

Bone turnover markers: Emerging tool in the management of osteoporosis

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ABSTRACT

Bone is a dynamic tissue which undergoes constant remodeling throughout the life span. Bone turnover is balanced with coupling of bone formation and resorption at various rates leading to continuous remodeling of bone. A study of bone turnover markers (BTMs) provides an insight of the dynamics of bone turnover in many metabolic bone disorders. An increase in bone turnover seen with aging and pathological states such as osteoporosis leads to deterioration of bone microarchitecture and thus contributes to an increase in the risk of fracture independent of low bone mineral density (BMD). These microarchitectural alterations affecting the bone quality can be assessed by BTMs and thus may serve as a complementary tool to BMD in the assessment of fracture risk. A systematic search of literature regarding BTMs was carried out using the PubMed database for the purpose of this review. Various reliable, rapid, and cost-effective automated assays of BTMs with good sensitivity are available for the management of osteoporosis. However, BTMs are subjected to various preanalytical and analytical variations necessitating strict sample collection and assays methods along with utilizing ethnicity-based reference standards for different populations. Estimation of fracture risk and monitoring the adherence and response to therapy, which is a challenge in a chronic, asymptomatic disease such as osteoporosis, are the most important applications of measuring BTMs. This review describes the physiology of bone remodeling, various conventional and novel BTMs, and BTM assays and their role in the assessment of fracture risk and monitoring response to treatment with antiresorptive or anabolic agents.

Key words: Bone turnover markers, osteoporosis, postmenopausal women

INTRODUCTION

Osteoporosis is the most common metabolic bone disorder characterized by a structural deterioration of bone tissue leading to an increased risk of fracture. Being an important public health problem of the elderly, it is expected to rise with an increased life span. India is one of the leading countries affected by osteoporosis, with one out of two Indian women above the age of 50 years and one out of five Indian men above the age of 65 years at risk of osteoporosis.^[1,2]

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Osteoporosis being a silent disease in many situations may present with a dreaded complication such as hip fracture with its associated increased morbidity and 4 times higher mortality in the elderly population.^[3]

Bone mineral density (BMD) assessment using dual-energy X-ray absorptiometry (DXA) scan is the current gold standard test for the diagnosis of osteoporosis.^[4] However, about 50% of women who had sustained osteoporotic fracture have BMD above the WHO definition of osteoporosis.^[5] DXA scan also has its own limitations being a static measure and an expensive investigation with limited availability in the many parts of India. Hence, there is a need for estimating the fracture risk using web-based tools

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such as FRAX which assess other risk factors in addition to BMD.^[6] However, FRAX does not incorporate all risk factors such as those associated with falls, markers of bone turnover, region-specific risk entities such as lower dietary calcium intake and Vitamin D deficiency.

Molecular markers of bone metabolism are novel tools which detect the dynamics of bone remodeling with respect to bone formation and resorption. The wider availability of reliable, cost-effective, sensitive, and specific assays for bone turnover markers (BTMs) would complement the measurement of BMD in the management of osteoporosis, especially in the follow-up of the patients who had been on antiresorptive or bone formation therapies.

BONE REMODELING – PHYSIOLOGY OF BONE TURNOVER MARKERS

Bone is a metabolically active structure which undergoes continuous remodeling throughout life. After attaining peak bone mass, bone undergoes constant remodeling through bone resorption followed by formation sequentially at basic multicellular unit of bone called “Bone remodeling unit.” Various biomolecules released into the circulation during bone resorption and formation are called BTMs [Figure 1].^[7] Under optimal physiological conditions, bone resorption takes place in around 10 days and bone formation takes about 3 months. Up to 20% of the skeleton, it may be replaced by remodeling every year.^[8]

The biochemical markers currently available for the assessment of bone turnover include enzymes and nonenzymatic peptides derived from the cellular and noncellular compartments of bone.

The BTMs are grouped into two categories based on the metabolic phase during which they are produced as:

1. Bone formation markers
2. Bone resorption markers.

International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine has proposed serum CTX-1 (sCTX) and serum P1NP to be used as reference markers of bone resorption and formation, respectively, for the assessment of fracture risk and monitoring therapy in clinical settings.^[9]

MARKERS OF BONE FORMATION

Bone formation markers are products of active osteoblasts expressed during different phases of their development and are considered to reflect different aspects of osteoblast

function and bone formation. All markers of bone formation are measured in serum or plasma.

Bone formation markers are categorized as:

1. By-products of collagen synthesis: Propeptides of type 1 collagen: (C-terminal: P1CP, N-terminal: P1NP)
2. Osteoblast enzymes: Alkaline phosphatase (ALP) (total and bone-specific)
3. Matrix proteins: Osteocalcin (OC).

PROCOLLAGEN TYPE I PROPEPTIDES

Procollagen Type I N-terminal propeptide (PINP) and procollagen type I C-terminal propeptide (PICP) are peptides derived from posttranslational cleavage of type I procollagen molecules by proteases at N- and C-terminal, respectively. PINP and PICP originate predominantly from proliferating osteoblasts and fibroblasts with small contributions from skin, tendon, dentin, and cartilage.^[10] PINP is preferred for clinical use as a marker of bone formation to PICP as PICP, unlike PINP, is cleared by the mannose receptor, which in turn can be regulated by growth hormone and thyroid hormones, thus complicating the interpretation in patients with pituitary or thyroid dysfunction. P1NP exists in serum as trimeric or monomeric form. Immunoassays detect either trimeric (automated IDS ISYSS assays) or both forms which are otherwise called as total P1NP assays (automated Roche Elecsys assay).^[11]

P1NP is proposed as a reference bone formation marker by IOF in view of its predictable response to treatment and the reliability of P1NP assays as evidenced by low intra-individual variability, smaller circadian variation, stability at room temperature, and a good assay precision.^[9]

SERUM ALKALINE PHOSPHATASE (TOTAL AND BONE-SPECIFIC ALKALINE PHOSPHATASE)

ALP is a ubiquitous, membrane-bound tetrameric enzyme present in the plasma membrane of the osteoblasts. It plays an important role in osteoid formation and mineralization by enzymatic degradation of the inhibitor of mineralization, pyrophosphate at an alkaline pH.^[12] ALP is the first BTM to be used in both clinical and research setting. Several isoforms of ALP have been identified in liver, intestine, placenta, and bone. Unlike the hepatic isoenzyme which is heat stable, that of bone origin is thermolabile.

OSTEOCALCIN

OC is a hydroxyapatite-binding protein exclusively synthesized by osteoblast, odontoblasts, and hypertrophic chondrocytes. It is also called as the bone GLA protein

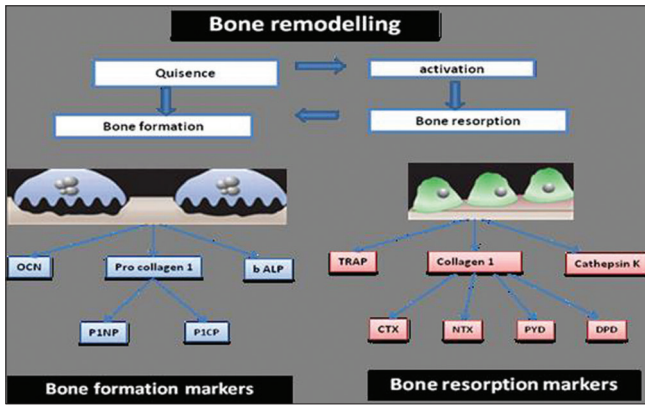


Figure 1: Bone turnover markers released during bone formation and resorption phase of bone remodeling

and constitutes 15% of the noncollagenous bone matrix.^[13] Mineral-binding of OC requires γ carboxylation of three glutamate residues of OC. The undercarboxylated OC has been shown to have a negative correlation with hip fracture in elderly women.^[14]

Being a late marker of osteoblastic activity, it has been used as a bone formation marker but is limited by its short half-life, unstable intact molecule, and the influence of Vitamin K status, renal function, and circadian rhythm. OC has been found to be a useful biomarker in steroid-induced osteoporosis.^[15]

MARKERS OF BONE RESORPTION

These markers which are formed during the bone resorption phase of bone remodeling include byproducts of osteoclasts activity released during bone resorption.

The bone resorption markers are categorized as follows:

1. Collagen degradation products:
 - Telopeptides of type 1 collagen (C-terminal: CTX-1 and CTX-matrix metalloproteinases [MMP], N-terminal: NTX-1)
 - Hydroxyproline
 - Pyridinium crosslinks (pyridinoline [PYD], deoxypyridinoline [DPD])
2. Noncollagenous proteins:
 - Bone sialoprotein
3. Osteoclastic enzymes:
 - Tartrate-resistant acid phosphatase
 - Cathepsin K
4. Osteocyte activity markers:
 - Receptor activator of nuclear factor kappa-B ligand (RANKL)
 - Osteoprotegerin (OPG)
 - Dickkopf-related protein 1
 - Sclerostin

CARBOXY TERMINAL CROSSLINKED TELOPEPTIDES OF TYPE 1 COLLAGEN (CTX-BETA CROSS LAP)

CTX are degradation products of Type 1 collagen of bone generated by the activity of the enzyme cathepsin K. The native CTX exists in two forms: α and β isomerized forms. These isomerized forms undergo further isomerization to form D and L forms. The spontaneous β isomerization of α isoforms occurs with protein aging. Hence, the altered ratio of α and β isomerized forms occurs with new bone formation as in physiological conditions such as growing children and pathological conditions such as malignant bone diseases and Paget's disease of bone and also in patients on parathyroid hormone treatment.^[16,17]

The sCTX1 has been recommended as reference bone resorption marker by IOF. The major problem with CTX measurement is its circadian variation, with peak in the second half of night and nadir in the afternoon. A study looking at circadian variation of CTX by Hannon and Eastell showed that the peak levels of CTX were seen at 05.00 h and of nadir were seen at 14.00 h.^[18,19]

Studies have also looked at the effect of food intake on CTX measurement and found lower postprandial levels by 20% as compared to the fasting state. Hence, to reduce this preanalytical variability, it is recommended to collect the sample in the morning after the overnight fast.^[20]

CARBOXY TERMINAL CROSSLINKED TELOPEPTIDES OF TYPE 1 COLLAGEN – MATRIX METALLOPROTEINASES

Crosslinked C-terminal telopeptide (CTX-MMP) is a variant a CTX which is generated by the cleavage of type 1 collagen by MMP at neutral PH. CTX-MMP may differ from CTX-I levels at presentation and during follow-up of different metabolic bone disorders and is not used as commonly as CTX-I.^[21]

AMINO TERMINAL CROSSLINKED TELOPEPTIDES OF TYPE 1 COLLAGEN (NTX)

NTX are generated from the amino terminus of the type 1 collagen by cleavage of N-terminal region by cathepsin K during the resorption phase of bone turnover. NTX is measured using monoclonal antibody against specific N-terminal epitope. This can be measured both in the serum and urine. NTX measurements are altered in liver and renal failure. Urine NTX exhibits less circadian and

postprandial variability as compared to CTX. However, the 24 h urine collection is more cumbersome.^[22]

3-HYDROXYPYRIDINIUM CROSSLINKS OF COLLAGEN PYRIDINOLINE AND DEOXYPYRIDINOLINE

PYD and DPD are covalent pyridinium compounds formed during the extracellular maturation of fibrillar collagens. During bone resorption, these crosslinked collagens are released into the circulation when mature Type 1 collagen is proteolytically degraded for mechanical stabilization of the molecule. As their measurements are not influenced by the degradation of newly synthesized collagens, their levels strictly reflect the degradation of mature, i.e. crosslinked collagens. In addition, the urinary excretion of pyridinium crosslinks is independent of dietary sources as they are not absorbed from the gut and exhibit high specificity for skeletal tissues. While PYD is found in cartilage, bone, ligaments, and vessels, DPD is almost exclusively found in bone and dentin. These can be measured in 24 h urine collection or as creatinine corrected spot urine measurements. Thus, the pyridinium crosslinks are currently viewed as one of the good indices for assessing bone resorption.^[23]

TARTRATE-RESISTANT ACID PHOSPHATASE (TRAP)

Tartrate-resistant acid phosphatase isoform 5b (TRAP5b) are isoform of acid phosphatase which is resistant to degradation by tartrate, cleaved by protease into isoform 5b, most specifically expressed in the ruffled border of osteoclast and cleaves type 1 collagen into fragments during bone resorption. Mainly used in research settings, it is typically increased in high bone turnover conditions, such as Paget's disease, bone metastases, multiple myeloma, and after ovariectomy.^[24]

CATHEPSIN K

Cathepsin K is an osteoclastic enzyme, cysteine proteinase present at active osteoclast ruffled border, which is a specific biomarker of bone resorption.^[24] Due to the fact that cathepsin K is expressed and secreted by osteoclasts during active bone resorption, cathepsin K, and specifically its circulating form, it may be a useful and specific biochemical marker of osteoclastic activity.^[25]

RECEPTOR ACTIVATOR OF NUCLEAR FACTOR KAPPA-B LIGAND

RANKL are osteocytes markers which reflect bone microenvironment. They are produced by osteoblasts and

activated by B and T cells and bind to RANK expressed on osteoclasts and their precursors stimulating their differentiation and activity. These are novel biomarkers of bone resorption currently used in research setting to study the efficacy, safety, mechanism, and mode of action of drugs used in osteoporosis and other metabolic bone diseases.

OSTEOPROTEGERIN

OPG is another novel biomarker of bone turnover which are markers of osteocyte activity and reflect bone microenvironment. They are synthesized by osteoblasts and act as decoy receptor to RANKL. They prevent osteoclastogenesis by binding to RANK and thus reduce bone resorption. They are used in research settings and require further clinical and analytical validation for clinical use.

DICKKOPF-RELATED PROTEIN 1 AND SCLEROSTIN

These novel biomarkers are markers of osteocyte activity used in research settings. These are secreted by osteocytes and inhibit bone formation by inhibiting WNT signaling by binding to LRP-5 in the osteoblasts.^[26]

BONE TURNOVER MARKER ASSAYS

Automated and manual immunoassay and multiplex microarrays are available for the analysis of BTMs for clinical and research purposes. Various methods commonly used for the measurements of BTMs include radioimmunoassay, immunoradiometric assays, enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassay, and electrochemiluminescent immunoassay. As BTMs assays are prone to analytical variations, uses of automated standardized assays according to international reference standards are recommended.

FACTORS DETERMINING PREANALYTICAL VARIABILITY OF BONE TURNOVER MARKERS

Most BTMs exhibit significant within-subject variability. This poses a major problem in the practical use of bone markers: Whenever a change in a bone marker level is observed in an individual patient (e.g., following an intervention), this change must be interpreted against the background of the respective marker's variability. Various factors determining preanalytical variability of bone turnover markers are shown in Table 1.^[27] Therefore, knowledge of the sources of variability and the strategies

Table 1: Factors determining preanalytical variability of BTMs**I. Modifiable factors**

Circadian variation
 Seasonal variation
 Fasting and food intake
 Physical activity
 Menstrual variation

II. Non modifiable factors

Age
 Sex
 Menopausal state
 Vitamin D deficiency
 Secondary hyperparathyroidism
 Chronic renal disease
 High bone turnover diseases: thyrotoxicosis, primary hyperparathyroidism, Acromegaly, bone metastasis, pagets disease
 Low bone turnover diseases: hypothyroidism, hypoparathyroidism, hypopituitarism, GH deficiency
 Dissociation between bone formation and resorption: cushing's disease and multiple myeloma
 Recent fracture
 Chronic diseases with limited mobility: stroke, paraplegia, hemiplegia, dementia, schizophrenia, depression
 Drugs: corticosteroids, oral contraceptives, aromatase inhibitors, antiepileptics, heparin, thiazolidinediones, vitamin K antagonist

used to cope with them are essential for the meaningful interpretation of bone markers.

In view of analytical and preanalytical variability of BTMs assays, appropriate sample collection and storage conditions are recommended. To reduce the biological variability, samples must be collected in the morning after an overnight fast. Subsequent serial measurements must be done on the same time of the day and preferably the same season to account for the seasonal variation.

Changes in the BTMs must be large to monitor clinical response in view of biological and analytical variations. While interpreting the BTM response, “least significant change” (LSC) for each BTM must be utilized which is derived by multiplying the each BTMs precision error provided by the laboratory by 2.77 (95% confidence interval).^[28]

ETHNICITY SPECIFIC REFERENCE RANGE FOR BONE TURNOVER MARKERS

Genetic, epigenetic, and environmental factors play an important role in determining peak bone mass as well as rate of bone loss of an individual.^[29] Studies have shown ethnicity based variations in the distribution of BTMs and the need for the establishment of ethnicity-specific reference range for each BTMs for clinical use in different population.^[30,31]

BONE MARKERS IN THE ASSESSMENT OF FRACTURE RISK

The high BTMs may predict the risk of sustaining osteoporotic fractures in postmenopausal women independent of BMD. Increased bone turnover leads to the deterioration of bone microarchitecture and thus contributes to increased risk of fracture in addition to low BMD. This microarchitectural alteration affecting bone quality can be assessed by BTMs and thus serves as complementary tool to BMD which can only assess the bone mass in fracture risk assessments. Increased BTMs suggest a decline in the structural integrity of bone as the newly synthesized bone may be less mineralized with decreased posttranslational modification in terms of decreased beta crosslinks and beta isomerization of the type 1 collagen.^[32,33]

Garnero *et al.* studied 435 healthy untreated younger postmenopausal women aged 50–89 years (mean, 64 years) from OFELY cohort comprising 1039 women age 31–89 years of age (OFELY study). Baseline bone markers were compared in 55 cases (women who had sustained fractures, 20 vertebral and 35 peripheral fractures) and 380 controls followed up for 5 years. Two-fold increased risk of fracture was seen in women with BTMs in highest quartile with relative risk (RR) of 1.8% (1–3.4) for urinary free PYD, 1.7 (0.9–3.2) for urinary NTX (uNTX), 2.3 (1.3–4.1) for urinary CTX, 2.1 (1.2–3.8) for sCTX and 2.4 (1.3–4.2) for serum bone alkaline phosphatase.^[34]

Another nested case–control study from The Netherlands by van Daele *et al.*, the urinary pyridinium crosslinks which included total pyrinoline, free pyrinoline, total deoxypyridinoline, and free DPD showed a significant association with hip fracture risk with age-adjusted RR of 3.3, 3, 2.2, and 1.8 respectively.^[35]

Vergnaud *et al.* studied 104 subjects with hip fracture versus 255 controls from a cohort of 7598 postmenopausal women over 75 years of age. They found that the undercarboxylated OC measured by ELISA predicted the risk of fracture with an odds ratio (OR) of 1.9 (1.2–3.0), which persisted even after adjusting for femoral neck BMD and mobility (adjusted OR - 1.8 [1.0–3.0]).

In another nested case–control study from EPIDOS cohort of postmenopausal women, which included 115 fracture subjects as cases and 293 controls, sCTX samples collected in the afternoon had a significant prediction of fracture, with relative hazard of 1.86 (1.01–3.76), unlike whole group CTX which was not predictive.^[36]

A study from Finland also showed that in addition to total and carboxylated OC, lower ratio of carboxylated to total serum OC also had a significant association with the risk of fractures (hazard ratio: 5.32 [3.26–8.68]).^[37]

BONE TURNOVER MARKERS AND OSTEOPOROTIC TREATMENT MONITORING

BTMs not only provide information on the ongoing bone remodeling but also provide pharmacodynamic information on the therapeutic response to osteoporosis treatment in an individual, thus aid in the optimization of therapy. They are mainly useful to assess the patient's compliance to medication in the chronic, asymptomatic diseases such as osteoporosis, where drug compliance may be a major issue.

Monitoring compliance and adherence to treatment represent a potentially useful application of BTMs, particularly in the case of bone resorption inhibitors. Adherence to oral medications is notoriously poor and represents one of the major challenges to reducing the incidence of fractures in the elderly. This problem is particularly relevant for the oral bisphosphonates which must be taken following a strict dosing procedure, and the introduction of weekly and monthly oral formulations has only slightly improved adherence or persistence. Thus, BTMs could be useful in identifying a less than expected suppression of bone turnover with a given treatment, which may suggest either persistence failure (i.e., the patient has discontinued treatment) or that the patient has not been fully compliant with the drug dosing regimen. Therefore, BTMs could be used to monitor biologic efficacy or adherence to treatment although this of course implies that BTMs be determined before initiation of treatment and during subsequent follow-up.

In a multinational prospective, open-label, cluster-randomized study of postmenopausal women (IMPACT study), uNTX and sCTX levels were assessed at baseline and weeks 10 and 22 of treatment with risedronate 5 mg/day in 2302 women and it was found that the responses beyond LSC in BTMs (uNTX and sCTX) and spine BMD were associated with a reduced risk of nonvertebral fractures (NVFs) and all fractures. The incidence of NVF was about 50% lower in patients with reductions of uNTX of 30% or more at 22 weeks (1.6%) than in those with < 30% reduction (3.2%, $P = 0.015$).^[38]

Chen *et al.* studied changes in five BTMs in a subset of women who received daily teriparatide therapy for postmenopausal osteoporosis enrolled in the Fracture Prevention Trial. Significant correlation was found between Lumbar Spine BMD response and BTMs with correlation coefficients of 0.41 for PINP, 0.40 for NTX, 0.36 for PICP, 0.28 for bone

ALP, and 0.23 for DPD. Among these, PICP increase at 1 month and PINP at 3 months correlated best with increases in LS BMD at 18 months (0.65 and 0.61, respectively; $P < 0.05$).^[39] Changes in BTMs have also been described with other antiosteoporosis treatments such as raloxifene and strontium.^[40,41]

This strong association of BTMs with fracture risk reduction in various studies on osteoporosis treatment complements the use of BTMs along with the assessment of BMD in the management of osteoporosis.

Limitations of bone turnover markers

- Preanalytical and analytical variability
- Inadequate appreciation of sources of variability of each BTMs
- Lack of standardization of the assays for BTMs
- Ethnic variations of BTMs and lack of ethnicity based reference interval for each population
- Nonavailability of data on response of various BTMs to different osteoporosis treatment and comparison between them.

CONCLUSION

BTMs are important tools for management of osteoporosis that are gaining acceptance in clinical practice worldwide. Estimation of fracture risk based on bone remodeling rates and monitoring the adherence and response to therapy is the most important application of BTMs. Large epidemiologic studies have demonstrated BTMs as an independent contributor to fracture risk. Understanding the biological and preanalytical variations and availability of reliable, rapid, cost-effective and standardized BTMs assays may help in better utilization of BTMs in the management of osteoporosis.

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Conflicts of interest

There are no conflicts of interest.

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