

Investigation of receptor activator of nuclear factor kappaB ligand and osteoprotegerin levels in multiple sclerosis patients

[Multiple skleroz hastalarında reseptör aktivatör nükleer faktör kappaB ligand ve osteoprotegerin seviyelerinin araştırılması]

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ABSTRACT

Aim: The aim of this study was to investigate the osteoprotegerin (OPG) and receptor activator of nuclear factor kappaB ligand (RANKL) levels and their correlation with bone mineral density (BMD) and levels of other bone turnover parameters such as serum osteocalcin, parathyroid hormone (PTH), calcium, bone alkaline phosphatase (bALP) and urinary deoxypyridinoline (DPD) in patients with multiple sclerosis (MS).

Methods: Forty six MS patients (30F, 16M, 33.48 ± 9.57 years old) and 24 healthy controls (14F, 10M, 33.04 ± 7.97 years old) were included in the study. Serum OPG, RANKL, bALP, osteocalcin, PTH, calcium, urinary DPD levels of all subjects, and BMD of 29 patients and all control subjects were measured. Osteoprotegerin and RANKL levels, bALP, osteocalcin and PTH levels, and urinary DPD levels were measured by ELISA method, chemiluminescent method, and HPLC technique. The BMD was measured by dual-X-ray absorptiometry.

Results: Serum OPG (p<0.01), RANKL (p<0.01), bALP (p<0.05), PTH (p<0.01) and calcium (p<0.05) levels were significantly higher in MS patients than in controls. There was no significant difference between serum osteocalcin, urinary DPD levels and BMD measures of the groups.

Conclusion: Increased RANKL levels associated with osteoclastogenesis suggests a tendency towards osteoporosis in MS patients. However, no significant change in BMD levels of the subjects shows that the effect of RANKL is compensated by increased OPG levels. OPG and RANKL levels are involved in the pathogenesis and regulation of bone turnover and thus, circulating levels of them may be useful markers to assess bone turnover and to develop new approaches in MS.

Conflict of Interest: The authors declare no conflict of interest.

Key words: Bone mineral density, Multiple sclerosis, Osteoporosis, Osteoprotegerin, Receptor activator of NF-kappaB ligand

ÖZET

Amaç: Bu çalışmanın amacı multiple skleroz (MS) hastalarında osteoprotegerin (OPG) ve nükleer faktör kappaB ligand reseptör aktivatörü (RANKL) seviyelerini ve onların kemik mineral dansiteleri (KMD) ve serum osteokalsin, parathormon (PTH), kalsiyum, kemik alkalen fosfatazı (kALP) ve idrar deoksipiridinolin (DPD) gibi diğer kemik turnover parametrelerinin seviyeleri ile ilişkisini araştırmaktır.

Yöntemler: Çalışmaya 46 MS hastası (30K, 16E, 33,48 ± 9,57 yaş) ve 24 sağlıklı kontrol (14K, 10E, 33,04 ± 7,97 yaş) dahil edildi. Tüm vakaların serum OPG, RANKL, kALP, osteokalsin, PTH, kalsiyum, idrar DPD seviyeleri ile 29 hastanın ve tüm kontrol vakalarının KMD ölçüldü. Osteoprotegerin ve RANKL seviyeleri ELISA metodu, kALP, osteokalsin ve PTH seviyeleri kemilüminesans metod ve idrar DPD seviyeleri HPLC tekniği kullanılarak ölçüldü. Kemik mineral dansitesi dual-X-ray absorptiometri ile ölçüldü.

Bulgular: MS hastalarının serum OPG (p<0.01), RANKL (p<0.01), kALP (p<0.05), PTH (p<0.01) ve kalsiyum (p<0.05) seviyeleri sağlıklı kontrollerden anlamlı olarak yüksek bulundu. Grupların serum osteokalsin, idrar DPD seviyeleri ve KMD ölçümleri arasında önemli farklılık yoktu.

Sonuçlar: Çalışma sonucunda osteoklastogenesis ile ilişkili RANKL seviyelerinin MS hastalarında artmış bulunması, osteoporoz eğilim olduğunu düşündürmektedir. Fakat olguların KMD değerlerinde değişiklik olmaması RANKL'nin etkilerinin OPG seviyelerinde artma ile kompanse edildiğini göstermektedir. Sonuç olarak, kemik turnover patogenezinde ve regülasyonunda rol oynayan OPG ve RANKL'nin dolaşımdaki seviyeleri MS'de kemik turnover değerlendirmede faydalı bir marker olabilir ve tedavide yeni yaklaşımlar geliştirilmesine katkı sağlayabilir.

Çıkar Çatışması: Yazarların mevcut olan konuyla ilgili herhangi bir çıkar çatışması bulunmamaktadır.

Anahtar Kelimeler: Kemik mineral dansitesi, Multiple skleroz, Osteoporoz, Osteoprotegerin, NF-kappaB ligand reseptör aktivatörü

Introduction

Multiple sclerosis (MS) is a progressive and inflammatory autoimmune disorder of the central nervous system that causes early disability in young adults [1]. Multiple sclerosis patients are at greater risk of osteoporosis and pathological fractures, the underlying mechanism of which is uncertain [2-7]. However, it has been reported that acute and long-term glucocorticoid use, early decrease of physical activity, motor disturbances caused by progressive pyramidal deficit and cerebellar dysfunction accompanied by ataxia with frequent falls, possible skeletal muscle atrophy and the activation of immunoregulatory mechanisms modulating cytokine pathways are likely to be determinants [3-5]. Also, MS patients have a high risk of low bone mineral density (BMD). Reduced BMD in MS patients is associated with high level of Expanded Disability Status Scale (EDSS) score, vitamin D deficiency and disease duration [2-7].

Bones are constantly remodelled through the synthesis of bone matrix by osteoblasts and the resorption of bone by osteoclasts. Thus, bone remodeling depends on a delicate balance between bone formation and bone resorption. Perturbations in inflammatory cytokines and hormones such as interleukin (IL)-1, IL-4, IL-6, transforming growth factor (TGF)- α , TGF- β , tumor necrosis factor (TNF)- α , TNF- β and colony stimulating factor (CSF)-1, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ and glucocorticoids cause an imbalance between osteoclast and osteoblast activities and can result in skeletal abnormalities such as osteoporosis and osteopetrosis [8-11].

The osteoprotegerin (OPG)/receptor activator of nuclear factor kappaB (RANK)/RANK ligand (RANKL) system, solved a long standing unresolved question in bone biology, namely the precise mechanisms by which preosteoblastic/stromal cells controlled osteoclast development. The identification of the OPG/RANK/RANKL system as the dominant, final mediator of osteoclastogenesis represents a major advance in bone biology. Osteoprotegerin, a soluble protein member of the TNF receptor family, and its ligand, RANKL, are recently identified cytokines that regulate osteoclastogenesis [9, 11-14]. They are essential paracrine mediators of bone metabolism and immune functions and have been clearly implicated in various skeletal and immune disorders and diseases at the interface between bone metabolism and the immune system [9, 10, 12]. Ligand of RANKL to its cognate receptor RANK, which is expressed by osteoclasts and their precursors, promotes osteoclast formation, fusion, differentiation, activation and survival, leading to enhanced bone resorption and bone loss. Osteoprotegerin binding to RANKL inhibits the osteoclastogenic interaction between RANKL and RANK and prevents its function. The secretion of OPG and RANKL from osteoblasts and stromal cells is regulated by numerous hormones, peptides and cytokines [9,

11-14]. Although OPG mRNA is not restricted to bone and is expressed in a number of tissues, RANKL is produced by osteoblastic lineage cells [9, 11-14].

The balance between OPG/RANK/RANKL signaling and the levels of biologically active OPG and RANKL regulate development and activation of osteoclasts and bone metabolism. Consequently, excess RANKL increases bone resorption, whereas excess OPG inhibits bone turnover by inhibiting osteoclast maturation and osteoclast activation [11-15]. Abnormalities of the OPG/RANK/RANKL system have been reported in various immune-mediated human diseases, such as rheumatoid arthritis [16], osteoarthritis [16], coronary artery disease [17], peripheral vascular disease [17], cancers which damage the human skeleton [18] and MS disease [19]. Further understanding of this system may lead to new therapeutic approaches in treating cancers that develop in the skeleton and postmenopausal osteoporosis [18].

Bone alkaline phosphatase (bALP) and osteocalcin are secreted by osteoblasts and thought to play a role in bone formation. Calcium (Ca) plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life. Long-term Ca deficiency can lead to osteoporosis, in which the bone deteriorates and there is an increased risk of fractures. While a lifelong deficit can affect bone and tooth formation, over-retention can cause hypercalcemia. Urinary deoxypyridinoline (DPD) is a degradation product of type I collagen and is a bone degradation marker [20].

Emerging data suggest a significant increase in the prevalence of osteoporosis and high risk of low BMD in MS patients compared to age-matched controls [3-7], but the molecular mechanism of osteoporosis in MS patients is not known and it may be due to increased osteoclast activity. Since the OPG/RANK/RANKL system appears to be the most relevant therapeutic target for all bone diseases, we aimed to investigate the serum OPG and RANKL levels and their correlation with BMD and levels of other bone turnover parameters such as serum osteocalcin, PTH, Ca, bALP and urinary DPD in patients with MS.

Materials and Methods

Patients

Forty six MS patients (30F, 16M) aged 20 to 53 years (mean 33.48 \pm 9.57 years) and 24 healthy controls (14F, 10M) aged 21 to 52 years (mean 33.04 \pm 7.97 years) were enrolled in the study. The patients were diagnosed as relapsing-remitting MS according to McDonald's criteria [21] and followed up by the Department of Neurology, Meram Medical Faculty, Selcuk University, Konya, Turkey. The functional status of MS patients was evaluated by using the EDSS [22], performed by a single neurologist, who was blind to the OPG and RANKL levels and BMD status. None of the patients had formerly diagnosed with any bone disease or disease that can affect

bone metabolism other than osteoporosis, neither were on any drug therapy affecting bone metabolism. Any MS patients who have a history of treatment for osteoporosis were also excluded from the study. Subjects with inflammatory diseases, any metabolic bone diseases or who were on treatment with any of the drugs that may affect bone metabolism were not included as controls.

The study protocol was approved by the Ethics Committee of Meram Medical School, University of Selcuk, Konya, Turkey (Date: 27 February 2007, number: 280-1255). All subjects were informed with the details of the study and the written consent of each subject was received.

Measurement of Biochemical Parameters

Blood samples were collected from each participant after an overnight (10 to 12h) fasting and sera were separated. Serum intact PTH, bALP, osteocalcin and Ca levels were studied immediately and serum samples were stored at -80°C until the day of RANKL and OPG analysis. Serum bALP (Ostase, Access, Beckman Coulter, Fullerton, CA, USA), osteocalcin and intact PTH (Immulite Diagnostic Products Co., Los Angeles, CA, USA) levels were measured by commercially available chemiluminescent immunoassays. Reference ranges of PTH, bALP, osteocalcin and Ca were 11-67 pg/mL, 5.8-17.90 $\mu\text{g/L}$, 2-21 ng/mL and 8.6-10.3 mg/dL, respectively. Serum Ca and urinary creatinine levels were measured by routine methods (Beckman Coulter, Fullerton, CA, U.S.A.). Urinary DPD levels (adjusted for creatinine excretion, normal range, 6-26 pmol/ μmol creatinine) in first morning urine specimens were analyzed using Chromsystems reagent kit for high-performance liquid chromatography (HPLC) technique on an Agilent 1100 series fluorescence detector on the same day.

The intra- and inter-assay coefficient of variations (CV) (%) of the parameters were as follows: PTH 4.7% and 6.8%, bALP 2.6% and 4.29%, osteocalcin 2.52% and 2.65%, Ca 1.33% and 1.64% and DPD 5.4% and 8.2%, respectively.

Serum total RANKL (sRANKL, free and unbound serum RANKL) and OPG levels were measured using commercially available kits based on sandwich enzyme-linked immunoassay (ELISA) method (Biovendor, Candler, NC 28715, USA). Reference range of OPG has been given as 1.8-6.4 pmol/L in the kit catalogue; whereas, it has not been mentioned for RANKL. The intra- and inter-assay CV (%) were 3.5 and 5.8 for OPG and 8.7 and 9.19 for RANKL, respectively. Limit of detection for RANKL was 0.2 pmol/L and that of OPG was 0.13 pmol/L.

Measurement of BMD

We have used BMD values of 29 MS patients which have been measured as a routine parameter. The BMD measurements of the other patients were not available. Also, BMD values of all control subjects were measured.

Bone mineral density measurements for the lumbar spine and femur were obtained by dual energy X-ray absorptiometry (DXA) using Hologic QDR 4500C. The CV (%) for the neck of femur and lumbar spine were 1.23 and 1.07%, respectively.

The z and t score calculations were done within the software used for DXA scans and obtained from the clinical reports. Patients were classified as normal with a BMD t score greater than -1.0 , as osteoporotic with a BMD t score less than or equal to -1.0 , but greater than -2.5 defines osteopenia, whereas t score less than or equal to -2.5 defines osteoporosis.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Version 16.0). The descriptive variables were defined as mean value \pm standard deviation (SD). Statistical differences between the groups were evaluated using a χ^2 test, independent T-test and Mann Whitney U-test. The correlations between variables were performed by Pearson's Correlation test for nominal data and Spearman Correlation analysis was performed for the relationship between ordinal data. Differences were considered significant at a probability level of $p < 0.05$.

Results

Demographic characteristics of the patients and the controls are shown in Table 1. There were no significant differences between the groups in terms of age, gender and body mass index (BMI). BMI is a parameter which is known to effect bone turnover [23].

The biochemical findings are presented in Table 2. Serum OPG ($p < 0.01$), RANKL ($p < 0.01$), bALP ($p < 0.05$), PTH ($p < 0.01$) and Ca ($p < 0.01$) levels of the patients were significantly higher than those of the controls (Figure 1). There were no significant differences between osteocalcin and DPD levels of the groups. Also, the lumbar spine (L1-L4) and femur neck BMD, t and z scores of MS patients were slightly but not significantly lower than those of the controls (Table 3).

There was a statistically significant positive correlation between EDSS and disease duration ($r = 0.394$, $p < 0.01$) and between EDSS and attack number ($r = 0.418$, $p < 0.01$) and a statistically significant negative correlation between EDSS and bALP levels ($r = -0.346$, $p < 0.05$) in the MS patients.

There was a statistically significant positive correlation between RANKL and PTH levels ($r = 0.357$, $p < 0.05$) and a statistically significant negative correlation between RANKL levels and neck BMD ($r = -0.412$, $p < 0.05$). A statistically significant positive correlation between DPD levels and disease duration was found ($r = 0.298$, $p < 0.05$). There were no correlations between any of the biochemical markers and BMD in the controls.

Table 1. Demographic characteristics of all patients and controls

Variables	MS patients (n=46)	Controls (n=24)	P value
Age (years)	33.48 ± 9.57 (ranges from 20 to 53)	33.04 ± 7.97 (ranges from 21 to 52)	0.850
Gender (M/F)	16/30	10/14	0.572
BMI (kg/m ²)	25.70 ± 4.22	25.04 ± 3.95	0.538
Age at onset (years)	28.30 ± 8.08 (ranges from 15 to 46)	–	–
Disease duration (years)	5.27 ± 4.07 (ranges from 1 to 16)	–	–
EDSS score	2.54 ± 1.49 (ranges from 0.5 to 6.0)	–	–

Data are presented as mean values ± SD (except gender). MS; multiple sclerosis, BMI; body mass index, EDSS; Expanded Disability Status Scale, SD; standard deviation

Table 2. Biochemical findings of the patients and the controls

Variables	MS patients (n=46)	Controls (n=24)	P value
RANKL (pmol/L)	673.75 ± 221.07	515.33 ± 221.10	0.007
OPG (pmol/L)	2.70 ± 1.09	2.04 ± 0.70	0.003
RANKL/OPG	283.64 ± 138.05	287.88 ± 172.73	0.889
bALP (µg/L)	11.98 ± 4.42	9.84 ± 3.05	0.022
Osteocalcin (ng/mL)	10.63 ± 8.00	8.08 ± 5.04	0.241
Intact PTH (pg/mL)	63.42 ± 37.70	36.51 ± 25.93	0.002
DPD (pmol/µmol urinary Creatinine)	11.62 ± 4.57	10.14 ± 3.49	0.186
Ca (mg/dL)	9.64 ± 0.39	9.39 ± 0.34	0.011

Data are presented as mean values ± SD. MS; multiple sclerosis, RANKL; receptor activator of NF-kappaB ligand, OPG; osteoprotegerin, bALP; bone alkaline phosphatase, PTH; parathyroid hormone, Ca; calcium, SD; standard deviation

Table 3. The BMD measures of 29 patients and controls

Variables	MS patients (n=29)	Controls (n=24)	P value
Age (years)	32.70 ± 8.12 (ranges from 22 to 50)	33.04 ± 7.97 (ranges from 21 to 52)	0.887
Gender (M/F)	11/18	10/14	0.539
BMI (kg/m ²)	25.96 ± 4.78	25.04 ± 3.95	0.465
Age at onset (years)	27.63 ± 7.97	–	–
Disease duration	5.30 ± 4.00	–	–
EDSS levels	2.41 ± 1.55	–	–
L1–L4 (BMD g/m ²)	0.96 ± 0.10	0.98 ± 0.14	0.717
L1–L4 (t score)	-0.88 ± 1.02	-0.82 ± 1.27	0.674
L1–L4 (z score)	-0.63 ± 1.12	-0.69 ± 1.25	0.941
Neck of femur (BMD g/m ²)	0.86 ± 0.12	0.92 ± 0.13	0.132
Neck of femur (t score)	-0.58 ± 1.12	-0.23 ± 1.15	0.177
Neck of femur (z score)	-0.96 ± 1.12	0.28 ± 1.21	0.265

Data are presented as mean values ± SD (except gender). BMD; bone mineral density, MS; multiple sclerosis, BMI; body mass index, EDSS; Expanded Disability Status Scale, L1–L4; lumbar spine, SD; standard deviation

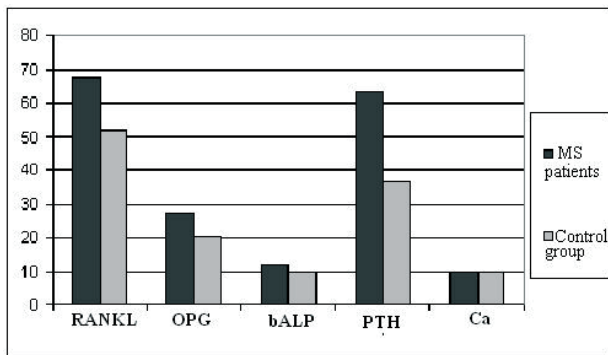


Figure 1. RANKL (pmol/L), OPG (pmol/L), bALP (µg/L), PTH (pg/ml) and Ca (mg/dl) levels of MS patients and the controls (RANKL levels were divided by 10 and OPG levels were multiplied by 10). MS; multiple sclerosis, RANKL; receptor activator of NF-kappaB ligand, OPG; osteoprotegerin, bALP; bone alkaline phosphatase, PTH; parathyroid hormone, Ca; calcium

Discussion

Elevated levels of RANKL and OPG in MS patients found in this study are consistent with the findings of our previous study [19]. This finding suggests that osteoclastogenic effect of RANKL was compensated by elevated level of OPG in these patients. To the best of our knowledge, correlation between OPG and RANKL levels and BMD in MS patients was not investigated previously.

Receptor activator of nuclear factor kappaB ligand is synthesized at highest levels in bone and bone marrow, as well as in lymphoid tissues [9, 11, 12]. Pro-inflammatory cytokines such as TNF- α , IL-1, IL-6 or IL-11 and PTH induce this synthesis [9, 11-14]. Elevated RANKL expression by T cells, as well as by osteoblasts play a role in bone loss in inflammatory diseases [13, 16-18]. Indeed, many reports have suggested that the RANKL/OPG ratio is a major determinant of bone mass [11, 13]. Therefore, it can be argued that significantly increased serum RANKL levels of MS patients induce osteoclastogenesis which can result in bone loss. However, lumbar spine and femur neck BMD of our patients were not changed in spite of increased levels of RANKL, a finding which shows the compensatory effect of OPG. It has been reported that osteoclastogenesis is under negative regulation of OPG, which functions as a decoy receptor for RANKL [9, 11-14]. By this way, OPG attenuates excessive RANKL signaling. Thus, OPG is regarded as a safeguard against excessive RANKL effects. This finding is in accordance with the findings of other investigators [15]. The seeming paradox of increased serum levels of OPG in patients with active osteoporosis has been interpreted as an incomplete regulatory mechanism to counteract disease progression [12]. Since pro-inflammatory cytokines are known to up-regulate OPG mRNA levels [9, 11-14], another possible mechanism of increased OPG levels can be explained by elevated serum level of pro-inflammatory cytokines in MS patients.

In addition, enhanced OPG level may be due to the binding of OPG on RANKL, which subsequently reducing OPG clearance [24].

Osteoprotegerin has been reported to block osteoclastogenesis stimulated by many hormones and cytokines, including PTH [14]. It has been shown that OPG blocks PTH induced osteoclastogenesis [25] and also prevents PTH induced bone resorption in organ cultures [26]. Thus, it was suggested that most or all factors which stimulate osteoclastogenesis do so through a pathway which can ultimately be blocked by OPG [25]. Furthermore, OPG has been suggested to increase bone mass and density independently from reduced osteoclastogenesis which is consistent with the possibility that OPG may directly affect bone metabolism beyond its known role as decoy receptor for RANKL [27].

Parathyroid hormone levels of our patients were higher than that of the controls but there was no significant difference between BMD values of the groups. We believe that this is also due to increased levels of OPG in MS patients. Some studies have shown that after PTH injection, RANKL expression is increased by osteoblasts [28], leading to activation of existing osteoclasts and release by them of a factor(s) that stimulates new bone formation [29].

Serum bALP levels of our patients were significantly increased; whereas, serum osteocalcin and urinary DPD levels were not significantly changed compared to those of controls. Since osteoclastic bone resorption normally initiates the bone turnover cycle, which is followed by osteoblastic bone formation [29, 30], increased serum bALP levels in our patients suggest deregulated bone turnover. In addition, the levels of bone formation and degradation markers are increased in disease states associated with bone loss [31]. Therefore, when the activation frequency is increased, the bone formation rate also increases. As a result, these provide an explanation for the increase in bALP levels in MS patients. Also, Grundt *et al.* [27] showed that OPG directly increased ALP activity in human osteoblastic cells. Thus, increased levels of OPG in our patients may result in increased activity. Although both bALP and osteocalcin are bone formation markers, they reflect different steps in the differentiation process of the osteoblastic process [32]. It has been reported that osteocalcin is less consistently raised in active disease compared with other formation markers such as ALP [33]. Also, a longer half-life of bALP in the circulation was thought to explain the discrepancy between markers [34].

Serum Ca levels of both groups were within reference ranges. However, Ca levels of our patients were significantly higher than that of control group, a finding which might be due to a compensatory mechanism against osteoporosis.

Although both OPG and RANKL levels were increased in our patients, RANKL/OPG ratio was not significantly

different from control group. RANKL/OPG ratio was reported to be decisive for clinical interpretation of bone mass findings [11, 13]. Thus, unchanged RANKL/OPG ratio of our patients provides another explanation for the lack of difference between BMD of the patients and the controls.

As it has been stated above, we have found no significant change in BMD levels of our patients. This could partly be explained by that all patients enrolled in our study were in relapsing remitting and all of them in remission period. Also, none of them were experiencing progression or developed new symptoms within six months prior to the onset of the study. Weinstock-Guttman et al. [4] have found that the lumbar and femur BMD were not statistically different between the relapsing remitting, secondary progressive and primary progressive groups, but there was a trend towards lower scores in the secondary progressive and the primary progressive groups compared to the relapsing remitting MS, a finding which supports our results.

In addition, the disease duration itself was reported to be an independent factor to affect BMD in MS patients [6]. Disease duration was about 5.3 years in our patients, a time span which is quite shorter compared to that of other studies [4, 5, 7].

Expanded Disability Status Scale was reported to be an important factor associated with low BMD [4, 5, 7]. It has been shown that mean EDSS score (6.79 ± 1.6) of the osteoporotic group was significantly higher than the EDSS score of the group with normal BMD scores (4.49 ± 2.4) and the group of osteopenic patients (5.79 ± 1.6) [4]. The mean EDSS score of our patients with BMD measures was 2.41 ± 1.55 . This value is lower than the findings of the above investigators. Therefore, we can suggest that the lower EDSS score found in our patients did not result in a significant change in BMD levels [4, 7].

We have found a statistically significant negative correlation between RANKL levels and neck BMD ($r = -0.412$, $p < 0.05$). Except this, there were no correlations between any of the biochemical markers we have measured and BMD in MS patients and in controls. Tuzun et al. [6] have found that there was no significant correlation between BMD and osteocalcin, PTH and urinary DPD levels. These findings are in agreement with our findings.

On the other hand, Stejskal et al. [35] have demonstrated that OPG concentrations were varied inversely with bone density values (correlation coefficient -0.31), a finding which is not in agreement with ours. The underlying mechanism of this inconsistency is not known.

In conclusion, our findings show that increased levels of RANKL, which is associated with osteoclastogenesis, is compensated by increased levels of OPG, a mechanism which seems to prevent a change in BMD of MS patients. Thus, evaluating OPG and RANKL levels in

these patients may be used as specific clinical tests and may be helpful in defining the appropriate therapeutic intervention. Also, further studies involving the primary progressive and secondary progressive MS patients may provide additional information regarding the biochemical mechanism of MS disease.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Meram Medical School, University of Selcuk, Konya, Turkey (Date: 27 February 2007, number: 280-1255). All subjects were informed of the details of the study and the written consent of each subject was received.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Rodriguez M, Siva A, Ward J, Stolp-Smith K, O'Brien P, et al. (1994) Impairment, disability, and handicap in multiple sclerosis: a population-based study in Olmsted County, Minnesota. *Neurology* 44:28-33.
- [2] Cosman F, Nieves J, Komar L, Ferrer G, Herbert J, et al. (1998) Fracture history and bone loss in patients with MS. *Neurology* 51:1161-5.
- [3] Formica CA, Cosman F, Nieves J, Herbert J, Lindsay R. (1997) Reduced bone mass and fat-free mass in women with multiple sclerosis: effects of ambulatory status and glucocorticoid use. *Calcif Tissue Int* 61:129-33.
- [4] Weinstock-Guttman B, Gallagher E, Baier M, Green L, Feichter J, et al. (2004) Risk of bone loss in men with multiple sclerosis. *Mult Scler* 10:170-5.
- [5] Khachanova NV, Demina TL, Smirnov AV, Gusev EI. (2006) Risk factors of osteoporosis in women with multiple sclerosis. *Zh Nevrol Psikhiatr Im S S Korsakova* 3:56-63.
- [6] Tuzun S, Altintas A, Karacan I, Tangurek S, Saip S, et al. (2003) Bone status in multiple sclerosis: beyond corticosteroids. *Mult Scler* 9:600-4.
- [7] Ozgocmen S, Bulut S, Ilhan N, Gulkesen A, Ardicoglu O, et al. (2005) Vitamin D deficiency and reduced bone mineral density in multiple sclerosis: effect of ambulatory status and functional capacity. *J Bone Miner Metab* 23:309-13.
- [8] Boyle WJ, Simonet WS, Lacey DL. (2003) Osteoclast differentiation and activation. *Nature* 423:337-42.
- [9] Hofbauer LC. (1999) Osteoprotegerin ligand and osteoprotegerin: novel implications for osteoclast biology and bone metabolism. *Eur J Endocrinol* 141:195-210.
- [10] Nakashima T, Takayanagi H. (2009) Osteoclasts and the immune system. *J Bone Miner Metab* 27:519-29.
- [11] Khosla S. (2001) Minireview: the OPG/RANKL/RANK system. *Endocrinology* 142:5050-5.

- [12] Schoppet M, Preissner KT, Hofbauer LC. (2002) RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. *Arterioscler Thromb Vasc Biol* 22:549-53.
- [13] Hofbauer LC, Schoppet M. (2004) Clinical implications of the osteoprotegerin/RANKL/ RANK system for bone and vascular diseases. *JAMA* 292:490-5.
- [14] Kostenuik PJ, Shalhoub V. (2001) Osteoprotegerin: a physiological and pharmacological inhibitor of bone resorption. *Curr Pharm Des* 7:613-35.
- [15] Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, *et al.* (1999) Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. *J Bone Miner Res* 14:518-27.
- [16] Skoumal M, Kolarz G, Haberhauer G, Woloszczuk W, Hawa G, *et al.* (2005) Osteoprotegerin and the receptor activator of NF-kappa B ligand in the serum and synovial fluid. A comparison of patients with longstanding rheumatoid arthritis and osteoarthritis. *Rheumatol Int* 26:63-9.
- [17] Semb AG, Ueland T, Aukrust P, Wareham NJ, Luben R, *et al.* (2009) Osteoprotegerin and soluble receptor activator of nuclear factor-kappaB ligand and risk for coronary events: a nested case-control approach in the prospective EPIC-Norfolk population study 1993-2003. *Arterioscler Thromb Vasc Biol* 29:975-80.
- [18] Buckle CH, Neville-Webbe HL, Croucher PI, Lawson MA. (2010) Targeting RANK/RANKL in the treatment of solid tumours and myeloma. *Curr Pharm Des* 16:1272-83.
- [19] Kurban S, Akpınar Z, Mehmetoglu I. (2008) Receptor activator of nuclear factor kappaB ligand (RANKL) and osteoprotegerin levels in multiple sclerosis. *Mult Scler* 14:431-2.
- [20] Endres DB, Rude RK. (2006) Mineral and Bone Metabolism. In: *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed. (Ed: Burtis CA, Ashwood ER, Burns DE) pg. 1891-943, St. Louis, MO: Elsevier Saunders.
- [21] Polman CH, Wolinsky JS, Reingold SC. (2005) Multiple sclerosis diagnostic criteria: three years later. *Mult Scler* 11:5-12.
- [22] Kurtzke JF. (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 33:1444-52.
- [23] Holecki M, Wiecek A. (2010) Relationship between body fat mass and bone metabolism. *Pol Arch Med Wewn* 120:361-7.
- [24] Browner WS, Lui LY, Cummings SR. (2001) Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *J Clin Endocrinol Metab* 86:631-7.
- [25] Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, *et al.* (1997) Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochem Biophys Res Commun* 234:137-42.
- [26] Tsukii K, Shima N, Mochizuki S, Yamaguchi K, Kinosaki M, *et al.* (1998) Osteoclast differentiation factor mediates an essential signal for bone resorption induced by 1 alpha,25-dihydroxyvitamin D₃, prostaglandin E₂, or parathyroid hormone in the microenvironment of bone. *Biochem Biophys Res Commun* 246:337-41.
- [27] Grundt A, Grafe IA, Liegibel U, Sommer U, Nawroth P, *et al.* (2009) Direct effects of osteoprotegerin on human bone cell metabolism. *Biochem Biophys Res Commun* 389:550-5.
- [28] Dai JC, He P, Chen X, Greenfield EM. (2006) TNF α and PTH utilize distinct mechanisms to induce IL-6 and RANKL expression with markedly different kinetics. *Bone* 38:509-20.
- [29] Li X, Qin L, Bergenstock M, Bevelock LM, Novack DV, *et al.* (2007) Parathyroid hormone stimulates osteoblastic expression of MCP-1 to recruit and increase the fusion of pre/osteoclasts. *J Biol Chem* 282:33098-106.
- [30] Eriksen EF. (1986) Normal and pathological remodeling of human trabecular bone: three dimensional reconstruction of the remodeling sequence in normals and in metabolic bone disease. *Endocr Rev* 7:379-408.
- [31] Weinstock-Guttman B, Hong J, Santos R, Tamano-Blanco M, Badgett D, *et al.* (2006) Interferon-beta modulates bone-associated cytokines and osteoclast precursor activity in multiple sclerosis patients. *Mult Scler* 12:541-50.
- [32] Aubin JE. (2001) Regulation of osteoblast formation and function. *Rev Endocr Metab Disord* 2:81-94.
- [33] Shankar S, Hosking DJ. (2006) Biochemical assessment of Paget's disease of bone. *J Bone Miner Res* 21 Suppl 2:P22-7.
- [34] Walton RJ, Preston CJ, Russell RG, Kanis JA. (1975) An estimate of the turnover rate of bone-derived plasma alkaline phosphatase in Paget's disease. *Clin Chim Acta* 63:227-9.[35] Stejskal D, Zurek M, Bartek J, Jedelsky L, Ruzicka V. (2001) Osteoprotegerin and bone density. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 145:75-6.