

Effect of Calcium -Vitamin D and Alendronate on serum Dickkopf-1(Dkk-1) and Osteoprotegerin(OPG) in women with osteoporosis

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Abstract: Introduction: The early diagnosis and treatment of osteoporosis is one of the priorities of medical sciences and so many complications can be prevent with early diagnosis of this disease. There are several markers characterized for bone formation and re absorption but none of these markers has direct relation with bone density. **Methods and Materials:** In a before after clinical trial, we enrolled 36 patients with osteoporosis and 36 healthy subjects in the form of two case and control groups and the efficacy of osteoporosis standard treatment protocol was assessed on Dkk-1 and OPG serum levels. The results were assessed with measuring the markers serum levels before and after the treatment. **Results:** The overall mean age of subjects was 60.2±6.8 years in the range of 40 to 68 years. The mean DKK-1 levels in patients, before the therapy was 3.01±1.27 which changed to 3.03±1.24 nano grams/ml after the treatment with no significant difference (P=0.92). However, the difference between the groups was meaningful (P<0.0001). The OPG levels, before the treatment was 4.44±1.12 which has changed to 4.42 ±1.10 nano grams/ml, the difference was not statistically different (P=0.74). Despite Dkk-1, the difference in serum OPG levels was not significant before the treatment (P=0.36). **Conclusion:** The Dkk-1 serum levels in osteoporotic patients were higher comparing to healthy subjects, but we can conclude that osteoporosis standard treatment has no significant effect on decreasing the Dkk-1 levels and increasing the OPG levels. Therefore, more multi central studies with more number of cases is necessary.

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1. Introduction

Osteoporosis is a generalized and metabolic bone disease that is characterized with reduces of bone strength and increases the trend to bone fractures. This condition is defined with DEXA method and T-Score <2.5 in healthy young women (Sweet, 2008).

Almost 50% of white women experience fractures due to the osteoporosis. The increase in mortality rate and the disability caused by these fractures is an important health subject. For example, the mortality of the neck of femur fracture increases by 10-20 % comparing to their similar subjects and increase the long-term home nurse cares in about 25% of patients (Ford, 2011).

Therefore, the early diagnosis and treatment of the osteoporosis is one of the priorities of medicine, which can prevent so many related complications. There are several markers which is identified as bone formation markers(osteocalcin and precollagen type1) or bone reabsorption markers(Hydroxy pyrolin and piridinolines) that have a role in controlling the osteoporosis, but none of these factors have a direct correlation with bone density(Kanis and Reginster, 2008).

Therefore, the need for some markers with near to direct correlation with bone density that gives almost exact information about the bone density, treatment course and the drug effect is avoidable (Pinzone, 2009).

Dkk-1 is one of the factors, which had a gain attention nowadays. This protein is classified with the WNT signal inhibitor proteins. Wnt proteins has role in cellular growth, proliferation, differentiation and apoptosis and does this by activating specific intracellular signals (Long, 2011).

OPG/RANKL/RANK system is another important pathway for bone reabsorption and resolution. RANKL/OPG ratio is introduced as a bone metabolic equation in several inflammatory disorders. RANKL is also characterized as OPG ligand or cytokine activity related TNF. OPG acts as a natural decoy receptor and blocks RANKL activity by interfering in the RANKL to RANK junction (Butler, 2011).

The laboratory and clinical studies has shown that, PTH acts in two-way procedures in inflammatory diseases such as rheumatoid arthritis, in one hand, PTH stimulates the production of the RANKL and in the other hand, it prevents the OPG

production, so RANKL/OPG cytokine system may have a role in the PTH functional changes (Camphenout and Gollodge, 2009).

There are limited studies on the effect of the osteoporosis classic treatment on the factors associated with bone resolution and formation. The present study is conducted with the aim of evaluating the 4 months Calcium, Vitamin D and Alendronate therapy on the Dkk-1 and OPG serum levels of women with osteoporosis. The findings of this study may provide some useful information regarding to the bone situation, treatment course and the disease condition. The aim of present study is the evaluation of effect of osteoporosis treatment with Calcium, Vitamin D and Alendronate on Dkk-1 and Osteoprotegerin serum levels in women with osteoporosis.

2. Material and Methods

In a before after clinical trial, in the internal medicine department of Tabriz university of medical sciences we evaluated the effect of osteoporosis treatment with Calcium, Vitamin D and Alendronate on Dkk-1 and Osteoprotegerin serum levels in women with osteoporosis.

A study that performed in the rheumatology department of the Imam Reza hospital and Sheikholaie clinic related to Tabriz university of Medical sciences in a 15 months period.

No additional interventions were administered to patient and all the data from patients was kept secret. Each of the treatments and provided services like densitometry and laboratory tests used in this study is based on valuable references and routine treatments administered by physicians. There was no other ethical limitations expect the rules related to administration of Calcium, Vit D and Alendronate. The written consent was taken from all the patients.

The study procedure was approved by Research Ethical committee of Tabriz University of Medical Sciences, and was registered as a clinical trial in IRCT website with the number of N12012012388.09.

All the referring patients in the 12 months period of this study was assessed in the form of two case and control groups, the patient were selected randomly.

The number of the study population was defined 60 patients with regard to the costs and facilities of the laboratory centers; we increased the number of cases to 36 cases to elevate the accuracy of the study.

The rheumatologists examined all patients and the exact history was obtained from the patients, then patient underwent the densitometry with hologic device. Patient with osteoporosis received Calcium 1000 mg daily, Vitamin D 600 units daily and Alendronate 70 mg weekly for 4 months. The treatment is the standard osteoporosis treatment regimen, so no other interventions added to this

treatment. The subjects in the control group did not receive any treatments.

The Dkk-1 and OPG was measured for patients before and after the treatment. The patients who needed drugs other than the standard osteoporosis regimen, such as anti-inflammatory agents were excluded from the study.

OPG, Dkk-1, Vitamin D, Calcium, Phosphorus and Alkaline phosphatase levels was measured by ELISA method and OPG and Dkk-1 was compared before and after the 4 months period.

Exclusion criteria:

- 1- Using the Glucocorticoids and other drugs with effect on the metabolism and bone density (cytotoxic and antithrombotic)
- 2- Patients under treatment with Calcium, Vitamin D and both of these agents.
- 3- Positive history of malignant diseases.
- 4- Disease, which secondarily cause osteoporosis like hepatic, renal, rheumatic or endocrine diseases.
- 5- Progressive osteoarthritis in clinical examination.

Statistical Analysis:

SPSS version 16 was used for data analysis, we used the SPSS version 16 for quantitative variables and Chi-square software for qualitative variables, the Fisher's exact test was used in cases of need. Pearson or Spearman test was used for evaluation of the correlation between parameters. The results were presented as mean \pm Standard deviation frequency and percent. The p value was considered meaningful in less than 0.05 levels.

3. Results

In a before after clinical trial we studied 36 patients with osteoporosis and 36 healthy subjects after achieving the inclusion criteria in the form of two case and control groups.

The mean age of the patients was 60.2 ± 6.8 years in the range of 40-68 years, the mean age of the patients with osteoporosis was 59.7 ± 7.5 years (40-68) and the mean age of subjects in control group was 60.7 ± 6 years (47-66). ($P=0.53$).

The description of par clinic findings such as calcium, Phosphorus, Alkaline phosphates, Vitamin D serum levels and iPTH levels before the intervention is shown on table 1.

BMD of hip and vertebrae in two-case and control group is shown on table 2.

The serum levels of bone markers in the patients with osteoporosis and healthy subjects before and after the 4 months period is shown in table 3.

The description of the changes of bone markers in the patients with osteoporosis and healthy subjects before and after the 4 months period is shown in table 3.

There was not a significant relationship between OPG and density of hip in the patients with osteoporosis (P=0.87, R=0.09) and healthy subjects (P=0.50 and R=-0.056).

There was not a significant relationship between OPG and density of Vertebrae in the patients with osteoporosis (P=0.87, R=0.004) and healthy subjects (P=0.29 and R=-0.13).

There was no significant correlation between Dkk-1 and density of Hip in the patients with osteoporosis (P=0.82, R=-0.45) and healthy subjects (P=0.26 and R=-0.10).

There was no significant correlation between Dkk-1 and density of Vertebrae in the patients with osteoporosis (P=0.81, R=-0.12) and healthy subjects (P=0.28 and R=-0.007).

Table 1: Laboratory finding of patients between two groups at begging of study

	Osteoporotic group	None osteoporotic group	P
Ca	9.1 ± 0.99	9.49 ± 0.58	0.44
P	4.12 ± 0.38	3.99 ± 0.17	0.002
ALP	185.7 ± 37.9	175.5 ± 56.4	0.001
Vit. D	18.6 ± 1.93	26.8 ± 4.71	0.77
iPTH	42.8 ± 6.2	24.4 ± 9.8	0.001

There was a reverse linear correlation between OPG and iPTH levels in patients with osteoporosis (P=0.02, R=-0.36) while this correlation was not meaningful in healthy subjects (P=0.16, R=0.34).

There was not significant relationship between OPG and CA, Phosphorus, Alkaline phosphates and Vitamin D.

There was no significant relationship between Dkk-1 and iPTH in patients with and without osteoporosis, and there was not meaningful relationship between Dkk-1 and Ca, P alkaline phosphates and Vitamin D.

4. Discussions

The early diagnosis and treatment of osteoporosis is one of the priorities of medical sciences and so many complications can be prevented with early diagnosis of this disease.

There are several markers characterized for bone formation and reabsorption but none of these markers has direct relation with bone density.

Therefore, the needs for the markers with a close relationship with bone density, which can provide almost exact information about the bone density, treatment course, and drugs effect is avoidable (Pinzone, 2009).

Table 2. Evaluation of Bone Mineral Density (BMD) between two groups

	Osteoporotic group	None osteoporotic group	P
Hip BMD(g/cm ²)	0.63 ± 0.11	0.89 ± 0.01	<0.0001
Vertebra BMD(g/cm ²)	0.71 ± 0.10	0.98 ± 0.01	<0.0001

Gurban and his colleagues in 2011 have introduced the Zn ions and OPG/RANKL system as a promotor of bone remodeling in the process of the osteoporosis (Gurban and Mederle, 2011).

The results of this study also reveal that OPG marker is slightly increased in osteoporotic patient after the treatment, it is noted that in the cases of the reduction of this marker, Apoptosis of osteoblasts occurs (Gurban and Mederle, 2011).

Even in post-menopausal patients, free Zn ions is reduced due to the reduced osteoblasts differentiation, following these processes the bone mineralization is interfered. In this study, there was a reverse significant relationship between the reduction of Zn of bone and the increase of RANKL while there is a meaningful correlation with OPG serum levels (Gurban and Mederle, 2011).

Table 3. Evaluation of bone marker of two groups at begging and after 4 months late

	Osteoporotic group	None osteoporotic group	P [¥]
OPG before	4.44 ± 1.12	4.79 ± 2.05	0.36
OPG after 4 month	4.42 ± 1.10	4.85 ± 0.32	0.25
P	0.74	0.40	
DKK-1 before	3.01 ± 1.27	1.97 ± 0.94	<0.0001
DKK-1 after 4 month	3.03 ± 1.24	1.98 ± 0.91	<0.0001
P*	0.92	0.55	

¥_P value between group

*_P value within group

In our study, there was not significant difference between OPG levels after the 4 months standard treatment despite the findings of Gurban et al.

This can be due to reduction of osteoblasts in osteoporotic patients, in our study the RANK and RANKL gene expression and its relationship with Zn ions in bony structures was not evaluated.

We did not find any significant relationship between the OPG and the density of hip in osteoporotic patients. In addition, there was not meaningful relation between OPG and the density of vertebrae ($P=0.87$, person's $r=-0.004$).

In another study by Long et al in 2010, Dkk-1 marker has been introduced as a potent marker in evaluation of the bone deconstruction in lupus disease (Long, 2011).

The results of this study presents that there is a significant increase in Dkk-1 in erosive arthritis, this study has recommended more study for better certification of these findings and revealed that OPG a serum level is elevated in rheumatoid arthritis patients (Long, 2011). In our study, the patients with evidences of rheumatic disease were excluded so the possibility of the increase in bone markers is eliminated.

Lane and colleague in a systematic review in 2009 have shown that a great progress is expectable in the field of osteoporosis prevention and treatment in the next 10 years, they noted to new identified receptors like Dkk-1 for therapeutic goals (Lane and Yao, 2009).

In 2009, Fulciniti and colleagues have used the monoclonal DKK-1 antibody as a new therapeutic modality in multiple myeloma and have concluded that Dkk-1 is a suitable therapeutic target in multiple myeloma; they support the theory that DKK-1 target therapy can prevent the progression of bone disease in Multiple Myeloma patient (Fulciniti, 2010).

In the study of butler et al, in 2010, it is concluded that Dkk-1 serum levels in osteoporotic patients is significantly higher than normal subjects. It is also show that Dkk-1 gene expression is in relation with bone mass changes (Butler, 2011).

Like the findings of the Butler and colleagues, Dkk-1 serum levels, before the intervention in osteoporotic patients was significantly higher than the subjects in control group, this was approximately 1.6 times more than control group. However, the difference between the two groups was not significant for serum OPG.

Unlike the Butler's study, in our evaluation there was no significant correlation between the Dkk-1 bone markers and hip and spine bone density in patients with osteoporosis.

In another study on postmenopausal women, Gurban and colleagues evaluated the OPG/RANKL system correlation with bone density and presented that SRANKL serum levels, in post-menopausal women with osteoporosis is meaningfully more than postmenopausal women without osteoporosis. OPG serum levels are also lower in patients with osteoporosis comparing with healthy subjects.

The serum OPG levels in individuals with osteoporosis compared with controls was low and declining serum OPG in patients with osteoporosis is secondary to stimulation of apoptosis of osteoblasts (Gurban, 2009). In our study, similar to the Gurban et al study baseline serum OPG in osteoporotic patients was lower than controls, but this difference was not statistically significant.

As noted above, in our study, there was no significant association between OPG bone marker and bone mineral density of hip and spine, this results is in line with the results of the of study Gurban et al.

Pinzone and colleagues in a study in 2009, has shown that Dkk-1 has a reverse correlation with bone density. Dkk-1 is also introduced as a strong reverse regulator for osteoblasts(Pinzone, 2009).

In another study by Juji et al, in 2002 in Japan, they stated that RANKL can be as a therapeutic target in producing a vaccine to prevent the bone deconstruction, they also concluded that the principles of bone destruction in osteoporosis, is similar to other collagen vascular diseases(Juji, 2002).

It is note that a novel approach and vaccination against RANKL system can prevent the bone destruction in related diseases (Juji, 2002).

In a study by Gaetti et al in 2010 in Italy, the effect of the treatment with bis propionates was assessed on patients with Dkk-1 in osteoporotic patients, the changes in alkaline phosphates serum levels was significant, while the changes in Dkk-1 was not significant(Gatti, 2011).

Our study conducted in a 4 months period, Dkk-1 serum levels was not statistically significant similar to Gaetti's findings, we did not evaluate the alkaline phosphates levels.

Gaetti and colleagues studied the effect of the treatment with Denosumab on serum Dkk-1 levels and stated that the long-term therapy with this agent can probably reduce the Dkk-1 levels in osteoporotic patients (Gatti, 2012).

In the study of Anastasilakis in 2010 in Greece, the treatment with Teriparatid has found to elevate the Dkk-1 in osteoporotic women (Anastasilakis, 2010).

Conclusion

According to these results, it can be stated that the standard treatment of osteoporosis does not have a significant role in reduction of Dkk-1 and increasing of OPG. To confirm the results more multi-center studies with a larger sample size is required.

Recommendations

There were some limitations in our study, and if these limitations can be overcome, these recommendations can be presented:

- 1 - Conducting a similar study with a larger sample size and effect on osteoporotic patients to evaluate the effect of Zoledronic acid with calcium - vitamin D as an osteoporosis treatment on serum levels of Dkk-1.
- 2 - Conducting a similar study in osteoporotic patients with larger sample size to study the effect of long-term treatment of osteoporosis on Dkk-1 and OPG.

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