



BONE TURNOVER MARKERS AND DIAGNOSIS OF OSTEOPOROSIS

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ABSTRACT

Osteoporosis is the most common systemic bone disease and cause of fragility fractures. Bone turnover markers (BTMs) measure the rate of bone remodeling allowing for a dynamic assessment of skeletal status and hold promise in identifying those at highest risk of rapid bone loss and subsequent fracture. Further, the use of BTMs to monitor individuals administered osteoporosis therapy is attractive as monitoring anti-fracture efficacy with bone mineral density has significant limitations. The risk of developing osteoporosis can be assessed by determining the maximum density and strength achieved at maturity (peak bone mass) and the rate and duration of age associated bone loss and the diagnosis of osteoporosis by various bone densitometers for the measurement of Bone Mineral Density (BMD).

Keywords: Osteoporosis, Risk factors, bone turnover markers (BTMs), Diagnosis.

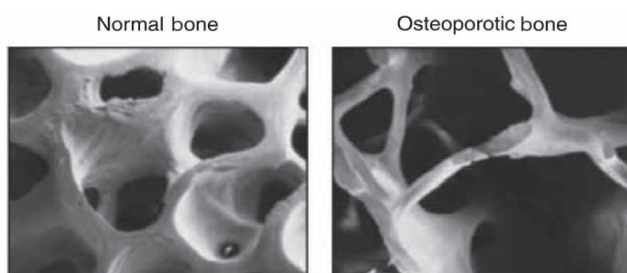
INTRODUCTION

Osteoporosis is a systemic skeletal disease, characterized by low bone mass and disorders in bone micro architecture which lead to increased risk of fractures¹. The bone mass decreases with increasing age and this may be due to increased bone resorption and to decreased bone formation².

Bone is a dynamic tissue, undergoing a continuous process known as bone remodeling. This is a coupled action of osteoclasts and osteoblasts, the former being the cells responsible for Bone resorption and the latter for bone formation. Bone cells participate in the growth, modeling and remodeling of bone although they account for only a small fraction of bone volume.¹ In osteoporosis, this equilibrium of osteoclastic-osteoblastic activity is compromised in favor of osteoclasts³.

Osteoporosis is widely recognized as a major worldwide health problem that affects many millions of people around the world. Osteoporosis affects 50% of females and 30% of males above 65 years of age. In India, about 50% of population above the age of 50 years is supposed to have osteopenia. It is revealed that 10% of people in India above 65 years of age and at least 5 crore people are at risk of osteoporotic fractures⁴.

Figure 1: Differences between Normal and Osteoporotic bone



It is classified as primary (idiopathic), types I and II, and secondary. Type I, It occur in postmenopausal women, characterized by disproportionate loss of trabecular bone with vertebral and distal forearm fractures. In contrast, type II, or senile, is associated with aging due to chronic deficiency of calcium, increase in parathormone activity and decrease in bone formation. The secondary type results of identifiable causes such as inflammatory processes, endocrine changes, multiple myeloma, and the overuse of drugs such as heparin, corticoids and alcohol.

Risk factors for osteoporosis⁵

- Advanced age
- White or Asian race
- Endocrine disorders (gonadal failure, hyperparathyroidism, hyperthyroidism, etc.)
- Excessive alcohol intake
- Family history of osteoporosis
- High/excessive intake of some vitamins and minerals (e.g. mega-doses of vitamin A may inhibit bone growth)
- History of previous fracture
- Impaired calcium and/or vitamin D absorption
- Lack of weight-bearing exercise
- Long-term use of specific drugs/hormones (corticosteroids, excess thyroid hormone, LHRH agonists, hyperprolactinemia, etc.)
- Low calcium and/or vitamin D intake or other minerals/vitamins (e.g., inadequate vitamin K intake)
- Low exposure to sunlight (sunlight activates the endogenous production of vitaminD)
- Low levels of testosterone/estrogen

- Propensity for falls (postural instability, neuromuscular impairment, poor vision, lower limb weakness, drugs that affect blood pressure, etc.)
- Renal disease and smoking.

Characteristically, osteoporosis is referred as a “silent disease” because bone loss occurs frequently without overt symptoms. In fact, most people are not aware of their condition until they experience a fracture. Consequently, social and economic burden of osteoporosis is important, since it expands as an epidemic disease in aging developed countries. Therefore, the disease carries a high cost for the diagnosis, treatment and rehabilitation of fractures^{6,7}. Consequently, osteoporosis constitutes an emerging question and challenge of Health Policy Institutes and understanding people’s knowledge about its risk factors .It is important for the development of the appropriate strategies for osteoporosis prevention and intervention.

BONE TURNOVER MARKERS

Bone turnover markers (BTMs) are emerging as promising tools in the management of osteoporosis as they provide dynamic information regarding skeletal status that is independent from, and often complementary to, BMD measurements.

During the last decade, biochemical markers of bone turnover (BTM) have been developed, that are more sensitive than conventional ones for detecting abnormalities or changes of bone turnover rate⁸. Increased levels of bone resorption, and short-term changes in BTM, have been shown to predict the risk of fracture, independently of the level of BMD in untreated individuals^{9,10}.

Dynamic changes in bone turnover, estimated by measurement of bone biochemical markers, such as breakdown products of type I collagen and proteins secreted by osteoblasts and osteoclasts in blood and urine, can also account for a major portion of antifracture efficacy of anti-resorptive or bone-forming agents¹¹.

Since BTM measurements are non-invasive, fairly inexpensive and can be repeated often, it seems important to provide some guidance about the role BTM can play to identify patients with rapid bone loss, to aid in therapeutic decision-making and to monitor therapeutic efficacy of various treatments.

Most of the currently marketed anti-osteoporosis medications induce prompt and significant changes in BTM. BTMs are valuable for assessing the dynamic nature of bone. When paired with static BMD data, BTMs may enhance the estimation of the future risk of fracture and may independently provide a valuable tool for the monitoring of therapy. BTMs are measured in the urine or serum, with urinary samples requiring correction for creatinine, adding an additional step to most procedures. During the remodelling cycle, active cells synthesize proteins or release degradation products which can be

measured as markers of bone resorption or formation. Bone turnover markers are mainly divided into two types.

1. Bone formation markers and
2. Bone resorption markers

There are 4 markers of bone formation, commonly measured in serum. Procollagen type I carboxy terminal peptide and procollagen type I amino terminal peptide are byproducts of collagen synthesis are shown in (fig. 2). There are also the bone matrix protein osteocalcin and the osteoblast enzyme bone-specific alkaline phosphatase. Serum levels of these markers indicate osteoblastic activity and, therefore, correlate with the rate of bone formation.

There are 6 commonly measured markers of bone resorption: 3 collagen degradation products (hydroxyproline, pyridinoline, and deoxypyridinoline); 2crosslinked telopeptides of type I collagen (amino terminal crosslinked telopeptide and carboxy terminal crosslinked telopeptide); and the osteoclast enzyme tartrate-resistant acid phosphatase. Hydroxyproline is measured in urine, and tartrate-resistant acid phosphatase is measured in serum are shown in (fig. 3); the other markers of resorption can be measured in either serum or urine. All of these markers are associated with osteoclast function and indicate catabolic activity¹².

Figure 2: Bone Formation Markers

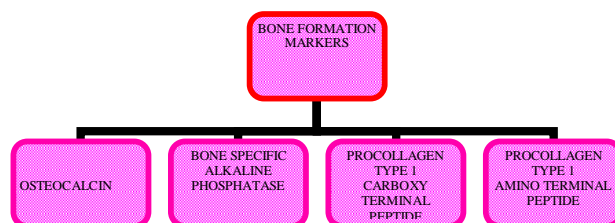
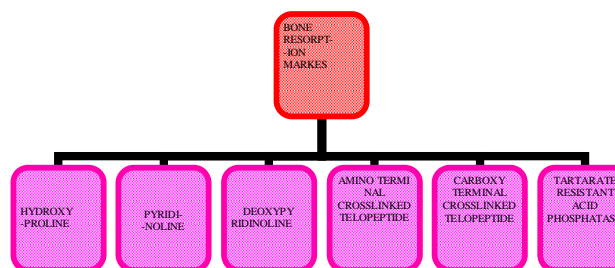


Figure 2: Bone Resorption Markers



BONE FORMATION MARKERS

Osteocalcin:

Osteocalcin, the most abundant non-collagenous protein found in bone. It is small, hydroxyapatite-binding protein synthesized by osteoblasts, odontoblast and to a lesser extent by hypertrophic chondrocytes. It is also known as bone Gla protein (BGP), is a bone specific protein, which has proven to be a sensitive and specific marker of osteoblast activity in a variety of metabolic bone diseases^{13, 14}, is a 49-amino acid peptide containing up to three gamma-carboxyglutamic acid residues which are

responsible for the calcium-binding properties of the molecule. During osteoid synthesis osteocalcin is released by osteoblasts. The precise function of osteocalcin is not known, but it is likely involved in influencing osteoid mineralization and providing negative feedback during the bone remodelling process. Newly synthesized osteocalcin is largely incorporated in the extracellular bone matrix; however a small fraction is released into the circulation providing a marker of bone formation. Osteocalcin is a late marker of osteoblast activity, compared to synthesis of bone alkaline phosphatase and type I collagen. Serum levels of osteocalcin correlates well with the bone formation rate as assessed by both bone histomorphometry and calcium kinetic studies^{15,16}. Osteocalcin is rapidly degraded in vivo and in vitro and the intact molecule and fragments coexist in the circulation. Some osteocalcin fragments are also released during bone resorption^{17,18}.

Procollagen peptides

Type 1 collagen is abundant in osteoblasts and secreted as procollagen into the extracellular space where it is cleaved at both its amino-terminal and carboxy-terminal ends to give rise to N-terminal (PINP) and C terminal (PICP) propeptides of Type I collagen. PINP is released into circulation as a trimetric form which is unstable at 37 °C and is rapidly converted to its monomeric form. In clinical studies of osteoporotic patients, PINP was decreased by up to 80% from baseline with antiresorptive agents^{19,20} or increased by up to 200% from baseline with parathyroid hormone three months after initiation of therapy^{21,22}.

Bone specific alkaline phosphatase

Alkaline phosphatase (ALP) is a ubiquitous ecto-enzyme which catalyzes the hydrolysis of monophosphate ester groups. The total ALP serum pool consists of several dimeric isoforms which originate from various tissues such as liver, bone, intestine, spleen, kidney, and placenta. In adults with normal liver function, approximately 50% of total ALP activity in serum is derived from liver, whereas 50% arises from bone.

There are four genes encoding the isoenzymes — intestinal, placental, germ cell and tissue non-specific (bone/ osteoblast, liver and kidney isoforms); the isoforms result from post-translational glycation and sialation. The bone isoform (BALP) is involved in skeletal calcification by various purported processes — increasing local concentrations of inorganic phosphate, destroying pyrophosphate which inhibits mineral crystallization or acting as a calcium-binding protein or Ca⁺⁺-ATPase. In serum, 95% of ALP is derived from bone and liver, in roughly equal amounts²³.

BONE RESORPTION MARKERS

C-telopeptide

The cross-link telopeptides of type I collagen are derived from specific non-helical amino (N) or carboxy-terminal

(C) regions of the collagen molecule. Serum C-telopeptide of type I collagen (s-CTX) is one of the resorption markers released into circulation as a result of the osteoclast mediated degradation of type I collagen. Bone consists of a calcified organic matrix, which is composed of 90% type I collagen. During bone resorption, molecule of type I collagen is degraded, and small fragments are liberated into the blood-stream. The amino acid sequence EKAHDGGR found in the C-terminal telopeptides of the 1 chain of type I collagen (CTX), which can undergo isomerization, has proven to be a sensitive marker of bone resorption²⁴.

Hydroxyproline

Hydroxyproline (Hyp) constitutes 12-14% of the total amino acid content of mature collagens, but only 10% of Hyp released during bone resorption reaches the urine in free or peptide-bound forms. The most widely used marker of bone resorption was the measurement of hydroxyproline in urine. Peptides containing hydroxyproline are released into urine from the proteolytic breakdown of collagen in bone and other tissues. Since hydroxyproline is one of the abundant amino acids in collagen, its measurement is logical, but hydroxyproline is also found in other proteins and is not specific for collagen in bone. Furthermore, significant amounts of hydroxyproline can be derived from dietary sources of collagen (gelatin), and there is extensive metabolism within the body. Hydroxyproline is considered a nonspecific index of collagen turnover and has been largely replaced by more specific techniques. The hydroxylysine-glycosides are integral parts of bone collagen and occur in two forms: glycosyl- galactosyl-hydroxylysine (Glc- Gal- Hyl) and galactosyl- hydroxylysine (Gal- Hyl). Both components are released into the circulation during collagen degradation and may be measured in urine by high performance liquid chromatography (HPLC). The measurements of hydroxyproline, particularly in early morning fasting urines, have proved to be useful in evaluating responses in trials of new therapies²⁵.

Pyridinoline crosslinks

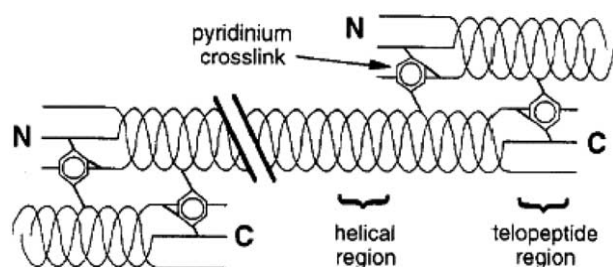
Pyridinoline (Pyr) and deoxypyridinoline (DPyr), also called hydroxylysyl pyridinoline (HL) and lysyl pyridinoline (LP) respectively, are currently receiving considerable attention as the most promising markers of bone resorption. Both are non-reducible crosslinks which stabilise the collagen chains within the extracellular matrix and are formed by the condensation of three lysine and/or hydroxylysine residues in adjacent alpha chains. Both Pyr and DPyr are present in bone, but DPyr appears to be found in significant quantities only in bone collagen, making it a potentially specific and more robust marker for bone resorption.

The free or total amount after acid hydrolysis are usually measured by reverse phase HPLC analysis with detection based on the intrinsic fluorescence of these compounds²⁶. Although these assays remain the reference methods,



these assays are cumbersome and labour-intensive. Fortunately, there has been recent progress in developing immunoassays against the free amino acids or against peptides containing the crosslinks, and these offer considerable hope for producing more rapid and specific assays²⁷.

Figure 4: Fibrils of collagen showing the N- and C terminal ends bonding to helical areas of adjacent fibrils by PYD and DPD.



Tartrate-resistant acid phosphatase:

Tartrate-resistant acid phosphatase (TRAP) is a lysosomal di-iron protein of osteoclasts and mononuclear phagocytes. The enzyme which exists in several forms in different tissues. The type 5 isoenzyme is the one found in osteoclasts, which appear to be released during bone resorption. TRAP is a binuclear iron-containing protein, capable of hydrolysing a wide range of phosphate substrates including nucleotides, aryl phosphates and phosphoproteins including osteopontin and bone sialoprotein²⁸. In addition it has the ability to generate oxygen radicals in the presence of hydrogen peroxide. TRAP activity can be detected in the serum of mammals where it exists as a complex with α_2 -macroglobulin. Enzyme activity is pathologically increased in the serum of patients suffering from conditions involving increased bone resorption, such as Gaucher's disease, Paget's disease, hyperparathyroidism and osteoporosis. Assays of total tartrate-resistant acid phosphatase (TRAP, Tartrate-Resistant Acid Phosphatase) in the circulation are moderately raised in disorders associated with increased bone resorption, but the assays are difficult to perform because of the instability of the enzyme and the relatively small changes observed in pathological states. The development of immunoassays specific for the type 5 form offer the potential of producing better assays for monitoring bone resorption²⁹.

DIAGNOSIS

Bone strength is determined by bone density and bone quality. In the absence of a fracture, the measurement of bone mineral density (BMD) is central to the diagnosis of osteoporosis as there is as yet no satisfactory clinical method to assess bone quality. The diagnosis of osteoporosis relies on quantitative measurement of the bone mineral content of the skeleton also has great importance in osteoporosis research, both in assessing the time of intervention and the effect of treatment.

Clinical trials, conducted since the early nineties, have unequivocally shown the ability of several anti-

osteoporosis medications to reduce fracture occurrence at various skeletal sites, including but not exhaustively the spine and hip³⁰. Whereas the operational definition of osteoporosis is still based on a low bone mineral density (BMD), because low BMD is known to contribute to increased fracture risk, changes in bone mass and density, in response to anti-resorptive therapy, account for only a small portion of the predicted fracture risk reduction [30]. BMD at the femoral neck provides the reference site. It is defined as a value for BMD 2.5 SD or more below the young female adult mean (T-score less than or equal to -2.5 SD). The bone mineral content or density may be determined at focal, axial, or appendicular skeletal sites (e.g., forearm, heel, spine), or in the total skeleton. All techniques have limitations that hamper measurement of apparent changes in bone mass³¹.

Over the last two decades, a variety of techniques have been developed, including Dual energy absorptiometry (isotope-based and X-ray based), quantitative computed tomography (QCT), and ultrasound.

Dual energy x-ray absorptiometry:

Dual energy X-ray absorptiometry (DXA) is a method of measuring bone mineral density (BMD). Dual X-ray beams each with different energy levels are directed at the patient. DXA provides a two dimensional measurement of the three dimensional skeleton and it is from this two dimensional representation, acquired during the scan, that a density can be estimated. The technique provides a measure of bone mineral content (BMC) and bone area (BA), which can then be used to calculate "areal" bone mineral density (aBMD) as BMC/BA, i.e. the amount of bone mineral per unit area (in g/cm²)

The trabecular bone in the spine seems to disintegrate faster just after the menopause than the peripheral bone. Since many osteoporotic women have spinal fractures, it is of high clinical value to assess spinal bone mass. It is complicated to determine bone mass in the spine because of the low ratio of bone to soft tissue, the varying thickness and composition of the soft tissue, and the irregular configuration of the vertebrae-but with dual energy X-ray absorptiometry (DEXA), precision errors of only 1%-2% are obtained. This makes the equipment suitable for measuring bone mass in the individual patient. The accuracy error of spine measurements using DEXA is about 10%. DEXA may be used to determine total body bone mass. Such measurements may have major interest for scientific purposes, but may only be justified in a subset of patients with diseases of Calcium metabolism³².

Quantitative X-ray computed tomography

Single and dual energy QCT (DQCT) is the only method measuring purely trabecular bone of the vertebral spongiosum or at other sites. This is in contrast to DPA and DEXA, which measure an integral of compact and trabecular bone. QCT provides a precision error of 2%-5%, and accuracy errors of at least 10%. DQCT uses the same



principle as DPA and DEXA, namely that the difference in attenuation between soft tissue and bone will be greater at a low photon energy than at a high one. DQCT provides a better accuracy than QCT; however, the precision error increases to approximately 10%. The relatively large radiation doses of QCT and DQCT make it difficult to perform repeated measurements. It is not clear whether measurement of integral vertebral bone mass or of only the trabecular core gives the best estimate of fracture risk³³.

Ultrasound

Ultrasound-based techniques for bone mass measurements in the patella and heel are still being investigated, but data suggest that if the method is optimized it will be useful in the diagnosis of osteoporosis. The use of ultrasound to measure bone properties both in vitro and in vivo studies seems to provide additional information on bone structure, elasticity, and bone strength. Two of the most commonly quoted parameters are broadband ultrasound attenuation (BUA) and the speed of sound (SOS), also known as ultrasonic transmission velocity (UTV). Quantitative ultrasound parameters have been found to be highly correlated with structural and architectural parameters, such as trabecular volume, trabecular number, strength, and load-bearing capacity.

Osteoporosis is diagnosed by above bone densitometers, which determines whether the bone density is sufficiently low to make the patient at risk for fracture. This approach has the advantage of leading to a diagnosis before the morbidity of a fracture ensues, thereby allowing the use of preventive therapy. The major problem with this diagnostic procedure is that it is not available to all individuals over 50 years of age, despite the fact that 40% of women who are over 50 years of age are destined to develop an osteoporotic fracture. Moreover, because of the costs, it seems unlikely that there would be universal screening with bone densitometers to diagnose osteoporosis in the foreseeable future³⁴.

CONCLUSION

Further, the use of BTMs to monitor the efficacy of osteoporosis therapies holds promise. Markers of bone resorption and bone formation may help to assess and assign fracture risk and to monitor the effects of osteoporosis therapy. It is important to remember that BTMs provide an estimate of skeletal turnover for the entire skeleton and that there are other organs that often contribute to a specific BTM, in addition to the proportion attributed to skeletal turnover. Data from the literature indicate that biochemical markers of bone turnover are significantly correlated with fracture risk. As awareness of the importance of osteoporosis increases, knowledge gained from research has allowed this problem to be addressed on many fronts. Although the challenge is to improve on the effectiveness of diagnostics.

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