CHAPTER 1

INTRODUCTION

VITAMIN D

Vitamin D is a sterol and a prohormone, present in nature in several forms, also called calciferol, anti-rickets factor and sunshine vitamin and has two common forms that are:

* Vitamin D2 (ergocalciferol): It is of plant origin, obtained from the ergot plant. It can also be synthesized in the laboratory, by irradiating ergosterol.

* Vitamin D3 (cholecalciferol): It is of animal origin, the natural substance obtained from fish oil or derived from 7-dehydrocholesterol by ultraviolet irradiation in the skin. Interestingly, vitamin D3 and its active metabolites have also been obtained from some plant sources, particularly from tomato leaves. Both vitamin D2 and D3 are seco-steroids and are metabolically activated to carry out their functions (1).

Provitamin D and Pre-vitamin D: 7-Dehydrocholesterol is a precursor of vitamin D3 in animals and it is synthesized in human beings in intestinal mucosa. By the action of UV-B light in the skin, 7-dehydrocholesterol is converted into an intermediate called provitaminD3 which is spontaneously isomerized to vitamin D3 (cholecalciferol), in this process (photobiogenesis), the UV-B range rays of sunlight with a wave length of 290–315 nm initiates photoconversion. On the other hand, ergosterol is the provitamin D2 in plants. In the presence of light, by the process of photolysis, ergosterol is converted to pre-vitamin D2 which is isomerized to ergocalciferol (vitamin D2)(1).

Vitamin D is a fat-soluble vitamin or nutrient that changes to a hormone within the body and has both skeletal and non-skeletal functions in the body. Vitamin D became a nutrient when, Sir Edward Mellanby in the twentieth century, demonstrated that rickets could be cured by the administration of cod liver oil (2). However, he was McCollum who later determined that a nutrient present in cod liver oil was responsible for its anti-rachitic activity and named this nutrient as vitamin D. Humans get vitamin D from exposure to sunlight and dietary supplements
During the exposure to sunlight the humans get ultraviolet-B (UVB: 295-310) rays which helps in synthesis of cholecalciferol (Vitamin D3). In liver vitamin D is hydroxylated to its circulating form, 25-hydroxy vitamin D [25(OH)D] which is converted in the kidney into the biologically active form that is 1,25-dihydroxy vitamin D [1,25(OH)₂D]. In response to serum calcium and phosphorus levels, parathyroid hormone produces 1,25(OH)₂D in the kidney (4). The main role of vitamin D is to maintain adequate intestinal absorption of calcium & phosphorus, and mineralization of bone (5). Impaired calcium metabolism, may be responsible for secondary hyperparathyroidism increases bone turnover, & progressive bone loss (6). Vitamin D plays many roles in our body for example in bones, it has many functions in cells, immune response, cell differentiation and proliferation and apoptosis (7). It may also decrease the risk of many diseases such as cancers, autoimmune disease, infectious disease and cardiovascular diseases (3).

Since last so many years, vitamin D is in interest of medical people for various etiological and broad clinical, biochemical and pathological purposes. The endogenous synthesis of vitamin D only requires the presence of basic molecule i.e.7-dehydrocholesrerol in the skin. When 25(OH)D was discovered in 1922, it was misnamed. It was considered as false vitamin because external nutrient source was not required to maintain normal levels in the body, and vitamin D was exactly categorized as a seco-steroid hormone. A hormone is a chemical substance that produced by one organ and then transported in the bloodstream to a target organ, where it causes a specific biological action (8).

Vitamin D has been conventionally understood as anti-ricketic factor or sunshine vitamin which is metabolized within the body and can function as a hormone too. Besides its important role in Calcium homeostasis & bone mineral metabolism, vitamin D has now been documented for its wide range of fundamental biological functions like cell differentiation, inhibition of cell growth as well as immune modulation (9,10,11,12,13,14,15,16).This steroid vitamin is known to regulate, a complex system of genomic functions and also helps in neo plastic transformation prevention. Results from genetic, nutritional & epidemiological studies state that vitamin D has preventive and curative roles in endocrine diseases
such as hypertension, myopathic disorders, autoimmune disorders & cancer (11,17,18,19,20,21).

Vitamin D is a group of anti-rachitic substances that are found in certain foods as well as synthesized in skin. Various forms of vitamin D are:

- Vitamin D₁,
- Vitamin D₂ (ergocalciferol),
- Vitamin D₃ (cholecalciferol),
- Vitamin D₄ (di-hydroergocalciferol),
- Vitamin D₅ (sitocalciferol).

In the human context, there are two important forms i.e. vitamin D₃ & vitamin D₂. Vitamin D₃ is derived from the precursor 7-dehydrocholesterol which is synthesized in skin, on the other hand vitamin D₂ is obtained from ergosterol found in foods like bread, milk, yeast, mushrooms and cod liver oil. Exposure of skin to the ultraviolet rays of sun is required for the conversion of these precursors into pre-vitamins. The synthesis of biologically active form from vitamin D₃ is a two-step process, first it is hydroxylated to 25(OH)D₃ in the liver & followed by hydroxylation in kidney to produce the final active form i.e. 1, 25 dihydroxy vitamin D [1,25(OH)₂ D (22,23,24)]. These active metabolites employ their effects through VDR (vitamin D receptor) which is a nuclear hormonal receptor that is expressed in most of the tissues (22).

Vitamin D is a collective representation for vitamin D₂, vitamin D₃, and their metabolites (5) Vitamin D₂ is found in the yeast and plant sterol and is widely used in pharmaceutical manufacturing. When exposed sunlight, UV-B photons (wavelength 290-315 nm) can penetrate skin where 7-DHC (7-dehydrocholesterol) absorption takes place and UV-B helps to breaking B and makes pre-D₃ (pre-cholecalciferol) which isomerizes the double bonds and makes 25(OH)D (4). In the liver, vitamin D is metabolized and circulated to tissues and stored for further activation. Some of these are converted in the kidney as hormone that is 1,25(OH)₂D (1, 25(dihydroxy) cholecalciferol) and this form of vitamin D is biologically active. In the kidney, various factors like serum phosphorus and PTH, are responsible for regulating the production of 1,25(OH)₂D which in turn, is responsible for regulating
calcium metabolism in tissues, bone and intestine (4). VDRs have been reported in various tissues like small intestine, osteoblast, brain, heart, skin, pancreas, breast, colon, and immune cells. 1,25(OH)_{2} cholecalciferol helps to maintain cell growth and maturation, it is also reported that vitamin D plays important role in insulin secretion, inhibition of renin production and regulation of activated T and B lymphocytes & macrophages (25,15,26).

**Factor affecting vitamin D synthesis**

Vitamin D (fat-soluble vitamin) is dependent on many factors like sun exposure, its duration and time of exposure, skin pigmentation, latitude, air pollution and clothing. As suggested by FAO (Food and Agriculture Organization) and WHO, the best latitude of the world for abundant sunshine is in between 42°N and 42°S which is good for synthesizing vitamin D subcutaneously from 7-dehydrocholesterol. Sun exposure for half an hour from 10 AM to 2 PM afternoon (maximum UV-B rays transmitted from the sun) falling on face and arms is adequate for Vitamin D deficiency prevention (27).

Vitamin D is one of the members of ‘Ca-25(OH)D-PTH’ endocrine axis (8) which is responsible for regulating normal Ca homeostasis in the body. Vitamin D deficiency which means low calcium intake causes osteoporosis, low bone mass, weak muscles and risk of fracture, long standing vitamin D deficiency cause secondary hyperparathyroidism, which also cause osteoporotic fractures (28,29). Adequate intake of calcium & vitamin D can reduce the risk of these problems and it is also necessary to maintain the bone mass. It is essential to check vitamin D readings with BMD (bone mineral density) simultaneously because studies of BMD without estimating 25(OH)-cholecalciferol concentrations can mislead the clinical interpretations. Various autoimmune illnesses like multiple sclerosis, rheumatoid arthritis and diabetes mellitus type 1, infections and danger of acquisition of the cancers of various organs like breast, colon, prostate and ovary have been reported to be connected with 25(OH)D concentrations (3).

Conclusion made from many studies reveals that vitamin D deficiency is common among nearly 1 billion populations around the globe. Indian population is also affected due to low dietary calcium intake (8) Apart from dietary factors peoples are suffering from different diseases like hepatic, renal and skin disorders,
and other factors are alcoholism and inflammatory rheumatological conditions. Other common factors in India are:

- Changing food habits contribute to low dietary calcium & vitamin D intake.
- High fiber diet containing phosphates and phytates which increase calcium requirement causes depletion of vitamin D stores (30).
- Genomic factors responsible for catabolizing 25-OH-cholecalciferol to inactive metabolites (31).
- Plasma 25-OH-vitamin D depends on the heritability of VDB (vitamin D binding protein) in response to treatment (32).
- With changing lifestyle and modernization, people nowadays spend their maximum time indoor and hence reducing the sun exposure time. This is common in the city regions of India.
- UVB is blocked due to increasing pollution and this leads to compromised synthesis of vitamin D in the epidermis (33).
- Burqa and the pardah like culturally significant clothing practices are responsible for low vitamin D.
- Recurrent and unintentional pregnancies among the ladies having insufficient dietary intake can also trigger suboptimal vitamin D status among the mother & the fetus.

**Vitamin D status**

25(OH)D is calculated for the measurement of total vitamin D status of the body (34,35). 25(OH)D level less than 30 ng/ml is an indicator of vitamin D deficiency whereas more than 30 ng/ml is considered to be sufficient (36). Studies on bone mineral density, fracture prevention, lower extremity function & cancer prevention intimate 30 ng/ml of 25(OH) D in the blood (37). The amount of vitamin D intake required to maintain optimal 25 (OH) D concentrations has not been defined yet. Many studies indicate that mean daily intake of vitamin D is required to be at least 500 IU for young adults in summer to optimize 25(OH)D statuses and prevent winter time bone loss (38). During the season when there is prolonged absence of sun exposure, the mean daily requirement for vitamin D is estimated to be at high as 2,000 IU.
Vitamin D deficiency

Not only in the Asian but Europe too is under the influence of suboptimal 25(OH)D effects. Vitamin D fortified milk and vitamin supplements usage are common practice among the people of European nations (33). India is a vast country having different zones with different temperature and humidity, and other climate changes which discourage the people to have proper sunlight, as other countries are using supplement in milk, it can be used in India, also it is a good option to manage this deficiency for Indian population.

During winter, epidermis production of vitamin D is compromised due to diminished exposure to the sunlight (39) since UVB is decreased during winter and at the high latitude. Skin pigmentation also plays role in vitamin D production in the epidermis as melanin is a natural sunscreen and blocks the UVB. Even though a little of vitamin D is naturally present in some foods, dietary supplementation is unable to compensate vitamin D requirement alone if epidermis synthesis is compromised and this truth is proved in the countries where mandatory food fortification, such as in milk consumption, vegetarian diet and limited use of dietary supplements is being implemented, but vitamin D deficiency is still reported in those nations (23).

Vitamin D is unique and has two forms vitamin D2 and D3, obtained from yeast sterol ergosterol, and sun-exposed mushrooms, and oil-rich fish such as salmon, mackerel, & herring contain vitamin D3 naturally(40,41,42,43). Vitamin D2, or D3, or both when ingested, are incorporated into chylomicrons for absorption into the lymphatic system and in this way, these metabolites enter the blood. Vitamin D derived from the epidermis or diet is biologically inert & requires its first hydroxylation in the liver by 25-hydroxylase to form 25(OH)D (40,44) and second hydroxylation of 25(OH)D at C-1 in the kidneys by 1alpha-hydroxylase (CYP27B1) to form 1,25-dihydroxy-vitamin D (biologically active)(40,44) which is responsible for all the actions of vitamin D (45). It is said that about 10-15 percent of dietary Calcium & only 60 percent of phosphorus are absorbed in the absence of active vitamin D (40,46)
Prevalence of vitamin D deficiency

Vitamin D deficiency, defined as low concentration of 25(OH)D in the plasma, is common around the globe irrespective of age, gender, country of origin, latitude, or dietary practices. The increasing incidence of vitamin D deficiency among healthy individuals has been reported in large-scale studies from all parts of the world; the US (47), Canada (48), South America (49), Europe (50), Australia (51), the Middle East (52), South Asia (53) and Africa (54). Severe deficiency of vitamin D is common in China, India, South America, and the Middle East. In India, the studies from the different regions have revealed the occurrence of vitamin D deficiency in varying degree from 30 percent to 100 percent (53,55,56). However, the cutoff value of vitamin D concentration to define its deficiency varies widely. The various studies have stated the cutoff value i.e. 30 ng/ml to define the deficiency of this vitamin among different age groups, pregnant and lactating women, newborns, adolescents and healthy professionals (56)

Indian latitude

Although Indian public (residing from 8.4°N latitude to 37.6°N latitude) is believed to get sun exposure throughout the year, the deficiency of vitamin D in India (57) among all sex and age group is very common (8,58,59).

Factors affecting vitamin D requirement and its deficiency

The factors that affect vitamin D requirement and its deficiency include; sunscreen use, obesity, skin pigmentation, atmospheric components, latitude, season, time of day, clothing, variation in sun exposure, age, and several chronic diseases etc. some of them are described here:

1. Use of sunscreen: The people used to sunscreen must avoid sunburn, skin cancer, and excessive skin aging. But in actual, sunscreen can increase the cancer risk due to its strong blocking action against vitamin D in human being (60) while blocking the ultraviolet-B rays, which are responsible for the production of vitamin D.

2. Obesity/over-weight: It is said that adipose tissues absorb vitamin D and act as a storage tank for this vitamin. Therefore, only necessary amount body fat might be helpful for adequate vitamin D levels throughout, regardless of supplementation (61)
3. **Skin pigmentation/skin Color**: The skin pigment; Melanin (a natural sun block) inhibits ultraviolet-B rays entry into the dermis thus halting vitamin D production. Therefore, darker skin containing more melanin pigment triggers the greater chance of vitamin D deficiency among dark complexes population (62) and these people are suggested to spend more time in the sunlight, or/and increase supplementation in order to optimize serum 25(OH)D status.

4. **Air pollution**: Increase in poor quality of air, results to increase in air pollution by any means causes absorption of ultraviolet-B rays making less availability and this results in decreased amount of UVB to skin thus, resulting in low vitamin D synthesis. The persons living in the city are as having poor air quality have presented with difficulties for optimum vitamin D synthesis (63).

5. **Location**: In winter season, ultraviolet-B rays exert less impact on the earth’s surface so there would be less production of vitamin D. Supplementation is required during winter season to achieve adequate amount of vitamin D in the blood. Shorter duration of days along with wearing full body covering clothes is also an important factor that limits the sunlight exposure leading to diminished synthesis of 25(OH)D (64).

6. **Aging**: When the age is compared, then elderly population has decreased concentration of precursor in the dermis that ultraviolet-B rays produce vitamin D as proven by the present study which indicate a big problem among older people (64).

7. **Health of the gut**: The vitamin D taken in the diet or as supplements that are captivated in small intestine timely downstream from the stomach. Vitamin D absorption is influenced by factors like intestinal cell wall, integrity of stomach juices, bile, and pancreatic secretions. Therefore, environments that influence the gut health and digestion (as in case of diseases like celiac disease, chronic pancreatitis, Cohn’s disease, and cystic fibrosis) have negative impacts in the absorption of vitamin D in the intestine (64).

8. **The health of liver and kidneys**: Some hepatic disorders may also have role in declined absorption of 25(OH)D because the ailing liver can not synthesize required quantity of bile juice and these result in incomplete vitamin D metabolism. The renal health determines 1,25-dihydroxy-cholecalciferol amount which can decrease due to
renal diseases and their condition, and vitamin D level diminishes as low as undetectable in end-stage renal diseases (64).

Studies showing that lower level of serum vitamin D has been linked to various non-skeletal diseases such as cancer (65,66), infections (67,68), autoimmune diseases (69) cardiovascular disease (70,71). Optimization of vitamin D may be helpful in decreasing the incidence of these diseases and decrease mortality rate (72,46). Worldwide one billion people have been reported to have been affected by hypovitaminosis D among all ethnicities and age groups (73,3,74).

**Effect of vitamin D on sex hormones**

Studies suggest that vitamin D facilitates reproductive processes in women as well as in men because infertility is a complex disorder with significant medical, psycho-social, and economic aspects (75), which affect about 15% of couples (76). The research is concerned with vitamin D status & fertility with endocrine disturbances in women including IVF (in vitro fertilization) outcome, polycystic ovarian disease and endometriosis. There is a significant association of vitamin D with; in pregnancy, the prenatal period and lactation has been found (77,78).

Studies indicate that in 30-40% of infertile couples, the underlying cause found the male factor (79) due to decreasing semen quality which might again be due to environmental factors (80) and research reveals 20% of young men having sperm count below WHO recommendation level (81). However, a lot of adverse aspects of male aging have been attributed to the decrease in testosterone levels (82) and evidences suggest androgen and vitamin D metabolism are linked to each other (83,84).

The effect of vitamin D on sex hormones is that may lower the risk of breast cancer via an effect on steroid hormones in women. Increased vitamin D exposure in young women might decrease progesterone & estrogen by providing a potential mechanism for reduction in breast cancer. Every increase of 10 nmol/l of vitamin D, progesterone correspondently is lowered by 10% and estrogen is decreased by 3 percent (85). Androgen levels & vitamin D levels are associated in men & reveal a concordant seasonal variation. Men with normal level of vitamin D (≥30 ng/ml) had significantly higher levels of testosterone and significantly lower levels of serum
hormone binding globulin (SHBG) when compared to vitamin D and vitamin D deficiency in men with probability (p<0.05) (83).

It has been observed that vitamin D deficiency has been a neglected disorder and not much work has been done on its demographic patterns, especially in the Indian context. Present study aims to evaluate the demographic pattern of vitamin D deficiency in the local population of north-east Haryana, India. Haryana has a flourishing middle-class society with a mix of agrarian, business, and service communities. Economic growth has brought about changes in the lifestyles as regards the dietary habits, work types and schedules. Whether these population characteristics influence the vitamin D levels need to be explored now.

Present topic was selected due to the increasing awareness of vitamin D deficiency among the Indian people mostly of Haryana. Various studies have published which are addressing the newly invented roles of vitamin D in the body. It has been suggested that vitamin D deficiency may increase risk for cancer, autoimmune diseases, and cardiovascular disease & bone disorders. Although it has been proposed that the current recommendation for vitamin D intake is far too low to prevent deficiency. Present study seeks to determine the prevalence of vitamin D deficiency among the Indian population Mostly of Haryana. It also addresses the interventions that can be taken to increase vitamin D status.