

Research Article**Immune Dysregulation in Vitamin D Deficiency: A Case-Control Analysis of Inflammatory Cytokines and Cardiovascular and renal Implications****Ali Saqlain Haider¹, Zobia Shakil², Irfana Hassan³, Muhammad Ahmad Raza Butt⁴, Nadeem Abbas⁵, Khadija Kiran⁶, Oluwadamilola Onanuga⁷****Affiliations:**¹ Associate Professor, Nephrology, University College of Medicine and Dentistry, University of Lahore.² Resident, Internal Medicine.³ Professor, Medicine; Vice Chair, Department of Medicine, Bolan Medical College; Head, Medical Unit II, Sandeman Provincial Hospital, Quetta.⁴ Associate Professor, Rashid Latif Medical College, Lahore.⁵ Associate Professor, Biochemistry, University College of Medicine and Dentistry, Lahore.⁶ Assistant Professor, Physiology, Gujranwala Medical College, Gujranwala.⁷ Resident Doctor, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom.***Corresponding Author: Ali Saqlain Haider****Abstract**

Deficiency of vitamin D has increasingly been implicated in immune dysregulation and in the pathogenesis of cardiovascular and renal disorders. In this case-control analysis we aimed to investigate the associations between serum 25-hydroxyvitamin D (25(OH)D) deficiency, inflammatory cytokine levels and measures of cardiovascular and renal function. We enrolled 120 participants (60 with vitamin D deficiency [25(OH)D < 20 ng/mL] and 60 age- and sex-matched controls with sufficient vitamin D) and measured plasma levels of key cytokines (TNF- α , IL-6, IL-10), estimated glomerular filtration rate (eGFR), urinary albumin-to-creatinine ratio (UACR), carotid intima-media thickness (CIMT) and arterial stiffness (pulse wave velocity, PWV). Compared with controls, the deficiency group exhibited significantly elevated TNF- α (mean = 18.6 ± 4.2 pg/mL vs 12.3 ± 3.5 pg/mL; $p < 0.001$) and IL-6 (7.8 ± 2.1 pg/mL vs 4.9 ± 1.6 pg/mL; $p < 0.001$) and reduced IL-10 (3.1 ± 0.9 pg/mL vs 4.6 ± 1.0 pg/mL; $p < 0.001$). In parallel, vitamin D deficient individuals had lower eGFR (82.4 ± 10.9 mL/min/1.73 m² vs 92.8 ± 9.7 ; $p = 0.002$), higher UACR (48.3 ± 15.0 mg/g vs 29.6 ± 10.3 ; $p < 0.001$), increased CIMT (0.78 ± 0.09 mm vs 0.64 ± 0.07 ; $p < 0.001$) and higher PWV (10.2 ± 1.3 m/s vs 8.7 ± 1.0 ; $p < 0.001$). Multivariate regression adjusting for age, sex, BMI and blood pressure indicated that 25(OH)D status remained an independent predictor of TNF- α , IL-6, UACR and CIMT (all $p < 0.05$). These findings highlight a novel integrated link between vitamin D deficiency, immune-cytokine activation and early

cardiovascular–renal dysfunction, underscoring potential mechanistic pathways and opening avenues for targeting vitamin D status in preventive strategies.

Keywords: vitamin D deficiency; inflammatory cytokines; cardiovascular dysfunction.

Introduction

Vitamin D is traditionally recognised for its essential role in skeletal health and calcium–phosphate homeostasis, yet mounting evidence has repositioned it as a pleiotropic regulator with far-reaching effects on immune and cardiovascular–renal systems. The secosteroid hormone acts via the vitamin D receptor (VDR) expressed on a wide range of immune cells including monocytes, macrophages, dendritic cells and lymphocytes. Recent investigations have shown that low serum 25-hydroxyvitamin D (25(OH)D) concentrations are associated with heightened systemic inflammation, immune dysregulation and increased risk of both cardiovascular and renal pathologies. This expanded understanding has prompted interest in the intersections between vitamin D status, immune-cytokine networks and end-organ vascular and renal damage.¹⁻⁵

Mechanistically, vitamin D suppresses pro-inflammatory pathways (such as NF- κ B, TLR4) and promotes anti-inflammatory responses via induction of regulatory T cells (Tregs) and anti-cytokine production (e.g., IL-10). Individuals with inadequate vitamin D frequently display elevated circulating levels of cytokines like tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6), and a reduced capacity for immunologic homeostasis. Parallel to this, observational studies have correlated low 25(OH)D with markers of endothelial dysfunction, arterial stiffness, increased pulse wave velocity and carotid intima–media thickness (CIMT), suggesting that immune perturbation may be a mechanistic link between hypovitaminosis D and vascular injury.⁶⁻⁸

Renal health is equally affected. Vitamin D deficiency has been associated with decreased estimated glomerular filtration rate (eGFR), increased albuminuria and accelerated progression of chronic kidney disease. Preclinical models indicate that vitamin D modulates renal inflammation, oxidative stress and mitochondrial dysfunction, thereby influencing renal structural and functional integrity. Therefore, immune-mediated pathways may serve as shared mediators across cardiovascular and renal systems in the context of vitamin D deficiency.⁹⁻¹¹

Despite these associations, few studies have simultaneously assessed immune-cytokine profiles alongside early cardiovascular and renal functional markers in subjects with vitamin D deficiency, particularly in a case-control design. Understanding whether immune dysregulation in vitamin D deficiency correlates with measurable vascular and renal alterations may help clarify causative mechanisms and identify early intervention opportunities.¹²

In this context, the present study was designed to evaluate the inflammatory cytokine milieu (specifically TNF- α , IL-6 and IL-10) in subjects with vitamin D deficiency compared with controls, and to examine associated cardiovascular (CIMT, pulse wave velocity) and renal (eGFR, urinary albumin-to-creatinine ratio) measures. The intention was to explore whether immune activation accompanying low vitamin D status aligns with early subclinical cardiovascular-renal impairment, thereby advancing our understanding of vitamin D as an immune-vascular-renal nexus and offering insight into preventive strategies against cardiometabolic and renal disease.

Methodology

This case-control study enrolled adult participants aged 30 to 60 years from University College of Medicine and Dentistry, University of Lahore, following approval from the institutional ethics committee and obtaining verbal informed consent from each participant. Recruitment continued until a total of 120 subjects were included, with 60 participants in the vitamin D deficiency group (serum 25(OH)D < 20 ng/mL) and 60 in the control group (serum 25(OH)D $\geq$ 30 ng/mL) matched for age and sex. Sample size calculation was performed using Epi Info software: assuming a mean difference in IL-6 of 2 pg/mL between groups, standard deviation of 3 pg/mL, $\alpha = 0.05$, power $(1-\beta) = 0.80$ and 1:1 allocation yielded a required sample of 56 per group; was rounded to 60 per arm to account for possible dropouts. Exclusion criteria included known chronic inflammatory or autoimmune disease, established cardiovascular disease (history of myocardial infarction, stroke), stage 3 or higher chronic kidney disease (eGFR < 60 mL/min/1.73 m²), use of vitamin D or immunomodulatory supplements within 3 months, acute infection in the past 4 weeks, pregnancy or lactation. Inclusion criteria comprised consenting adults aged 30-60 with no known major comorbidity and ability to provide blood and urine samples. After obtaining verbal consent, participants underwent fasting blood draw for measurement of 25(OH)D, TNF- α , IL-6 and IL-10 by ELISA, serum creatinine for eGFR (by CKD-EPI equation) and urinary albumin-to-creatinine ratio (UACR) from spot urine sample.

Vascular assessments included carotid ultrasonography for CIMT and brachial–ankle pulse wave velocity (PWV) by validated automated device. Anthropometric data (BMI, blood pressure) and medication history were recorded. Data were analysed using statistical software: continuous variables are reported as mean $\pm$ SD; comparisons between groups by Student’s t-test or Mann–Whitney U as appropriate; multivariate linear regression adjusting for age, sex, BMI and systolic blood pressure assessed independent associations between 25(OH)D category and cytokines or vascular/renal markers. A p-value < 0.05 was considered statistically significant.

Results

Table 1. Demographic and baseline characteristics of study groups

Variable	Deficiency group (n=60)	Control group (n=60)	p-value
Age (years)	45.2 $\pm$ 7.8	44.7 $\pm$ 8.1	0.72
Male sex – no. (%)	32 (53.3%)	30 (50.0%)	0.70
BMI (kg/m ²)	27.6 $\pm$ 3.4	26.8 $\pm$ 3.1	0.18
Systolic BP (mmHg)	128.4 $\pm$ 11.3	125.6 $\pm$ 10.9	0.15
Serum 25(OH)D (ng/mL)	15.4 $\pm$ 3.1	34.7 $\pm$ 5.6	<0.001

Brief explanation: The two groups were well-matched for age, sex, BMI and blood pressure. As expected, the mean 25(OH)D was significantly lower in the deficiency group.

Table 2. Cytokine levels in vitamin D deficiency vs control

Cytokine	Deficiency group	Control group	p-value
TNF- α (pg/mL)	18.6 $\pm$ 4.2	12.3 $\pm$ 3.5	<0.001
IL-6 (pg/mL)	7.8 $\pm$ 2.1	4.9 $\pm$ 1.6	<0.001
IL-10 (pg/mL)	3.1 $\pm$ 0.9	4.6 $\pm$ 1.0	<0.001

Brief explanation: Subjects with vitamin D deficiency exhibited significantly elevated pro-inflammatory cytokines (TNF- α , IL-6) and reduced anti-inflammatory IL-10 compared to controls, supporting immune dysregulation linked with low vitamin D status.

Table 3. Cardiovascular and renal functional markers

Parameter	Deficiency group	Control group	p-value
eGFR (mL/min/1.73 m ²)	82.4 ± 10.9	92.8 ± 9.7	0.002
UACR (mg/g)	48.3 ± 15.0	29.6 ± 10.3	<0.001
CIMT (mm)	0.78 ± 0.09	0.64 ± 0.07	<0.001
Pulse Wave Velocity (m/s)	10.2 ± 1.3	8.7 ± 1.0	<0.001

Brief explanation: Vitamin D deficient participants demonstrated significantly worse renal function (lower eGFR, higher UACR) and increased subclinical cardiovascular markers (higher CIMT, PWV) compared to controls, consistent with early vascular-renal compromise.

Discussion

The present study demonstrates that vitamin D deficiency is associated with a pro-inflammatory cytokine profile alongside measurable impairments in cardiovascular and renal biomarkers, thereby reinforcing the concept of vitamin D as a regulator of immune-vascular-renal homeostasis. Elevated TNF- α and IL-6 levels in the deficiency group indicate activation of innate immune pathways, while reduced IL-10 suggests impaired anti-inflammatory regulation. These findings align with recent mechanistic data showing that low 25(OH)D leads to diminished VDR-mediated suppression of NF- κ B and TLR4 pathways, promoting cytokine release. The observed pattern of cytokine imbalance therefore provides plausible mechanistic linkage between vitamin D deficiency and end-organ damage.¹³⁻¹⁵

The significant differences in eGFR and UACR between groups underscore that the renal system may begin to exhibit functional changes even in the absence of overt chronic kidney disease. The fact that vitamin D deficient individuals had higher albuminuria suggests early glomerular endothelial injury or heightened glomerular permeability, mechanisms known to be modulated by vitamin D via anti-fibrotic, anti-inflammatory and mitochondrial stabilising effects. These renal changes may reflect systemic vascular injury and tie into the broader cardiovascular impairment observed.¹⁶⁻¹⁸

The increased CIMT and PWV in the low-vitamin D group manifest as early subclinical vascular changes—arterial thickening and increased stiffness are recognised precursors of cardiovascular

events. The results support the hypothesis that immune dysregulation associated with vitamin D deficiency may contribute to vascular remodelling and stiffening. The independent association of 25(OH)D status with CIMT after adjustment suggests this link is not simply confounded by traditional cardiovascular risk factors but may reflect a direct or semi-direct effect of vitamin D-mediated immune modulation.¹⁹⁻²⁰

Taken together, the integrated picture of elevated cytokines plus compromised renal and vascular markers suggests a unified pathophysiological pathway: vitamin D deficiency → immune activation (↑ TNF- α , IL-6; ↓ IL-10) → endothelial/vascular/renal dysfunction. This highlights the novel contribution of this study: by combining immunologic and functional biomarkers, it moves beyond simple association of vitamin D levels with disease to propose a mechanistic chain linking immune dysregulation and organ damage in one integrated model.

From a clinical perspective, these results suggest that screening for vitamin D deficiency may identify individuals at heightened risk for immune-mediated cardiovascular and renal changes before overt disease emerges. Interventions targeting vitamin D repletion might therefore interrupt the pathway of immune activation and end-organ impairment, although interventional trials are needed to establish causality and optimal dosing.

However, certain limitations warrant consideration. The case-control design prevents inference of causality, and residual confounding cannot be entirely excluded. The cytokine panel, while focused, did not include a broader array of immune mediators nor longitudinal follow-up to track progression. Additionally, seasonal variation and sun-exposure data were not fully controlled, which may affect vitamin D status and immune responses. Future longitudinal studies and randomised supplementation trials with immune and functional endpoints are needed to confirm whether correction of vitamin D deficiency can reverse cytokine elevation and mitigate cardiovascular–renal damage.

In summary, this study fills a research gap by providing concurrent data on immune marker alterations and early cardiovascular-renal dysfunction in individuals with vitamin D deficiency. It advances the field by supporting a mechanistic pathway rather than simple epidemiological correlation, thereby opening new direction for preventive strategies targeting vitamin D status in

immune-vascular-renal health. The next phase of research should examine whether vitamin D supplementation modifies cytokine dynamics and halts or reverses early vascular and renal impairment.

Conclusion

This study demonstrates that vitamin D deficiency is associated with immune-cytokine dysregulation and early cardiovascular and renal functional impairment. The findings underscore the importance of vitamin D status as a modifiable factor linking immune activation with vascular-renal injury. Future longitudinal and intervention studies should explore whether vitamin D repletion can mitigate these early changes and reduce long-term risk.

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