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Review Article

The guiding role of bone metabolism test in osteoporosis treatment

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Abstract: Osteoporosis (OP) and osteoporotic fractures are becoming a serious health care issue in the world. Calcium and vitamin D are the basic treatment for osteoporosis. Nonetheless, they do not effectively reduce the incidences of fracture. Currently approved treatments for osteoporosis include selective estrogen receptor modulators (SERMs), bisphosphonates, denosumab, teriparatide, calcitonin and others. However, the appearance of some adverse effects including atypical fracture and breast cancer has limited long-term treatments above mentioned. Therefore, treatment decision should be made on an individual basis, taking into account the relative benefits and risks in different patients. Bone metabolism test helps to assess the patient's condition, which may ultimately lead to therapeutic options and better clinical outcomes.

Keywords: Osteoporosis, calcium, 25(OH)D, bone turnover markers, estrogen, androgen, calcitonin, parathyroid hormone, cytokines, Tregs

Introduction

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue that increases bone fragility and increases the risk of bone fractures [1]. Osteoporosis is affiliating with around 6.6% of Chinese population. Osteoporosis is classified into primary and secondary one on the basis of the precipitating factors [2]. This disease is often associated with inadequate calcium intakes, whereas insufficient vitamin D contributes to osteoporosis by reducing calcium absorption. Adequate storage levels of vitamin D maintain bone strength and might help prevent osteoporosis in older adults [3]. However, calcium and vitamin D may not prevent bone loss and fracture completely. Hormone therapy with estrogen and progesterone might be able to delay the onset of osteoporosis, nonetheless, hormone therapy has evident side effects, not every patient can tolerate. Thus, bone metabolism test that assesses the condition of patients may ultimately lead to therapeutic options.

The presence of a fragility fracture or bone mineral density (BMD) measurements form the basis of diagnostic techniques that guides targeted intervention strategies. The levels of blood calcium and vitamin D are used to guide and determine doses of the supplemental calcium and vitamin D. 24 hours urine calcium is used to determine the intestinal calcium absorption capacity. Bone resorption and bone conversion indicators are used to determine whether osteoporosis is osteoclast or osteoblast dysfunction. Calcitonin, parathyroid hormone, androgens and estrogen, are used to determine primary or secondary osteoporosis. Cytokines and Treg are used to analyze the causes of osteoporosis to find new targets for the treatment of osteoporosis.

Calcium and phosphorus in body metabolism

Bone and blood calcium changes constantly and dynamically, and constitutes the physiological balance together. Bone calcium in the role of osteoclasts, comes from the bone into the

blood to form blood calcium. The blood calcium is deposited on the bone to form bone calcium, in the presence of osteoblasts. This function is regulated by many hormones and enzymes in the body. Among them, vitamin D, calcitonin, parathyroid hormone and their active metabolites are the major humoral regulatory factors. Bones, intestines and kidneys are the three main target organs. Vitamin D and its active metabolites promote the absorption of intestinal calcium. Calcitonin has the effect of inhibiting the dissolution of bone salt and reducing the blood calcium [3]. Parathyroid hormone has the effect of promoting osteolysis and elevating the blood calcium [4].

When there is a disease or metabolic disorder, it can destroy the blood calcium balance system and leads to wither too high or too low in the levels of blood calcium. Hypercalcemia is common in primary hyperparathyroidism and malignancy, and it also appears in unsuitable use of estrogen and thiazide diuretics [5]. Hypocalcemia can be seen in the insufficiency of calcium intake, calcium absorption disorder, hypoparathyroidism and other diseases [5]. It is noted that hypocalcemia is not a necessary indicative of calcium deficiency in the body, and high blood calcium does not represent the excess calcium deposits in the body. Blood calcium content can help to analyze the etiology of osteoporosis but not as a basis for diagnosing osteoporosis.

The moderate ratio of calcium and phosphorus is beneficial to the absorption of calcium. But studies showed that elevated tissue phosphate concentrations have been shown to increase oxidative stress in endothelial cells [6]. Phosphorus intakes exceed the needs of a healthy population may significantly disrupt the hormonal regulation between phosphate, calcium, and vitamin D, which contributes to disordered mineral metabolism, bone loss, vascular calcification and impaired kidney function. Katsumata [7] suggests that dietary calcium supplementation prevents the bone loss and decline in kidney function induced by a high phosphate diet.

Accurate estimates of dietary phosphorus intake are essential to assess the contributions of serum phosphate to health. Nevertheless, using serum phosphate as a measure of dietary phosphate has many confounders. Such as pronounced circadian rhythm and the body's ability to correct the elevated serum phosphate to fasting concentrations with high dietary phosphorus loads. Future studies using serum phosphate as a measure of dietary burden will need to be designed in a way to control these confounders [8, 9].

24-hour urine calcium demonstrates calcium absorption

Hypercalciuria (HC) is frequently present in patients with OP regardless of the underlying bone turnover status. This may suggest the presence of a bone-derived renal calcium regulating factors. Further studies are needed to understand the exact mechanism and the potential consequences of HC in OP patients [10]. Impaired intestinal calcium absorption contributes to osteoporosis. Hanseree [11] suggests that 24-hour urine calcium (24HUC) values can diagnose low fractional calcium absorption (FCA). When 24HUC value <150 mg demonstrated a high negative predictive value (NPV) for calcium malabsorption. They suggest that 24HUC levels can exclude calcium malabsorption in postmenopausal women.

25(OH)D and 1,25$(OH)_2$D promote calcium absorption

Vitamin D promotes calcium absorption in the gut. It maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is essential for bone growth and bone remodeling by osteoblasts and osteoclasts [12, 13]. Without sufficient vitamin D, bones will become thin, brittle, or misshapen. Vitamin D sufficiency prevents Rickets in children and Osteomalacia in adults [12]. Together with calcium, vitamin D also helps prevent older adults from osteoporosis.

Serum concentration of 25-hydroxyvitamin D [25(OH)D] is the best indicator of vitamin D status. It has a long circulating half-life of 15 days [14]. It reflects vitamin D that obtained from food or supplements. It has functions as a biomarker of exposure, but it is not clear to what extent 25(OH)D levels also serve as a biomarker of effect (i.e., relating to health status or outcomes) [12]. Serum 25(OH)D levels do not directly indicate the amounts of vitamin D stored in body tissues.

In contrast to 25(OH)D, circulating $1,25(OH)_2D$ is not a good indicator of vitamin D status generally, because it has a short half-life of 15 hours and serum concentrations are closely regulated by calcium, phosphate and parathyroid hormone [14]. Levels of $1,25(OH)_2D$ do not typically decrease until vitamin D deficiency is severe [13, 15].

Although there are considerable discussions of the serum concentrations of 25(OH)D associated with adequacy for bone health, and optimal overall health, the consensus has not yet been reached by a scientific community. A committee of the Institute of Medicine concludes that persons are at risk of vitamin D deficiency if the serum 25(OH)D concentration is less than 30 nmol/L (<12 ng/mL). Some are potentially at risk for inadequacy when 25(OH)D =30-50 nmol/L. Practically all people is sufficient when 25(OH)D≥50 nmol/L. But serum concentrations >125 nmol/L are associated with potential adverse effects [12].

Nonetheless, the study of Zhao [16] enrolled adults older than 50 years who were living in their communities. They had found there are no significant associations between calcium, vitamin D, or combined calcium and vitamin D supplements and the incidences of nonvertebral, vertebral, or total fractures. Their subgroup analyses showed that these results were generally consistent regardless of the calcium or vitamin D dose, sex, fracture history, dietary calcium intake, and baseline serum 25(OH)D concentration. As a result, the relationships between the supplement of vitamin D, calcium and the incidences of fracture are still controversial in different studies, we need to study more mechanisms about the causes of osteoporosis.

Bone turnover markers (BTMs) are tools for management of osteoporosis

Bone remodeling requires a precise balance between resorption and formation. The OPG/RANKL/RANK system plays an important role in osteoclastogenesis and represents a great progress in bone biology. RANKL, which expresses on the surface of osteoblast/stromal cells and activated T cells, binds to RANK on the osteoclastic precursors or mature osteoclasts, and promotes osteoclastogenesis and bone resorption. While osteoprotegerin (OPG), which is expressed by osteoblasts/stromal cells, strongly inhibits bone resorption by binding to its ligand RANKL and thereby blocks the interaction between RANKL and RANK. A number of cytokines and hormones regulate the OPG/RANKL ratio in the bone marrow microenvironment. Modulation of these systems can provide a novel therapeutics to inhibit bone loss in osteoporosis [17].

An increase in bone turnover developed with aging and pathological states, and an increase in the risk of fracture independent on low bone mineral density (BMD). Because osteoporosis leads to deterioration of bone microarchitecture, alterations affecting the bone quality can be assessed by bone turnover markers (BTMs) and thus may serve as a complementary tool to BMD in the assessment of fracture risk. Bone formation markers are expressed during different phases of their development and are considered to reflect different aspects of osteoblast function and bone formation.

Markers of bone formation include propeptides of type 1 collagen (P1CP and P1NP), alkaline phosphatase (ALP), matrix proteins and osteocalcin (OC). P1NP is proposed as a reference bone formation marker by International Osteoporosis Foundation (IOF) because of its predictable response to treatment. P1NP assay is also reliable by low intra-individual variability, smaller circadian variation, stability at room temperature, and a good assay precision [18]. OC has been found to be a useful biomarker in steroid-induced osteoporosis [19].

Markers of bone resorption include telopeptides of type 1 collagen (CTX-1 and MMP and NTX-1), pyridinoline (PYD), deoxypyridinoline (DPD), Bone sialoprotein, Osteoclastic enzymes, and osteocyte activity markers(RANKL and OPG). CTX is degradation products of Type 1 collagen of bone generated by the activity of the enzyme cathepsin K. According to the sample collection difficulty and the accuracy of the results, the CTX-1 has been recommended as reference bone resorption marker by IOF [20, 21].

Some novel biomarkers of bone resorption currently used provide new tools to study the efficacy, safety, mechanism, and mode of action of drugs used in osteoporosis and other metabolic bone diseases. RANKL and OPG are novel

osteocyte markers to reflect bone microenvironment. BTMs are important tools for management of osteoporosis, which are reliable, rapid, cost-effective. It changes precede BMD during treatment. But there was insufficient evidence to inform which bone turnover marker can be chosen to use in routine clinical practice to monitor osteoporosis treatment response [22].

Bisphosphonates, calcitonin, estrogen, SERMs and RANKL inhibitor are the inhibitors of osteoclast functions, only PTH is to promote bone formation. Under the current evidence, bisphosphonates reduce the risk of vertebral and possibly nonvertebral fractures for men with osteoporosis [23]. Zoledronic acid is the most effective in preventing vertebral fractures, and risedronate is selected in the first line to prevent non-vertebral fracture in men with primary osteoporosis [24]. While osteonecrosis has emerged as a significant complication induced by bisphosphonates, which is called bisphosphonate-related osteonecrosis of the jaw (BRONJ) [25]. The inhibition of osteoclast function can also inhibit normal bone turnover, preventing local micro-damage from normal mechanical loading or injury being repaired, this ultimately results in bone necrosis.

With the deepening research of the bone metabolism, new drugs are invented. Denosumab is a human monoclonal IgG2 antibody which has high affinity and specificity to RANKL, it works as a RANKL inhibitor that creates a new category of antiresorptive agent. In physiological conditions, denosumab is binding to RANKL as OPG does that inhibits the osteoclast activation. Bone resorption markers were decreased rapidly after denosumab injection, and bone turnover suppression appeared to be dose dependent [26]. It is very different from bisphosphonates. Compared with alendronate and placebo, denosumab was shown to be the more efficient treatment in improving bone mechanical properties of the femoral neck [27]. In terms of safety and tolerability, less risk of jaw osteonecrosis disabling adverse event was reported under denosumab [28]. Strontium ranelate, which has proven to be effective on the RANKL/OPG pathway, induced a concentration-dependent increase in OPG mRNA expression as well as OPG secretion [29].

Estrogen and androgen have pleiotropic effects on bone metabolism

It has been generally accepted that sex hormones play an important role in primary osteoporosis. The abrupt loss of estrogen at the menopause is considered the major reason for Type 1 primary osteoporosis in women [30]. Menopause is characterized by an estrogen deficiency leading to bone loss.

Estrogen clearly has pleiotropic effects on bone metabolism, the mechanisms are complex. Recent studies have demonstrated that the osteoclast is a direct target for estrogen though estrogen receptors [31]. Estrogen deficiency is associated with the increases of pro-resorptive cytokines, including TNF-α, IL-1 α, IL-6, IL-7 and others [32, 33]. These cytokines expand the pool of osteoclast precursor cells, and increase the key molecules to regulate osteoclast development, activity, and lifespan [32].

Estrogen replacement therapy has been used effectively by suppression of osteoclast activity. At the clinical level, treatment of postmenopausal women with estrogen leads to a marked and sustained reduction in urine or serum markers of bone resorption [34]. However, estrogen is not available in every patient because of unacceptable efficacy, safety and tolerability profiles, such as risk of blood clots, endometrial cancer and breast cancer.

SERMs bind to the estrogen receptor (ER) with high affinity, and work as an agonist to mediate transcriptional events. SERMs are used in postmenopausal women of younger age and are particularly recommended if there is a family history of invasive breast cancer. Raloxifene is approved for the prevention and treatment of postmenopausal osteoporosis and vertebral compression fracture [35-38]. Other new SERMs have been developed in recent years, such as bazedoxifene and lasofoxifene. Clinical studies are now to test the comparative advantages of these SERMs [35].

Male osteoporosis is different from postmenopausal osteoporosis. There was no correlation between serum testosterone levels and patient age, body-mass index, or any measured BMD values in postmenopausal women [39]. Men do not have a dramatic loss of androgens, but most reports have shown that serum testoster-

one levels decline with aging [40]. Androgens may prevent the loss of cancellous bone and stimulate periosteal cortical bone apposition [41]. Studies on bone metabolism in castrated mice indicated that testosterone increased bone number, width, volume and BMD. In contrast, it reduces bone turnover in an androgen receptor (AR)-dependent manner. Some selective androgen receptor modulators (SARMs) have been proposed as choice treatments for osteoporosis in man [42, 43]. The study from this group [44] suggests that 5α-DHT exhibits outstanding potential for promoting proliferation and differentiation in osteoblasts, it could be chosen to treat male osteoporosis who is androgen-deficient. Clinical studies are going on now to study the adverse reaction of these SARMs.

Calcitonin inhibit osteoclastic activity though receptors on osteoclasts

Calcitonin (CT) can decrease cytosolic Ca^{2+} along with hypocalcaemic and hyperphosphoric effect. CT has antiresorptive property, which is effected by intracellular second messengers following binding to specific G protein-coupled receptors on osteoclasts, resulting in nonapoptotic inhibition of osteoclastic activity [45]. CT treatment was shown to increase spine BMD and significantly reduce spinal fracture risk [46]. Until now, the complications of CT associated with suppression of bone remodeling such as osteonecrosis of the jaw (ONJ) and atypical femoral fracture (AFF) have never been reported. However, some adverse events (AEs) with CT also appeared, such as nausea, vomiting and abdominal pain, as well as hot flushes [47]. Expression of CT and its receptor (CTR) is frequently elevated in prostate cancers (PCs) and activation of CT-CTR axis in non-invasive PC cells induces an invasive phenotype [48]. Additionally some experts recommend against CT-salmon for postmenopausal osteoporosis [49]. Interestingly, a meta-analysis demonstrated that no data to document that CT can induce carcinogenic transformation. Data about the ability of CT to promote tumor aggressiveness are inconsistent and contradictory [50]. All of the available data suggest that the association between CT and cancer is weak.

Parathyroid hormone plays a dual effect on bone metabolism

Parathyroid hormone (PTH), a peptide hormone that is secreted from the parathyroid glands, regulates calcium homeostasis. PTH can response to low extracellular calcium physiologically, reduced binding of ca^{2+} to the calcium sensing receptor stimulates PTH synthesis and release.

PTH can regulate bone homeostasis but it plays a dual effect on bone metabolism. It can be either an anabolic one or a catabolic one [51]. PTH influences bone remodeling by increasing osteoblast proliferation and decreasing osteoblast apoptosis. Moreover PTH also increases osteoclast activity by enhancing RANKL production from osteoblasts [52]. When administered intermittently, PTH increases bone mass, but when present continuously and in excess bone loss ensues, such as hyperparathyroidism. This two-way role in PTH depends not only on the dosing regimen, continuous or intermittent, but also on how the PTH molecule interacts with its receptors, influencing downstream signaling pathways differentially.

Circulating PTH is rapidly metabolized by renal and hepatic tissue. Therefore serum levels of PTH have great extent, which are determined by the production rate of the hormone [53]. PTH demonstrates a very short half-life which is indeed administered as continuous intravenous infusion. In humans, the estimated half-life of PTH(1-34) is about 10 min [54]. PTH analogues are the only compounds that stimulate bone formation. Treatment with teriparatide [PTH(1-34)] generally shows an increase of BMD at the lumbar spine and hip [55-58]. The group of Sugimoto used once-weekly teriparatide injections for 24 weeks in postmenopausal women with osteoporosis. After 24 weeks, the bone formation marker and serum OC increased significantly, but P1NP did not. Bone resorption markers decreased or remained the same [59]. Considering the time of drug metabolism and side effects, teriparatide is recommended to use no more than 2 years [60].

Cytokines and Tregs participate in bone immunology

The skeletal system interacts with the immune system, and the relationship between osteoclasts and T cells is called bone immunology. The T cells cause osteoclasts to be generated through the RANK/RANKL pathway, which promotes bone absorption [61]. OPG/RANK/RANKL is also an immune molecule, which plays an important role in the differentiation

and development of lymphocytes and lymphoid organs. T, B cells and other cytokines were also involved in bone reconstruction. TNF-α, IL-1, IL-6, IL-17 can promote bone absorption, IFN-γ has a certain anti-bone absorption effect [62, 63].

Tregs is known as a suppressor T cell which is a potent immunomodulatory T-cell subset [64-66]. Major function of Tregs played is to restore immune homeostasis after inflammatory responses in order to limit inflammation and prevent chronic inflammatory diseases [67-69]. Tregs inhibits osteoclasts, controls bone absorption in the body, maintains bone mass and reduces the occurrence of osteoporosis [61]. In addition to $CD4^+$Treg cells, Zachary [70] used $CD8^+$T cell defect mice and $CD8^+FoxP3^+$T cell transfer method to confirm that $CD8^+$Treg could control bone loss by inhibiting RANKL. The increase of human peripheral blood memory $CD8^+$T cells delayed the healing of bone wound surface, while $CD4^+$Treg cells promoted healing [71, 72]. Tregs inhibits RANKL to regulate the formation of osteoclasts in a dose-dependent manner. Moreover, Tregs affects bone metabolism directly that does not need other T cell lineages [73]. Tregs can also inhibit the differentiation of osteoclasts by inhibiting TGF-β1, GM-CSF, IFN-γ, IL-5 and IL-10 [74]. Tregs inhibit the formation and differentiation of osteoclasts, and its number and function changes can affect bone metabolism. The study on bone metabolism is helpful to understand the mechanism of bone reconstruction and the pathogenesis of bone loss, and provide new targets for the prevention and treatment of OP.

Conclusion

Disruption of the cooperative balance between osteoblasts and osteoclasts causes various bone disorders, some of which are related to the abnormal osteoclast recruitment. Osteoporosis is one of the bone disorders. The above tests were the important technical tools for monitoring the level of bone metabolism and the disease diagnosis. First, BMD is used to diagnose osteoporosis. Second, bone metabolism tests are used to classify osteoporosis into primary and secondary one. Third, they could guide the selection of medications. Rare adverse events become apparent with calcium and vitamin D treatments for osteoporosis with long-term therapy. So calcium and vitamin D are the basic treatment for osteoporosis.

Currently approved treatments for osteoporosis include SERMs, bisphosphonates, denosumab, teriparatide, calcitonin and others. Nonetheless, some adverse effects such as atypical fracture with bisphosphonates and breast cancer with estrogen/progestin therapy exist, particularly when they are used long-term by older women. Calcitonin is associated with an increased risk of cancer than bisphosphonate, supporting the recent warning issued by the European Medicines Agency and US Food and Drug Administration. SERMs is found to be associated with an increased risk of cancer than bisphosphonate [75, 76]. Due to the probability of adverse effects, no single antiosteoporosis agent is appropriate for all patients. Treatment decisions should be made on an individual basis, taking into account the relative benefits and risks in different patient populations. In addition to the development of novel drugs, the exploitation of novel compounds for preventive treatment is important in an aging society.

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None.

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