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Araştırma Makalesi/Research Article

Vitamin D levels in experimental osteoporosis model treated with denosumab

Denosumab ile tedavi edilen deneysel osteoporoz modeli çalışmasında D vitamin düzeyleri

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Öz

Amaç: Kemik, yaşam boyu metabolik aktivitesini devam ettiren ve yıkım/yapım şeklinde dinamik özelliğe sahip bir endokrin doku olarak kabul edilmektedir. Osteoporoz sistemik bir iskelet bozukluğu ve metabolik kemik hastalıklarının en yaygın tiplerinden biridir. D vitamini, normal kalsiyum ve kemik homeostazı için hayati önem taşıdığından, kemik sağlığı ile güçlü bir şekilde bağlantılıdır. Erişkinlerde vitamin D eksikliği, osteopeni ve osteoporozu kolaylaştırır ve de kırık riskini artırır. Denosumab, kemik rezorpsiyon mediyatörü olan nükleer faktör-B ligandı hedefleyen bir insan monoklonal (IgG2) antikoru olup osteoporotik kırıkların tedavisinde kullanılan antirezorptif bir ajandır.

Gereç ve Yöntem: Osteoporoz çalışmaları için yaygın olarak ovariektomi yapılmış hayvan modeli kullanılmaktadır. Ovariektomi ve kortikosteroid tedavisinin kombinasyonu ile osteoporoz geliştikten sonra tavşanlar denosumab ile tedavi edilmiştir.

Bulgular: Osteoporoz gelişimi sonunda D vitamin düzeyleri ortalama 31.3 ng/mL iken denosumab ile tedavi sonrasında ortalama 49.7 ng/mL olmuştur.

Sonuç: Günümüzde yaygın olarak görülmesi sebebiyle osteoporoz için tanı, tedavi ve tedavinin izlenmesi kritik öneme sahiptir. Fizyolojik konsantrasyonlarda aktif D vitamini, RANKL/OPG sinyali yoluyla normal bir kemik yapım-yıkım hızını korur. Osteoporotik koşullar altındaki gereksinimler ve etkiler henüz kesin olarak belirlenmemiştir. Normal fizyolojik koşullarda vücutta gerekli olan seviyeler sentezlenmektedir. Patolojik koşullar için Vit D seviye takibi yaparak değerlendirmek önemlidir.

Anahtar Kelimeler: Osteoporoz, Denosumab, D Vitamini

Abstract

Objective: Bone is considered to be an endocrine tissue that continues its metabolic activity throughout life and has a dynamic property in the form of destruction /construction.
Osteoporosis is a systemic skeletal disorder and one of the most common types of metabolic bone diseases.
Vitamin D is strongly linked to bone health, as it is vital for normal calcium and bone homeostasis. Vitamin D deficiency in adults facilitates osteopenia and osteoporosis, and also increases the risk of fractures.
Denosumab is a human monoclonal (IgG2) antibody that targets the nuclear factor-B ligand, a bone resorption mediator, and is an antiresorptive agent used in the treatment of osteoporotic fractures.

Material and Methods: Ovariectomized animal models are commonly used for osteoporosis studies.
Rabbits were treated with denosumab after developing osteoporosis with a combination of ovariectomy and corticosteroid therapy.

Results: While vitamin D levels were 31.3 ng/mL osteoporosis stage, it was 49.7 ng/mL after treatment with denosumab.

Conclusions: Diagnosis, treatment and follow-up of treatment are critical for osteoporosis because it is common today. Active vitamin D at physiological concentrations maintains a normal rate of bone resorption and formation via RANKL/OPG signaling. The requirements and effects for vitamin D under osteoporotic conditions have not yet been conclusively established. Under normal physiological conditions, the necessary levels are synthesized in the body. It is important to evaluate by monitoring Vit D level for pathological conditions.

Key Words: Osteoporosis, Denosumab, Vitamin D

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INTRODUCTION

Bone is an endocrine tissue that continues its metabolic activity throughout life and has a dynamic feature in the form of destruction /construction. Bone homeostasis is a complex process that is closely connected with the functions of many organisms. Disturbances in these processes are associated with many chronic or acute diseases that seriously affect quality of life and even threaten life.

Under physiological conditions, it cycles through bone loss through osteoclast-mediated bone destruction, followed by bone replacement through osteoblast-mediated bone formation, which maintains bone structure. Imbalances in this ongoing interaction between osteoblasts and osteoclasts can lead to pathological conditions such as osteoporosis, rheumatoid arthritis, periodontitis, osteolytic bone metastases, Paget's disease of bone. Osteoporosis is a systemic skeletal disorder and one of the most common types of metabolic bone diseases[1,2].

Vitamin D, a steroid hormone, is very important for skeletal health and mineral metabolism. The forms of Vitamin D, ergesterol of plant food origin, 7-dehydrocholesterol of animal food origin and provitamin D3 (7-dehydrocholesterol) synthesized from cholesterol in the body follow a similar pattern in their metabolism. Vitamin D3 is converted to the form of 25-hydroxyvitamin-D (25(OH)D, Calcidiol) in the liver. The 25(OH)D formed is converted into the active form 1,25 dihydroxycholecalciferol (1,25(OH)2D, Calcitriol) or the inactive metabolite 24,25 dihydroxyvitamin D [24,25(OH)2D] in the kids [3,4]. The active form of vitamin D, 1,25(OH)2D, acts through the nuclear vitamin D receptor, a receptor found in almost all nucleated cells, and is involved in cell proliferation, differentiation and apoptosis, immune and hormonal regulation and other processes related to our body[5]. Vitamin D plays an important role in skeletal development, maintaining bone health and neuromuscular functions[6].

Vitamin D deficiency in adults facilitates osteopenia and osteoporosis and increases the risk of fractures[7–9].

The most important effect is to increase the absorption of calcium and phosphorus from the intestines[3]. 1,25 dihydroxycholecalciferol controls its own synthesis by stimulating the activity of 24-hydroxylase and suppressing the activity of 1-hydroxylase [4,10].

Vit D and PTH have synergistic effects on bone. In high concentrations, it stimulates the differentiation of stem cells into osteoclasts in the bone marrow and also causes the synthesis of cytokines and other stimuli that affect osteoclastic activity in osteoblasts, leading to the dissolution of the organic and inorganic phase of the bone[4].

Vitamin D affects the activity of osteoblasts, osteoclasts and osteocytes, suggesting that it affects bone formation, bone resorption and bone quality. Active vitamin D at physiological concentrations maintains a normal rate of bone resorption and formation via RANKL/OPG signaling[11].

For the treatment of osteoporosis, bisphosphonate group drugs are used, which have a traditional and widespread use.

However, common side effects can be encountered in the use of bisphosphonates [12] . Denosumab or intravenous zoledronic acid are used in patients who cannot take oral bisphosphonates due to contraindications and intolerance or who are at high risk of fracture[13].

Denosumab; It is a human monoclonal antibody (IgG2) that targets RANKL and binds to it with high affinity and specificity. It prevents the RANKL/RANK interaction, which inhibits the activation of its receptor, RANK, on the surface of osteoclast precursors and osteoclasts. It inhibits osteoclast formation, function and survival, thereby reducing resorption in cortical and trabecular bone.

The fact that the formation and treatment period of the disease is very long complicates human studies and pushes researchers to experimental studies. Although the experimental animal model frequently used in osteoporosis studies is the ovariectomized rat. There are also many disadvantages of this model, such as its small size, incomplete closure of the epiphyseal plate, and bone differences with humans. For this reason, there are studies on different animal groups and the use of the rabbit model comes to the fore in this field.

MATERIALS AND METHODS

Experimental animal model. A total of 9 healthy adult female New Zealand white rabbits aged about 3 months old with a mean weight of

2.0±0.5 kg were purchased from the Animal Center of Gazi University (Ankara, Turkey) and were used in the present study.

Guiding principles for experimental procedures found in Gazi University Council and Declaration of Helsinki of the World Medical Association regarding animal experimentation were followed in the present study. Standardized OECD and Turkish National regulation for static bioassays were applied.

A combination of ovariectomy and corticosteroid therapy was used to create an osteoporotic animal model. First of all, bilateral ovariectomy was performed on rabbits under general anesthesia. One week after the operation, methylprednisolone succinate was injected at a dose of 1 mg/kg/day for 8 weeks and the formation of osteoporosis was observed. After determining the development of osteoporosis in the animal, the treatment stage was started. In the treatment, Denosumab was administered subcutaneously at a dose of 1 mL 8mg/mL. A 14-week period was determined for the treatment. Blood samples were collected every 2 weeks during all this disease formation and treatment.

Blood samples were collected and centrifuged at 3200×g for 10 min for serum separation. Serum samples were aliquoted into Eppendorf tubes and were stored at − 80 °C until analyses were performed.

25(OH)D analyses were done at Gazi University Central Biochemistry Laboratory. Serum 25(OH)D levels were studied with chemiluminescent method by using auto-analyzer (Beckman Coulter DXI 800) and ready to use kits (Beckman Coulter).

The results are shown in the table1.

Table 1: Results of Vitamin D level of each animal after ovx every 2 weeks for 6 measurements and the levels after treatment by denosumab every 2 weeks for 7 measurements.,

Vitamin D level													
After OVX every 2 weeks						After Denosumab every 2 weeks							
1st measurement	2nd measurement	3rd measurement	4th measurement	5th measurement	6th measurement	1st measurement	2nd measurement	3rd measurement	4th measurement	5th measurement	6th measurement	7th measurement	
59,15	74,95	44,18	51,91										
49,85	108,56	75,64	63,74	62,37	62,1	45,94	71,96	26,19	40,85	27,55	27,25	50,62	
33,81	92,17	62,02	66,28	17,05	27,73	52,64		53,73	29,61	32,5	49,97	33,11	
59,26	90,27	62,95	23,11	34,95	22,21	36,49	26,87	30,68	36,9	31,74	76,22	72,01	
60,52	96,16	36,25	31,57	55,17	28,38	55,96	34,4	40,47	32,15	32,28	69,92	30,92	
	49,25	77,22	32,83	28,41	37,56	18,27	39,46	49,23	34,94				
17,92	34,83	29,91	41,5	41,27	33,6								
21,83	33,84	48,71	26,14	34,78	21,99	21,02	19,42	21,37	47,61	12,75	49,87	57,41	
43,69	61,36	39,21	41,25	38,87	16,83	17,6	37,83	37,79	32,44	40	32,37	54,33	

DISCUSSION and CONCLUSION

Osteoporosis is defined as a systemic skeletal disease characterized by increased fragility as a result of decrease in bone mass per unit volume, microarchitecture of bone tissue and deterioration of bone quality. Osteoporosis has a high prevalence rate [14]. With prolongation of life expectancy and the increasing aging population, has become an increasingly important health problem[15].

Vitamin D deficiency in adults facilitates osteopenia and osteoporosis, and also increases the risk of fractures[7,8].

Vitamin D regulates calcium and phosphorus metabolism with its physiological effects on bones, intestines, kidneys and parathyroid glands[7]. Its active form, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], regulates many genes related to cell differentiation and proliferation through the nuclear vitamin D receptor. Vitamin D plays an important role in skeletal development, maintaining bone health and neuromuscular functions[6].

Currently, it is critically important to monitor the diagnosis, treatment and treatment of osteoporosis due to its widespread occurrence. A major challenge is that osteoporosis is asymptomatic until it presents with a fracture; laboratory experimental models are often needed for biomarker and therapeutic agent studies in this disease, which is defined as a silent disease. With the experimental model we constructed, starting from the first occurrence of the disease and then following it throughout the entire treatment period we showed changes in serum Vitamin D levels, which is thought to be an important component of osteoporosis.

In osteoporosis studies; Rabbits were preferred because of complete closure of the epiphyseal plate at 6-8 months, active Haversian remodelling similar to that observed in humans, and faster bone turnover than rodents and primates[16,17]. Calcium is the most abundant mineral in the human and animal body and is an essential nutrient for achieving optimal bone mass and preventing bone loss at all ages. It is known that in rabbits, similar to humans, insufficient calcium intake affects bone mass[16,18–20].

Because of all these results, the New Zealand White rabbits were chosen.

Vitamin D, which is transported to the liver by binding proteins, is converted to 25-hydroxy vitamin D (25(OH)D) by the enzyme 25-hydroxylase (CYP27A1) in the liver[10,21].

25-hydroxy vitamin D3, transported to the kidneys, forms the active metabolite 1,25 dihydroxy cholecalciferol by the enzyme 1-α-hydroxylase (CYP27B1). 1,25-dihydroxy cholecalciferol controls its own synthesis by stimulating the activity of 24-hydroxylase and inhibiting the activity of 1-α-hydroxylase[4,21].

Calcium, 1,25(OH)2D suppresses it, while PTH and hypophosphatemia are the main inducers of this microsomal enzyme[22–24].

When the absorption of Ca from the intestines is insufficient, the level of parathormones increases, and under the influence of this hormone, the enzyme 1-hydroxylase is activated and the level of 1,25 dihydroxy vitamin D3 increases. In this case, the calcium-mobilizing effect of vitamin D from the bones is revealed. Since the serum Ca level is more important in the organism, the Ca level in the serum is tried to be kept at its normal value by mobilizing Ca from the bones. As a result of the deficiency of Ca or vitamin D that occurs in this way, the mineralization of the bones is disrupted[10,25].

Osteoclasts are multinuclear cells responsible for bone resorption.

PTH and 1,25 (OH)2 vitamin D3 stimulate osteoclastic activity. Formation, activation and resorption of osteoclasts are regulated by RANKL/OPG ratio, IL-1 and IL-6, CSF, PTH, 1,25 (OH)2 vitamin D3 and calcitonin[26]. OPG acts as a trap receptor by binding to RANKL and prevents it from binding to the receptor activator nuclear factor-kappa B (RANK). As a result, osteoclast differentiation and activation are inhibited and RANKL cannot form bone resorption[27].

Receptor activator factor kappa B (NF-kB) ligand (RANKL) is an essential molecule for the development, function and survival of osteoclasts[28,29]. In order for osteoclast differentiation to be completed, RANKL expression is required from osteoblastic stromal cells, and RANK expression is required from osteoclast precursors [30]. RANKL; stimulate these cells by binding to RANK, which is its own receptor located on the surface of precursor and mature osteoclasts, stimulated T and dendritic cells[26,31,32].

Denosumab; It is a human monoclonal antibody (IgG2) that targets RANKL and binds to it with high affinity and specificity. It prevents the RANKL/RANK interaction, which inhibits the activation of its receptor, RANK, on the surface of osteoclast precursors and osteoclasts. It inhibits osteoclast formation, function and survival, thereby reducing resorption in cortical and trabecular bone.

In the pharmacological treatment of OP, drugs that reduce bone destruction and increase bone construction are used. Oral bisphosphonates are frequently used in the treatment of OP.

A wide range of drug groups such as calcium, vitamin D, bisphosphonates, selective estrogen receptor modulators, calcitonin, parathormone, strontium ranelate, denosumab are included in the treatment of osteoporosis[33].

However, dosing of drugs and drug-related side effects may reduce compliance. The lack of compliance with the drug also causes a decrease in the effectiveness of the drug [34].

Denosumab is used in patients who cannot take oral bisphosphonates due to contraindications and intolerance or who are at high risk of fracture.

Considering that OP is a chronic process and long-term drug use is involved, the importance of patient compliance and satisfaction in terms of continuing treatment will be understood.

In clinical studies, denosumab was found to be slightly more effective in terms of increasing bone density compared to oral bisphosphonates and zoledronic acid[13,35,36].

The values of 25-hydroxyvitamin D3 [25-(OH)D3] were examined before and after treatment of patients receiving Ca and D vit Therapy in combination with denosumab therapy. It was found that the average at the end of treatment was higher[13].

While vitamin D levels were 31.3 ng/mL osteoporosis stage, it was 49.7 ng/mL after treatment with denosumab (Table1, Figure1). In our study, vitamin D levels increased with Denosumab treatment, without an extra vitamin D supplement.

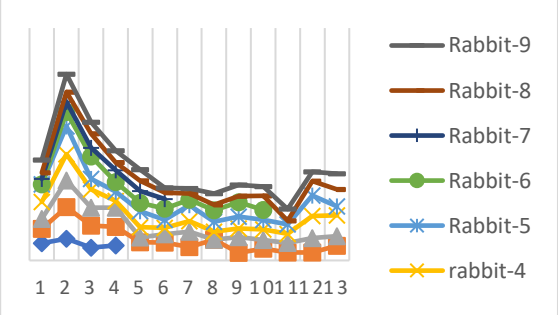

Figure 1: Variation of vitamin D levels during disease formation and treatment.

It is not clear, however, if vitamin D supplementation is directly related to the treatment of osteoporosis largely because vitamin D is usually accompanied by pharmacologic treatment (steroids, bisphosphonates, etc.), which can obscure any possible net effect[37–39].

The requirements and effects under osteoporotic conditions have not yet been definitively established[40]. The National Osteoporosis Risk Assessment (NORA) study[41] concluded that higher calcium and vitamin D intake can significantly counteract BMD-defined osteoporosis without reducing the likelihood of a possible fracture. However, discontinuation of combined calcium vitamin D supplementation resulted in a return to pre-treatment levels of bone turnover[42].

The fact is that low Vitamin D levels are observed with many diseases, including OP. It is still unclear whether this is a cause or an effect. In normal physiological conditions, the necessary levels are synthesized in the body. It is important to evaluate by monitoring Vit D level for pathological conditions.

Ethical Approval: The protocol (Gazi University G.Ü.ET-21.001) for using rabbit in the experiments was reviewed and approved by the Gazi University (Ankara, Turkey) Animal Experiments Local Ethical Committee.

Conflict of Interest: Author declared no conflict of interest.

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