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Bone Turnover Markers and their Utility in Osteoporosis

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ABSTRACT

Osteoporosis is a major health problem worldwide and is projected to increase exponentially due to the aging of the population. Fractures, the most dreaded complication of bone disease are associated with high co-morbidity and mortality. The consequent public health and socioeconomic burden thus warrant timely diagnosis, treatment and follow-up of these disorders. Biochemical markers of bone turnover are convenient as the changes in their levels readily reflect bone physiology. Currently literature data advocate bone biomarkers as best used in monitoring anti-osteoporosis therapy efficacy and compliance, however there is abundant data supporting their role in predicting bone loss and fracture risk. Although dual energy x-ray absorptiometry (DEXA) is the gold standard to assess bone mass it does not account for fracture risk. Bone mineral density (BMD) measurement by DEXA has its own limitations being invasive, expensive and un- availability of machines at the primary level. Moreover changes in BMD measurements are static, reflecting at a particular site and observed after a year unlike changes in the bone turnover makers (BTMs) which occur within months of therapy. Due to these limitations, measurement of biomarkers from blood samples is an attractive alternative to evaluate bone turnover. The pre and post-analytic implications of bone turnover markers is mandatory in correct interpretation of test results. This mini review summarizes the most widely used BTMs, a look at more novel markers and discusses their strengths, weaknesses, feasibility of development of immunoassays, pre analytical issues and their clinical utility in the management of osteoporosis.

Keywords: Bone turnover markers, Hormones, Cytokines, Osteoporosis

Abbreviations: BMD: Bone Mineral Density; BSAP: Bone-Specific Alkaline Phosphatase; BTM: Bone Turnover Maker; Cr: Creatinine; CTX: C-Terminal Telopeptide of Type I Collagen; NTX: N-Terminal Telopeptide of Type I Collagen; DEXA: Dual Energy X-Ray Absorptiometry; DPD: Deoxypyridinoline; E1G: Estrone Glucuronide; ELISA: Enzyme Linked Immunosorbent Assay; FSH: Follicle Stimulating Hormone; HRT: Hormone Replacement Therapy; IL: Interleukin; OB: Osteoblast; OC: Osteoclast; OPG: Osteoprotegerin; OSC: Osteocalcin; PICP: C-Terminal Propeptide of Type I Collagen; PINP: N-terminal Propeptide of Type I Collagen; POF: Premature Ovarian Failure; PTH: Parathyroid Hormone; PyD: Pyridinoline; RANK: Receptor Activator of Nuclear Factor-Kappa B; RANKL: Receptor Activator of Nuclear Factor-Kappa B Ligand; TRAP: Tartrate-Resistant Acid Phosphatase

INTRODUCTION

Bone remodeling requires a precise balance between resorption and formation. It is a complex process that involves numerous factors: hormones, growth factors, vitamins, and cytokines osteoprotegerin (OPG) and receptor activator for nuclear factor-B (RANK) ligand. The signaling pathway OPG/RANK/RANKL is a key to regulation for maintaining the balance between the activity of osteoblasts (OBs) and osteoclasts (OCs) in order to prevent bone loss and ensure a normal bone turnover. Since all metabolic bone diseases usually present with alterations in OBs and OC activity, bone markers reflecting these activities mimic real bone turnover. Any imbalance in their functioning ultimately disrupts bone turnover which is a characteristic feature in metabolic bone disease [1].

BTMs are a series of protein or protein derivatives released during bone remodeling by OBs or OCs, which offer

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prognostic information on fracture risk with radiographic measures of bone mass [2,3]. Although DEXA to measure (BMD) is the gold standard, it is well known that decrease in bone mass does not solely account for fracture risk [4,5]. However, one has to take into account the large number of pre analytical factors and comorbid clinical conditions influencing the bone biomarkers. BTMs respond rapidly to changes in bone physiology, hence they have utility in determining patient response and compliance to therapies for osteoporosis [6]. Furthermore, they have widespread clinical utility in osteoporosis, renal osteodystrophy, and certain oncological conditions and rheumatic diseases [7-9].

The review highlights our studies on some of the prominent bone biomarkers, establishment of reference Indian database, clinical and diagnostic utility of these biomarkers, their analytical variability and development of assays with utility in assessment of bone health from Indian perception.

BONE TURNOVER MARKERS

BTMs are collagen degradation products or enzymes reflecting OC activity or collagenous and non-collagenous proteins produced by the OBs. All these markers can be quantitated from blood samples, serum being the preferred sample of choice. The most commonly used bone markers are listed in **Table 1**.

Table 1. Bone turnover markers.

Bone Formation Markers	Bone Resorption Marker
Osteocalcin	C-terminal telopeptide of Type I collagen crosslinks
Bone Specific Alkaline Phosphatase	N-terminal telopeptide of Type I collagen crosslinks
Carboxy terminal propeptide of Type I collagen	Pyridinoline
Amino terminal propeptide of Type I collagen	Deoxypyridinoline
	Tartarate Resistant Acid Phosphatase

BONE FORMATION MARKERS

Osteocalcin (OSC)

OSC the most abundant non-collagenous protein of 49 amino acids, secreted by the OBs also known as bone gamma-carboxyglutamic acid-containing protein with 3 glutamic acids at positions 13 17 and 20 that undergo gamma carboxylation in a vitamin-K dependent fashion. Now considered as a bone turnover marker OSC reflects both formation and resorption. Recently OSC has gained importance due to its role as a bone derived hormone influencing male fertility, glucose metabolism and its actions on the central nervous system and muscle in animal experiments [10-12]. Assays for intact OC are available, however, there have been reports indicating the development of new immunoassays detecting the more stable N-mid terminal region of OSC [13].

Bone-specific alkaline phosphatase (BSAP)

BSAP is a sensitive marker in monitoring disease progress in patients suffering from Paget's disease. Due to its cross reactivity with liver alkaline phosphate BSAP measurements have limited applicability.

N- and C-terminal propeptides of type I collagen (PINP, PICP)

OBs secrete type I collagen as a procollagen which form a triple helix (containing two identical α1-chains and a α2-chain) that contain the N- and C-terminal propeptides (PINP and PICP). These propeptides are immediately cleaved, eventually entering the blood circulation as N- and C-terminal propeptides - the bone formation markers. The cleaved products initially in trimeric form eventually break down to the monomeric form in the circulation. The trimeric PINP is cleared by hepatic uptake, while the monomeric form is cleared via the kidneys. The monomeric and trimeric forms (total PINP) or only the trimeric form (intact PINP) are measured by assays [14,15].

BONE RESORPTION MARKERS

Collagen crosslinks (Pyd, DPD)

Urinary pyridinoline (Pyd) and deoxypyridinoline (DPD) are more specific markers of bone resorption. Pyd are cross-linking amino acids that strengthen collagen fibrils in the extracellular matrix. DPD is abundant in bone collagen as a selective bone marker [16]. Since Pyd is not metabolized and excreted as small peptides during bone resorption, immunoassays developed to date selectively measure cross-link-containing peptide fragments. However, urinary hydroxyproline, the oldest test of bone resorption, is no longer used lacks specificity as it comes from other tissues [17].

C- and N-terminal telopeptide of type I collagen (CTX, NTX)

OC derived tartrate-resistant acid phosphatase (TRAP) and cathepsin K breakdown the bone matrix, including the triple helices of the mature type I collagen, to release CTX and NTX during bone resorption. CTX fragments are either non-

isomerized (α) or β-isomerized. β-CTX is considered better marker of bone resorption as it is associated with aging bones. Both CTX and NTX can be measured from urine as well as serum samples [18]. However, serum NTX cannot be used for diagnosis of osteoporosis. Though TRAP, PyD and DPD, hydroxyproline and bone sialoprotein measure OC activity they have been superseded by the more sensitive and specific CTX. Thus CTX has gained popularity as it can be measured from blood samples on automated platforms and has turned out to be the biomarker of choice [19,20].

PRE-ANALYTICAL AND ANALYTICAL CONSIDERATIONS

Normal serum levels of BTMs are high in children, multifold in adult population and decline in the elderly. They increase in women following menopause indicating a high bone turnover and are usually higher in men as compared to women [21]. Moreover, Caucasians have lower levels compared to their age and sex matched adult counterparts, making ethnic background of the patient essential while determining their levels. BTM levels are elevated during pregnancy and lactation and tend to normalize after a few months following weaning [22]. Marked elevations in the levels are seen in immobilized or bedridden patients following a fracture. Patient with concomitant comorbidities such as primary hyperparathyroidism, Paget's disease, multiple myeloma and metastatic prostate and breast cancer and abnormal kidney function usually present with higher levels, particularly PINP, CTX and OSC which mainly undergo renal clearance [23,24]. BTMs are quite sensitive to issues pertaining to urine sample collection. Hence, blood is the preferred mode of sample collection [25].

Clinicians using BTMs for management of osteoporosis should be aware of the factors that influence their levels. BTM levels vary due to endogenous factors such as circadian rhythm, phase of the menstrual period and exogenous factors such as seasonal variation, physical exercise and diet. Bone formation markers are elevated following ovulation, i.e., during the luteal phase while resorption markers are elevated during the follicular phase of the menstrual period [26,27]. Resorption markers follow a circadian rhythm where the peak levels are observed in early morning hours which taper off during the day. Thus, factors that influence assay results such as diurnal variability, Ca intake and sample handling needs to be taken into consideration. Although these markers have been used in research for a long time they are now being recognized as tools in the clinical management of bone disease [28,29]. In this review we highlight our earlier studies on osteoporosis.

AGE RELATED CHANGES AND REFERENCE LEVELS OF BTMS IN INDIAN WOMEN

Earlier we had reported the reference database for the markers OSC, BSAP, urinary CTX and DPD in healthy Indian women and age related changes in pre and postmenopausal women by correlating with BMD measurements [30,31]. A decline in estrone glucuronide (E1G) levels the principal metabolite of estradiol in urine; rise in urinary FSH (uFSH) with increased bone turnover was observed during menopausal transition. Established cut off levels of OSC, uCTX, uFSH and (E1G) suggested that simultaneous measurements of BTMs and ovarian hormones are desirable while screening peri and post- menopausal women at risk for osteoporosis **(Figures 1 and 2)**.

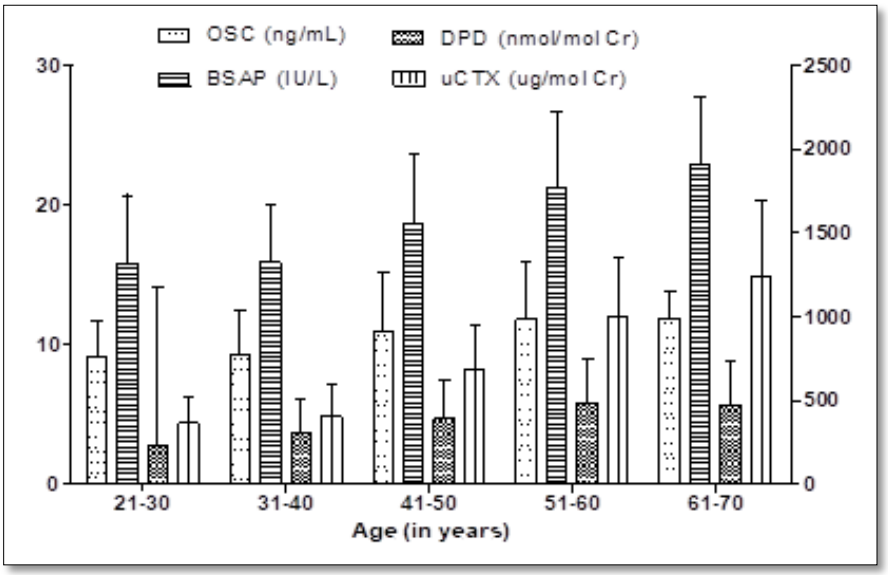

Figure 1. Reference values for bone markers.
OSC: Osteocalcin; DPD: Deoxypyridinoline; BSAP: Bone Specific Alkaline Phosphatase; uCTX: urinary C-Terminal Telopeptide of Type I Collagen Crosslinks
Refer left Axis for OSC, BSAP, DPD and right axis for uCTX

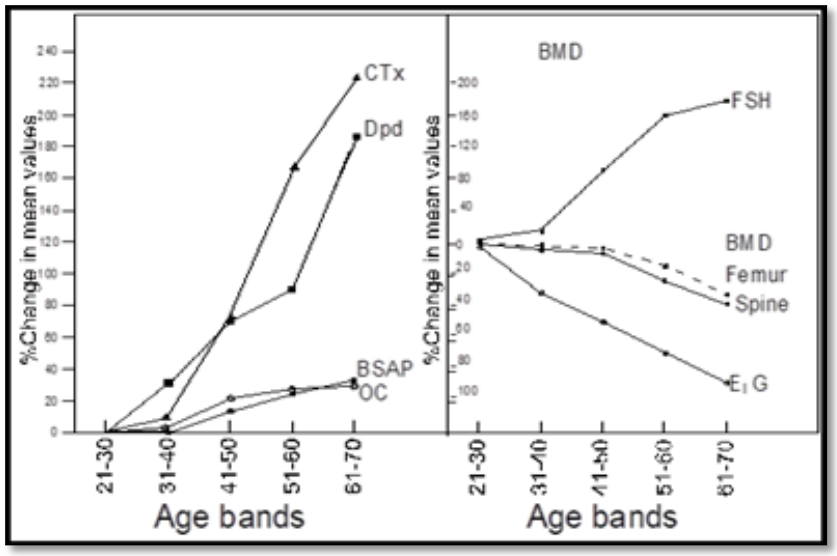

Figure 2. Percentage change in the mean levels of BTMs across the age bands.
OSC: Osteocalcin; DPD: Deoxypyridinoline; BSAP: Bone Specific Alkaline Phosphatase; uCTX: urinary C-Terminal Telopeptide of Type I Collagen Crosslinks; BMD: Bone Mineral Density; FSH: Follicle Stimulating Hormone; E1G: Estrone Glucuronide

A rapid rise in the bone resorption markers (CTX-I, DPD) was observed after the age band of 30-40 years reflecting a high bone turnover wherein resorption and formation are uncoupled leading to bone loss. In women above the age 40, a rapid increase in CTX-I, DPD (50 to 200%) followed by marginal increase in the bone formation markers OSC, BSAP (0 to 25%) was observed. This imbalance remains in late postmenopausal women as the negative correlation between BMD and the BTMs is more evident with advancing age documented in the study.

Bone loss commences before the onset of menopause and estrogen deficiency. The increase in FSH precedes the estrogen decline and may be the cause of bone loss before menopause in women [32].We also studied the changes in estrone glucuronide (E1G), urinary FSH and BTMs levels in pre, peri and postmenopausal women. A significant decline in the E1G levels with a rise in uFSH levels >40 mIU/mg Cr and the bone resorption uCTx>500 ug/mol Cr was reported in peri and postmenopausal women indicating higher FSH and CTX is associated with poor bone health in these women **(Figure 3)**.

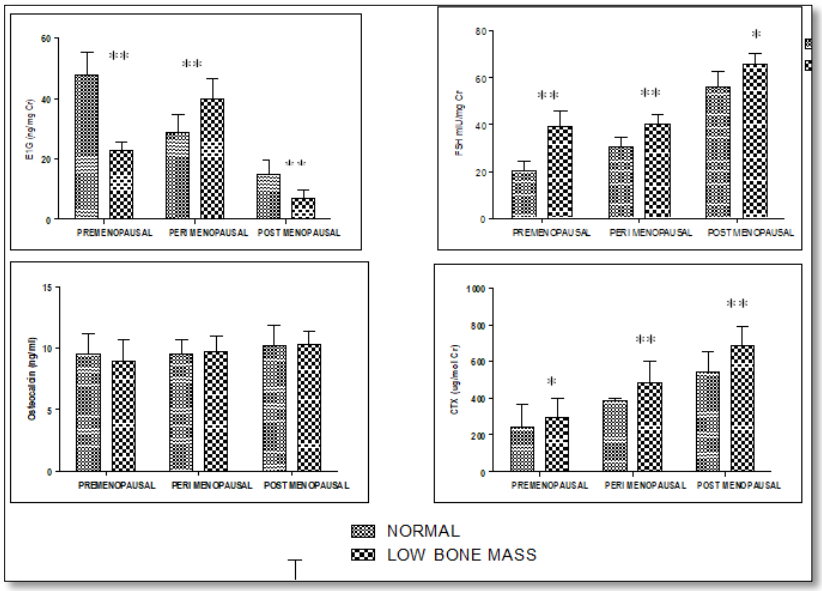

Figure 3. Levels of hormones (E1G and FSH) and bone markers (OSC and CTX) in pre, peri and postmenopausal women.
**p value<0.05; **p value<0.001*

UTILITY OF BONE MARKERS IN ASSESSING THERAPY

Clinical utility of the prominent markers OSC, BSAP, CTX and DPD was assessed in small group of women for the response to therapy. BTMs were estimated at baseline, 3 months, 6 months and 12 months following therapy **(Figure 3)** in three groups of women. Group I (n=20) women on Calcium and Vitamin D supplementation, Group II (n=20) women on HRT, calcium and Vitamin D supplementation and Group III (n=20) women on Bisphosphonate, calcium and Vitamin D supplementations.

The change in the level of BTMs was evident within 3 months of therapy indicating that the BTMs give a better picture of the overall skeletal status in assessing therapy thus having an advantage over the BMD measurements wherein the changes in bone mass are seen after a year [33].

HORMONES, CYTOKINES AND BTMS DURING MENOPAUSAL TRANSITION

Changes in systemic hormones and cytokines IL1β, IL6 and TNF α during menopausal transition are associated with the progression of postmenopausal osteoporosis. The postmenopausal drop in estrogen leads to increased cytokine production especially IL1, IL6 and TNF α from bone cells. IL6 a potent stimulator of bone loss negatively correlated with the estrogen levels and positively with the bone resorption markers **(Figure 4)**. The study suggested that IL6 levels, in conjunction with CTX, DPD and estrogen improved the prediction of bone loss in menopausal women [34].

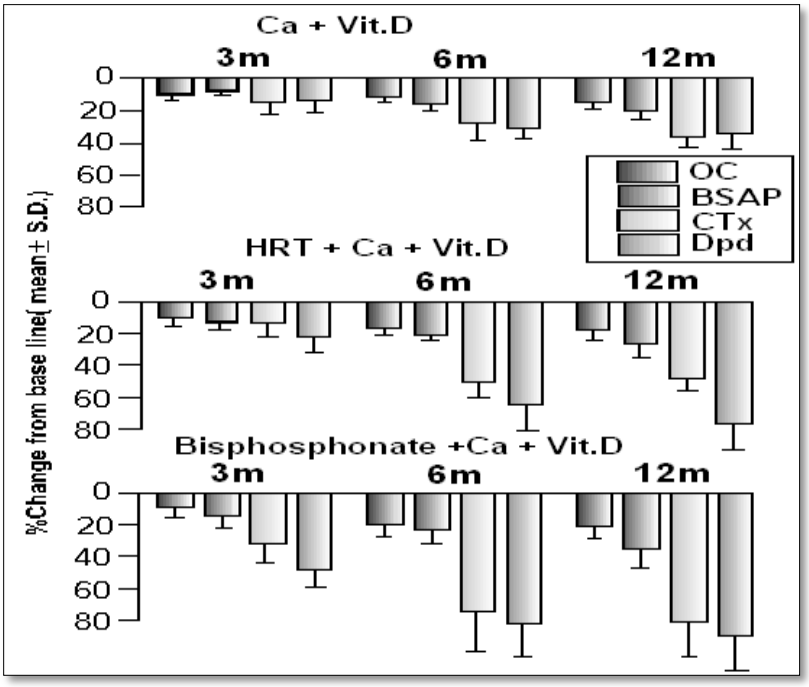

Figure 4. Monitoring women on therapy with bone markers.
OSC: Osteocalcin; DPD: Deoxypyridinoline; BSAP: Bone Specific Alkaline Phosphatase; CTX: C-Terminal Telopeptide of Type I Collagen Crosslinks; Ca: Calcium; Vit D: Vitamin D; HRT: Hormone Replacement Therapy; 3m: 3 months; 6m: 6 months; 12m: 12 months

BTMS, HORMONES AND BMD IN WOMEN WITH PREMATURE OVARIAN FAILURE (POF)

Premature ovarian failure (POF) defined as ovarian failure in women under the age of 40 years affects 1% of general population. Due to reduced estrogen at early age these women are at increased risk of developing osteoporosis [35]. In women with POF elevated FSH, PTH and resorption markers CTX and DPD were associated with low bone mass suggesting the need of assessing bone health **(Figures 5 and 6)**.

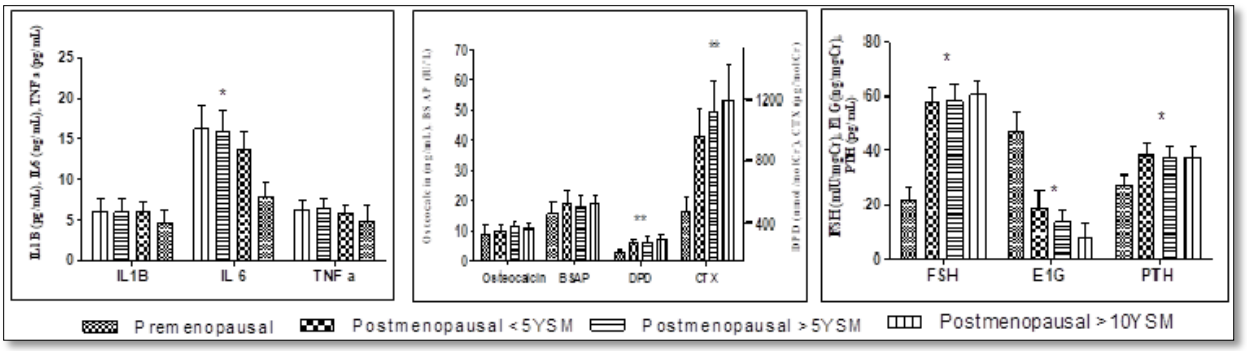

Figure 5. Cytokines, hormones and bone turnover markers in pre and postmenopausal women.
IL: Interleukin; TNF a: Tumor Necrosis Factor a; OSC: Osteocalcin; BSAP: Bone Specific Alkaline Phosphatase; DPD: Deoxypyridinoline; CTX: C-Terminal Telopeptide of Type I Collagen Crosslinks; FSH: Follicle Stimulating Hormone; E1G: Estrone Glucuronide; PTH: Parathyroid Hormone
** p value<0.05; ** p value<0.001*

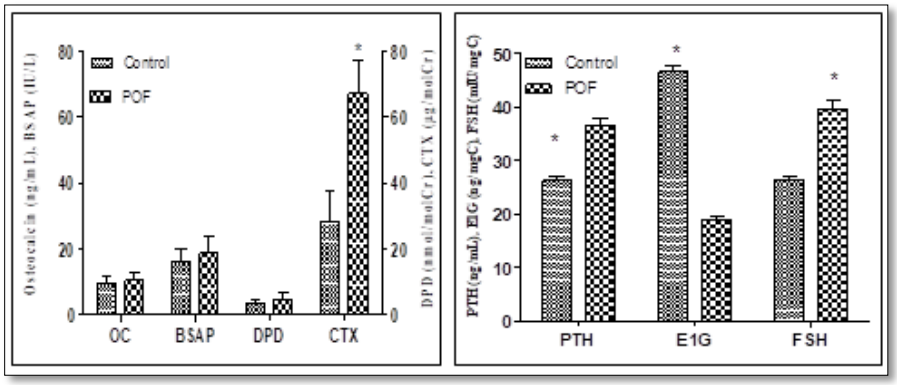

Figure 6. BTMs and hormonal levels in women with premature ovarian failure.
OSC: Osteocalcin; BSAP: Bone Specific Alkaline Phosphatase; DPD: Deoxypyridinoline; CTX: C-Terminal Telopeptide of Type I Collagen Crosslinks; PTH: Parathyroid Hormone; E1G: Estrone Glucuronide; FSH: Follicle Stimulating Hormone
**p value<0.05; **p value<0.001*

ASSAYS FOR BTMs

We reported an ELISA for human intact OSC after documenting the utility of BTMs and establishing the reference database of some of the prominent bone markers in identifying women with low bone mass and high bone turnover. The Sandwich ELISA reported was precise accurate matrix free, sensitive and cost effective. The OSC levels were significantly different in women with osteopenia and with osteoporosis as compared to the women with normal BMD [36]. ROC curve analysis demonstrated cut off values of OSC>11.9 ng/ml for osteopenia and >14.9 ng/ml for osteoporosis. The OSC prototype was validated and compared with a commercial ELISA kit. The diagnostic sensitivity, specificity and accuracy of the prototype was >85% [37]. The cost effective prototype can be used for the screening and management of Indian women with low bone mass. In addition, we are in the process of multiplexing the prominent bone markers in an ELISA array format that can aid in the assessment of bone health [38]. International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recommended one bone formation marker (serum-PINP) and one bone resorption marker (serum CTX) as reference markers in studies to assess their clinical performance [39,40]. However this does not exclude the use of other BTM as OSC or BSAP but possibly used in parallel with PINP and CTX. Standardization on patient condition (fasting status, etc.) and control of sample timing, handling and storage are important aspects to decrease variability and increase the accuracy by which BTM reflect the rate of bone remodelling [41,42].

CONCLUSION

BTMs reflect the activity of OBs and OCs in both physiological and pathophysiological conditions and are used in monitoring anti-osteoporosis therapy efficacy. The norms were established with strict inclusion exclusion criteria taking into consideration the sample timing handling and storage to decrease the variability in the estimation of the BTM levels as well as increase their accuracy. Measurement of BTMs is currently not included in algorithms for fracture risk calculations due to the lack of

data. However a combination of BMD measurement by DEXA with BTM levels at least one bone resorption and one bone formation marker, may potentially improve early detection of individuals at increased risk for bone loss.

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