Immunomodulatory Effect of 1,25-Dihydroxyvitamin D3 in-vitro on Interleukin-10 Secretion by Peripheral Blood Mononuclear Cells in Normal Population

Thesis

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Nora Hafez

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List of Abbreviations

Abb.		Full term
25(OH) D	**:**	25-hydroxyvitamin D
1,25(OH)$_2$D	**:**	1, 25-dihydroxyvitamin D
1α hydroxylase	**:**	1 alfa hydroxylase
ALT	**:**	Alanine aminotransferase
ANOVA	**:**	Analysis of variance test
APCs	**:**	Antigen presenting cells
AST	**:**	Aspartate aminotransferase
BMI	**:**	Body mass index
CD	**:**	Cluster of differentiation
CYP27B1	**:**	Cytochrome P450 family 27 subfamily B member 1
DCs	**:**	Dendritic cells
DNA	**:**	Deoxyribonucleic acid
EDTA	**:**	Ethylene diamine tetra acetic acid
ELISA	**:**	Enzyme-linked immunosorbent assay
FGF-23	**:**	Fibroblast growth factor-23
IBD	**:**	Inflammatory bowel disease
IDDM	**:**	Insulin-dependent diabetes mellitus, type 1 diabetes
IFN-γ	**:**	Interferon gamma
Ig	**:**	Immunoglobulin
IL	**:**	Interleukin
IL-10 R1	**:**	Interleukin 10 receptor 1
IL-10 R2	**:**	Interleukin 10 receptor 2
JAK	**:**	Janus kinase
LPS	**:**	Lipopolysacchaide

Abb.		Full term
MHC	**:**	Major histocompatibility complex
MS	**:**	Multiple sclerosis
NK	**:**	Natural killer cells
PAMPs	**:**	Pathogen associated molecular patterns
PBMCs	**:**	Peripheral blood mononuclear cells
PBS	**:**	Phosphate buffer saline
PHA	**:**	Phytohemagglutinin
PTH	**:**	Parathormone
RPMI	**:**	Roswell Park Memorial Institute
RXR	**:**	Retinoic X receptor
SA-HRP	**:**	Streptavidin-horseradish peroxidase
SD	**:**	Standard deviation
SLE	**:**	Systemic lupus erythematosus
SPSS	**:**	Statistical package for Social Science
STAT	**:**	Signal transducer and activator of transcription
TGF- β	**:**	Transforming growth factor beta
Th	**:**	T-helper lymphocytes
TLR	**:**	Toll-like receptors
TMB	**:**	Tetramethylbenzidine
TNF- α	**:**	Tumor necrosis factor alpha
TNF- β	**:**	Tumor necrosis factor beta
T-reg	**:**	T-regulatory lymphocytes
Tyk2	**:**	Tyrosine kinase 2
UVR	**:**	Ultraviolet rays
VDR	**:**	Vitamin D receptor
VDREs	**:**	Vitamin D response elements

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Abstract

Vitamin D has two forms: the inactive form 25(OH) D which is the main circulating form that undergoes hydroxylation in the kidney under the influence of 1α-hydroxylase enzyme to yield the biologically active form 1, $25(OH)_2D$. Vitamin D plays a primary physiological role in maintaining extracellular calcium ion levels in the human body, primarily by controlling the absorption of calcium from the intestine, through direct effects on bone and also through its effects on PTH secretion.

Vitamin D has an important role in both innate and adaptive immune responses as receptors for vitamin D are present in various immune cells, including monocytes, macrophages and dendritic cells, as well as T and B lymphocytes. Vitamin D exerts an inhibitory action on the immune system. In the adaptive immune system, 1, $25(OH)_2$ D suppresses proliferation of B cells and immunoglobulin production and retards the differentiation of B cell precursors into plasma cells. It also inhibits T cell proliferation, in particular the Th1 cells capable of producing IFN-γ and IL-2. These actions prevent further antigen presentation and recruitment of T lymphocytes (role of IFN-γ), and T lymphocyte proliferation (role of IL-2). In contrast IL-4, IL-5, and IL-10 production can be increased, shifting the balance to a Th2 cell.

Methods

All subjects in the study were subjected to the following:

1. History taking laying stress on residence, occupation, sun exposure and previous intake of vitamin D or treatment.
2. Laboratory investigations:

- Assessment of Serum 25(OH) vitamin D level using enzyme immunoassay kit (Immunodiagnostic, Mainzer Landstrasse 49 60329 Frankfurt, Germany).

- Serum PTH level by Electrochemiluminescence approach using cobas e 411 analyzer (Roche Diagnostics, Germany).
- Serum calcium level, creatinine, AST and ALT using Beckman Synchron CX9 ALX Chemistry analyzer (Diamond Diagnostics, USA).
- Peripheral blood mononuclear cell separation, stimulation by Phytohemagglutin (PHA) and culture in absence and presence of vitamin D in culture ***(Jing et al., 2013, with minor modifications)***.
- IL-10 assessment in culture supernatant in absence and presence of vitamin D in culture using enzyme-linked immunosorbent assay (ELISA) kit (Wkea med supplies, 206 No.6 Building, Chenguang Gardon, Qianjian Street, Chaoyang District, Changchun 130012, China).

Results

The present study demonstrated a high prevalence of low vitamin D status among the study group (80% of our study candidates) ranged from 5 to 65 ng/ml with 21.32 mean±14.98 2SD. Moreover, it defined some risk factors for hypovitaminos D as working indoors and absence of outdoor activities (60% of our study candidates). It also showed a high significant differences in the supernatant IL-10 levels between cultures in absence and presence of vitamin D. Also, there was significant negative correlation between sufficient serum 25(OH) D levels group and IL-10 levels in culture supernatant pretreated with vitamin D. There was significant negative correlation between serum 25(OH) D and PTH levels. On contrast, it revealed no significant correlation between serum 25(OH) D levels and age, sex and serum calcium levels.

Key words: **Vitamin D**, **IL10:** interleukin 10, **DC:** denteritic cells, **ELISA:** Enzyme-linked immunosorbent assay, **T-reg:** T-regulatory lymphocytes, **VDR: vitamin D receptor .**

Introduction

Vitamin D refers to a group of fat-soluble secosteroid 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 or calcitriol) 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 extra skeletal 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, colon cells, 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-dihydroxyvitaminD3 [1,25$(OH)_2$D] 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 and inflammatory diseases as well as cancers ***(Pludowski et al., 2013)***.

Furthermore, vitamin D may also influence circulating cytokine levels and cytokine production. Within the general population it has been reported that circulating pro-inflammatory cytokine concentrations, such as Tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and Interleukin 2 (IL-2), were significantly lower in vitamin D sufficient adults compared with those who were vitamin D insufficient. Also, the circulating interleukin-10 (IL-10) concentrations were significantly higher in the vitamin D sufficient athletes ***(He et al., 2014).*** Moreover, calcitriol effectively up-regulates the synthesis of the anti-inflammatory cytokine IL-10 and induces IL-10 receptor expression in-vitro ***(Schleithoff et al., 2006).***

Aim of the work

The aim of this study is to assess the role of vitamin D3 in modulating the in-vitro production of IL-10 by peripheral blood mononuclear Cells (PBMCs) in normal population.

Vitamin D

I. Introduction

Vitamin D is a fat-soluble secosteroid that is naturally present in very few foods and also available as a dietary supplement ***(Autier et al., 2014)***. In humans, the most important compounds in this group are vitamin D_2 (ergocalciferol) and vitamin D_3 (also known as cholecalciferol) ***(Norman, 2008)*** that differ chemically only in their side-chain structure ***(Holick, 2007).*** Ergocalciferol and Cholecalciferol can be ingested from the diet and from supplements ***(Nair and Maseeh, 2012).***

II. Sources of Vitamin D

A. Food

Very few kinds of foods in nature contain vitamin D. The flesh of fatty fish (such as salmon, tuna, and mackerel) and fish liver oils are among the best sources. Small amounts of vitamin D are found in beef liver, cheese, and egg yolks. Vitamin D in these foods is primarily in the form of vitamin D_3 (cholecalceferol) ***(Ovesen et al., 2003)***. Some mushrooms provide vitamin D_2 (ergocalciferol) ***(Calvo et al., 2004).***