Effect of Vitamin D Supplementation on Patients with Graves disease

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Abstract :

Background: Graves disease (GD) is an autoimmune disease characterized by hyperthyroidism secondary to circulating auto-antibodies. It has become apparent that multiple factors contribute to the etiology of (GD), including genetic and environmental factors.The role of vitamin D is well known in calcium metabolism and skeletal homeostasis. Vitamin D has also been shown to be a modulator in both innate and adaptive immunity **.**There is a well-established link between vitamin D deficiency and various autoimmune diseases,including type 1 diabetes mellitus,systemic lupus erythematosus, rheumatoid arthritis,inflammatory bowel disease, and multiple sclerosis.The prevalence of vitamin D deficiency was reported to be common in patients with GD**.**Interestingly,vitamin D deficiency is found to be associated with higher thyroid volume in patients with newly onset GD**.**Whether vitamin D deficiency has a causal relationship with GD remains a controversial issue.Objective:to evaluate the effect of vitamin D supplementation in patients with GD with and without opthalmopathy Subjects and methods:A randomized prospective study was conducted on 60 adult patients with Graves' disease aged (20-40) years**Group 1**: 20 patients with Graves' receiving daily dose of 30mg of methimazole alone.**Group 2**: 40 patients with Graves' receiving same dose of methimazole supplemented with vitamin D3 200.000IU/month for 3 months.Patients were followed up over (3months duration).Results: 40% of patients in group 1 and 72.5% in Group 2 were vitamin D deficient,Vitamin D was significantly correlated with thyroid volume and degree of exophthalmos .On Vitamin D supplemention,Group 2 had significant lower

thyroid volume and better effect on the degree of exopthalomos.Conclusion:Vitamin D suuplementation for GD has a favourable effect on thyroid volume and on the degree of exophthalmos

.Key words: GD, Exophthalmos, Vitamin D

INTRODUCTION

Graves'is an autoimmune disease characterized by hyperthyroidism secondary to circulating auto-antibodies. It has become apparent that multiple factors contribute to the etiology of Graves' Disease (GD), including genetic and environmental factors. The pathophysiology of GD involves the infiltration of T cells in the thyroid gland. These activated T cells in turn increase the secretion of thyroid-specific auto-antibodies from B cells ***(Alhuzaim and Aljohani, 2014).***

The role of vitamin D is well known in calcium metabolism and skeletal homeostasis. Vitamin D has also been shown to be a modulator in both innate and adaptive immunity ***(Adams and Hewison, 2008).*** There is a well-established link between vitamin D deficiency and various autoimmune diseases, including type 1 diabetes mellitus (T1DM), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and multiple sclerosis (MS). Furthermore, it has been found that the supplementation of vitamin D can prevent the onset and/or development of different kinds of autoimmune disorders in human beings and animal models ***(Baeke et al., 2010).***

The prevalence of vitamin D deficiency was reported to be common in patients with GD ***(Yamashita et al., 2001).*** Interestingly, vitamin D deficiency is found to be associated with higher thyroid volume in patients with newly onset GD

(Yasuda et al., 2012). Whether vitamin D deficiency has a causal relationship with GD remains a controversial issue. It has been recently discovered that vitamin D-receptor gene and vitamin D-binding protein gene polymorphisms are associated with GD ***(Zhou et al., 2001).***

The role of vitamin D in GD has been investigated in several studies. Misharin et al. observed that vitamin D deficiency was found to modulate Graves' hyperthyroidism induced in BALB/c mice by thyrotropin receptor immunization. In this study, BALB/c mice on a vitamin D deficient diet were more likely to develop persistent hyperthyroidism than other mice receiving adequate vitamin D supply ***(Misharin et al., 2009).*** In another study, combination treatment with methimazole and vitamin D3 (1, 25 $(OH)_2D$) in patients with GD has more rapid euthyroidism achievement compared with patients receiving methimazole alone ***(Fukawa et al., 1997).*** In addition, vitamin D supplementation has been shown to inhibit inflammatory responses in human thyroid and T cells ***(Borgogni et al., 2008).***

AIM OF THE WORK

In a previous work by ***Sheriba et al. (2016)***; vitamin D proved to be lower in GD patients than in healthy people and the degree of exophthalmos was inversely correlated with the degree of vitamin D deficiency. There was also a negative correlation between vitamin D status and FT_3 and FT_4 and a positive correlation with TSH level.

We aim to evaluate the effect of concomitant administration of vitamin D3 in treating hyperthyroidism in patients with Graves' disease.

Chapter 1
VITAMIN D PHYSIOLOGY AND STRUCTURE

Vitamin D, the fat-soluble vitamin, is natively found in little foods and obtainable as a dietary supplement. Vitamin D is synthesized endogenously when ultraviolet (UVB) rays from sunlight hit the skin ***(Institute of Medicine, 2010).***

Vitamin D enhances calcium absorption in the gut to keep sufficient level of calcium and phosphate in the serum for normal bone mineralization and prevention of hypocalcemic tetany. Vitamin D is also involved in bone growth and bone remodeling by osteoblasts and osteoclasts ***(Cranney et al., 2007).***

Vitamin D has also extra skeletal effects in the body such as; modulation of cell growth, immune function and neuromuscular and antinfamatory effects ***(Holick, 2006).*** Several genes coding for proteins that regulate cell proliferation, differentiation, and apoptosis are regulated to some extent by vitamin D ***(Institute of Medicine, 2010).***

Many forms (vitamers) of vitamin D are present (**Table 1**). The two main forms are ergocalciferol (vitamin $D_{2)}$ and cholecalciferol (vitamin D_3), vitamin D without an index means either D_2 or D_3 or both. Both are named calciferol ***(Dorland's Illustrated Medical Dictionary, 2013)***.

Table (1): Forms of vitamin D *(Dorland's Illustrated Medical Dictionary, 2013)*:

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)

Sources of vitamin D

Sunlight, supplements and diet are the major sources of vitamin D ***(Holick, 2007)***. When UVB radiation (wavelengths: 290–315 nm) hits the human skin, the 7-dehydrocholesterol is converted to previtamin D_3 in the skin by temperature- and membrane-dependent processes the previtamin D_3 is then converted to vitamin D_3 (cholecalciferol) ***(Holick et al., 1995).***

Unfortunately, vitamin D rich foods are very few involving, oily fish such as salmon, sardines and tuna, and oils of the liver of some fish such as cod besides sun-exposed mushrooms (***Holick, 2007***).

The Recommended Dietary Allowance (RDA)

The RDA for vitamin D is listed in the (**table 2**) by life stage and gender ***(Holick et al., 2011).***

Table (2): Recommended Dietary Allowance (RDA) for Vitamin D *(Holick et al., 2011):*

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

Vitamin D Bioactivation

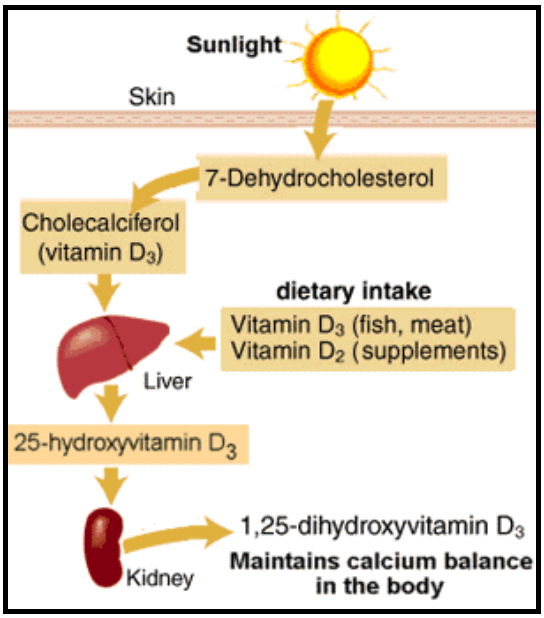

Fig. (1): Steps of vitamin D bioactivation ***(Ramos et al., 2010).***

Vitamin D_2 is available in a specific food but mainly in supplements. Vitamin D_3 is present in both food and supplements, but is mainly produced by exposed skin to UVB radiation: 7- and 8-dehydrocholesterol are changed by photolysis to pre- Vitamin D_3 and then by thermal isomerization, to Vitamin D_3 ***(Ramos et al., 2010).***

Vitamin D_3, the pro-hormone, is then changed to 25-hydroxycholecalciferol [25(OH) D] in the liver, various hepatic cytochrome P-450s have been found to 25-hydroxylate vitamin D compounds, CYP2R1 is the most important one as it was found to 25-hydroxylate both vitamin D_3 and vitamin D_2. CYP2R1 presents principally in the liver and testis ***(Motola et al., 2003)*** and alterations in the 2R1 gene have been found in a patient with low 25(OH) D levels and rickets ***(Cheng et al., 2004)***. Thus CYP2R1 seems to be the major 25-hydroxylase included in vitamin D metabolism ***(Holick et al., 1981)***.

25(OH)D is often circulating in the serum bound to vitamin D-binding protein (DBP), which is filtered by the glomerulus to be reabsorbed by the proximal tubules through megalin-mediated endocytosis ***(Ramos et al., 2010)***. In proximal tubules, 1-α-hydroxylase or (CYP27B1) activates the 25(OH) D to the 1, 25-dihydroxycholecalciferol [1, 25(OH)D] or (calcitriol), an activity highly modulated by the Ca^{+2}–phosphorus–parathyroid hormone (PTH) axis ***(Ramos et al., 2010).***

Despite the strict control of vitamin D3 activation in the renal proximal tubules, many other cell types can generate 1α hydroxylase, permitting extrarenal activation of circulating 25-OH-D3 to the active form.These involve dermal and intestinal epithelial cells, macrophages, and monocytic cell lines.whilst, kidney-generated 1, 25(OH)D acts as a hormone, the peripheral tissue-generated 1, 25(OH)D has autocrine/paracrine effects ***(Richart et al., 2007).***

Vitamin D transport

Vitamin D metabolites are characterized by being lipophilic with low aqueous solubility so in order to be transported in the circulation they must be bound to plasma proteins. Vitamin D binding protein (DBP), is the major of these carrier proteins which binds the metabolites with high affinity as in the order 25(OH)D equal to 24, $25(OH)_2D$ but more than 1, $25(OH)_2D$ which also more than vitamin D ***(Cooke and Haddad, 1989).***

DBP Plasma levels are 20 times higher than the total levels of vitamin D metabolites, and more than 99% of circulating vitamin D metabolites are protein bound, principally to DBP (85-88%) whilst albumin and lipoproteins contribute to lesser extents (12-15%). DBP-bound vitamin D metabolites have a longer circulating half-life owing to limited access to target cells and, so, less susceptibility to hepatic metabolism and subsequent biliary excretion ***(Cooke and Haddad, 1989).***

Only the minor fraction of unbound vitamin D metabolites can passively enter the target cells to be further metabolized or to exert their biological action. Studies revealed that biological action of active vitamin D correlated with the concentration of free hormone ***(Brown et al., 1993).*** Accordingly, DBP is the buffer of the free levels of active vitamin D molecules, preventing vitamin D intoxication ***(Bouillon et al., 1981).***

Liver synthesizes DBP and albumin, and these proteins may be wasted in protein losing enteropathies or the nephrotic syndrome. Therefore, patients with liver, intestinal, or renal disorders that cause low levels of transport proteins usually have low total vitamin D levels despite being vitamin D sufficient due to normal free or unbound fraction ***(Bikle et al., 1985).***

Mechanism of action

Vitamin D mediates its actions through the nuclear transcription factor, vitamin D receptor (VDR) ***(Sutton and MacDonald, 2003)***. The affinity of 1, 25(OH)D to VDR is 100-fold more than the 25(OH)D ***(Bikle et al., 1985).***

On entering the nucleus of a cell, 1, 25(OH)D binds to the VDR and enhances this bond with the retinoic acid X receptor (RXR). With the association of 1, 25(OH)D the VDR/RXR complex binds to vitamin D response elements

(VDREs) which is a small sequences of DNA and then commences a series of molecular interactions that attenuate the transcription of certain genes. More than 50 genes in tissues all through the body are modulated by 1, 25(OH)D ***(Guyton et al., 2003)***.

1, 25$(OH)_2D_3$ Metabolism

1, 25$(OH)_2D_3$ is highly potent in elevating serum calcium and phosphate levels, that is why a feedback mechanism is needed to attenuate its activity. This is achieved within mostly all target cells by vitamin D 24-hydroxylase enzyme which is induced by 1, 25$(OH)_2D_3$. Vitamin D 24-hydroxylase stimulates a cascade of oxidation reactions at carbons 24 and 23, resulting in side chain cleavage and vitamin D inactivation. Mice deficient in functional 24-hydroxylase gene have high serum 1, 25$(OH)_2D_3$ levels due to the diminished capacity to catalyze Vitamin D ***(Messerlian et al., 1997).*** 24-Hydroxylase regulation is reciprocal to 1α-hydroxylase, owing to being stimulated by phosphate ***(Tangpricha et al., 2002)*** and suppressed by PTH ***(Henry and Norman, 1984).***

Biological actions of vitamin D

Classic Vitamin D-Responsive Tissues

Intestine

Vitamin D is necessary to stimulate the small intestine to absorb dietary calcium and phosphate ***(Hoenderop et al., 2000).***

Skeleton

Vitamin D is necessary for the growth and maintenance of a mineralized bone. Vitamin D inadequacy causes rickets in growing children and osteomalacia in adults ***(Amling et al., 1998)***.

Kidney

The role of 1, $25(OH)_2D_3$ in the renal handling of calcium and phosphate is controversial due to the concurrent effects of 1, $25(OH)_2D_3$ on serum PTH and on intestinal calcium and phosphate absorption, which influences the filter load of both ions.1, $25(OH)_2D_3$ stimulates renal calcium reabsorption and calbindin expression and enhances PTH-dependent calcium transport in the distal tubule which is the main determinant of the final excretion of calcium into the urine and the site with the highest VDR content. The role of 1, $25(OH)_2D_3$ in enhancing 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).***

Ingesting 1, $25(OH)_2D_3$ as a supplement decreases the incidence of glomerulosclerosis and the progression of albuminuria via PTH-independent antiproliferative actions ***(Russell et al., 1998).*** 1, $25(OH)_2D_3$-induced decrease podocyte loss and podocyte hypertrophy are also may also contribute to the less pronounced albuminuria and glomerulosclerosis ***(Kuhlmann et al., 2004).***

Parathyroid glands

PTH enhances the production of 1, 25(OH) $_2$D which in turn hinders PTH production ***(Cantley et al., 1985).*** The regulation of this process occurs at the transcriptional level ***(Mackey et al., 1996).*** Serum Calcium level modifies the effect of 1, 25$(OH)_2$ D on PTH gene expression. Calcium strongly inhibits PTH production and secretion, probably by acting through the calcium receptor on the plasma membrane of the parathyroid cell. Low dietary calcium enhances calreticulin levels in the parathyroid gland ***(Sela et al., 1996),*** the protein calreticulin binds to nuclear hormone receptors involving VDR and hinders their activity ***(Wheeler et al., 1995).***

Nonclassic Vitamin D Actions

Epidemiological, genetic and nutritional evidence relates vitamin D endocrine system abnormalities with diseases not related to calcium homeostasis, ranging from hypertension and disturbed muscle function to vulnerability to infections, cancer and autoimmune diseases ***(Holick, 2004)***.

Modulation of immune responses

The role of vitamin D in the innate immune system

Vitamin D and anti-microbial peptides

Innate immunity acts as the first barrier of defense against the attacking microorganisms like bacteria, viruses, protozoa, and fungi ***(Trinchieri and Sher, 2007).*** The first duty of the innate immunity is to identify foreign organisms and to activate a cascade of reactions that eventually lead to the elimination and/or damage of the attacking organism ***(Trinchieri and Sher, 2007).***

The innate immune system cells are responsible for expression of Pattern recognition receptors in order to be able to recognize molecular patterns of different classes of pathogens, which called pathogen-associated molecular patterns (PAMPs) ***(Medzhitov, 2007).*** PAMPs involve flagellin, lipopolysaccharide (LPS), viral proteins and single- and double-stranded RNA. Toll-like receptors (TLRs) the sub-class of pattern recognition receptors are expressed principally on the cell membrane or on endosomes ***(Trinchieri and Sher, 2007)***.

The response of innate immunity is influenced by the specific TLR and/or combination of TLRs that are activated by PAMPs. The TLR signaling response consists of the generation of anti-microbial peptides and cytokines and apoptosis of the host cells along with other responses ***(Medzhitov, 2007)***.