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Diagnostic use of bone biomarkers and drugs affecting bone remodelling

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Abstract

Bone is a dynamic organ made up of mostly collagen that provides the structure to the skeleton and calcium phosphate that gives strength and hardens the skeleton structure. Bone is constantly renewed by remodeling, a lifelong process consisting of bone resorption (osteoclasts) and formation (osteoblasts) to repair skeletal damage and maintain calcium homeostasis. Several hormones like calcitonin, parathyroid hormone, vitamin D, estrogen, testosterone etc. regulate resorption and formation. Several factors like age, low diet calcium, smoking, certain medications, - glucorticoids, aromatase inhibitors, proton pump inhibitors, immunosuppressant, selective serotonin inhibitors and antiepileptic drugs can influence bone health which are used for long term treatment. Imaging techniques like dual X-ray absorptiometry (DEXA), Quantitative computerized tomography (QCT) are available for exact measurement of bone mineral density which helps identifying the fracture risk. Newly, bone biomarkers are known to increase their demand and play an important role in detection of bone loss at early stage. Recent evidence suggests that these markers may be useful in monitoring the response to anti-resorptive therapy. The potential for these markers used along with imaging techniques helps to diagnose bone loss, possibly prior to overt clinical signs. Hence, the aim of the article was to review the diagnosis of bone loss using specific bone biomarkers which can strongly correlate to these classes of drugs causing bone loss at early stage with possible duration of the treatment.

Keywords: Diagnostic marker, Bone biomarkers, Bone health, Medications.

Introduction

Bone is a rigid organ composed of mineral and protein matrix of collagen and non-collagenous substances in which type I collagen is the main organic constituent. The non-collagenous protein substances are osteocalcin, glycoproteins and proteoglycans. Bone is metabolically active tissue that undergoes remodeling process which is mediated by the bone formation and bone resorption via osteoblasts and osteoclasts respectively. They result in release of different molecules like osteocalcin (OC), procollagen type I propeptide (PINP), C-terminal telopeptide of type I collagen (CTx), N-terminal telopeptide of type I collagen (NTx), tartrate resistant acid phosphatase (TRAP) & parathyroid hormone (PTH).(1)

In worldwide, it is estimated that over 200 million people suffer from osteoporosis. Approximately, 30% of the women and 15-30% of men have osteoporosis in the United States and in Europe(2) while 20% women and 10-15% of men would be osteoporotic in India.(3) Hip fracture is increasing exponentially with age associated with 20% mortality and 50%permanent loss in function.

Several risk factors are usually associated with decrease bone turnover and they include age, family history, prior disease, low calcium diet, vitamin D deficiency, smoking, excess alcohol consumption, genetics, environmental factors, disease conditions and use of certain medications.(4) Several classes of drugs are known to induce the bone loss, reduce the bone mineral density and increase the risk of fractures. The prevalent classes of drugs that affect bone health are glucocorticoids, antiepileptic drugs, antacids, anticonvulsants, immunosuppressants, aromatase inhibitors, proton pump inhibitors, selective serotonin reuptake inhibitors and thiazolidenediones. These drugs are known to alter the cellular functions of osteoclasts and osteoblasts leading to osteopenia or osteoporosis.(5)

Identifying these alterations at very early stage is now a challenging task. We know that there are different kinds of imaging techniques which are important in identifying the individuals who are at risk of osteoporotic fractures and require pharmacotherapy. Significantly, imaging techniques are advantageous over other diagnosis and these methods are ideal to determine the current status of the bone. Several biomarkers are used as indicative for alteration in bone turnover rate and can be measured in serum and urine which help to evaluate or monitor the bone turnover. Various biochemical markers namely bone formation markers like osteocalcin, procollagen type I propeptide, bone alkaline phosphatase (BLP) and bone resorption markers such as C-terminal telopeptide of type I collagen, N-terminal telopeptide of type I collagen, tartrate resistant acid phosphatase and deoxypyridinoline are now available to detect the specific and sensitive rate of bone formation and bone resorption.(6,7) Biochemical bone markers do not substitute the well-known imaging methods such dual energy X-ray absorptiometry (DEXA), computerized tomography (CT), magnetic resonance image (MRI) and ultra sound which are widely used for measuring bone mineral density. However, it provides the repository information to the diagnosis at early stage.

Bone mineral density (BMD) is an important predictor for fracture risk. The importance of bone

biomarkers are increasing, as it provides a dynamic understanding of spectrum of disease, elucidation of specific indication, characterization of analytical features, possible to get different markers prognosis and diagnosis. It can also be useful to improve evaluation of individual fracture risk when BMD measurement by itself does not provide an exact indication.[8]

In this article we are focusing mainly on different classes of drugs that affect the bone health, specific bone biomarkers which can strongly correlate to these classes of drugs causing bone loss and possible duration of treatment of such drugs causing bone loss.

Aromatase inhibitors: The aromatase inhibitors (AIs) areused in the treatment of cancer chemotherapy. These drugs act by blocking aromatase enzymeand inhibit the conversion from androgen to estrogen. When compared to the first and second generation, third generation AIs like anastrozole,[9] letrozole[10] and exemestane[11] are known to cause bone loss. These drugs inhibit the endogenous production of estrogen by 50-90%. Estrogen regulates the physiologic bone remodeling by suppressing osteoclast mediated bone resorption. Treatment with aromatase inhibitors is associated with estrogen deficiency leading to imbalance in bone resorption and osteoblast mediated bone formation leading to net bone loss.[12,13]

Several trials lik eanastrozole, tamoxifen alone combination (ATAC), Australian breast cancer study group (ABCSG) have shown that patients treated with anastrozolehad significant increase in risk of fracture at lumbar spine and reduce hip bone mineral density with 2 years of treatment compared with tamoxifen. Forbes et al 2008,[14] Eastell et al 2006,[15] Julie et al 2013[16] reported that fracture risk was high in anastrozole group compared to tamoxifen group. The non-steroidal letrozole is another superior AI and is more effectively used in the treatment of breast cancer. While a trial by breast international group (BIG1-98) summarized that letrozole is superior over tamoxifenin treating breast cancer but it is associated with increased risk of fracture by 8.6% compared to tamoxifen group (5.8%) within 1 year of treatment.[17] MA-17 trial concluded that patients treated with letrozole had a significant decrease in BMD at 24 months in both lumbar spine and hip.[18]

Exemestane is another aromatase inhibitor which is extensively protein bound metabolized in liver to inactive metabolites. A key metabolite 17-hydroxy exemestane is formed by reduction of 17-oxo group via 17-β hydroxyl steroid dehydrogenase which has weak androgenic activity and also contributes toanti-tumor activity. It might result in less adverse effects on bone health and improves disease free survival.[19] Intergroup exemestane study (IES) showed increase in the risk of bone loss in patients receiving exemestane for a period of 2 years by 7% when compared to tamoxifen group (4.9%).[20]

Aromatase inhibitor leads to change in the bone mineral density and bone turnover markers which are detectable in the serum and urine for first 3 to 24 months. ATAC trail demonstrated that, there was a significant increase in the bone resorption marker; serum C-terminal telopeptide of type I collagen (CTX) or serum cross laps, N-terminal telopeptide (NTX); Bone formation marker bone alkaline phosphatase (ALP) and procollagen type-I N-propeptide (PINP) within one year of treatment.[21] MA-17 bone study showed increase in the bone resorption markers NTXand CTX in patients treated with letrozole at 24 months respectively.[22] BMD was also reduced in women who received treatment with letrozole for a period of 4 years. Several studies reported that 1year treatment with exemestane increased in CTX, bone ALP and decrease in BMD parameters both at lumbar spine and at femoral neck.[23] Aromatase inhibitors can cause changes in increased bone turnover and it is associated with higher risk of fracture. These findings suggested that the issue of bone loss prevention among breast cancer patients treated with AI with help of biochemical bone markers and also along with fracture risk assessment include evaluation of bone density. Further studies should assess the long-term effects of AI treat menton bone health.

Glucocorticoids: Glucocorticoids (GCs) are steroids that reduce inflammation. Synthetic glucocorticoids like prednisone, methyl prednisone, dexamethasone & hydrocortisone are used in therapeutics. On continuous administration of these drugs for more than 3 months they can cause rapid loss in bone mineral density and incidence of fractures which can be as high as 17%.[24] Corticosteroid inhibits formation of new osteoblasts by reducing the synthesis of bone collagen, osteocalcin and promotes apoptosis of osteoblasts, osteocytes and osteoclasts. GCs also decrease the function and differentiation of the osteocytes and osteoblasts. These mechanisms eventually lead to increased bone resorption and decreased bone formation leading to fractures. GCs also reduce intestinal calcium absorption and increase renal calcium excretion resulting in bone loss.[25]

Observational studies reported that fractures are often asymptomaticoccur in 30-50%of patients depending on dosage and duration of treatment.[26] A study by Robert et al (2011) shows that daily dosage of 7.5mg of prednisone for 3 months or more causes bone damage and with increase in the dose greater than 20 mg/day, bone mineral density decreases drastically irrespective of age, gender or menopausal status.[27] Pulsed intravenous high dose corticosteroids such as 1g methyl prednisolonehave less deleterious effect on bone mineral density, but increase the risk of osteonecrosis.[28,29,30]

A short term dose of 5mg/day prednisolone showed an immediate and significant fall in osteocalcin and propeptide of type I collagen (PINP), while Bone alkaline phosphatase showed only a modest decrease and

serum CTX levels showed a progressive increase with a subsequent decrease, at the end of the treatment. There was a highly significant correlation between the two markers (PINP, CTx) throughout the 2 year follow up period. TRAP and CTX are two bone resorption markers useful for identification of number of osteoclasts.[31,32] Prospective studies are needed for persisting biomarker on a long term basis.

Proton pump inhibitors: Proton pump inhibitors (PPIs) reduce the acid production by blocking H+/K+ ATPase. PPIs are used to treat conditions such as duodenal stomach ulcers, non-steroidalanti-inflammatory drugs (NSAIDs) associated ulcer, gastroesophageal reflux disease (GERD). PPI sinclude omeprazole, esomeprazole, lansoprazole, dexlansoprazole, rabeprazole and pantoprazole. However, long term use of omeprazole, lansoprazole and esomeprazole can increase the risk leading to osteoporosis.

The pathophysiologic mechanism of PPI induced bone loss is not clear but possible mechanism could be that PPI increase gastric pH which may impair dietary calcium release and thereby calcium absorption. This further may lead to compensatory secondary hyperparathyroidism causing decreased osteoclastic activity, decrease BMD causing fracture risk.[33] Long-term use of PPIs can reduce vitamin B^{12} absorption leading to increase inhomocysteine levels which interferes with collagen cross-linking and weakens the bone.[34] PPIs act by irreversibly binding to H^{+}/K^{+}ATPase on the parietal cells.

A study by Gray et al (2010) concluded that the use of omeprazole and lansoprazole for ≥3 years is associated with increased risk of fractures at spine, forearm and wrist.[35] Recent study by Yunju Jo et al (2015) investigated the association between pantoprazole and revaprazan on bone health. Authors reported that patients who received pantoprazole 40mg and revaprazan 200mg daily for a period of 8 weeks had significant alteration in the bone biomarkers such as urine deoxypyridinoline (DPD), intact parathyroid hormone (iPTH), osteocalcin, serum corrected calcium. This study suggested that PPIs might directly alter bone metabolism via vacuolar type-H^{+}-ATPase on the osteoclasts.[36] A study by Sharara et al (2013) demonstrated the effect in healthy human young males aged between 18-50 years receiving PPIs for 12 weeks. They summarized that the biochemical markers of calcium and bone metabolism had no significant effect on intact parathyroid hormone (iPTH), ionized calcium, osteocalcin, or serum C-terminal cross-linked telopeptide of type I collagen.[37]

An animal study by Yanagihara(2015) investigated the effects of long term administration of omeprazole(300 μmoL/kg/day) in adult rats for a period of 90 days. They concluded that omeprazole use was associated with decrease bone mineral density and increased risk of fracture.[38] Another animal study conducted by Joo (2013) demonstrated the effect of omeprazole 30mg/kg for 8 weeks using osteoclast differentiation and turnover markers in ovariectomized rats. They concluded that the expression levels of osteocalcin were decreased, while the levels of serum C-terminal cross-linked telopeptide of type I collagen were increased in the group with a low calcium diet and omeprazole administration.[39] However, further studies are needed to identify the risk of PPIs with duration and specific biochemical bone markers for PPIs induce bone loss.

Immunosuppressants: Immunosuppressants are drugs that reduce body's ability to reject a transplanted organ and these are also known as anti-rejection drugs. They are classified as induction drugs and maintenance drugs - which are used as powerful anti-rejection agents at the time of transplant. Immunosuppressants are also used to treat other diseases like psoriasis, multiple sclerosis, rheumatoid arthritis and crohn's disease. Certain immunosuppressive agents like tacrolimus, cyclosporine and sirolimus contribute further to bone loss. The immunosuppressants drugs which affect bone health are calcineurin Inhibitors (tacrolimus and cyclosporine), anti-proliferative agents (azathioprine) and mTOR inhibitor (sirolimus).

Calcineurin inhibitors have adverse effects on osteoblasts and osteoclasts but the mechanism is not well understood. Calcineurin inhibitors – CsA and tacrolimus may cause imbalance between receptor activator nuclear factor kappa ligand (RANKL) and osteoprotegerin. These drugs increase the levels of RANKL whereas sirolimus increases the level of osteoprotegerin and protein.[40]

In experimental animal models, male wistar rats received daily dose of 10mg/kg cyclosporine and 1mg/kg tacrolimus for 120 days. This was associated with increase in the bone resorption, decrease bone density and increase osteoclastic activity. The tacrolimus greatly reduces bone mineral density at femoral, lumbar & spine region. Cyclosporine (CsA) has shown to accelerate bone resorption leading to severe osteopenic with increased level of osteocalcin and calcitrol.[41] Another study conducted by Ruberta et al (2015) demonstrated the effects of CsA (2 mg/kg/day) rapamycin(1.25 mg/kg/day) and tacrolimus(3 mg/kg/day) for 3 months in male wistar rats. They observed that there was increase in the level of osteocalcin, CTx and TRAP in animals with tacrolimus, while there was decrease in the level of PINP in all other groups.[42] Experimental model conducted by Jager et al (2012) demonstrated the effects of CsA (5 mg/kg) three times per week for 2 months. They observed that bone alkaline phosphatase (BALP) and TRAP-5b was significantly elevated in CsA treated male wistar rats when compared to female rats.[43] Cross sectional studies were conducted with high doses of sirolimus, cyclosporine, tacrolimus and mycophenolatemofetil and

the effects of these drugs were compared. They observed that sirolimus promotes osteoclastic activity and thereby accelerates bone turnover in patients undergoing renal transplantation.(44)

Study conducted in patients by Ballanti et al (2006) reported elevated level of TRAP-5b with 1-2 years of cyclosporine (80-120 ng/mL) treated for renal transplant when compared with sirolimus and tacrolimus group. These finding identifies the fact that TRAP-5b may be a more specific marker for bone resorption and BALP for bone formation.(42,45) The disadvantage of such studies with regards to immunosuppressants is homogeneity in the population, age, type of organ transplant where the treatment, its dose and duration differs. More clinical studies are required in order to identify the effects of immunosuppressants on bone.

Selective serotonin reuptake inhibitors: Selective serotonin reuptake inhibitors (SSRIs) are widely used antidepressants which act by blocking or delaying the reabsorption of the neurotransmitter serotonin at presynaptic nerve endings and thereby increasing the levels of serotonin at synapses. Currently, newer generation antidepressants available are selective serotonin reuptake inhibitors (SSRIs) like citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, and serotonin nor-epinephrine reuptake inhibitors (SNRIs) such as venlafaxine, duloxetine. Among these antidepressants mostly SSRIs are known to increase fracture risk by two fold.(46) The mechanism by which SSRIs affect bone health is unknown but the effect of SSRIs on bone formation and resorption appears to be governed by the activation of number of 5-HT receptors on osteoblasts and osteoclasts via endocrine, paracrine and neuronal pathways. Use of citalopram, fluoxetine, and sertraline is associated with dose dependent increase in fracture risk.(47) Some observational studies reported increased risk of fracture with the use of SSRIs.(48)

In vivo studies could be attributed that mice treated with fluoxetine 10mg/kg/day delivered subcutaneously for 5 days per week, for a period of4weeks reported with significant reduction in bone density, bone formation and increasing bone resorption.(49) Recent animal study conducted by Folwarczna et al (2009)demonstrated the use of antidepressants bupropion in a dose of 30-60 mg/kg/day for a period of four to six weeks in ovariectomized rats. They concluded that there is significant alteration in biochemical bone markers with increased levels of urinary DPD and serum BALP, osteocalcin, TRAP, CTX and RANKL and decreased bone mineral density after six weeks of treatment. Unlike bupropion other antidepressant such as fluoxetine, sertraline and fluvoxamine found alteration of bone markers in previous experimental studies.(50,51)

Clinical studies investigated the use of antidepressants in patients receiving venlafaxine 150-300mg daily for 12 weeks with age group of 60 years which showed significant increase in resorption marker-CTX resulting in bone loss.(52) The evidence suggests that the antidepressants contribute to bone loss based on dose dependent. Further studies are necessary to evaluate the bone markers and effects of SSRIs in humans.

Antiepileptic drugs: Antiepileptic drugs (AEDs) are used to prevent rapid, repetitive, stimulation of brain causing seizure activity such as epilepsy. These drugs are also used in non-epileptic conditions like trigeminal neuralgia,(53) diabetic neuropathy,(54) migraine prophylaxis(55) and bipolar disorders.(56) Antiepileptic drugs include carbamazepine, valproic acid, primidone, benzodiazepines, phenytoin, phenobarbital, gabapentin, oxcarbazepine and zonisamide which contribute to bone loss.(57) Several literatures indicates that AEDs are at increased risk for low bone mineral density and metabolic bone diseases including changes in bone turn over, alteration in bone quality and osteoporosis. It is said that AEDs act by inducing hepatic cytochrome P450 enzymes which causes increase in the conversion of vitamin-D to inactive metabolites in the liver thereby reducing vitamin-D levels leading to hypocalcemia. Other possible mechanisms are direct effect on bone cells including impaired absorption of calcium, inhibition of response to PTH, hyperparathyroidism and calcitonin deficiency.(58) Fractures are more important manifestations of bone mineral density.

Animal studies investigated by Sofie et al demonstrated that 75 mg/kg of carbamazepine for 5 weeks, 50 mg/kg of Levetiracetam, valproic acid 300mg/kg and phenytoin sodium50mg/kg received female wistar ratsfor 3 months had significant adverse effects on bone health and calcium metabolism.(59,60) Long term use of these drugs for more than 3 months had shown significant reduction in bone mineral density, decrease in vitamin-D level and increase the PTH level leading to bone loss,(59,61) The drug induce bone loss is dependent on duration and dosage of the treatment. The extent to which the newer AEDs influence bone metabolism remains to be determined. Conversely an experimental study demonstrated oral administration of valporic acid to epileptic rats for 6 months resulted in a significant increase of bALP, osteocalcin, NTx compared with control group,(62)

A study by Mintzer and colleagues (2010) showed that older men and women receiving valproate, carbamazepine, levetiracetam, phenobarbitone, vigabatrin, lamotrigine and oxcarbazepine had significant increase in bone loss at proximal femur while decreased BMD was observed at the spine and hip. Longitudinal studies evaluated the effects of phenytoin and carbamazepine for 1 year. This study results showed risk of osteopenia and osteoporosis leading to significant effects on bone density with bone loss at the femoral neck.(63)

Recently, few studies have been evaluated the effects of AEDs on bone turnover and increase in these markers of bone turnover has been correlated with the

use of AEDs. Patients treated with classical AEDs such as benzodiazepines and carbamazepine have shown increase in the levels of bone turn over markers like osteocalcin, bALP, carboxy terminal telopeptide of type-I collagen and NTx in 3-6 months of treatment.(64)

A recent longitudinal study in patients aged between 18 to 50yrsreceiving lamotrigine monotherapy was evaluated before and after 6 months of treatment. It indicated that there was increase in the level of PTH and osteocalcin resulting in bone loss by increase in bone formation and it does not lead to osteopenia.(65) In another study reported by Mintzer et al (2006) patients treated with oxcarbazepine monotherapy for 18 months showed increase in the level of BALP, PTH and decrease in vitamin Dlevels. They concluded that act by oxcarbazepine monotherapy may directly affect osteoblasts proliferation.(63,66) In 2011, Imran Ali and his colleagues conducted a study on female patients with migraine receiving topiramate. They evaluated patients bone health by examining biochemical and radiological markers of bone metabolism, in these women with migraine. They noted that 53% of patients were osteopenic, while this was associated with the duration of exposure to topiramate.(67) Another similar study by Heo and his colleagues in 2011, investigated the effect of topiramate on bone mass and metabolism in thirty six (n=36) premenopausal women with epilepsy for a period of one year. These patients were compared with women receiving carbamazepine (n=36), valproate (n=32), and 36 age and sex matched controls. They analyzed BMD and serum for indices of bone metabolism. Their results demonstrated that use of topiramate was associated with lower parathyroid hormone and bicarbonate concentrations along with mild hypocalcemia and increased bone turnover, which suggests that topiramate may have long-term effects on bone.(68)

Moreover, antiepileptic drugs can increase the bone turn over markers based on the duration of the treatment. The treatment of epilepsy adversely affects the bone metabolisms. Some of the newer drugs have exhibited with low effects of bone loss but the studies are remains controversial. One of our own study conducted by Dwajani et al (2015) concluded that in patients receiving levetiracetam and topiramate, for a period of 6 months, did not show any significant change in bone remodeling.(69) It is necessary that more such studies are required on long term basis and further evidence is required on safety of those antiepileptic drugs in future.

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