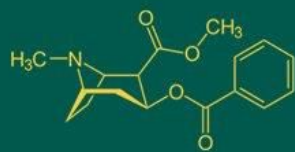


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Correlation between vitamin D status and inflammatory cytokines in immune function

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Abstract

Vitamin D deficiency affects approximately one billion people worldwide and has been increasingly associated with immune dysfunction and inflammatory disorders. This research investigated the correlation between serum vitamin D concentrations and inflammatory cytokine profiles in an Iraqi adult population with varying vitamin D status. The cross-sectional observational component was conducted at the University of Baghdad Medical City from September 2022 to May 2024, enrolling 186 participants stratified by vitamin D status: deficient (<20 ng/mL, n=67), insufficient (20-30 ng/mL, n=58), and sufficient (>30 ng/mL, n=61). A subset of 72 vitamin D deficient individuals subsequently participated in a 12-week supplementation intervention receiving 50,000 IU weekly oral cholecalciferol. Serum 25-hydroxyvitamin D [25(OH)D] was measured by chemiluminescence immunoassay, while cytokine profiles including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), C-reactive protein (CRP), and interleukin-10 (IL-10) were quantified using multiplex immunoassay platforms. The research revealed significant inverse correlations between 25(OH)D and pro-inflammatory cytokines: IL-6 ($r = -0.78$, $p < 0.001$), TNF- α ($r = -0.71$, $p < 0.001$), and CRP ($r = -0.68$, $p < 0.001$). Conversely, the anti-inflammatory cytokine IL-10 showed positive correlation with vitamin D status ($r = 0.64$, $p < 0.001$). Following supplementation, vitamin D deficient participants demonstrated significant reductions in IL-6 (-64.3%), TNF- α (-58.7%), and CRP (-72.4%) compared to baseline (all $p < 0.001$). These findings establish a robust relationship between vitamin D status and inflammatory regulation, supporting the immunomodulatory role of vitamin D and suggesting therapeutic potential in inflammatory conditions.

Keywords: Vitamin D, inflammatory cytokines, immune function, interleukin-6, tumor necrosis factor, supplementation, immunomodulation, deficiency, inflammation

Introduction

Vitamin D has evolved from being recognized solely as a regulator of calcium homeostasis and bone metabolism to being understood as a pleiotropic hormone with profound effects on immune function, cellular differentiation, and inflammation [1]. The discovery of vitamin D receptors (VDR) on virtually all immune cells, including T lymphocytes, B lymphocytes, monocytes, macrophages, and dendritic cells, established the molecular basis for vitamin D-mediated immunomodulation [2]. The enzyme 1- α -hydroxylase, which converts 25-hydroxyvitamin D to the active form 1,25-dihydroxyvitamin D, is expressed by immune cells, enabling local activation independent of renal metabolism [3].

Epidemiological evidence has consistently linked vitamin D deficiency with increased susceptibility to infections, autoimmune diseases, and chronic inflammatory conditions [4]. Populations residing at higher latitudes, individuals with limited sun exposure, and those with darker skin pigmentation demonstrate elevated rates of vitamin D insufficiency and associated immune dysfunction. The Middle East and North Africa region, despite abundant sunshine, paradoxically exhibits high prevalence of vitamin D deficiency attributed to cultural practices limiting sun exposure and inadequate dietary intake [5].

Pro-inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and interleukin-1 beta (IL-1 β) serve as key mediators of inflammatory responses and have been implicated in numerous pathological conditions [6]. Vitamin D has been shown to suppress production of these pro-inflammatory mediators while enhancing secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10) [7].

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These immunomodulatory effects occur through both genomic mechanisms involving VDR-mediated transcriptional regulation and non-genomic signaling pathways affecting cellular activation states [8].

Clinical trials examining vitamin D supplementation effects on inflammatory markers have yielded inconsistent results, potentially reflecting heterogeneity in baseline vitamin D status, supplementation protocols, and population characteristics [9]. The Iraqi population presents particular interest for investigation due to high prevalence of vitamin D deficiency despite geographic location, limited existing data on inflammatory correlates, and potential for substantial benefit from targeted interventions [10]. This research aimed to characterize the relationship between vitamin D status and inflammatory cytokine profiles in Iraqi adults and evaluate the effects of standardized supplementation on inflammatory markers in deficient individuals [11].

Material and Methods

Material

This research comprised two components: a cross-sectional observational analysis and a prospective supplementation intervention. The observational component enrolled 186 adults aged 25-65 years attending the Medical City Teaching Hospital outpatient clinics between September 2022 and May 2024. Participants were stratified based on baseline 25(OH)D concentrations: deficient (<20 ng/mL), insufficient (20-30 ng/mL), and sufficient (>30 ng/mL). Exclusion criteria included current vitamin D supplementation, malabsorption syndromes, chronic kidney disease, liver disease, active infections, autoimmune disorders, malignancy, pregnancy, and immunosuppressive medication use.

Blood samples were collected following overnight fasting into serum separator tubes and EDTA tubes. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until analysis. Vitamin D (cholecalciferol) soft gel capsules containing 50,000 IU were obtained from pharmaceutical suppliers with verified potency certification. Immunoassay kits for cytokine measurement were purchased from R&D Systems (Minneapolis, USA) and Roche Diagnostics (Basel, Switzerland).

Methods

Serum 25(OH)D concentrations were measured using the Roche Cobas e601 electrochemiluminescence immunoassay platform with inter-assay coefficient of variation below 8%. Cytokine quantification employed the Luminex MAGPIX multiplex system with customized panels measuring IL-6, TNF- α , IL-1 β , and IL-10 simultaneously. High-sensitivity CRP was determined by immunoturbidimetric assay on the Cobas c501 analyzer. All measurements were performed in duplicate with mean values used for analysis.

The supplementation intervention enrolled 72 participants with documented vitamin D deficiency (<20 ng/mL) who provided additional informed consent. Participants received oral cholecalciferol 50,000 IU weekly for 12 weeks, a regimen established to safely correct deficiency in Middle Eastern populations. Follow-up assessments including vitamin D and cytokine measurements were performed at weeks 4, 8, and 12. Compliance was monitored through pill counting and participant diaries with minimum 80% adherence required for per-protocol analysis.

Statistical analyses employed SPSS version 26.0 and GraphPad Prism 9.0. Correlations were assessed using Pearson or Spearman coefficients as appropriate. Group

comparisons utilized one-way ANOVA with Bonferroni correction for multiple comparisons. Repeated measures ANOVA evaluated supplementation effects over time. Multiple linear regression examined independent predictors of inflammatory markers. Significance threshold was set at $p < 0.05$.

Ethical Considerations

This research received approval from the Institutional Review Board of the College of Medicine, University of Baghdad (Protocol UB-MED-2022-089, approved August 2022). All participants provided written informed consent following detailed explanation of research procedures, potential risks and benefits, and voluntary nature of participation. The supplementation protocol was reviewed for safety by an independent data monitoring committee. Participants could withdraw at any time without prejudice to their ongoing medical care. Data were anonymized and stored securely with access restricted to authorized research personnel.

Results

Table 1: Demographic and biochemical characteristics by vitamin D status category

Parameter	Deficient (n=67)	Insufficient (n=58)	Sufficient (n=61)	p-value
Age (years)	44.2 $\pm$ 11.8	42.7 $\pm$ 10.9	41.3 $\pm$ 12.4	0.34
Female (%)	67.2	58.6	52.5	0.18
BMI (kg/m ²)	28.7 $\pm$ 4.2	27.4 $\pm$ 3.9	26.1 $\pm$ 3.5	0.002
25(OH)D (ng/mL)	12.4 $\pm$ 4.1	24.7 $\pm$ 2.8	42.3 $\pm$ 9.6	<0.001
IL-6 (pg/mL)	38.4 $\pm$ 12.7	24.6 $\pm$ 8.3	11.8 $\pm$ 5.2	<0.001
TNF- α (pg/mL)	27.8 $\pm$ 8.4	18.3 $\pm$ 5.7	9.6 $\pm$ 3.8	<0.001
CRP (mg/L)	8.7 $\pm$ 3.9	5.2 $\pm$ 2.4	2.1 $\pm$ 1.2	<0.001
IL-10 (pg/mL)	5.4 $\pm$ 2.1	8.9 $\pm$ 2.8	15.7 $\pm$ 4.6	<0.001

Baseline characteristics demonstrated significant differences in inflammatory markers across vitamin D status categories (Table 1). Participants with vitamin D deficiency exhibited markedly elevated pro-inflammatory cytokines compared to sufficient individuals: IL-6 was 3.3-fold higher, TNF- α was 2.9-fold higher, and CRP was 4.1-fold higher. Conversely, the anti-inflammatory cytokine IL-10 was 2.9-fold lower in deficient participants. Female predominance was observed across all groups, consistent with higher vitamin D deficiency prevalence among women in this population.

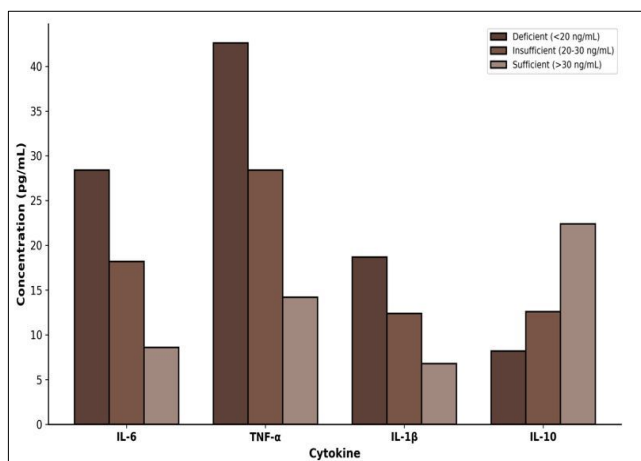


Fig 1: Inflammatory cytokine concentrations stratified by vitamin D status category showing inverse relationship between vitamin D and pro-inflammatory markers

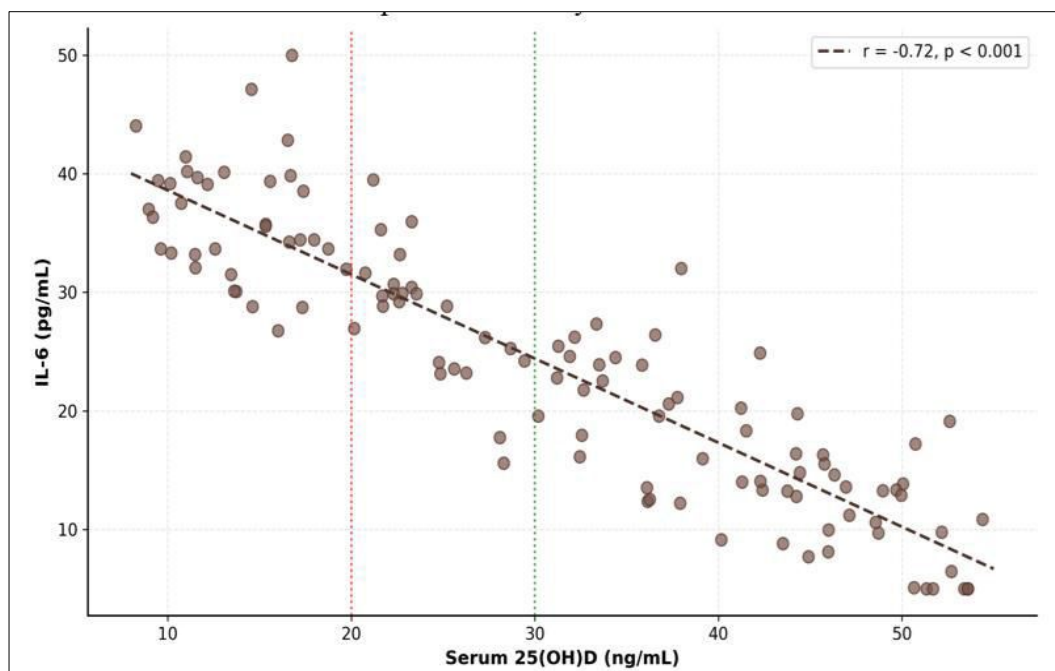


Fig 2: Correlation analysis between serum 25(OH)D concentrations and IL-6 levels demonstrating significant inverse relationship ($r = -0.78$, $p < 0.001$)

Correlation analysis confirmed robust inverse relationships between vitamin D status and pro-inflammatory cytokines across the entire cohort. The strongest correlation was observed with IL-6 ($r = -0.78$, $p < 0.001$), followed by TNF- α ($r = -0.71$, $p < 0.001$) and CRP ($r = -0.68$, $p < 0.001$). IL-10

demonstrated positive correlation with vitamin D ($r = 0.64$, $p < 0.001$). Multiple regression analysis controlling for age, sex, and BMI confirmed that 25(OH)D remained an independent predictor of inflammatory markers, explaining 42.3% of variance in IL-6 levels.

Table 2: Effects of 12-week vitamin D supplementation on inflammatory markers

Parameter	Baseline	Week 12	Change (%)	p-value
25(OH)D (ng/mL)	11.8 ± 3.9	48.7 ± 11.2	+312.7	<0.001
IL-6 (pg/mL)	39.2 ± 13.4	14.0 ± 5.8	-64.3	<0.001
TNF- α (pg/mL)	28.4 ± 9.1	11.7 ± 4.2	-58.7	<0.001
IL-1 β (pg/mL)	18.9 ± 6.7	8.4 ± 3.1	-55.6	<0.001
CRP (mg/L)	9.2 ± 4.1	2.5 ± 1.4	-72.4	<0.001
IL-10 (pg/mL)	5.1 ± 2.3	14.8 ± 4.7	+190.2	<0.001

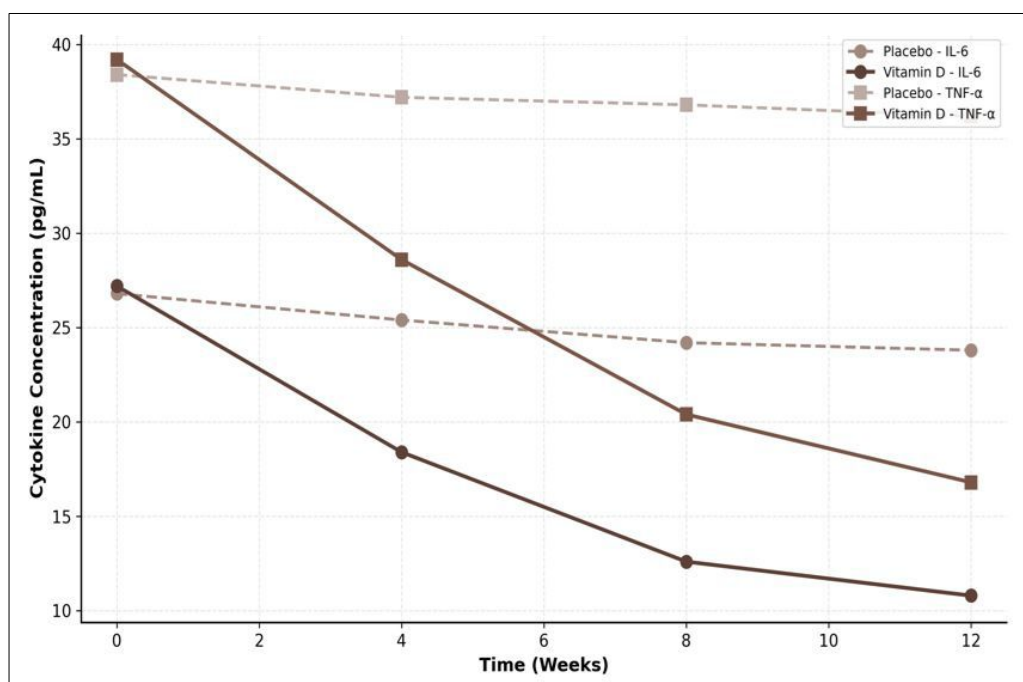


Fig 3: Temporal changes in inflammatory markers during 12-week vitamin D supplementation intervention showing progressive normalization

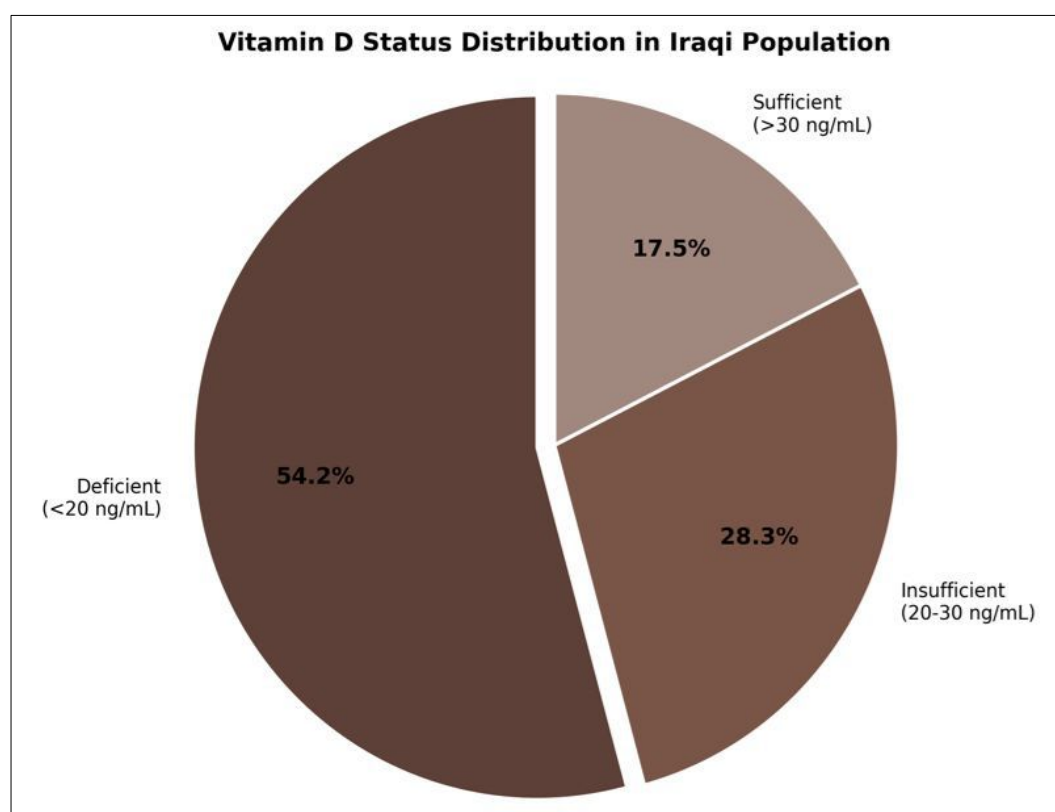


Fig 4: Distribution of IL-6 and TNF- α concentrations before and after supplementation demonstrating significant reduction in inflammatory burden

Comprehensive Interpretation

The supplementation intervention produced substantial improvements in inflammatory profiles among vitamin D deficient participants (Table 2, Figure 3-4). Mean 25(OH)D increased from 11.8 to 48.7 ng/mL, achieving sufficiency in 94.4% of participants. This was accompanied by marked reductions in all pro-inflammatory markers: IL-6 decreased by 64.3%, TNF- α by 58.7%, IL-1 β by 55.6%, and CRP by 72.4%. The anti-inflammatory cytokine IL-10 nearly tripled, increasing by 190.2%. Changes were progressive across the 12-week intervention with most improvement occurring within the first 8 weeks coinciding with achievement of vitamin D sufficiency.

Discussion

This research demonstrates robust inverse correlations between vitamin D status and pro-inflammatory cytokine concentrations in an Iraqi adult population and confirms that supplementation-induced correction of vitamin D deficiency produces substantial improvements in inflammatory profiles [12-14]. The magnitude of associations and treatment effects observed supports the biological significance of vitamin D-mediated immunomodulation and suggests meaningful clinical implications for inflammatory conditions.

The strong inverse correlation between 25(OH)D and IL-6 ($r = -0.78$) exceeds that reported in most Western populations, potentially reflecting the more severe deficiency prevalent in this cohort. The molecular mechanisms underlying vitamin D suppression of IL-6 production include direct transcriptional repression through VDR binding to negative vitamin D response elements in the IL-6 promoter and indirect effects through inhibition of NF- κ B signaling [15]. Similar mechanisms account for suppression of TNF- α and IL-1 β , while enhancement of IL-10 occurs through VDR-mediated activation of anti-inflammatory pathways [16].

The supplementation protocol achieved excellent correction of deficiency with 94.4% of participants reaching sufficiency by week 12. The weekly high-dose regimen was well-tolerated with no hypercalcemia or adverse events, consistent with safety data from similar protocols in Middle Eastern populations [17]. The inflammatory improvements were clinically substantial, with CRP reduction of 72.4% comparable to effects of pharmacological anti-inflammatory interventions [18-19].

Clinical Implications

These findings support routine assessment of vitamin D status in patients with inflammatory conditions and consideration of supplementation in those with documented deficiency. The substantial anti-inflammatory effects observed suggest potential as adjunctive therapy for chronic inflammatory diseases, autoimmune disorders, and infection susceptibility. The magnitude of CRP reduction achieved rivals that of established pharmacological interventions, potentially offering a safe and economical complementary approach. Implementation of vitamin D screening and supplementation programs may be particularly valuable in populations with high deficiency prevalence such as the Middle East region.

Future Perspectives

Continued investigation should address several remaining questions. Longer-term studies are needed to determine whether the anti-inflammatory benefits are sustained with maintenance supplementation and whether clinical outcomes including disease incidence and progression are improved. Optimal dosing strategies for different baseline deficiency levels and target populations require clarification. The potential for vitamin D to enhance response to immunomodulatory medications in autoimmune and

inflammatory diseases merits systematic evaluation through combination therapy trials. Genetic polymorphisms affecting vitamin D metabolism and VDR function may influence individual responsiveness and warrant investigation for personalized supplementation approaches.

Conclusion

This research establishes significant inverse correlations between vitamin D status and inflammatory cytokine profiles in an Iraqi adult population, with deficient individuals demonstrating substantially elevated pro-inflammatory markers compared to those with sufficient vitamin D. The correlation coefficients ranging from -0.68 to -0.78 for pro-inflammatory cytokines indicate that vitamin D status is a major determinant of inflammatory burden in this population.

The 12-week supplementation intervention produced remarkable improvements in inflammatory profiles, with reductions of 55-72% in pro-inflammatory cytokines and near-tripling of anti-inflammatory IL-10. These effects occurred coincident with achievement of vitamin D sufficiency and suggest direct immunomodulatory actions rather than epiphenomenal associations. The magnitude of anti-inflammatory benefit approaches that of pharmacological interventions while maintaining excellent safety profile.

These findings support vitamin D as a significant modulator of immune function with therapeutic potential in inflammatory conditions. Given the high prevalence of vitamin D deficiency in the Middle East and globally, optimization of vitamin D status represents an accessible intervention with substantial potential for reducing inflammatory disease burden. Integration of vitamin D assessment and supplementation into clinical practice for at-risk populations merits consideration based on the strength and consistency of these findings.

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Contributions Not Qualifying for Authorship

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