CHAPTER 2

REVIEW OF LITERATURE

History of vitamin D

Two scientists Whistler (86) and Glisson (87) were first to explain the biochemical cause of rickets. During mid-sixteenth century, they separately described that it was low vitamin D which caused rickets but they did not say anything about the role of diet or sun-exposure to treat the illness. Another important observation was made by Sniadecki (Polish scientist) in 1840 AD. He observed that children in the country outside Warsaw showed no rickets suffering, while children in the industrial area of Warsaw did suffer from the disease. Then, he assumed that sun exposure might have something to do with the disease. He assumed that lack of sun light exposure along with substantial air pollution in the industrial area of Warsaw might have caused the sickness. But his assumption was not well acknowledged at that time and this led to increase in frequency of rickets incidence among the children of the polluted industrial areas as population and pollution increased during 19th century. Nearly ninety percent children were affected with bone disease in Europe. Similar picture was seen during late 1800s in Boston and New York City. Eighty percent of children were stated to suffer from this disease in Boston by 1900 AD.

In 1918 Sir Edward Mellanby discovered that beagles housed exclusively indoors and fed a diet of oatmeal, developed rickets but that the addition of cod liver oil to the food treated the disease successfully (88). He wrote in 1921 "The action offal in rickets was due to a vitamin or accessory food factor which they contain, probably identical with the fat-soluble vitamin". Various experiments by Hess, Steenbock and Black in the 1920s followed in which excised pieces of rat skin were UV-irradiated or rat food was UV-irradiated. It must have been extremely surprising at the time to establish that both could be used as a dietary source to treat rats suffering from this bone disease. During the same time, vitamin A (first fat soluble vitamin) and vitamins B and C (water soluble) were being reported by scientists and it was considered that rickets preventing agent to be soluble in fat and the scientists gave ‘D’ alphabet which is after A, B and C. It was classified as a vitamin although
it was recognized from the beginning that it was not necessarily required as a dietary constituent. The chemical structures of the many forms of vitamin D were determined in the 1920s and 1930s by Windaus and colleagues (89) in Germany. Windaus was awarded the Nobel Prize in chemistry in 1928 “for his research work into the constitution of the sterols & their connection with the vitamins”. The biologically active form of vitamin D, found in the skin & called 1,25 di-hydroxy vitamin D3, was characterized in 1936 and was shown to result from the ultraviolet B (UV-B) radiation of 7-dehydrocholesterol. So vitamin D was established as a steroid. Very soon after this time the component in cod liver oil that prevented rickets was identified as vitamin D3.

No evidences have been documented when the role of vitamin D in calcium metabolism and bone health was considered to be important, but the evidences do show that more than 750 million years ago there was existence of phytoplankton capable of photo-synthesizing vitamin D (90,91,4). Calcium is being crucial element for regulating many biological functions, the vertebrates and the invertebrates obtained calcium from ocean environment for their elements of endoskeletons and exoskeletons respectively. When the vertebrate began to live on the land, the calcium on the behalf on which they become dependent was present in the soil, but they had no resolution to extract it.

While the unearthing of vitamin D and the rickets was cured, it was animmense problem in medical science as one of great success (92). In 1913, McCollum and Davis (93) reported first vitamin in their research and vitamin D and its importance in bone formation were discovered by 1940. Sir Edward Mellanby, demonstrated that he could produce rickets in dogs by feeding them oatmeal, but he was unaware of the fact that those dogs were not exposed to sunlight. Mellanby attributed the capability of cod liver oil to treat the rachitic condition in dogs as being another property of vitamin A (88). McCollum smartly demolished the vitamin A action of cod liver oil by adding oxygen through the solution & heating it, but the capability to treat rickets persisted in the preparation. McCollum correctly named a new vitamin as vitamin D (94). Huldshinsky (95) and Chick et al (96) independently established that sunlight exposure or/ and artificially produced ultraviolet light cured rachitic children. The major issue was almost answered when
Steenbock and Black (97) revealed that the skin of animals and the food they consumed comprise of anti-rachitic activity to either the animals or in their food. Moreover, Goldblatt and Soames (98) demonstrated that livers obtained from irradiated mice helped to cure rickets. Steenbock and Black (97) said that vitamin D irradiated food when consumed, could help to disappear rickets. Another discovery emphasized that the irradiation of fat-soluble elements taken from cells might be helpful for producing large amounts of vitamin D for further use.

**Vitamin D physiology**

When vitamin D was discovered, while discovering in 1922 by McCollum, it was termed “D” because it was the number fourth known vitamin (27). Nowadays it is clear that vitamin D and its metabolites would be rather classified as pro-hormones than as vitamins (3,99). Vitamin D has a unique metabolism and its major source is endogenous production in the skin (3,99,100). About eighty to ninety percent of vitamin D is obtained when skin is exposed to sunlight where as only a minor amounts of vitamin D is obtained from food (3,99,101). This process is mainly regulated by mineral metabolism by which calcium and phosphorus levels are within physiological limit (3,99).

Askew et. al. (102) suggested the chemical structure of ergocalciferol (vitamin D2) while Windaus et al (103) demonstrated the chemical construction of cholecalciferol (vitamin D3). Scientists during the second half of the 20th century stated that vitamin D is evenly a pro-hormone not only a vitamin. Vitamin D is generally absent from the food products and plant materials (eg, vegetables, fruits, or grains) or may be present in very low amount. It is found abundantly in meat & other animal food sources except in some cases like fish liver oils and plants like waxy-leaf nightshade “Solanum glaucophyllum”.

**Cutaneous production of vitamin D**

Cholecalciferol synthesis is dependent on the number of ultra violet B (wave length 280-315 nm) photons entering the epidermis layer of skin and penetrating the epidermis triggers the photochemistry essential for making pro-vitamin D3. The ultraviolet-B photons also signal to upsurge the synthesis of melanin which is a natural sunscreen. Melanin proficiently packaged into melanosomes and migrates
upward to the upper layer of the epidermis where it absorbs ultraviolet-B (290-315) and ultraviolet-A (316-400 nm) radiation (4).

**Source of vitamin D**

Vitamin D is very important fat-soluble nutrient naturally found in very few foods like mushrooms & fatty fish that is sardines, salmon and mackerel which contain a large amount. Other foods like milk and milk products. The body can produce large amounts of vitamin D when skin comes directly exposed to sunlight so referred to as the “sunshine vitamin”. However, increased sun exposure may increase the risk of skin cancer because vitamin D which carries health risks in spite of this dieticians and doctors usually advise supplements of different doses of vitamin D. It is not only required for the formation of bone but also plays an important role in several other physiologic processes. The use of vitamin D may prevent many degenerative disorders and play a role as an anticancer agent.

Vitamin D3 itself is a physiologically inactive as cited by genetic defects that result in rickets despite normal consumption of vitamin D (104). In 1967 it was thought, vitamin D being converted to active form (105,106), and by 1969; the circulating form of vitamin D had been chemically recognized & synthesized (107,108). The active hormone form which is [1, 25(OH)2D3], was isolated & identified in 1971 and its structure was determined as 1α, 25-dihydroxyvitamin D3 [1, 25(OH)2D3] (109,110). For nearly two decades it was understood that more than one hormone was obtained from vitamin D and 33 metabolites were known (111) but after some time it was made clear that not all metabolites were active rather less active or intermediates in the degradation process. The most common are 24, 25-dihydroxyvitamin D3 and 1α, 24-dihydroxyvitamin D3, 25-trihydroxyvitamin D3 produced by the enzyme CYP24 which itself is induced by the vitamin D hormone (112).

Due to traditional, cultural and lingual diversity India is a vast tropical country. It is not believed that vitamin D deficiency is common in India even after, mostly of its population living in area receiving enough sunlight exposure throughout the year. But contrary to that it is common in all the age groups & both sexes across the country (113).
The vitamin D deficiency pandemic

In 1940 it was considered that 100 IU of vitamin D per day was sufficient to prevent overt skeletal deformities associated with rickets (3). Regulatory agencies in the United State and Europe increased the dose twice and recommended that 200 IU of vitamin D per day required for children and supposed that the same dose was good for adults. It was conducted by the healthcare and regulatory agencies and physicians and general public that rickets is not commonly seen in these days and concluded that vitamin D deficiency was controlled. The number of incidence of rickets was reported to be increased in Montreal hospitals once the regular vitamin D fortified milk was stopped in Canada after the end to Second World War. Then compulsive vitamin D fortified milk was re-introduced by federal regulation in 1979. Outbreak of hyper-calcemia caused by vitamin D intoxication was reported in Great Britain in 1950 AD and this led to stop vitamin D fortification of dairy products expect cereals and margarine. In the 1970 blood from healthy individuals who were supposed with sufficient level of vitamin D in serum, was collected to assess the normal reference range and test was performed for vitamin D [25(OH) D] estimation and the mean ± two standard deviations were considered as the normal reference reading (10-55 ng/ml). It was year 1998 when healthy subjects having vitamin D [25(OH) D] level between 11 & 25ng/ml were said to be sufficient when they consumed 50,000 IU of vitamin D2 every week for 8 weeks and this resulted in comparative fall in their parathyroid hormone level in blood. Individuals with <20 ng/ml vitamin D level showed 35 percent fall in parathyroid hormone values. Keeping these results in mind, vitamin D deficiency was defined as a serum vitamin D [25(OH)D] level <20 ng/ml (114). Scientists noticed that vitamin D level between 30-40 ng/ml kept parathyroid hormone at plateau state (115). It was also reported in a study that women in their postmenopausal state having vitamin D 20-30 ng/ml, showed 65% enhance (on average) in efficient absorption in Calcium in GIT (116). Collective conclusion from these combined data is that vitamin D deficiency is less than 20 ng/ml of vitamin D while 21-29 ng/ml reading is said to be vitamin D insufficiency (3,35). Data available from the past and as per new criteria for vitamin D deficiency & insufficiency, it is suggested that more than a billion population around the globe fall in the above mentioned categories (3,35,117).
An assembly of many researches has documented that 50-100% of the elderly population (either sex) in United State and Europe are vitamin D deficient (3) while high risk population include children, pregnant & lactating ladies and even young adults. 76 percent of mother and 81 percent of newborns are vitamin D deficient at the time of birth even though, these mothers were said to have been taking 600 IU/day of vitamin D during pregnancy (3). In the US, there are more than 50 million teen-age population suffering from vitamin D deficiency or insufficiency (118) and similarly 50 percent of children between age group of 1-5 and seventy percent of children between age group of 6-11 years are reported to have suboptimal vitamin D status in the US (119). Vitamin D deficiency among children residing in India, China and Saudi Arabia is nearly 30-50 percent (3). Thirty-two percent of healthy physicians & medical residents at a Boston hospital were vitamin D deficient although they consumed a multivitamin which also contained 400 IU of vitamin D every day & also drank a glass of vitamin D fortified milk daily. Twenty percent fall in serum vitamin D reading was reported by NHANES-III (National Health and Nutrition Examination Survey-III) during the year from 1994 to 2004 in the US. This fall was explained as obesity, changes in the lifestyle, decreased milk consumption and increase in use of sun-block lotion being deciding factors (120).

This vitamin D deficiency might be pandemic since the deficiency has been reported from various nations like Austria, Finland, Germany, New Zealand, Great Britain and India (121,122,123,124,125,126). Even though Australia is a sunny country, thirty to fifty percent of children and adults have been reported to be vitamin D deficient (3). These studies from various nations include various age groups including young as well as elderly population (127). Although countries like the US and Canada are practising vitamin D fortification for decades but population in these countries have low vitamin D which might be related to season, latitude, age, gender and socio-economic status (128). Satisfactory sunlight exposure during summer was indispensable way to optimize vitamin D, but oral intake was amplified by both fortification & supplementation to maintain optimal level.

Now-a-days vitamin D has become popular public health interest because its deficiency is very common and associated with musculo-skeletal diseases (3,129,130). These skeletal effects of vitamin D are closely associated with its role in
regulating bone and mineral metabolism by calcium absorption in the gut and supplying sufficient calcium for bone mineralization (131,99). The increasing interest has been noted since last few years regard to the relationship of vitamin D with non-skeletal diseases (3,129,130,99) after the documentation of VDRs (vitamin D receptors) in various tissues (99). Many researchers have indicated link between vitamin D deficiency with a many chronic non-skeletal illnesses like cardiovascular and renal diseases, cancer, autoimmune, neurological and infectious diseases (3,99,132,133,134,135,136,137,138). It is understood from the past studies that vitamin D was classified as a vitamin during the early twentieth century and pro-hormone (conditional vitamin) was the name given to vitamin D during the second half of the twentieth century (3,139). Two hydroxylation processes are required to form 1,25-di-hydroxy vitamin D from 25(hydroxy)D. The first hydroxylation take place in the liver & second in the kidney to finally produce active vitamin D. 25(OH)D (less biological active) form is blood circulating form and is normally considered as body status of vitamin D (3,139). As soon as the circulating form of vitamin D reaches optimal level in the blood then the kidney starts synthesizing other hydroxylated metabolites such as 24,25(OH)2D or 1,24,25(OH)3D from 25(OH) D which are less biological active instead of 1,25-dihydroxy-D. Renal production of cholecalciferol is closely controlled by the level of parathyroid hormone in the blood along with serum Ca (calcium) and P (phosphorus) levels. Various tissues like prostate, colon, mammary gland, osteoblasts, macrophages, antigen-presenting cells & keratinocytes, express the enzyme for catalyzing the formation of 1, 25-di-hydroxy vitamin D from 25-hydroxy vitamin D & the enzyme is 25-hydroxy-vitamin D3-1-α-hydroxylase (140).

**Synthesis and terminology of vitamin D**

Vitamin D coordinate with the “Calcium-25(OH) D-Parathyroid hormone endocrine axis” and one needs to be familiar with the various forms of vitamin D, as cholecalciferol, calcidiol and calcitriol. Cholecalciferol is the naturally occurring form of vitamin D. It is made in huge quantities in skin exposed to sunlight rays (UV–B rays of 290 to 310 nm). Cholecalciferol is transported to liver where it metabolized into calcidiol. Calcidiol is a pre-hormone which is directly made from cholecalciferol. The importance of calcidiol is that it is the storage form of vitamin
D. Serum 25(OH) D is the most reliable pointer of vitamin D adequacy of an individual (141). The production of 25(OH) D & the concentration in serum both reflects cutaneous synthesis & absorption from diet. After the hepatic conversion of cholecalciferol to calcidiol, calcidiol divert in two pathways, one is responsible for bone & other for cellular effects. Calcitriol is produced from calcidiol in both the kidneys & in other tissues and is the most potent steroid hormone derived from cholecalciferol.

Cod and fish liver oils are the much better sources of vitamin D. Egg yolk of hens and oily fish are also supposed to be good sources while livers from mammals and milk are poor in vitamin D sources. Vitamin D concentrate in milk and eggs usually rely on sunlight exposure and the kind of diets cow and hens are provided. Milk from human is also poor source compared to milk obtained from cows. There is almost absence of vitamin D in plant source and therefore, it can only be derived from animal sources. It is clear that most natural foods including those derived from animal source are low in vitamin D concentration. Humans try to get much sunlight as possible to activated pro-vitamin D3 to vitamin D3. If sunlight exposure is filtered through smoke and dust or through window glasses its effectiveness is decreased.

**Vitamin D analogs**

Five natural and four synthetic analogs of vitamin D have been reported. The natural analogs are called vitamers and artificial analogs are synthesized chemically. These analogs are categorized as seco-steroids when these steroids have one broken bond.

**Natural analogs (40)**

- Vitamin D1: chemically composed of ergocalciferol (D2) and lumisterol in equal proportion.
- Vitamin D2: also known as ergocalciferol and is produced by invertebrates, some plants & fungi. Biologically, ultraviolet light triggers the vitamin D2 synthesis.
- Vitamin D3: another name is cholecalciferol and is produced in the skin when UV radiation reaches to epidermis to chemically activate 7-dehydrocholesterol.
- Vitamin D4: scientifically called 22-dihydroergocalciferol.
- Vitamin D5: also called sito-calciferol and is also generated from 7-dehydrosito-sterol.

**Synthetic analogs**

- Maxacalcitol: is the first analog having a wider therapeutic window than 1,25(OH)2D3 and is also known as 22-oxacalcitriol or OCT (142).
- Calcipotriol: this is obtained from calcitriol and was first uncovered during trials which used vitamin D for osteoporosis treatment.
- DHT (Di-hydro-tachy-sterol): this synthetic form is thought to be superior as compared to natural D2& D3. It becomes biologically active by the liver even without undergoing any hydroxylation in the kidneys.
- Paricalcitol: chemically it is neither 19-nor D2 and can be obtained from calcitriol and is first vitamin D analogs approved for 20 hyperparathyroidism treatments and differs from calcitriol by lacking the exocyclic carbon 19 and has a vitamin D2 side chain instead of a vitamin D3 side chain (143).
- Tacalcitol: a derivative of vitamin D3 and is established as hinder keratinocytes in the skin.
- Doxicalciferol: chemically is 1α (OH) D2 and is a pro-drug. It is less harmful than 1α (OH) D3 (144) when administered frequently.
- Falecalcitriol: chemical nomenclature is 1, 25(hydroxy)26,27-F6-D3 and is approved for 20 hyperparathyroidism in Japan (143) and is more active than calcitriol, it catabolizes slowly (145).

**Vitamin D from sunlight**

Humans do indeed to synthesize vitamin D in the skin from cholesterol by the action of sunlight. But it is difficult to obtain even a small amount of vitamin D with a brief change into the sunlight waves (146,147). Ultraviolet light is divided into three wavelength ranges (148) as:

- UV-C (100-290 nm)
- UV-B (290-315 nm)
- UV-A (315-400 nm)
**UV-C:** Among the ultraviolet bands, it is the shortest and highly energetic. It can burn skin rapidly even if the dose is extreme low but, luckily ozone layer blocks this radiation. However, it is thought that some lights might produce this radiation. That is why, fluorescent and halogen and other special lights might have role in cancer to dermis.

**UV-B:** The ultraviolet wavelength that stimulates our bodies to produce vitamin D is ultraviolet-B (151). It is also called "burning ray" because it is the primary cause of erythema (sunburn). However, ultraviolet-B initiates beneficial responses by stimulating the production of vitamin D that the body uses in many important processes. Although this radiation causes sunburn, it also causes special skin cells called melanocytes to produce melanin which is protective. Ultraviolet-B also stimulates the production of MSH (melanocyte stimulating hormone), an important hormone in weight loss & energy production (152).

**UV-A:** It is mainly accountable for deepening the pigment in skin and is also called the ‘tanning ray’. Tanning bulbs have great amount of ultraviolet-A rays output while there is low percentage of ultraviolet-B rays output. This radiation is less energetic compared to ultraviolet-B radiation therefore, ultraviolet-A exposure will not result in a burn until and unless the skin is photosensitive or there are excessive doses. Due to longer wavelength this radiation can pierce dermis deeper than ultraviolet-B. Ultraviolet-A was considered to be unblocked by sunscreens (149) until recently and now is considered as a chief donor to the high incidence of non-melanoma skin cancers (150). 78 percent of this radiation penetrates glass and therefore, glass windows cannot block this radiation.

Only ten minutes of daily exposure to the sunlight to the arms and legs can produce the required amount of vitamin D. At higher latitudes, ultraviolet-B is present only during mid-day hours and only has significant intensity in temperate or tropical latitudes. Only 5% of the ultraviolet-B light range goes through glass (153) and it does not penetrate clouds or fog. Sun exposure at higher latitudes before 10 am or after 2 pm will cause burning from ultraviolet-A before it will supply adequate vitamin D from ultraviolet-B. Therefore, vitamin D synthesis would occur only when exposure to sun is between these hours when it is said to avoid sun exposure. Depending upon the complexion of skin, exposure to sunlight between ten
to two pm during summer for 20-120 minutes would be enough to acquire adequate vitamin D (154).

In this way produced vitamin D may last, in the blood, for at least twice the duration when compared orally ingested vitamin D (155). A naked adult when exposed to one minimal erythemal dose of ultraviolet radiation (a slight pinkness to the skin 24 hours after exposure), his/her body can produce vitamin D equivalent to ingesting between 10,000 and 25,000 IU (35). There are various factors that can reduce the efficiency of skin's production of vitamin D3 and these factors may be increased skin pigmentation, aging, and the topical application of a sun block lotion (3,156,157). An alteration in the zenith angle of the sun caused by a change in latitude, season of the year, or time of day can also dramatically affect production of vitamin D3 in the skin (3,35).

**Vitamin D-metabolites**

The two forms of vitamin D; cholecalciferol (produced from the conversion of 7-dehydrocholesterol in the epidermis & dermis in humans) and ergocalciferol (produced in mushrooms & yeast) have been reported. The chemical and structural difference between both the metabolites is in the side chain (158); vitamin D2 has a double bond between carbons 22 and 23 and a methyl group on carbon 24 in contrast to vitamin D3.

**Vitamin D metabolism**

7-dehydrocholesterol (vitamin D precursor) present in the skin (3) is an intermediate formed in the cholesterol pathway. The conversion of 7-dehydrocholesterol to pro-vitamin D3 that spontaneously isomerizes to cholecalciferol (vitamin D3) is induced by Ultraviolet-B radiation. Vitamin D3 is then released into blood and carried by the VDBP (vitamin D-binding protein). Nearly 80-90 percent is synthesized in the epidermis when sunlight reaches into the skin and a small amount can also be obtained from diet and/or supplements. The dietary sources may be from plants or fungi containing ergol-calciferol (vitamin D2) or fatty fish or cod-liver oil those contain vitamin D3 (3). The enzyme 25-hydroxylase (encoded by CYP2R1) metabolizes vitamin D (from the skin and diet) to 25(OH)D in the liver and this is the major circulating form used to determine
blood vitamin D status and the normal level is 30-100 ng/ml. 25(hydroxy) D is further hydroxylated at carbon 1 in the kidneys by the enzyme 1-α-hydroxylase to form 1, 25-dihydroxyvitamin D3 or 1,25(OH)2D3; biologically active vitamin D as shown in figure 2.1. This enzyme i.e. 1-α-hydroxylase has been reported in many other tissues and this allows the local synthesis of 1,25(OH)2Dcholecalciferol from 25(OH) cholecalciferol (3).

**Half-life of vitamin D**

Calcitriol (the active vitamin D) has half-life of nearly 15 hours and that of calcidiol is 15 days (159). It binds with receptors located throughout the body. Renal enzyme 1α-hydroxylase transfer 25(OH)D into 1,25(OH)2D, which circulates in blood with very low concentrations than 25(OH)D, but it has a higher binding capacity to the vitamin D receptor (160). Many research reveal that many tissues like vascular wall express 1α-hydroxylase with subsequent intracellular formation of 1,25(OH)2D from 25(OH)D and this active vitamin D plays its role at the cellular level before it is catabolized to inactive calcitroic acid (3,161,99). Fibroblast growth factor 23 & Klotho like factors (can suppress 1α-hydroxylase expression) have also been studied for their role to control the renal formation of 1,25(OH)2D from 25(OH)D (162). It has also been reported the expression of 1α-hydroxylase in various cells other than kidneys and this extra renal expression motivates numerous regulatory pathways. Some of the extra renal 1,25(OH)2D synthesis are in macrophages (stimulated by Toll-like receptor) for innate immunity against intracellular bacteria (163). Another site for extra renal production of 1,25(OH)2D is keratinocytes (164) It is understood that vitamin D can enter the brain crossing the blood-brain barrier and VDRs are found across the brain, but the role of vitamin D is still unknown.
Units of vitamin D

The World Health Organization had responsibility for defining the ‘International Unit’ of vitamin D3 and their most recent definition was provided in 1950 which states that "the International Unit of vitamin D recommended for adoption is the vitamin D activity of 0.025 micrograms (25 nanograms) of the international standard preparation of crystalline vitamin D3". Thus, 1.0 IU of vitamin D3 is 25 nanograms, which is equivalent to 65.0 pmoles. While the discovery of the metabolism of vitamin D3 to other active seco-steroids, particularly
1-α-25(OH)_{2}D3, it was recommended that 1.0 unit of 1-α-25(OH)_{2} D3 be set equivalent in molar terms to that of the parent vitamin D3. Thus, 1.0 unit of 1-α-25(OH)_{2}D3 has been operationally defined to be equivalent to 65 pmoles. For conversion of 25(OH)D ng/ml to nmol/l multiply by 2.496 (165). Vitamin D levels as mentioned by different studies found in various units i.e. ng/ml, μg/dl, μg/l and ng/dl to nmol/l. The transfer formula was 1 ng/ml = 2.5 nmol/l, IU was taken as the standard unit & all other units were converted using a standard formula. The transfer formula for micrograms is 1 μg= 40 IU and units for vitamin D, 1 IU = 25ng, 40 IU = 1mg (166).

**Vitamin D receptor**

Vitamin D receptor (VDR)is expressed in various cells like parathyroid glands, skeletal muscles, is responsible for physiological actions of vitamin D (3,167), after VDR forms chemical bond with vitamin D, the complex then heterodimerizes with retinoid X receptor. Thus formed complex further combines with vitamin D responsive element which is situated in the promoter area of the target genes (112). The VDR can also intermingle with other RNA synthesizing factors as well. These factors may be co-activator proteins and transcription integrators like calcium-binding-proteins (168). This genetic process to happen can take up-to days (99). The other way is the communication with a cell surface receptor and 2nd messengers which lead to quicker response within a minute (3.99). 24-hydroxylase catalyzes the breakdown of 1,25(hydroxy)2 cholecalciferol & 25(hydroxy)D to biologically inactive calcitroic acid (3).

VDRs are present almost in all the tissues as shown in table 2.1 and cells of our body (169,170). A wide range of biological actions such as inhibition of cellular proliferation & inducing terminal differentiation, inhibiting angiogenesis, stimulating insulin production, inhibiting renin production and stimulating macrophage cathelicidin production can be exhibited by active vitamin D (169,170,171,172). The local synthesis of 1,25(OH)_{2} cholecalciferol might be accountable for controlling up to 200 genes (173) that may simplify many of the pleio-tropic health benefits (174,41,42,170). The VDR is also thought to be present with the plasma membrane of the target cells where it can induce rapid responses like opening of chloride or calcium channels or stimulating exocytosis (165).
Table 2.1: Tissue and organ with vitamin D receptor

<table>
<thead>
<tr>
<th>Adipose tissue</th>
<th>Osseous tissue</th>
<th>Lymphocytes B and T</th>
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<tbody>
<tr>
<td>Cartilaginous tissue</td>
<td>Smooth muscles</td>
<td>Cardiac muscles</td>
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<tr>
<td>Fetal muscles tissue</td>
<td>Adrenal gland</td>
<td>Cancer cell</td>
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<tr>
<td>Stomach</td>
<td>Small intestine</td>
<td>Large intestine</td>
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<tr>
<td>Hair follicle</td>
<td>Kidney</td>
<td>Lungs</td>
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<tr>
<td>Brain</td>
<td>Parathyroid gland</td>
<td>Pituitary gland</td>
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<tr>
<td>Thymus gland</td>
<td>Thyroid gland</td>
<td>Mammary gland</td>
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<tr>
<td>Placenta</td>
<td>Uterus</td>
<td>Testicle</td>
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<tr>
<td>Ovary</td>
<td>Epididymis</td>
<td>Parotid gland</td>
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<tr>
<td>Retina</td>
<td>Bone marrow</td>
<td>Pancreatic B cells</td>
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<tr>
<td>Osteoblasts</td>
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</tbody>
</table>

About 1 billion people worldwide, across all ethnicities & age groups are affected by vitamin D deficiency (3,74,174). Lifestyle & environmental factors that can reduce exposure to sunlight can be responsible for hypovitaminosis D. Dark pigmented people absorb more ultraviolet-B in their skin compared to others & therefore more sun exposure is required to produce the same amount of vitamin D (175).

Aging, evenly affects on health of an individual. Reduction in the muscle mass and muscle strength is evident among elderly population (176,177,178). This subsequent loss of muscle strength might result in functional weakness (179,180) and this requires assistance for performing daily works (181,182) otherwise the risk of falling and non-vertebral fractures is inevitable (183). Suboptimal vitamin D is related to muscle weakness (184) and is common among old age population (185). Other risk factors to develop vitamin D deficiency among older people are: diminished sunlight exposure, decreased dietary intake, impaired intestinal absorption, reduced skin thickness, & impaired hydroxylation in the liver and kidneys (186,127,187). Aging also decrease the capacity of the skin to produce vitamin D3; this capacity is reduced after 70 years of age. Sunscreens can reduce or prevent cutaneous production of vitamin D3 by absorbing the solar radiation responsible for pre-vitamin D3 synthesis in the skin. Other factors that affect the cutaneous synthesis of vitamin D3 include latitude, time of day, geographic location,
Latitude has profound effects on the cutaneous synthesis of vitamin D (188).

**Vitamin D endocrine system**

Calcium sensing proteins capable of sensing plasma calcium level are present in the parathyroid gland (189,190). Even slight fall in calcium concentrations triggers these trans-membrane proteins, coupled with G protein system to stimulate the secretion of PTH which then acts to maintain Ca homeostasis mobilizing 1α-hydroxylase enzyme to synthesize vitamin D which in turn increase intestinal absorption of Ca, mobilization of bone Ca and Ca re-absorption in the kidney (190,191). The increase in serum Ca levels huts down the parathyroid gland-induced cascade of actions. If the Ca level in the blood is high, then the C-cells of the thyroid gland secrete calcitonin which stops bone calcium mobilization (192). Calcitonin also inspires the renal 1α-hydroxylase to provide the vitamin D for non-calcemic requirement (193).

The important aspect of the vitamin D endocrine system is that dietary calcium is favored to support serum calcium concentrations under normal conditions but when calcium concentrations fails, the system mediates calcium mobilization from bone and re-absorption in the kidney to stable the needs of the organism that will results in loss of calcium from the skeleton and can ultimately lead to osteoporosis. Another important aspect is that except for stimulating mineralization of the skeleton, the vitamin D hormone has not been found to be anabolic on bone by itself.

**Role of vitamin D in human metabolic processes**

Calcium and phosphorus are desirable for the normal mineralization of bone, muscle contraction, nerve conduction & general cellular function in all the cells and vitamin D maintains those micronutrients at optimal level. This is achieved via conversion to 1,25-dihydroxyvitamin D form. This is the form responsible for regulating the transcription of many vitamin D dependent genes coding for calcium carrying proteins and bone matrix proteins.

The transcription of cell cycle proteins is modulated by vitamin D. This protein helps in decreasing cell proliferation while helps in increasing cell differentiation of various specific cells of the body such as enterocytes,
keratinocytes & osteoclastic precursors. This is how vitamin D might be responsible for bone re-sorption, intestinal calcium transport. Vitamin D also holds immune-modulator characteristic that may alter responses to infections. These cell differentiating and immune-modulatory properties of vitamin D are now utilized in successful treatment of psoriasis & other skin disorders.

All vitamin D metabolites are available in the blood bound to a specific globulin called VDBP (vitamin D binding protein). Calcitriol behaves as steroid hormone and enters through the plasma membrane and intermingles with VDR; a specific nuclear receptor and this receptor is a DNA-binding, zinc-finger protein (194). This ligand-receptor complex chemically reacts with a specific vitamin D-responsive element and with allied transcription factors (e.g. retinoid X receptor) and in this way, improves the transcription of mRNAs which code for various proteins like calcium-transporting proteins, bone matrix proteins or cell cycle-regulating proteins (112). 1,25(OH)2 cholecalciferol also excites absorption of calcium & phosphorus in the small intestine & also mobilizes these elements via bone re-sorption stimulation (195). From both the above-mentioned activities calcium and phosphorus levels are maintained in the blood. Calcitriol also suppresses interleukin-2 production in activated T-lymphocytes (196,197), an effect which suggests the hormone might be playing an important role in immune-modulation in vivo. Other tissues like skin are directly affected by exogenous administration of vitamin D, though the physiologic significance of these effects is poorly understood. The pharmacologic effects of 1,25(OH)2D are profound & have resulted in the development of vitamin D analogues which are approved for use in hyper-proliferative conditions like psoriasis (198).

**Calcium, parathyroid hormone and vitamin D**

During calcium homeostasis, 1, 25-dihydroxy cholecalciferol co-operated with PTH (parathyroid hormone) to yield its advantageous effects on ionized calcium and phosphate levels in the blood (112,199). When there is decrease in ionized calcium level in the blood, then PTH is secreted and this hormone excites 25(OH)D-1-alpha-hydroxylase to synthesize 1,25(OH)2 vitamin D. This leads to increase in calcium transport within the intestine, bone, & kidney (200) and
ultimately lead to normalize calcium level. PTH secretion is regulated by feedback mechanism of calcium. Vitamin D function is to maintain calcium which is also required for cellular metabolic processes and neuromuscular activities. Intestinal calcium absorption is influenced by vitamin D by increasing the expression of the epithelial calcium channel protein that in turn helps in the transportation of calcium through the cytosol & across the basolateral membrane of the enterocyte. 1, 25(OH)2 vitamin D indirectly influences bone mineralization by maintaining plasma calcium and phosphorus concentrations and subsequently extra cellular calcium & phosphorus concentrations. It has yet to be understood whether 1,25(OH)2 vitamin D directly influences bone mineralization. A variety of other tissues and cells are influenced by vitamin D. Many biological systems have vitamin D receptors & are responsive to 1, 25(OH)2 cholecalciferol (201). Therefore, the biological effects of 1,25(OH)2 cholecalciferol are diverse such as helpful in insulin secretion, innate immunity & inhibits cell proliferation & stimulates their differentiation (202).

**Biological mechanism of vitamin D**

The biological action has been performed through its receptor among various tissues including skeleton & reproductive tissue as well as parathyroid gland (167). It joins with its nuclear receptor which hetero-dimerizes to the retinoid X receptor. After this, it binds to the vitamin D responsive element present in the promoter side of the target genes (112). Vitamin D receptor binds with other factor (transcription) like co-activator protein and integrators like calcium binding proteins which consume hours-to-day (160, 99). Another way to interact with second messenger and cell surface receptors, leads to quick response within second to minutes (99). Catabolism 1,25(OH)2D3 and 25(OH)D to biologically active calci troic acid is catalyzed by 24-hydroxylase activity.

Vitamin D target organs have been expanding and the re-productive role of vitamin D is noteworthy since expression of VDR & enzymes metabolizing vitamin D in testicular tissue, male re-productive tract and human spermatozoa are documented. The expression of VDR and cytochrome P450 (mitochondrial enzyme) encoded by CYP24A1 gene in human spermatozoa assist as positive predictive indicator of semen quality & vitamin D also helps in induction of sperm motility with the help of calcium. It shows the positive association between serum 25(OH) D
levels & sperm motility in both fertile & infertile men. Expression of vitamin D receptor & enzymes that metabolize vitamin D in fetal testis indicates undefined role during development which may be extrapolated from invasive testicular germ cell tumors where 1-α-25-dihydroxyvitamin D induces a meso-dermal differentiation of the pluripotent testicular cancer cells. Taken together, vitamin D signaling has a positive effect on semen quality that increases estrogen responsiveness & differentiates germ cell tumors (203).

**Vitamin D and other diseases**

Vitamin D is thought to play role in vitamin D binding proteins and some calcium-binding proteins regulation. Calcium binding proteins are responsible for transporting calcium to correct place and shielding cells from injury by free calcium (204). The high dietary calcium and low vitamin D in blood may promote calcification of the arteries, joints, kidney & even the brain (205,206,207). Diminished vitamin D level may be a reason for syndrome X and connected to obesity, diabetes, heart disease and hypertension (208).

Supplementation of vitamin D & calcium has shown promise in the treatment of PCOS (Polycystic Ovarian Syndrome) (209). Vitamin D plays an important role in regulation of immune system (210). Sub-optimal vitamin D is linked to numerous auto-immune diseases like multiple sclerosis, thyroiditis, rheumatoid arthritis & Crohn's disease (211,212). Osteoporosis is significantly connected to low vitamin D (213). Infertility is also associated with hypovitaminosis D (214). Vitamin D supports synthesis of estrogen in men & women (167). Pre-menstrual syndrome has been seen to be completely reversed by addition of calcium, magnesium and 25(OH) D (215). Similarly, menstrual migraine is also due to low vitamin D & calcium (216). Breast, prostate, skin & colon cancer have a strong relation with low 25(OH) D (217,218,219,220,221). Tyrosine hydroxylase is also found to be regulated by activated vitamin D in adrenal gland; the enzyme necessary to produce dopamine, epinephrine and nor-epinephrine. Sub-optimal vitamin D may also contribute to depression and chronic fatigue (222). In tuberculosis (TB), vitamin D provides immunity by enhancing the macrophage phagocytosis of *Mycobacterium tuberculosis*. It is often associated with low levels of serum 25(OH) D (223,224). In researches vitamin D shows anti-cancer benefits (225), increased sunlight exposure or dietary intake reduce the colorectal cancer
The study shows 25(OH) D is a strong anti-cancer cell in prostate cancer (227). In a study vitamin D has been examined with diabetes mellitus type 1 (228). Studies have also been conducted relating vitamin D deficiency with increased prevalence of diabetes mellitus type 2 (229). 1,25-dihydroxy-cholecalciferol is one of the most important factor which adjusts and suppresses renal rennin (blood pressure hormone) (230). Adequate level of 25(OH)D plays a chief part in hypertension treatment via in appropriate renin-angiotensin system stimulation. 1,25(OH)₂ cholecalciferol intermingles with its receptors present in the immune cells and plays significant role in minimizing the risk for acquiring autoimmune illnesses as a solid immune-modulator. It is now recognized through various researches that people at latitude above 37°are at danger of acquiring multiple sclerosis during the life period by 100 percent (231), and supplementing 400 IU dose of vitamin D, decreases the hazard of getting multiple sclerosis by 40 percent (232). A study carried out among the women showed that those women taking 400 IU dose of vitamin D supplement in the form of multivitamin, showed diminished risk of rheumatoid arthritis by 40 percent (233).

**Vitamin D therapy**

Single, infrequent and intense exposure to ultraviolet-B radiation not only causes sunburn but also suppresses the immunity and low-level exposure normalizes immune function increasing NK-cell & T-cell production, reducing abnormal inflammatory responses typical of autoimmune disorders & reducing of infectious disease (211,234,235,236,237,238). So it is important to sunbathe repeatedly for short periods of time rather than spent, long hours in the sun. No history of liver or kidney diseases along with the diet having adequate calcium, magnesium & other minerals make supplementation safer (239).

**Major function of vitamin D**

The major functions of vitamin D are:

- Promotion of calcium absorption in the gut.
- Calcium transportation across cell membranes thus helping in strong bones & a calm, contented nervous system formation.
- 25(OH) D also favors the absorption of the other micronutrients like magnesium, iron and zinc.
Vitamin D regulates calcium homeostasis. Low calcium in the diet compels vitamin D to draw calcium from the bone. Vitamin D intoxication de-mineralizes bone if optimum calcium is not available in blood. The uptake of toxic metals like aluminum, lead, cadmium & strontium, if magnesium, calcium and phosphorus are not present in proper amounts is assisted by vitamin D (240). Vitamin D deficiency decreases biosynthesis and release of insulin hormone (241). Glucose intolerance has been found inversely associated with the concentration of vitamin D in the blood. Therefore, vitamin D might help to protect against both Type I & Type II diabetes (242). The risk of senile cataract is reduced in persons with optimal vitamin D & carotenoids (243).

**Function of vitamin D other than calcium**

After discovery of the receptors, it was noted that receptor was not only present in the target cells of enterocytes, osteoblasts, and distal renal tubule cells but also in various cells like parathyroid gland cells, skin keratinocytes cells, pro-myelocytes cells, lymphocytes, colon cells, pituitary gland cells, and ovarian cells etc (112). The expression of VDRs in these cells suggests that they must have some functions in those cells (112).

Suda et. al. (244) verified that the vitamin D has a significant role in the terminal differentiation of pro-myelocytes to monocytes, which are precursors of the bone formation process. They also stated that the cells differentiated into a functional cell line and growth stopped. Later, it was found to be fundamental to vitamin D-induced synthesis of osteoclasts through the receptor activator of RANKL (nuclear factor kappa-B ligand) system (244). One of the great importances is the finding of the VDR in the parathyroid glands (112). An important function of 25(OH) D is to sustain the synthesis of the pre-pro-parathyroid gene under control & reasonably suppressed (112,245). Furthermore, 25(OH) D via its receptor, functions to prevent spread of parathyroid gland cells and hence, this vitamin helps to maintain normal parathyroid functioning. Among kidney failure patients, the place for production of the vitamin D in the kidney is damaged & this leads the parathyroid gland to become low in vitamin D. Vitamin D is required for the treatment of secondary hyperparathyroidism.
Immune system; mostly T cell-mediated immunity is affected due to low vitamin D whereas vitamin D toxicity, over-powers certain features of the immune system (246,247). This characteristic due to high vitamin D led scientists to treat certain autoimmune diseases such as multiple sclerosis and experimental autoimmune encephalomyelitis. Another example of an autoimmune disease that can be controlled by 25(OH)D is diabetes mellitus type 1 (248). Among non-obese diabetic rats, low vitamin D triggered a notice-able increase in occurrence and a notice-able decline in the lag time for the diabetes initiation. Investigations have revealed that large doses of 25(OH) D could diabetes mellitus type-1 (248) and also found to prevent the islet cells destruction. Other disorders treated by vitamin D are systemic lupus (249), rheumatoid arthritis (250) and inflammatory bowel disease (251).

The suppression of these autoimmune disorders might be due to interaction of vitamin D with T helper lymphocytes, which in turn defeat the inflammatory responses of T helper type 1 lymphocytes. As alternative ideas scientists believe that vitamin D suppresses the dendritic cells that present antigens to the T cells (252). Although the processes of this regulation of autoimmune diseases are not well understood, the results are promising for the use of vitamin D for the treatment of such disorders. 1, 25(OH)2 cholecalciferol has also been shown its role in preventing vascular and nonvascular transplant rejections (253).

**Requirements for vitamin D:**

Cholecalciferol is synthesized in the epidermis after exposure of 7-dehydrocholesterol to sunlight which helps to produce enough vitamin D as per required by the body. Tendency to wear clothes, to live in cities where tall buildings block adequate sunlight from reaching the ground, to live indoors, to use synthetic sunscreens that block ultraviolet rays and to live in geographical regions that do not receive adequate sunlight, all these factors downfall the ability of the skin to synthesize enough vitamin D (187). This makes vitamin D as an important nutritional factor when there is sub-optimal sunlight exposure and this makes it a true vitamin when supplied in the diet regularly. Since vitamin D can be produced within the body and can be retained for long time, it is difficult to govern with precision the minimum daily requirements of this micronutrient (254).
Vitamin D is a vital fat-soluble vitamin which regulates calcium homeostasis and is essential for muscle and bone health, all include as under (256):

**In infant:** At birth, infants acquired the vitamin D, in uterus and carry them through the first month of life. Breast-fed infants are particularly at high risk since there is low vitamin D content in human milk (155). This problem is further seen in some infants when there is restriction in sun exposure which may be due to seasonal, latitudinal, cultural or social reasons. Infants born especially during winter at extreme latitudes are at high risk because they spend the first 6 months of their life indoors and therefore have a little chance to synthesize vitamin D in their skin (257,258,259).

**In adolescents:** Another period of rapid growth of the skeleton occurs during puberty and the requirement for 1, 25(OH)2D is high. This requirement is met when there is increased conversion of 25(OH)D to 1,25(OH)2D (260). Unlike infants, adolescents usually are exposed to ultra-violet light and this might help in synthesizing vitamin D for their requirement. Excess production of vitamin D can be stored mainly in the adipose tissue (261) & can be made available during the winter. In sufficient vitamin D stores during this period of increased growth can lead to vitamin D insufficiency in the blood (262).

**In pregnant and lactating women:** the changes happened in calcitropic-hormones during gestation period and lactation has exposed a role for vitamin D in the latter. During pregnancy there is a change in vitamin D metabolism. This change in metabolism leads to increase the maternal plasma levels of active vitamin D (263) and this may be due to putative placental production of the hormone (264). This compensates the maternal vitamin D requirements because transfer of vitamin D from mother to fetus is important for the neonate’s growth rate.

**In elderly:** there is evidence that vitamin D deficiency backs to declining bone mass and increases the occurrence of hip fractures (265).

**Vitamin D effects in reproductive tissues in females**

Vitamin D is said to have biologically important role in female reproductive system. In human ovarian tissue, 1, 25(OH)2 cholecalciferol is understood to stimulate progesterone secretion by 13 percent, estradiol production by 9 percent
and progesterone synthesis by 21 percent (266). It was demonstrated that P450 aromatase activity (catalyzing the synthesis of estrogens) in a chorio-carcinoma cell and expression are stimulated by 1,25-dihydroxy cholecalciferol and that an atypical vitamin D response element has been cited in CYP19 (CYP19A1) (encoding P450 aromatase) promoter (267). 1, 25(OH)₂ vitamin D controls HCG (human chorionic gonadotropin) expression & production in human syncytiotrophoblasts (268) and increases placental sex steroid production (269). Biological active vitamin D encourages calcium transport in the placenta (270), inspires placenta lactogen expression (271), and controls HOXA10 gene expression in human endometrial stroma tissues (272). HOXA10 expression is significant for the growth of the uterus and vital for endometrial advancement, allowing uterine accessibility to implantation (273). In males the precise method by which vitamin D impacts male re-production is still unknown.

**Supplementation of vitamin D**

The RDA (recommended dietary allowance) of 25(OH) D is between 400-800 IU/day and during advancement of age the needs increasing but it has been challenged by many authors who recommend a higher allowance of 800-1000 IU per day for all ages in the absence of adequate sun exposure (274,35). Also to treat vitamin D deficiency, a higher dose of at least 50,000 IU per week is recommended to achieve optimal levels (35). The Indian Council of Medical Research (ICMR) has stuck to the lower allowance of 400 IU per day by emphasizing a greater exposure to sunlight as a result to improve the levels (275). However, it is difficult to ensure a higher sunlight exposure for every individual across the country. In spite of greater annual sunshine time in the region, the commonness of suboptimal vitamin D level is high as stated in some studies (276,277).

**Storage of vitamin D**

Adipose tissue stores vitamin D which has probability to represent “non-specific” stores sequestered because of the hydrophobic nature of vitamin D but the extent to which the processes of mobilization or accumulation are regulated by normal physiological mechanisms (278).

Vitamin D stored in the adipocytes is not readily accessible thus, fatty people need higher doses of vitamin D supplements to achieve a serum vitamin D levels.
relative to that of normal weight individuals. This hypothesis can also be supported from the observation that serum vitamin D levels rise when obese people lose body fat indicating that vitamin D could be stored in adipose tissues (279).

**Vitamin D Toxicity**

Potential vitamin D toxicity can be caused if the intake of vitamin D is not monitored properly and the toxicity might result into: hypercalcemia, hyperphosphatemia, nephrocalcinosis & soft tissue calcification these all might show increased danger of death. Various studies suggest that daily dose of 10,000 IU of vitamin D for at least 5 months may not alter serum calcium or urinary calcium excretion (37) Studies among children and adults have shown that those receiving daily dose of 2000 IU of vitamin D have not reported any toxicity. Vitamin D toxicity is one of the rarest medical complications that could be due to excessively high vitamin D concentration acquired by consuming an average of more than 10,000 IU of vitamin D daily for more than 24 weeks (3).

**Excretion**

The products of vitamin D metabolism are eliminated through the bile into the feces & very little is excreted through the urine. It is in part due to renal re-uptake of vitamin D metabolites bound to vitamin D binding protein as mediated by the cubilin-megalin receptor system (280).

**Prevention & treatment for vitamin D deficiency**

Two reports have come into light citing that vitamin D2 is less operative than vitamin D3 when it comes in maintaining vitamin D level. The claim has also been made that vitamin D2 leads the demolition of vitamin D3. Various other researches have also claimed that vitamin D2 is as operative as vitamin D3 in keeping vitamin D concentration within normal range among children as well as adults (3,281,282). In children daily dose of 2000 IU of vitamin D2 was found to as effective as daily dose of 2000 IU of vitamin D3 for achieving normal serum 25(OH) D level (282). Among healthy adults, 1000 IU of vitamin D2 or 1000 IU of vitamin D3 or 500 IU of vitamin D2 combined with 500 IU of vitamin D3 were effective to increase blood level of 25(OH) D by 10 ng/ml.
It has been noted that level of 25(OH) D in blood will rise by 1 ng/ml with every 100 IU of daily dose of vitamin D (3,281). To overcome low vitamin D in blood, pharmacologic dose i.e. 50,000 IU of vitamin D2, once a week for eight weeks of vitamin D is recommended. This is equal to supplementing 6000 IU of vitamin D daily. However, to avoid return of low vitamin D problem, it is suggested to supplement 50,000 IU of vitamin D2, once every 2 weeks permanently (283).

Many studies have reported that children and adults getting 2000 IU of vitamin D daily for up to one year, have blood level of 25(OH) D more than 30 ng/ml. A study in pre-pubertal and teenage girls supplementing 2000 IU of vitamin D3 daily for 12 months indicated improvement in bone mineral density (284). Adults getting at least 800 IU of vitamin D each day have shown decrease in fracture risk by more than fifty percent (285,286).

Vitamin D deficiency should not be over treated without actual monitoring of vitamin D levels. People having hypervitaminosis D can show the complications like hypercalcemia, decreased appetite, constipation, dehydration, lethargy, polydipsia, polyuria, abdominal pain, vomiting, and headache and nephrocalcinosis. In a previous study on mice reports that premature aging in mice (287) may be due to an increased 1-alpha-hydroxylase activity during hyper-vitaminosis D. The excess quantity (which is defined as intake more than 40,000 IU/day) of vitamin D can also cause severe hypercalcemia and this can only be treated by medication of intravenous fluids, oral prednisolone, and restriction of calcium diet while some people may need pamidronate infusion in order to correct high blood Ca level (288). High and low vitamin D status can both be avoided with proper vitamin D utilization and supplementation. Therefore, it is suggested that consuming 1-1.5 gm of dietary Calcium & 2000 IU of Vitamin D every day in our diet enough for preventing suboptimal vitamin D status among the people residing in India.

**Consequences of suboptimal vitamin D on health**

Osteoporosis and increased danger for fractures especially of the hip, vertebrae & forearm with thoughtful effect on quality of life, might be the skeletal consequences of having sub-optimal level of vitamin D (3,35). Suboptimal vitamin D concentration might also be responsible for muscle weakness, increasing risk of falling, thus further increasing risk of fracture in older population (3,35).
Vitamin D deficiency has been associated with increased risk for cancer including the breast, prostate, colon and ovaries (3,160,35,117,289). Women who had a blood level of 25(OH) D = 48 ng/ml reduced their risk of developing breast cancer by 50% (290). This was with the observation that women who had the ample sun exposure as teenagers & young adults reduced their risk of developing breast cancer later in life by more than 60% (291), A retrospective analysis of postmenopausal women receiving 1500 mg of calcium and 1100 IU of vitamin D per day revealed a decrease of more than 60% in the development of all cancers compared to women who either took calcium without vitamin D (46). The link between increased risk for colorectal cancer and colorectal adenoma with low serum 25(OH)D levels in human has been cited by the IARC (International Agency for Research on Cancer) (292).

Various chronic disorders such as: heart disease, diabetes mellitus type-2, autoimmune diseases, infectious diseases, asthma have said to be associated with low vitamin D in serum. Studies have shown that there is increase danger for acquiring diabetes mellitus type-1 and multiple sclerosis among those who reside in upper and lower latitudes. Research has shown that 42 percent of ladies having optimal vitamin D concentration have shown reduced risk of developing multiple sclerosis (3,35). Similarly, another research among ladies who were on highest intake of vitamin D, have shown 40 percent of reduced risk of developing rheumatoid arthritis (3). A report from Finland stated that children taking 2000 IU of vitamin D daily during their first year of life and took vitamin D for 31 years and these people showed reduced risk of developing type-1 diabetes by 78 percent (293). Vitamin D deficiency has also reported to be associated with increased risk of developing myocardial infarction and the risk is increased by 50 percent (173). The other disorders like peripheral vascular disease, hypertension and congestive heart failure have been said to be linked with vitamin D deficiency (3). VDRs have been reported in the tissues like vascular smooth muscle & cardio-myocytes and 1,25(OH)2 cholecalciferol is said to directly or indirectly influence 200 genes related to cardiovascular health (3).

Activated macrophages are reported to synthesize 1,25-dihydroxy vitamin D from 25(hydroxy) D. When the macrophage does this, it also directs the cell to yield
cathelicidin (a member of the defensin protein which functions to destroy infective agents such as tuberculosis) (3,163). This is the reason behind upper respiratory tract infections & influenza during the winter season when 25 (OH) cholecalciferol concentrations is below normal. A similar observation was reported the adults of the US. Those with highest serum vitamin D level showed lower risk of acquiring upper respiratory tract infections compared to those with lower vitamin D level (294). Similar observations were seen among postmenopausal ladies who showed 90 percent of reduced risk of acquiring upper respiratory tract infections when they took 2000 IU of vitamin D dose on daily basis (3).

People when advised to prevent from the risk of developing skin cancers; they tend to reduce sun exposure. Office works are mainly done indoors while children and adolescents spend a lot of times indoors playing with electronic gadgets instead of going outdoors. An attractive advertisement to promote use of sun block lotions is also a factor that might have resulted in a marked increase in vitamin D deficiency among various populations (295).

Ca (CALCIUM)

Ca is a very common and important mineral in human metabolism in addition to its well-known role in bone formation. Ca helps to control muscle and nerve function as well as to control acid and base balance in our blood and it builds up about 1.9% of the total body by weight (296). It is believed that 99% of the body Ca is present in the skeleton and the remaining one percent is being distributed in the teeth and soft tissues with only 0.1 percent in the extracellular fluid (297).

Calcium absorption and excretion

Ca is absorbed in the GITby two mechanisms:

- The first mechanism which is also called as vitamin D-dependent active transport mechanism, takes place in the duodenum and jejunum and the part of the small intestine just below the stomach. This area contributes the highest rate of absorption that is about three times that of the remainder of the gut (298)

- The second mechanism is diffusion which occurs throughout the small & large intestine (299)
PHOSPHORUS (P)

Phosphorus contributes up to 1 percent of the total body by mass and present in each cells of the body. Most of P is originated in the bones & teeth in the body and plays a significant part in the creation of bones and teeth. It also plays an important role in how the body uses carbohydrates and fats and needed for the body to make protein for growth, maintenance and renovate of cells & tissues. Phosphorus also helps in ATP formation (300).

Daily requirement of phosphorus is 800 mg but the average American diet constitutes about 1000-400 mg phosphorus daily. About two-third of the ingested phosphorus is excreted through urine and the rest one-third through stool & this phosphorus excretion is highly dependent on function of the kidneys. When phosphorus concentration in the blood falls, this will stimulate the renal enzyme 1-alpha hydroxylase, and this will start formation of calcitriol from calcidiol and this in turn, will increase absorption of phosphorus in intestine and reduction in excretion of phosphorus in the urine (301)

ALKALINE PHOSPHATASE

Alkaline phosphatase (ALP) is a membrane-bound metallo-enzyme falls in the category of hydrolyze-enzyme responsible for extracting phosphate groups from many types of molecules including proteins, nucleotides, & alkaloids. The process of extracting phosphate group is called de-phosphorylation and alkaline phosphatases are most effective in alkaline environment (302). Alkaline phosphatases are a group of enzymes found in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2) and a small amount is produced by the cells lining the intestines (isoenzyme ALP-3), the placenta & the kidney (in the proximal convoluted tubules). Alkaline phosphatase measured in the serum sample that is released from these tissues into the blood. The enzyme works better at alkaline pH at 10 and in humans alkaline phosphatase is present in all tissues throughout the body, but its concentration found particularly is in liver, bile ducts, kidney, bone, intestinal mucosa and the placenta.

The elevated levels serum alkaline phosphatase may be due to the obstruction of the biliary tract which can occur within the liver, the ducts passing
from the liver to the gall bladder and or the duct passing from the gall bladder through the pancreas that empty into the duodenum (small intestine). The organs liver, gall bladder, pancreas and duodenum may be involved. Alkaline phosphatase has become a useful tool in molecular biology and it’s another important use is as a label for enzyme immunoassay. Alkaline phosphatase is commonly used in the dairy industry as an indicator of proper pasteurization (303).

**ESTROGEN, PROGESTERONE AND TESTOSTERONE:**

These three hormones fall in the category of sex hormones, male and female all have three hormones (304), although the concentration of these three hormones may differ gender-wise. The two hormones (estrogen and progesterone) are ascendant hormone in female whereas testosterone in male. Estrogen is synthesized in the ovaries. In human, the three main known estrogen molecules are estriol, estradiol an estrone. Estradiol is the most biologically active kind of estrogen produced by the ovaries, the corpusluteum and adrenal gland fat cells. Estrogen and progesterone are antagonists and their actions are designed to balance each other & keep each other in check (305). Progesterone acts as precursor for most of the sex hormones such as estrogen, testosterone, androgens & other adrenal hormones as well as. Progesterone in the breast and uterus plays an imperative role for stimulating cell growth, which is directly regulated by estrogen. It activates the progesterone receptor which in-turn down-regulates the estrogen receptor since progesterone acts as suppressor for estrogen driven cell proliferation, progesterone in the natural state helps keep breast cell growth in healthy balance (306).

Many studies performed by many researchers, are mentioned here which are agreed and disagreed with the present study are as follow:

**Chapuy MC et. al.** (1997 in France), reported statistically significant variation in 25-hydroxy-vitamin D concentration based on geographical regions, they found lower vitamin D concentration in North while higher readings in the South with a significant sunlight effect (p=0.03) and latitude effect (p=0.01). In their healthy adult population, 14 percent of subjects were having 25(OH) D values less than 12 ng/ml which denoted the lower reference (2SD) for a normal adult population estimated in winter with the same technique. A significant negative correlation was found between PTH and serum 25(OH) D reading (p<0.01) (115).
Harinarayan CV et al. (2004), stated that Ca and 25(OH)D sub-optimal concentrations could adversely affect the bone mineral metabolism. Calcium, phosphorus and vitamin D levels were estimated in serum of 191 rural and 125 urban subjects. About 31% of the subjects had normal vitamin D levels, 54 percent had insufficient vitamin D and 15 % had deficient vitamin D concentrations (307).

A study carried by Arya et al., in 2004 at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow and showed that 78.3 percent subjects were affected by low vitamin D while serum 1,25(OH)$_2$ cholecalciferol level was within the normal range (40.6±20.1 pg/ml; mean ± SD.) They reported a significant correlation between serum vitamin D level & daily sun exposure ($p<0.001$) (308).

Gordon CM et al. in 2004: carried a research at Boston, concluding no statistically significant difference in the occurrence of low vitamin D among adolescent girls & boys. They also found a positive correlation between vitamin D deficiency and consumption of soft drinks, fruit juice, & iced tea, while they reported negative correlation between the deficiency and consumption of milk & cold cereal (fortified with vitamin D). No significant association between vitamin D deficiency & consumption of yogurt, cheese, or ice cream was reported by the authors (309)

Zargar AH et al. (in 2007) made a conclusion from their study carried on 92 healthy subjects (64 men and 28 women) having age 18-40 years residing in Kashmir and they observed 83 percent of the participants had lower vitamin D concentration than normal. They observed that 69.6 percent employed subjects and up to 100 percent house-hold participants had sub-optimal vitamin D concentration. The incidence of vitamin D deficiency among rural and urban population was calculated to be 80 percent and 85.7 percent respectively (277).

Mahmood et al. (in 2009) found in their study that the frequency of hypovitaminosis D was 79% among females. These subjects were predominantly married (72%), almost living in apartments (47.5%) and among of them (41.8%) exposed their face and hands. Duration of sun exposure was 1-2 hour /day (42%). Majority used clothes of variable color (72%) & fabric (41%). 76.2% subjects were found with deficiency of vitamin D and significantly associated with duration of sunlight exposure, large area of skin exposed, vitamin D consumed in diet and color of
cloths. Vitamin D was significantly negatively correlated with serum phosphorus & ALP whereas serum calcium correlated positively (310).

**Chittari V et al. (in 2009)** studied for the concentrations of serum calcium, phosphorus, alkaline phosphatase, vitamin D and found that calcium & phosphorous were significantly lower in rural adults compared to urban (p=0.0001). The dietary phytate-to-calcium ratio was higher in rural subjects than in urban subjects (p=0.0001). Vitamin D concentrations of the rural subjects were higher than urban subjects (p=0.001) in both men & women. Low dietary calcium intake & vitamin D levels were associated with bone mineral homeostasis (311).

**Mansoor S et al. (in 2010)** carried a research in Karachi, Pakistan and observed that the mean value of vitamin D concentration among the study population was calculated to be 41.1±9.6 nmol/l. 90 percent of the participants had sub-optimal serum vitamin D concentration; 69.9 percent were deficient while 21.1 percent were insufficient in vitamin D concentration (312).

**Knight JA et al. (in 2010)** concluded from a study that progesterone multiplicatively decreased by 10 percent (95% CI 5-14%, p<0.001) for every 10 nmol per liter of vitamin D increment, and decrease estradiol was observed by 3 percent (95% CI 0-6%, p=0.04). Higher levels of vitamin D might have decreased progesterone & estradiol, providing a potential mechanism for reduction in breast cancer risk from increased vitamin D exposure in young women (85).

**Shivane VK et. al, 2011,** found in their study that serum vitamin D concentration in study population was 17.4±9.1 ng/ml and men & women participants had 18.9±8.9 ng/ml and 15.8±9.1 ng/ml, respectively. Seventy percent of the study population had hypovitaminosis D; 76% of women had higher frequency of lower vitamin D (<20 ng/ml). Mean dietary calcium intake of the study subjects was 322.92±135.17 mg/day & was very low when compared with the recommended dietary allowance (400 mg/day for adults of both sexes) issued by the Indian Council of Medical Research. Dietary phytate was much higher than calcium intake with a dietary phytate to calcium ratio of 2.25±0.76. Serum PTH had significant negative correlation with vitamin D (p<0.001) (313).
Nimptsch K et al.; 2012, estimated the levels of vitamin D and testosterone in man, and found that there was positively association between vitamin D and testosterone levels (p=0.003) (314).

Shaheen et al., 2012, made their study on 110 subjects and found that more than 90 percent of their participants had deficiency of vitamin D. 23% had mild, 55% had moderate and 19.1% had severe vitamin D deficiencies. All study subjects who divided in three groups were with normal reference range of alkaline phosphatase and the total mean value of alkaline phosphatase was 135.97±68.14I U/L. They found the correlation coefficient of alkaline phosphatase & serum vitamin D3 levels (p=0.593) and conclude serum vitamin D3 levels may not be correlated with increased serum alkaline phosphatase levels. Therefore, alkaline phosphatase may not be used as a screening test to rule out deficiency of vitamin D (315).

Sherman et. al.; 2013, lides a study at Baltimore, Maryland and observed in a healthy population of 167 men & 114 women, aged between 20-94 years that serum vitamin D & its biological active form, did not decline with advancement of age in either sex. Non-linear regression using a shine function showed a significant seasonal association in 25(OH)D & 1,25(OH)2D in both sexes (p<0.005). Serum intact PTH increased significantly by 35 percent over the age span in both sexes (p<0.005). In women serum phosphorus and total & ionized calcium remained constant with advancement of age. In sharp contrast males had a marked 25 percent down fall in phosphorus across the age span (p<0.0001) and a slightly significant association, 4 percent decline in total and ionized calcium was observed (316).

Gaafar M. and Badr S. (in 2013) came to a conclusion after studying 365 subjects, the age of 18 years and above that 61.4% vitamin D deficiency was significantly prominent among female in age group 60 years & more with probability (p<0.05), they also concluded highly significant negative correlation between duration of daily sunlight exposure & vitamin D deficiency & insufficiency with probability (p<0.0001) The study population consuming milk, eggs, fish, liver & cheese, had normal vitamin D level while those who were not consuming these food items, had deficiency in vitamin D concentration with the probability (p<0.05) (317).
Baig et al., 2013 carried out a study on population of 176 individuals (50.57% males and 49.43% females). They compared BMI, vitamin D, PTH, calcium, & phosphorus among vegetarians & non-vegetarians with respect to the samples taken from urban and rural regions using student’s t-test and the probability p≤0.05 was considered to be significant. There was comparison between vegetarians belonging to urban and rural areas for BMI, calcium, vitamin D, and phosphorus and found significant association (t=2.09, df=81; t= -2.44, df=81; t= -2.71, df=81; & t= -3.25, df=81) with the probability p<0.05 and p<0.01 respectively. Among urban and rural non-vegetarians, the differences in BMI, PTH & calcium were (t= -6.69, df=91; t= -4.52, df=91; &t=4.37, df=91) respectively with the probability p<0.001 were significant. Differences for vitamin D (t=2.48, df=91) with the probability p<0.05 were significant. There was no significant difference in between serum phosphorus levels and in two groups (318).

Kiran B et al., 2014, led an investigation on 81 healthy subjects and performed vitamin D, calcium, phosphorus & alkaline phosphatase estimation in the serum and found, 73.91% subjects were vitamin D deficient. Serum vitamin D was found to have no significant correlation with serum phosphorus, serum alkaline phosphatase, skin color & living condition. But they found positive correlation between serum calcium, sun light exposure with vitamin D levels. Subject’s age was from 20-72 years with a mean of 41.93±13.02. Mean of serum vitamin D, calcium, phosphorus & alkaline phosphatase levels were 15.49±7.58, 8.95± 2.03, 4.70±0.87 and 126.54±64.25 respectively. They observed that serum calcium and serum phosphorus mean values were high among females relative to males. They also reported significant negative correlation between socio-economic status & sunlight exposure with the probability (p=0.001). Significant difference was found between males and females with sun light exposure with the probability (p<0.05). The mean of sun light exposure was more in males (31.90±52.37) as compared with females (10.16±7.47). Among males, serum vitamin D was significantly positively correlated with serum calcium, sun light exposure and living condition whereas it was negatively correlated with serum phosphorus and socio-economic status. The p value was significant for serum calcium, serum phosphorus, sun light exposure, socio-economic status & living condition. Correlation was significant with the probability (p<0.05). Among females, serum vitamin D was positively correlated
with serum calcium & negatively correlated with socio-economic status and milk consumption and the p value was found significant for all these three parameters at the levels (p< 0.05)(319).

Mehlawat U, et al. (2014) while working at AIIMS, New Delhi, found that vitamin D deficiency population was 50-90 percent among all the age groups. The most affected population was above 50 years as they reported (320).

Vasudevan J et al., 2014 assessed in their study that the utility of serum alkaline phosphatase as a screening test to identify vitamin D deficiency that it was not a useful screening tool for diagnosis (321).

Bachhel R, et al., 2015, Found 90% prevalence of vitamin D deficiency in the north-west Punjab population among 150 healthy volunteers of both sex. There was a significant difference in the prevalence of vitamin D deficiency between rural & urban subjects with the probability (p<0.05). A significant difference was observed in gender in 25(OH) D levels that women were showing higher prevalence of deficiency as compared to men (p<0.05). Lower prevalence was displayed by those subjects who have greater opportunities for sunlight exposure like rural residencies. Frequency of the subjects enrolled was as: 50% were males, 68% were from urban background, and 32% from rural background and the subjects in the age range of 51-60 years shows the least prevalence (25%) of vitamin D deficiency followed by the age range of 41-50 years (76.92%). Highest prevalence was seen in subjects of age less than 40 years as well as in subjects more than 60 years (95.83-100%) (p<0.05) (322).

Abdulrahman Saleh Al Mulhim, et al, 2015; done a study at Kingdom of Saudi Arabia and found in their study the majority of subjects had Vitamin D deficiency (43%) and insufficiency (57%). None was with normal concentration of vitamin D that is 75 nmol/l (323).

Shukla K et al, 2016; observed in their research that the prevalence of vitamin D deficiency in different age groups. There were 26,346 apparently healthy subjects, enrolled under executive health check-up at Medanta the Medicity, Gurgaon, vitamin D deficiency was found in 93 percent of the subject population. Maximum number of the subjects belongs to the age group of 41-60 years. 59 % had
frank 25(OH) D deficiency when cut off level was <20 ng/ml. The mean value of vitamin D in their subjects was 21.4±14.4 ng/ml. They observed a significant difference in vitamin D levels in between male & female study subjects. (324).

**Fentaw Yet al., 2017;** pursued a study on 118 adult patients with fractures participated in this study. The prevalence of patients with decreased serum levels of vitamin D at post test was 63.6% [95% CI; (0.551-0.720)]. Inadequate intake of milk & milk products in the 1st week of fracture [AOR = 95%CI: 0.20 (0.05-0.90)], Poor Dietary Diversity Score [AOR = 95% CI: 29.1 (2.27-371.65)], & ossified bone [AOR =95% CI: 4.10 (1.12-14.95)] showed statistically significant association with decreased serum level of vitamin D (325).

**Misra P et al., 2017;** conducted a study to estimate the prevalence of vitamin D deficiency among adult females aged 20-60 years residing in a rural community of North India, of rural Ballabgarh (Haryana) and found the prevalence of vitamin D deficiency, that was 90.8 percent with (95% confidence interval CI - 87.5-93.3), while that of vitamin D insufficiency was 8.9 percent with (95% CI - 6.4-12.2) (326).

In 2016 a study conducted by **Afifa Jahan** at Telangana state University to compare serum vitamin D levels to varying degree of sunlight exposure and food habit in age group of 22-60 years of different status and living conditions. She concluded that the vitamin D status of the participants completely exposed to sunlight and the participant with the habit of moderately exposed to sunlight was better than the participant with the habit of zero exposure of sunlight (354).

A study conducted by **Bani-issa W. et.al. 2017** on 18-64 years, age group population and found the deficiency of vitamin D 74% in Adults (355).

A study conducted by **Lhamo Y et. al. in 2017** and found vitamin D deficiency and management in majority, among Indian undergraduate medical student due to lack of awareness (356).

A study analyses done by **Robert Scragg in New Zealand; 2018,** from publications that clinical trials and observational studies are in favor of thresholds in vitamin D status. Vitamin D deficiency in population increase the risk of diseases, hence vitamin D supplementation has beneficial effects (357).