Vitamin D Supplementation Reduce Serum Inflammatory Biomarker (TNF-α) Levels in Male Albino Mice on High Fat Diet

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Abstract

Objective: The objective of this study was to determine whether vitamin D supplementation affects the levels of inflammatory biomarker (TNF- alpha) in mice (male albino) or not?

Material and Methods: This was a Quasi experimental study. Sample size was 90. Consecutive, nonprobability sampling technique was used. Mice were randomly divided into three groups, each group containing 30 mice. This study was carried out for 6 weeks. Normal diet was administered to mice of group A. High fat diet was administered to group B mice. High fat diet & vitamin D supplementation were given to group C mice. Vitamin D was administered to group C mice through oral gavage (100ng/kg/day) for 6 weeks. Terminal blood sampling technique was used to collect blood sample. ELISA technique was used to determine levels of TNF-α (Tumor necrosis factor alpha). SPSS version 20 was used for analysis of data.

Results: Group B mice had significantly raised serum TNF-α levels as compared to group A mice. Group C mice had significantly reduced levels of tumor necrosis factor-alpha as compared to mice of group B and mice of group A.

Conclusion: Vitamin D supplementation might be beneficial by reducing levels of serum TNF-α levels in mice on high fat diet.

Keywords: Vitamin D, High fat diet, Hyperlipidemia, TNF-α.

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Introduction

High fat diet induces inflammation throughout the body like brain (in hypothalamus by blunting insulin sensitivity and leptin pathway), liver, fat cells, intestine and skeletal muscles. HFD alters gut microbiota and decreases its diversity. It increases bacteroides and decreases fermicutes in the gut. This shift of gut microbiota activates toll like receptors (TLR) signaling pathway, so, there is increased permeability of intestinal epithelium to bacterial endotoxins like lipopolysaccharides (LPS) which are then delivered to circulation.[1] Dietary free fatty acids also activate intestinal epithelial calls. Lipopolysaccharides (LPS), free fatty acid (FFA) stimulate intestinal cells and lead to the production of TNF-α. All these substances (LPS, FFA, inflammatory cytokines) are delivered through circulation to various parts of body, thus inducing inflammation there.[1] These substances activate toll like receptors (TLR) in macrophages and convert them to M1 type macrophages, which release inflammatory cytokines like TNF-α, IL-12 and IL-6.[1] Activated macrophages reach adipose tissues, muscles, blood vessels and pancreas and induce inflammation there. Increased CD8+-T cells in adipose

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tissues lead to macrophages accumulation there, so adipose tissue don't store lipids because of HFD induced stress rather lipids are accumulated in the peripheral tissues. This ectopic accumulation of lipids leads to increased expression of inflammatory mediators and macrophage recruitment there thus further promoting inflammation.[1] T cells ($CD4^+$ and $CD8^+$) are abundant in obese adipose tissues. $CD4^+$T (cluster of differentiation 4^+T cells) cells are destined to become Th1 and Th17 cells which are proinflammatory.[2] Adipose tissue Th2 cells attenuate inflammation though they are less in number.[3] New studies have identified a hematopoietic stem cell (HSC) derived cell called circulating fibroblast precursors (CFP) which have increased expression of CD45 and discoidin domain receptor 2 (DDR2). DDR2 has a role in the activation of immune cells. $CD45^+$ $DDR2^+$ cells reside inflammatory cells like adipocytes. They elicit increased production of inflammatory cytokines like interferon gamma (INF-γ), IL-6 and TNF-α by $CD4^+$ T cells.[2]

Expansion of adipose tissue results in hypoxia due to increased distance between adipose tissue and blood vessels. This hypoxia induces the expression of a factor (HIF-1, hypoxia inducible factor-1). HIF-1 is responsible for negative effects of obesity. Hypoxia leads to cell death and fibrosis. Macrophages infiltrate to eat up dead cells. There is abundance of M1 type macrophages which lead to production of TNF-α and IL-6.[3] Vitamin D binds with vitamin D receptors in nucleus. Vitamin D and VDR bind with retinoid X receptors. This whole complex induces or suppresses the activation of various genes on DNA.4 Vitamin D has an immune modulatory & anti-inflammatory role too[5,6]. Many immune cells express vitamin D receptors (VDR). Vitamin D increases regulatory T cell production and polarizes the differentiation of T-helper cells into type-2 helper cells rather than Th1 and Th17 types.7 Helper-2 cells have anti-inflammatory properties while helper-1 cells are pro-inflammatory. Dendritic cells modify antigen and present it to T cells. T lymphocytes become either inflammatory or anti- inflammatory cells depending upon the cytokines secreted by dendritic cells. Vitamin D converts dendritic cells (DC) to tolerant dendritic cells which produce anti-inflammatory cytokines (IL-10) rather than inflammatory ones (TNF-α, INF-γ). Metabolic reprogramming by switching towards glycolysis and PI3K/Akt/mTOR pathway (phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin) are necessary for the production of tolerant dendritic cells. Indole amine 2,3 dioxygenase (IDO) induction on dendritic cells is also necessary for the production of tolerant dendritic cells. All these mechanisms are regulated by vitamin D.[8] Vitamin D promotes polarization of macrophages to M2 phenotype (anti-inflammatory phenotype which produces IL-10) rather than M1 phenotype. These M1 type macrophages release inflammatory cytokines like IL-6, IL-12, TNF-α and differentiate Th cells to Th1 and Th17 subtypes. In macrophages, nuclear factor kappa B (NFκB) up regulates miR-155 which inhibits suppressors of cytokine signaling (SOCS) proteins to induce inflammation. Vitamin D activates thioestrase superfamily member 4 gene (THEM4) and results in the inhibition of NF-κB signaling pathway to activate suppressor of cytokine signaling (SOCS) and reduces inflammation by decreasing the production of various inflammatory cytokines.[8] (Fig-1) Vitamin D is required for keeping CD8+ T cells quiescent. Thus, decreased activation of CD8+ T lymphocytes leads to decreased production of inflammatory cytokines (TNF-α and INF-γ).[8] (Fig-2)

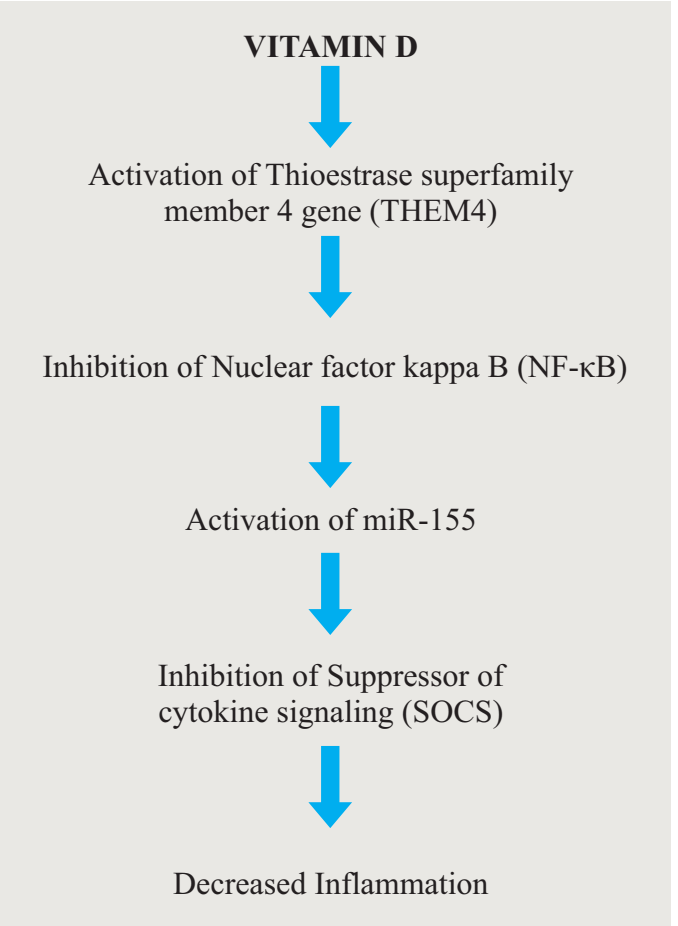

Figure 1: *Anti- inflammatory role of vitamin D*[8]

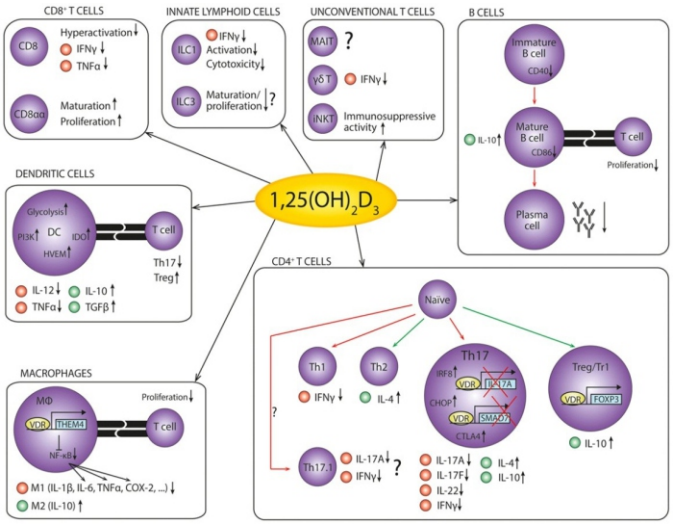

Fig-2: *Immune modulation by vitamin D*[8]

- *red dots are for down regulation*
- *green dots are for up regulation*

Material and Methods

It was a Quasi experimental study conducted at Department of Physiology Akhtar Saeed Medical and Dental College (AMDC), Lahore from February 2021 to November 2021. After taking approval from Ethical Committee IRB No 207 dated 22-11-2017. Non probability consecutive sampling technique was used. University of Veterinary and Animal Sciences, Lahore provided 90 male albino mice. Mice were 8-10 weeks of age. One week was given for acclimatization. Lottery method was used to divide mice into 3 groups randomly.

Group A (Normal diet group, n=30) mice were raised on normal diet for 6 weeks. (11% kcal from fat)

Group B (High fat diet group, n=30) Mice were administered high fat diet9 for a duration of 6 weeks. (44% kcal from fat)

Group C (high fat diet plus vitamin D group, n=30) mice were administered high fat diet and vitamin D for a duration of 6 weeks.

100ng/kg/day[10,11] was the dose of vitamin D. It was administered through oral gavage tube for 6 weeks. Weight was recorded once every week to calculate the weekly dose of vitamin D.

Bood samples were collected by terminal cardiac puncture method. Blood samples were centrifuged to separate the serum and were kept at -20°C until ELISA technique was applied.

SPSS version 20 was used for analysis of data. Mean values and standard deviations were recorded. One way ANOVA was used to compare the results of three groups. Post hoc Tukey's test was used to see the significance of difference amongst three groups.

Results were scribed as mean ± SD.

p value ≤ 0.005 was taken significant.

p value ≤ 0.001 was taken highly significant.

Results

Group B mice had significantly raised serum TNF-α levels as compared to group A mice. Group C mice had a significant reduction in the levels of tumor necrosis factor alpha as compared to group B and group A mice. (Table-1&2)

Table 1: *Serum TNF-α levels of normal diet group (A), high fat diet group (B) and high fat diet plus vitamin D group(C)-One way ANOVA*

Parameter	Group A (normal diet) (number=30)	Group B (high fat diet) (number=30)	Group C (high fat diet +vitamin D) (number=30)	p value
Serum TNF _α (pg/ml)	23.55±2.39	46.11±7.45	18.14±4.84	0.000 **

*** p value ≤ 0.001 was taken highly significant.*

Table 2: *Serum TNF-α levels among normal diet group (A), high fat diet group (B) and high fat diet plus vitamin group (C)- Post hoc Tukey's test*

Parameter	Comparison of the groups		p value
Serum tumor necrosis factor alpha (pg/ml)	A	B	0.000**
	B	C	0.000**
	C	A	0.000**

*** p value ≤0.001 was taken highly significant.*

Discussion

Vitamin D has anti-inflammatory effect. In one study, vitamin D reduced cardiac hypertrophy by decreasing TNF-α expression in rats' heart and blocking NF-KB/P65 signalling pathway.[12]

El-Boshy et al[13] showed that vitamin D reduced inflammation throughout the body and it had anti oxidative effects too in para-cetamol toxicity in rats. They showed that paracetamol toxicity caused markedly elevated levels of hepatorenal markers and increased level of inflammatory biomarkers including TNF-α, whereas anti-inflammatory and anti-oxidative biomarkers were reduced significantly. Vitamin D, when taken prophylactically or therapeutically reduced inflammation. Results were more marked in prophylactic vitamin D

group than therapeutic vitamin D group. In our study, we used high fat diet model to induce inflammation and vitamin D was used to reduce fat induced inflammation. High fat diet elevated TNF-α levels while vitamin D reduced it.

In another study[14], macrophages were treated with vitamin D and then exposed to lipopolysaccharide (LPS) challenge. RNA expression of VDR, tumor necrosis factor-alpha and another nuclear factor (NF-κB) in macrophages was reduced in vitamin D treated unstimulated cells as well as vitamin D treated LPS stimulated cells. TNF-α production was also reduced in both groups. This study showed that vitamin D had anti-inflammatory action both in normal and pro-inflammatory conditions by reducing TNF-α production by macrophages and decreased expression of signaling proteins.[14]

Dadaei et al[15] showed that vitamin D supplementation in inflammatory bowel disease (IBD) patients for 12 weeks reduced serum TNF-α levels but results were not significant statistically. Oliveira Brito et al[16] demonstrated that vitamin D had a beneficial effect in reducing inflammation induced by monocytes in chronic kidney disease patients. They incubated monocytes with or without vitamin D for 24 hours, then monocytes were treated with serum of healthy or uremic patients. In uremic monocyte pool treated with vitamin D, expression of toll like receptor (TLR4), monocyte chemo attractant protein (MCP-1) and cathelicidin protein levels were decreased. Uremic pool had increased levels of TNF-α and other inflammatory cytokines but vitamin D in the presence of uremia did not affect TNF-α levels. There was no difference in the activity of nuclear factor kappa B (NF-κB) in both groups. Vitamin D decreased TLR4 expression but it had no effect on tumor necrosis factor-alpha levels or NF-κB levels probably due to a very short duration of incubation period (24 hours). In another study, WD obese rats had increased weight, BMI and high levels of TNF- α and leptin. Vitamin D supplementation reduced body weight, BMI, TNF- α and leptin levels.[17]

Conclusion

Vitamin D supplementation may prove beneficial by lowering levels of inflammatory cytokine TNF-α in male albino mice on high fat diet.

Conflict of Interest *None*

Funding Source *None*

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Authors Contribution

CN: Conceptualization of Project
CN: Data Collection
MS: Literature Search
MN: Statistical Analysis
NN: Drafting, Revision
ZH: Writing of Manuscript