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Osteoimmunological Insight in to Vertebral Fractures in Osteoporosis

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Abstract

Background: The aim of this study was to investigate the relationship among circulating levels of OPG, RANKL, cytokine profiles, bone mineral density (BMD) and vertebral fractures in pre and postmenopausal women and comparing these finding in three groups including osteoporotic patients with and without fracture and healthy women.

Methods: In a cross-sectional study, 215 women who attended the BMD unit of Endocrinology & Metabolism Research Center (EMRC) of Tehran University of medical sciences were recruited. Serum Osteoporotegerin and sRANKL were measured. In addition, cytokines profile evaluated. Lumbar radiographs in the antero-posterior and left lateral projections were acquired following a standardized protocol and bone mineral densitometry was performed.

Results: In X-ray study, 65.2% of postmenopausal women and 34.8% of pre menopausal women had at least one vertebral fracture (*P*= 0.04). Serum OPG and TNFα concentration significantly correlated with age (OPG: *P*= 0.001, r= 0.22, TNFα: *P*=0.04, r= 0.15). In logistic regression model, RANKL/OPG ratio independent of age and BMD was predicted vertebral fractures.

Conclusion: Osteoimmunological insight in to vertebral fracture indicated that important role of proinflammatory cytokines and RANKL/OPG pathway in bone remodeling**.**

Keywords: *Vertebral fractures, Osteoimmunology, Osteoprotegerin, RANKL, Bone turnover*

Introduction

Osteoporosis is defined as a metabolic bone disorder characterized by waning bone strength predisposing a person to an increased risk of fracture. Osteoporosis is the most common metabolic bone disorder in the developed countries. In this condition, bone mass reduces in association with advancing age; the lifetime risk for a fragility fracture in a 50 yr old white US woman is approximately 40%, whereas that in a white US man is 13% (1).

The term 'osteoimmunology' was first used for highlighting the intrgrated communication between the immune and skeletal systems (2). Investigations in this field have lead to the revelation of molecular mechanisms and various cytokines and signaling pathways contribute to the regulatory interactions between immune cells and bone cells.

Many of the cytokines produced by osteoblasts such as IL-1 (3, 4), tumor necrosis factor alpha and beta (TNFα, TNFß) are potent stimulators of bone resorption in vivo (5, 6) and in vitro (7) and known as important mediators of bone turnover.It has been reported that there is elevated production of interleukin1 (IL1) and TNF α by circulating mononuclear cells in women with postmenopausal osteoporosis (8, 9). The role of IL-1 was further supported by study that mice lacking the IL1 receptor are protected from bone loss resulting from ovariectomy (10). The role of TNF in the pathogenesis of estrogen deficiency-induced bone loss has also been reported (11).Also the production of a wide range of cytokines by human osteoblasts in vitro has been demonstrated (12, 13). Other study reported that the production of IL-6 by osteoblasts of mouse, rat and human in response to IL1 and

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TNFα is suppressed by 17b estradiol. It proposes a role for IL6 in augmentation of bone loss associated with osteoporosis in postmenopausal women (14). Other investigators have been unable to confirm the inhibitory effects of estrogen on IL-6 secretion (12).

The concept that stimulation of bone resorption requires an interaction between cells of the osteoblastic and osteoclastic lineages was proposed many years ago, but its molecular mechanism has been identified recently (15-17). Receptor activator of nuclear factor κB ligand (RANKL) is the main regulator of osteoclastogenesis. RANKL is a member of the TNF and TNF receptor superfamily and is a ligand for the receptor activator of NF-κB (RANK) on hematopoietic cells. It activates the differentiation of osteoclasts, maintains their function, and is an important molecule in augmentation of activation of osteoclasts. Osteoblasts also produce and secrete Osteoporotegerin (OPG), a decoy receptor that blocks RANKL/RANK interactions. Stimulators of bone resorption have been found to decrease OPG expression (15, 17).

Some study indicated that the surface expression of RANKL on marrow stromal cells, B cells and T cells was significantly higher in early postmenopausal when compared to premenopausal or estrogen-treated women (18). These findings suggest that estrogen deficiency may lead to upregulation of RANKL on stromal cells and lymphocytes in the bone marrow that mediate increased bone resorption. Whereas the study of Eghbali-Fatourechi et al. refers to the early, rapid phase of postmenopausal bone loss, there are data that indicate a role of the RANKL/ OPG pathway also in fracture susceptibility (19).

Abdallah et al demonstrated an increased RANKL/ OPG mRNA ratio in bone biopsies from women with hip fractures (20). In contrast to studies on surface expression or mRNA levels of RANKL and OPG, the measurement of these markers in serum has produced contradicting results. With regard to OPG, most studies found elevated OPG serum levels in patients with osteoporosis (21–24) whereas one study reported decreased OPG levels in osteoporotic patients with vertebral fractures (25). Liu et al. found no differences of serum OPG and RANKL levels as well as the RANKL/OPG ratio among normal, osteopenic and osteoporotic women (26). Nevertheless, Schett et al. showed that low levels of RANKL are a predictor of an increased risk of non traumatic fracture (27).

However, despite extensive cross regulation between bone metabolism and the immune system, the clinical aspect of osteoimmunology, especially in osteoporotic fractures are poorly understood.

The aim of this study was to investigate the relationship among circulating levels of OPG, RANKL, cytokine profiles, bone mineral density (BMD) and vertebral fractures in pre and postmenopausal women and comparing these finding in three groups including osteoporotic patients with and without fracture and healthy women.

Material and Methods

In a cross-sectional study, 215 women who attended the BMD unit of Endocrinology & Metabolism Research Center (EMRC) of Tehran University of Medical Sciences were recruited. The women were selected consecutively if they fulfilled the criteria and if they were willing to participate in the study. After interview, a general physical examination by a physician was conducted and informed consent was acquired. Blood samples were drawn and centrifuged for 30 minutes Samples were frozen at -80C in the Hormone Laboratory of EMRC. The study protocol was approved by the research ethics committee of EMRC.

Measurements

Serum concentration of Interleukin1beta (IL-1β) was measured by immunoassay (ELISA) using a R&D system kit (R&D system, USA); intra- and inter-assay coefficients of variation (CV) were 4.8% and 4.1%, respectively. Serum Interleukin 6(IL-6) was also detected using a R&D system kit (R&D system, USA), with intra- and

inter-assay CV of 2.4% and 4.7%, respectively. Serum TNFα was measured by immunoassay (ELISA) using a R&D system kit (R&D system, USA); intra- and inter-assay coefficients of variation (CV) were 4.8% and 6.1%, respectively. Serum Osteoprotegerin was measured by ELISA using a Immunodiagnostic kit. The intra- and inter-assay CV were 6.6% and 5.7%, respectively. Serum sRANKL was measured by immunoassay (ELISA) using a Biomedica kit, with intra- and inter-assay CV of 4.1% and 5.1%, respectively.

Questionnaire

The questionnaire administered at baseline contained questions on demographics, medical history, fracture history, gynecological information, physical activity, and lifestyle variables. To assess fracture history, participants were asked if they had ever suffered from a broken bone, and if so, to give details on which bone, age at first fracture, and level of trauma experienced. The fracture type choices given were vertebral, hip, rib, forearm, and other. Daily intake of dietary calcium and vitamin D was calculated from a food frequency questionnaire that was approved by the nutrition group of EMRC.

Spinal radiography

Radiograph images were taken by a professional X-ray technician using standard, proven safety precautions.

Lumbar radiographs in the antero-posterior and left lateral projections were acquired following a standardized protocol (28). For the lateral views, subjects were positioned in their left side with knees and hips flexed. Tube-to-film distance was set at 115 cm and films were centered at L3 for lumbar views.

The spinal radiographs were assessed independently by two expert observers (who were both medically qualified) for evidence of osteoporotic vertebral fracture.

BMD measurements

Using DPX Lunar, postero-anterior scans of the lumbar spine (from L1 to L4) and left hip were also acquired to measure BMD. Based on their bone mass, patients were classified as normal, osteopenic or osteoporotic; according to the WHO criteria (29). T-score of vertebral height was calculated.

Visual semiquantitative assessment (SQ)

Conventional radiographs were examined first for quality and then for fractures by an experienced radiologist. According to Genant et al. (30), reductions in the anterior, middle or posterior vertebral heights were classified as mild (20-25% reduction), moderate (25-40% reduction), or severe (> 40% reduction).

Statistical analysis

Data were analyzed by means of a personal computer implemented with dedicated software (SPSS 11.5), to obtain mean±SD values, correlation matrix, Student's *t*-test, analysis of variance and/or χ^2 tests, as appropriate. The level of significance was settled at < 5%, as usual.

Results

Totally 215 women (129 postmenopausal and 86 pre menopausal women) were recruited in the study. The characteristics of participants summarized in Table1. Postmenopausal women had higher serum OPG, IL1 and TNFα comparing to pre menopausal group. There were no significant differences in menarche age, body mass index, serum concentrations of RANKL and IL6 between pre and postmenopausal women. Serum OPG and TNFα concentration significantly correlated with age (OPG: P= 0.001, r= 0.22, TNFα: P= 0.04, r= 0.15).

In X-ray study 65.2% of postmenopausal women and 34.8% of pre menopausal women had at least one vertebral fracture (P= 0.04).

BMD of lumbar spine and total hip in postmenopausal women were lower than pre menopausal women (Table 1).

All participants based on osteoporosis status and vertebral fractures were classified in three groups that included healthy women, osteoporotic patients without fracture and osteoporotic patients with fracture (Table 2).

There were no significant differences in menarche age and BMI bvetween healthy women, osteoporotic patients without fracture and osteoporotic patients with fracture.

In osteoporotic patients with and without fracture, BMD of lumbar spine and total hip were significantly lower in comparison with healthy women (P= 0.001). In these two groups, serum concentration of cytokine profile were higher than healthy women. Also in osteoporotic patients with and without fracture, RANKL/OPG ratio was higher than healthy women (Table2). In logistic regression model, RANKL/OPG ratio independent of age and BMD was predicted vertebral fractures (P= 0.006).

Table 1: Characteristics data of study population

Characteristic	Pre menopausal	Postmenopausal	*P*
Age(years)	48.65 ±6.83	58.62 ±7.49	0.001
BMI(Kg/m^2)	27.37 ±5.1	27.80±5.23	0.6
Menarche age(years)	13.54 ±1.57	13.32±1.26	0.3
Hip BMD(gr/cm^2)	0.94±0.16	0.87 ±0.12	0.001
Spine BMD(gr/cm2)	1.09±0.18	0.98±0.15	0.001
Serum RANKL(pmol/L)	0.069 ± 0.12	0.107 ±0.19	0.07
Serum OPG(pmol/L)	5.49 ± 1.6	6.12 ± 2.1	0.02
Serum IL1 (ng/mL)	0.65± 0.84	1.02± 1.36	0.01
Serum IL6(ng/mL)	1.59 ± 2.02	2.11±3.08	0.2
Serum TNFα (ng/mL)	0.52± 0.97	1.17± 1.53	0.001

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

Table 2: Measurements data of study population with respect to osteoporosis and fractures

Characteristic	Healthy	Osteoporotic without fracture	Osteoporotic with fracture	*P* value ANOVA
Age(years)	54.2 ±8.04	56.08 ±8.84	58.9 ±9	0.002
BMI(Kg/m^2)	28.59 ±4.7	26.70±3.9	26.83±5.89	0.1
Menarche age(years)	13.4 ±1.48	13.57±1.44	13.34±1.21	0.7
Hip BMD(gr/cm^2)	0.99±0.13	0.8 ±0.08	0.83 ±0.11	0.001
Spine BMD(gr/cm2)	1.13±0.15	0.9±0.09	0.91±0.14	0.001
Serum RANKL (nmol/L)	0.052 ± 0.06	0.091 ±0.21	0.125 ±0.18	0.05
Serum OPG(pmol/L)	5.83 ± 2.1	6.17 ± 2	6.02 ± 2.1	0.7
Serum IL1 (ng/mL)	0.6± 0.95	0.74± 0.93	1.24± 1.49	0.003
Serum IL6(ng/mL)	1.29 ± 1.88	1.65±1.63	2.75±4.81	0.015
Serum TNFα (ng/mL)	0.49± 0.9	0.87±1.01	1.39± 1.83	0.001
RANKL/OPG Ratio	0.009 ± 0.01	0.015 ±0.035	0.025 ±0.046	0.04

Values are expressed as mean±SD
ANOVA, analysis of variance

Discussion

Osteoporosis is the most common metabolic bone disorder in the world. Recent studies indicated the high prevalence of osteoporosis and related fractures in some developing countries (31, 32). Osteoporosis is characterized by a reduced bone mass vulnerable it to fracture following minimal trauma.

The most common type of osteoporotic fractures is spine fractures (33). We also found high prevalence of vertebral fractures in postmenopausal women in this study. The prevalence of

vertebral fractures among similar age varies in other studies (32-35).

Regulation of osteoclastic activity is critical for understanding bone loss associated with the postmenopausal period. Estrogen deficiency has been proposed as a modulator of cytokine production, which plays critical role in postmenopausal osteoporosis (36). This study demonstrated that serum concentration of TNFα and IL1β in postmenopausal women were higher than in premenopausal women. Zheng and colleagues observed increased production of TNFα, IL-1β and IL-6 in postmenopausal women with osteoporosis (37). It has been suggested that there is elevated production of IL1 and TNFα by circulating mononuclear cells in women with postmenopausal osteoporosis (8, 9). The role of IL-1 was further supported by recent findings that mice undergone ovariectomy and lacking the IL-1 receptor are protected from bone loss (10). A role for TNF in the pathogenesis of oestrogen deficiency-induced bone loss has also been reported (11). On the other hands, Cell signaling molecules such as the inflammatory cytokines are expressed during fracture healing. Previous studies have shown that they are involved in the regulation of the repair process (38-44). Our result showed that cytokine profile including IL1, IL6 and TNFα in osteoporotic patients with vertebral fracture were higher than healthy women. TNFα and IL-1 play important roles in type I collagen remodeling in fracture healing (39). It seems that other cytokines such as TNF-α could compensate for the absence of IL-6 in the fracture healing process, similar to the redundancy in cytokine stimulate osteoclastogenesis, angiogenesis, and endochondral tissue remodeling (45).

IL-6 expression has been found to peak at day 3 of the fracture healing process in a rat with femoral fracture (46). However TNF-α and IL-1 were expressed most strongly later in the healing process (38). Clinical studies also have found elevated serum IL-6 levels immediately after long-bone fracture (46). The potential roles of IL-6 in angiogenesis and osteoclastogenesis could clarify the importance of IL-6 signaling in early stages of fracture healing (45).

This study demonstrated that serum OPG level positively correlated with age in pre and postmenopausal women. Previous reports have presented similar results about the association between serum OPG level and the age in women and men (47-49).

In the present study, the mean serum OPG level was about 11% greater in postmenopausal women than in pre menopausal women, which is consistent with the results reported by other reports (47-50).

Osteoprotegerin acts as a decoy receptor of the receptor activator of nuclear factor kappa B ligand (RANKL), which is a key regulator of osteoclastogenesis and is known to inhibit osteoclastogenesis by binding to RANKL, thus, preventing RANKL from binding to the receptor activator of NF-kappa B on osteoclasts (50-52). Osteoprotegerin completely blocked TNF mediated bone loss by increasing bone mineral density and bone volume in a transgenic mouse model (53).

Recent studies have shown that OPG not only protects osteoblasts from TNF induced apoptosis (54) but also demonstrated a strong immunoreactivity for OPG apart from in osteoblasts, also in osteocytes and mineralized bone matrix (55). If low serum OPG levels reflect decreased deposition into the bone matrix by osteoblasts then possible detrimental effects on osteocyte/ osteoblast survival rates or differences in bone mineralization may add new explanations, in addition to the anti-resorptive effects of OPG, to why patients with lower OPG levels may be more prone to eventually develop fractures (56).

Further results of our study indicated that RANKL/ OPG ratio independently of age and bone density is predictive of vertebral fracture status.

Whereas the study of Eghbali-Fatourechi et al. refers to the early, rapid phase of postmenopausal bone loss, there are data that indicate a role of the RANKL/OPG pathway also in frac-

ture susceptibility (19). Abdallah et al. demonstrated an increased RANKL/OPG mRNA ratio in bone biopsies from women with hip fractures (20). An intervention trial that proposes RANKL/ OPG pathway as a promising target for the treatment of osteoporosis, provides further evidence for the critical role of this pathway (57).

In conclusion, osteoimmunological insight into vertebral fracture indicated the important role of proinflammatory cytokines and RANKL/OPG pathway in bone remodeling.

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