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Research Article

# Effect of Vitamin D Supplementation on Serum Cytokines Expression in Lymphatic Filariasis Patients with Type 2 Diabetes Mellitus: A Pilot Study

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## A B S T R A C T

*Introduction:* Introduction: More than 100 million population worldwide are infected with one of the three lymph-dwelling filariae (Wuchereria bancrofti, Brugia malayi or B. timori), the major causative agents of lymphatic filariasis (LF). The disease burden from LF is concentrated in tropical and sub-tropical countries (such as India) where the prevalence of type 2 diabetes (T2DM) is the greatest. Uncontrolled T2DM manifests as chronic low-grade inflammation and leads to alteration of the functioning of the innate immune system. Active vitamin D hormone promotes innate immune response in antigen-presenting cells.

*Objective:* Our aim was to evaluate circulating levels of TNF-α and IL-6 in LF patients with T2DM before and after vitamin D supplementation thereby unveiling the possible role of vitamin D in regulation of TNF-α and IL-6.

*Methodology:* 31 LF patients with T2DM were enrolled in the study. Clinical data and demographic details were recorded. Each patient was supplemented with a 300000 IU dose of vitamin D per oral. Serum concentrations of circulating 25 (OH) vitamin D and cytokines (TNF-α, IL-6) at day 0 and at day 30 were estimated by enzyme-linked immunosorbent assay (ELISA) method.

*Results:* A significant increase in serum levels of 25(OH) vitamin D was noticed post-supplementation of vitamin D. Counts of total leukocytes, neutrophils and lymphocytes were reduced. Levels of circulating serum cytokines after vitamin D supplementation were significantly reduced.

*Conclusion:* We observed a favourable effect of vitamin D on the inflammatory response to LF infection with T2DM.

**Keywords:** Lymphatic Filariasis, Type 2 Diabetes Mellitus, Vitamin D, IL-6, TNF-α

## Introduction

Lymphatic filariasis commonly known as elephantiasis is a neglected tropical disease. 863 million people in 47 countries worldwide remain threatened by lymphatic filariasis (LF) and require preventive chemotherapy to stop the spread of this parasitic infection.[1] It is caused by infection with parasites classified as nematodes of the family Filariodidea. These thread-like filarial worms are of three types: *Wuchereria bancrofti*, (responsible for 90% of the cases), *Brugia malayi* and *Brugia timori*. Adult worms nest in the lymphatic vessels and disorder the normal function of the lymphatic system. Lymphatic filariasis is transmitted by different types of mosquitoes, for example by the *Culex* mosquito (mainly spread in urban and semi-urban areas), Anopheles (mainly found in rural areas) and *Aedes*, mainly in endemic islands in the Pacific.

Diabetes is a chronic, metabolic disease attributed to elevated levels of blood glucose that may lead to serious damage to the heart, blood vessels, eyes, kidneys and nerves. It is manifested either by insufficient insulin production by the pancreas or the inability of the body to effectively use the produced insulin. In comparison to high-income countries, the prevalence of diabetes has been rising more rapidly in low- and middle-income countries. Worldwide, about 422 million people have diabetes.[2] It is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation.[2] There was a 3% increase in diabetes mortality rates by age between 2000 and 2019.[2]

Type 2 diabetes mellitus (T2DM), when poorly controlled, attributes chronic low-grade inflammation and alters the innate immune system.[3] LF is concentrated in tropical and sub-tropical countries (such as India) where the prevalence of type 2 diabetes (T2DM) is greatest.[4] Filarial parasites induce immunoregulatory effects on the host immune system with both parasite-antigen-specific and more generalised levels of immune modulation. The main mechanisms of immune evasion are immunosuppression, immunological tolerance and modification of stereotypical Th2 responses.[5]

Tumour necrosis factor alpha (TNF-α) is secreted by macrophages and adipocytes, involved in systemic inflammation and stimulation of the acute phase reaction, and plays a role in the peripheral tissue (primarily muscle) resistance to insulin in patients with T2DM. Interleukin-6 (IL-6) is a pleiotropic cytokine, produced by adipocytes, fibroblasts, endothelial cells, and activated leukocytes and monocytes. An elevated level of IL-6 is associated with an increased risk of T2DM.

Despite its pivotal role in calcium and bone metabolism, the active vitamin D hormone, 1, 25-D stimulates the innate immune response in antigen-presenting cells. We aimed to evaluate circulating levels of TNF-α and IL-6 in LF patients with T2DM before and after vitamin D supplementation thereby unveiling the possible role of vitamin D in regulating the expression and concentrations of TNF-α and IL-6.

## Materials and Methods

### Patients

Newly diagnosed patients with lymphatic filariasis with a history of T2DM attending the outdoor patient clinic at our institute were enrolled. A total of 31 patients were enrolled. All enrolled patients were supplemented with a 300000 IU dose of vitamin D per oral at the enrollment (day 0, baseline). Detailed clinical history including age, sex, grade of LF, duration of T2DM, and concomitant and anti-diabetic medications was recorded. Guidelines from the National Centre for Vector Borne Diseases Control for the classification of different grades of LF were employed for grading LF. Patients taking immune suppressants and calcium supplements, having nephrological issues were excluded from the study. Written informed consent was obtained from each participant.

### Sample Collection

Blood samples of patients of both groups at day 0 (baseline) and at day 30 (follow-up) were collected into a plain vial and into an anticoagulant EDTA vial. Coagulated blood was centrifuged at 1800g for 10 minutes, serum was separated and aliquots of 300 µl were stored frozen at -80°C until analysis. Blood samples with EDTA were used for estimating glycosylated haemoglobin (HbA1c) using the DS5 system (Drew Scientific Inc. Dallas, Texas) based on ion exchange chromatography.

### Serum 25 (OH) Vitamin D and cytokine estimation

The serum concentration of 25 (OH) vitamin D was estimated by ELISA (enzyme-linked immunosorbent assay) using commercial kits (Sigma-Aldrich) as per the manufacturer's instructions. Serum concentrations of cytokines IL-6 and TNF-α were estimated by ELISA (enzyme-linked immunosorbent assay) using commercial kits (BD Biosciences, USA) as per the manufacturer's instructions.

### Statistical analysis

Data were mentioned as mean (± standard deviation) unless otherwise indicated. Statistical analysis was conducted by using SPSS 16.0 (SPSS, Chicago, USA). Parametric and non-parametric tests were used as per applicability. Independent t-test was used for the comparison of means of different variables at baseline and follow-up (before and after vitamin D supplementation) (Tables 1 and 2). Z-test was used to compare the mean values (± SD) of serum cytokines, vitamin D and other biochemical parameters at baseline and follow-up levels.

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**Table 1.Biochemical Characteristics of Lymphatic Filariasis Patients with T2DM: Baseline and Follow-up Study After One Month Post Vitamin D Supplementation**

| Biochemical Characteristics | Baseline (Day 0) | Follow-up (Day 30) | p Value |
|---|---|---|---|
| N (no. of subjects) | 31 | 31 | - |
| Age (years) | 47.41 ± 5.17 | 47.51 ± 5.39 | NS |
| Sex (male/ female) | 20/11 | 20/11 | - |
| BMI ($kg/m^2$) | 20.14 ± 2.37 | 20.07 ± 2.91 | NS |
| Duration of T2DM (years) | 2.5 ± 1.63 | 2.6 ± 1.74 | NS |
| Blood sugar (fasting) (mg/dl) | 202.19 ± 18.43 | 88.84 ± 29.43 | < 0.0001 |
| Blood sugar (post-prondial) (mg/dl) | 305.19 ± 16.13 | 137.52 ± 31.69 | 0.0006 |
| Hb $A_{1c}$ (%) | 9.6 ± 2.3 | 9.6 ± 2.1 | NS |
| Blood urea (mg/dl) | 44.31 ± 20.56 | 42.11 ± 19.17 | NS |
| Serum creatinine (mg/dl) | 1.44 ± 0.71 | 1.17 ± 0.53 | NS |
| Total leukocyte count | 6300.45 ± 1767 | 5608.28 ± 2708 | NS |
| Differential leukocyte count | $N_{71.12 \pm 19.76}$ $L_{15.34 \pm 7.31}$ | $N_{68.67 \pm 17.32}$ $L_{13.18 \pm 9.94}$ | NS |
| 25OH vitamin $D_3$ (ng/dl) | 14.31 ± 7.26 | 43.97 ± 63.14 | < 0.0001 |

*BMI: Body mass index, T2DM: Type 2 diabetes mellitus, $HbA_{1c}$: Glycosylated haemoglobin, NS: Non-significant.

**Table 2.Mean Levels of Circulating Inflammatory Cytokines in Lymphatic Filariasis Patients with T2DM at Baseline and One-month Post Vitamin D Supplementation**

| Immunological Characteristics | Baseline (Day 0) | Follow-up (Day 30) | p Value |
|---|---|---|---|
| TNF-α (pg/ml) | 216.46 ± 54.31 | 144.42 ± 29.18 | 0.0001 |
| IL-6 (pg/ml) | 133.7 ± 9.1 | 95.8 ± 17.3 | 0.0018 |

*TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6.

## Results

Age, sex, body mass index (BMI), grade of LF, duration of T2DM, fasting and post prondial blood sugar, glycated haemoglobin (HbA1c), blood urea, serum creatinine, total leukocyte count (TLC), differential leukocyte count (DLC) and serum 25-OH vitamin D levels at baseline (day 0) and post vitamin D supplementation (day 30) have been summarised in Table 1. All enrolled patients had Grade 1 of lymphatic filariasis as per national guidelines (mild lymphoedema, characterised by reversible swelling that subsided with limb elevation, pitting was present).

Mean levels of circulating serum inflammatory cytokines TNF-α and IL-6 at baseline and one month after vitamin D supplementation have been summarised in Table 2.

No significant difference was noticed in the mean (± SD) values of age, BMI, duration of T2DM, HbA1c, serum creatinine and blood urea between baseline and follow-up (one month after vitamin D supplementation. Mean (± SD) values of fasting blood sugar and post-prandial blood sugar levels at baseline and follow-up were found to be significantly different ($p < 0.05$).

Post vitamin D supplementation, a significant increase in serum levels of 25(OH) vitamin D was noticed (baseline value was 14.31 ± 7.26 ng/dl, follow-up value was 43.97 ± 63.14 ng/dl; p value < 0.0001).

Counts of total leukocytes, neutrophils and lymphocytes were reduced post vitamin D supplementation. Baseline and follow-up values of IL-6 were 133.7 ± 9.1 pg/ml and 95.8 ± 17.3 pg/ml (p value 0.0018). Baseline and follow-up values of TNF-α were 216.46 ± 54.31 pg/ml and 144.42 ± 29.18 pg/ml (p value 0.0001). Thus, a significant reduction in levels of circulating serum cytokines IL-6 and TNF-α after vitamin D supplementation was found.

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## Discussion

Vitamin D receptors are expressed on immune cells like macrophages, T lymphocytes, dendritic cells and monocytes.[6] Interaction of Vitamin D with vitamin D response elements in the promoter region of cytokine genes interferes with nuclear transcription factors implicated in cytokine generation and action.[7] It supports our finding that is, the expression of cytokines IL-6 and TNF-α was downregulated after vitamin D supplementation was given to the patients as it was evident from a significant reduction in serum concentrations of these cytokines post-supplementation in the present study. Filarial antigen triggers innate immune responses which ultimately lead to the activation of vascular endothelial growth factors (VEGF), thus promoting lymph vessel hyperplasia as a first step to lymphedema development. Elevated levels of lymphangiogenic factors are associated with the severity of lymphatic pathology.[8] Inflammatory cytokines, such as TNF-α and IL-6 in human patients are known to augment the expression of VEGF-C/ VEGFR3, presumably by the lymphatic endothelial cells of host lymphatics.[9] Thus, we propose the possible role of vitamin D in controlling the innate immune responses of LF patients by actively monitoring the expression of inflammatory cytokines in the host body.

Vitamin D also up-regulates the expression of calbindin, a cytosolic calcium-binding protein found in pancreatic β-cells.[10] Vitamin D deficiency is known to be related to insulin secretion, insulin resistance, and β-cell dysfunction in the pancreas.[11] It is evident from previous studies that vitamin D deficiency inhibits the secretion of pancreatic insulin in the diabetic animal model.[12] Further, studies have established that administration of vitamin D restores glucose-stimulated insulin secretion and promotes β-cell survival by modulating the generation and effects of cytokines.[13] Our study also supports the therapeutic role of vitamin D in controlling T2DM.

## Conclusion

Severe vitamin D deficiency is known to be associated with elevated inflammatory cytokine concentration in T2DM patients.[14] A significant reduction in circulating serum cytokines concentrations in LF patients with T2DM was noticed after vitamin D supplementation suggesting a favourable effect of vitamin D on the inflammatory response to LF infection with T2DM.

There was a reduction in counts of total leukocytes and polymorphonucleocytes from baseline to follow-up (post vitamin D supplementation) indicating a possible role of vitamin D supplementation over containment of systemic infection. We suggest the need for extensive research to unveil the therapeutic and immunomodulatory effect of vitamin D molecules in lymphatic filariasis.

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**Conflict of Interest:** None

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