CHAPTER 2
REVIEW OF LITERATURE

Thinning bones or osteoporosis can result in a painful fracture. It includes aging, being female, low body weight, low sex hormones or menopause, smoking, socio economic status, nutrition, sun exposure and some medications etc. as risk factors. Prevention and treatment popularly include calcium and vitamin D supplementation, exercise, and osteoporosis medications. India, having all the risk factors functional at their peak and encompass a burst in the number of elderly population alongside made osteoporosis to be prevalent in the country to the fullest; hence roused encountering osteoporosis as the need of the hour. Besides, with today's fast life and limited resources have made medication, of all, the first line therapy. Supplementation of Ca and vitamin D has grabbed the spot light. But often, different types of doses are being recommended without considering the other risk factors especially age and gender. Thus, finding a most effective therapeutic regime or combined regimes that will treat osteoporosis targeting the risk factors in conjunction with.

The categories o literature search available under this chapter is comprehended as follows:

2.1 Old age – the delicate later part of life

Old age is the later part of life; the period of life comes after middle age; usually with physical deterioration (Oxford English Dictionary Online, 2013). The beginning point of old age cannot be universally defined because it shifts according to the context. In India 60 years is officially marked as old age (https://en.wikipedia.org/wiki/Elder_law_in_India). The United Nations has agreed that 60+ years may be usually denoted as old age (World Health Organization, 2013). Moreover, most of the developed Western countries have set the age of 60 to 65 years for retirement and the beginning of old-age. Besides, in Africa, the World Health Organization (WHO) has set 50 years as the beginning of old age. Whereas, the developing world often defines old age, not by years, but by new roles, loss of previous roles, or inability to make active contribution to society (World Health Organization, 2013). Old age also can comprise of three sub-populations commonly referred as the young old (65-74 years), the old (74-84 years) and the oldest-old (85+ years) (Transgenerational.org, 2009).

Marks of old age

The distinctive signs of old age consist of both physical and mental characteristics (Salokangas R. K. and Joukamaa M., 1991). These marks do not always occur at the same chronological age for everyone. Rather they may occur at different rates and order for different people (ohioline.osu.edu/ss-fact/0101, 2013). Moreover the marks of old age may vary from person to person, even those of the same chronological age, because each person is unique (Ageing: Myth and Reality, 2013, The Department of Communities, Child Safety and Disability Services, Australia, 12-13). But a basic mark of old age affects both body and mind is slowness in behavior (Birren JE. and Fisher LM., 1995). This slowing down process finds a correlation between advancement in age and slowness of reaction and task performance, both physical and mental (Donald H. *et. al*., 2007).

Physical marks of old age:

Physical marks of old age include the following:

- **Bone and joint:** Thinning and shrinkage of the body are the two very important marks of old age. This leads to a loss of height (about two inches by the age of 80),

a stooping posture in many people, and a greater susceptibility to bone and joint diseases such as osteoarthritis and osteoporosis (documbase.com, 2013).

- **Chronic diseases:** Generally an old person has been observed to have at least one or multiple chronic conditions. In 2007-2009, the most frequently occurring conditions among older persons in the United States were uncontrolled hypertension (34%), diagnosed arthritis (50%), and heart disease (32%) (U.S. Department of Health and Human Services, 2011). In India a study conducted in rural Pondicherry reported pain in joints and joint stiffness in 43.4% elderly, hypertension in 14%, heart disease in 9% and diabetes in 8.1% (Purty AJ *et. al.* 2006).
- **Other health issues among elderly:** In old age chance of developing tooth decay and infection increases because of less saliva production and poor oral hygiene (Zukerman R., 2014). Besides, problems with digestive system and difficulty in swallowing, inability to eat enough and to absorb nutrition, constipation and bleeding (Johns Hopkins Health Alerts, 2013). Reading becomes more difficult in low lighting and in smaller print because of diminished eyesight (American Psychological Association, 2014), impaired in hearing (American Psychological Association, 2013), rheumatological or malignant pains (King SA., 2009; Cleane GM. and Smith H., 2006), daytime sleepiness and troubled sleep at night (Jana R. Cooke, 2008; McCall, WV., 2004) etc. are the most common health issues prevalent amongst elderly people.
- **Falls:** elderly have a greater risk of injury from falls that might not cause injury to a younger person (Centre for Healthy Aging, 2013). Every year, about one-third of 65 years old and over half of 80 years old experience fall (learnnottofall.com, 2013). Falls are the leading cause of injury and infirmity followed by death among old people (Falls among older adults: an overview, 2015).

Mental marks of old age:

Mental marks of old age include adaptability in elderly people, caution**,** depression, fear, mental disorders, reduced mental and cognitive ability to name a few.

2.2 Elderly population of the world:

For the first time in human history our planet has contained so many older people— or such a large percentage of them. This was not the case always. In the year 2000, people 65+ years represented 12.4% of the population and expected to swell to 19% of the population by 2030. This dramatic growth is believed to be because of increased life expectancy, energetic life styles, and better healthcare facilities, declined fertility rates and declined mortality rates, which apparently now enables us to live 20 to 25% of our lives in active retirement (Transgenerational.org, 2009).

Future predictions for worldwide elderly population (*Transgenerational.org, 2009****)***

- By 2050, the 60 and older population will increase from 680 million to 2 billion—increasing from 11 to 22 percent of the world's population.
- From 1950 to 2050, the world population will have increased by a factor of 3.6; those 60 and over will have increased by a factor of 10; and those 80 and over by a factor of 27.
- By 2050, Europe will continue to be the world's oldest region with its elder population increasing more than fivefold—from 40 million to 219 million.
- Only 5 percent of Africa's population is projected to be 65 and older by 2050, with sub-Saharan Africa remaining the world's youngest region.
- China and India have the largest older populations. By 2050, China will see its number of elders grow 30% from 109 million to 350 million—India, from 62 million to 240 million.
- Japan, with today's largest share of the world's old-age population, will see its percentage of those 60 and over rise from 27 percent to 44 percent in 2050.
- By 2050, more than 70 countries, representing about one third of the world's population, will surpass Japan's present old-age share of 27 percent.
- In the coming decades, all regions of the globe will experience population aging. Today's 5-22 percent range will become an 11-34 percent range in 2050 (UN, 2009)

Elderly population in India:

In India the size of the elderly population, i.e. above the age of 60 years is growing fast although it constituted only 7.4% of total population at the turn of the new millennium and a big portion of the same i.e. 75% reside in rural areas of India (Central Statistics Office, Ministry of Statistics, 2011). There had been a firm rise in the share of elderly population (aged 60 years or above) in the total population over the decades. As against 5.6% in 1961, the proportion goes up to 7.4% in 2001. For males the rise was a bit decent i.e. from 5.5% to 7.1%, while for females there had been a steep rise from 5.8% to 7.8% during the five decadal Censuses from 1961 to 2001. The decadal growth rate of India's elderly population and of the general population, for the period 1951 to 2011, as shown in the table below, revealed once again that the aged population in India had grown very steadily since 1951 at a much faster rate as compared to that of general population (Population Census Data, 2011). For a developing country like India, this may pose an increasing pressure on various socio economic fronts such as pension outlays, health care expenditures, fiscal discipline, savings level etc. Again this segment of population faces multiple medical and psychological problems. There is an emerging need to pay greater attention to ageing-related issues and to promote holistic policies and programs to deal with the ageing society.

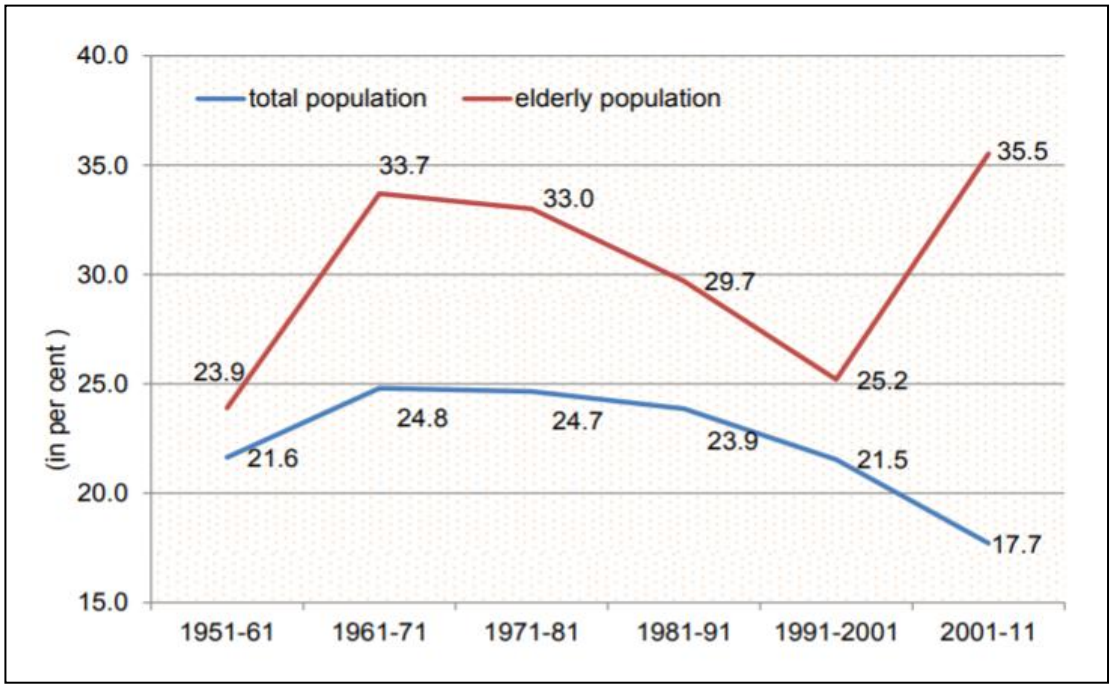
Table 2.2.1 Growth of Elderly Population (60+) by Gender, India Population 60+ (millions)

Year	Total Population	Males	Females
1901	12.06	5.50	6.56
1911	13.17	6.18	6.99
1921	13.48	6.48	7.00
1931	14.21	6.94	7.27
1941	18.04	8.89	9.15
1952	19.61	9.67	9.94
1961	24.71	12.36	12.35
1971	32.70	16.87	15.83
1981	43.98	22.49	21.49
1991	55.30	28.23	27.07

2001	75.93	38.22	37.71
2011	35.5	51.1	52.8

Source: Ageing in India: Occasional Paper No.2 of 1991, Office of the Registrar General & Census Commissioner, India, and Population Census Data, 2011

Figure 2.2.2: Decadal growth in elderly population vis-à-vis that of total population



Source: Population Census Data, India, 2011

According the census 2015, at the National level, percentage of aged (60+) population is 8.3 millions. Composition of 60+ aged females is higher in all of the bigger States/UTs except Andhra Pradesh, Assam, Bihar, Odisha, Telangana and West Bengal. In rural areas population in the age group 60+ constitutes 8.3 percent of the total population and variation in aged population ranges from 6.4 percent in Assam to 13.3 percent in Kerala. The urban proportion of aged population in most of the states is lower than the corresponding rural share (except Assam, Delhi, Jammu & Kashmir, West Bengal, Jharkhand, Bihar and Rajasthan). Residence-wise percentage of old age population in bigger States/UTs is given below.

Table 2.2.3 Percentage of Population in the age group 60 years and above to the total population by sex and residence, India and bigger States/UTs, 2015

India and bigger States/UTs	Total	Total male	Total female	Total rural	Total urban
Andhra Pradesh	9.8	10.1	9.4	10.5	8.4
Assam	6.8	7.0	6.5	6.4	8.1
Bihar	6.8	6.9	6.7	6.6	8.6
Chhattisgarh	7.1	6.6	7.7	7.3	8.3
Delhi	6.9	6.5	7.3	6.6	6.5
Gujarat	8.6	7.8	9.4	8.9	6.9
Haryana	7.6	7.1	8.2	7.7	8.2
Himachal Pradesh	11.2	10. 9	11.5	11.4	7.4
Jammu & Kashmir	9.7	9.5	9.9	9.2	11.0
Jharkhand	6.7	6.6	6.8	6.5	7.2
Karnataka	8.3	7.8	8.8	8.7	7.6
Kerala	13.1	12.4	13.8	13.3	12.9
Madhya Pradesh	7.2	7.0	7.4	7.2	7.2
Maharashtra	9.3	8.9	9.7	10.2	8.0
Odisha	9.8	9.9	9.6	10.0	8.8
Punjab	10.3	9.7	11.0	11.1	9.2
Rajasthan	7.4	6.7	8.2	7.4	7.6
Tamil Nadu	10.5	10.3	10.7	10.8	10.2
Telangana	8.5	8.5	8.4	9.9	6.2
Uttar Pradesh	7.0	6.7	7.4	7.1	6.9
Uttarakhand	9.1	8.4	9.8	9.6	7.6
West Bengal	9.0	9.1	8.9	7.8	11.6

Source: Population Census, India, 2015

2.3 Bone – the protective and structural support of body

Bone is basically connective tissue that comprises the skeleton of vertebrate. Bones have a numerous significant functions in the body such as supporting and protecting various organs of the body, producing red and white blood cells, storing minerals, providing structure and support to the body, and enable mobility.

Bones have a variety of shapes and sizes and have a complex internal and external structure. Bone tissue is hard and a type of dense connective tissue having a honeycomb-like matrix internally, which helps to give the bone rigidity. Bone tissue is made up of different types of bone cells (Cavendish M., 2010; wikipedia.org). Osteoblasts and osteocytes are such bone cells involved in the formation and mineralization of bone. Osteoclasts are involved in the resorption of bone tissue and flattened osteoblasts become the lining cells that form a protective layer on the bone surface. The mineralized matrix of bone tissue has an organic component (mainly collagen) called ossein and an inorganic component of bone mineral made up of various salts. The mineralized bone tissues are of two types, cortical and cancellous bone (Cavendish C., 2010; wikipedia.org).

Structure of bone:

Bone is an irregularly distributed solid but a tough matrix. This matrix makes up about 30% of the bone and the other 70% is made up of salts that give strength to it. The matrix is made up of 90-95% collagen fibres, and the remaining part is ground substance (John H., 2011). The primary tissue of bone (osseous tissue) is relatively hard and light in weight. Bone's matrix is mostly made up of hydroxylapatite – a composite material (this is the bone mineral that gives bones their rigidity), collagen, an elastic protein which improves fracture resistance (Schmidt N. and Knut, 1984). Bone is formed by the hardening of this matrix around entrapped cells. When these cells become entrapped from osteoblasts they become osteocytes.

Functions of bone: Bones serve a variety of functions.

Mechanical functions

- Bones together the in the body form the skeleton and provide a frame to support the body.
- It attaches the skeletal muscles, tendons, ligaments and joints together, and function together to generate and transfer forces, so that individual body parts or the whole body can be manipulated in three-dimensional space.
- Bones protect internal organs i.e. skull protects the brain, ribs protect the heart and lungs.
- Bone has a high compressive strength of about 170 MPa (1800 kgf/cm²) (Schmidt-Nielsen and Knut, 1984), but a poor tensile strength of 104–121 MPa, and a very

low capacity to bear shear stress i.e. 51.6 MPa (Vincent K., 2013; Turner CH. *et. al.*, 2001). Because of its structural characteristics bone can resist pushing (compressional) stress well, pulling (tensional) stress less well, but shear stress (torsional loads) poorly.

- Bone is brittle in nature, made of collagen chiefly and does not have a significant degree of elasticity (Levrero F. and Margetts L. *et. al.*, 2016).

Synthetic functions

- The cancellous part of bones contains bone marrow. In bone marrow hematopoietic cells produces red blood cells, erythroblasts produce white blood cells and megakaryocytes produces platelets (Fernández, KS.; Alarcón, PA., 2013; Deakin 2006).
- Bone marrow is the site where red blood cells are produced as well as defective or aged RBCs are destroyed.

Metabolic functions

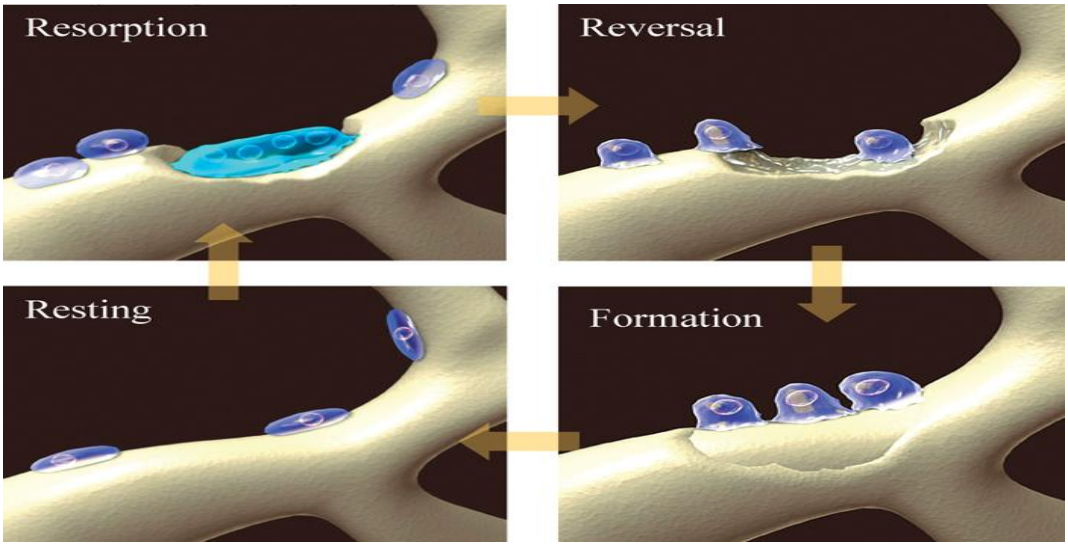
- **Mineral storage:** Bones reserve minerals important for the body, mostly calcium and phosphorus (Doyle ME. *et. al*., 2008).
- **Growth factor storage:** Important growth factors such as insulin-like growth factors, transforming growth factor, bone morphogenetic proteins etc. are stored in mineralized bone matrix (en.wikipedia.org).
- **Fat storage:** Fatty acids are stored in the yellow bone marrow (en.wikipedia.org).
- **Acid-base balance:** Alkaline salts are released by bones to buffer the blood in a condition of excessive pH changes (en.wikipedia.org).
- **Detoxification:** Bone tissues remove heavy metals and other foreign elements from blood and reducing their effects on other tissues. These can later be gradually released for excretion (en.wikipedia.org).
- **Endocrine organ:** Fibroblast growth factor – 23 (FGF-23) is released by bone which controls phosphate metabolism, and acts on kidneys to reduce phosphate re-absorption. A hormone called osteocalcin is released from bone cells, which contributes to the regulation of blood sugar (glucose) and fat deposition. Osteocalcin increases both the insulin secretion and sensitivity, in addition to boost the number of insulin-producing cells and reduce stores of fat (Na Kyung L; *et al.*, 2007).

- **Calcium balance:** During the bone resorption, osteoclasts releases stored calcium into the systemic circulation and is an important process in regulating calcium balance. In its mineral form bone formation actively maintains the circulating calcium by removing it from the blood stream. Whereas, resorption by actively unfixing it increases circulating calcium levels. These processes occur in tandem at site-specific locations (en.wikipedia.org).

Remodelling function

Bone remodelling is a constant process of creating and replacing the bone tissues. This ongoing turnover process of resorption follows a replacement of bone with little change in shape. This process is accomplished by osteoblasts and osteoclasts. These two cells are stimulated by a variety of signals, and together referred as a remodelling unit. Approximately 10% of the skeletal mass of an adult is remodelled each year (Manolagas SC., 2000). The purpose of remodelling is regulating the calcium homeostasis, repairing micro damages in bones caused by everyday stress, and shaping the skeleton during growth.

Figure 2.2.4 Bone remodelling



Source: Advances in the use of bisphosphonates in the prostate cancer setting. J P Coxon, G M Oades, K W Colston and R S Kirby.

2.4 Osteoporosis – the silent disease

Definition

Osteoporosis is a disease of bone characterized by low bone mass and micro-architectural deterioration of bone tissue, that leads to enhanced bone fragility and

increased likelihood of fractures (World Health Organization, 1994; Dey, 2003). Osteoporosis may be caused due to lower than the normal peak bone mass and greater than the normal bone loss, thus osteoporosis can be defined as the evaluation of bone mineral density (BMD). Osteoporosis is operationally defined as the BMD T-score of <-2.5 SD for young healthy men and women (a) (World Health Organization, 1994). Osteoporosis is also called as a "silent disease" because until and unless a fracture occurs, low bone mass density is not revealed. It is a disease that weakens the skeleton and breaks the bones (National Institute of Health, Osteoporosis in men, January 2012). The National Institute of Health (United States) consensus conference in the year 2000 has modified the definition of osteoporosis as "a skeletal disorder characterized by compromised bone strength predisposing a person to increased risk of fracture. Bone strength reflects the integration of 2 main features: bone density and bone quality" (National Institute of Health C. S., 2000). Hence, the quality of bone is greatly affected by the quantity of the bone mass. Low bone mass results in faster rate of bone porosity and predisposes to osteoporotic fractures (Heaney RP., 2012). Thus, poor bone mass density is the most common reason for a broken bone among the elderly especially among the post menopausal women due to lower levels of estrogens.

Signs and symptoms of osteoporosis

In general osteoporosis itself has no symptoms; its main consequence is the increased risk of bone fractures. Osteoporotic fractures or fragility fractures occur in situations where healthy people would not normally break a bone. Very often a typical fragility fractures occurs in the vertebral column, rib, hip and wrist.

Types of osteoporosis

Four types of osteoporosis are there, such as primary, secondary, osteogenesis imperfecta and idiopathic juvenile.

Primary osteoporosis

Primary osteoporosis is the most common one. A person reaches peak bone mass (density) at about age 30 years. After that, the rate of bone loss slowly increases and rate of bone building decreases. Primary osteoporosis also depends on the thickness of the bones in early life as well as health, diet, and physical activity at all ages. More over females have an additional factor to accelerate primary

osteoporosis i.e. menopause (webmd.com, 2017). Ibandronate, denosumab, and strontium ranelate etc. are few popular first line therapies to treat primary osteoporosis.

Secondary osteoporosis

Secondary osteoporosis has more or less similar symptoms as primary osteoporosis. It occurs as a result of having certain medical conditions, such as endocrine disorders (hyperthyroidism, Cushing's syndrome, hypogonadism in males); gastrointestinal disorders like inflammatory bowels disease causing malabsorption; cancer chemotherapy, multiple myeloma, chronic renal failure, prolonged immobilization, osteogenesis imperfecta, inflammatory arthritis (ankylosing, rheumatoid arthritis), barbiturates, prolonged consumption of drugs like corticosteroids, aluminum – containing antacids, gonadotropin- releasing hormone therapy and malnutrition (webmd.com, 2017). Treatment of secondary osteoporosis is way more critical than the treatment of primary osteoporosis. Treating the underlying cause is first step to treat secondary osteoporosis.

Osteogenesis imperfecta

This is a rare form of osteoporosis that is acquired by birth. This form of osteoporosis is causes fracture apparently for no reason (webmd.com, 2017). Genetic reasons generally cause this type of osteoporosis and their care included strengthen the muscles, care of broken bones, physiotherapy, surgery etc.

Idiopathic juvenile osteoporosis

Idiopathic juvenile osteoporosis is pretty rare and it occurs among children of 8-14 years or during the times of rapid growth. There is no specifically known cause for this type of osteoporosis, but it is assumed that either there is too little bone formation or excessive bone loss. But the condition definitely increases the risk of fractures like other forms of osteoporosis (webmd.com, 2017).

Risk factors of osteoporosis

Risk factors for osteoporosis can be divided between modifiable and non-modifiable (potentially).

Non-modifiable risk factors

- **Age:**

As age increases, there is a risk for osteoporosis and increase incidence of fracture in both men and women equally (Liu A., 2004).

- **Female gender:**

  Estrogen deficiency after menopause or surgical removal of the ovaries is correlated with a rapid reduction in bone mineral density (bone turnover process). While in men, a decrease in testosterone levels has comparatively less hazardous impact (Sinnesael M. *et. al*., 2013; Sinnesael M. *et. al*., 2011).

- **Menopause:**

  Postmenopausal osteoporosis is a very common cause of morbidity and mortality (Shah SR., 2005; Brown LB, 2005). The average age for women reaching menopause is around 52 years, but in India onset of menopause takes place early. Therefore it leads to early reduction in bone mass and thus increases the risk of early fractures.

- **Race:**

  Though, osteoporosis has affected every part of the world; European and Asian ancestry predisposition of osteoporosis (Melton LJ., 2003).

- **Heredity:**

  According to Cooper GS (1999), 50% of the peak bone mass, bone geometry, bone strength and bone architecture depends on genetic predisposition. Thus, individual with a family history of fracture or osteoporosis is at an increased risk of developing the heritability of the fracture and low bone mineral density up to 25 to 80% (Rodriguez, V., 2008). At least 30 genes are associated with the development of osteoporosis (Raisz L., 2005). Also, those who have already experienced a fracture are at least twice at risk to have another fracture compared to someone of the same age and sex (Ojo F. *et. al.*, 2007).

- **Build:**

  A small stature is also a non-modifiable risk factor associated with the development of osteoporosis (Alldredge BK. *et. al.*, 2009).

Potentially modifiable risk factors:

- **Type 2 diabetes Mellitus:**

  Type 2 diabetes mellitus (DM) is also associated with low bone mineral density (BMD) and paradoxically with increased fracture risk. When low BMD score

among older adults with type 2 DM was analyzed; femoral and neck BMD T score and FRAX score were found to be associated with hip and non-spine fracture risk. (Melton LJ, 2008).

- **Peak bone mass development:**

  To prevent osteoporosis achieving and maintaining the peak bone mass is most important factor. Though the signs of osteoporosis appear later in life but it is a cumulative result of the accumulation of bone mass in childhood and adolescent period i.e. 40% of bone mass develops between from the late childhood and adolescence period (Cummings SR., 2002). Now the accomplishment of peak bone mass is further influenced by dietary calcium and vitamin D intake, physical activity at the childhood and adolescent age (Pattif AM., 1982). An Indian study showed that peak bone mineral density is attained in healthy males at the age of 25 years and in females at 28 years (Indian Council of Medical Research, 2007).

- **Parity- lactation:**

  Studies form developing countries like Morocco, Vietnam, and Korea showed that parity and lactation have a detrimental effect on Bone Mineral Density (Memom A., 1998). Studies from South America showed that osteopenia and osteoporosis are more prevalent among nulliparous women than non-nulliparous women (Black DM., 1999). Studies also have shown that prolonged lactation, period of amenorrhea and pre pregnancy weight have a vital role to loss of calcium and decrease bone density (Grimes JP., 2003).

Medical factors

- Disease conditions like gastrointestinal disorders (Malabsorption, Inflammatory bowel Syndrome), haematological disorders (Thalassemia, Pernicious anaemia) and hypo gonadal states, thyrotoxicosis, anorexia nervosa etc. are the leading causes for secondary osteoporosis in men and women (National Osteoporosis Foundation, 2011).
- Regular and excess usage of steroids in various medical aliments may trigger to reduced bone mass and lead to increased risk of fracture (National Institute Health, 2001). A recent meta-analysis from Cochrane showed the use of steroidal contraceptive like Depot medroxyprogesterome acetate (DMPA) reduced bone mineral density and increased the risk of fractures.

Nutritional factors

- Vitamin D plays an important role in calcium absorption in body and overall bone health. Vitamin D supplementation combined with calcium is beneficial for fractures; however, vitamin D alone is not beneficial to improve bone health (DIPART, 2010; Avenell A. *et. al.,* 2014, Holick, 2007)
- Malnutrition is the eternal chronic health problem in Asian and African countries. Inadequate intake of protein in diet delays the building of peak bone mass at childhood and adolescence and increase the risk of developing osteoporosis, osteoporotic fractures later in life (Rizzoli R., 1999). At the same time it has also been believed that high intake of calcium and protein in diet provokes the negative calcium balance in body and decrease the bone mineral density.
- Several cross-sectional studies had shown that adequate intake of calcium in diet during the childhood and adolescent helps to achieve the peak bone mineral density (Yousef FMA, 2015). Along with dietary calcium deficiency and low body mass index (BMI) plays an important role in reduction on BMD (Mazoccoa L *et. al.* 2017). Having said that, it must be mentioned here; Ca works together with vitamin D to build bone mass.
- Peak bone mass at the childhood and adolescent age is highly affected by the state of protein supply and intake. Indeed, the need for protein is increased in childhood and adolescence, to optimize the body growth. Protein malnutrition rather can be considered as a risk factor for hip fracture because, to increase the chances of fall by impairing movement coordination (Shatrugna V *et. al.* 2005) had evidenced that 98.82% of the study population who were vegetarian had osteoporosis (Gandhi AB., 2005). Vegetarian diet very often contains less calcium, besides, mal-absorption of calcium and poor availability of the same in blood may be the contributing factors for low BMD and risk for osteoporotic fractures among vegetarians (Barr SJ., 1998).

Physical activity

Exercise increases bone strength, as measured by bone mineral density (BMD), in people of all age groups. The improvement in bone strength, however, also depends on some other factors including age, reproductive hormone status, nutritional status, and the nature of the exercise. Exercise combined with the above mentioned factors minimize bone loss in adulthood, improve peak bone mass, and reduce the risk of falling. A cohort study showed that the physical inactivity

among the old age population is responsible for declining BMD and was the major risk factor for osteoporotic fractures (Coup CL., 1993).

Low body weight and body mass index

Relationship between body mass index (BMI), weight, height, and BMD was well established for different populations (Nguyen TV. *et. al.,* 2000). Body weight or BMI has been found to be inversely related to the risk of osteoporotic fractures (Ravn P. *et. al.,* 1999; Nguyen TV. *et. al.,* 2000; Siris ES., 2001). An epidemiological study had reported that low body weight is one of the important determinants and risk factor for hip fractures (Ensurd KE., 1997).

Low exposure to sunlight

Vitamin D stimulates the absorption of calcium and magnesium; they both are essential to maintain strong and healthy bones. But surprisingly dietary sources (like food and supplements) alone accounts for partial amount of vitamin D circulates in our blood; and here the sun shine enters! Sunlight is an excellent natural source of the best form of Vitamin D_3- Cholecalciferol. It is very unique about Vitamin D_3 that with the help of sun rays, human body can synthesis it in kidneys. We have few scientific evidences on sunshine and osteoporosis. One such multi centric and cross sectional study showed a strong association between less sun exposure and osteoporosis, which decreased the fracture threshold among men and women of $\geq$ 50 years (Johnell O., 1995). Being a tropical country India has enough sun exposure but few unavoidable factors like lifestyle, wearing sunscreen, not letting the body exposed to sun etc. are few of those reasons behind the decreased serum vitamin D and BMD.

Smoking

Studies had shown a relationship between tobacco use and decreased bone density but it is a bit complicated to analyse its impact on bone health. It is hard to determine whether a decrease in bone density occurs due to smoking itself or because of the other risk factors common among smokers. For example, in many cases smokers are thinner than non-smokers, tend to drink more alcohol, may be less physically active, and have poor diets. Women who smoke also tend to have an earlier menopause than non-smokers. Such factors place many smokers at an increased risk for osteoporosis apart from their tobacco use. A case control study

from USA showed that the smokers had comparatively high risk of hip fracture OD 2.27 (95% CI 1.22-4.21) (Krall EA., 1991).

Alcohol consumption

In presence of alcohol stomach does not absorb calcium adequately and also interferes with the pancreas and its absorption of vitamin D. Alcohol also affects the liver, which is important for activating vitamin D and calcium absorption. Alcohol decreases the circulating estrogen which leads to an irregular periods, slow bone remodeling and bone loss. More over alcohol increases the amount of two potentially bone-damaging hormones, cortisol and parathyroid hormone. High level of cortisol decreases bone formation and increase bone breakdown and high level of parathyroid hormone leaches calcium out from the bone. Also, excess alcohol kills osteoblasts- the bone-making cells increases risk of hip fractures up to five times in men and women (Hoidrup S., 1999).

2.5 Prognosis of osteoporosis

Although osteoporotic patients have an increased mortality rate due to the complications of fracture but it is rarely lethal. Fractures can lead to decreased mobility, prolonged morbidity and additional risks of numerous complications (such as deep venous thrombosis and/or pulmonary embolism, and pneumonia) (Melton LJ., 2001). However, it is important to recall that both bone strength and the amount of force applied to the bone contribute to the occurrence of fracture in an individual. Several studies said that there is an inverse relation among bone density and fractures and reduced BMD (Marshall D, 1996). A perspective population based study of Rotterdam, showed a decline of age adjusted Hazard ratio with per SD decrease in Bone Mineral Density at femoral neck i.e.1.5 for women and 1.4 for men at the age of 50 years. The study also showed that the osteoporotic fractures occur mainly in the trabecular bone areas of the skeleton (Holoroyld C, 2008). Also a meta-analysis showed that previous history of fracture is one of the major risk factors for osteoporotic fracture and chances to get another fracture are doubled in such cases (Bonaiuti, 2002; Ross PD., 1993).

Hip fractures

The most serious consequence of osteoporosis is hip fracture. Hip fracture is the international barometer of osteoporosis, disability, and needs more costs to repair

(Cummings SR., 2002). Treatment of osteoporotic fractures invole prolonged hospital stay (30 days generally); pretty similar to breast cancer, cardiovascular diseases, and chronic obstructive pulmonary diseases etc. (Kanis JA., 1994). The incidence of hip fractures is increasing each decade for both women and men for all populations (Kanis JA., 2002). The highest incidence of fracture is found among men and women aged ≥80 years (Brenneman SK. *el. al.,* 2006). In the United States, more than 250,000 hip fractures annually take place due to osteoporosis (Hannan EL. *et. al.,* 2001). In India osteoporotic hip fractures have affected both the genders equally and mostly occurring at the age of 60-70 years. Moreover studies had shown that osteoporotic fractures are prevalent among the elderly belonged to the low socio economic status up to 29% in northern India (Shatruguna V., 2005). Race or genetic influences also affect the risk of hip fractures sometimes. A study showed that the lifetime risk of fracture at the proximal femur for a 50-year-old white woman is estimated to be 17.5%.

Vertebral fractures

The impact of vertebral fracture is comparatively low but these fractures cause loss of height, kyphosis, back pain, loss of mobility and increases the risk of further fractures (Neil O., 1996). Data showed that in the United States, 700,000 vertebral fractures occur annually, but only about one third is recognized. In other study 9704 women aged 68.8 were studied for 15 years and it was observed that 324 had already suffered a vertebral fracture before being enrolled in the study and 18.2% experienced vertebral fracture during the study. But that risk of experiencing more fractures rose to 41.4% in women with previous history of vertebral fracture (Riggs BL. and Melton LJ, 1995). Osteoporotic vertebral fractures are prevalent among Asians similar to Caucasians. Majority of these fractures take place because of falls or during performing the routine activities like lifting of light objects, and heavy loads.

Wrist fractures

This osteoporotic wrist fracture occurs more often in the middle age and takes place due to simple fall in the out stretched hands; and is left as the leading cause for future osteoporotic fractures (World Health Organization, 1994). Wrist

fractures are the third most common type of osteoporotic fractures. In the United States, 250,000 wrist fractures take place annually because of osteoporosis (Hannan EL. *et. al.*, 2001). By the time women reach 70 years of her age, about 20% have experienced at least one wrist fracture (Brenneman SK. *el. al.*, 2006).

Rib fractures

Fragility fractures of the ribs are pretty much common in men as young as age 35 years. But unfortunately these are often overlooked as a sign of osteoporosis, as these men are often physically active and suffer the fracture in the course of physical activity. An example would be as a result of falling while water skiing or jet skiing. However, a quick test of the individual's testosterone level maybe performed during the course of diagnosis of the fracture to reveal readily whether that individual is at risk of osteoporosis.

2.6 Pathogenesis of osteoporosis:

An inequity between bone resorption and bone formation is the basic mechanism of osteoporosis. Matrix remodeling of bone is constant in normal bone and up to 10% of all bone mass may undergo remodeling process at any point in time. The process takes place in the bone multi cellular units (BMUs) and it was first described by Frost & Thomas in 1963 (Frost HM. *et. al.*, 1963). Osteoclasts and osteoblasts are the two types of cells involved in this process. Osteoclasts with the help of transcription factor PU.1 degrade the bone matrix, while osteoblasts rebuild the bone matrix. Low bone mass density is the outcome of a situation when osteoclasts are degrading the bone matrix faster than the osteoblasts are rebuilding the bone (Shuyan Wu., 2013).

Following are the three main mechanisms by which osteoporosis develop:

- An inadequate peak bone mass (the skeleton develops insufficient mass and strength during growth).
- Excessive bone resorption.
- Inadequate formation of new bone during remodelling.

These three mechanisms interplay to develop fragile bone tissue (Raisz L., 2005). The rate of bone resorption is strongly determined by hormonal factors; lack of estrogen (e.g. as a result of menopause) increases bone resorption, as well as decrease the deposition of new bone cells that normally takes place in weight-

bearing bones. The α-form of the estrogen receptor appears to be the most important element in regulating bone turnover (Raisz L., 2005). Apart from estrogen, calcium metabolism plays a significant role in bone turnover, and deficiency of the same leads to impaired bone deposition. In addition, the parathyroid glands react to low calcium levels in blood by secreting parathyroid hormone, which further increases bone resorption to maintain sufficient calcium in the blood. On the contrary, a hormone named calcitonin, generated by the thyroid gland increases bone deposition, but the activity of this hormone is less clear and probably not as significant as that of PTH (Raisz L., 2005).

Regulation of bone turnover

RANKL (receptor activator of nuclear factor kappa-B ligand) is one of the best molecular signals to activate the osteoclasts. Osteoclast is produced by osteoblasts and lymphocytes, which stimulates RANK (receptor activator of nuclear factor κB). RANKL is then gets bound with osteoprotegerin (OPG) before it gets a chance to bind to RANK, thus its ability to increase bone resorption gets suppressed. Local production of eicosanoids and interleukins is supposed to participate in the regulation of bone turnover, and excess or reduced production of these mediators may underlie the development of osteoporosis (Raisz L., 2005).

A bone is constituted with two parts i.e. trabecular bone: the sponge-like bone in the ends of long bones and vertebrae (or cancellous bone); and cortical bone: the hard outer shell of bones and the middle of long bones. As osteoblasts and osteoclasts inhabit the surface of bones, trabecular bone is more active and is more involved in bone turnover and remodeling. The common osteoporotic fracture sites are wrist, hip and spine, which have a relatively high trabecular bone compared to cortical bone. These sites pretty much rely on the trabecular bone for strength. So, the intense remodeling insists these sites to degenerate most when the remodeling is imbalanced. Around the ages of 30–35 years, cancellous or trabecular bone loss begins. Women may lose as much as 50%, while men lose about 30% at the same time, age and same circumstances (Alldredge BK. *et. al.,* 2009).

Diagnosis of osteoporosis

The diagnosis of osteoporosis is based on demonstration of low bone mineral density (BMD) by bone densitometer. The three most popular tools for diagnosis

are conventional radiography, dual-energy X-ray absorptiometry and Quantitative ultrasound. World Health Organization (WHO) has standardized the interpretation of BMD based on a comparison of a patient's BMD with the mean for a normal young adult population of the same sex and race (Kanis JA, 1994). In addition to the detection of abnormal BMD, the diagnosis of osteoporosis requires investigations into potentially modifiable underlying causes such as serum Ca and vitamin D levels, and other related diseases. To measure the BMD, the subject is assigned a "T-score" which is the number of standard deviations above or below the mean BMD for normal young adults, i.e.

Table 2.6.1 BMD cut offs

Category	**T-score range**
Normal	T-score ≥ -1.0
Osteopenia	$-2.5 <$ T-score < -1.0
Osteoporosis	T-score ≤ -2.5
Severe osteoporosis	T-score ≤ -2.5 with fragility fracture

Source: WHO, 1994

The WHO criterion has been derived from Caucasian postmenopausal women, thus the cut off is applicable to the Indian population too. Standards for osteoporosis given by WHO is been widely used in clinical practices worldwide. In India no normative data is available for the gross population. So, it is possible that if WHO criteria are used, substantial number of subjects may be over or under diagnosed and over or under treated. Thus, it is crucial that the normative data for major Indian ethnic groups should be established or alternative methods to measure the bone mass should be developed.

Conventional radiography

Conventional radiography can be used independently or coupled with CT or MRI, to detect complications of osteopenia, such as fractures (for differential diagnosis of osteopenia), follow-up in particular clinical conditions such as soft tissue calcifications, osteomalacia in renal osteodystrophy, secondary hyperparathyroidism etc. However, radiography is relatively insensitive to detect early stage of osteoporosis and requires a substantial amount of bone loss (about 30%) to be apparent on X-ray images. Cortical thinning and increased

radiolucency are the main radiographic features of generalized osteoporosis. A spinal radiography can help considerably in diagnosis and follow-up of osteoporosis induced vertebral fractures (en.wikipedia.org, 2017).

Dual-energy X-ray

Dual-energy X-ray absorptiometry (DXA) is considered as the gold standard for the diagnosis of osteoporosis and perfect tool to diagnose at the low BMD induced fractures especially among the elderly (Kuttikat A. *et. al*., 2004). Osteoporosis is diagnosed when the bone mineral density is less than or equal to 2.5 standard deviations below that of a young (30–40-year-old) (WHO, 2003; Henwood MJ, Binkovitz L, 2009), healthy adult women reference population. Though this is the commonly used investigational technique it has got some limitations too. It is poorly available in hospitals especially in the remote areas because of its high expenditure. Exposure to radiation is another notable drawback of DEXA.

Quantitative ultrasound (QUS)

QUS, to measure the mineral density in the peripheral skeleton has grabbed a considerable interest in recent years (Guglielmi G, Scalzo G., 2010). Quantitative ultrasound assesses the calcaneus site of skeleton because it has a high percentage of trabecular bone that is replaced more often than cortical bone, providing early evidence of metabolic change. Also, the calcaneus is fairly flat and parallel that reduces the repositioning of errors (FDA Cleared, 2016). QUS is an intriguing method to characterize the mineral properties of the bone in a non- invasive, non-ionizing, non radiation, non destructive method, relatively accurate and most importantly it portable. The primary advantage of QUS is that it is capable of measuring not only bone quantity (e.g. BMD), but also bone quality. Preliminary results for predicting osteoporosis using QUS are promising, and it has a great potential for wide spread application. However, there are few noted limitations with QUS too, which includes the tissues boundary interaction, the nonlinear function of density associated with bone ultrasonic attenuation, the single index covering a broad range of tissues (including the cortical and trabecular regions), and the interpolation of the results.

Biomarkers

Bone degradation can also be detected using chemical biomarkers. The enzyme cathepsin K breaks down type-I collagen protein, an important constituent in bones. Prepared antibodies can recognize the resulting fragment, called a neoepitope, as a way to diagnose osteoporosis (Yasuda Y. *et. al.*, 2005). Besides, increased urinary excretion of C-telopeptides, a type-I collagen breakdown product, serum calcium and vitamin D levels also serve as a biomarker for osteoporosis (Pierre M., 1998).

2.7 Epidemiology of osteoporosis:

Osteoporosis is the second largest, after cardiovascular diseases, global health problem and one of the leading causes of morbidity among elderly population of both the sexes in most part of the World (Malhotra N. and Mithal A., 2008; Ojo F. and Edwards BJ., 2003; Cashman, 2002). Worldwide more than 33.6 million have low bone mass density, which is placing them at an increased risk of osteoporotic fractures (National Osteoporosis Foundation, 2011). Epidemiologically osteoporotic fractures have turned out to a major public health problem (National Institute of Hhealth C. S., 2000). To support the same evidences were given by Nguyen T.V. (2004) and RACGP-Guidelines (2012). According to them considering the WHO cut offs, approximately 11% men and 27% women aged 60 years and older were documented osteoporotic; and approximately 42% men and 51% women were osteopenic in the Osteoporosis Epidemiology Study. An overall picture of the prevalence of poor BMD and osteoporotic fractures worldwide is given next.

Global scenario of osteoporosis among the elderly population

Worldwide, osteoporosis causes fracture in every 3 seconds (Gullberg B. *et. al.* 1997). Osteoporosis, currently is affecting more than 200 million women worldwide, which is approximately the one-tenth of the women aged 60 years globally, one-fifth of women aged 70 years, two-fifths of women aged 80 years and two-thirds of women aged 90 years (Kanis JA. 2007). Data from the different corner of the world had reported a high prevalence of osteoporosis. Osteoporosis affects almost 44 million U.S. women and men aged 50 and older, and had estimated the number to reach to >61 million by 2020 (www.nof.org, 2011). In Europe, India, Japan and the USA alone, there are an estimated 125 million people

with osteoporosis. Countries in Middle East and Africa, despite of ample sunshine the mortality rate after a hip fracture was higher by 2-3 folds than the western populations (Baddoura R. *et. al*., 2011). Moreover the number of people living with osteoporosis in all regions of the world is set to increase dramatically in the coming decades, due to the increased number of ageing populations and changed lifestyle (International Osteoporosis Foundation, 2014). Osteoporosis alone consumes a big proportion of any country's economy. The total direct costs of treatment of osteoporosis in Europe were estimated at €31.7 billion which were expected to increase to €76.7 billion in 2050 (Kanis JA and Johnell O., 2005). It is estimated that more than 50% of global osteoporotic hip fractures will occur in Asia by the year 2050 (Gullberg B, 1997; Cooper C. *et. al.*, 1993). Osteoporosis is greatly under diagnosed and undertreated in Asia especially in rural areas. Treatment is relatively expensive and is not widely available in most developing Asian countries (International Osteoporosis Foundation, 2009).

Scenario of osteoporosis among the Indian elderly population

Old history of osteoporosis and osteoporosis induced fractures

- In past also we had few studies mentioning that osteoporosis as a growing health problem in the medical history of India. Though the exact figures on the prevalence of osteoporosis neither were nor are available yet, but an extended estimation by Joshi VR. *et. al.* in 1998 said that more than 61 million Indians had osteoporosis with women accounting for 61% of them.
- Another similar study was conducted in a hospital in New Delhi in the year 1974. In this study Vaishnava and Rizvi studied 421 patients with hip fractures, of which 33% were caused by osteoporosis.
- Peak incidence of osteoporosis in most of the western countries, occurs at about 70-80 years of age, but in India it afflicts about 10-20 years younger, at the age of 50-60 years (Damodaran P. *et. al.,* 2000).
- In a study carried out in Medical College Kanpur 1965, Out of 53 cases (≥50 years) 89% showed evidence of osteoporosis. This data was compared with the data of western countries and concluded that hip fractures occur ten times earlier among the Indian males and females (average 56 years) and are relatively more frequent among males (Samuel K. *et. al*., 1967).

Recent history of osteoporosis and osteoporosis induced fractures

- Osteoporosis is greatly under diagnosed and undertreated in India and particularly acute in rural areas, even in the most high risk patients who already have experienced a fracture (Giangregorio L., 2006). Thus the exact number of prevalence is unavailable since most patients with osteoporotic fractures, with the exception of hip fracture are not hospitalized. Approximately, the incidence of hip fracture is 1 woman to 1 man in India (Damodaran P. *et. al.*, 2000).
- In India, the approximate number of osteoporotic sufferers was 26 million (2003 figures) with an expected rise up to 36 million by 2013 (Osteoporosis Society of India, 2003).
- In the Institute of Medical Sciences, Karad, 288 patients were screened by Rao H. *et. al.* (2003), and the results showed that 103 (35.76%) was osteopenic and 60 (20.83%) was osteoporotic. According to the study menopause and chronic bone and joint complaints had a significant effect on the BMD ($p<0.001$).
- Figures investigated in 2004, in India, 1 out of 8 males and 1 out of 3 females suffered from osteoporosis and this unfortunate medical condition is making India one of the largest affected countries in the world (Outlookindia, 2004).
- In a study among Indian women aged 30-60 years from low income groups, a very high prevalence of osteopenia (52%) and osteoporosis (29%) and its strong correlation with low socio economic status was reported. It also reported that the BMD at all the skeletal sites of the study subjects were much lower than values reported from developed countries, thought to be due to inadequate nutrition (Shatrugna V. *et. al.*, 2005).
- In a community-based cross-sectional study conducted in a semi urban region of Vellore, osteoporosis was reported at the lumbar spine among 48%, at the femoral neck among 16.7%, and at any site among 50% of the 150 female study subjects aged ≥ 50 years (Paul TV. *et. al.*, 2008).
- In another study in Delhi, one thousand and sixty subjects (≥ 50 years) were evaluated for bone mineral metabolic parameters. A high prevalence of osteoporosis (35.1%; male- 24.6%, female- 42.5%) and osteopenia (49.5%; male- 54.3%, female- 44.9%) was observed in the population under observation (Marwah RK. *et. al.*, 2011).

- A study conducted by Aggarwal N. *et. al.* (2011), in PIMER, Chandigarh evidenced that 53% of the study population (females of ≥ 50 years) had poor BMD.
- A multi-centric study by Paul T. *et. al.* (2012) involved more than 3,500 subjects in South India and reported a prevalence of osteoporosis at the spine and hip as 42.7% and 11.4%.
- With the increasing longevity a greater proportion of the Indian population over the age of 50 years are likely to be affected by osteoporosis. In 2013, estimates suggested that ~50 million people in India had T-scores of <−1 (Mithal A. *et. al*. 2012).
- A very recent study by Gopinathan NR. *et. al.* (2016) evidenced that out of 100 female subjects, 18 had a "t" score of − 2.5 and below (osteoporotic) and 55 had "t" score of 1 to − 2.5 (osteopenic).

Thus, observing the available data since 1967, a small summary of the enormity of osteoporosis amongst Indian elderly can be drawn. In 1967 a study evidenced a dominance of 98% of 53 study population as osteoporotic. In a study in 1974, 33% elderly was showed as osteoporotic amongst 421. In 1998 estimation was derived as high as 61 million elderly to be affected by osteoporosis by the next decade. In 2003, estimation was made as 26 million elderly to be affected by osteoporosis. Besides, a study in 2003 came up with an occurrence of 76% (n=288) osteoporosis among Indian elderly; 52% osteoporosis and 29% osteopenia were reported by another study in 2005. In 2008, 50% (n=150) prevalence of osteoporosis was accounted in a study. In 2011, 35% osteoporosis and 49.5% osteopenia were reported in one study and 53% osteoporosis was reported in another study in the same year. In 2012, 53% of 3500 elderly subjects were reported as osteoporotic. Also an extremely high estimation was made in 2012 and in 2013 as 36 million and 50 million elderly to be affected by osteoporosis. The very recent data arrived in 2016 which suggested a 73% prevalence of osteoporosis (n=100) amongst Indian elderly. Therefore, the prevalence of osteoporosis showed a unswerving increase, be it a study with a small segment of population or a nation-wise estimation. The increasing fashion of osteoporosis decade by decade coupled with other risk factors especially old age, sedentary lifestyle, over protection against sun and apparently less known age specific targeted

supplementing doses of Ca and vitamin D puts an ethical responsibility on health professionals to investigate the same, with a rather effective and updated methods.

2.8 Role of nutrients on BMD

The peak attainment and the rate of subsequent loss of bone mass depend on the partial interaction of nutritional factors along with genetic, hormonal and environmental factors. Several major and minor nutrients play beneficial role in the process of attaining the peak bone mass, maintaining and minimizing the loss of it. Chronically low intake of calcium, vitamin D and possibly magnesium, boron, fluoride and vitamins K, B12, B6 and folic acid; particularly if their deficiency co-exist, may pre-dispose to osteoporosis. On the other hand, persistent high intake of protein, sodium chloride, alcohol and caffeine may also sharply affect on bone health. For an example, a typical Western diet which is high in protein, salt and refined, processed foods combined with a sedentary lifestyle may contribute to the increasing incidence of osteoporosis in the elderly (Bunker VW., 1994). So, the nutrients of greatest importance in prevention and treatment of osteoporosis are calcium, vitamin D, vitamin K, phosphorus and magnesium; they will be discussed elaborately next in this chapter.

Interaction of Ca and vitamin D with osteoporosis

A diet inadequate in either of calcium, vitamin D or both will influence calcium-regulating hormones. Deficiency of either calcium or vitamin D or both will raise a condition of reduced calcium absorption and a lower concentration of circulating ionized calcium. In such condition, parathyroid hormone (PTH) secretion is stimulated and results into an increased PTH level in blood. The cumulative effect of higher PTH levels, secondary to poor calcium and vitamin D nutrition (secondary hyperparathyroidism), gives a hike in bone remodeling which leads to significant loss of bone tissues and an increased fracture risk (Nieves JW., 2005).

Nutrients of great importance in osteoporosis

Calcium

In the history of osteoporosis research a considerable epidemiological data has been accumulated to evaluate the relation between calcium intake and bone density. Peak bone mass; attained during adolescence/young adulthood, can be maximized by reaching the adequate intake of Ca recommended by the experts or

Food and Nutrition Board. Out of 86 observational epidemiologic studies, 64 studies showed that higher dietary calcium intake had been related to higher bone mass in children, young adults, and in postmenopausal women (Heaney RP., 2000). Calcium is a threshold regulated nutrient, i.e. Ca can only be stored as bone and increasing Ca intake beyond that which produces optimal bone mass will not result in more bone. Ca contains about 1–2% of body weight of an adult human body; of this, 99% is found as Ca phosphate (together with a small component of Ca carbonate) in mineralized tissues i.e. bones and teeth; providing rigidity and structure (Cashman, 2002). The remaining 1% mediates vascular contraction and vasodilation, nerve transmission, glandular secretion, muscle contraction and is distributed in blood, extracellular fluid (ECF), muscle, and other tissues (Medicine, 1997). Calcium is always under close homeostatic control with processes such as absorption, excretion and secretion and storage in bone being involved in maintaining the concentration of ionized Ca in the plasma within a tightly regulated range (1·1–1·3 mmol/l) (British Nutrition Foundation, 1989). This regulation of plasma Ca concentration is achieved through a complex physiological system. The system comprises the interaction of the calcitropic hormones (parathyroid hormone- PTH), 1,25 dihydroxycholecalciferol (1,25 (OH)2D3) and calcitonin with specific target tissues (kidney, bone and intestine); which increase or decrease the entry of Ca into the extracellular space. Apart from the above role, Ca is engaged in other important physiological functions in body. It is required for normal growth and development of the skeleton. Until the age of the early twenties Ca accumulates in the skeleton at an average rate of 150 mg per day. During maturity, the body and the skeleton more or less maintain Ca equilibrium. The Ca equilibrium becomes negative generally after the age of 50 years among men and among women after the menopause. This negative equilibrium leads to bone loss and fracture in both sexes, but particularly in women. Hence, the requirement of Ca also varies at different point of life of an individual, especially during the periods of rapid growth in childhood and adolescence, during pregnancy and lactation, and in old age. For biological reasons elderly people become weaker to absorb calcium from food. Besides, a rise in PTH pulls Ca from bone to maintain the Ca equilibrium in blood stream, leaving the bones porous.

Phosphorus

Phosphorus is a mineral that contributes 1% of a person's total body weight. It is the second most abundant mineral in the body and is present in every cell of the body. Bones and teeth are the major sites where phosphorus is found in the body (U.S. National Library of Medicine, 2017). Phosphorus is essential for bone building during growth and low serum phosphate will limit mineralization of bone and uncompromised function of osteoblast (Heaney RP., 2004; Lippincott W., 1999). There is very little evidence to supports the fact that inadequate dietary phosphorus influences the risk of developing osteoporosis among individuals. On the other hand, a high intake of phosphorous especially in relation to the consumption of carbonated drinks alters calcium metabolism and increases parathyroid hormone secretion. Also an effect negligible though, on calcium excretion was observed (Heaney RP., 2001).

Magnesium

Magnesium is involved in bone and mineral homeostasis and is important in bone crystal growth and stabilization. Though the influence of magnesium on osteoporotic fracture risk is unclear, but it has been found to play a role in the vitamin D and parathyroid hormone axis. In a limited number of studies, magnesium supplementation had a short-term increase in BMD among the middle-aged women (Lindberg S., 1993).

Potassium

The main importance of potassium in relation to bone health is its influence on calcium homeostasis, particularly the urinary conservation and excretion of calcium. Low potassium diets increase urinary calcium losses (Nieves JW., 2005).

Vitamin D

To maintain a healthy mineralized skeleton of most land vertebrates including humans, vitamin D plays an essential role. In presence of the sunlight photo production of vitamin D_3 takes place in the skin. Then vitamin D_3 is metabolized sequentially to the liver and kidney to convert into 1, 25-dihydroxyvitamin D. The major biological functions of 1, 25-dihydroxyvitamin D are to help calcium get absorbed in the body and to keep the serum calcium and phosphorus concentrations within the normal range. This is important to maintain essential

cellular functions and to promote mineralization of the skeleton. Thus, it is essential to fulfill the daily requirement of vitamin D in the blood. But unfortunately most of the foods do not contain considerable amount of vitamin D. Though some fortified foods contain a variable amount of vitamin D but cannot be depended on as a sole source of vitamin D nutrition. Therefore, as an effective alternative exposure to sunlight completes most humans with their vitamin D requirement. Sun exposure to the arms and legs for 10-15 minutes will provide 3000 to 20000 IU vitamin D. The amount of vitamin D produced depends on the UVB index and also a dark skinned needs 5-10 extra minutes to get same amount of a white skinned. Despite of abundant sunlight and is readily available in nature. But, there also are few other factors which sometimes might dramatically affect the subcutaneous production of vitamin D_3 such as aging, use of sunscreen and the change in the zenith angle of the sun. Vitamin D insufficiency (VDI) and vitamin D deficiency (VDD) are now has been marked as a major cause of metabolic bone disease among elderly. In general it has been accepted that a calcium intake of 1000-1500 mg/d along with a minimum amount of 400 IU/d vitamin D is required to maintain good bone health. The daily requirement of Ca and vitamin D is different for population and communities though (Holick MF., 1996).

Vitamin K

Vitamin K is a fat-soluble vitamin that works as a cofactor in enzymes involved in the synthesis of blood coagulation, bone metabolism, reduces urinary calcium excretion and it also facilitate carboxylation of proteins such as osteocalcin (involved in bone formation) (Booth SL., 1997; Vermeer C. *et. al.,* 1992; Meunier, 1999). Vitamin K deficiency can be considered as a predictor of hip fractures by measuring the carboxylated fractions of osteocalcin in body. However, a state of PEM is associated with multiple deficiencies. The particular contribution of vitamin K deficiency to the bone loss and sustain a hip fracture among undernourished patients is not clear (Nieves JW, 2005).

Vitamin C

Vitamin C is known an essential cofactor for collagen formation and synthesis of hydroxyproline and hydroxylysine. Epidemiologic studies showed a positive association between vitamin C and bone mass. A low intake of vitamin C is associated with a faster rate of bone mineral loss and a higher vitamin C intake

was associated with lower risk of fractures; however, there are no randomized clinical trials available to establish the same (Hall SL. and Greendale GA, 1998; Kaptoge S. *et. al.,* 2003).

Protein:

Adequate nutrition plays an important role in resistant to usual mechanical stresses. In addition to calcium in the development and maintenance of bone structures, vitamin D and dietary proteins play a key role to prevent osteoporosis. Studies point a positive effect of high protein intake on bone mineral density and a significant reduction in hip fracture incidence among postmenopausal women. Besides, low protein intake (< 0.8 g/kg body weight/day) is often observed among the patients with hip fractures (Bonjour JP, 2011).

2.9 Bone health of elderly in relation to dietary calcium and vitamin D intake:

Global scenario of Bone health in relation to dietary calcium and vitamin D intake:

Calcium intake is positively correlated to bone mass at all ages. Ca requirement is different at different phase and condition of life. But the Ca intake and storage as bone mass during childhood and adolescent age have always a long term effect on overall bone health; even extended in old age as well. We have pretty good scientific evidences of the same and have been discussed next.

Studies have shown that higher intake of dietary calcium at different ages are associated with higher bone mineral density compared with the bone mass of those with lower calcium intakes (Nieves JW., 2002). Foods rich in Ca are milk, cheese, yogurt, kale, okra, spinach, soybeans, collards, white beans and some fish like salmon, sardines, rainbow trout and perch etc. Foods fortified with calcium are orange juice, oatmeal, and breakfast cereals etc. Few of these food sources must be included in daily diet. A nutritionally balanced diet including proper amount of vitamins, minerals and other nutrients is required to help the body absorb and utilize calcium optimally. For example, a low calorie diet is often low in calcium and vitamins too; thus even the small available amount of Ca doesn't get used optimally for the poor amount of vitamin D, phosphorus and fat present in such diet. Similarly, a research had shown that about 25% to 35% calcium present in

dairy products is absorbed in normal healthy people (Kuttikat A. *et. al.,* 2004). According to International Osteoporosis Foundation (2009) nearly all Asian countries fall far below than the FAO/WHO recommendations for calcium intake i.e. 1000-1300 mg/day. The median dietary calcium intake by the adult Asian population is approximately 450 mg/day, with a potential detrimental impact on bone health in the region. From different parts of the world, studies have evidenced a positive association between low bone mineral density and poor dietary intake of Ca and calcium-rich foods (Liu H, 2008; Holick, 2007; Cranney A., 2007; Fatima M. *et. al.,* 2009; Akhter P. *et. al.,* 2004). An adequate vitamin D intake was associated with a lower risk of hip fracture in a follow-up cohort i.e. Nurses' Health Study (Feskanish D. *et. al*., 2003). Thus, to fulfill the required amount of Ca and vitamin D focus should be more on foods rich in Ca and vitamin D and include them in daily diet. Fruit and vegetables are another food groups that hold a very important place in daily diet; and intake of the same was positively associated with bone density in a study. Minimum 5 servings of fruits and vegetables should be included in daily diet to optimize the micronutrients required for bone health and to prevent falls and fractures (Jeri W Nieves, 2005). The RDA of calcium and vitamin D for elderly is quite high but the dietary intake in reality is almost half of it. Thus, an attempt should be made to achieve the recommended total daily calcium intake levels for maximum benefit for bone health (ILSI/FAO/WHO, 2002). On the contrary, dietary vitamin D intake was evidenced as of minor importance for the occurrence of fractures and osteoporosis among the study subjects in the population-based Swedish Mammography Cohort (61433 women followed for 19 years), (Snellman G. *et. al*., 2014).

Scenario of Bone health in relation to dietary calcium and vitamin D intake among Indian elderly

In many developing countries calcium intake is much lower (500 mg/day) in almost all the age groups. After examining the evidence for nutritional status and Ca intake of the Indian population, experts suggested an upward revision of RDA for calcium (Indian Council of Medical reaserch report, National Institute of Nutrition, 2009). In another study the current level of Ca intake was less than 400 mg Ca/ day, which was not enough to prevent development of poor bone health (NNMB, 2006). Similarly, a decreased level of calcium intake was associated with

decreased bone mineral content. It also suggested that a minimum intake of 800 mg calcium/day is associated with better bone health status (ICMR, NIN, 2009). Moreover, in developing countries milk intake is as low as 344 ml/ day (average), compared to developed countries (average of 850 ml). In such case majority of the dietary calcium comes from cereals. Since cereals are moderate source of Ca, the daily intake of Ca in country like India remains at a very low range (Gopalan S. and Ramachandran P., 2008). In the year 2009, ICMR Task Force report was able to find a relationship out between socio-economic status and bone health. It said that after the age of 50 years, the extent of osteoporosis in the spine was only 16% in HIG group (with higher calcium intakes of around 1g/day) compared to the LIG group with 65% osteoporosis (calcium intakes around 400 mg/day). Thus, with decreasing income, there was a decrease not only in anthropometry but also in BMD and daily calcium intake (ICMR, NIN, 2009).

2.10 Vitamin D deficiency among the geriatric population

Global scenario of vitamin D deficiency among the geriatric population

Sunlight or ultraviolet B (UVB) radiation is absorbed by the 7- dehydrocholesterol that resides in the skin to form pre-vitamin D_3. Pre-vitamin D_3 is an unstable compound and quickly converted to vitamin D_3 in presence of heat. Then the pre-vitamin D is drawn into the capillaries by vitamin D-binding protein. Through the capillaries, vitamin D is transported to the liver where it undergoes hydroxylation to form 25-hydroxyvitamin D [25(OH) D]. With the help of vitamin D-binding protein 25-hydroxyvitamin D is transported and released into the renal tubule cell and hydroxylated to form 1, 25-dihydroxyvitamin D [1,25(OH)2D], the active form of vitamin D (Laughlin M. *et. al,*. 1982; Holick, 2005; DeLuca, 2004). In the maintenance of bone health vitamin D is one of the very important nutrients. The primary functions of vitamin D are the regulation of intestinal calcium absorption and the stimulation of bone resorption, to maintain the serum calcium concentration (Reid *et. al.,* 2003). Sources of vitamin D include sunlight, diet, and supplements; but very often people especially elderly people remain unable to reach the daily requirements and develop vitamin D deficiency. According to Moore *et. al*. (2004) majority of Americans do not achieve adequate vitamin D levels. In fact, it is estimated that 90% of adults between 51 and 70 years of age do not get enough vitamin D from their diet. Moreover, a very high prevalence of

VDI and VDD has been reported from United States (Blacks, Hispanics, and Asians) UK, and Saudi Arabia where approximately 90% people above the age of 65 years had vitamin D level < 30ng/ml. (Adams JS., Hewison M., 2010; Hirani V. *et. al.*, 2010; Sedrani SH. *et. al.*, 1983). A very high prevalence of VDD among postmenopausal women >50 years has been reported in recent times from Croatia (92.5%) (Laktasic ZN., 2010) and France (89.9%) (Cock DC., 2008). VDD and VDI have been reported to be associated with increased risk of fracture (Hollick MF. 2007). The recent statistics in the United States demonstrate that more than 90% of the Blacks, Hispanics, and Asians were suffering from vitamin D insufficiency (25-hydroxyvitamin D30 ng/ml), with nearly three fourths of the white population in this country also being vitamin D insufficient (Adams S. and Hewison M., 2010).

Scenario of vitamin D deficiency among the Indian geriatric population:

The Indian subcontinent is situated between 8.4° N and 37.6° N latitude and has adequate sunshine throughout the year. Despite of being a tropical country and having abundant sun throughout the year, osteoporosis is highly prevalent among Indian males and females of almost all age groups (Harinarayan CV. and Joshi SR., 2009; Harinarayan CV., 2005; Marwaha RK., 2005; Seth A., 2009). A recent study showed that vitamin D deficiency is wide spread major public health problem and responsible for osteoporotic fractures all across the world including India (Mithal A., 2009). In a study 643 males and 703 females from north India (58.0 ± 9.5 years) were examined for the serum vitamin D level in 2011 and it revealed that VDD (< 20 ng/ml) was present among 1228 (91.2%) and VDI (20-<30 ng/ml) in 92 (6.8%) subjects (Malhotra N., 2008). Another study from north India showed that 91.2% subjects (>50 years) had VDD and 6.8% subjects had VDI (Marwaha *et. al.*, 2011). Harinarayan CV. (2009) documented a high prevalence of VDD (14.6±7 ng/ml) and VDI (20.85±8.63 ng/ml) among postmenopausal women (50-67 years) in southern India. In another study in southern India, among 100 post menopausal women 47% had VDD (<20 ng/ml) and 31% had VDI (Vasundhara K. *et. al.*, 2016). VDD has been reported to be prevalent among post menopausal women by Tondon VR. *et. al*. (2014). Among 310 post menopausal women VDD was prevalent among 53.35% and VDI among 19.48%. As a result of subclinical VDD or VDI decrease in overall bone mass has

been documented (Arya V. *et. al.,* 2004; Bischoff-Ferrari HA. *et. al.,* 2004). Vitamin D deficiency is not a problem only in India but also in countries like Pakistan, China, middle-East and Africa. It is relatively less common in Japan, USA, Canada and South-east Asia (Londhey V. 2011).

2.11 Calcium and vitamin D supplementation among the geriatric population

Global scenario of Ca and vitamin D supplementation among the geriatric population

Based on the scientific literature it is now certain that VDI is common in older men and women; and low vitamin D level is linked with low BMD and an increased risk of falls (Cranney A., 2007). Thus, prevention of VDI and VDD should be taken more seriously and intake of Ca and vitamin D or supplementation should be focused more. The recommended daily intake of vitamin D was around 10 μg (400 IU/day) until 1997 (Whiting SJ. *et. al.,* 2005) but further recent studies had shown that this daily allowance was not sufficient (Bouillon R. 2007; Holick MF. 2007; Aloia JF. 2008). However, few prospective studies have addressed a combination of calcium and vitamin D supplementation instead of Ca or vitamin D alone. According to Kuttikat A. *et. al.,* (2004) and Cannell J. (2008) the maximum effective dose of Vitamin D was uncertain but thought to be around 400 – 1000 IU per day should be able to reduce the vertebral fracture by 24%. Later on, a number of studies suggested higher doses of Ca and vitamin D to maintain serum level of the same and to prevent osteoporosis. Such studies suggested that, to achieve 25(OH) D level of 30ng/ml, the dose of vitamin D supplementation needs to be increased to 1,000– 4,000 IU/day (Bouillon R. *et. al*., 2007; Vieth R. *et. al.* 2001). In 2011, 3,432 women of 60-70 years in Eastern Finland were supplemented daily with 800 IU cholecalciferol and 1000 mg calcium carbonate, and reported a decreased risk of multiple falls requiring medical attention; recommended a higher daily dose of vitamin D for postmenopausal women (Veli-Matti K., 2011; Sminth H., 2007; Nieves JW, 2003; DIPART, 2010; Avenell A. *et. al.,* 2014). Khir AS. in 2006 had suggested that all the elderly should be encouraged to remain physically active and consume 800 IU vitamin D and 1500 mg calcium daily. Studies also have evidenced that a high and prolonged dose of vitamin D (250 μg/ 10,000 IU/ day) is safe and likely to pose no risk of adverse effects (Veith R. 2007).

Scenario of Ca and vitamin D supplementation among the Indian geriatric population

VDD is very common in Northern Europe, Middle East, India, China and Japan; and intend to be a major public health problem soon. But it is comparatively less common in Northern America and South East Asia (Johnell O., 2005; Malhotra N., 2008). Thus, in India since long scientists have been tried and supplanted different experimental doses to figure out a safe and treatable dose of Ca and vitamin D for osteoporosis. In 2011 one such study was done by Londhey V.; and there a daily dose of 2000 IU of Vitamin D was recommended to avoid VDD among the Indian population. Another study from northern India reported a requirement of 60,000-120,000 IU per month to achieve vitamin D level of > 30ng/ml (Malhotra N., 2009). Another study reported an increase in serum vitamin D level after 8 weeks of supplementation with 60,000 IU/ week (Goswami R., 2008). In New Delhi 100 adult males and females having either VDI or VDD were supplemented with 60,000 IU oral cholecalciferol/month during summer and 120,000 IU/month during winter for a period of 9 months; and it was observed that the doses safely increased vitamin D level almost near to normal (Malhotra N. *et. al.,* 2009). Controversy is still there between the high dose and low dose of calcium and vitamin D supplementation to treat osteoporosis.

2.12 Hemoglobin and bone health

Hypoxemia has been recognized as a risk factor for the loss of bone mass. Moreover, several scientific reports in specific condition are available in the literature and suggest a direct relationship of anemia and hemoglobin levels with bone density (Espallargues M. *et. al*., 2001; Vichinsky EP. 1998). However, very few studies have been done in this area. Anemic post menopausal Turkish women had significant lower femur t score, femur BMD, femur Z score, spinal t score, spinal BMD and spinal Z score ($p<0.001$). Additionally, the ratio of subjects with low bone mass in the femur and spine were significantly high in anemic patients ($p<0.002$) (Korkmaz U. *et. al.,* 2012). In the year 2005, 950 participants (75 years) were enrolled in “Invecchiare in Chianti” study. In the study a significant association was found between

hemoglobin levels and trabecular bone density (beta = 0.112, SE = 0.049; P = 0.02). Besides, the association was also significant among total bone density (beta = 0.101, SE = 0.046; P = 0.03), cortical bone density (beta = 0.100, SE = 0.046; P = 0.03) and the ratio between cortical bone and total area (beta = 0.092, SE = 0.045; P = 0.04) (Cesari M. *et. al.,* 2005). Besides, poor hemoglobin level affects the work efficiency badly and poor work efficiency may contribute to mobility related problems and diminished BMD.

2.13 Physical activity and Bone health:

Global scenario of life style, exercise and bone health of geriatric population

Exercises known as "Weight bearing" or "load bearing" exercises like jogging, walking, exercise by using external weights etc. help to stimulate bone cells to multiply, increase muscle strength and balance and reduce the risk of falls and fractures (Kuttikat A. *et. al.,* 2004; Vuori IM. 2001). In another study the adult gymnasts showed higher BMD than age- matched sedentary controls, which clearly indicated the benefits of physical activity (Bass, 1998). Encouraging physical activity at all ages is therefore a top priority to prevent osteoporosis (Chan K., 2003). Maintaining a standard BMI (not less than 19 kg/m2) is also a matter of concern to maintain BMD (World Health Organization, 1994). Evidence showed that low body weight and excessive dieting is associated with low bone mineral status and increased fracture risk (Nguyen, TV., 1998). Moreover, according to Campbell AJ. *et. al*. (1997) 5 minutes of walking per day, can reduce the risk falls in women age 80+ without previous history of falls. According to Iqbal SP. *et. al.,* 2004 and Fatima M. *et. al*., 2009, 72% Pakistani women lead a sedentary lifestyle and high prevalence of osteopenia and osteoporosis was observed amongst them. WHO (2011) has given an elaborated guidelines of physical activities for older adults. According to the guidelines older adults should do at least 150 minutes of moderate-intensity aerobics throughout the week or do at least 75 minutes of vigorous intensity aerobics throughout the week or an equivalent combination of moderate- and vigorous-intensity activity. Aerobic activity should be performed in bouts of at least 10 minutes duration. For additional health benefits, older adults should increase their moderate intensity aerobic physical activity to 300 minutes per week, or engage in 150 minutes of vigorous-intensity aerobic physical activity per week, or an equivalent combination of moderate-and vigorous-intensity

activity. Older adults, with poor mobility, should perform physical activity to enhance balance and prevent falls on 3 or more days per week. Muscle-strengthening activities, involving major muscle groups, should be done on 2 or more days a week. When older adults cannot do the recommended amounts of physical activity due to health conditions, they should be as physically active as their abilities and conditions allow (WHO, Global Recommendations on physical activty, 2011).

Scenario of life style, exercise and bone health of geriatric population in India

Calcium and vitamin D along with lifestyle modifications has always played an important role in determining the bone health. It is said that there is no age bar for exercise and getting its benefits (Kuttikat A. *et. al*., 2004). According to Bonaiuti D., *et. al*., 2002 and Ahuja M. 2008 aerobics, weight bearing, and resistance exercises are effective to increase the bone mineral density of the spine in postmenopausal women. In another study Mehta G., (2004) the paradox of lower fracture rates among the Indo-Asian population than those in caucasian women, despite of lower skeletal mass at maturity in the former Indo-Asian group was also noticed. This suggested the potential need of measuring bone mass density and physical activity as two vital risk factors for the bone health in Indian women (Vaidya R. and Shah R., 2010).

2.14 Departmental studies done on bone health of elderly

In 2010 a study was conducted on the nutritional and health profile of depressed elderly men of Baroda. The results revealed that bone related problem was widely prevalent among the elderly and the percentage was 36, 42 & 56 among 50-59 years, 60-69 years, and +70 years respectively. (Mehta and co-workers, 2010). Mehta and Patel, (1999) studied few factors such as lifestyle factors, nutritional status and dietary profile of elderly women and men in relation to osteoporotic fractures and concluded that few dietary and non-dietary factors were related to the occurrence of osteoporosis. About 90% subjects (60-90 years) suffered an osteoporotic fracture. The activity pattern showed that 80% osteoporotic males were involved in sedentary activities. A study conducted in tribal areas of Godhra in 2012, reported that the total prevalence of osteopenia and osteoporosis among

94 elderly was 47.9% and 41.5% (Gandhi H. Pareekh (S., 2012). In another study, 53.8% rural elderly were osteopenic and 28.8% of the subjects were diagnosed as osteoporotic (H Gandhi SP., 2012). In 2012, a study on various nutritional and dietary aspects in relation to bone disorder was conducted in urban Vadodara (n=250). The prevalence observed for osteoporosis and osteopenia among the females above 60 years was 21.6% and 40.4% respectively. Among males the prevalence of osteopenia was 17.6% and osteoporosis was 5.2% (Chauhan K., Maity A. and Panwar N, Mistry P, 2015).

2.15 Management of osteoporosis

Osteoporosis is a condition which can be prevented before it sets in and it can be improved at any point of time. The progress of osteoporosis can be slowed down or even stopped before permanent structural defects occur. There are various factors which can be considered for the prevention of osteoporosis which can be summarized as follows:

- Adequate dietary calcium and vitamin D
- Weight bearing exercise
- Regular physical activity
- Avoidance of heavy alcohol consumption and smoking
- Minimizing exposure to glucocorticoid therapy
- Preventive use of bio phosphates during glucocorticoids therapy

The treatment for osteoporosis can be classified into the following

- Non-pharmacologic therapies
    - Weight bearing or balance exercises
    - Calcium and vitamin D rich food
    - Fall prevention measures
- Pharmacologic therapies
    - Calcium with Vitamin D
    - Bisphosphonates (alendronate, risedronate, zoledronic acid, ibanddronate)
    - Selective oestrogen receptor modulators
    - Oestrogen and progesterone therapy
    - Parathyroid hormone

The following are the few lifestyle modifications which can lead to improved bone health

- Stop smoking
- Get adequate exposure to sunlight
- Drink alcohol in moderation
- Limit the consumption of caffeinated drinks
- Exercise regularly
- Prevent falls

Thus, increased longevity of life, expansion in elderly population, alteration in life style and food habits together pushing the elderly population towards an aging with osteoporosis; and ultimately imposing a greater burden on the country's economy. It is the need of the hour to understand the epidemiology, pathogenesis, diagnosis, prevention and treatment of osteoporosis in elderly men and women. To execute the same the current study entitled "An investigation into bone mineral density and its correlation with calcium and vitamin d supplementation to the geriatric population of urban Vadodara: evaluation of dietary intake and impact of exercise on bone health" had been undertaken. Tools and techniques used in the present study have been discussed in the next chapter.