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# Evaluation of serum vitamin D levels in patients with X syndrome

F.N. TURHAN CAGLAR[1], I. UNGAN[1], V. KSANSKI[1], S. OPAN[1], S. ÇIFTÇI[1], A. KURAL[2], A. KOYUNCU[1], F. AKTURK[1], O. KARAKAYA[1]

[1]Cardiology Department, Dr. Sadi Konuk Bakırkoy Education and Research Hospital, Bakırkoy, Istanbul, Turkey
[2]Biochemistry Department, Dr. Sadi Konuk Bakırkoy Education and Research Hospital, Bakırkoy, Istanbul, Turkey

**Abstract. – OBJECTIVE: Cardiac X syndrome is defined in patients with normal coronary angiogram who has typical chest pain and objective myocardial ischemia evidence. Recent studies have evaluated the association between vitamin D deficiency (vit D def) and cardiovascular diseases. Our aim of this study was to compare serum vit D levels in patients with syndrome X and controls.**

**PATIENTS AND METHODS: We included 66 patients (49 women, 17 men) with syndrome X and 47 (30 women, 17 men) healthy controls. All of the patients' demographic features, laboratory analysis and medications are recorded. Vit D is measured quantitatively by paramagnetic particle chemiluminescence method.**

**RESULTS: Mean age of the syndrome X group was higher than controls (56 ± 9.2 *vs*. 49 ± 9.6 years $p < 0.001$). Body mass index was higher in the patient group than controls (31.2 ± 5.6 *vs*. 29.1 ± 4.7 kg/m$^2$ $p$: 0.011). Vit D levels were significantly lower in the syndrome X group than controls (6 ± 5.2 *vs*. 11.9 ± 7 ng/ml, $p < 0.001$). Parathormone levels were significantly higher in the syndrome X group than the control group (38.3 ± 23.4 *vs*. 28 ± 17.2 pg/ml, $p$: 0.014). hsCRP levels were higher in the syndrome X group than controls (3.1 ± 5.4 *vs*. 1.8 ± 2.4 mg/L, $p$: 0.042).**

**CONCLUSIONS: Our study demonstrated significantly lower vit D levels in patients with CSX. This finding is correlated with previous studies showing an inverse correlation with lower serum vit D levels and different types of cardiovascular diseases. Vit D def may be a risk factor for syndrome X. Vit D def related increased inflammation may lead to the development of endothelial dysfunction and microvascular angina.**

*Key Words:*
Vitamin D, Inflammation, Cardiac syndrome X, Endothelial dysfunction.

## Introduction

Coronary angiograms emerged the fact that not all of the patients with clinical suspicion of coronary artery disease (CAD) have obstructive epicardial CAD[1]. Cardiac syndrome X (CSX) is defined in patients with normal coronary angiograms who have typical chest pain and objective myocardial ischemia evidence[1]. It was first defined by Kemp et al[2] as the "unknown reason of chest pain" in 1973. Although the pathogenesis of CSX is not elucidated yet; previous studies have demonstrated the significant role of impaired coronary microcirculation and endothelial dysfunction[1,3].

Vitamin D (vit D) is a hormone with significant cardiovascular effects[4]. Vitamin D deficiency (Vit D def) is related to increased vascular inflammation and endothelial dysfunction via positive regulation of renin-angiotensin-aldosterone system (RAS)[5,6]. Vit D def and associated increased parathormone may lead to increased insulin resistance; which is also associated with endothelial dysfunction[5,7]. Recent studies revealed an association between vit D def and CAD, heart failure and stroke, and determined vit D def as a risk factor for diabetes mellitus, hypertension, dyslipidemia, endothelial dysfunction, subclinical and clinical atherosclerosis[6,8].

Based on the evidence above our aim of this study was to compare serum vit D levels in patients with CSX and controls.

## Patients and Methods

This study was planned as a cross-sectional study conducted in 66 patients (49 female, 17

*Corresponding Author:* Fatma Nihan Turhan Caglar, MD; e-mail: nhnturhan@gmail.com

male) with CSX and 47 (30 female, 17 male) healthy controls. Patients with typical angina pectoris, normal rest electrogram, positive myocardial perfusion scintigraphy test as the instrumental evidence of ischemia and normal coronary angiogram (CAG) were considered as having CSX. Demographic features like age, sex, smoking, family history, health history and medications were recorded. Body mass index (BMI) was calculated due to World Health Organization criteria.

All patients gave written informed consent, and this study complies with the Declaration of Helsinki. Our study was approved by the Institutional Review Board and Ethics Committee.

Patients with known CAD, left ventricular hypertrophy, coronary artery spasm, parathyroid disease disorders, hyper/hypocalcemia, estimated glomerular filtration rate (eGFR) < 60 ml/min, serious valvular disease, congenital cardiac disease, pregnancy, uncontrolled arterial hypertension, heart failure, serious hepatic failure, acute/chronic infection, fever, immune disease, rheumatic disease, cancer, osteoporosis, older than 75 years old and under vit D treatment were excluded.

All of the patients' laboratory findings were recorded. Electrocardiogram (ECG) and transthoracic echocardiography (TTE) was performed before CAG to all of the patients. Patients who were under antihypertensive treatment or had blood pressure higher than 140 mmHg systolic or 90 mmHg diastolic measured from any arms were considered as hypertensive. Plasma creatinine levels higher than 1.5 mg/dl were considered as a renal failure. Patients with known diabetes, taking antidiabetic medication or who had fasting plasma glucose levels higher than 126 mg/dl were considered as diabetics. Patients who had antihyperlipidemic medication in the last 6 months or had fasting plasma total cholesterol levels ≥ 200 mg/dl, low-density lipoprotein (LDL) levels ≥ 130 mg/dl were considered as hyperlipidemic. TTE recordings were performed while the patients were at left lateral decubitus position using Vivid 7 (GE Healthcare Medical Systems, Wauwatosa, WI, USA) device and 1.5-3.3 Mhz transducer.

### Coronary Angiography (CAG)

CAG was performed using the femoral approach with standard Judkins method. Siemens, Axiom Artis was used as cineangiography device. All of the angiograms were recorded to compact discs in DICOM format and evaluated 'off-line' and visually later. All of the records were analysed by 2 independent cardiologists.

### Vitamin D and Parathormone (PTH)

- **Vit D**: Blood samples collected from all patients were centrifuged and then put into Eppendorf upon protection from light, and stored until studied at -80 degree. All of the samples were studied quantitatively by paramagnetic particle chemiluminescence method at UniCel DxI800 Immunoassay analyser (Beckman Coulter Inc, Brea, CA, USA). Results were expressed as ng/ml.
- **PTH:** Blood samples collected from all patients were centrifuged immediately to separate the serum sample and stored until studied at -80 degrees. All of the samples were studied quantitatively by chemiluminescence method at Immulite 2000 model analyser using original kits (Siemens Healthcare GmbH, Henkestr, Erlangen, Germany). Results were expressed as pg/ml.

### Statistical Analysis

Comparisons of numerical variables; including age, BMI, serum vitamin D levels and other laboratory parameters; were analysed by independent samples *t*-test for normally distributing groups and Mann-Whitney U-test for non-normally distributing groups. Categorical variables were compared by using Chi-Square or Fisher's exact test where appropriate. Spearman test was used to investigate the associations between non-normally distributed numerical variables. A $p$ value less than 0.05 was accepted as statistically significant. Statistical analysis was carried out using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

## Results

Demographic and clinical features of patients with CSX and controls are given in Table I. Mean age of the CSX group was higher than controls (56 ± 9.2 *vs.* 49 ± 9.6 years, $p < 0.001$). BMI was higher in the patient group than controls (31.2 ± 5.6 *vs.* 29.1 ± 4.7 kg/m$^2$, $p$: 0.011). Hypertension was higher in CSX group than controls ($p$: 0.009). Biochemical parameters are given in Table II. Vitamin D levels were significantly lower in the CSX group than controls (6 ± 5.2 *vs.* 11.9 ± 7 ng/ml, $p < 0.001$) (Figure 1).

**Table I.** Clinical and laboratory features of patient and control groups.

| | Patients n (%) | Controls (%) | *p*-value |
|---|---|---|---|
| Number of cases | 66 (58) | 47 (42) | |
| **Age (median ± SD) (years)** | **56 ± 9.2** | **49 ± 9.6** | **< 0.001*** |
| **BMI (median±SD) (kg/m²)** | **31.2 ± 5.6** | **29.1 ±4.7** | **0.011*** |
| Gender | | | 0.23 |
| Female n (%) | 49 (74.2) | 30 (63.8) | |
| Male n (%) | 17 (25.8) | 17 (36.2) | |
| Smoking | | | 0.06 |
| Yes n (%) | 10 (15.2) | 14 (29.8) | |
| No n (%) | 56 (84.8) | 33 (70.2) | |
| Diabetes | | | 0.41 |
| Yes n (%) | 17 (25.8) | 9 (19.1) | |
| No n (%) | 49 (74.2) | 38 (80.9) | |
| Hypertension | | | 0.009 |
| Yes n (%) | 39 (59.1) | 16 (34) | |
| No n (%) | 27 (40.9) | 31 (66) | |
| Hyperlipidemia | | | 0.21 |
| Yes n (%) | 18 (27.3) | 18 (38.3) | |
| No n (%) | 48 (72.7) | 29 (61.7) | |
| Family history | | | 0.47 |
| Yes n (%) | 17 (25.8) | 15 (31.9) | |
| No n (%) | 49 (74.2) | 32 (68.1) | |

Chi-square test, *Student *t*-test: BMI: body mass index.

Parathormone levels were significantly higher in CSX group than the control group (38.3 ± 23.4 *vs*. 28 ± 17.2 pg/ml, *p*: 0.014) (Table II). hsCRP levels were higher in the CSX group than controls (3.1 ± 5.4 *vs*. 1.8 ± 2.4 mg/L, *p*: 0.042) (Table II). Neutrophil/lymphocyte ratio was significantly lower in the CSX group than controls (1.67 ± 0.72 *vs*. 1.98 ± 0.66, *p*: 0.021) (Table II).

Correlation analysis revealed no correlation between vit D levels and hsCRP levels (Ro=-0.11, $p = 0.22$), lymphocyte count (Ro = -0.07, $p = 0.4$), neutrophil count (Ro = 0.036, $p = 0.7$) and neutrophil/lymphocyte ratio (NLR) (Ro = 0.06, $p = 0.47$) (Spearman test) (Table III).

**Table II.** Comparison of serum levels of vitamin D and other laboratory parameters

| | Patients (median ±SD) | Controls (median ± SD) | *p*-value |
|---|---|---|---|
| **Vitamin D ng/ml** | **6 ± 5.2** | **11.9 ± 7.8** | **< 0.001**** |
| **hsCRP mg/L** | **3.1 ± 5.4** | **1.8 ± 2.4** | **0.042**** |
| **PTH pg/ml** | **38.3 ± 23.4** | **28 ± 17.2** | **0.014** |
| Urea mg/dl | 30 ± 8.8 | 28 ± 9.8 | 0.66 |
| Creatinine mg/dl | 0.7 ± 0.2 | 0.7 ± 0.1 | 0.16 |
| LDL mg/dl | 131.5 ± 34.6 | 118 ± 30 | 0.15 |
| Total Cholesterol mg/dl | 204.5 ± 45.8 | 201 ± 37.3 | 0.33 |
| HDL mg/dl | 48 ± 11.4 | 48 ± 10.5 | 0.95 |
| Triglyceride mg/dl | 137 ± 78.4 | 151 ± 72 | 0.89 |
| Uric acid mg/dl | 5.1 ± 1.2 | 5.3 ± 1.4 | 0.93 |
| WBC $10^3/\mu$L | 6750 ± 1510 | 6700 ± 1500 | 0.94 |
| Hemoglobin g/dl | 13.5 ± 1.5 | 13 ± 1.6 | 0.49 |
| Platelet/$\mu$L | 266000 ± 63400 | 276000 ± 48900 | 0.39 |
| Lymphocyte /$\mu$L | 2000 ± 540 | 2110 ± 690 | 0.055 |
| Neutrophil /$\mu$L | 3810 ± 1110 | 3690 ± 1000 | 0.38 |
| **NLR** | **1.98 ± 0.66** | **1.67 ± 0.72** | **0.021**** |

Student *t* test, *Mann-Whitney U test, SD: standart deviation. hsCRP: high sensitive C-reactive protein, PTH: parathormone, LDL: low density lipoprotein, HDL: high density lipoprotein, WBC: white blood cell NLR: neutrophil/lymphociyte ratio.

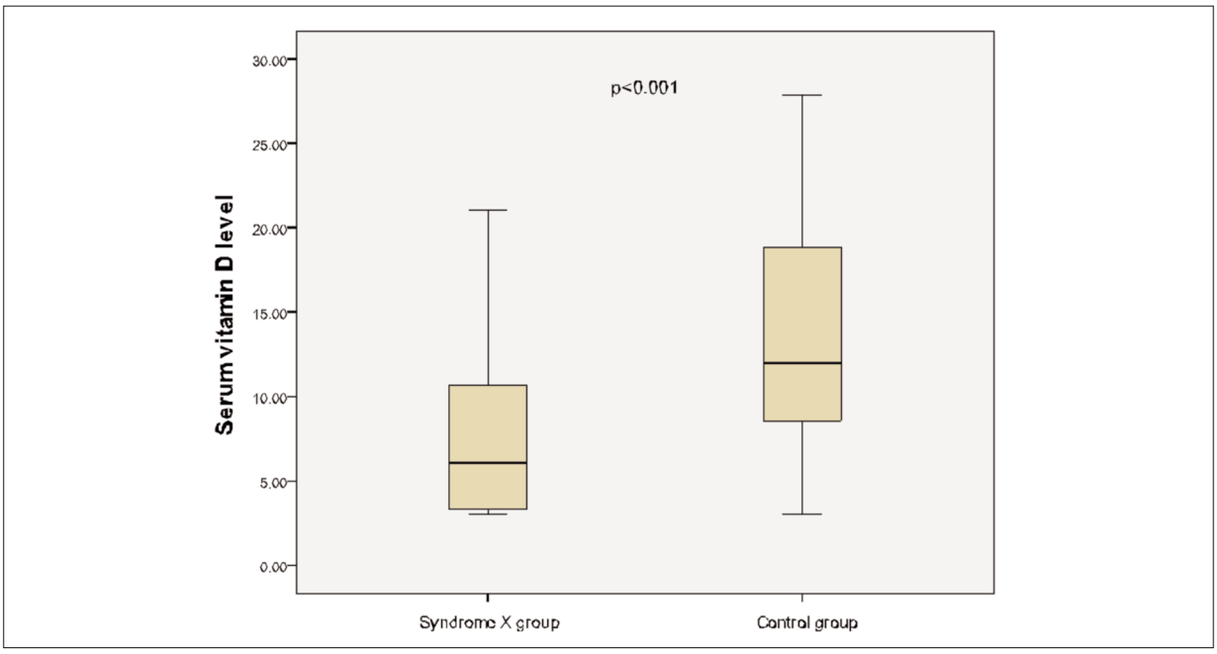


**Figure 1.** Distribution of serum vitamin D levels in syndrome X patients and controls (Box-plot).

## Discussion

Our study demonstrated significantly lower vit D levels in patients with CSX. This finding is correlated with previous studies showing an inverse correlation with lower serum vit D levels and different types of cardiovascular diseases[5,7].

CSX is considered as a type of cardiovascular disease although the underlying mechanism of chest pain in patients with normal coronary arteries is not fully understood yet[1]. Microvascular angina, myocardial ischemia, impaired coronary blood flow, increased pain threshold and endothelial dysfunction have been proposed as the pathophysiological mechanism of CSX[9-14].

Vit D is a hormone that is effective on cardiovascular system through unclear mechanisms[4].

**Table III.** Correlation analysis between vit D and some other biomarkers.

| | Vit D |
|---|---|
| hsCRP | Ro = -0.11, $p = 0.22$ |
| lymphocyte | Ro = -0.07, $p = 0.4$ |
| neutrophil | Ro = 0.036, $p = 0.7$ |
| NLR | Ro = 0.06, $p = 0.47$ |

Vit D: vitamin D, hsCRP: high sensitive C-reactive protein, NLR: neutrophil/lymphocyte ratio.

Vitamin D receptors are located in all tissues including vascular smooth muscle cells and cardiomyocytes[15]. Jablonski et al[16] demonstrated for the first time that endothelial function is inversely associated with serum vit D levels in asymptomatic middle-aged/older adults. They also revealed an association between vit D status and pro-inflammatory $NF_kB$-IL-6 activation in vascular endothelial cells[16]. Yiu et al[17] showed endothelial dysfunction in vit D deficient patients with type 2 diabetes. Tarcin et al[7] not only demonstrated an association between vit D def and endothelial dysfunction; but also showed improvement of endothelial function after vit D replacement therapy in their study. Scragg et al[18] suggested that the lower vit D levels may explain the higher cardiovascular disease incidence observed in winter than summer. Framingham Offspring study[19] revealed 53-80% higher major cardiovascular event risk in asymptomatic patients with low vit D levels after 5.4 years of follow-up.

Although some of the studies demonstrated higher female prevalence in CSX population, the number of female patients in the CSX group was similar to the control group in our study. Nevertheless, our finding is similar to Pasternak et al[20]and Kemp et al's[21] reports in which neither of them could not show such a difference in their series.

Traditional CAD risk factors are known to be similar in patients with CSX and general popu-

lation[1]. However, the number of hypertensive patients were higher in CSX group than controls in our study. This result can be related to the findings of previous studies demonstrating an inverse association between serum vit D status and hypertension[5]. Active vit D is an endogenous inhibitor of RAS; therefore, vit D def is associated with excessive RAS activation and hypertension[15].

hsCRP synthesis is regulated by cytokines such as IL-6, IL-10 and TNF-□ and, on the other hand, vit D may suppress the release of these cytokines[5]. In this context, Müller et al[22] demonstrated an inverse correlation between serum vit D and TNF-□ levels. Although hsCRP was not correlated with serum vit D levels in our study, hsCRP levels were significantly higher in CSX group than controls in our study. Similarly, Luo et al[23] and Sakr et al[24] also showed higher hsCRP levels in patients with CSX than controls. Endothelial dysfunction and systemic inflammation related with elevated serum hsCRP levels may play a role in CSX pathogenesis[23].

Yurtdas et al[25] and Okyay et al[26] showed higher NLR in patients with CSX than controls. Similarly, NLR was higher in CSX group than controls in our study. As NLR is a surrogate marker of inflammation, this finding is consistent with our hypothesis suggesting increased inflammation and endothelial dysfunction via vit D def, and consequent CSX development.

To our knowledge, our study is the first to evaluate vit D levels in patients with CSX. In a similar manner, Andishmand et al[3] revealed clinical improvement after vit D hormone replacement in CSX patients with vit D def. However, they only included patients with CSX and low serum vit D levels. They did not have a control group[3].

## Conclusions

Our findings suggest that Vit D def may be associated with CSX. Vit D def related increased inflammation may lead to the development of endothelial dysfunction and microvascular angina. Further larger randomized placebo-controlled studies are needed to confirm our results for reproducibility.

A limitation of our study is the small and limited nature of our study. Other markers of endothelial dysfunction were not evaluated in this cross-sectional study. Although vit D status is affected by seasonal fluctuations and dietary intake we could only measure vit D once in this cross-sectional study. We would like to prescribe vit D replacement therapy in our CSX patients with low vit D levels; however, we did not have ethical approval.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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