INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, and altered intermediary metabolism of major food substrates ***(Scoppola et al., 2001)***.

Vitamin D is a fat-soluble vitamin that is naturally present in very few foods, and available as a dietary supplement. It is also produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis ***(Holick et al., 2006).***

Vitamin D is a pleiotropic hormone known to play an immunomodulatory role ***(Baeke et al., 2007)*** in addition to Ca and bone metabolism, Receptors for its activated form have been identified on pancreatic Beta-cells and immune cells ***(Chiu et al., 2004; Veldman et al., 2000);*** Different studies have shown that deficiency of vitamin D leads to immune cell dysfunction, Beta cell damage and impaired insulin production ***(Hayes et al., 2003; Mattila et al., 2007)***. Evidence is available linking vitamin D deficiency with bacterial and viral infections ***(Kawarau et al., 2006; James et al., 2010).***

Immunological defects in addition to neuropathy and vascular abnormality are the prime contributors in the pathogenesis of diabetic foot and subsequent infections ***(Geerlings et al., 1999).***

Diabetic foot ulcers and their complications are an important cause of morbidity and mortality in patient with DM. These ulcers tend to heal slowly, need intensive care and healing can be complicated by infection and gangrene, leading to long term hospital treatment and /or amputation ***(Apelqvist et al., 2000)***.

In 2013 ***Tiwari et al.*** studied circulating plasma levels of 25(OH) D in 162 diabetic patients with ulcer and another 162 without ulcer in a prospective cohort hospital based study, Subjects with diabetic foot ulcer showed lower median plasma level of 25(OH) D.

In recent years, there has been an effort to understand possible roles of 25(OH)D, including its role in the immune system particularly on T cell medicated immunity, pancreatic insulin secretion and insulin action. 25(OH)D stimulates the cell differentiation and reduces cell proliferation, which is essential for cell growth and wound healing. However, data on the association between low level of plasma 25(OH)D and diabetic foot syndrome are scarce, so further trials are needed to evaluate if an association does exist.

AIM OF THE WORK

To study the relationship between Vitamin D level and risk of developing diabetic foot ulcer.

VITAMIN D

Introduction:

Vitamin D is a fat-soluble vitamin which is different from all other fat soluble vitamins, in that the body can synthesise it with the help of sunlight from a precursor that the body makes from cholesterol ***(Holick et al., 2006).***

The main biological function of vitamin D is to maintain normal blood levels of calcium and phosphate. This in turn sustains the normal mineralisation of bone, muscle contraction, nerve conduction and general cellular function in all cells of the body. The active form of vitamin D, 1, 25-dihydroxyvitamin D or calcitriol, also regulates the transcription of a number of vitamin D-dependent genes coding for calcium transporting proteins and bone matrix proteins ***(Norman et al., 2008).***

It also seems to have some anti-inflammatory and immune-modulating properties. In addition, recent epidemiologic studies have observed relationships between low vitamin D levels and multiple disease states. Low vitamin D levels are associated with increased overall and cardiovascular mortality, cancer incidence and mortality, and autoimmune diseases such as Rheumatoid arthritis, Inflammatory bowel disease and multiple sclerosis. Although it is well known that the combination of vitamin D and calcium is necessary to maintain bone density as people age, vitamin D may also be an independent risk factor for falls among the elderly ***(Brannon et al., 2008)***.

Structure and Forms of vitamin D:

Table (1): Forms of vitamin D:

Name	Chemical composition
Vitamin D_1	Molecular compound of ergocalciferol with lumisterol
Vitamin D_2	Ergocalciferol (made from ergosterol)
Vitamin D_3	Cholecalciferol (made from 7-Dehydrocholesterol in the skin).
Vitamin D_4	22-dihydroergocalciferol
Vitamin D_5	Sitocalciferol (made from 7-dehydrositosterol)

Several forms (vitamers) of vitamin D exist (see table 1). The two major forms are vitamin D_2 or ergocalciferol, and vitamin D_3 or cholecalciferol, vitamin D without a subscript refers to either D_2 or D_3 or both. These are known collectively as calciferol ***(Hodgkin, 1957).***

Chemically, the various forms of vitamin D are secosteroids, i.e., steroids in which one of the bonds in the steroid rings is broken. The structural difference between vitamin D_2 and vitamin D_3 is the side chain of D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24 ***(Hodgkin et al., 1957).***

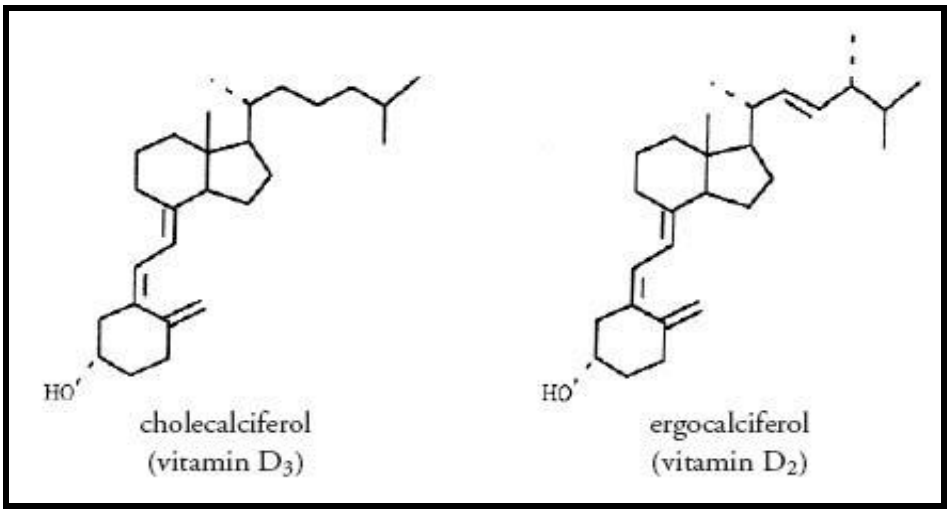

Figure (1): The two major forms of vitamin D ***(Ikekawa et al., 1993).***

Sources of vitamin D:

The main sources of vitamin D are sunlight, supplements and diet ***(Holick et al., 2007)***. Exposure of human skin to solar UVB radiation (wavelengths: 290–315 nm) leads to the conversion of 7-dehydrocholesterol to previtamin D_3 in the skin. Previtamin D_3 is then rapidly converted to vitamin D_3 (cholecalciferol) by temperature- and membrane-dependent processes ***(Holick et al., 1995).***

The number of foods naturally containing vitamin D in significant amounts is very limited. Among these are oily fish such as salmon, sardines and tuna, and oils of the liver of some fish such as cod as well as sun-exposed mushrooms ***(Holick et al., 2007).***

The Recommended Dietary Allowance (RDA):

The RDA for vitamin D is listed in the table below by life stage and gender ***(Holick et al., 2011).***

Table (2): Recommended Dietary Allowance (RDA) for Vitamin D.

Life Stage	Age	Males mcg/day (IU/day)	Females mcg/day (IU/day)
Infants	0-12 months	400 IU	400 IU
Children and Adolescents	1-18 years	600 IU	600 IU
Adults	19-50 years	600 IU	600 IU
Adults	51-70 years	600 IU	600 IU
Adults	71 years and older	800 IU	800 IU
Pregnancy and lactation	all ages	-	1500-2000 IU

Vitamin D metabolism:

A- Storage, Circulation and Excretion:

Previously believed to be biologically inert at physiological levels, 25(OH)D is the major storage and circulating form of vitamin D and frequently measured as an index of vitamin D status. In the human body, the highest concentration of 25(OH)D is noted in the plasma (usually measured in the serum as 20–150 nmol/L or 8–60 ng/mL), but the largest pool of 25(OH)D is in adipose tissue and muscle Hence,

although a circulating half-life of 25(OH)D is approximately 10–15 days ***(Holick, 2007)***.

Renally produced 1, 25$(OH)_2$D circulates in the blood at levels in the picomolar range, about one thousandth those of 25(OH)D. Contrasting with the relative lack of regulation of 25-hydroxylation, 1α-hydroxylation is under control by serum parathyroid hormone (PTH) and fibroblast growth factor 23 in response to serum calcium and phosphate and represents the rate-limiting step in the synthetic pathway.

Metabolites in the vitamin D pathway are transported in the circulation predominantly (about 85–90%) bound to vitamin D-binding protein (DBP, also known as group-specific component globulin, Gc-globulin) and albumin (about 10–15%), with <1% in the free form ***(Powe et al., 2011).***

DBP is a liver-derived, 58 kDa glycosylated α-globulin structurally similar to albumin, which circulates at concentrations of 0.6–11 μmol/L. The affinity of DBP for 25(OH)D is 5×10^8 mol/L, about an order of magnitude greater than that for vitamin D (1×10^5 to 1×10^7mol/L) or 1, 25$(OH)_2$D (2×10^7 mol/L). This difference in affinity partly accounts for the shorter plasma half-life of vitamin D (~4–6 h) and 1, 25$(OH)_2$D (~4–20 h) ***(Otterbein et al., 2002)***.

Tight regulation of 1α-hydroxylation and the short half-life mean than the serum concentration of 1, 25$(OH)_2$D is not

an accurate measurement of total body vitamin D status, and measurement is of most use in altered states of 1α-hydroxylation such as chronic kidney disease (reduced) or granulomatous disease (increased) ***(Zimmerman et al., 2001).***

The catabolic enzyme 24-hydroxylase (CYP24A1) is responsible for the conversion of both 25(OH)D and 1, $25(OH)_2D$ to the inactive metabolites, 24, $25(OH)_2D$ and 25(OH)D-26, 23-lactone, and via a multistep pathway to the water soluble calcitroic acid (1α-hydroxy-23 carboxy-24, 25, 26, 27-tetranorvitamin D3), which undergoes urinary and biliary excretion ***(Plum et al., 2011)***.

B- Vitamin D synthesis Regulation:

The 25-hydroxylation of vitamin D is poorly regulated ***(Holick et al., 1981)***. The production of 1, $25(OH)_2D$ in the kidney is tightly controlled, being stimulated by parathyroid hormone (PTH), and inhibited by calcium, phosphate, and fibroblast growth factor 23 (FGF23) ***(Bikle, 2012).***

Extrarenal production of 1, $25(OH)_2D$ as in keratinocytes, macrophages, and osteoblasts is under different control, being stimulated primarily by cytokines such as tumor necrosis factor-α, interferon-γ, and interleukin (IL)-1β ***(Bikle, 2009).***

1, $25(OH)_2D$ reduces 1, $25(OH)_2D$ levels in cells by decreasing production or by stimulating its catabolism through the induction of CYP24A1, the 24-hydroxylase ***(Munson et al., 2002).***

Mechanism of action of vitamin D:

The mechanism of action of the active form of vitamin D, 1, 25(OH) D is similar to that of other steroid hormones. The intracellular mediator of 1, 2S(OH) D function is the VDR.

1, 2S(OH) D, binds stereospecifically to VDR, which is a high affinity, low-capacity intracellular receptor that has extensive homology with other members of the superfamily of nuclear receptors including receptors of steroid and thyroid hormones ***(Christakos et al., 2003)***.

Upon entering the nucleus of a cell, 1, 25-dihydroxy vitamin D associates with the VDR and promotes its association with the retinoic acid X receptor (RXR). In the presence of 1, 25-dihydroxyvitamin D the VDR/RXR complex binds small sequences of DNA known as vitamin D response elements (VDREs) and initiates a cascade of molecular interactions that modulate the transcription of specific genes. More than 50 genes in tissues throughout the body are known to be regulated by 1, 25-dihydroxyvitamin D ***(Guyton et al., 2003).***

1, 25(OH)D has 100-fold more affinity for the vitamin D receptor (VDR) than 25(OH)D ***(Bikle et al., 1985).***

Classical role of Vitamin D:

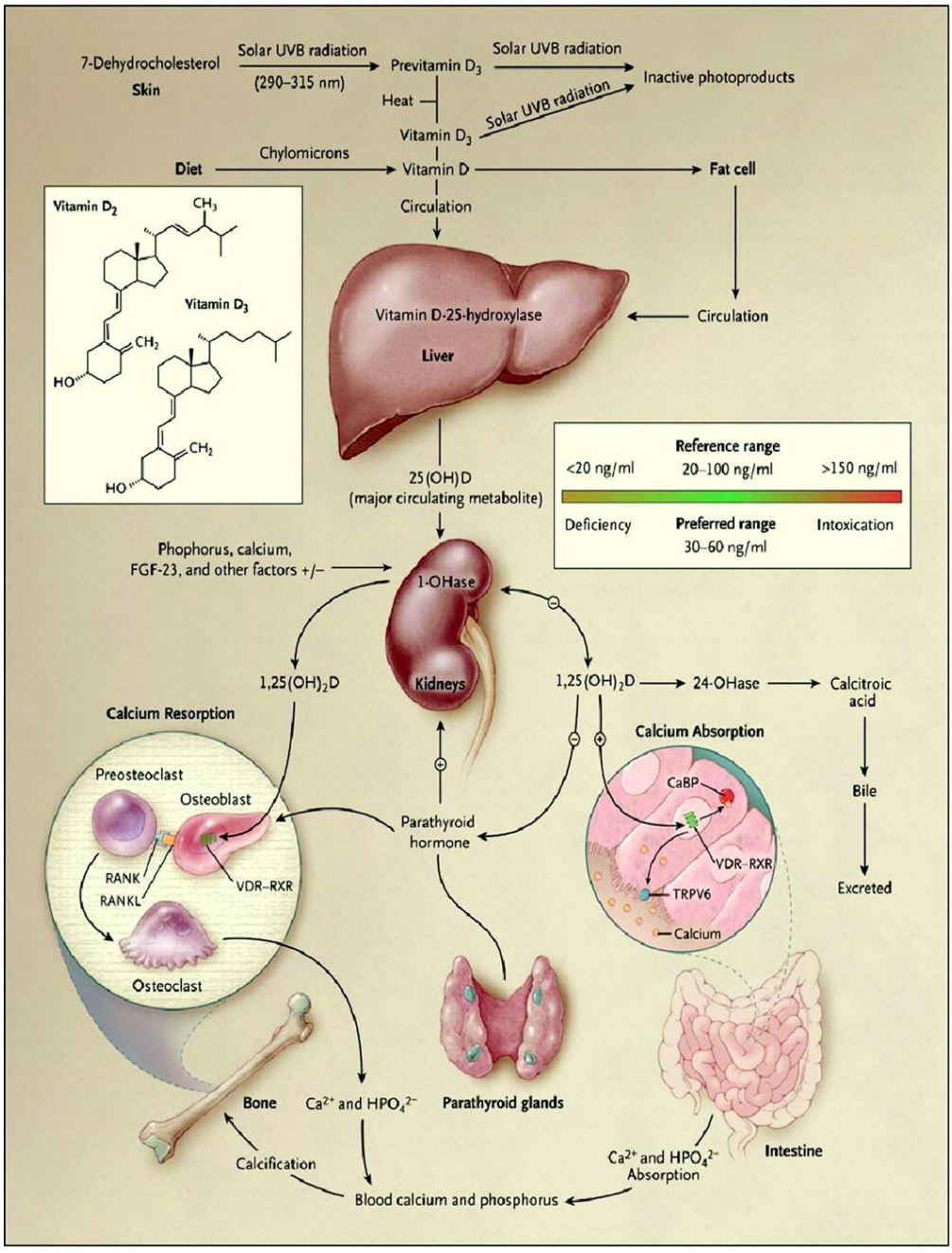

Figure (2): Classical role of vitamin D ***(Holick, 2007).***

A- Intestine:

Vitamin D is essential to enhance the efficiency of the small intestine to absorb dietary calcium and phosphate. Epithelial calcium transport is stimulated by 1, $25(OH)_2D_3$ by induction of ***(Hoenderop et al., 2000).***

The initial calcium uptake is the rate-limiting step in intestinal calcium absorption and highly dependent on vitamin D ***(Vesely and Juan, 1980).***

1, $25(OH)_2D_3$ also increases active phosphate transport through stimulation of the expression of the Na-P_i cotransporter ***(Yamamoto et al., 1999)*** and changes in the composition of the enterocyte plasma membrane that increase fluidity and phosphate uptake. Little is known, however, concerning the molecular mechanisms involved in the extrusion of phosphate across the basolateral membrane into the circulation ***(Kurnik and Hruska, 1985).***

B- Skeleton:

Vitamin D is essential for the development and maintenance of a mineralized skeleton. Vitamin D deficiency results in rickets in young growing children and osteomalacia in adults ***(Amling et al., 1998).***

Furthermore, the 1, $25(OH)_2D_3$-VDR system was revealed to be critical for the normal coupling of bone remodeling. Both osteogenesis and osteoclastogenesis were

impaired in the 1, 25$(OH)_2D_3$-VDR-defective mutants ***(Panda et al., 2001).***

C- Kidney:

The most important endocrine effect of 1, 25$(OH)_2D_3$ in the kidney is a tight control of its own homeostasis through simultaneous suppression of 1α-hydroxylase and stimulation of 24-hydroxylase and very likely through its ability to induce megalin expression in the proximal tubule ***(Liu et al., 1998).***

1, 25$(OH)_2D_3$ involvement in the renal handling of calcium and phosphate continues to be controversial due to the simultaneous effects of 1, 25$(OH)_2D_3$ on serum PTH and on intestinal calcium and phosphate absorption, which affect the filter load of both ions.1, 25$(OH)_2D_3$ enhances renal calcium reabsorption and calbindin expression and accelerates PTH-dependent calcium transport in the distal tubule, the main determinant of the final excretion of calcium into the urine and the site with the highest VDR content. The effect of 1, 25$(OH)_2D_3$ in improving renal absorption of phosphate in the presence of PTH may not be due to a direct action of the sterol on the kidney ***(Kitazawa et al., 2003).***

1, 25$(OH)_2D_3$ administration attenuates the development of glomerulosclerosis and the progression of albuminuria through PTH-independent antiproliferative actions ***(Russell et al., 1998).*** 1, 25$(OH)_2D_3$-induced decreases in podocyte loss and podocyte hypertrophy may also contribute to the less

pronounced albuminuria and glomerulosclerosis ***(Kuhlmann et al., 2004).***

D- Parathyroid glands:

PTH stimulates the production of 1, 25(OH) $_2$D. In turn 1, 25$(OH)_2$ D inhibits the production of PTH ***(Cantley et al., 1985).*** The regulation occurs at the transcriptional level. Within the promoter of the PTH gene is a region that binds the VDR and mediates the suppression of the PTH promoter by 1, 25$(OH)_2$D ***(Mackey et al., 1996).***

Non Classic Actions of Vitamin D:

Genetic, nutritional, and epidemiological evidence links abnormalities in the vitamin D endocrine system with disorders unrelated to calcium homeostasis, ranging from hypertension and disturbed muscle function to susceptibility to infections, autoimmune diseases, and cancer ***(Holick, 2004).***

1-Regulation differentiation of proliferation:

Cells that are dividing rapidly are said to be proliferating. Differentiation results in the specialization of cells for specific functions. In general, differentiation of cells leads to a decrease in proliferation. While cellular proliferation is essential for growth and wound healing, uncontrolled proliferation of cells with certain mutations may lead to diseases like cancer. The active form of vitamin D, 1, 25-dihydroxyvitamin D, inhibits

proliferation and stimulates the differentiation of cells ***(Holick, 2006).***

2- Modulation of immune responses:

Vitamin D in the form of 1, 25-dihydroxyvitamin D is a potent immune system modulator. The vitamin D receptor (VDR) is expressed by most cells of the immune system, including T cells and antigen-presenting cells, such as dendritic cells and macrophages ***(Abramovits, 2009)***. Under some circumstances, macrophages also produce the 25-hydroxyvitamin D_3-1-hydroxylase enzyme that converts 25-hydroxyvitamin D to 1, 25-dihydroxyvitamin D ***(Gerritsen et al., 2001).*** There is considerable scientific evidence that 1, 25-dihydroxyvitamin D has a variety of effects on immune system function, which may enhance innate immunity and inhibit the development of autoimmunity ***(Rizova and Corroller, 2001).***

3-Control of the nervous system:

The involvement of vitamin D in the function of the central nervous system is supported by the presence of the enzyme 25(OH)D3-1a-hydroxylase, responsible for the formation of the active form of vitamin D, as well as the presence of vitamin D receptors in the brain, mainly in the hypothalamus and dopaminergic neurons of the substantia nigra ***(Eyles et al., 2005).***