Could decreased vitamin D levels be related with impaired cardiac autonomic functions in patients with chronic heart failure: An observational study

Metin Çetin, Güliz Kozdağ, Dilek Ural*, Göksel Kahraman*, İrem Yılmaz*, Yaşar Akay*, Raşit Onuk*, Nigar Dursun***

Clinic of Cardiology, Çerkezköy State Hospital; Tekirdağ-*Turkey*
Department of *Cardiology, **Physical Medicine and Rehabilitation, Faculty of Medicine, Kocaeli University; Kocaeli-*Turkey*

Abstract

Objective: Vitamin D status has been implicated in the pathophysiology of heart failure (HF). The aim of this study was to investigate the association between vitamin D levels with heart rate variability and heart rate turbulence in patients with heart failure whom had ischemic and non-ischemic dilated cardiomyopathy.

Methods: Study designed as an observational cross-sectional study. Seventy-one patients [36 non-ischemic dilated cardiomyopathy (NIDCM), 35 ischemic dilated cardiomyopathy (IDCM)] with chronic heart failure and 25 control subject were included. It was evaluated the association between 25 hydroxyvitamin D [25(OH)D] and calcitriol levels with heart rate variability time domain (SDNN, SDANN, RMSSD) and heart rate turbulence [turbulence onset (TO), turbulence slope (TS)] parameters. Statistical analysis was performed using Kruskal-Wallis test and ANOVA.

Results: Calcitriol levels in NIDCM patients with abnormal TO and TS were significantly lower than NIDCM patients with normal TO (17.1±11.3 vs. 27.6±15.5 pg/mL, p=0.05) and TS (16.6±9.1 vs. 29.4±16.9 pg/mL, p=.018). There was a positive correlation between 25 (OH) D with heart rate variability parameters SDNN (r=0.368, p=0.027) and SDANN (r=0.360, p=0.031). It was not found any association between vitamin D and parameters of heart rate variability and heart rate turbulence in IDCM patients.

Conclusion: Insufficiency of vitamin D may have deleterious effects on cardiac autonomic functions which were showed with heart rate turbulence and heart rate variability in patients with NIDCM. Vitamin D levels might be a predictor to determine the sudden cardiac death in patients with non-ischemic etiology. *(Anadolu Kardiyol Derg 2014; 14: 434-41)*

Key words: 25 hydroxy vitamin D, calcitriol, dilated cardiomyopathy, heart rate variability, heart rate turbulence, sudden cardiac death

Introduction

Congestive heart failure (HF) is a chronic disease that incidence is growing in the population (1). Despite recent advances in therapy, HF carries an unacceptably high mortality rate (2, 3). There is an accumulating body of evidence that vitamin D insufficiency is a frequent finding in patients with HF and its insufficiency plays an important role in the etiology and pathogenesis of congestive HF. Low vitamin D status is associated with increased activity of the renin-angiotensin-aldosterone system causing arterial hypertension and myocardial hypertrophy. Inflammatory actions significantly increase with poor vitamin D status· Vitamin D metabolites have direct effects on cardiomyocytes including anti-hypertrophic actions, regulation of extracellular matrix turnover, and modulation of contractility (4-6). In addition vitamin D insufficiency has effect on HF prognosis. Several further studies have shown associations of low vitamin D concentrations with cardiovascular events including sudden cardiac death and mortality with HF patients (4, 5, 7, 8).

Heart rate variability (HRV) (9-11) and heart rate turbulence (HRT) (12-14) that reflect cardiac autonomic imbalance have been introduced as useful techniques in identifying patients at high risk for cardiac events including sudden cardiac death with HF. There is not enough data about relationship with vitamin D status and cardiac autonomic function. The aims of this study were to determine whether a low plasma vitamin D levels is associated with an impaired cardiac autonomic functions in patients with HF or not.

Address for Correspondence: Dr. Metin Çetin, Çerkezköy Devlet Hastanesi, 59500 Çerkezköy, Tekirdağ-*Türkiye*
Phone: +90 532 410 33 77 E-mail: metincetin81@yahoo.com
Accepted Date: 13.12.2013 **Available Online Date:** 26.05.2014
©Copyright 2014 by Turkish Society of Cardiology - Available online at www.anakarder.com
DOI:10.5152/akd.2014.4869

Methods

Study design: an observational cross-sectional study.

Patient population

Seventy-one patients with chronic heart failure and 25 healthy controls were included into the study between November 2009-May 2011 at Kocaeli University, Hospital of Medical Faculty. Thirty-six of patients had non-ischemic dilated cardiomyopathy (NIDCM), thirty-five of them had ischemic dilated cardiomyopathy (IDCM). Patients who recruited into the study were hospitalized due to decompensated chronic heart failure. DCM was diagnosed based on left ventricular systolic dysfunction (LVEF ≤45%) and left ventricular dilatation [left ventricular diastolic dimension (LVDD) >55 mm]. Ischemic DCM was determined if any of the following criteria were met: (1) stenosis of a major coronary artery of ≥50% on angiography; (2) a history of percutaneous coronary revascularization; (3) a history of coronary artery bypass graft surgery or (4) a documented history of myocardial infarction. All other etiologies were considered to be non-ischemic. All patients had sinus rhythm, available 24 hours Holter recording, normal blood calcium levels. The bone mineral densitometry was done to determine of the osteoporosis in the study group. Exclusion criteria's were as follows: chronic kidney disease, chronic liver disease, diabetes mellitus, atrial fibrillation, thyroid or parathyroid disease or recent surgery, cancer, using drugs effect on autonomic nervous system and current smokers.

This study conforms to the Declaration of Helsinki, local Ethics Committees approved the study, and all patients provided written informed consent. The clinical characteristics of the patient population are listed in Table 1.

Vitamin D analysis

Venous blood samples were obtained non-fasting in first three days during the hospitalization. 25 hydroxyvitamin D [25 (OH) D] and its acting form calcitriol were measured. 25 (OH) D was measured in ng/mL by enzyme-linked immunosorbent assay (EIA; Immuno Diagnostic Systems, Boldon, UK). Patients with 25 (OH) D levels ≤20 ng/mL are considered vitamin D insufficient. Calcitriol was measured in pg/mL by enzyme-linked immunosorbent assay (EIA; Immuno Diagnostic Systems, Boldon, UK).

Measurement of heart rate variability

Heart rate variability (HRV) was assessed from 24-hour Holter ECGs recordings using previously described and validated methods (15, 16). Holter recordings were performed after hemodynamic stabilization of patients. Holter recordings were analyzed using the Cardio Navigator Holter system (Del Mar Reynolds Medical Ltd, UK). After the labeling process, the data file was verified, manually over read, and corrected where appropriate. Heart rate variability was analyzed using the HRV analysis module in Cardio Navigator Holter system. For the purposes of this study, 3 different HRV indices were measured: (1) the standard deviation of all normal-to-normal RR intervals (SDNN), (2) the standard deviation of the average normal to normal interval (SDANN) and (3) the square root of the mean of the squares of the differences between adjacent normal-to-normal RR intervals (RMSSD).

Measurement of heart rate turbulence

Heart rate turbulence was calculated from the same Holter recordings. HRT was automatically measured using software HRT, view application. HRT parameters included turbulence onset (TO) and turbulence slope (TS), which were determined according to a previously published method (14-17). The TO is defined as the difference between the mean of the first two sinus RR intervals after a ventricular premature beat (VPB) and the last two sinus RR intervals before the VPB divided by the mean of the last two RR intervals before the VPB [(RR1 +RR2)-(RR-2 +RR-1)/(RR-2 +RR-1)], thus, expressing the proportional RR interval decrease immediately after the compensatory pause of the VPB. Positive values of TO reflect sinus rhythm deceleration after VPB, whereas negative values indicate acceleration of the sinus rhythm. The TS is defined as the steepest slope of a regression line assessed over any sequence of 5 subsequent RR intervals within the first 15 sinus rhythm intervals after the VPB (unit: ms/RR interval) thus expressing the subsequent RR interval increase. The TO and TS were dichotomized at predefined cut-off points (TO=0%, TS=2.5 ms/RR interval) (14). HRT abnormal patients defined as when TO or TS were abnormal (TO≥0% or TS≤2.5 ms/RR interval) in this study.

Statistical analysis

The SPSS 13.0 (SPSS Inc., an IBM company; Chicago, Ill) statistical software package was used for statistical analyses. Results are presented as mean±SD or as percentages and numbers for categorical data. Normality tests were used for all variables. Continuous variables that were normally distributed were analyzed with using *t*-test for Independent samples, and unequally distributed variables were analyzed with Mann-Whitney U test. To compare values within the 3 groups, a 1-way ANOVA analysis of variance was used and Kruskal-Wallis test was applied for analysis of abnormally distributed variables. Homogeneity of variances was tested for all variables with Levene's test. If equal variances were assumed, Tukey's HSD posthoc test was applied; if not, the Tamhane T2 test was used to compare the parameters within groups. The Bonferroni correction was used to determine statistically significant values among patient groups. Categorical data and proportions were analyzed using the κ^2. Correlations between 25 (OH) D and calcitriol with other variables were determined by Spearman correlation analysis.

Results

According to baseline characteristics there were no difference with age, sex (NIDCM group; mean age 59±14 years, 22

Table 1. The clinical characteristics of the patient and control population

	NIDCM (n=36)	IDCM (n=35)	Control (n=25)	Chi-square	f
Main characteristics					
Age, years	59±14	62±10	56±9	31.07	33.57
Sex, Male/Female	22 (61%)/14 (39%)	26 (74%)/9 (26%)	12 (48%)/13 (52%)	5.44	3.00
BMI, kg/m^2	28±4	28±5	27±4	2.83	1.45
NYHA class, I - III	2.1±0.5*	2.2±0.5**	1.0±0.2	41.67	54.81
Sun exposure, hour/day	3.8±2.1	2.4±2.3	4.6±2.2	2.37	1.47
Hypertension	3 (8%)	1 (3%)	0 (0%)	3.12	1.86
Systolic BP, mm Hg	110±15	102±11	119±16	17.63	14.86
Diastolic BP, mm Hg	69±10	64±9	75±10	15.89	11.73
LVEDD, mm	67.2±8.6*	64.0±7.8**	48.0±3.7	36.82	54.85
LVEF, %	21.6±7.1*	22.4±7.3**	67.2±6.7	37.08	35.29
Labarotory parameters					
Hemoglobin, g/dL	13.2±1.6	13.3±1.7	13.9±1.4	0.02	1.97
Calcium, mg/dL	9.3±0.4	9.0±0.6^{++}	9.4±0.3	1.88	1.61
Creatinin, mg/dL	0.9±0.2^{+}	1.0±0.1**	0.8±0.2	18.65	13.08
eGFR, mL/min/1.73 m^2	82±17*	77±18**	100±17	16.70	12.90
hs-CRP, mg/dL	0.484±0.430	0.934±1.233^{++}	0.345±0.299	10.01	5.25
BNP, pg/mL	473±825^{+}	498±683^{++}	29±20	29.83	4.32
ECG Holter parameters					
Mean heart rate	76±12	73±11^{++}	80±9	4.86	2.20
Corrected QT duration, ms	436±36	414±72	418±40	0.01	1.59
Sustain VT	4 (11%)	2 (6%)	0 (0%)	1.00	5.50
Non-sustain VT	4 (11%)	3 (9%)	0 (0%)	6.09	2.74
Medication					
Aspirin	27 (75%)	35 (100%)$^{\#\#}$	No drug use	45.23	67.20
Beta blocker	30 (83%)	25 (71%)		25.24	35.49
ACE-inhibitor	18 (50%)	21 (60%)		10.56	6.57
Angiotensin-II-antagonist	17 (47%)	6 (17%)$^{\#\#}$		4.26	11.20
Statins	9 (25%)	27 (77%)$^{\#}$		32.66	35.43
Fibrats	1 (3%)	3 (5%)		2.04	1.02
Diuretics	27 (75%)	25 (71%)		27.56	34.30
Nitrats	1 (3%)	9 (26%)$^{\#\#}$		9.33	8.55
Digoxin	7 (19%)	0 (0%)$^{\#\#}$		0	7.57
Spironolactone	20 (56%)	11 (31%)		9.33	8.02
Calcium channel blocker	4 (11%)	8 (23%)		1.46	0.73

Statistics were done using by One-Way ANOVA and Kruskal-Wallis test, posttest for pairwise comparisons: if 2 group variables carry the same superscript (*,$^{+}$,**, $^{++}$) it means that the difference between these two variables significant after posttest pairwise comparison. Statistics were done by κ^2 test: if 2 group variables carry the same superscript ($^{\#}$, $^{\#\#}$) in this Table
*Significant difference between NIDCM with control group (p<0.001); $^{+}$Significant difference between NIDCM with control group (p<0.05); **Significant difference between IDCM with control group (p<0.001); $^{++}$Significant difference between IDCM with control group (p<0.05); $^{\#}$Significant difference between NIDCM with IDCM group (p<0.001); $^{\#\#}$Significant difference between NIDCM with IDCM group (p<0.05)
ACE - inhibitor: angiotensin-converting enzyme inhibitor; BMI - body mass indeks; BNP - brain natriuretic peptide; BP - blood pressure; eGFR - estimated glomerular filtration rate; hs-CRP - high sensitive C-reactive protein; IDCM - ischemic dilated cardiomyopathy; LVEDD - left ventricular end-diastolic diameter; LVEF - left ventricular ejection fraction; NIDCM - non-ischemic dilated cardiomyopathy; VT - ventricular tachycardia

male, 14 female, IDCM group; mean age 62±10 years, 26 male, 9 female, control group; mean age 56±9 years, 12 male, 13 female), BMI and sun exposure within three groups. There were no significant differences with other clinical variables between two DCM groups except medications. The number of patients who were using angiotensin-II-antagonist and digoxin were

Table 2. Vitamin D status of the patient and control population

	NIDCM (n=36)		IDCMP (n=35)		Control (n=25)	Chi-square	f
25 (OH) D, mean, ng/mL	14.3±6.2*		15.8±6.5**		33.6±14.3	22.32	29.87
<10 ng/mL	14 (39%)		8 (23%)**		1 (4%)		
10-20 ng/mL	13 (36%)		19 (54%)**		3 (12%)		
21-30 ng/mL	8 (22%)	*	6 (17%)**	**	8 (32%)	19.18	22.06
>30 ng/mL	1 (3%)		2 (6%)**		13 (52%)		
Calcitriol, pg/mL	21.6±13.8		20.3±12.5		31.0±9.8	0.17	0.11

Statistics were done using by Kruskal-Wallis test, One-Way ANOVA posttest for pairwise comparisons: if 2 group variables carry the same superscript (*, **) it means that the difference between these two variables significant after posttest pairwise comparison in this table.
*Significant difference between NIDCM with control group (p<0.001); ** Significant difference between IDCM with control group (p<0.001)
IDCM - ischemic dilated cardiomyopathy; NIDCM - non- ischemic dilated cardiomyopathy

Table 3. HRV and HRT values of patients and control

	NIDCM (n=36)	IDCM (n=35)	Control (n=25)	Chi-square	f
SDNN, ms	94.5±41.8*	86.2±39.9**	130.2±26.2	18.98	11.13
SDANN, ms	79.6±27.9*	70.0±33.7**	121.2±28.3	28.98	22.18
RMSSD, ms	23.2±13.5	21.1±11.9++	30.2±9.7	12.08	4.27
TO, %	-0.0051±0.0457	-0.0167±0.480	-0.0668±0.1998	8.87	1.75
TS, ms/RR	2.3922±1.8890*	2.3580±2.0948**	4.6350±1.7615	15.43	11.90
TO abnormal	20 (56%)*	15 (43%)++	3 (12%)	4.99	6.03
TS abnormal	20 (56%)*	20 (57%)**	1 (4%)	21.77	16.58

Statistics were done using by Kruskal-Wallis test and One-Way ANOVA posttest for pairwise comparisons: if 2 group variables carry the same superscript (*, **,+) it means that the difference between these two variables significant after posttest pairwise comparison in this table
*Significant difference between NIDCM with control group (p<0.001); **Significant difference between IDCM with control group (p<0.001); ++Significant difference between IDCM with control group (p<0.05)
IDCM - ischemic dilated cardiomyopathy; NIDCM - non - ischemic dilated cardiomyopathy, SDNN - standard deviation of all normal-to-normal RR intervals; SDANN - standard deviation of the average normal to normal interval; RMSSD - squares of the differences between adjacent normal-to-normal RR intervals; TO - turbulence onset; TS - turbulence slope

much more in NIDCP group, number of patients using aspirin, statins and nitrates were much more in IDCM group (Table 1). There was no statistical difference of HRV and HRT values among patients who were taken digoxin, ARB, diuretic, statin and nitrates and those who were not taken above mentioned medications (digoxin, ARB, diuretic, statin and nitrates) within three groups.

Outcome of analysis of vitamin D

Baseline characteristics according to 25(OH) D and calsitriol are presented in Table 2. Mean 25 (OH) D levels were lower both in NIDCM (14.3±10.6 ng/mL) and IDCM (15.8±6.5 ng/mL) groups and it was significantly different when compared with control (33.6±14.3 ng/mL) group (p<0.001). 27 (75%) of NIDCM, 25 (71%) of IDCM patient and 4 (16%) of control subjects had deficient levels of 25 (OH) D concentration that described by most guidelines [<20 ng/mL (50 nmoL/L)] (18). Although calcitriol levels of two patients group were lower compared to control group, there were no statistically differences in calcitriol levels within three groups.

Outcome of measurement of HRV and HRT

Comparisons of mean values of HRV and HRT indices in patients and control groups were demonstrated in Table 3. Patients with NIDCM and IDCM as compared with control subjects had reduced HRV (SDNN, SDANN and RMSSD). Reduction on SDNN and SDANN indices in both DCM groups were statically significant (p<0.001) when compared with control. Within RMSSD values there was significant difference only between IDCM and control (p<0.05). There were not any differences with HRV indices between DCM groups.

Average TS values were significantly lower in DCM groups compared with control (p<0.001). There were no differences within average TO values. 20 (56%) patient with NIDCM, 15 (43%) patient with IDCM, 3 (12%) of control subjects had abnormal TO, 20 (56%) patient with NIDCM, 20 (57%) patient with IDCM, 1 (4%) of control subjects had abnormal TS, respectively. Abnormal TO and TS ratios were same in DCM groups but significantly more than control group.

Association of vitamin D with HRV and HRT

All groups were divided in two subgroups of 25 (OH) D concentration <20 ng/mL and >20 ng/mL. Association between deficiency of 25 (OH) D level and HRV indices were evaluated. In NIDCM patients with 25 (OH) D deficiencies SDNN, SDANN and RMSSD parameters were lower than others but not statistically significant. In IDCM and control groups there were no association with 25 (OH) D levels and HRV. We did not find any statistical

Table 4. The association between 25 (OH) vitamin D level with HRV

25 (OH) D, ng/mL		n	SDNN	SDANN	RMSSD
NIDCM (n=36)	≤20	27	90.2±36.3	78.5±29.8	22.6±13.2
	>20	9	107.4±56.0	83.0±22.2	25.2±15.1
IDCM (n=35)	≤20	27	86.3±44.4	69.5±6.8	21.7±13.0
	>20	8	83.0±20.2	67.8±23.0	19.8±9.0
CONTROL (n=25)	≤20	4	134.0±11.5	124.2±16.5	31.5±9.8
	>20	21	129.5±28.3	120.6±30.3	30.0±10.0

Statistics were done using by Student t-test and by Mann-Whitney U test in this table. There were not significant differences between HRV indices in groups
IDCM - ischemic dilated cardiomyopathy; NIDCM - non-ischemic dilated cardiomyopathy; SDNN - standard deviation of all normal-to-normal RR intervals; SDANN - standard deviation of the average normal to normal interval; RMSSD - squares of the differences between adjacent normal-to-normal RR intervals; TO - turbulence onset; TS - turbulence slope

differences in SDNN, SDAN and RMSSD when we compared those in patients who had level of 25OH D level below 20 ng/mL and higher 20 ng/mL. Details are shown in Table 4. There was a positive correlation between 25 (OH) D levels with SDNN (r=0.368, p=0.027), SDANN (r=0.360, p=0.031) and negative correlation with QTc durations (r=-0.340, p=0.042) in patient with NIDCM (Table 5). In all groups there was no association of calcitriol with HRV.

Comparison of vitamin D levels with abnormal TO and TS showed that, calcitriol levels were significantly lower in NIDCM patients with abnormal TS (p=0.018) and abnormal TO (p=0.05) than with normal TS and normal TO. There was not same association in IDCM and control groups. 25 (OH) D levels did not show any association with HRT. Details are listed in Table 6.

Discussion

In this study, as expected the patients with ischemic and non-ischemic DCM had lower levels of 25 (OH) D and the reduced HRV indices (such as SDNN and SDANN) than the control subjects. Although TS values were statistically lower in the patient groups than the control group, TO values were not statistically different among three groups. In patients with NIDCM, 25 (OH) D were positively correlated with SDNN and SDANN. The calcitriol levels in NIDCM patients with abnormal TO and TS were significantly lower than NIDCMP patients with normal TO and TS.

In previous studies it was reported that patients with heart failure had decreased vitamin D levels (19-21). The lowest values were between 9.6±5.8 ng/mL and 11.4±6.6 ng/mL determined by Zitterman et al. (19) Our findings confirm and extends the results of previous studies that patients with HF exhibit low vitamin D status.

Recent studies demonstrated the association of low vitamin D concentrations with cardiovascular events and all-cause mortality (4-6, 22). However there are only two studies those showed the association of low levels of 25(OH) D and calcitriol level with sudden cardiac death (SCD) (7, 8). First, Pilz et al. (7)

Table 5. Correlations between 25 (OH) vitamin D with SDNN, SDANN and QTc

		25 (OH) D	
		r	*P*
NICMP (n=36)	SDNN	0.368	0.027
	SDANN	0.360	0.031
	QTc (ms)	-0.340	0.042
ICMP (n=35)	SDNN	-0.025	NS
	SDANN	-0.030	NS
	QTc (ms)	-0.054	NS

Statistics were done using by Spearman correlation analysis in this table.
NS - not significant; ICMP - ischemic dilated cardiomyopathy; NIDCM - non-ischemic dilated cardiomyopathy; SDNN - standard deviation of all normal-to-normal RR intervals; SDANN - standard deviation of the average normal to normal interval; RMSSD - squares of the differences between adjacent normal-to-normal RR intervals; TO - turbulence onset; TS - turbulence slope

evaluate the risk of SCDs in cohort of the patients who referred for coronary angiography. During median follow-up time of 7.7 years, after adjustment for cardiovascular risk factors, risk for death due to SCDs were higher [HR: 5.05, 95% confidence interval (CI): 2.13-11.97] when comparing patients with severe vitamin D deficiency [25 (OH) D <25 nmoL/liter)] with people in the optimal range [25 (OH) D >75 nmoL/liter]. Results were similar with both 25 (OH) D and calcitriol. This finding was supported by Drechsler, et al. (8) in the hemodialysis patients. 25 (OH) D was measured in 1108 diabetic patients who have had hemodialysis with followed up for a median of 4 years. According to baseline 25 (OH) D levels, the vitamin D deficiency had a 3 fold higher risk of SCDs compared with those with sufficient 25 (OH) D levels [HR: 2.99, 95% confidence interval (CI): 1.39-6.40] (8). The underlying pathways of SCD in patients with vitamin D deficiency are still unclear. Altered myocardial calcium flux and increased risk of SCD related to a poor vitamin D status suggest a link to cardiac arrhythmias (23-25). This notion is in line with the observations in hemodialysis patients showing that calcitriol treatment reduced a prolonged QTc dispersion (23), which is a risk factor for SCD and this relationship might be causal. Our findings supported the positive correlation between 25 (OH) D with QTc, in NIDCM patients.

Our study is the first to highlight the relation between vitamin D deficiency and autonomic imbalance. We demonstrated that, 25 (OH) D and calcitriol were related with HRV and HRT indices that reflect the activity of the autonomic nervous system. NIDCM patients with abnormal HRT had low calcitriol levels and 25 (OH) D levels were positively correlated with HRV. As we know HF is characterized by the autonomic dysfunction that cause increased risk for the arrhythmias, sudden death, and increased mortality (26-30). According to our data, low concentrations of vitamin D may have negative effects on autonomic balance and cause ventricular arrhythmias. Association of low levels of vitamin D with autonomic imbalance might be one of the possible causal mechanisms for the pathogenesis of SCD. Interestingly, the risk for the SCD was higher for study partici-

Table 6. Association between 25 (OH) vitamin D, Calcitriol with HRT

		TS	N	Mean±SD	*P*
NIDCM (n=36)	25 (OH) D	Normal	16	13.8±6.8	NS
		Abnormal	20	15.5±12.9	
	Calcitriol	Normal	20	29.4±16.9	0.018
		Abnormal	16	16.6±9.1	
IDKMP (n=35)	25 (OH) D	Normal	15	15.2±7.6	NS
		Abnormal	20	16.3±6.2	
	Calcitriol	Normal	24	26.0±16.8	NS
		Abnormal	11	17.7±8.4	
Control (n=25)	25 (OH) D	Normal	24	33.2±14.5	NS
		Abnormal	1	42.6	
	Calcitriol	Normal	24	31.8±9.7	NS
		Abnormal	1	20.0	
		TO			
NIDCM (n=36)	25 (OH) D	Normal	16	15.7±6.6	NS
		Abnormal	20	14.2±13.0	
	Calcitriol	Normal	21	27.6±15.5	0.050
		Abnormal	15	17.1±11.3	
IDCMP (n=35)	25 (OH) D	Normal	20	80.8±52.9	NS
		Abnormal	20	15.5±6.1	
	Calcitriol	Normal	15	16.2±7.4	NS
		Abnormal	25	22.7±12.1	
Control (n=25)	25 (OH) D	Normal	10	19.6±13.5	NS
		Abnormal	15	68.6±38.9	
	Calcitriol	Normal	22	35.2±14.5	NS
		Abnormal	3	21.6±3.4	

Statistics were done using by κ^2, in this table.
NS - not significant; IDKMP - ischemic dilated cardiomyopathy; NIDCM - non-ischemic dilated cardiomyopathy; SDNN - standard deviation of all normal-to-normal RR intervals; SDANN - standard deviation of the average normal to normal interval; RMSSD - squares of the differences between adjacent normal-to-normal RR intervals; TO - turbulence onset; TS - turbulence slope

pants without coronary artery disease than for those with coronary artery disease observed by Pilz et al. (7) Our results were in line with Pilz et al. (7) This finding may suggest that vitamin D may be more important for the physiology of the cardiomyocytes and less for the coronary circulation. And the vitamin D deficiency could be more closely related to the pathogenesis of "non-ischemic" myocardial diseases compared with those with an ischemic origin.

After this study we suggested that; vitamin D status should detect routinely in all NIDCM patients. It could be an easy and simple way to determine the high risk patients. At this point the effects of the treatment with vitamin D on autonomic dysfunction are not known. Witham et al. (31). showed that, vitamin D replacement has no effect on HRT values during 16 weeks on patients who had previously suffered a stroke. The above mentioned study was the single one with the small population which focused on this topic. A-sixteen week treatment with vitamin D couldn't be enough to observe the treatment effect of vitamin D in patients with low vitamin status. Another point, we do not know yet which vitamin D level is enough to see an effect on HRT in patients with HF. This issue needs more research to find out the best vitamin D levels for this patient population. Our results could not be generalized for all HF patients because of small groups. Presented study could be a trigger for other researches on this topic. It seems that we need to do more researches to understand the effects of vitamin D treatment on autonomic dysfunction with HF patients.

In our study we couldn't observe the same results with the ischemic DCM patients. It was thought that the specific pathogenesis of ischemic DCM might be the reason of this difference. In other words; the relation between vitamin D levels and autonomic function might be disintegrated by common atherosclerosis and inflammation. The medications of two DCM groups were different. It is well known that medication influence HRV and HRT. Beta-blockers inhibit sympathetic activity and improve HRV (32, 33) also angiotensin converting enzym inhibitors and angio-

tensin receptor blockers enhance the vagal stimulations and baroreflex sensitivity so that positively effects HRV and HRT (34, 35). In our study, the number of patients who were using angiotensin-II antagonist and digoxin were much more in NIDCM group and the number of patients using aspirin, statins and nitrates were much more in IDCM group than the other group. These drugs may change the hemodynamics with the different ways that unknown. Heterogenic distribution of drugs using and their effects might be the other reason of the difference between two DCM groups.

Study limitations

There are several limitations of our study. Firstly, the population size was small because our exclusion criteria. We did not include patients with diabetes chronic kidney disease, atrial fibrillation, thyroid disease and current smokers. Secondly using the less reliable time domain HRV parameters, instead of frequency domain HRV parameter is another limitation Also, we do not have follow-up and mortality data for the patients groups therefore, and additional analyses of the end-points were not performed.

Conclusion

This was the first study which evaluated the relation between the vitamin D status and autonomic function indices as HRV and HRT in patients with DCM. It was shown in this study; the lower vitamin D levels may have deleterious effect on HRT indices in patients with NIDCM. The low vitamin D level could be a trigger for sudden cardiac death in this patient population. This topic needs a lot of research to find out the relation between vitamin D levels and autonomic dysfunction and sudden cardiac death. There is another question to be answered that the appropriate vitamin D level for patients with NIDCM is another question to be answered.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - G.K., M.Ç.; Design - G.K., M.Ç.; Supervision - G.K., N.D., D.U.; Resource - M.Ç., G.K., D.U., G.K., İ.Y., Y.A., R.O., N.D.; Materials - G.K., D.U., G.K., İ.Y., Y.A., R.O.; Data collection &/or processing - M.Ç., R.O., G.K., N.D.; Analysis &/or interpretation - M.Ç., G.K.; Literature search - M.Ç., G.K., G.K, R.O.; Writing - M.Ç., G.K.; Critical review - G.K., M.Ç., D.U., R.O., G.K., N.D.

References

1. Hobbs FD. The scale of heart failure: diagnosis and management issues for primary care. Heart 1999; 82: 8-10.
2. Bleumink GS, Knetsch AM, Sturkenboom MC, Straus SM, Hofman A, Deckers JW, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J 2004; 25: 1614-9. [CrossRef]
3. Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, et al. Long-term trends in the incidence of and survival with heart failure. N Engl J Med 2002; 347: 1397-402. [CrossRef]
4. Cozzolino M, Ketteler M, Zehnder D. The vitamin D system: a crosstalk between the heart and kidney. Eur J Heart Fail 2010; 12: 1031-41. [CrossRef]
5. Pilz S, Tomaschitz A, Drechsler C, Dekker JM, Marz W. Vitamin D deficiency and myocardial diseases. Mol Nutr Food Res 2010; 54: 1103-13.
6. Liu LC, Voors AA, van Veldhuisen DJ, van der Veer E, Belonje AM, Szymanski MK, et al. Vitamin D status and outcomes in heart failure patients. Eur J Heart Fail 2011; 13: 619-25. [CrossRef]
7. Pilz S, Marz W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, et al. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab 2008; 93: 3927-35. [CrossRef]
8. Drechsler C, Pilz S, Obermayer-Pietsch B, Verduijn M, Tomaschitz A, Krane V, et al. Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. Eur Heart J 2010; 31: 2253-61. [CrossRef]
9. Nolan J, Batin PD, Andrews R, Lindsay SJ, Brooksby P, Mullen M, et al. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-Heart). Circulation 1998; 98: 1510-6. [CrossRef]
10. Guzzetti S, La Rovere MT, Pinna GD, Maestri R, Borroni E, Porta A, et al. Different spectral components of 24 h heart rate variability are related to different modes of death in chronic heart failure. Eur Heart J 2005; 26: 357-62. [CrossRef]
11. Bilchick KC, Fetics B, Djoukeng R, Fisher SG, Fletcher RD, Singh SN, et al. Prognostic value of heart rate variability in chronic congestive heart failure (Veterans Affairs' Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure). Am J Cardiol 2002; 90: 24-8. [CrossRef]
12. Koyama J, Watanabe J, Yamada A, Koseki Y, Konno Y, Toda S, et al. Evaluation of heart-rate turbulence as a new prognostic marker in patients with chronic heart failure. Circ J 2002; 66: 902-7. [CrossRef]
13. Cygankiewicz I, Zareba W, Vazquez R, Vallverdu M, Gonzalez-Juanatey JR, Valdes M, et al. Muerte Subita en Insuficiencia Cardiaca Investigators: Heart rate turbulence predicts all-cause mortality and sudden death in congestive heart failure patients. Heart Rhythm 2008; 5: 1095-102. [CrossRef]
14. Bauer A, Malik M, Schmidt G, Barthel P, Bonnemeier H, Cygankiewicz I, et al. Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. J Am Coll Cardiol 2008; 52: 1353-65. [CrossRef]
15. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet 1998 ; 351: 478-84. [CrossRef]
16. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996; 93: 1043-65. [CrossRef]
17. Schmidt G, Malik M, Barthel P, Schneider R, Ulm K, Rolnitzky L, et al. Heart-rate turbulence after ventricular premature beats as a

predictor of mortality after acute myocardial infarction. Lancet 1999; 353: 1390-6. [CrossRef]
18. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? J Am Coll Cardiol 2008; 52: 1949-56. [CrossRef]
19. Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Korfer R, Stehle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? J Am Coll Cardiol 2003; 41: 105-12. [CrossRef]
20. Zittermann A, Schleithoff SS, Koerfer R. Vitamin D insufficiency in congestive heart failure: why and what to do about it ? Heart Fail Rev 2006; 11: 25-33. [CrossRef]
21. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006; 83: 754-9.
22. Schierbeck LL, Jensen TS, Bang U, Jensen G, Kober L, Jensen JE. Parathyroid hormone and vitamin D – markers for cardiovascular and all cause mortality in heart failure. Eur J Heart Fail 2011; 13: 626-32. [CrossRef]
23. Kim HW, Park CW, Shin YS, Kim YS, Shin SJ, Kim YS, et al. Calcitriol regresses cardiac hypertrophy and QT dispersion in secondary hyperparathyroidism on hemodialysis. Nephron Clin Pract 2006; 102: 21-9. [CrossRef]
24. Thomasset M, Parkes CO, Cuisinier-Gleizes P. Rat calcium-binding proteins: distribution, development, and vitamin D dependence. Am J Physiol 1982; 243: 483-8.
25. Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. Int J Epidemiol 1990; 19: 559-63. [CrossRef]
26. Meredith IT, Broughton A, Jennings GL, Esler MD. Evidence of a selective increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. N Engl J Med 1991; 325: 618-24. [CrossRef]
27. Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA, Korner PI. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. Circulation 1986; 73: 615-21. [CrossRef]
28. Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984; 311: 819-23. [CrossRef]
29. Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J Am Coll Cardiol 1992; 20: 248-54. [CrossRef]
30. Eisenhofer G, Friberg P, Rundqvist B, Quyyumi AA, Lambert G, Kaye DM, et al. Cardiac sympathetic nerve function in congestive heart failure. Circulation 1996; 93: 1667-76. [CrossRef]
31. Witham MD, Dove FJ, Sugden JA, Doney AS, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. Nutr Metab Cardiovasc Dis 2012; 22: 864-70. [CrossRef]
32. Praskurnichii EA, Shevchenko OP, Makarova SV, Zhukova VA. Antihypertensive activity and effect on parameters of heart rate variability of beta-blockers carvedilol and metoprolol. Kardiologiia 2005; 45: 17-23.
33. Saxon LA. Improvement in heart rate turbulence as a measure of response to beta-blocker therapy for heart failure. J Cardiovasc Electrophysiol 2004;15: 757-8. [CrossRef]
34. Lin LY, Lai LP, Lin JL, Du CC, Shau WY, Chan HL, et al. Tight mechanism correlation between heart rate turbulence and baroreflex sensitivity: sequential autonomic blockade analysis. J Cardiovasc Electrophysiol 2002; 13: 427-31. [CrossRef]
35. Özdemir M, Arslan U, Türkoğlu S, Balcıoğlu S, Çengel A. Losartan improves heart rate variability and heart rate turbulence in heart failure due to ischemic cardiomyopathy. J Card Fail 2007; 13: 812-7. [CrossRef]