Dickkopf-1 levels and serum Osteoprotegerin in women with Osteoporosis

Mehrzad Hajialilo[1], Vahid Rameshknia[2], Amir Ghorbanihaghjo[1], Nadereh Rashtchizadeh[3], Alireza Khabbazi[4], Susan Kolahi[5], Mohammad Reza Jafari-Nakhjavani[6], Bahman Yousefi[7], Sina Raeisi[7], Maryamolsadat Shahidi[7], Bahareh Amanifar[8]

Original Article

Abstract

BACKGROUND: In bone the Wnt signaling pathway has diverse roles in bone modeling and remodeling. Dickkopf-related protein 1 (DKK-1), as an endogenous inhibitors of the canonical Wnt/β–catenin pathway specific to bone and Osteoprotegerin (OPG), have been demonstrated to be key molecules involved in bone erosion and bone remodeling. The present study aimed to evaluate DKK-1 and OPG in women with osteoporosis to predict activity and severity of this common disease.

METHODS: The study population included 44 women with osteoporosis and 44 controls with normal bone mineral density (BMD). Serum levels of DKK-1 and OPG were measured by standard methods.

RESULTS: The serum Dkk1 concentration in the osteoporosis group (2.91 ± 1.27) was significantly increased compared to the control group (2.07 ± 0.87) ($P < 0.010$). The serum concentration of OPG was significantly higher in control group than patients (4.70 ± 2.16 vs. 4.56 ± 1.21; $P = 0.005$).

CONCLUSIONS: Although the results of this study indicate that DKK-1 and OPG may play different roles in the pathogenesis of osteoporosis, the increase of DKK-1 level and its correlation with FN-BMD might be related to disease activity and bone remodeling in osteoporosis.

KEYWORDS: Osteoporosis, Dickkopf-1, Osteoprotegerin, Bone Mineral Density

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Introduction

Osteoporosis is a common disease characterized by a systemic impairment of bone mass that results in fragility fractures.[1,2] The World Health Organization (WHO) defined osteoporosis as a systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture.[3-5] It occurs most commonly in postmenopausal women and prevalence increases markedly with age from 2% in women at 50 years to more than 25% at

1- Associate Professor, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2- Biochemistry Student, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3- Professor, Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4- Assistant Professor, Tabriz Rheumatology Research Team, Connective Tissue Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5- Associate Professor, Tabriz Rheumatology Research Team, Connective Tissue Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
6- Assistant Professor, Department of Rheumatology, Tabriz University of Medical Sciences, Tabriz, Iran
7- Biochemistry Student, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
8- Medical Student, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Corresponding Author: Amir Ghorbanihaghjo, PhD, Email: ghorbaniamir@hotmail.com

80 years of age.[6,7] More than 200 million people are affected by osteoporosis worldwide, as estimated by 2 million annual hip fractures and other debilitating bone fractures.[8] Osteoporosis is a multi-factorial disease with potential contributions from genetic, endocrine functional, exercise related and nutritional factors.[9,10] Usually osteoporosis is defined according to the WHO criteria, i.e. a T-score below -2.5 SD and/or a prevalent fragility fracture, a large proportion of fractures occur at T-scores above -2.5 SD and in patients without prior fractures.[11,12]

There are several risk factors for screening osteoporosis such as age, low body mass index (BMI), previous fragility fractures, a family history of fractures, the use of glucocorticoids and active cigarette smoking.[13-15] Bone mineral density (BMD) can be assessed with dual X-ray absorptiometry (DXA), and DXA is a valid method to diagnose osteoporosis and to predict the risk of fracture.[16] Bone remodeling is a continuous process by which old bone is removed by bone-resorbing cells, the osteoclasts, and is replaced by the new bone synthesized by bone forming cells, i.e. the osteoblasts.[17] Several key molecules conduct the coordinated activities of osteoblasts and osteoclasts during bone remodeling such as RANKL (Receptor Activator of Nuclear factor κβ Ligand), a member of the TNF (Tumor Necrosis Factor) cytokines.[18-20] RANKL is abundantly expressed by bone-forming osteoblasts as well as bone marrow stromal cells (T and B lymphocytes), and activates its receptor. RANK is expressed on osteoclasts leading to their proliferation, maturation, activation, survival and ultimately resulting in increased bone resorption.[21] Osteoprotegerin (OPG), a soluble decoy receptor of RANKL, which is also produced by the osteoblasts, acts as its natural antagonist.[22]

Looking beyond the RANK/RANKL/OPG axis, as recently discovered, osteoblastic differentiation and the number of osteoblast precursor cells recruited is regulated by a core molecular pathway, the canonical Wnt/β-catenin pathway.[23]

Wnt proteins are a family of secreted cysteine-rich glycoproteins with functions relating to cell specification, formation of the body plan, cell growth, proliferation, differentiation, and apoptosis.[24,25] In bone, the Wnt signaling pathway has diverse roles in bone modeling and remodeling plays a key role in osteoblast differentiation so that agents directed against endogenous Wnt inhibitors specific to bone, might selectively permit accelerated osteoblast differentiation and thus increase the bone formation rate.[26-28] Sclerostin (SOST) and dickkopf-1 (dkk1) are endogenous inhibitors of the canonical Wnt/β–catenin pathway specific to bone. In the presence of these inhibitors, the osteoblast precursors are not exposed to a Wnt signal, so that β-catenin is degraded and osteoblast differentiation and recruitment is stopped.[29] Dickkopf-1 is a protein encoded by the dkk1 gene which is a regulator of bone mass with increased expression linked to osteopenia and decreased expression to high bone mass.[30] DKK-1 may also play a role in osteoarthritis, metabolic bone disease (osteoporosis and Paget's disease), as well as multiple myeloma-associated bone disease and prostate cancer bone metastases.[31,32] Serum dickkopf-1 concentrations are significantly higher in patients with low BMD and in women with postmenopausal osteoporosis.[29,30] Serum dickkopf-1 levels may serve as markers of bone metabolism and disease.[33]

In this study, we evaluated the association between serum Dickkopf-1 levels and serum Osteoprotegerin with bone density in women with osteoporosis.

Methods

Patients

This was a case-control study performed in the Department of Biochemistry in Tabriz University of Medical Sciences (TUMS).

Recruitment of the patients occurred during the period from September 2012 to July 2013. All patients diagnosed with osteoporosis were subsequently referred to a Rheumatologist at Sheikholrrais Clinic. Ethical approval was obtained from the Medical Ethics Committee of Tabriz University of Medical Sciences and written informed consent was received from all patients according to the tenets of the Declaration of Helsinki.

The study population included 44 women with osteoporosis. Exclusive criteria included prior corticosteroid use, hormone replacement therapy, calcium or vitamin D supplementation, or any medication altering bone metabolism, patients with diseases that can be a secondary cause of osteoporosis, including intestinal and kidney disease, rheumatic diseases (rheumatoid arthritis, lupus, etc.), and endocrine disorders. Our control group was postmenopausal women with mechanical pain who needed to perform BMD based on national osteoporosis foundation (NOF) recommendations, but the results of the BMD were normal.

From all the participants in this study, approximately 10 ml of venous blood in tubes without anticoagulant was obtained at 8:00 a.m. after overnight fasting. Serum separated with centrifuging at 2500 rpm for 10 min and maintained in -70 C until the relevant tests.

DEXA Scanning

BMD of hip with femoral neck, and lumbar spines over L2-L4 regions were measured with Dual-Energy X-ray Absorptiometry (DEXA; Hologic QDR 4500, USA) and expressed as g/cm²using. The difference between an individual's BMD and the mean BMD for a reference population is expressed in standard deviation (SD) units. Z- and T-scores were calculated where the Z-score is the SD of the individual's BMD compared to the mean BMD score of a similar sex-, age-, weight- and height matched population and the T-score is the SD of the individual's BMD compared to the mean BMD score in a young healthy population.

Determination the levels of DKK-1 and OPG

Serum levels of DKK-1 and OPG were measured by commercially available ELISA kits. Human Dkk1 ELISA kit (BOSter BioLogical Technology: cat N 86 China) was obtained to analyze Dkk1 protein concentration in human serum samples. ELISA was performed in accordance with manufacturer's protocols.

Measurement of OPG was performed using human commercially available kits (R and D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Statistical analysis

All statistical analyses were completed using the SPSS for Windows 17.0 (SPSS Inc., Chicago, IL, USA). All results are expressed as the mean ± SD. Pearson's correlation test was used to analyze the association between bone mineral density and serum Dkk1 and serum OPG levels. Significant differences in differentiation between the control and patients were tested with the use of the independent samples t test. P-Values < 0.05 were considered statistically significant.

Results

The biochemical data of the control and postmenopausal patients are presented in table 1. There was no statistical significant difference between the age of two groups (58.85 ± 7.51 vs. 59.06 ± 7.81; P = 0.990). The serum concentration of iPTH was significantly higher in patients than control group (42.11 ± 38.12 vs. 24.42 ± 19.82; P = 0.001). Osteoporosis patients had increased values of L_1-L_4 BMD (0.71 ± 0.10 vs. 0.98 ± 0.0; P < 0.001), (Hip) FN-BMD (0.62 ± 0.10 vs. 0.89 ± 0.00; P < 0.001) compared to the controls. The serum concentration of OPG was significantly higher in patients than control group (4.70 ± 2.16 vs. 4.56 ± 1.21; P = 0.005). The serum Dkk1 concentration in the osteoporosis group (2.91 ± 1.27) was significantly increased compared to the

control group (2.01 ± 0.87; P < 0.010). There was no statistical significant difference in serum DKK1 levels between osteopenia and osteoporotic patients (1.34 ± 0.25 vs. 1.10 ± 0.35; P = 0.926). The serum concentration of OPG was significantly higher in control group than patients (4.70 ± 2.16 vs. 4.56 ± 1.21; P < 0.010). There was no statistical significant difference between the serum OPG levels in osteopenia and osteoporotic patients. (1.12 ± 0.21 vs. 1.19 ± 0.38; P = 0.497). There was a negative statistical significant association between Dkk1 levels and (Hip) FN-BMD (r = -0.48, P < 0.010) in the osteoporotic patients (Figure 1). No significant association was found between the (Hip) FN-BMD and serum OPG levels in the osteoporotic patients (r = 0.04, P = 0.790) (Figure 2).

Table 1. Biochemical data of the control and patients group

Parameters	Control (Mean ± SD)	Patients (Mean ± SD)	P
Age (year)	58.85 ± 7.51	59.06 ± 7.81	0.990
iPTH (pg/dl)	24.42 ± 19.82	42.11 ± 38.12	0.001
Calcium (mg/dl)	9.49 ± 0.58	9.14 ± 0.52	0.450
Phosphorus (mg/dl)	3.99 ± 0.40	4.12 ± 0.31	0.002
Alk (Iu/l)	175.52 ± 56.48	190.67 ± 25.30	0.001
VitD (ng/ml)	26.82 ± 4.71	20.15 ± 4.96	0.771
FN-BMD (g/cm^2)	0.62 ± 0.10	0.89 ± 0.00	0.001
L_1-L_4 BMD (g/cm^2)	0.71 ± 0.10	0.98 ± 0.00	0.001
OPG (ng/ml)	4.70 ± 2.16	4.56 ± 1.21	0.005
DKK-1 (pmol/l)	2.07 ± 0.87	2.91 ± 1.27	0.009

Differences among groups were assessed by independent samples t-test
iPTH: Intact parathyroid hormone; Alk: Alkanin; FN-BMD: Femoral neck-Bone mineral density; OPG: Osteoprotegerin; DKK-1: Dickkopf-related protein-1

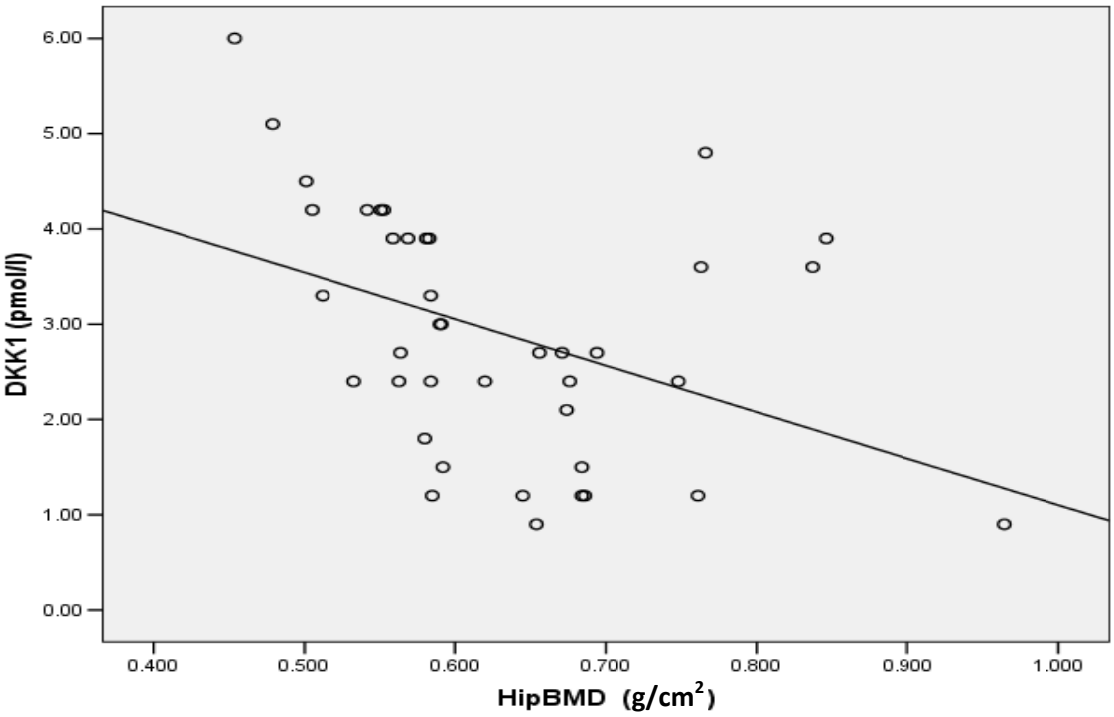

Figure 1. Correlation and linear regression analysis performed between serum Dkk1 levels and the (Hip) FN-BMD (Femoral neck-Bone mineral density) (r = -0.48; P < 0.010)

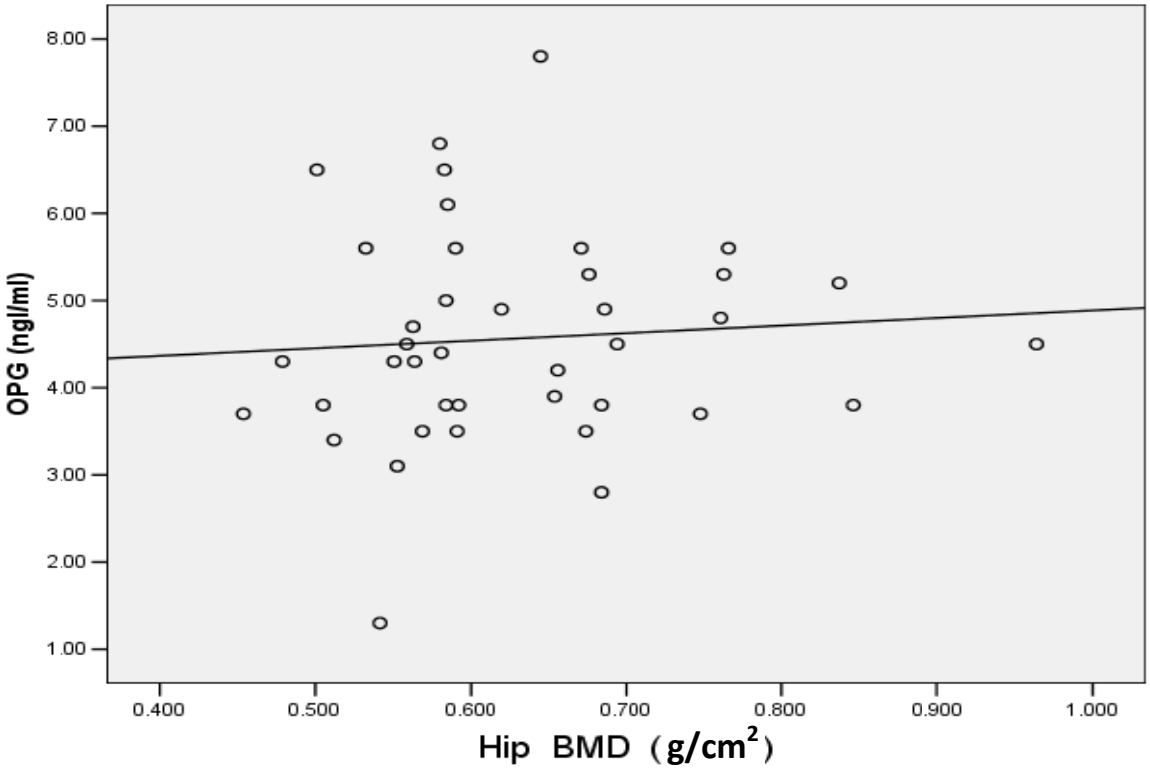

Figure 2. Correlation and linear regression analysis performed between serum OPG levels and (Hip) FN-BMD (Femoral neck-Bone mineral density) (r = 0.04; P = 0.790)

Discussion

Osteoporosis is a common a skeletal disorder characterized by a systemic impairment of low bone mass and progressive micro-architectural deterioration resulting in fragility fractures.[34,35] With the ageing population, the medical impact of osteoporosis in general and postmenopausal osteoporosis in particular, will be exacerbated. Reduced bone mass is the result of an imbalance tightly regulated processes of bone formation and bone resorption.[36] Recent investigation has led to the identification of novel therapeutic targets. Based on it, therapeutic strategies have been developed with the purpose of inhibiting excessive bone resorption and by increasing bone formation.

Wnt-dependent nuclear accumulation of β-catenin is a major trigger of osteoblastic differentiation and bone formation. Wnt signaling plays a key role in regulating bone development and remodeling, with aberrations in signaling resulting in disturbances in bone mass.[36] Dkk1 protein is implicated in osteoblast differentiation and bone remodeling in healthy people and in patients with bone disorders. Dkk1 has been shown to inhibit canonical Wnt/b-catenin signaling pathway. Our results demonstrated that serum Dkk1 was significantly increased in patients with osteoporosis compared to control group. We also observed that Dkk1 was highly correlated with FN-BMD but there was no statistical significant correlation between Dkk1 with L_1-L_4-BMD, which proved the results reported by Butler et al.[37] They showed serum DKK 1 increased in patients with osteoporosis when compared to controls

Osteoblasts produce OPG and RANKL. RANKL binds to RANK and enhance osteoclast differentiation/activity. OPG, a soluble decoy receptor for RANKL, competitively inhibits RANKL, RANK interaction; therefore, it is the OPG: RANKL ratio that determines the net effect on osteoclasts.[38,39] Studies have shown that the

effects of Dkk1 on bone were mediated by inhibition of Wnt signaling, which directly impaired new bone formation and limited OPG expression, thereby shifting the OPG:RANKL ratio to favor bone resorption.[40]

Our results show that OPG is increased in serum of patients with osteoporosis. We did not find any association between serum OPG and L_1-L_4 or FN-BMD. These results are in agreement with those reported by Yano et al. who reported that circulating OPG was significantly elevated in postmenopausal women with osteoporosis. These findings suggest that circulating OPG levels may regulate by osteoporotic factors and that the increase in serum concentration may be a compensatory response against enhanced osteoclastic bone resorption.[41].

Conclusion

The results of this study indicated that there are statistically significant increases in serum levels of DKK1 and OPG compared with the control group. Moreover, we found that Dkk1 was highly associated with FN-BMD. Future studies would clarify the beneficial role of OPG and DKK1 for diagnosis or treatment of osteoporosis.

Conflict of Interests

Authors have no conflict of interest.

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