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Author: Fazil Choban Almammadov

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Association of vitamin D deficiency with inflammatory cytokines and disease severity in chronic heart failure

Fazil Choban Almammadov

Department of Family Medicine, Azerbaijan Medical University, Baku, Azerbaijan

Corresponding author: Fazil Choban Almammadov, e-mail: xeyalcafarov4@gmail.com

ORCID

FCA: https://orcid.org/0009-0004-4041-6905

ABSTRACT

Introduction and aim. Vitamin D deficiency has been associated with impaired cardiovascular dysfunction and adverse outcomes in chronic heart failure (CHF); yet the connection between inflammatory and cardiac biomarkers in different disease severities remains incompletely understood. Therefore, this study aimed to investigate the role of vitamin D and related biochemical parameters in the etiopathophysiology and severity of chronic heart failure.

Material and methods. A total of 219 people diagnosed with chronic heart failure, aged 30 and 89 years, were enrolled and stratified according to the functional class (I–II, n=123; III–IV, n=96). An age and sex-matched control group of 51 apparently healthy individuals was included. Serum levels of vitamin D, natriuretic peptide (BNP), N-terminal pro–brain natriuretic peptide (NT-proBNP), interleukin-6 (IL-6), interleukin-18 (IL-18) and tumor necrosis factor-alpha (TNF-α), galectin-3 and fibroblast growth factor-23 (FGF-23) were quantified using validated immunoassays. Comparisons between groups were performed using nonparametric statistical tests. Associations between variables were evaluated using Spearman's correlation analysis. The diagnostic utility of the selected biomarkers for different levels of severity of CHF was evaluated using receiver operating characteristic (ROC) curve analysis.

Results. Vitamin D deficiency was associated with CHF severity. Across increasing NYHA functional classes, serum levels of IL-6, IL-18, TNF-α, BNP, NT-proBNP, galectin-3, and FGF-23 differed significantly ($p<0.001$). Vitamin D concentrations were positively associated with left ventricular ejection fraction and inversely related to BNP, NT-proBNP, IL-6, IL-18, TNF-α, galectin-3, and FGF-23. ROC analysis indicated that IL-6 (AUC 0.799, sensitivity 75%, specificity 78%) and galectin-3 (AUC 0.791, sensitivity 72%, specificity 80%) were the most discriminative biomarkers in patients with CHF with vitamin D deficiency, supporting their clinical utility for risk stratification and disease monitoring.

Conclusion. This study provides novel evidence by integrating inflammatory cytokines, galectin-3, FGF-23, and natriuretic peptides in patients with vitamin D-deficient CHF, identifying IL-6 and galectin-3 as the most discriminative biomarkers of disease severity in this clinical setting.
Keywords. chronic heart failure, cytokines, FGF-23, galectin-3, vitamin D

Introduction

Chronic heart failure (CHF) continues to pose significant challenges in contemporary medicine.[1,2] Its prevalence is expected to increase due to increasing rates of primary etiologies, including arterial hypertension, ischemic heart disease, and type 2 diabetes mellitus. Despite advances in treatment and preventive strategies, patients with CHF continue to face high mortality, frequent hospitalizations, and multiple comorbidities.[3] Therefore, there is a continued need for therapeutic approaches that improve clinical outcomes and quality of life in these patients.

In recent years, vitamin D deficiency has attracted growing attention in the context of CHF. Globally, approximately 40.4% of people exhibit vitamin D deficiency.[4] Multiple studies have shown links of vitamin D deficiency with various cardiovascular conditions, such as heart failure, arterial hypertension, and atrial fibrillation.[1,5] Insufficient vitamin D can promote activation of the renin–angiotensin–aldosterone system, trigger inflammatory pathways, and affect endothelial function.[6] Production of pro-inflammatory cytokines, including interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) is up-regulated, promoting oxidative stress. Vitamin D modulates the NF-κB pathway, thus reducing the production of interleukin-6 (IL-6), interleukin-12 (IL-12), interferon-γ, and TNF-α.[7-9] This pro-inflammatory environment coexists with neurohormonal activation and endothelial dysfunction, creating conditions favorable for adverse ventricular remodeling.[10,11]

Fibro-inflammatory remodeling is also reflected in galectin-3, which increases with macrophage activation and interstitial fibrosis, correlated with adverse structural remodeling and poorer results in CHF.[12,13] CHF is also associated with activation of mineral metabolism pathways. Fibroblast growth factor-23 (FGF-23) levels increase in patients experiencing systemic inflammation and cardiorenal stress, and evidence suggests that FGF-23 contributes to the progression of cardiovascular disease.[14,15] Within the mineral–bone–cardiac axis, FGF-23 typically increases along with inflammatory activity and renal dysfunction. Experimental and clinical evidence indicates a bidirectional link between inflammatory signaling and FGF-23 expression, positioning FGF-23 as both a mediator and a marker of maladaptive remodeling in CHF, although its direct myocardial effects remain debated.[16,17] Hemodynamic overload and neurohormonal activation are reflected by natriuretic peptides, such as natriuretic peptide (BNP) and N-terminal pro–brain natriuretic peptide (NT-proBNP), which increase with stress in the ventricular wall and correlate with inflammatory activity. These markers serve as complementary indices to cytokines and fibrosis biomarkers in vitamin D–deficient CHF phenotypes.[18,19]

The selected biomarker panel integrates inflammatory, fibrotic, mineral metabolism, and natriuretic markers to comprehensively assess disease severity in patients with vitamin D-deficient CHF.[20] While prior studies have examined single biomarkers or limited pathways, the present study provides novel insights by evaluating this panel, identifying key pathophysiological links, and supporting the evaluation of disease progression.

Aim

The aim of this study was to investigate the role of vitamin D and related biochemical parameters in the etiopathophysiology and severity of CHF.

Material and methods

Study design and setting

This was a multicenter, cross-sectional observational study**.** Patients were consecutively enrolled during routine visits to outpatient clinics or inpatient departments at three tertiary care centers in Baku, Azerbaijan: Republican Clinical Center, Sabunchu Clinical Center, and 1st Clinical Medical Center, between 2023 and 2024. Seasonal variations in vitamin D levels were taken into account during recruitment to minimize confounders.

Participants

A cohort of 219 people diagnosed with chronic heart failure (CHF), aged 30 to 89 years (mean 61.5±0.7 years), was recruited. The classification was performed according to the New York Heart Association (NYHA) functional scale: 123 in classes I-II (mean age 60.4±1.0 years) and 96 in classes III-IV (mean age 62.9±1.0 years). All CHF patients were clinically stable at enrollment, without recent hospitalization or acute decompensation, to minimize variability in biomarker levels, particularly BNP and inflammatory markers.

Heart failure phenotype

The cohort consisted of patients who presented primarily with heart failure with reduced ejection fraction (HFrEF, EF <40%). However, the median EF in NYHA I–II patients was greater than 40% (Me=68.0%, Q1–Q3: 62.0–71.0), indicating that this subgroup included a few patients with HFmrEF (EF 40–49%). The median EF for NYHA III–IV was 40.0% (Q1–Q3: 33.0–46.0). All analyzes were performed on the combined HF population and results are interpreted accordingly. The main etiologies of CHF were coronary artery disease, arterial hypertension, and dilated cardiomyopathy.

Control group

The study included 51 healthy volunteers, matched by age and sex, who were recruited from hospital personnel in the same clinical centers as the patient. The controls were verified to have normal vitamin D levels and were free of cardiovascular, renal, or inflammatory diseases, with no acute infections at the time of blood collection. Potential bias related to occupational health, lifestyle, and socioeconomic differences is acknowledged.

Exclusion criteria

Exclusion criteria included acute or chronic kidney failure, liver failure, infectious or inflammatory diseases, recent use of diuretics or steroids, vitamin D and/or calcium supplementation within the previous two months, hypoparathyroidism and hypothyroidism. Patients with eGFR <60 ml/min/1.73 m² or known chronic kidney disease were excluded to ensure normal kidney function at baseline.

Potential confounding factors

Baseline assessments included variables that could potentially confound the results, such as body mass index (BMI), smoking habits, concomitant medications (ACE inhibitors, ARNI, beta-blockers, statins), exposure to sunlight, and seasonal variations. These factors were recognized as potentially influencing vitamin D and related biomarker levels; No multivariate adjustment was performed, which represents a limitation inherent in the observational design**.**

Echocardiography

Two-dimensional Doppler imaging using the Simpson's biplane method (GE Vivid E95, Philips EPIQ 7) was performed by experienced cardiologists following standardized protocols to minimize variability between and intra-operator variability. Echocardiography was performed the same day as blood collection.

Blood collection and biomarker analysis

Morning blood samples were obtained after patients fasted overnight. Vitamin D, BNP, and NT-proBNP were analyzed immediately; samples for cytokines and galectin-3 were stored at -70 ° C until assay. Each assay was carried out in batches, with samples from patients and controls analyzed simultaneously and blinded to NYHA classification to reduce variability between assays. Calibration between laboratories ensured methodological consistency.

Creatinine was measured using a human immunoassay (mg/dL). BNP and NT-proBNP were analyzed with Roche Elecsys immunoassays (pg/mL). Cytokines IL-6, IL-18 and TNF-α were measured using Vector Best ELISA kits (pg/mL). Galectin-3 concentrations were determined by RayBiotech ELISA (ng/mL), and FGF-23 was assessed using a Human FGF-23 ELISA (pg/mL). Serum 25-hydroxyvitamin D [25(OH)D]

was measured with a Bioaktiva Diagnostic immunoassay (ng/mL). All assays were performed in batches, with patient and control samples analyzed concurrently and blinded to NYHA class, and interlaboratory calibration was conducted to ensure consistency across measurements. The coefficients of variation within and between the assays were less than 10%, and the sensitivity of the assay fell within the ranges recommended by the manufacturer.

Vitamin D measurement

Serum 25-hydroxyvitamin D [25(OH)D] was measured using a validated immunoassay (Bioaktiva Diagnostic). Concentrations are reported in nanograms per milliliter. Vitamin D deficiency: <20 ng/mL; insufficiency: 20–30 ng/mL; sufficiency: >30 ng/mL, in accordance with current clinical guidelines**.**

Statistical methods

Data are expressed as median (Me), 25th (Q1) and 75th (Q3) percentiles. Nonparametric comparisons between two groups were made using the Mann-Whitney U test, and differences between three groups were assessed with the Kruskal-Wallis test. Fold increases or decreases reported in the results section were calculated as the ratio of median values. Spearman's correlation evaluated the relationships between variables. The diagnostic ability of inflammatory and fibrosis-associated biomarkers was evaluated in CHF patients with vitamin D deficiency using a receiver operating characteristic (ROC) curve. The outcome variable was CHF severity, classified according to NYHA functional class **(**I–II vs III–IV**),** with thresholds determined using the Youden index to maximize sensitivity and specificity. The area under the receiver operating characteristic curve (AUC) was determined with 95% confidence intervals (CI) and the variability of estimates was expressed as standard errors. The sample size was justified based on previous CHF biomarker studies, ensuring adequate power for detecting significant differences. Data gaps were minimal and handled by list deletion**.** A p-value of less than 0.05 was considered statistically significant and all analyses were performed using IBM SPSS Statistics version 26 (Armonk, NY, USA).

Ethics

The study was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Azerbaijan Medical University, approval No. 3/2018, dated 16.09.2018.

Results

According to echocardiographic data, the median EF was 40.0% (Q1-Q3: 33.5-46.0%) in patients in NYHA class I-II and 37.5% (Q1-Q3:31.5-41.5%) in NYHA class III-IV, compared with 68.0% (Q1-Q3: 62.0-71.0%) in the healthy control group. EF was significantly lower in both NYHA I–II and III–IV patients

compared to controls (p<0.001). No significant differences in blood creatinine levels were observed between CHF patients, indicating preserved renal function (p=0.283) (Table 1).

Table 1. Changes in functional and biochemical parameters of patients with chronic heart failure according to the stage of the disease*

Parameters	Groups												p#
	Control				NYHA class I-II				NYHA class III-IV				
	M	Me	Q1	Q3	M	Me	Q1	Q3	M	Me	Q1	Q3	
Ejection fraction	66.9	68.0	62.0	71.0	39.5	40.0	33.0	46.0	36.8	37.5	31.5	41.5	<0.001
p					<0.001				<0.001				
p1									0.003				
Creatinine, mg/dL	0.86	0.88	0.76	0.96	0.90	0.92	0.79	1.00	0.89	0.89	0.76	1.01	0.283
p					0.112				0.488				
p1									0.376				
Vit. D, ng/mL	43.5	42.0	38.0	48.0	28.6	29.0	21.0	37.0	20.5	17.5	12.5	28.0	<0.001
p					<0.001				<0.001				
p1									<0.001				
BNP, pg/mL	46.2	50.0	29.0	63.0	541.1	538.0	423.0	628.0	663.7	634.0	557.5	759.5	<0.001
p					<0.001				<0.001				
p1									<0.001				
NT-pro-BNP, pg/mL	72.5	65.0	46.0	96.0	1270.1	1285.0	954.0	1485.0	1662.0	1688.5	1556.5	1845.5	<0.001
p					<0.001				<0.001				
p1									<0.001				
IL-6, pg/mL	2.26	2.00	1.60	2.90	3.59	3.50	3.10	4.10	4.74	4.80	4.00	5.30	<0.001
p					<0.001				<0.001				
p1									<0.001				
IL-18, pg/mL	197.0	210.0	146.9	243.5	366.9	368.0	295.0	430.0	409.9	409.5	350.0	485.5	<0.001
p					<0.001				<0.001				
p1									0.003				
TNF-α, pg/mL	4.9	5.0	4.6	5.3	7.3	7.3	6.5	7.9	8.2	8.1	7.3	8.9	<0.001
p					<0.001				<0.001				
p1									<0.001				
Galectin-3, ng/mL	7.3	7.0	5.5	9.6	14.2	14.4	11.8	16.3	18.1	18.1	15.4	19.4	

p						<0.001				<0.001			
p1										<0.001			
FGF-23, pg/mL	48.3	48.2	39.1	61.2	55.0	53.4	48.9	62.9	58.2	56.4	47.1	65.9	0.001
p						0.003				<0.001			
p1										0.194			

***** M – mean, Me – median, Q1 – first quartile (25th percentile), Q3 – third quartile (75th percentile), p – significance versus controls, p1 – significance versus NYHA I–II, p-values were determined using the Mann-Whitney U test, while # – indicates comparisons across all NYHA classes using the Kruskal-Wallis test

There were no statistically significant differences in serum creatinine concentration between the patients. The median BNP concentration was markedly elevated, showing a 10.8-fold increase in CHF patients of the NYHA functional class I–II (median 538.0 pg/mL; Q1–Q3: 423.0–628.0; p<0.001) and a 12.7-fold increase in those with NYHA class III–IV (634.0; 557.5–759.5 pg/mL, p<0.001) compared to the control group (50.0; 29.0–63.0 pg/mL). Similarly, the median concentration of NT-proBNP was significantly higher, 19.8 times higher in patients with NYHA class I–II (1285.0; 954.0–1485.0 pg/mL, p<0.001) and 26.0-fold higher in those with NYHA class III–IV (1688.5; 1556.5–1845.5 pg/mL, p<0.001) relative to controls (65.0; 46.0–96.0 pg/mL). BNP and NT-proBNP levels increased further in NYHA class III–IV compared to class I–II, by 17.8% (p<0.001) and 31.4% (p<0.001), respectively (pH=0.001 between groups). Vitamin D deficiency in CHF patients worsened progressively with advancing NYHA functional class. In class I–II, the median vitamin D concentration was approximately 50% lower (29.0; 21.0–37.0 ng/mL, p<0.001) than in the control group (42.0; 38.0-48.0 ng / mL), while in class III–IV, this decrease was 2.5 times (17.5; 12.5–28.0 ng/mL, p<0.001). Vitamin D levels in class III–IV were 65.7% lower (p<0.001) compared to class I–II, indicating a more severe deficiency in advanced stages of CHF (pH=0.001 between groups).

Inflammatory cytokine levels increased in accordance with the NYHA functional class. IL-6, IL-18, and TNF-α showed stepwise elevations: IL-6 increased by 75.0% in class I–II (3.50; 3.10–4.10 pg/mL, p<0.001) and 2.4 times in class III–IV (4.80; 4.0–5.30 pg/mL, p<0.001), compared to controls (2.00; 1.60–2.90 pg / ml). IL-18 increased by 75.2% (368.0; 295.0–430.0 pg/mL, p<0.001) in class I–II and 95.0% (409.5; 350.0–485.0 pg/mL, p<0.001) in class III–IV (controls: 210.0; 146.9–243.5 pg/mL). TNF-α increased by 46.0% in class I–II (7.3; 6.5–7.9 pg/mL, p<0.001) and 62.0% in class III–IV (8.1; 7.3–8.9 pg/mL, p<0.001), compared to controls (5.0; 4.6-5.3 pg / ml). In class III-IV compared to class I–II, IL-6, IL-18, and TNF-α concentrations were significantly higher by 37.1% (p<0.001), 11.3% (p=0.003) and 11.0% (p<0.001), respectively (pH=0.001 between groups).

Galectin-3 levels were elevated in both functional classes compared to the control group (7.0; 5.5 to 9.6 ng / ml), showing a 2.0-fold increase in class I–II (14.4; 11.8–16.3 ng/mL, p<0.001) and a 2.6-fold increase in

class III–IV (18.1; 15.4–19.4 ng/mL, p<0.001). Median galectin-3 concentrations further increased with disease progression, showing a 25.7% increase in class III–IV compared to class I–II (p<0.001) (p_H<0.001 across groups).

FGF-23 levels increased by 10.8% in NYHA class I–II (53.4; 48.9-62.9 pg / ml, p=0.003) and by 20.0% in class III–IV (56.4; 47.1–65.9 pg/mL, p<0.001), compared to the control group (48.2; 39.1-61.2 pg / ml). This suggests a mechanism of myocardial remodeling potentially associated with vitamin D deficiency and phosphate dysregulation. However, FGF-23 concentrations did not differ significantly between NYHA class III–IV and class I-II (p=0.119) (pH=0.001 between groups).

The study identified statistically significant correlations between vitamin D deficiency and several biochemical parameters in CHF patients. Serum vitamin D concentrations exhibited a weak but statistically significant inverse correlation with NT-proBNP levels (ρ=−0.136; p=0.044). Furthermore, vitamin D levels were inversely correlated with key inflammatory mediators, including IL-6 (ρ=−0.255; p<0.001) and TNF-α (ρ=−0.282; p<0.001). A significant negative association was also observed between vitamin D concentrations and galectin-3 levels (ρ=−0.275; p<0.001). In contrast, no statistically significant relationships were detected between vitamin D levels and IL-18 (ρ=−0.122; p=0.070) or FGF-23 (ρ=−0.079; p=0.242).

Several inflammatory and fibrotic biomarkers demonstrated strong positive associations with markers of cardiac neurohormonal activation. IL-6 showed significant positive correlations with both BNP (ρ=0.366; p<0.001) and NT-proBNP (ρ=0.444; p<0.001). Similarly, IL-18 was strongly correlated with BNP (ρ=0.490; p<0.001) and NT-proBNP (ρ=0.464; p<0.001).

TNF-α also exhibited significant positive correlations with BNP (ρ=0.465; p<0.001) and NT-proBNP (ρ=0.454; p<0.001). Among the biomarkers evaluated, galectin-3 demonstrated the strongest associations with natriuretic peptides, showing robust correlations with BNP (ρ=0.732; p<0.001) and NT-proBNP (ρ=0.764; p<0.001). FGF-23 concentrations were positively correlated with BNP (ρ=0.381; p<0.001) and NT-proBNP (ρ=0.403; p<0.001). Furthermore, a significant positive association was observed between FGF-23 and IL-6 levels (ρ=0.363; p<0.001). Strong positive interrelationships were identified among inflammatory and fibrotic biomarkers. IL-6 demonstrated significant positive correlations with IL-18 (ρ= 0.691; p<0.001), TNF-α (ρ=0.557; p<0.001), and galectin-3 (ρ=0.555; p<0.001). Moreover, TNF-α was positively correlated with galectin-3 (ρ=0.655; p<0.001) and FGF-23 (ρ=0.640; p<0.001).

ROC curve analysis was performed to assess the ability of inflammatory and fibrosis-related biomarkers to discriminate disease progression in patients with chronic heart failure and concomitant vitamin D deficiency. The ROC curves for IL-6, IL-18, FGF-23, galectin-3, and TNF-α are presented in Figure 1. Among the biomarkers analyzed, IL-6 demonstrated the highest diagnostic precision, with an area under the curve (AUC) of 0.799 (95% CI: 0.739–0.859, p<0.001). At the selected cut-off value of 18.5 pg/mL, IL-6 showed a sensitivity of 75% and a specificity of 78%. Similarly, galectin-3 exhibited a strong

discriminative performance with an AUC of 0.791 (95% CI 0.731–0.851, p<0.001). Using a cut-off value of 16.0 ng/mL, sensitivity and specificity were 72% and 80%, respectively. TNF-α showed moderate diagnostic ability, resulting in an AUC of 0.689 (95% CI 0.617–0.761, p<0.001). At a cut-off value of 12.5 pg/mL, sensitivity and specificity were 65% and 75%, respectively. On the contrary, IL-18 demonstrated limited discriminative capacity, with an AUC of 0.618 (95% CI 0.542–0.693, p=0.003). The selected cutoff of 210.0 pg/mL provided a sensitivity of 60% and a specificity of 62%. FGF-23 showed poor diagnostic performance, with an AUC of 0.551 (95% CI: 0.473–0.630, p=0.194). At the cut-off value of 145.0 pg/mL, the sensitivity and specificity were 55% and 57%, respectively, indicating a limited ability to discriminate disease progression (Table 2).

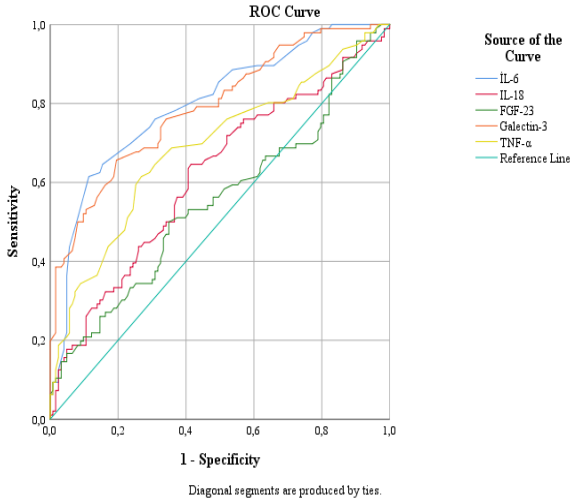

Fig. 1. ROC curves of inflammatory and fibrosis-related biomarkers in patients with chronic heart failure

Table 2. ROC analysis results of inflammatory and fibrosis-related biomarkers in patients with chronic heart failure

Test result variable(s)	AUC (%95)	Cut-off	p	Sensitivity (%)	Specificity (%)
IL-6, pg/mL	0.799 (0.739–0.859)	18.5	<0.001	75	78
IL-18, pg/mL	0.618 (0.542–0.693)	210.0	0.003	60	62
FGF-23, pg/mL	0.551 (0.473–0.630)	145.0	0.194	55	57
Galectin-3, ng/mL	0.791 (0.731–0.851)	16.0	<0.001	72	80
TNF-α, pg/mL	0.689 (0.617–0.761)	12.5	<0.001	65	75

Discussion

Vitamin D deficiency appears to be involved in multiple pathophysiological pathways associated with the severity and progression of CHF. In this observational study, reduced serum vitamin D concentrations were correlated with higher NYHA functional classes, indicating a possible relationship of vitamin D status with

the severity of clinical manifestations. These results reinforce the idea that vitamin D could affect cardiovascular function in ways that extend beyond its traditional role in calcium–phosphate balance. Experimental and clinical data suggest that vitamin D signaling influences myocardial architecture, intracellular pathways, immune responses, and neurohormonal regulation through the participation of the vitamin D receptor (VDR).[21,22] The biologically active form, $1,25(OH)_2D_3$, has been shown to modulate the renin–angiotensin–aldosterone system (RAAS), oxidative stress pathways, and inflammatory responses, all of which are central mechanisms in CHF progression.[23,24]

Although the correlations between vitamin D levels and natriuretic peptides (BNP and NT-proBNP) were modest, their statistical significance suggests a biologically relevant association between vitamin D status and neurohormonal activation. In particular, the inverse relationship observed between vitamin D concentrations and NT-proBNP levels may reflect lower tension in the left ventricle wall tension and reduced neurohormonal activation in individuals who exhibit elevated vitamin D levels. Our results align with previous studies showing that vitamin D insufficiency is associated with elevated NT-proBNP levels, impaired cardiac function, and less favorable outcomes in heart failure populations.[25-27] However, given the observational design of the present study, these associations should be interpreted cautiously and cannot be considered evidence of a causal relationship.

Chronic systemic inflammation represents a key feature of CHF and contributes to myocardial dysfunction and adverse ventricular remodeling. Vitamin D exerts immunomodulatory effects by suppressing macrophage activation and downregulating the synthesis of pro-inflammatory cytokines while improving anti-inflammatory responses.[28,29] In the present investigation, lower vitamin D levels were inversely related to IL-6 concentrations, indicating a possible link between vitamin D deficiency and increased systemic inflammatory activity. Elevated IL-6 levels have been implicated in cardiomyocyte apoptosis, myocardial fibrosis, endothelial dysfunction, and left ventricular systolic impairment through mechanisms mediated by oxidative stress. These observations are consistent with previous studies reporting elevated IL-6 concentrations among patients with insufficient vitamin D and coexisting cardiovascular or metabolic conditions.[30-33]

TNF-α is a key inflammatory mediator involved in myocardial fibrosis, cardiomyocyte apoptosis, negative inotropic effects, and adverse ventricular remodeling. The observed negative correlation of vitamin D concentrations with TNF-α in this study indicates that maintaining sufficient vitamin D can help reduce TNF-α–driven inflammatory responses. Experimental data support this hypothesis, demonstrating that calcitriol, the active form of vitamin D, can inhibit TNF-α expression and release.[28,34,35] However, these findings should be interpreted as associative rather than mechanistic. For comparison, vitamin D concentrations did not show notable associations with IL-18 or FGF-23. This lack of association may indicate that these pathways are less directly influenced by vitamin D status in CHF or are regulated through alternative or more complex mechanisms. IL-18 has been associated with endothelial injury, cardiomyocyte

apoptosis, and ventricular hypertrophy,[35,37] while FGF-23 has emerged as an important mediator of myocardial stress, fibrosis, and maladaptive remodeling.[38-40] Our findings indicate a relationship between higher concentrations of FGF-23 and natriuretic peptides as well as inflammatory markers, highlighting its involvement in a wider network of neurohormonal and inflammatory dysregulation. Experimental and clinical studies suggest that FGF-23 interacts with RAAS activation and inflammatory signaling, leading to a self-reinforcing cycle of left ventricular hypertrophy, fibrotic remodeling, and endothelial impairment.[39-42] Furthermore, concurrent higher levels of FGF-23 and lower Klotho expression have been associated with greater cardiovascular risk and worse outcomes in patients with heart failure.[43]

Galectin-3, a well-established biomarker of cardiac fibrosis, demonstrated significant associations with neurohormonal markers in the present study, indicating that fibrotic remodeling is closely related to disease severity in CHF.[13,44,45] Previous studies have reported elevated levels of galectin-3 in heart failure patients, reflecting sustained macrophage activation and remodeling of the extracellular matrix, even in the absence of a direct relationship with vitamin D status.[46] Consistent with these observations, the inverse associations identified between vitamin D levels and IL-6, TNF-α, and galectin-3 in the present cohort suggest that vitamin D deficiency may contribute indirectly to improved inflammatory and fibrotic signaling in CHF. In line with these associations, ROC curve analysis further demonstrated that IL-6 and galectin-3 provide the highest discriminatory value for disease progression in patients with CHF and vitamin D deficiency. On the contrary, IL-18 and FGF-23 showed limited diagnostic utility, while TNF-α exhibited moderate prognostic performance. These results indicate that IL-6 and galectin-3 could function as informative biomarkers for functional stratification and monitoring of disease progression in this clinical context. Loss of vitamin D-mediated immunomodulatory effects can facilitate persistent macrophage activation and cytokine-driven myocardial injury, thus amplifying galectin-3–associated adverse remodeling associated with galectin-3.[29,47]

Taken together, the observed interrelationships among inflammatory cytokines, fibrotic biomarkers, mineral metabolism, and natriuretic peptides highlight the integrated and multifactorial nature of CHF pathophysiology.[32] These pathways appear to act in concert, contributing to progressive myocardial injury, fibrosis, and neurohormonal activation.[48-50] Within this complex framework, vitamin D deficiency may act as an upstream modulator that amplifies inflammatory and fibrotic responses.

Current results show that lower vitamin D levels correlate with more pronounced inflammatory and fibrotic alterations in CHF patients, highlighting the close link of vitamin D deficiency to key pathophysiological mechanisms of the disease.[26,45,46] Insufficient vitamin D appears to exacerbate systemic inflammation, as reflected in elevated cytokines such as IL-6, IL-18 and TNF-α, which contribute to myocardial injury, apoptosis, and adverse remodeling.[32,35] These observations underscore the relevance of assessing vitamin D levels as part of a comprehensive clinical evaluation.

This study provides a new integrated perspective on chronic heart failure by simultaneously evaluating vitamin D status, inflammatory cytokines (IL-6, TNF-α), galectin-3, FGF-23, and natriuretic peptides. Unlike previous reports that focused on single biomarkers, this comprehensive assessment highlights the interplay between mineral metabolism, systemic inflammation, fibrotic remodeling, and hemodynamic stress. Importantly, IL-6 and galectin-3 demonstrated the highest discriminative value for disease severity, representing a significant clinical addition. Overall, these findings uniquely contribute to understanding how vitamin D deficiency may amplify key pathophysiological mechanisms, providing information for grouping patients according to the severity of the disease and guiding clinical management.

Study limitations

The observational design of this study limits the interpretation of causal relationships between vitamin D deficiency and the severity of the disease in chronic heart failure. Additional limitations include that the study was conducted at a single site, the small number of participants, and the absence of interventional data on vitamin D supplementation, which may affect the applicability of the results.

Subsequent research should assess the potential therapeutic effects of correcting vitamin D deficiency on myocardial remodeling, inflammatory mediators, and long-term clinical outcomes, and additionally clarify the mechanistic connections between vitamin D levels, neurohormonal activation, and fibrotic pathways in chronic heart failure.

Conclusion

Vitamin D deficiency in chronic heart failure was associated with greater disease severity and adverse changes in key biomarkers, including BNP, NT-proBNP, IL-6, TNF-α, galectin-3, and FGF-23. These findings provide novel clinical and pathophysiological insights into how vitamin D status, inflammation, fibrosis, and hemodynamic stress interact. In particular, IL-6 and galectin-3 showed the highest discriminative value for disease severity, supporting their use in biomarker-based risk assessment and clinical monitoring of patients with vitamin D deficiency.

Declarations

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Authors' contributions

Conceptualization, A.F.; Methodology, A.F.; Software, A.F.; Validation, A.F.; Formal Analysis, A.F.; Investigation, A.F.; Resources, A.F.; Data Curation, A.F.; Writing – Original Draft Preparation, A.F.;

Writing – Review & Editing, A.F.; Visualization, A.F.; Supervision, A.F.; Project Administration, A.F.; Funding Acquisition, A.F.

Conflicts of interest

The author declares no competing interests.

Data availability

The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

All subjects provided informed consent prior to participation. The study was carried out in accordance with the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the Ethics Committee of Azerbaijan Medical University (Approval No: AMU-2018/09, Date: 9 September 2018).

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