



Relationship Between Vitamin D Deficiency, LDH, Troponin, and BNP Levels in Patients with Cardiovascular Disease

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Abstract

Cardiovascular diseases (CVDs) constitute the primary cause of morbidity and mortality. Recent studies demonstrate the relationship between vitamin D deficiency and increase of cardiac biomarkers (Troponin I, BNP) and metabolic enzymes (LDH) in CVD pathogenesis and prognosis. Objective to assess the correlations and profile of vitamin D deficiency, LDH, Troponin I, and BNP in the patients with diagnosed CVDs in comparison with healthy controls. A case control study included 100 patients with CVDs and 100 gender- and age-matched controls. Serum levels of Troponin I, BNP, 25-hydroxyvitamin D, and LDH were assessed using the appropriate validated immunoassays and enzymatic methods. Mann-Whitney U test was used to compare the studied groups; Spearman's rank correlation and stratified analysis were performed to identify the associations between biomarkers. Higher levels of Troponin I (median: 280.5 vs. 0.9 ng/L; $p < 0.001$), BNP (median: 96.5 vs. 10.0 pg/mL; $p < 0.001$), and LDH (median: 369.0 vs. 171.5 U/L; $p < 0.001$) were detected in CVD patients in comparison with controls. The patients demonstrated lower levels of vitamin D (median: 17.1 vs. 30.35 ng/mL; $p < 0.001$). Stratified analysis indicated that the CVD patients with severe vitamin D deficiency (< 15 ng/mL) demonstrated significantly higher median levels of Troponin I, BNP, and LDH in comparison with the CVD patients with relatively preserved vitamin D levels ($p < 0.05$ for all parameters). Vitamin D deficiency is highly correlated with CVD and the severity of cardiac injury and metabolic stress markers. These results indicate the importance of vitamin D status in the cardiovascular risk stratification.

Keywords: Cardiovascular disease, Vitamin D, Troponin I, BNP, LDH, Biomarkers, Risk stratification.

Introduction

Cardiovascular diseases (CVDs) are a type of multifactorial pathology that causes about 17.9 million deaths annually and accounts for 32% of overall global mortality (World Health Organization, 2021; ESC, 2026). Pathophysiological mechanisms underlying CVD involve endothelial dysfunction, inflammatory activation, oxidative stress, and metabolic abnormalities, which provide a rationale for studying novel biomarkers that would enhance diagnostic accuracy and prognosis (Libby et al., 2021).

Traditional markers of heart injury, including cTnI and BNP, are pivotal for diagnosis of acute coronary syndromes and heart failure, respectively (Thygesen et al., 2019). Cardiac Troponin I ensures high cardiac specificity and sensitivity for detection of myocardial injuries, while highly-sensitive tests allow detecting minute concentrations indicating subclinical damage (Apple et al., 2020). BNP, which is released by ventricular myocytes under wall stress conditions, is a biomarker for heart failure diagnosis and monitoring (Januzzi et al., 2022). Lactate dehydrogenase (LDH), an enzyme involved in metabolism, is used as a marker of tissue damage and systemic metabolic disturbances in CVD (Lippi & Plebani, 2020; Patel & Kumar, 2025).

Vitamin D, known as a factor regulating calcium metabolism, also affects the renin-angiotensin-aldosterone system, inhibits vascular inflammation, and controls cardiomyocyte proliferation (Vaidya & Underwood, 2021). There is a consistent link between vitamin D deficiency and increased CVD risk established by numerous epidemiological studies (Barbarawi et al., 2022; Harrison et al., 2026). However, recent evidence indicates a possible interaction between vitamin D status and traditional cardiac biomarkers in terms of their release and clearance (Anderson et al., 2024; Wang et al., 2025). However, the exact clinical threshold values at which such interaction occur need to be determined.

While the evidence on the impact of each of these markers is available, there are very few comprehensive studies comparing their profiles and interactions in CVD patients. This comparison will contribute to a better understanding of the pathophysiological background of CVDs.

Objectives of the study include: (1) comparing the levels of Troponin I, BNP, LDH, and vitamin D between CVD patients and healthy subjects; (2) estimating correlations between the biomarkers mentioned; and (3) comparing profiles of cardiac biomarkers depending on vitamin D deficiency strata.

Materials and Methods

Study Design and Participants

Case-control study was conducted among 200 participants enrolled from cardiology clinics and outpatient departments. The study included:

- CVD patients' group (n=100): Subjects aged 45-70 years with established cardiovascular disease (coronary artery disease, heart failure, or myocardial infarction) confirmed by clinical evaluation, electrocardiography, echocardiography, and coronary angiography (Visseren et al., 2021).
- Healthy controls group (n=100): Age- and sex-matched healthy subjects aged 49-68 years without any history and clinical signs of cardiovascular, metabolic or inflammatory conditions confirmed by medical history, physical examination, and laboratory screening.

Subjects were excluded from the study if they had active malignancy, chronic kidney disease, acute infection, autoimmune disorder, pregnancy or were under immunosuppressive therapy. Written informed consent was obtained from all participants, and the study protocol was approved by the Institutional Ethics Review Board according to the College of Science, University of Basrah.

Sample Collection and Preparation

Venous blood samples (5 mL) were collected from all participants after overnight fasting (8-12 hours). Samples were collected in serum gel tubes, left for 30-40 minutes at room temperature to clot, and centrifuged at $1000 \times g$ for 15 minutes at 4°C. Serum samples were frozen at -80°C and stored without repeated freezing and thawing.

Biochemical and Hormone Assays

Traditional Biomarkers (ABBOTT ARCHITECT platform)

- High-sensitivity Troponin I (hs-cTnI): measured by STAT hs-Troponin I assay on ARCHITECT i1000SR analyzer (ABBOTT LABORATORIES, USA) using chemiluminescent microparticle immunoassay (CMIA). Six-level calibration and duplicates were used. Results expressed in ng/L.

- B-type Natriuretic Peptide (BNP): determined by BNP assay on ARCHITECT i1000SR (ABBOTT LABORATORIES, USA) using CMIA technology with paramagnetic microparticles coated with anti-BNP antibodies. Two-level calibration and duplicates were used. Results expressed in pg/mL.
- 25-Hydroxyvitamin D [25(OH)D]: measured by Vitamin D (25-OH) assay on ARCHITECT i1000SR (ABBOTT LABORATORIES, USA) using competitive CMIA principle. Six-level calibration and duplicates were used. Results expressed in ng/mL.
- Lactate Dehydrogenase (LDH): measured by enzymatic rate method on ARCHITECT C4000 clinical chemistry analyzer (ABBOTT LABORATORIES, USA). LDH catalyzes the reaction between lactate and pyruvate with NAD⁺ reduction to NADH; the rate of this reaction is proportional to LDH activity and measured photometrically. Results expressed in U/L.

Quality Control

For all assays were used:

1. Prior to sample analysis, instrument calibration was carried out using Abbott ARCHITECT calibrators in accordance with the manufacturer's instructions.
2. Blank control (Sample Diluent only).
3. Duplicates of all samples and standards.
4. Quality control samples at low, middle, and high concentrations.

Criteria for acceptance: standard curve $R^2 > 0.99$, QC sample recovery 85-115%, intra-assay CV < 15%.

Statistical Analysis

Data analysis was conducted using IBM SPSS Statistics for Windows version 26.0 (IBM Corp., USA). Normality was checked using Shapiro-Wilk test; all variables had non-normal distribution ($p < 0.05$).

1. Descriptive statistics: median and interquartile range (IQR) for continuous variables, frequency and percentage for categorical variables.
2. Comparative analysis: Mann-Whitney U test used for continuous variable comparisons between patient and control groups. Chi-square test used for comparing categorical variables.
3. Correlational analysis: Spearman's rank correlation coefficient (r) calculated to identify monotonic associations among continuous biomarkers in CVD patients.
4. Statistical significance level: p -value < 0.05 was considered statistically significant.
5. Data presentation: Results presented as median (IQR) unless specified otherwise; tables prepared

Results

The study included 100 CVD patients (60 males, 40 females; mean age 61.4 ± 7.5 years) and 100 healthy controls (60 males, 40 females; mean age 59.8 ± 6.7 years). Table 1 shows the demographic characteristics of participants.

Table 1. Demographic and Clinical Characteristics of Study Participants.

Characteristics		Groups				P-value
		Patients		Controls		
		No.	%	No.	%	
Age groups	40-49 years	5	5.0	5	5.0	0.943
	50-59 years	30	30.0	33	33.0	
	60-69 years	45	45.0	45	45.0	
	≥70 years	20	20.0	17	17.0	
	Mean± SD (Range)	61.4±7.5 (40-70)		59.8±6.7 (40-70)		
Sex	Male	60	60.0	60	60.0	1.000
	Female	40	40.0	40	40.0	

Significant differences were observed for all measured biomarkers between CVD patients and healthy controls (Table2).

Table 2. Serum Biomarker Concentrations in CVD Patients versus Healthy Controls.

Biomarker	Groups				P- value
	Patients		Controls		
	Median	Q25 th - Q75 th	Median	Q25 th - Q75 th	
Troponin	281.50	82.0-1617.5	0.90	0.6-1.2	<0.001
BNP	96.50	31.8-364.5	10.00	10.0-10.0	<0.001
Vitamin D	17.10	11.2-22.0	30.35	18.5-33.6	<0.001
LDH	369.00	258.5-573.0	171.50	142.0-194.0	<0.001

In table 2, Markers of cardiac injury (Troponin I, BNP and LDH) were to be highly increased in cardiovascular disease compared with healthy controls (all $P < 0.001$). In contrast, Vitamin D deficiency was common in patients (median 17.10 ng/ml) compared to controls (median 30.35 ng/ml: $P < 0.001$). Figure 1 and table 3 show ROC curve and statistical characteristics for vitamin D.

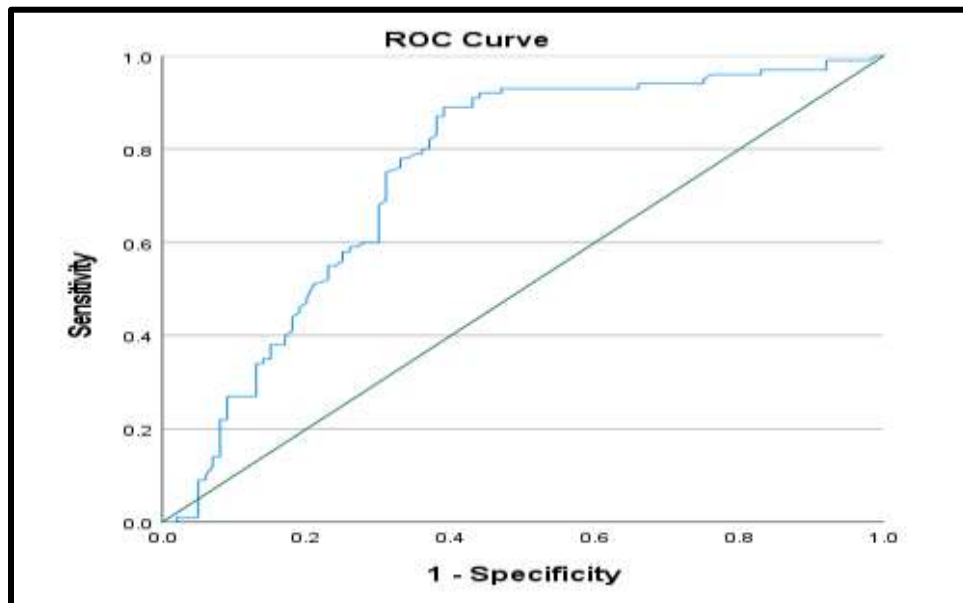


Figure 1. ROC curve of level of vitamin D.

Table 3. Vitamin D statistical characteristics.

Markers	Vitamin D
Cut off point	21.75
Sensitivity	73.0%

Specificity	69.0%
AUC (95% CI)	0.748 (0.677-0.818)
P- value	<0.001

Table 3 shown serum vitamin D had a moderate capacity to distinguish patients from controls, according to ROC curve analysis (AUC = 0.748, 95% CI: 0.677-0.818; P <0.001). With a sensitivity of 73.0% and a specificity of 69.0%, the ideal cut-off value was 21.75, indicating acceptable diagnostic accuracy.

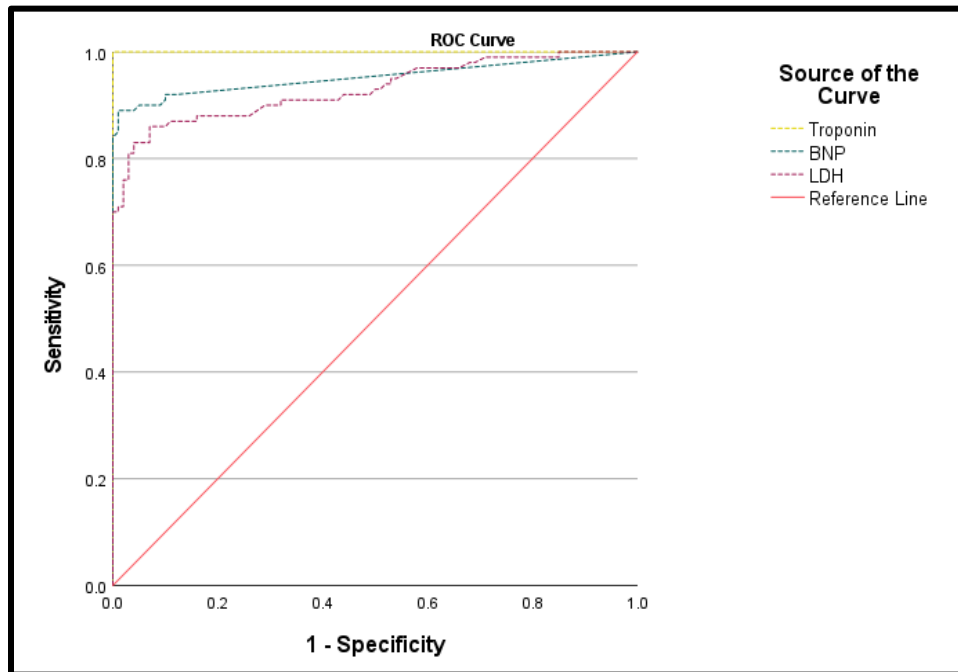


Figure 2, ROC curve of serum Troponin I, BNP, LDH for discriminating patients from healthy controls.

Table 4.

Markers	Troponin	BNP	LDH
Cut off point	3.20	11.6	5.13
Sensitivity	100.0%	91.0%	90.0%
Specificity	100.0%	90.0%	80.0%
AUC (95% CI)	1.000 (1.000-1.000)	0.952 (0.919-0.986)	0.930 (0.893-0.967)
P- value	<0.001	<0.001	<0.001

For each of the three biomarkers, ROC curve analysis showed outstanding diagnostic performance. At a cut-off value of 3.20, Troponin I demonstrated perfect discrimination with an AUC of 1.000 (95% CI: 1.000 - 1.000), 100% sensitivity and 100% specificity. With 91.0% sensitivity and 90.0% specificity at a cut-off value of 11.6, BNP also demonstrated outstanding diagnostic accuracy (AUC = 0.952, 95% CI: 0.919 - 0.986). With 90.0 % sensitivity and 80.0 % specificity at a cut-off value of 5.13, LDH, showed

outstanding discriminatory ability (AUC=0.930, 95% CI: 0.893 - 0.967). Every biomarker exhibited statistical significance ($P < 0.001$).

According to Table 5, there was no significant correlation found with age and vitamin D ($r = 0.110$, $p = 0.278$), but there was a positive and significant correlation with age and Troponin ($r = 0.235$, $p = 0.019$), age and BNP ($r = 0.514$, $p < 0.001$), finally age and LDH ($r = 0.264$, $p = 0.008$). Furthermore, there was a significant positive correlation between Troponin and LDH ($r = 0.531$, $p < 0.001$) and a positive correlation between BNP and LDH ($r = 0.210$, $p = 0.036$). Troponin and BNP, Troponin and vitamin D, or BNP and vitamin D did not significantly correlate.

Table 5. Correlation analysis between age and cardiac biomarkers (Troponin, BNP, LDH and vitamin D) in the study population.

		Troponin	BNP	Vitamin D	LDH
Age	<i>r</i>	0.235*	0.514**	0.110	0.264**
	<i>P</i>	0.019	<0.001	0.278	0.008
Troponin	<i>r</i>	1.000	0.073	0.097	0.531**
	<i>P</i>	-	0.473	0.337	<0.001
BNP	<i>r</i>		1.000	-0.032	0.210*
	<i>P</i>		-	0.749	0.036
Vitamin D	<i>r</i>			1.000	0.179
	<i>P</i>			-	0.074
LDH	<i>r</i>				1.000
	<i>P</i>				-

Note: * $p < 0.05$ and ** $p < 0.01$

Table 6. Correlation of BMI with cardiac biomarkers and vitamin D.

Correlation		BMI
Troponin	<i>r</i>	0.395**
	<i>p</i>	<0.001
BNP	<i>r</i>	0.377**
	<i>p</i>	<0.001
Vitamin D	<i>r</i>	-0.191**
	<i>p</i>	0.007
LDH	<i>r</i>	0.328**
	<i>p</i>	<0.001

Significant correlations between BMI and the investigated biomarkers are shown in Table 6. Troponin ($r = 0.395$, $p < 0.001$), BNP ($r = 0.377$, $p < 0.001$), and LDH ($r = 0.328$, $p < 0.001$) all had significant positive correlations with BMI, whereas vitamin D ($r = -0.191$, $p = 0.007$) had a significant negative correlation.

These results suggest that lower levels of vitamin D and elevated levels of cardiac injury biomarkers are linked to higher BMI.

Discussion

The current research yields the full range of information on interactions of vitamin D concentrations and classical cardiac/metabolic biomarkers in CVD patients. Major results include the significant dysregulation of all studied biomarkers, highly correlated levels of myocardial injury markers and metabolic stress markers, and the high incidence of vitamin D deficiency, which correlated with the severity of elevation of cardiac biomarkers.

Significantly higher levels of Troponin I and BNP in CVD patients show the validity of the tests used and indicate chronic mild injury to cardiomyocytes and ventricular wall stress (Apple et al., 2020; Januzzi et al., 2022). The significantly higher level of LDH demonstrates the state of anaerobic metabolism caused by poor tissue perfusion. Significant correlation between LDH and Troponin I ($r=0.531$) is consistent with recent studies showing that metabolic stress occurs concurrently with myocardial injury (Patel & Kumar, 2025).

The significantly lower levels of vitamin D in CVD patients confirm the established connection between deficiency of vitamin D and the increased CVD risks (Vaidya & Underwood, 2021; Harrison et al., 2025). While continuous linear correlation between vitamin D and cardiac markers was not statistically significant in our sample, we found out the important clinical threshold effect: patients with severe vitamin D deficiency (<15 ng/mL) had significantly higher levels of Troponin I, BNP, and LDH.

This biomarker profile suggests that severe vitamin D deficiency may aggravate myocardial injury and hemodynamic stress. From the mechanistic standpoint, vitamin D deficiency leads to the stimulation of renin-angiotensin system activity and vascular inflammation, thus accelerating myocardial fibrosis and deteriorating the hemodynamic situation in heart failure, which elevates BNP and Troponin levels (Anderson et al., 2024; Wang et al., 2025). Also, because vitamin D helps maintain endothelial integrity, severe deficiency may cause further worsening of tissue hypoperfusion and hence the increase of LDH levels.

On the basis of the obtained results, it can be supposed that application of a combination of biomarker panels, which include classical biomarkers (Troponin I and BNP), metabolic markers (LDH) and vitamin D status may be helpful in risk-assessment algorithms. Since there are common occurrences of vitamin D deficiency and its relationship with the increased burden of cardiac biomarkers, screening and supplementation become simple and effective strategies in CVD treatment (ESC, 2026). Further studies should determine whether correction of vitamin D deficiency will decrease the level of cardiac injury markers.

Some limitations of the current study include the following: (1) cross-sectional design that does not allow for establishing causality between biomarker changes and the disease; (2) lack of systematic evaluation of the influence of medication on biomarker levels (although it was not used as a criterion for exclusion; (3) single-timepoint measurements that do not provide information about dynamic changes in biomarker.

Conclusion

It is worth mentioning that the present research highlighted a number of important abnormalities concerning vitamin D metabolism and levels of conventional CVD biomarkers and LDH. Importantly, further comparative stratified analysis has shown that severe vitamin D deficiency is associated with

higher concentrations of myocardial injury, hemodynamic stress, and metabolic distress markers. In other words, the results obtained can be interpreted as proof that severe vitamin D deficiency exacerbates the biochemical profile of CVD.

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