



REVIEW ARTICLE

RANK LIGAND-OSTEOPROTEGERIN SYSTEM AS A DIAGNOSTIC TOOL IN OSTEOPOROSIS

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ABSTRACT

Background: Osteoporosis is a systemic disease of the skeletal system, characterized by low mineral density and the deterioration of the micro-architecture of the bone tissue, with a consequent increase in bone fragility which has a significance for dentists and TMJ specialists as well.

Aim: The purpose of this study was to analyze the importance of the Receptor Activator of Nuclear (RANK) ligand-osteoprotegerin system in bone metabolism and evaluate what may be predicting factors of early diagnosis while taking into consideration the impact of gender.

Methods: In this study diagnostic strategies are proposed in situations predisposing to osteoporosis. The RANK ligand-osteoprotegerin system could be an excellent tool for prevention.

Conclusion: Prevention remains the best strategy in avoiding the development of this disorder which can be disabling but also it could be leading to lethal consequences if neglected. The use of specific screenings such as those using RANK-ligand osteoprotegerin can be useful to reduce the onset of the disease. Furthermore, due to its significantly different impact in males vs. females.

Key words: rank ligand system, osteoprotegerin, menopause, gender medicine, bone mineral density, periodontitis and dental movement, osteoporosis, metabolism bone

INTRODUCTION

Osteoporosis is one of the main metabolic pathologies of the bone characterized by bone loss and de-mineralization and although it is mistakenly considered a pathology of the elderly, it can also be found in young subjects as well¹. Estrogen deficiency leads to a reduction in bone mineral density in women, during both fertile years (hypoestrogenic amenorrhea) and during menopause, while androgen deficiency induces an increase in RANKL for men also, with consequent loss of bone mass².

The predisposing factors are many: family history of osteoporosis, early menopause, low testosterone levels, amenorrhea, anorexia or bulimia, thyroid disease, rheumatoid arthritis, diseases involving

blocked intestinal absorption of calcium, use of corticosteroid drugs, use of anticonvulsant drugs, low-calcium diet, lack of exercise, smoking, consumption of alcohol, unhealthy eating habits³. Osteoporosis is a disease that cannot always be diagnosed in time, it is often diagnosed after a fracture (the most frequent hip, vertebra, wrist fractures or a condyle fracture) and for this reason prevention screening must not be neglected. It is estimated that 40% of elderly women and 15% of elderly men are prone to skeletal fractures⁴⁻⁶.

According to data from the World Health Organization (WHO), osteoporosis affects more than 75 million people in the United States, Europe and

Japan. In the United States and the European Union, about 30% of postmenopausal women have osteoporosis and it is estimated that more than 40% will have a fracture sometime after menopause. It is estimated that about 4 million women in Italy have osteoporosis^{8,9}. For a man, the risk of suffering a fracture over the course of his life can be up to 27% more in those over 50s, which is higher than the risk of developing prostate cancer (11.3%), but few people are aware of it. As for women, the mortality rate after a hip fracture increases with age, and it is higher in the 12 months after the event. However, in the first 6 months. In general, although fragility fractures are more common among women, mortality rates following a fracture are higher in men and they are more likely to suffer serious consequences after the event.^{11,12} Men, therefore, should be aware of their risk factors and try to prevent the disease, by reducing modifiable risk factors (such as proper nutrition) and manage those that cannot be modified¹³. The risk factors for osteoporosis in men are largely comparable to those in women: testosterone deficiency (primary or secondary hypogonadism) and the possible use of androgen deprivation therapy (ADT), a treatment used in some cases to fight prostate cancer. In the presence of these risk factors, a man between 50 and 69 years old should undergo a DEXA test to assess whether he has osteoporosis. The examination to evaluate bone density should be performed in any case once age 70 is reached and in particular if the man has already suffered a fracture following a fall after age 50, if he is being treated with steroids or ADT, and if he has low testosterone levels. Diagnosis is currently made with laboratory and diagnostic tests, such as the computerized bone mineral density (BMD) test. There are many examination instruments for the study of bone computed tomography (CT), scintigraphy and positron emission tomography/computed tomography (PET/CT)⁷. Several studies support the application of ¹⁸F-sodium fluoride (NaF) PET-CT to evaluate benign bone diseases, although its clinical use is currently limited to the detection of skeletal metastases. In particular bone turnover can be measured through NaF uptake to diagnose osteoporosis and evaluate the efficacy of therapeutic treatment with bisphosphonates⁵. But despite being a disabling disease and with serious consequences, usually it is diagnosed late: during menopause in women and often only after the fracture in men. Estrogen deficiency negatively affects other structures as well, purview of the dental profession, such as the alveolar bone, oral mucosa, and salivary glands¹³. This review aims to describe the RANK-ligand (RANKL) osteoprotegerin (OPG) as a screening test

for the evaluation of bone metabolism, to prevent conditions such as osteoporosis.

The limitation of this review is the scarce number of studies in the literature on this topic.

Nevertheless, we aim to draft the guidelines to carry out the analysis of bone metabolism to prevent a late diagnosis.

Factors affecting bone metabolism

Gender studies

The gender studies in relation to bone metabolism are particularly interesting. It is known that men have a greater bone mass than women, making osteoporosis less frequent in them, with a ratio of 1:4 for women and 1:10 for men. This data has concentrated the focus mostly on female osteoporosis both in terms of diagnosis and in terms of prevention. Many more women take calcium and vitamin D supplements in menopause and there are many more therapeutic strategies for women, starting with the correction of estrogen deficiency in menopause with hormone replacement therapy. Unfortunately, the so-called "gender blindness" has impacted males and so inevitably there are a greater number of men with undiagnosed advanced osteoporosis. About 20% of all hip fractures occur in males and the incidence of vertebral fractures is about half that of females. However, mortality and morbidity from vertebral and femoral fractures are higher in males than in females. Therefore, it is necessary for both women and men to find a way to diagnose osteoporosis as early as possible^{18,19}.

Besides laboratory tests [calcium (Ca), phosphorus (P), calcitonin (CT), parathyroid hormone (PTH), 25OH Vitamin D, osteocalcin (OC), pyridinoline or telopeptide (CTX)] and radiological tests (DEXA), a specific system to evaluate the metabolism of the bone is proposed. The dentist can represent the sentinel doctor because he often has to request orthopantomographic radiological examinations where the quantity of bone can be highlighted^{15,17}.

Estrogens have a protective effect on bone, and there are both in vitro and in vivo studies that show that these effects are mediated by the RANKL/OPG system. In particular, estrogens increase the production of OPG in osteoblastic cells in vitro¹⁶, and in young women who use oral estrogen-progestins the serum levels of OPG are significantly increased compared to a control group consisting of young women who did not use it¹⁶. This increased free OPG/RANKL ratio seems to justify the positive effect of estrogen-progestogens on the skeleton^{20,21}.

Drugs that interfere with bone metabolism

There are numerous drugs that interact with bone metabolism through three main mechanisms

1. increased osteoclast activation and induction of high bone turnover

2. direct suppression of osteoblastic new bone formation
 3. inhibition of normal osteoid mineralization
- Drug-induced bone loss can occur with varying frequency but it can be prevented or treated if the potential osteopenic effects that these drugs exert are known, which are altering the homeostasis of calcium, vitamin D and PTH, local factors and endocrine and paracrine hormones.

The use of these drugs, by reducing bone mass, would cause fractures, therefore it is important to recommend prudent use of the following drugs, using the minimum effective doses and/or limiting their administration to the strictly necessary time frame¹⁷⁻¹⁹.

MATERIAL AND METHODS

This study received approval from the Ethic committee of Cattolic University Rome(n.30759/19) ID : 2700

In this study we propose diagnostic strategies in situations predisposing to osteoporosis. The RANK ligand-osteoprotegerin system could be an excellent tool for prevention of osteoporosis²⁸⁻³⁰.

Role of RANKL/OGP system

In a complex system such as that of bone metabolism in which calcium, magnesium, vitamin D and the hormones estrogens, androgens, calcitonin and parathyroid have an important role, the RANKL/OPG has been emerging as a fundamental physiological system in recent years⁹. Under physiological conditions, the balance between bone resorption and neo-apposition is regulated by the expression of two proteins: the RANKL (Receptor Activator of Nuclear NF- κ B Ligand, secreted by activated T and B lymphocytes and osteoblasts) and the OPG (osteoprotegerin, secreted by fibroblasts of the periodontal ligament and osteoblasts) (Lu et al., 2006)⁵. Basically, OPG prevents RANKL from binding to its true receptor (RANK), creating a real competitive inhibition most of the secreted RANKL is saturated by OPG, which constitutes a false target for RANKL, while a small fraction of RANKL interacts with its natural receptor RANK (Receptor Activator of Nuclear Factor Kappa B) present on osteoclastic precursors, with consequent maturation of the osteoclast and bone resorption. In the presence of inflammation, the secretion of inflammatory cytokines causes activated T and B lymphocytes and osteoblasts to produce massive amounts of RANKL¹⁰. Thus, there is an up-regulation of RANKL associated with a down-regulation of OPG which will be produced in minimal quantities. The RANKL produced in excess,—will therefore bind the RANK present on the osteoclastic precursors, resulting in substantial bone resorption. The same unbalanced

mechanism towards bone resorption occurs in menopause causing osteoporosis. In fact, estrogen deficiency determines an activation of bone remodeling processes, in which osteoclastic activity leads to an increase in bone resorption processes, not balanced by an adequate increase in osteoblastic functions, with a significant reduction in typical bone mineral density¹⁰.

In addition to the serum level, the RANKL/OPG system has also been detected in gingival tissue, crevicular fluid and in saliva in various studies published in the last 10 years. For example, an altered ratio of the RANKL/OPG system, with an increased expression of the mRNA for RANKL, has been demonstrated in the gingival tissues of patients suffering from periodontal disease associated with local osteolysis²⁹⁻³².

It is an immunoenzymatic assay for the measurement in biological fluids, such as serum and saliva, of a protein that uses a specific antibody to detect the substance of interest in the biological fluid tested and a colorimetric enzymatic reaction produced by an enzyme linked to the antibody used that can be read and therefore quantified with a spectrophotometer, the intensity of the color will be directly proportional to the total concentration in the sample of the substance of interest.

DISCUSSION

The details of bone metabolism still require a lot of in-depth studies and we consider a great limitation that often the study of bone metabolism is done only in some conditions such as menopause.

However, it would be desirable to do so too in amenorrhea or fractures. Meantime, although laboratory and other tests are available, the diagnosis of osteoporosis is often late. MOC is recommended in all subjects between 50 and 69 and currently the densitometric criteria for the diagnosis of osteoporosis in males are not based on levels of evidence equal to those for females.

The goal is to prevent osteoporosis in the future, so it is necessary that all specialists cooperate in diagnosing bone dysfunction as early as possible. Even the dentist can notice local signs and symptoms of osteoporosis, thanks to a study by Taguchi (2015) on menopausal women suggesting that the orthopantomogram is a sufficiently sensitive investigation tool to draw a diagnosis of osteoporosis, therefore allowing the dentist often to be the first to notice bone defects. Plus tooth displacements may suggest bone metabolism problems³⁴⁻³⁶.

Proposal for future studies

If much is known about the behavior of the femoral and vertebral bones in conditions of

hypoestrogenism, on the other hand the data relating to the behavior of the bones of the oral cavity are decidedly scares. Only in recent years have specific studies been carried out to investigate the pathophysiological mechanisms that regulate the functions of the oral cavity in this phase of life. The available data indicate that sex hormones play a role in the physiology of oral mucosa, the salivary glands and the bones that support the teeth, and there is also a clear deterioration in the state of oral health in menopause which has a significant impact on the quality of life of the woman²⁰⁻²³.

Several markers of bone remodeling have been used so far to study bone metabolism in menopause, but in recent years the RANK/OPG system is assuming an increasingly important role in this area 21-24.

This system regulates the balance between bone resorption and neo-positioning. Most of the secreted RANKL of bone remodeling have been used so far to study bone metabolism in menopause, but in recent years the RANKL-OPG system is assuming an increasingly important role in this area 21-28). This system regulates the balance between bone resorption and neo-positioning. Most of the secreted RANKL (NF- κ B Receptor Activating Ligand, secreted by activated T and B lymphocytes and osteoblasts) is saturated by OPG (Osteoprotegerin secreted by fibroblasts, also of the periodontal ligament, and osteoblasts) which is a false target of RANKL, the unbound portion interacts with its natural receptor RANK, present on osteoclast receptors, resulting in osteoclast maturation and bone resorption. Moreover, this system appears to be modulated by estrogens. Several studies have highlighted the presence of this system in different tissues of the oral cavity, trying to define its role both in physiological and pathological conditions or during tooth movement induced with orthodontic treatments(38,39,40) The role of sex hormones in influencing the health of all oral tissues and the positive role of HRT in the oral area in menopause, as well as the pathophysiological mechanisms that determine these conditions are not yet well defined^{33,34}. Therefore, we need to acquire new data in order to be able to formulate recommendations regarding the promotion of oral health in menopausal women²⁵⁻³².

Furthermore, for males it is necessary to make an early diagnosis for example by screening for osteoporosis all the patients who have used corticosteroids for extended periods of time to control asthma or other disorders³⁵⁻³⁷.

The role of the dentist is very important to evaluate bone metabolism and can be a reference figure as a sentinel doctor for the early recognition of these problems: the oral cavity is affected by the hormonal impact in fact the estrogen deficiency negatively affects the alveolar bone, the oral mucosa the salivary glands and with menopause it is possible to find an

alteration of periodontal health with xerostomia, increased incidence of caries, atrophic gingivitis, periodontitis, resorption of alveolar bone, crowding or opening of spaces (diastemas) and dental movement during orthodontic treatment requires active bone remodeling, and that the speed of movement is inversely proportional to bone density, estrogen decreases the rate of tooth movement during orthodontic treatment, in fact young girls under PE show a reduction in orthodontic movement²⁴. In the future, it is desirable that the dentist who normally sees patients for periodic checks in the event of alterations in the oral cavity advises the patient for an in-depth diagnostic study⁴¹.

CONCLUSION

Although the medical literature is still very lacking on this subject, from the results of the current investigation, future studies will give the possibility of new early diagnosis and treatment of osteoporosis. Another important aspect is that in the field of gender medicine, males are less favored in the diagnosis of osteoporosis, therefore it is necessary to sensitize patients regarding the need to carry out diagnostic screening, while research on the impact of osteoporosis on non-binary gender is currently absent. Rank-ligand studies would open new frontiers on the study of bone metabolism and in the prevention of osteoporosis. Prevention remains the best weapon in avoiding the development of this disorder which can be disabling but also it could be leading to lethal consequences if neglected. The use of specific screenings such as those using RANK-ligand osteoprotegerin can be useful to reduce the onset of the disease.

DECLARATIONS

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Conflict of interest

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Ethical Approval

Not applicable

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