1. Introduction

Osteoporosis has existed throughout human history but recently it has become a major clinical problem due to lifestyle modifications and dietary habits [1, 2]. In the early 19th century, Sir Astley Cooper, an English surgeon, noted that bones acquire “the lightness and softness during the advanced stages of life” [3]. It is a systemic skeletal disease characterized by a progressive loss of bone mass (or density) and disturbed microarchitecture of bone tissue leading to increased risk of fracture. Unfavorable macroarchitecture, disturbed microarchitecture, cortical porosity, decreased viability of osteocytes are some of the factors contributing to decreased bone strength and quality [4]. Figure (1.1).

![Microarchitecture of healthy and osteoporotic bone](image)

**Figure 1.1 Microarchitecture of healthy and osteoporotic bone**

1.1 Incidence and prevalence of osteoporosis

Osteoporosis affects over 200 million people worldwide. One in three females and one in eight males develop osteoporosis. The combined lifetime risk of hip, forearm, and vertebral fractures drawing to clinical attention is around 40%, which is equivalent to the risk of cardiovascular diseases. In women above 45 years of age, osteoporosis accounts for more days of stay in hospital than diseases such as diabetes mellitus, myocardial infarction, and breast cancer. In the United States, osteoporosis was the commonest cause of more than 44 million patient-days in nursing home. Vertebral fractures are a significant component of osteoporosis, because the first osteoporotic fracture typically
occurs in the central region of the spine (thoracic and lumbar vertebrae) during the early stages of the disease. The prevalence of osteoporosis in developing countries such as Asia and Africa varies from 5% to 68% among women aged greater than 50 years and osteoporotic fractures estimation in south East Asia was 17.4%, given by WHO (World Health Organization) and International Osteoporosis Fact Sheet [5]. In India very few studies have been carried out to determine the prevalence of osteoporosis among post-menopausal women. The prevalence of osteoporosis (very low bone mass) and osteopenia (low bone mass) in Indian women over the age of 50 years is approximately 46 million (year 2015) and 50 million women having osteopenia; this was reported in Khadilkar and Mandlik et al [6]. 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 women above the age of 65 years are at the risk of osteoporosis.

### 1.2 Classification of osteoporosis

Four categories of osteoporosis have been identified: primary osteoporosis, secondary osteoporosis, Osteogenesis imperfecta and Idiopathic juvenile osteoporosis.

#### 1.2.1 Primary osteoporosis

Primary osteoporosis is the most common type of osteoporosis. It is more common in women than men. A person reaches peak bone mass (density) around 30 years of age. After that, the rate of bone loss slowly increases, while the rate of bone formation decreases. Whether a person develops osteoporosis depends on the thickness of the bones in early life as well as health, diet, and physical activity at all ages. In women, accelerated bone loss usually begins after menopause. This happens when the production of estrogen slows down (usually between the ages of 45 and 55). In men, gradual bone thinning starts at about 45 to 50 years of age, when the production of testosterone slows down. Osteoporosis usually does not have an effect on people until they are 60 years or older. Women are usually affected at an earlier age than men, because of the already
existing lower bone mass. Loss of bone mass is related to aging and unassociated with other illnesses. Its etiology is considered multifactorial and polygenic. This is a growing health problem due to contemporary environmental conditions of modern civilization. Risk factors that are considered as “modifiable” also play an important role and include physical activity, dietary habits and eating disorders. There are “non-modifiable” risk factors such as gender, age, race, personal and/or family history of fractures, which in turn indirectly reflect the genetic susceptibility to this disease.

1.2.2 Secondary osteoporosis

Secondary osteoporosis occurs at any age. It has the same symptoms as primary osteoporosis. But it occurs as a result of having certain medical conditions, such as hyperparathyroidism, hyperthyroidism, or leukemia. It may also occur as a result of taking medicines known to decrease bone mass, such as oral or high-dose inhaled corticosteroids, very high dose of thyroid replacement, or aromatase inhibitors (used to treat breast cancer).

1.2.3 Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) means "imperfectly formed bone." People with OI have a genetic defect that impairs the body's ability to make strong bones. It is a relatively rare condition. Some people have a more severe form of the disorder in which their bones break easily. They may have broken hundreds of bone fractures during their live times. Many people, however, have a milder form of OI, and sustain very few fractures.

In people with OI, one of the genes involved in the production of type I collagen does not function. Type I collagen is a major component of the connective tissues in bones. Type I collagen is also important in forming ligaments, teeth, and sclera. As a result of the defective gene, not enough type I collagen is produced, or the collagen that is produced is of poor quality. In either case, the result is fragile bones that break easily but can heal at a normal rate.
In most cases of OI; children inherit the defective gene from one of their parents. But, the child's symptoms and the degree of disability can be very different from that of the parent. In some children, neither parent has OI.

1.2.4 Idiopathic juvenile osteoporosis

Idiopathic juvenile osteoporosis is rare. It occurs in children between the ages of 8 and 14 years especially during times of rapid growth. There is no known cause for this type of osteoporosis, in which there is too little bone formation or excessive bone loss. This condition increases the risk of fractures [7].

1.3 Factors influences low and high bone mass

Bone mass is affected by genetic (60%~80% contribution) and other factors (20%~40% contribution) including exercise, sunlight, nutrition, smoking, alcohol, medication and ageing. Factors along the up-line (regular exercise, good sunlight exposure and proper nutrition) potentially contribute to higher bone mass and along the downline (person with chronic smoking and alcohol, steroid medication and aging) cause lower bone mass [8, 9]. (Figure 1.2)

![Figure 1.2 Factors contribution for bone mass](image)
1.4 Risk factors for osteoporosis [10]

Age-Related:
- Each decade beyond the fourth decade increases the risk by 1.5-fold
- Reduction in absorption of dietary calcium
- Decline in calcitonin

Gene-Related:
- White, Asian, Latino, and black (in order of high risk)
- Women more than men
- High concordance in monozygotic twins
- Hypogonadism: Turner & Klinefelter syndromes

Nutrition-Related:
- Low calcium intake
- High caffeine intake
- High sodium diet
- Malnutrition
- Malabsorption syndrome
- Anorexia nervosa

Behavior-Related:
- Cigarette use
- Alcohol abuse
- Low physical activity
- Depression

Endocrine-Related:
- Menopause
- Obesity
- Exercise-induced amenorrhea
- Diabetes mellitus
- Hyperparathyroidism
- Hyperthyroidism

Associated with other disorders:
- Nephropathies
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- Neoplasms
- Rheumatoid arthritis
- Ankylosing spondylitis
- Multiple sclerosis

**Drugs associated with osteoporosis**

- Thyroid hormones
- Glucocorticoids
- Anticoagulants
- Chronic lithium therapy
- Chemotherapy (breast cancer or lymphoma)
- Gonadotropin-releasing hormone
- Anticonvulsants
- Chronic phosphate binding antacid use
- Extended tetracycline use
- Diuretics producing calciuria
- Phenothiazine derivatives
- Cyclosporin A
- Antidepressants
- Antacids with aluminium
- Aromatase inhibitors
- Cimetidine
- Oral contraceptive pills
- Loop diuretics

1.5 Pathogenesis of osteoporosis

The pathogenesis of osteoporosis as a primary disorder is complex and is the consequence of genetic, hormonal, dietary, and behavioral factors. Adult bone is the result of the peak bone mass, acquired early in life and the maintenance of bone density and skeletal architecture in adult age. Impairment of bone accrual during skeletal growth and/or imbalance of factors favoring bone resorption versus bone formation in adult bone.
may be responsible for skeletal fragility which leads to increase in fracture risk (Figure 1.3). Genetics factors influence mainly bone accrual and the peak bone mass, while systemic hormones (mainly parathyroid hormone [PTH] excess and withdrawal of estrogens) or local cytokines are mostly responsible for bone remodeling imbalance [11,12].

Figure 1.3 Osteoporosis pathogenesis

1.6 Clinical manifestations of osteoporosis

Osteoporosis being a silent disease; in many situations may present with a dreadful complications such as hip fracture with its associated increased morbidity and four times higher mortality in the elderly population. The main clinical problem with osteoporosis is the occurrence of fractures. Low bone density by itself causes no symptoms. Osteoporotic fractures occur most commonly at the wrist, humerus and the hips especially after a fall. However fractures at the vertebral bodies can occur spontaneously without trauma. A hip fracture is probably the most serious of all osteoporotic fractures. It requires hospitalization and surgery to correct the fracture. Fractures of the spine can cause considerable pain or sometimes cause no pain, but alter the shape of the spine. The acute pain of a spine fracture often settles over a two-
month period. Multiple vertebral body fractures lead to decrease in height and a forward stoop known as a kyphosis or Dowager’s hump. The altered shape of the spine may lead to chronic back pain [13].

1.7 Bone remodeling

Bone is a dynamic connective tissue which is constantly remodeled by an active process coupled with mineralization (formation) and demineralization (resorption). This coupling process is typically mediated by the action of osteoblasts and osteoclasts. In healthy individuals, bone formation and bone resorption are maintaining an equal balance through the action of various chemical messengers and local mediators (Figure 1.4). In contrast, aging, metabolic bone diseases and therapeutic interventions are characterized by imbalance in bone turnover. This unregulated bone turnover leads to changes in the morphology, strength and mass of the bone [14, 15].

1.8 Bone densitometry

Bone mineral density (BMD) is a major factor, which determines the strength and quality of bone; any degree of decline in the normal level of BMD leads to future risk. BMD measurements by DEXA scan is considered to be the Gold standard for the
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Study of Biomarkers and Gene Polymorphism and their Association with Bone Mineral Density in Patients with Osteoporosis

The diagnosis of osteoporosis in post-menopausal women. This is major supporting evidence for the clinician in the diagnosis of osteoporosis. The BMD results obtained by DEXA scan are interpreted as T score. This T score was compared and calculated from the reference standard of gender and age specific young normal adult skeletal status [16]. According to the WHO criteria, osteoporosis is identified by “T-score value of bone mineral density more than 2.5 SD below the mean for young healthy normal adult women (T-score < -2.5). Patients with BMD values between 1 and 2.5 SD below the mean for young adults (T-score between -1.0 and -2.5) are classified as having osteopenia” [17].

Figure (1.5)

At the present time, BMD is the best estimate of bone strength. Bone density can easily be measured at the hip and spine using a DEXA machine. This is a painless procedure and takes no more than twenty minutes to perform. Bone density does not fully explain an individual’s risk of sustaining a fracture. Many other factors are important which include life style factors, such as smoking and excess alcohol intake, taking certain medications such as corticosteroids, older age, a history of previous fracture, female sex, family history of hip fracture, inflammatory joint disease such as rheumatoid arthritis, premature menopause, low body weight, type 1 diabetes mellitus, overactive thyroid gland, liver disease, and bowel disorders that may impair absorption of nutrients. Taking these risk factors into account, with or without a bone density reading, allows the clinician to predict an individual’s ten year probability of sustaining a fracture. Using such probabilities we can then decide on whether or not an individual needs treatment to

Figure 1.5 Schematic view of bone density report

-4 -3 -2 -1 0 +1 +2 +3 +4

Osteoporosis
Porous bone that can lead to fractures
Low Bone Density
(osteopenia)
Normal
As compared to an average 30 year old
reduce their risk of fractures in the future. This analysis is now done using a program developed by WHO called FRAX. This model requires an individual’s age, weight, height, and answers to 8 additional questions to allow the doctor to calculate the 10 year probability of having a fracture [10].

**Limitations of BMD measurements by DEXA:**

- A single measurement lacks sensitivity and upto 50% of patients with a known osteoporotic fracture may have a normal BMD.
- WHO criteria are based on data obtained from white, postmenopausal women, employing dual energy X-ray absorptiometry (DXA) of the axial skeleton (spine and hip) and cannot be extrapolated to other populations (male, young individuals and blacks) or other techniques to measure BMD (eg. QCT, ultrasound)
- Causes of low BMD other than osteoporosis (primary hyperparathyroidism, osteomalacia) are not considered.
- Extra skeletal risk factors for fractures (eg. propensity for falls) are not addressed.
- Qualitative bone changes are not addressed [18].

Although BMD is used in the diagnosis of osteoporosis, a low BMD does not identify all individuals who are at high risk for fractures. At a population level, more fractures occur in those with osteopenia than in those with osteoporosis simply because there are a much larger number of people with osteopenia than with osteoporosis. The results of the contribution of Bone Turnover Markers (BTMs) to fracture risk have been inconsistent, due to the use of different markers and different methodologies for their assessment. This has led to the recommendation for measurement of BTMs in osteoporosis. Most of the positive results with BTMs were for bone resorption markers, with increased resorption marker predicting an increased fracture risk. BTMs can predict fracture risk independently of BMD. Fracture leads to an increase in BTMs which are evident even 6 months after the event; bone formation markers may remain elevated even at 12 months after the fracture [19].
1.9 Biochemical markers of bone turnover

Increasing evidence suggests that a high rate of bone turnover is associated with low BMD and is strongly linked to fracture risk. Measurement of biochemical markers of bone turnover is therefore becoming a more widely used end-point in clinical trials in postmenopausal osteoporosis. Biochemical markers of bone resorption and formation can be measured in the blood and urine. Frequently used markers of resorption include deoxypyridinoline, which is measured in urine, and amino- and carboxy-terminal cross-linked telopeptides of type I collagen (NTX- I, CTX- I), which may be measured in serum or urine. Bone formation markers include osteocalcin, bone-specific alkaline phosphatase and amino-terminal propeptide of type I collagen (PINP) and carboxy-terminal propeptide of type I collagen (PICP). Early changes in biochemical markers of bone turnover predict BMD response to antiresorptive therapy and may potentially identify non-responders to therapy. Significant decreases in both type I collagen N-telopeptide and osteocalcin are evident in women treated with antiresorptive agents as early as 3 months while the percent change of N-telopeptide at 3 months has been shown to correlate with change of spinal BMD at 12 months of treatment [20].

Most biochemical indices of bone resorption are related to collagen breakdown products such as hydroxyproline or the various collagen cross-links and telopeptides (Figure 1.6). Other markers of bone resorption include non-collagenous matrix proteins such as bone sialoprotein (BSP), or osteoclast-specific enzymes like tartrate-resistant acid phosphatase or cathepsin K. In contrast, markers of bone formation are either by-products of collagen neosynthesis (e.g. propeptides of type I collagen [PINP and PICP]), (Figure1.7) or osteoblast-related proteins such as osteocalcin (OC) and alkaline phosphatase (ALP). For clinical purposes, markers of bone formation are distinguished from indices of bone resorption. This distinction, however, is not as sharp as it may appear. For example, some marker components reflect, at least in part, both bone formation and bone resorption (e.g. hydroxyproline, certain OC fragments). Finally, changes in markers of bone turnover are not disease specific but reflect, as an integral measure, alterations in the metabolism of the entire skeletal envelopments independently
of the underlying cause. Hence, results of bone marker measurements should always be interpreted against the background of the clinical picture [21].

![Type 1 collagen degradation](image1.png)

**Figure 1.6 Type 1 collagen degradation**

![Procollagentype 1 propeptide cleavages](image2.png)

**Figure 1.7 Procollagentype 1 propeptide cleavages**

### 1.10 Interaction of the Wnt, BMP, and sclerostin pathways

Differentiation of osteoblasts during both development and remodeling is dependent on the activity of both the Wingless/Integrated (Wnt) and Bone Morphogenetic protein (BMP) pathways. Wnt signaling requires the interaction of the LDL Receptor Related Protein 5 (LRP5) and frizzled receptors (Frz) and can be inhibited by Dickkopf (DKK; an inhibitor of LRP5) and secreted frizzled-related protein (SFRP). Antagonists such as sclerostin can block both BMP and Wnt signaling. The mediator of the canonical Wnt pathway, β-catenin, can synergize with BMP2 to enhance osteoblast differentiation.
and bone formation. Consistent with these interactions are the findings that high bone mass can result both from activating mutations of the Wnt pathway and deletion of SOST, the gene encoding sclerostin [22, 23]. (Figure 1.8)

![Figure 1.8 Schematic view of Wnt β catenin pathway](image)

### 1.11 Interactions between adipocytes and bone turnover

Mesenchymal stem cells (MSCs), where osteoblasts originate, are precursors for adipocytes. Differentiation of MSCs into either adipocytes or pre-osteoblasts is regulated by a complex process involving many growth and transcription factors. MSC differentiation is thought to be affected by the normal aging process favoring adipogenesis due to, in part, physiologic declines in growth factor secretion as well as oxygen tension and blood supply within the bone marrow. As a result, more adipose tissue is stored in the bone cavity with advancing age. This mechanism is also supported by histomorphometric studies on iliac crest biopsies where a positive correlation was observed between BMF and age. However, it remains unclear whether marrow adipocytes induce the reduction in bone formation and increase in bone resorption that leads to age-related bone loss in older individuals or whether BMF merely occupies the empty spaces created by the reduced osteoblastogenesis process. More importantly, to what extent BMF actually affects bone metabolism requires further investigation [24, 25].