COMPARISON OF THE BONE TURN-OVER MARKERS IN PATIENTS WITH MULTIPLE SCLEROSIS AND HEALTHY CONTROL SUBJECTS

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One of the major concerns for patients with multiple sclerosis (MS) is developing osteoporosis, especially when corticosteroid treatment is used. The aim of the present study is to compare the bone turn-over markers in patients with multiple sclerosis and healthy control subjects. A total of 176 subjects were enrolled in this case-control. Ninety-one MS patients with mean age of 35.26 ± 8.76 yrs were randomly selected from the Committee on Multiple Sclerosis Registry. The control group was composed of 85 healthy subjects who were recruited from the Iranian Multicenter Osteoporosis Study (IMOS). Fasting serum levels of parathyroid hormone (PTH), 25 (OH) D3, osteocalcin and cross laps were measured in two groups. Hip and spine BMD were measured using DXA. Our findings showed significant differences in hip BMD and its T-score and Z-score values between MS patients and the control group. Osteoporosis prevalence at hip area of the MS patients was almost 5 times higher than the control group [OR=4.66, (95% CI 0.97 to 22.27), RR=4.29, (95% CI 0.95 to19.32), p value=0.03]. No significant difference was found in BMD L2-L4, BMD T-score and BMD Z-score of lumbar area between two groups. The PTH and cross laps serum concentrations in MS patients were significantly higher than the control group. We did not find significant difference in serum osteocalcin level between the two groups. We concluded that in our study the serum levels of bone resorbtion markers in MS patients were significantly higher than the healthy control group. This may explain, at least in part, the elevated susceptibility of MS patients for developing osteoporosis.

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease that affects the central nervous system (CNS) by perivascular T cell and macrophage infiltration leading to demyelination (1). Multiple sclerosis is the most common demyelinating disorder of the CNS, affecting 2.5 million people worldwide (2). Prevalence of MS is varied from 20 to 150 cases per 100,000 people, depending on the population type and the geographic region. This disease usually is diagnosed among people between 20 and 50 years of age (3).

Bone metabolism disorder is one of the main complications in MS patients. Although the primary pathophysiology of the bone disease is hesitant, besides immobilization and vitamin D deficiency, corticoid therapy might be one of the main contributing causes of bone disorder to predisposition toward osteoporosis and other bone problems in MS patients (4). Bone mineral density (BMD) is a factor that establishes bone strength

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(5). This factor has been widely used as an estimate of the latter through measurement by dual-energy X-ray absorptiometry (DXA) (6). Although, many previous studies have reported low BMD in MS and its relation with corticosteroid treatment, but ongoing evidence has demonstrated beneficial effects of some treatment methods (7), however the risk of developing osteoporosis in MS patients treated with these methods has not yet been clarified (8).

An early prediction of skeletal dynamics with measurement of biochemical markers of bone metabolism is useful for complementing static measurements of BMD (9). Two main bone markers are osteocalcin and cross laps (10). Circulating osteocalcin is a biological marker for osteoblastic function (11) and CrossLaps (C-terminal cross-linked telopeptide of type I collagen) is a biological marker for osteoclastic function (12).

We therefore designed a case-control study to evaluate and compare the BMD and bone turnover markers in patients with multiple sclerosis and a healthy control group.

MATERIALS AND METHODS

Patient Selection

MS patients were invited from the Committee on Multiple Sclerosis Registry to participate in the study. Patients aged 20-45 yrs with diagnosed MS of 1–10 years (according to McDonald's criteria (13), made by a neurologist) were recruited in the study Exclusion criteria were: history of bone affecting diseases, prolonged immobilization (>3 weeks) and/or treatment with any drug documented to influence bone metabolism in humans such as hormone replacement therapy, statins or anticonvulsivants in the previous 6 months, diagnosis of any bone disease apart from osteoporosis Any MS patients who had a history of treatment for osteoporosis were also excluded. Healthy subjects were enrolled from a social program of osteoporosis agenda. Approval was obtained from the Endocrinology and Metabolism Research Center of Medical Sciences University of Tehran Ethics Committee In the control group, informed consent was also obtained from all the participants. Volunteers with any metabolic bone diseases or who were on treatment with medication affecting bone metabolism were excluded from the control group.

Clinical status of the patients was collected, including duration of MS, number of relapses, history of corticosteroid therapy, type and duration of IMT[1], and level of disability according to EDSS.

BMD measurements

All the subjects had undergone BMD measurements by dual energy X-ray absorptiometery (DXA) of the lumbar spine (vertebrae L2–L4) and hip. The coefficient of variation for longitudinal BMD measurements in the DEXA machine averaged at 1.04%. Normal bone mass was defined as BMD measurements at or above -1 standard deviation (S.D.) from the optimal peak bone density (T-score) of healthy young adults of the same sex. BMD measurement at or below -2.5 S.D. from the optimal peak bone density of healthy young adults of the same sex was osteoporotic, as per World Health Organization standard definitions. The results are presented as absolute values in g/cm^2 and as standard deviation units (Z-score) based on comparison with age and sex-matched BMD.

Laboratory measurements

Blood samples were collected in the morning, after overnight fasting. The blood was centrifuged and serum was separated for measurement of fasting serum parathyroid hormone, 25 hydroxy vitamin D, and PTH, osteocalcin and cross laps.

Serum concentration of 25-hydroxy vitamin D3 was measured using by a Biosource kit (Biosource Europe S.A, Belgium); intra- and inter-assay coefficients of variation (CV) were 5.2% and 5.7%, respectively. Serum PTH was also detected using a Biosource kit (Biosource Europe S.A, normal range: 13-66 pg/ml), with an intra- and inter-assay CV of 6.3% and 5.7%, respectively.

Osteocalcin was measured by immunoassay (ELISA) using a Bioscience kit (Nordic Bioscience Diagnostic A/S, Denmark). The intra- and inter-assay CV were 2.6% and 4.7%, respectively. Serum Cross Laps was measured by immunoassay (ELISA) using a Bioscience kit (Nordic Bioscience Diagnostic A/S, Denmark), with intra- and inter-assay CV of 5.1% and 6.6%, respectively.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 15, Chicago, IL, USA). For comparison of the groups, Student *t*-test, the one-way analysis of variance (ANOVA) or non-parametric tests were used, depending on whether data were or were not normally distributed. The risk of developing osteoporosis and osteopenia was assessed calculating the odds ratio (OR) and relative risk (RR) and the 95% confidence interval (CI).

RESULTS

All the subjects (91 MS patients and 85 healthy subjects) participated in this case-control study. The MS patients group contained 64 women and 27 men.

Table I. *The comparison of demographic characteristics and bone mass density values between MS patients and controls.*

Characteristic	participants		P value**
	MS * (N=91)	Control group* (N=85)	
Age (years)	35.26±8.76	38.87±11.21	0.1
BMD (total hip, g/cm2)	0.91±0.13	0.95±0.12	0.04
Hip T-score	-0.8±1.1	-0.3±1.01	0.005
Hip Z-score	-0.67±1.02	-0.1±0.9	0.001
BMD (L2-L4, g/cm2)	1.09±0.17	1.11±0.15	0.7
L2-L4 T-score	-0.85±1.30	-0.81±1.4	0.9
L2-L4 Z-score	-0.7±1.23	-0.56±1.23	0.4

**Values are expressed as mean ±Standard Deviation*
***comparing of variables means in two groups performed by Student T test*

Table II. *Prevalence of osteoporosis and osteopenia at different bone regions of the MS and control group.*

percent / Region	Group	Osteoporosis (%)	Osteopenia (%)	Normal (%)
Hip	MS patients	10.1	38.2	51.7
	Control	2.4	27.1	70.6
Lumbar	MS patients	21.2	34.4	44.4
	Control	15.6	27.1	57.3

The mean±SD of age in this group was 35.26 ± 8.76 yrs. The number of intravenous methylprednisolone pulses received by participants was 1-15 courses with a mean±SD of 2.98±2.59. Fifty percent of patients take vitamin D- calcium supplement (400 IU, 1000 mg) daily.

Table I demonstrates the comparison of demographic characteristics and bone mineral density values between MS patients and controls. As shown in this Table, there were significant differences in hip BMD, T-score and Z-score values between MS patients and control group. Our results show no significant differences in BMD of spine (L2-L4), T-score and Z-score of the lumbar area between the two groups. We estimated the osteoporosis, osteopenia and normal status of bone in hip and lumbar region in all participants, as shown in Table II. Our findings demonstrate significant differences

Table III. *Bone remodeling markers in MS and control group.*

Characteristic	participants		P value
	MS * (N=91)	Control group* (N=85)	
Serum 25-hydroxyvitamin D3 (nmol/dl)	41.68±35.90	15.23±19.18	0.001
Serum PTH (pmol/L)	54.99±29.49	32.25±32.46	0.001
Ostocalcin (ng/mL)	15.23±6.05	15.34±9.34	0.9
Cross Laps (ng/mL)	0.79±0.33	0.63±0.34	0.004

**Values are expressed as mean ±SD*
***comparing of variables means in two groups performed by Student T test*

between BMD values in the hip area (p value=0.015) and non-significant differences in the lumbar area (p value=0.1) between MS patients and the control group. Osteoporosis prevalence at the hip area was estimated to be almost 5 times higher in MS patients compared to the control group [OR=4.66, (95% CI 0.97 to 22.27), RR=4.29, (95% CI 0.95 to19.32), p value=0.03].

The prevalence of lower hip Z-score (less than -1) in MS patients was 2 times higher than in healthy subjects [OR=2.43 (95% CI 1.23 to 4.72), RR=1.85 (95% CI 1.14 to 3.1)].

Table III shows the serum concentrations of bone remodeling markers of MS patients and control group. The serum PTH and cross laps concentrations in MS patients were significantly higher than in the healthy control group. However, the osteocalcin level was not significantly different between the two groups. Based on serum cross laps concentration as bone resorbtion marker, we estimated that bone resorbtion in MS patients was 25% higher than healthy control .

DISCUSSION

The results of this study demonstrate significant lower total hip BMD and its T-score and Z-score values in MS patients in comparison to control group. However, no significant differences between BMD L2-L4 or T-score and Z-score at the lumbar area among the two groups were observed.

Regarding the possible effects of prescribed medicine on bone status in MS patients, Shuhaibar et al. (14) evaluated the BMD at both spine and femur in MS patients who had been treated with immunomodulatory therapy (IMT), and 80% of subjects had received contemporaneously intermittent corticosteroid therapy for over 3 years. They found patients with MS, even in the presence of steroid therapy, had significantly greater than zero in Z-score at the spine and femur areas which might be explained by the beneficial effects of other prescribed medicine in these patients. This unexpected finding was similar to our findings in Z-score at the lumbar area.

The significant difference observed in BMD hip area between MS and the healthy group in our results may be at least partly be explained by the proposed mechanism by Schwid Zorzon et al. (15). Their study demonstrated that the change in bone density in patients may have been related to inactivity, stage of disease and ambulatory state. This finding supports the idea that disability and immobilization along with pathological bone loss seen in MS could contribute to the development of osteoporosis even more than the steroid. The controversial reports about BMD changes in MS patients (15-16) may be due to the different characteristics of the studied populations, such as severity of disability, age, gender and the patient's status. Therefore, based on our understanding of the above evidence of previous literature and our own preliminary results, it appears that the most important reason may be explained by

the conflicting BMD changing among MS patients such as duration of steroid therapy, medicine used by patients, length of disease diagnosis and stage of disease. Our observation regarding loss of BMD at the hip area may explain the higher prevalence of fracture among MS patients compared to healthy subjects (17) which is not even resolved with vitamin D supplementation.

Due to vitamin D3 supplementation by the majority of patients, its concentration was significantly higher in MS patients compared to the control group. The PTH concentration in MS patients was also significantly higher than the control group. Vitamin D is essential for bone health and immune regulation, and its concentration is lower in MS. The higher concentration of 25(OH) D3 may be explained by administration of high-dose glucocorticoids in multiple sclerosis patients as previously reported by Cosman et al. (18). It has been reported that the total body and spine BMD increases with D3 treatment in osteoporotic women. This could be one of the reasons which explain the equal BMD lumbar values in MS patients and control groups (19).

Existing evidence has shown the development of the secondary hyperparathyroidism in response to negative calcium balance which was produced by glucocorticoid therapy. This could result in augmentation of the cortical porosity and increased bone fragility (20). It may be one of the probable mechanisms in significant lower hip BMD in MS patients in compared to healthy subjects in this study. Bonadonna et al. (21) showed that glucocorticoid treatment induces redistribution in dynamic of spontaneous secretory PTH by reducing the tonic release and increasing the pulse release. This change in secretory rhythm might partially elucidate the higher PTH levels in MS patients.

Our results demonstrate that MS patients had significantly higher concentrations of cross laps compared to the control group, though there were no significant differences in osteocalcin levels between the two groups. Cross laps is a marker for osteoclastic function. The osteoclasts are the only bone-resorbing cells and it has been shown that proinflammatory cytokines could enhance the osteoclast recruitment. The osteoclasts are known to be central to the pathogenesis of inflammatory osteolysis (22). The significant difference in cross laps concentration between two groups is explicable according to the inflammatory pathogenesis of MS. In agreement with our study the results of Stepan et al. (4) evaluated the markers of bone remodeling [type 1 collagen cross-linked C-telopeptide, aminoterminal propeptide of type I procollagen, and N-MID osteocalcin] in patients treated with low doses of glucocorticoids, and showed that the bone-turnover markers were not different in the two groups. Their results also demonstrated that bone resorbtion and not formation marker was higher compared to controls 2 years later. A more recent animal model study by Yao et al. (23) showed that excess glucocorticoid is associated with early activation of the genes associated with osteoclastogenesis and late suppression of the genes associated with osteogenesis and mineralization. This was consistent with the Weinstein et al. report (24) which showed that glucocorticoids may act directly on murine osteoclasts to prolong their lifespan. Moreover, an *in vitro* study by Kim et al. (25) demonstrated that glucocorticoids could inhibit the proliferation of osteoclasts from bone marrow macrophages in a dose-dependent manner. In addition to innovative proposed mechanism, they confirmed that glucocorticoids could increase the bone resorption by stimulating osteoclastogenesis mediated by increasing the RANK ligand expression and decreasing the decoy receptor, osteoprotegerin expression (26).

We concluded that there was active bone resorbtion process and lower BMD at the hip area, and also increased susceptibility of bone fracture at the hip area in MS patients. It appears that bone metabolism disorders should be considered as an elevated hazard for concomitant MS complications which necessitate precise consideration in future. The evaluation of the effect of combination therapy as well the anti-resorbtive treatment in osteoporosis prevention might be helpful in MS patients.

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