Immunomodulatory Effect of 1,25-Dihydroxyvitamin D3 on Interferon Gamma (INFγ) Secretion by TH1 Lymphocyte in Normal Population in-vitro

Thesis
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Enayat Abd El Razek

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Abb.	Full term
25(OH)D	25-hydroxy vitamin D
1,25(OH)2D	1,25-dihydroxyvitamin D
APCs	Antigen presenting cells
BMI	Body mass index
CDK	Cyclin-dependent kinase
CDKI	Cyclin-dependent kinase Inhibitor
CYP	Cytochrome P450
DC	Dendritic cell
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosrbent assay
FGF-23	Fibroblast growth factor
IBD	Inflamatory bowel disease
IFNAR	Interferon alpha receptor
IFNGR1	IFN- γ receptor chain 1
IFN-γ	Gama interferon
Ig	Immunoglobulin
IGTP	Inducible GTP-binding protein
IL	Interleukin
JAK	Janus tyrosine kinases
Lfα	Lymphotoxin α
MS	Multiple sclerosis
NFκB	Nuclear factor kappa B
NK	Natural killer
NR1I1	Nuclear factor subfamily 1, group I, member1
PBMC	Peripheral blood mononuclear cells
PG	Prostaglandin
PHA	Phytohaemagglutinin
PTH	Parathyroid hormone
RA	Rhematoid arthritis
RPMI	Rosewell park memorial institute
SLE	Systemic lupus erythrromatosus.
SP	Streptavidin-Peroxidase

List of Abbreviations (Cont...)

Abb.	Full term
STAT	Signal transducer and activator of transcription
T1D	Type 1 diabetes
TCR	T-cell receptor
TGF-α	Transforming growth factor-alpha
Th	T helper
TNF	Tumor necrosis factor
TLR	Toll like receptor
Treg	Regulatory T cells
UV-B	Ultra violet
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor

Abstract

Background: Vitamin D as 1,25(OH)2D functions by binding to a nuclear vitamin D receptor VDR and retinoid X receptor to regulate gene transcription. **Aim of the Work:** The aim of this study is to assess the role of Vitamin D3 in modulating the in-vitro, production of IFNγ by Th1 lymphocytes in normal Egyptian population. **Subjects and Methods:** The study was conducted at Clinical Pathology Department, Ain Shams University Hospitals. Thirty healthy individuals were enrolled in the study. They were 23 males and 7 females with age ranges from 25 to 50 years old (29.57 mean±10.02 2SD years old). Exclusion criteria for the subjects included abnormal liver and/or kidney functions. **Result:** IFN-γ levels in culture supernatants in presence of vitamin D ranged from 0.07 to 0.64 ng/ml with 0.37 mean±0.16 2SD. This difference proved to be highly statistically significant ($p<0.001$) being lower in presence of vitamin D. **Conclusion:** a highly significant decrease in the culture supernatant IFN-γ levels in presence of vitamin D which may explain the role of vitamin D as immunomodulatory agent that can suppress Th1 cytokine production in-vivo and polarize adaptive immune system away from Th1 towards Th2 responses. **Recommendations:** Assessment of vitamin D as an immunomodulatory agent and role of vitamin D supplements in prevention and therapy of related diseases.

Key words: vitamin D, 1,25(OH)2D, lymphocytes, VDR, immunomodulatory agents

INTRODUCTION

Vitamin D refers to a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc. In humans, the most important compounds in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol) ***(Norman, 2008)***. Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. The body can also synthesize vitamin D (specifically cholecalciferol) in the skin, from cholesterol, when sun exposure is adequate (hence its nickname, the "sunshine vitamin") ***(Nair and Maseeh, 2012)***.

While it was long held that vitamin D acted only at the intestine, kidney, and skeleton, and that its function was limited to calcium homeostasis, the possibility of extraskeletal effects has been considered for decades as a result of the discovery of the Vitamin D receptor (VDR) in tissues that have no involvement in calcium homeostasis (e.g., skin, placenta, pancreas, breast, prostate and activated T cells). Discovery of the VDR in these tissues led to exploration of the roles and

mechanisms of vitamin D function in each ***(Christakos et al., 2013)***.

In recent years, research efforts were also focused on understanding the immunomodulatory properties of vitamin D3. 1,25-dihydroxyvitamin D [(1,25 (OH)2D] has been shown to influence the growth and differentiation of both the innate and acquired immune cells, as well as their functions such as cytokine production ***(Khoo et al., 2012)***. As such, there has been much interest to identify its therapeutic potential in autoimmune or inflammatory diseases ***(Pludowski et al., 2013)***.

Early studies of vitamin D and the immune system demonstrated VDR expression on both T and B cells. Notably, VDR expression by these cells was only immunologically functional in active, proliferating cells, suggesting an antiproliferative role for 1,25(OH)2D on these cells. T helper (Th) cells appear to be the principal target for 1,25(OH)2D which can suppress Th cell proliferation as well as modulating cytokines production by these cells ***(Hewison, 2012)***.

Activation of naive Th cells by antigen in turn leads to the generation of Th subgroups with distinct cytokine profiles: Th1 [interleukin (IL)-2, interferon (IFN) gamma, tumour necrosis factor (TNF) alpha] and Th2 (IL-3, IL-4, IL-5, IL-10) that

respectively support cell-mediated and humoral immunity ***(Barbara et al., 2013)***.

1,25(OH)2D suppresses Th-1 cell proliferation leading to lowered production of IFNγ and IL-2. Lower levels of circulating cytokines leads to less antigen presentation by dendritic cells, in addition to less T lymphocyte recruitment and proliferation. Expression of Th2 associated cytokines, including interleukin-4 are increased by 1,25(OH)2D. Overall, vitamin D polarizes the adaptive immune system away from Th-1 and toward Th-2 responses ***(Jeremy et al., 2011)***.

AIM OF THE WORK

The aim of this study is to asses the role of Vitamin D3 in modulating the in-vitro, production of IFNγ by Th1 lymphocytes in normal Egyptian population.

Chapter One

I. VITAMIN D

A. Introduction:

Vitamin D is a secosteroid hormone which is produced mainly in the skin on exposure to ultraviolet B radiation (UVB). Vitamin D can also be supplied by the diet or by supplements ***(Audran and Briot, 2010)***.

B. Vitamin D Metabolism:

1- Synthesis and Activation:

The two biologically relevant forms of vitamin D are ergocalciferol or vitamin D2, found in a number of plants and mushrooms, and cholecalciferol or vitamin D3 which can be ingested or produced in the skin upon exposure to UVB radiation ***(Holick, 2007)***.

Vitamin D, as either D_3 or D_2, does not have significant biological activity. Rather, it must be metabolized within the body to the hormonally-active form known as 1, 25-dihydroxycholecalciferol. This transformation occurs in two steps, as depicted in Figure (1).

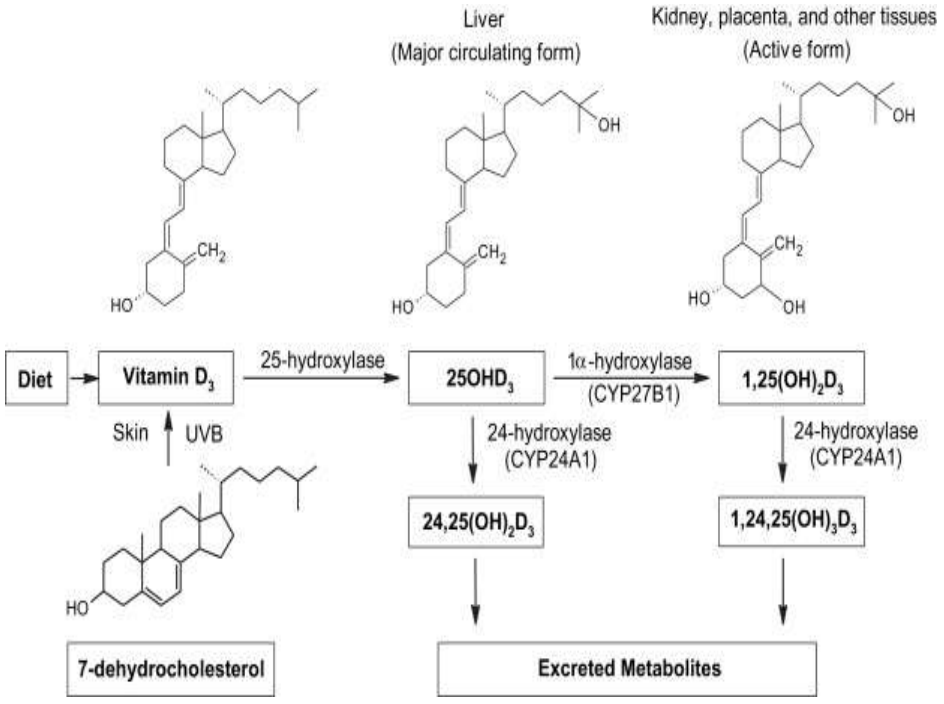

Fig. (1): Metabolism of vitamin D ***(How et al., 1994).***

In the human skin, cholecalciferol is synthesized from 7-dehydrocholesterol when exposed to UVB. Cholecalciferol is biologically inactive and immediately binds to vitamin D binding proteins (VDBP) or albumin allowing its translocation into the general circulation. In addition to cutaneous synthesis, vitamin D can be obtained from the diet in the form of vitamin D3 (cholecalciferol) or occasionally as vitamin D2 (ergocalciferol). Before entering the circulation, ingested vitamin D is absorbed and then transported in chylomicrons. Once in the circulation, it binds VDBP. Within the liver,