV= Mean cell volume; MCH= Mean cell hemoglobin; MCHC= Mean cell hemoglobin concentration; PLT= Platelet

Table 2. Correlation between DKK1, ferritin and osteoporosis indicators in the case and control groups

Osteoporosis index		T-score (spine)	Z-score (spine)	BMD-s (g/cm^2)	T-score (femoral)	Z-score (femoral)	BMD-f (g/cm^2)
DKK1	Correlation coefficient(r)	-0.52	-0.51	-0.48	-0.53	-0.53	-0.42
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ferritin	Correlation coefficient(r)	-0.42	-0.43	-0.36	0.48	0.48	-0.31
	P-value	<0.001	<0.001	<0.001	0.001	<0.001	0.002

DKK1=Dickkoopof-1; BMD= Bone mineral densitometry

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Discussion

As has been mentioned recently, introducing a new factor to investigate osteoporosis in BTM patients soon as possible has become a critical issue. In recent years, many studies have been performed on the DKK1 protein to identify its relationship with osteoporosis. Following the proposed aims, this study was designed.

In this study, mean of DKK1 was 34447.1 pg/ml in the case group and 15252.2 pg/ml in the control group. A significant increase was observed in the amount of DKK-1 in BTM patients. Butler et al. (2011) investigated the relationship between bone lytic lesions of multiple myeloma patients and their DKK1 protein level. Resultantly, DKK1 protein was significantly higher in osteoporotic multiple myeloma patients in comparison with controls; this may be due to the inhibitory role of DKK1 on osteoblastic differentiation and function [5]. Feng et al. studied the relationship between DKK1 protein and bone disease in multiple myeloma patients too. They discoverd that DKK1 is an important factor in the progression of bone disease and can be used as a target therapy to heal bone disorders. Since thalassemia patients are prone to osteoporosis, DKK1 increment may lead to osteoporosis development in these patients. DKK1 controlling and monitoring will help treat BTM patients [9].

In 2015, Rossini et al. investigated the relationship between DKK1 protein levels and bone mineral density in ankylosing spondylitis patients. The results showed the important role of DKK1 in the osteoporosis of ankylosing spondylitis patients [10]. The results of our study were in line with those of Rossini and the coworkers. DKK1 protein was associated with Z-score index ($r=-0.051$, $p<0.0001$). Moreover, the increase of DKK1 protein was shown to have correlation with low bone mineral density index in the spine, femur and ulnar. There was an inverse association between bone mineral density index and DKK1 protein ($r=-0.29$, $p=0.022$) in the lumbar spine L1-L4. In our study, a significant increase was observed in the mean serum levels of DKK1 protein in patients (34447.1 pg/ml) compared to the healthy subjects (15252.3 pg/ml) ($p<0.001$). A relation was found between DKK1 and osteoporosis indicators, too. These two findings are also supported by Voskaridou's study [11]. The correlation between DKK1 protein and bone mineral density index was reverse and negative in L1-L4 lumbar spine ($r=-0.48$, $p<0.001$); it means that DKK1 protein reduces bone density index in patients. Indeed, the relationship between DKK1 protein and bone mineral density index (g/cm2) was indirect in the femoral region ($r=-0.42$, $p<0.001$).

Valizadeh et al. [12] evaluated bone density of BTM patients who took regular transfusions. The average of hemoglobin and ferritin was reported 9.5 g/dl and 1421 ng/ml, respectively. After evaluating Z-score index of lumbar spine of 10 BTM patients, loss of bone density and normal bone density were observed in 80.0% and 20.0% of patients, respectively. Totally, 30% of patients showed low bone density in femoral region. In our

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study, the average of hemoglobin and ferritin were reported 8.6 g/dl and 3808.6 ng/ml, respectively. According to T-score index of femoral and lumbar spine of patients, 34.0% of them were osteopenic and 52.0% were osteoporotic. On the whole, 86% of the patients showed reduction of bone mineral density index. According to the studies conducted and the results obtained, we should pay attention to two important points: 1) elevation of DKK1 serum protein observed in osteoporotic thalassemia patients and 2) thesignificant and indirect correlation between the lumbar spine, femoral osteoporosis indicators and DKK1 serum levels. It seems that DKK1 protein should be considered as a pathogenesis agent or a risk factor in osteoporosis of adult BTM patients. In addition, high levels of DKK1 protein and ferritin (iron overload) are completely crucial due to their direct and indirect effects on bone density.

On the other hand, a direct and positive relationship was observed between serum ferritin level and DKK1 protein. It could be hypothesized that DKK1 elevation is due to iron overload which is the result of transfusion and ineffective hematopoiesis. These results were matched with previous findings that showed DKK1 protein increment in thalassemia patients.

Conclusions

According to the results of the present and other studies, it seems that DKK1 protein can be used as an applicable factor for monitoring and follow-up treating of BTM patients affected with osteoporosis. It seems that DKK1 is a suitable marker for early diagnosis of osteoporosis. It is also recommended to perform other studies on larger populations and evaluation of the DKK1 protein in longer periods of time.

Conflict of Interest

There is no conflict of interest to declare.

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