



# **Bone, it's Architecture, Markers of Resorption and Their Role in Osteoporosis in India: A Detailed Review**

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## **Authors' contributions**

*This review article was written in collaboration with all three authors. Author TR designed the study and wrote the study protocol. Author JD drafted the manuscript. Author PS managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Bone is a dynamic tissue that undergoes constant remodeling throughout the life span. Bone turnover is an equilibrium between the rates of bone formation and resorption. Assay of bone turnover markers (BTMs) is very important as they provide an insight in to 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. Osteoporosis is a major health problem in India with 230 million people getting affected. Women get more crippled as

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they show an additional accelerated phase of bone loss, which occurs 10 years earlier in India. Bone health in Indian women is more appalling with deficient nutritional status as compared to their counterparts. Biochemical assays are non-invasive, not site specific, reflect the turnover of entire skeleton, can detect early changes in the bone turnover. The present review was aimed to discuss the normal architecture of bone, markers of its turnover and their role in osteoporosis in India.

**Keywords:** Bone turnover markers; BTM; osteoporosis; India.

## 1. INTRODUCTION

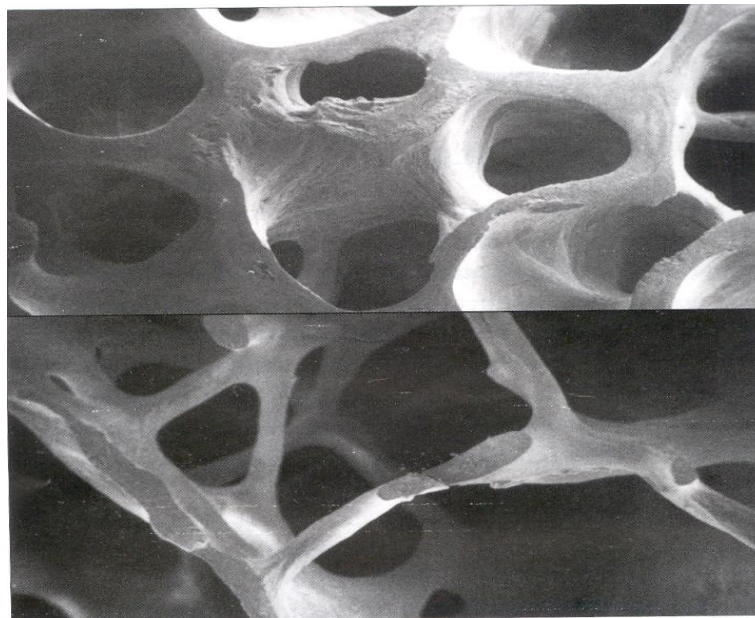
Osteoporosis is a progressive metabolic or skeletal disorder, leading to a consequent increase in fracture risk due to deterioration in bone mass and imperfection in its microarchitecture. Osteoporosis is defined as bone mineral density that is less than 2.5SD below the reference range in young adults of the same race and sex (t score of  $-2.5$ ) [1].

Bone grows in size during the first two decades of life as anticipated, with an accelerated growth during adolescence precisely related to estrogen in females and testosterone in males. In the third decade individuals attain maximum bone mass [1].

Approximately 3 % of cortical bone is replaced each year and 25% of trabecular bone is resorbed and replaced each year. (Fig 1) Women of all ethnic groups show an additional accelerated phase of bone loss, which occurs for

about 10 years after the cessation of ovarian function. Total bone loss in osteoporosis may exceed 30 to 40%. In women there is a 15 to 18% lifetime risk of hip fracture after the age of 50 years, versus 6% risk in men [3]. 230 million people in India are over the age of 50 and vulnerable to fragility fractures [4].

Normal bone density is defined as  $-1$  standard deviation or greater than the mean at 30 – 40 years (peak bone mass). Bone density between  $-1$ SD and  $-2.5$  SD of peak bone mass is osteopenia and less than 2.5 SD of peak bone mass is osteoporosis. For the diagnosis of osteoporosis, it has been recommended by the international osteoporosis foundation (IOF) to measure Bone mineral density (BMD) by dual X ray absorptiometry at the hip and lumbar region, and by this definition general prevalence of osteoporosis rises from 5% in women at the age of 50 years to 50% at the age of 85years and in men the comparable figures are 2.4% and 20% [5].



**Fig. 1. Normal trabecular bone (top) / Osteoporotic trabecular bone (bottom) [2]**

Osteoporosis is a silent killer, affecting more than one million women over the age of 50. Approximately 75% of the population faces the risk of fractures. Every eighth man and every third females in India suffers from osteoporotic fractures ten years earlier as compared to other developed countries, making India one of the largest affected countries globally. Life expectancy in India is up to 67 years which is expected to increase to 71 years in 2025. With increase in longevity there will be a 34% escalation in elderly population making them more susceptible to osteoporotic fractures. Osteoblasts, osteoclasts and osteocytes play a crucial role in bone remodeling [6]. This is a highly complex process comprising of coordinated action of these bone cells. In osteoporosis, the coordination is hampered and the basic multicellular unit (BMU) is influenced. This in turn is measured by bone turnover markers (BTM). Recent studies have suggested BTMs to outshine as markers for diagnosis of osteoporosis, these biochemical reflect the turnover of entire skeleton and can detect early changes in bone turnover [7]. The present nonsystematic review was aimed to discuss the normal architecture of bone, markers of its turnover and their role on osteoporosis in Indians.

## 2. BONE ARCHITECTURE

### 2.1 Histology of Bone

Normal bone is lamella. It can be cortical or cancellous. Cortical bone makes up 80% of the skeleton and is composed of tightly packed osteons that are connected by Haversian canals. These canals contain arterioles, venules, capillaries and nerves. Interstitial lamellae lie between the osteons. Cement lines define the outer border of the osteon, cortical bone is characterized by slow turnover rate and cancellous bone is less dense and undergoes more remodeling according to the lines of stress. Periosteum is a thick connective tissue comprising of blood vessels, nerves and osteogenic cells and they pack the cortical bone, whereas endosteum is a thin layer of connective tissues covering the cavities [8]. It has a higher turnover rate and is more elastic than cortical bone. During osteoporosis both cortical and trabecular bone are affected making the bones brittle and more prone to fractures. The trabecular bone is composed of plates and rods providing strength. As age progresses plates become more rod like and the connectivity

between them is lost, which lowers the stiffness and bone strength. This arrangement is evident in femoral neck. Inferior cortex provides resistance to compressive arcade and superior cortex resists tensile load. This conjunction provides flexibility and strength [9]. Failure of this interconnectivity is the reason for femoral neck fractures [10]. Cortical porosity also defines bone strength and is independent of cortical size. Large macropores are created by increased osteoclastic activity by expanding the Haversian canal. This will lead to incapability in bearing loads by thinning the cortical tissue. This mechanism with increased spaces and macropores is commonly seen in old age. The proximity in these macropores minimizes the impact on bone strength [11].

### 2.2 Cellular Biology [Fig 2]

**Osteoblasts:** Osteoblasts are derived from undifferentiated mesenchymal cells. These cells form bone. These cells have more endoplasmic reticulum, Golgi apparatus, and mitochondria. More differentiated, metabolically active cells, line bone surfaces and less active cells in the resting regions maintain the ionic milieu of bone. Disruption of the lining layer activates osteoblasts. This osteoprogenitor lineage is activated by gene expression, timely programmed steps such as formation of bone morphogenetic protein (BMPs) [12]. Gene expression of Runt related transcription factor 2(Run X2) is the most crucial gene of osteoblast differentiation. RunX2 devoid mice have demonstrated to down regulate osteoblast related gene CollA1, Cbf alpha and Cbf beta. Cbf beta is the most recent gene noticed in bone marrow and plays a vital role in osteoblast differentiation, followed by proliferation phase where, osteoblasts show high alkaline phosphatase activity, produce type I collagen, respond to parathyroid hormone and produce osteocalcin [13][14]. Osteoblasts have receptor - effector interactions for parathyroid hormone, vitamin D3, glucocorticoids, prostaglandins and estrogen.

**Osteocytes:** Make up 90% of the cells in the mature skeleton and serve to maintain bone. These cells represent former osteoblasts that have been trapped within newly formed matrix. The embedded osteocytes differ in their morphology depending on the bone type. trabecular bone osteocytes are round whereas osteocytes from cortical bone are comparatively flatter [16]. They have an important role in

controlling the extracellular concentration of calcium and phosphorous. They are directly stimulated by calcitonin and inhibited by parathyroid hormone. Once the osteocyte gets trapped within the mineralized bone matrix, the osteoblast markers such as osteocalcin and alkaline phosphatase are downregulated while expression of osteocyte markers such as dentine matrix protein1 (DMP1) and sclerostin are increased [17]. Osteocytes detect mechanical pressures and loads due to the interconnected network, thereby helping the bone to adapt to daily mechanical forces. The regulation of osteoblasts and osteoclasts mechanism depicts osteocytes as orchestrators of bone remodelling [18].

**Osteoclasts:** These multinucleated, irregularly shaped giant cells originate from hematopoietic tissues involved in resorption of bone. Multiple factors promote activation of gene expression in osteoclasts, factors such as macrophage colony-stimulating factor (M-CSF) and RANK ligand which is secreted by osteoblasts, osteocytes, and stromal cells play a crucial role in osteoclastic activity [19]. Osteoprotegerin (OPG), which is produced by osteoblasts, stromal cells, gingival and periodontal fibroblasts bind to RANKL, preventing osteoclastogenesis by inhibiting the interaction of RANK/RANKL. These interactions further promote gene expression of DC-STAMP, which plays a critical role in fusion of osteoclastic cells [20]. Bone resorption occurs in depressions known as Howships lacunae by osteoclasts that synthesize tartrate resistant acid phosphate and cathepsin K. Osteoclasts produce hydrogen ions via carbonic anhydrase to lower

the pH, which increases the solubility of Hydroxyapatite crystals, and organic matrix is removed by proteolytic digestion. Osteoclasts also have specific receptors for calcitonin to allow them to directly regulate bone resorption [21].

### 3. OSTEOPROGENITOR CELLS

These local mesenchymal cells line the haversian canals and the periosteum, awaiting the stimulus to differentiate into osteoblasts. In bones that have stopped displaying bone remodeling or when matured are spindle shaped and are "inactive osteoblasts". They are exhibited frequently and in enormous numbers in developing bones. These cells also reside in perichondrium and escalate the bone morphogenetic proteins that independently help in formation of bone matrix. Recent studies have confirmed that osteoprogenitor cells that originate from hypothalamus increase osteogenesis and inhibitors of proline rich tyrosine kinase 2 regulates early osteoprogenitors and can be used in the treatment of osteoporosis [22].

**Cells:** These are narrow, flattened cells that form an envelope around the bone. Functions of bone lining cell is not well understood. However, they prevent the interaction of osteoclasts and bone matrix during bone formation and produce OPG and RANKL that participate in osteoclast differentiation. Lining cell equally play a pivotal role in bone remodeling as other bone cells [23-24].

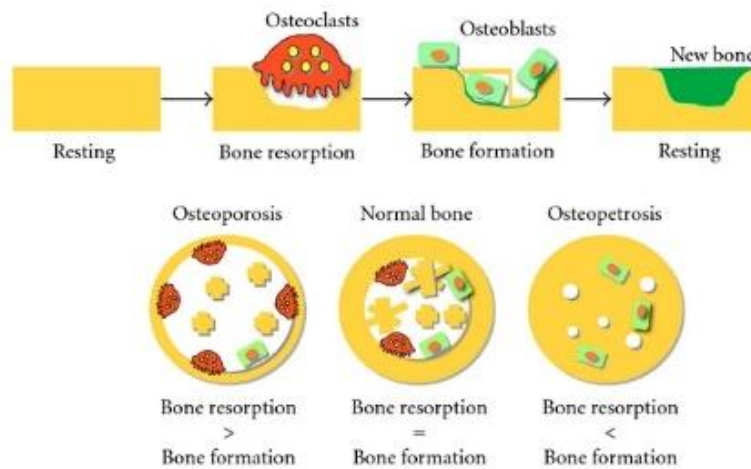


Fig. 2. Schematic diagram of bone turnover [15]

#### 4. BONE MATRIX

**Organic components:** Organic components make up 40% of the dry weight of bone. They include Collagen, Proteoglycans, Non collagenous matrix proteins and Growth factors and Cytokines. Collagen is responsible for the tensile strength of the bone and makes up 90% of the organic matrix of bone. It is composed primarily of type I collagen. Collagen structure consists of a triple helix of two  $\alpha 1$  and one  $\alpha 2$  chains. Small leucine rich proteoglycans like decorin, biglycan, lumican, osteoaderin, and seric proteins are partially responsible for the compressive strength of the bone [25]. There are a number of non-collagenous matrix proteins that promote mineralization and bone formation. They include Osteocalcin which is produced by osteoblasts, is directly related to the regulation of bone density. The synthesis of osteocalcin is inhibited by PTH and stimulated by Vit D3. Osteocalcin levels can be measured in the serum or urine as a marker of bone turnover. Osteonectin is secreted by platelets and osteoblasts. It is postulated to play a role in the regulation of calcium metabolism. Osteopontin is a cell binding protein and Growth factors and cytokines: Transforming growth factor  $\beta$ , Insulin like growth factor, Interleukins 1 & 6 and bone morphogenic proteins [26].

**Inorganic components:** Make up 60% of the dry weight of bone. Calcium hydroxyapatite makes up most of the inorganic matrix of bone and is responsible for the mineralization of the matrix. Osteocalcium phosphate {BRUSHITE}, makes up the remaining inorganic matrix. It also has significant amount of bicarbonate, sodium, potassium, citrate, magnesium, carbonate, fluorite, zinc, barium, and strontium are also present. The conjunction of these inorganic components with collagen and non-collagenous matrix forms a hydroxyapatite, providing strength and mechanical support to the bone matrix. These complexed frame work executes an important role in bone homeostasis and prediction of bone fractures. Age, nutrition, disease, and osteoporotic treatments have exhibited diverse effects on bone matrix proteins that cause detrimental effects on bone structure. Bone matrix contributes to bone quality [27].

#### 5. BONE CELLS AND BONE MATRIX

Bone matrix acts as a ground substance over which the bone cells anchor. They regulate the activity of bone cells through adhesion molecules

like integrins [28]. These cause osteoblasts to interact with bone matrix by binding to proteins like fibronectin, Osteopontin, collagen etc. integrins of osteoblasts are  $\alpha 1\beta 1$ ,  $\alpha 2\beta 1$ , and  $\alpha 5\beta 1$  and they play a pivotal role on organisation of osteoblasts on bone matrix during osteoid synthesis. The interaction between osteoclasts and bone matrix is very essential for exhibiting its role. Thus, during osteoclastic activity  $\alpha \nu \beta 3$  and  $\alpha 2\beta 1$  integrins bind to bone-enriched proteins, such as bone sialoprotein and Osteopontin. Despite these bindings, cadherins that provide constant contact between osteoclast and growth factors required for osteoclast differentiation fail to express.  $\beta 3$  and  $\beta 1$  integrins are involved in interaction of osteocytes to bone matrix. These are crucial as they induce signals and amplify them on detecting bone tissue deformity [29].

#### 6. OSTEOPOROSIS IN INDIA

##### 6.1 Risk Factors for Osteoporosis

The data obtained is mainly done by studies scattered in different parts of India.

In 2015, 230 million Indians are expected to be over 50 years with 20% sustaining osteoporotic fractures. Women of all ethnic groups are more prone to low bone mass after menopause. Estrogen hormone is required for bone formation and growth. Age of menopause in Indian women is ten years earlier as compared to Caucasian women [30-31]. Genetic factors also correlate strongly with bone mass. Indian women have shown lower BMD than other ethnic counterparts. It is also thought that vitamin D receptor polymorphism is responsible for varied results [32].

Even though India has ample sunlight, vitamin D deficiency is observed in Indians regardless of their gender. Diet, increase melanin production and attire in India may hinder the formation of Vitamin D. As the importance of vitamin D in bone growth is discussed earlier, osteoporosis is strongly influenced by this hormone. Thus, indirectly leading to demineralization and tampering the bone matrix. Indians also lack the daily recommended intake of calcium. The Indian diet hinders calcium absorption as it is rich in phytates. Thus, hindering the formation of bone matrix [33].

Sedentary life style in elders decreases exposure to sunlight and lack of exercise decreases muscle activity and acts as stressor to escalate

bone formation, thus improving bone quality [34]. Alcoholism and cigarette smoking can also cause detrimental effect on bone health. Smoking increases the lifetime risk of developing fragility fracture is 13% and 32% in women and men respectively [35]. However, significant relation of this parameter to osteoporosis in India is questionable, as the frequency of smokers in India are comparatively less. Long term treatment on glucocorticoids is a major contributor to osteoporosis in all ethnic groups [36]. Body mass index is a crucial risk factor for osteoporosis. However, it is controversial and contradicting. Obese women are said to have a protective effect on osteoporosis. Adipose tissue and BMD are positively correlated in many studies. However poor nutritional status can have detrimental effect on bone mass [37].

## 7. CLINICAL FEATURES OF OSTEOPOROSIS

It is noticed only after the first fracture. Most common fractures associated with osteoporosis in India are.

**Vertebral fracture:** Majority of vertebral fractures result from compressive loading associated with activities such as lifting, changing position, coughing sneezing or a simple fall from standing height. However only one third of new vertebral fracture relates to fall. The earliest and the major symptom of vertebral compression fracture is acute back pain and the most routine activities could be restricted. 17.1% of vertebral fractures in 415 female subjects in India were reported at the Delhi vertebral osteoporosis study [38].

**Hip fracture:** The incidence of hip fractures increases exponentially with age and is expected to escalate to 6.3 million in 2050. Over the age of 50, hip fractures were estimated to be 159/1000 women. Indian studies revealed that men had higher chance of sustaining hip fractures as compared to the preponderance of these fractures in females [39]. Hip fractures mainly involve the femoral neck and intertrochanter fractures in the elderly osteoporotic population, which are caused by low trauma. These fracture cause excruciating pain, loss of mobility and excess mortality. Nearly all are hospitalized and most undergo surgery. It is estimated that medical expenditures for hip fractures are exponentially high. However, non-operative treatment can lead to disastrous complications and operative treatment like arthroplasty and

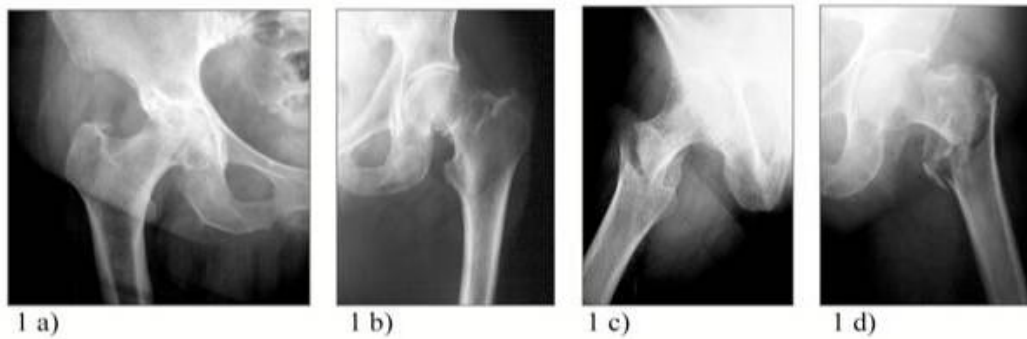
internal fixation are prone to failures that can cause irreparable damage.

## 8. BONE TURNOVER MARKERS

Measurement of Bone mineral density is at present the method of choice in the evaluation of fracture risk. However, they are influenced by multiple factors. Rural India has limited set ups for DEXA in government hospitals therefore suggesting scanty access to these machines [43]. Increasing evidence suggest that the risk of osteoporosis and fracture is associated with increase bone turnover as measured by markers of resorption and formation, independently. At present, the markers reflecting bone formation with acceptable specificity and sensitivity are osteocalcin, bone specific alkaline phosphatase and procollagen type 1 N-terminal pro-peptide. For evaluating bone resorption, pyridinoline, deoxy pyridinoline, and N and C terminal type 1 collagen telopeptide, urinary hydroxyproline, urinary excretion of the pyridinium cross-links (pyridinoline and deoxypyridinoline), and plasma tartrate-resistant acid phosphatase activity [44].

### 8.1 Biochemical Markers of Bone Formation

**Serum alkaline phosphatase:** Serum alkaline phosphatase activity is the most commonly used marker of bone formation, but it lacks sensitivity and specificity, especially in patients with osteoporosis. In an attempt to improve the specificity and the sensitivity of serum alkaline phosphatase measurement, techniques have been developed to differentiate the bone and the liver isoenzymes, which differ only by posttranslational modifications since they are coded by a single gene. These techniques rely on the use of differentially effective activators and inhibitors (heat, phenylalanine, and urea), separation by electrophoresis, and separation by specific antibodies. In general, these assays have slightly enhanced the sensitivity of this marker, but most of them are indirect or technically cumbersome or both. A real improvement should be obtained by using a monoclonal antibody recognizing the bone but not the liver and kidney isoenzyme. Serum total ALP has revealed interaction with spinal bone matrix. Recent study depict ALP as a marker for osteoporosis in men. Indian studies also revealed the same with significant higher levels in osteoporotic population [45].



**Fig. 3. X rays showing osteoporotic fracture of proximal femoral neck (1a), middle femoral neck (1b), basilar femoral neck (1c) and inter and subtrochanteric femoral regions. (1d) [40]**  
**Distal forearm fracture:** among the fractures sustained in osteoporosis, this fracture seems to be economically and emotionally less effective. However, it is speculated that these fractures can lead to fragility fractures in future, which is economically draining. Most distal forearm fractures occur in women. The age adjusted female to male ratio being 4: 1. there is a rapid increase in the incidence after menopause which plateaus at about 65 years. [41]



**Fig. 4. X ray showing distal radius fracture [42]**

**Serum osteocalcin (OC):** Osteocalcin, also called bone gla protein (BGP), is a small, non collagenous protein that is specific for bone tissue and dentin, but its precise function remains unknown. Osteocalcin is synthesized predominantly by the osteoblasts and incorporated into the extracellular matrix (ECM) of bone, They are gene mediated and the gene encoding for OC is the BGLAP gene. The physiological role of osteocalcin is glucose and calcium homeostasis, bone mineralisation and bone remodelling. Osteocalcin originally is

synthesized as a prohormone with 95 amino acids and then cleaved to form a mature polypeptide consisting of 49 amino acids in humans. Comprising of three gamma carboxy glutamic acid residues at positions 17, 21 and 24. This binds the osteocalcin to calcium and exhibits a helical structure with compact calcium molecules embedded forming an abundant molecule in the extracellular matrix of the bone [46]. Osteocalcin is activated by Vitamin K, a co factor for gamma carboxylation. OC circulates in the blood stream in two forms carboxylated and

uncarboxylated OC but a fraction of newly synthesized carboxylated OC is released into the circulation, where it is predominantly measured by radioimmunoassay. Uncarboxylated OC requires sophisticated techniques. Depending on the epitopes recognized by the antibody, monoclonal antibodies are used against OC fragments such as N-mid and N terminal OC fragments and in addition intact OC fragments of osteocalcin. Some of these fragments may be released during bone resorption in an acidic condition during high turnover state, but some of them are unrelated to resorption and their significance is not known. In most cases, however, serum osteocalcin is a valid marker of bone turnover when resorption and formation are coupled and is a specific marker of bone formation whenever formation and resorption are uncoupled. Osteocalcin is dependent on post-translational carboxylation for its hydroxyapatite affinity, a process that has been shown to decrease with age. High levels of under carboxylated osteocalcin are seen in hip fracture patients. OC is cost effective and can be used in screening and management of low bone mass in Indian population, as they are >85% sensitive, accurate and precise in diagnosing [47].

**Procollagen type I propeptides (PINP):** They are derived from collagen type I, in which they form amino- (N-) and carboxy- (C-) terminal extension peptides. Since both the carboxy- and the amino-terminal propeptides of type I collagen are generated in a stoichiometric fashion, the propeptides are considered quantitative measures of newly formed type I collagen. Both propeptides may be measured by specific, polyclonal-based immunoassay. PINP originates from fibroblasts and proliferating osteoblasts, also derived from skin, tendon, dentin, and cartilage. PINP is a better bone formation marker than its counterpart and is proposed as a novel bone formation marker by International osteoporosis foundation in account of its response to treatment, precision and reliability as evidenced by low variability and longer stability at room temperature. A study correlating PINP levels with age and sex matched Indians suggested the same with no gender difference and no variations with vitamin D deficiency [48].

## 8.2 Bone Resorption Markers

Most biochemical markers of bone resorption are degradation products of bone collagen, but non collagenous proteins such as bone sialoprotein and tartrate-resistant acid phosphatase are also used as bone resorption marker.

**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. Urinary Hyp has long served as the only marker of bone resorption, despite the fact that significant amounts of urinary Hyp are derived from the degradation of newly synthesized collagens, from collagens of tissues other than bone, and from the diet. Today, Hyp 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 ratio of the two glycosides may allow for the recognition of tissue specificity. Although the hydroxylysine have potential as markers of bone resorption, their major disadvantage is presently the absence of a convenient immunoassay format. They are the first to the excreted in the urine during bone resorption [49].

The hydroxypyridinium crosslinks of collagen, pyridinoline (PYD) and deoxy pyridinoline (DPD), are formed during the extracellular maturation of fibrillar collagens and are released upon the degradation of mature collagens. The measurement of PYD and DPD is not influenced by the degradation of newly synthesized collagens and independent of dietary sources. In addition, the two components show a high specificity for skeletal tissues. While PYD is found in cartilage, bone, ligaments and vessels, DPD is found in bone and dentin only [50].

Both crosslink components may be measured by a reverse-phase ion-paired HPLC technique. In urine PYD and DPD are present both as free moieties (about 40%) and peptide-bound (about 60%). In addition, the free (non-peptide-bound) forms can be detected by direct immunoassays (free DPD, 'Pyrilinks-D'). Sensitive immunoassays are available for the measurement of type I collagen telopeptides in urine and serum. Currently, these include a serum radioimmunoassay (RIA) for the carboxy-terminal type I collagen telo-peptide generated by matrix metalloproteases (CTX-MMP, also called 'ICTP') in serum, several immunoassays involving a synthetic octapeptide from the C telopeptide of type I collagen containing the



crosslinking site (CTX-I, 'Cross laps') and an enzyme-linked immunosorbent assay (ELISA) for the cross-linked N-terminal telopeptide of type I collagen (NTX-I, 'Osteomark'). The pyridinium crosslinks and the collagen telopeptide involving the crosslinking site are currently considered the best indices for the assessment of bone resorption. Their urinary levels need to be corrected by creatinine excretion. However a study in Manipur claimed that DPD did not correlate well with DEXA and thus implying DPD as not a good indicator for osteoporosis [51].

**Carboxy terminal crosslinked telopeptide (CTX- Beta Cross Lap):** Cathepsin K degrades type I collagen of bone to form (CTX). They exist in two forms:  $\alpha$  and  $\beta$ , in growing children the ratio is of the isoforms are altered. It is recommended as the marker for resorption by international osteoporosis foundation. They are altered in other metabolic disorders of bone [52]. However, there are studies that also claim CTX as not a good indicator for detecting osteoporosis.

**Amino Terminal Crosslinked Telopeptides (Ntx):** NTX like CTX are degraded from type 1 collagen by cleavage of N-terminal region by cathepsin K. This mechanism occurs during bone resorption. NTX is measured both in urine and serum using monoclonal antibody against specific N-terminal epitope.

**Bone sialoprotein (BSP)** accounts for 5-10% of the non-collagenous matrix of bone. The glycoprotein has been shown to be a major synthetic product of active osteoblasts and odontoblasts. BSP may play an important role in cell-matrix adhesion processes and in the supramolecular organization of the extracellular matrix of mineralized tissues. Immunoassays have been developed for the measurement of the immunoreactive form of BSP in serum. Based upon clinical data and the rapid reduction of serum BSP levels following intravenous bisphosphonate treatment, it is assumed that serum BSP reflects processes mainly related to bone resorption, they are overexpressed in malignant tissues and its role in cancer management is well known but data are lacking to assess the clinical utility of this new marker in osteoporosis [53].

**Tartrate-resistant acid phosphatase (TRACP)** exists in two sub-isoforms named 5a and 5b, of which only TRACP-5b has been shown to be characteristic for osteoclasts. Recently,

immunoassays for TRACP-5b have been described and preliminary clinical results indicate that this marker may be useful to assess osteoclast activity [54].

## 9. RECENT MARKERS

**Receptor activator nuclear factor kappa -B ligand:** RANKL reflects the activity in bone osteoclasts in a microenvironment. The activity of RANKL and RANK is explained earlier and as they increase osteoclastic role and its mechanism as a resorption marker is still not well understood. These are currently under research setting to be proven as novel biomarkers of bone resorption in osteoporosis.

**Osteoprotegerin:** This marker also depicts bone cell mechanism. Unlike RANKL they are synthesized by osteoblasts and inhibit the binding of osteoclasts to RANK thus inhibiting bone resorption. This marker requires further analysis on its role in osteoporosis.

## 10. BIOLOGICAL VARIATIONS IN BONE TUMOR MARKERS

### 10.1 Uncontrollable Biological Variation

**Renal Function:** In the elderly gradual renal impairment may lead to an increase in osteocalcin and in other markers metabolized and/or excreted by the kidney (pyridinoline crosslinks and related peptides); care should be taken in the interpretation of these bone markers when the creatinine clearance decreases below 30 ml/min [55].

**Pregnancy and lactation:** Pregnancy and lactation place a considerable burden on the maternal skeleton to provide calcium for the growing fetus and infant. The greatest demand from the fetus for calcium comes in the third trimester, but an anticipatory mechanism results in a gradual increase in bone resorption from the sixteenth week of pregnancy onward, followed by an increase in bone formation [56]. Contrary to this overall pattern, serum levels of Osteocalcin appear to decrease and may even be undetectable during pregnancy. It has been suggested that this is due to placental clearance of Osteocalcin. However, it may also be due the type of assay used and to the increase in renal function during pregnancy [57]. At term, markers of bone resorption such as NTX, PINP and CTX increase and return to normal after delivery.

Suggesting imprecision in BTM values during pregnancy.

**Drugs:** Corticosteroid treatment are misused thoroughly in India as they are freely available. They significantly reduce OC values [58]. Long term anticonvulsant therapy and GnRH agonist treatment both result in significant increases in markers of bone turnover. In contrast thiazide diuretics decrease bone turnover [59]. The effect of oral contraception (OCP) on bone turnover appears to be age-dependent. Intake of OCP in pre menopause has given a protective effect to bone thus reducing the bone turnover markers in post-menopausal women. Studies on the effect of oral contraception give inconsistent results [60].

**Immobility:** Bed rest results in a very rapid increase in markers of bone resorption. Urinary excretion of PYD and DPD are significantly increased after only 2 days and by 40% after a week. Markers of bone formation change little or remain unchanged during bed rest or immobility. In elderly, partially immobile subjects, the increase in urinary HYP is related to the degree of immobility. Once remobilization occurs resorption markers gradually return to initial levels, although paradoxically PICP may increase [61].

## 10.2 Controllable Sources of Biological Variability

**Circadian Rhythm:** Circadian variability has more impact on markers of bone turnover than most other sources of variability. Most markers of bone turnover are increased at night, reaching a peak between 0200 and 0800 hours, after which they decrease rapidly and reach a nadir between 1300 and 2300 hours. The amplitude of the rhythm is considerably greater for resorption markers than for formation markers. Serum OC are increased by 20% at night compared with their nadir in the early afternoon. CTX also observes circadian variation as they peak in the second half of night. 20% lower levels are observed post prandially. Hence fasting samples are preferred. Bone ALP has a somewhat different circadian rhythm with a peak between 1100 and 1400 hours and possibly another peak at 2330 hours. Calcium supplementation taken at night and bisphosphonate treatment can both suppress the circadian rhythm of markers of bone resorption [62].

**Exercise:** Exercise may affect the variability of markers of bone turnover in two ways: the effect

of persistent exercise and the acute effect of a bout of exercise within a day of the sample collection. Sub-acute exercise results in an increase in bone formation markers and a decrease in bone resorption markers. In most studies the acute effect of exercise is to increase markers of collagen formation and degradation by 15-40%. These increases persist for 24 hr and possibly for as long as 72 hr. It is therefore important to enquire about regular exercise and ask the subject to refrain from exercise for at least 24 hr before samples are collected [63].

**Diet:** Serum and urinary levels of most markers of bone turnover are unaffected by diet, with the exception of HYP and CTX. Before samples are collected for HYP and CTX measurements, subjects must have an overnight fast. Specific dietary restrictions are therefore only applicable to HYP measurements.

**Reference Ranges:** Each laboratory should establish its own reference ranges. Age, gender, menopausal status and race all affect levels of markers of bone turnover. Therefore, separate reference ranges should be established for men, premenopausal women and postmenopausal women. Because markers are still elevated in the third decade the male and premenopausal reference ranges should only include subjects over the age of 30 years. Standardized time and conditions for sample collection must also be defined for each reference range.

## 11. ASSESSMENT OF BONE TURNOVER MARKERS IN OSTEOPOROSIS IN INDIA

Sachdeva.A et al observed an increased excretion of hydroxyproline in postmenopausal women as compared to the premenopausal women. This increase was statistically highly significant ( $p < 0.001$ ) serum alkaline phosphatase and Calcium was used and was found to be significantly increased in postmenopausal women reflecting increased bone activity (osteoclastic and osteoblastic) as compared to premenopausal women. A study demonstrated significantly lower serum calcium level in the post-menopausal group, while the ALP level was significantly lower in premenopausal women suggesting increase in bone turnover with accelerated loss of bone mass in post-menopausal women. [64] Mean serum values of NTX increased significantly in post-menopausal women with increase in the duration of menopause and BMD values

decreased and correlated well with NTX suggesting increased bone resorption in women with low BMD [65]. Bone specific ALP, deoxypyridinoline (DPD) and correlation of BMD was assessed in chronic kidney disease (CKD) patients in India. Osteoporotic subjects had higher BAP, and DPD, suggesting higher bone turnover in osteoporosis in CKD patients. 80% were having vitamin D deficiency and 13% had insufficiency [66]. A negative correlation of serum OC and BMD was seen in osteoporotic post-menopausal women, suggesting BTMs and BMD together are better predictors of osteoporotic fractures [67-70] However, Rai et al and gurban et al proposed low serum OC levels in osteoporotic post-menopausal women as compared to healthy post-menopausal woman, suggesting low bone formation is prone to fractures [71]. Serum BSP was significantly elevated in postmenopausal osteoporosis and positively correlated with NTX & other resorption markers as compared to healthy perimenopausal controls, suggesting elevated bone resorption markers in osteoporosis. Studies showed increased bone turnover markers in post-menopausal women as compared to pre-menopausal women, implying higher bone turnover immediately after menopause [72-74]. Similar results were seen with serum Osteoprotegerin in the previous age group.

A study in Sikkim proposed NTX and urinary HYP as markers in diagnosing osteoporosis in men whereas osteocalcin as an indicator in women. They concluded that bone turnover markers are able to predict low bone mass and will help to predict the risk of osteoporosis [75]. Abraham. S et al did a study on osteoporosis and osteopenia in India and found that osteopenia was more in the age group of 40 – 49 years and osteoporosis was more in the age group of 60 – 69 years [76].

## 12. CONCLUSION

Unfortunately, rural India still is lagging behind socio- economically and in health care, DEXA is not accessible to many living in India. Thus, biochemical assays were studied as they are affordable, non-invasive, not site specific, reflects the turnover of entire skeleton and can detect early changes in the bone. Thus, concluding that bone turnover markers act as important tools in the management of osteoporosis irrespective of the risk factors. Fracture risk can be predicted based on remodeling rates as epidemiological studies demonstrated them as independent

contributors of fracture risk. Understanding the variations and availability of reliable, rapid and standardized turnover assays assists in better utilization of these markers in the management of osteoporosis.

## CONSENT

Not applicable

## ETHICAL APPROVAL

Not applicable

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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