

## Impaired Cardiac Autonomic Functions in Apparently Healthy Subjects with Vitamin D Deficiency

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**Objectives:** Vitamin D (VitD) deficiency affects the cardiovascular system via endocrine, paracrine, and autocrine pathways. Limited data are available regarding cardiac autonomic dysfunction in VitD deficiency. The aim of this study was to assess the cardiac autonomic functions by using heart rate recovery index (HRR1) and heart rate variability (HRV) in apparently healthy subjects with VitD deficiency.

**Methods:** A total of 24 VitD deficient and 50 age-, gender-, and body mass index-matched VitD sufficient healthy participants who admitted to outpatient clinics at a tertiary centre were enrolled. All study participants underwent Treadmill exercise test and 24-hour Holter recording to assess cardiac autonomic functions. HRR1s were calculated by subtracting first, second, and third minute heart rates during recovery period from maximal heart rate.

**Results:** Mean HRR1 ( $28.0 \pm 8.3$  vs  $42.8 \pm 6.4$ ,  $P < 0.001$ ), HRR2 ( $41.1 \pm 11.2$  vs  $60.8 \pm 10.4$ ,  $P < 0.001$ ), and HRR3 ( $44.9 \pm 13.3$  vs  $65.9 \pm 9.8$ ,  $P < 0.001$ ) were significantly higher in VitD sufficient group compared to VitD deficient group. HRV parameters as, SDNN ( $P = 0.040$ ), SDANN ( $P < 0.001$ ), RMSSD ( $P < 0.001$ ), PNN50 ( $P < 0.001$ ), and HF ( $P < 0.001$ ) were significantly decreased in patients with VitD deficiency; but LF ( $P < 0.001$ ) and LF/HF ( $P = 0.003$ ) were significantly higher in VitD deficient group. Serum 25(OH)D level was positively correlated with HRR1s ( $P < 0.001$ ), PNN50, RMSSD, SDANN, and HFnu; negatively correlated with LFnu and LF/HF ( $P < 0.05$ ). Also, multivariate linear regression analysis showed that serum 25(OH)D level was significantly associated with HRR1s and HRV parameters ( $P < 0.001$ ).

**Conclusion:** Our study results suggest that cardiac autonomic functions are impaired in patients with VitD deficiency despite the absence of overt cardiac involvement and symptoms. Further studies are needed to elucidate the prognostic significance and clinical implications of impaired autonomic functions in patients with VitD deficiency.

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vitamin D deficiency; cardiac autonomic function; heart rate recovery; heart rate variability

Besides its classical hormonal role in bone metabolism, vitamin D (VitD) metabolites also display physiological and pathological effects in nonskeletal tissues by paracrine and autocrine mechanisms.<sup>1</sup> The 25-hydroxyvitamin D (25(OH)D) is usually used as a circulating biomarker of VitD status.<sup>2</sup> There were various

studies investigating the cardiovascular effects of VitD deficiency.<sup>3–7</sup> It has been known that cardiac autonomic tone was impaired both in cardiac and noncardiac conditions and it was linked to poor prognosis.<sup>8–11</sup> Heart rate recovery index (HRR1) and heart rate variability (HRV) are simplistic techniques to evaluate cardiac autonomic functions

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in daily practice.<sup>12</sup> To our knowledge, there was no study evaluating the association of cardiac autonomic tone assessed by HRRi with VitD deficiency. Additionally, the association of HRV with VitD deficiency has been shown in a few studies.<sup>13,14</sup> Therefore, in this study we aimed to evaluate the cardiac autonomic functions by using HRRi and HRV in apparently healthy subjects with VitD deficiency.

## METHODS

### Study Population

In this cross-sectional study, we enrolled a consecutive subset of 74 apparently healthy subjects (34 [45.9%] female; mean age  $37.5 \pm 6.7$  years) who were admitted for routine check-up and examined at outpatient clinics between June 2013 and August 2013 in Ankara. VitD deficiency is defined as a 25(OH)D below 20 ng/mL (50 nmol/L) according to The Endocrinology Society guidelines.<sup>15</sup> Study population was divided into two as VitD sufficient ( $n = 50$ , 25(OH)D  $\geq 30$  ng/mL) and VitD deficient ( $n = 24$ , 25(OH)D  $< 20$  ng/mL) groups. Participants with hepatic, renal, or gastrointestinal dysfunction (inflammatory bowel disease and malabsorption), diabetes mellitus, hypertension, alcoholism, malignancy, pregnancy, rhythm abnormalities and structural heart disease including ischaemic heart disease and cardiomyopathy or patients taking drugs that can influence the autonomic nervous system, uncontrolled thyroid or parathyroid disease and individuals using calcium or VitD supplementation or therapies that interfere with VitD metabolism were excluded from the study. Informed consent was taken from each patient before enrolment. The study was in compliance with the principles outlined in the Declaration of Helsinki and approved by local ethics committee.

The study was performed in Ankara, Turkey which has a continental climate, with cold, snowy winters (December, January, February) and hot, dry summers (June, July, August). Rainfall occurs mostly during spring and autumn. The average temperature is 28°C (82°F) in summer and the temperatures as high as 42°C (108°F) were recorded.

Demographic and clinical information was recorded on the day of autonomic function assessment. The direct questioning of the

symptoms which associated with dysautonomia were orthostatic hypotension, erectile disorder, decreased sweating, abnormal gastrointestinal motility, and impaired urine voiding. Body weight (kg) and height (m) were determined, and body mass index (BMI;  $\text{kg}/\text{m}^2$ ) was calculated. Additionally, in order to properly demonstrate other factors that may have an impact on VitD deficiency, all subjects were enquired about age, weight, height, wearing style, physical activity (by using subject responses in regard to hours spent on exercise, at work or leisure time), current smoking habits and sunlight exposure (by calculating the average time spent in the sun per day) by a questionnaire.

### Assays

Blood samples were taken before the Treadmill test and at the same day through venipuncture and centrifuged within 2 hours after withdrawal. Serum was stored at  $-80^\circ\text{C}$ . The 25(OH)D concentration was measured with direct ELISA (Immunodiagnostik, Bensheim, Germany) with intraassay and interassay coefficient of variations were  $< 4.1\%$  and  $< 3.9\%$ . The concentration of iPTH was determined by use of immunoradiometric assay (Immulite 2000, Diagnostic Products, Los Angeles, CA, USA) with a reference range of 9.5–75 pg/mL. The average intra- and interassay CV for iPTH were  $\leq 4.6\%$  and  $\leq 3.8\%$ , respectively.

### Electrocardiography and Echocardiography

Twelve lead electrocardiography (ECG) at 25 mm/s (paper speed), treadmill exercise testing, and transthoracic echocardiography equipped with a 3.5-MHz transducer (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway) were done in each patient.

### Assessment of Autonomic Functions

#### Heart Rate Recovery Index

To calculate HRRIs, all patients underwent a symptom limited exercise stress test in accordance with the Bruce protocol reaching at least 85% of the age-predicted heart rates. After achieving peak workload, all patients spent at least 3 minutes of recovery without cool-down period. The participants maintain the standing position during the recovery period. Heart-rate recovery indices were calculated by subtracting first-, second-, and third-minute

heart rates from the maximal heart rate obtained during stress testing; these results were designated HRR1, HRR2, and HRR3, respectively.<sup>16</sup>

#### Heart Rate Variability

Using the ELATEC Holter system, 24-hour Holter recordings were obtained by using three-channel analogue recorders. In the heart rate variability (HRV) analysis, the Standard parameters obtained from the time-domain analysis of HRV including SDNN (Standard deviation of all NN intervals for a selected time period), SDANN (SD of the 5-minute mean RR intervals tabulated over an entire day), RMSSD (square root of the mean of the sum of the squares of differences between adjacent RR intervals), and PNN50 (the proportion of differences in successive NN intervals greater than 50 ms) were used. Spectral analysis of HRV is obtained by summing powers of each frequency band; high-frequency (HF) component (0.15–0.40 Hz); low-frequency (LF) component (0.04–0.15 Hz); and very low-frequency component (VLF) (0–0.04 Hz). In all patients the LF power/HF power was calculated.<sup>17</sup> In the calculation and analysis of HRV parameters, ELATEC Holter software was used.

#### Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL, USA). Normal distribution of variables were evaluated with Kolmogorov–Smirnov test. Numerical variables with a normal distribution were presented as the mean  $\pm$  standard deviation and numerical variables with a skewed distribution were presented as the median (minimum and maximum) and categorical variables were presented as percentages. For numerical variables, an independent sample t test and Mann–Whitney U test were used for intergroup comparisons. Chi-square test and Fisher's exact chi-square test were used for comparisons of categorical variables. Univariate linear regression analysis was used to evaluate association of HRRIs and HRV parameters with several clinical and echocardiographic variables. Stepwise multivariate regression analysis was used to evaluate independent association of HRRIs and HRV parameters with VitD deficiency. Correlation was done using Pearson's correlation coefficient (r).

A two-tailed  $P < 0.05$  was considered statistically significant.

## RESULTS

### Demographic Characteristics

Baseline characteristics of the participants according to VitD status were shown in Table 1. Both study groups were similar in regard to age, BMI, fasting plasma glucose, cholesterol levels, physical activity, sun exposure, and wearing clothes which restrict exposure to sunlight. All subjects were normotensives, and no significant differences were observed in resting systolic/diastolic blood pressures and resting heart rates between two groups. Echocardiographic parameters including biventricular and valvular functions were also in normal limits for both study group.

### Exercise Test Parameters

All participants were in sinus rhythm and normal 12-lead ECG results at rest. Study participants completed the Treadmill exercise test without any complications, ischemic changes and rhythm abnormalities. The parameters used in Treadmill exercise test including HRR indices of the study groups were shown in Table 2. Mean HRR1 ( $28.0 \pm 8.3$  vs  $42.8 \pm 6.4$ ,  $P < 0.001$ ), HRR2 ( $41.1 \pm 11.2$  vs  $60.8 \pm 10.4$ ,  $P < 0.001$ ) and HRR3 ( $44.9 \pm 13.3$  vs  $65.9 \pm 9.8$ ,  $P < 0.001$ ) were significantly higher in VitD sufficient group compared to VitD deficient group (Fig. 1). Also, all study participants were asymptomatic during the postexercise period.

There were remarkably positive correlations between the 25(OH)D and HRR1 ( $r = 0.851$ ,  $P < 0.001$ ), HRR2 ( $r = 0.787$ ,  $P < 0.001$ ) and HRR3 ( $r = 0.685$ ,  $P < 0.001$ ) (Fig. 2). Additionally, there was no correlation between 25(OH)D and HRR1 with BMI ( $P > 0.05$ ).

### HRV Parameters

The time-domain and frequency-domain parameters of HRV were compared between the VitD deficient and VitD sufficient groups; SDNN ( $58.3 \pm 15.2$  vs  $83.1 \pm 18.4$ ,  $P = 0.04$ ), SDANN ( $86.2 \pm 36.1$  vs  $179.2 \pm 33.2$ ,  $P < 0.001$ ), RMSSD ( $19.9 \pm 6.2$  vs  $70.3 \pm 18.4$ ,  $P < 0.001$ ), PNN50 ( $3.5 \pm 1.1$  vs  $33.4 \pm 9.8$ ,  $P < 0.001$ ), and HFnu ( $18.8 \pm 9.6$  vs  $36.6 \pm 13.4$ ,  $P < 0.001$ ) were significantly decreased in

**Table 1.** Baseline Clinical and Laboratory Characteristics of the Study Population (n = 74)

	VitD sufficient (n = 50)	VitD deficient (n = 24)	P
Age, years	37.2 ± 6.7	38.2 ± 6.6	0.540
Gender, female, n (%)	22 (44%)	12 (50%)	0.804
BMI, kg/m <sup>2</sup>	22.4 ± 1.3	22.0 ± 1.8	0.350
Current smokers, n (%)	9 (18%)	8 (25%)	0.543
Physical activity, n (%)			0.806
<15 min/day	13 (26%)	8 (33%)	
15–30 min/day	21 (42%)	9 (37.5%)	
>30 min/day	16 (32%)	7 (29.2%)	
Sun exposure (hours/week)			
Summer	1.44 ± 0.85	1.35 ± 0.85	0.687
Wearing clothes which restrict exposure to sunlight, n (%)	2 (4%)	2 (4.2%)	0.698
Heart rate (bpm)	85.5 ± 12.9	86.8 ± 10.9	0.670
Systolic blood pressure (mmHg)	115.6 ± 8.4	118.7 ± 7.0	0.123
Diastolic blood pressure (mmHg)	71.0 ± 8.2	73.3 ± 7.0	0.235
LVEF (%)	65.9 ± 3.4	66.9 ± 2.7	0.207
LVEDD (mm)	47.7 ± 3.5	48.1 ± 2.9	0.596
LVPWT (mm)	8.3 ± 0.8	8.4 ± 1.0	0.799
LVSWT (mm)	8.0 ± 0.7	8.1 ± 0.9	0.667
Total cholesterol (mg/dL)	177.3 ± 29.5	169 ± 9.6	0.182
Triglyceride (mg/dL)	131.5 ± 13.5	131.2 ± 18.3	0.957
LDL-cholesterol (mg/dL)	111.4 ± 10.8	112.1 ± 8.4	0.780
HDL-cholesterol (mg/dL)	45.7 ± 8.3	48.6 ± 6.3	0.130
25-OH Vitamin D (ng/mL)	34.0 ± 6.5	13.8 ± 2.3	<0.001
iPTH (pg/mL)	32.3 ± 5.7	59.5 ± 7.6	<0.001

Numerical variables were presented as the mean ± standard deviation or median (range), categorical variables were defined as percentage. BMI = body mass index; HDL = high-density lipoprotein; iPTH = intact-parathyroid hormone; LDL = low-density lipoprotein; LVEDD = left ventricle end-diastolic diameter; LVEF = left ventricle ejection fraction; LVPWT = left ventricle posterior wall thickness; LVSWT = left ventricle septal wall thickness.

patients with VitD deficiency compared with VitD sufficient group, as illustrated in Table 2. However, LFnu (68.6 ± 10.8 vs 55.6 ± 12.7,  $P < 0.001$ ) and LFnu/HFnu (6.37 ± 2.14 vs 1.91 ± 1.15,  $P = 0.003$ ) were significantly higher in VitD deficient patients (Fig. 1).

Additionally, there was significantly positive correlation of 25(OH)D with PNN50 ( $r = 0.719$ ,  $P < 0.001$ ), RMSSD ( $r = 0.674$ ,  $P < 0.001$ ), SDANN ( $r = 0.617$ ,  $P < 0.001$ ), and HFnu ( $r = 0.522$ ,  $P < 0.001$ ); negative correlation with LFnu ( $r = -0.419$ ,  $P < 0.001$ ) and LF/HF ( $r = -0.278$ ,  $P = 0.018$ ) (Fig. 2).

### Predictors of HRR1 and HRV

In univariate linear regression analysis, age, maximum systolic blood pressure and 25(OH)D levels were associated with HRR1 and HRV parameters ( $P < 0.05$ ). BMI was not associated with HRR1 and HRV parameters ( $P > 0.05$ ). However, in stepwise multivariate linear regression analysis, only 25(OH)D level was significantly associated

with HRR1 ( $\beta = 0.835$ ,  $P < 0.001$ ), HRR2 ( $\beta = 0.769$ ,  $P < 0.001$ ), and HRR3 ( $\beta = 0.689$ ,  $P < 0.001$ ). For HRV parameters, only 25(OH)D level was significantly associated with LFnu ( $\beta = -0.445$ ,  $P < 0.001$ ), HFnu ( $\beta = 0.539$ ,  $P < 0.001$ ), and LFnu/HFnu ( $\beta = -0.255$ ,  $P = 0.030$ ), age and 25(OH)D level were significantly associated with PNN50, SDANN, and RMSDD ( $P < 0.05$ )

### Discussion

Main findings of our study included; (a) cardiac autonomic tone assessed by using HRR1 and HRV parameters was significantly impaired in apparently healthy subjects with VitD deficiency, (b) serum 25(OH)D levels were positively correlated with HRR1s, PNN50, RMSDD, SDANN, and HFnu; there was significantly negative correlation of serum 25(OH)D levels with LFnu and LFnu/HFnu, (c) serum 25(OH)D level was found as the significant predictor of HRR1s and HRV parameters in the study population. To the best of our

**Table 2.** Comparison of Cardiac Autonomic Function Parameters between Study Groups (n = 74)

	VitD sufficient (n = 50)	VitD deficient (n = 24)	P
<i>Treadmill exercise test parameters</i>			
Baseline heart rate (bpm)	85.5 ± 12.9	86.8 ± 10.9	0.670
Maximum SBP (mmHg)	157.3 ± 12.8	153.3 ± 14.5	0.227
Maximum DBP (mmHg)	81.2 ± 6.3	83.5 ± 11.1	0.250
Exercise test duration (min)	7.4 ± 2.2	7.4 ± 1.5	0.990
Maximum heart rate (bpm)	155.2 ± 9.8	157.6 ± 9.4	0.325
Peak exercise capacity (METS)	9.3 ± 2.5	9.0 ± 1.8	0.646
HRR1	42.8 ± 6.4	28.0 ± 8.3	<0.001
HRR2	60.8 ± 10.4	41.1 ± 11.2	<0.001
HRR3	65.9 ± 9.8	44.9 ± 13.3	<0.001
<i>HRV parameters</i>			
Mean RR	758 ± 78	777 ± 106	0.389
Mean heart rate (bpm)	70.3 ± 9.5	71.4 ± 10.5	0.656
SDNN (ms)	83.1 ± 18.4	58.3 ± 15.2	0.040
SDANN (ms)	179.2 ± 33.2	86.2 ± 36.1	<0.001
RMSDD (ms)	70.3 ± 18.4	19.9 ± 6.2	<0.001
PNN50 (%)	33.4 ± 9.8	3.5 ± 1.1	<0.001
LF n.u. (%)	55.6 ± 12.7	68.6 ± 10.8	<0.001
HF n.u. (%)	36.6 ± 13.4	18.8 ± 9.6	<0.001
LF/HF	1.91 ± 1.15	6.37 ± 2.14	0.003

Numerical variables with a normal distribution were presented as the mean ± standard deviation.

DBP = diastolic blood pressure before exercise; HF = high-frequency component; HRR = heart rate recovery; LF = low-frequency component; pNN50 = the number of pairs of adjacent NN intervals differing by more than 50 ms divided by the total number of all NN intervals; RMSDD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals; SBP = systolic blood pressure before exercise; SDNN = standard deviations of all NN intervals; SDANN = standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording.

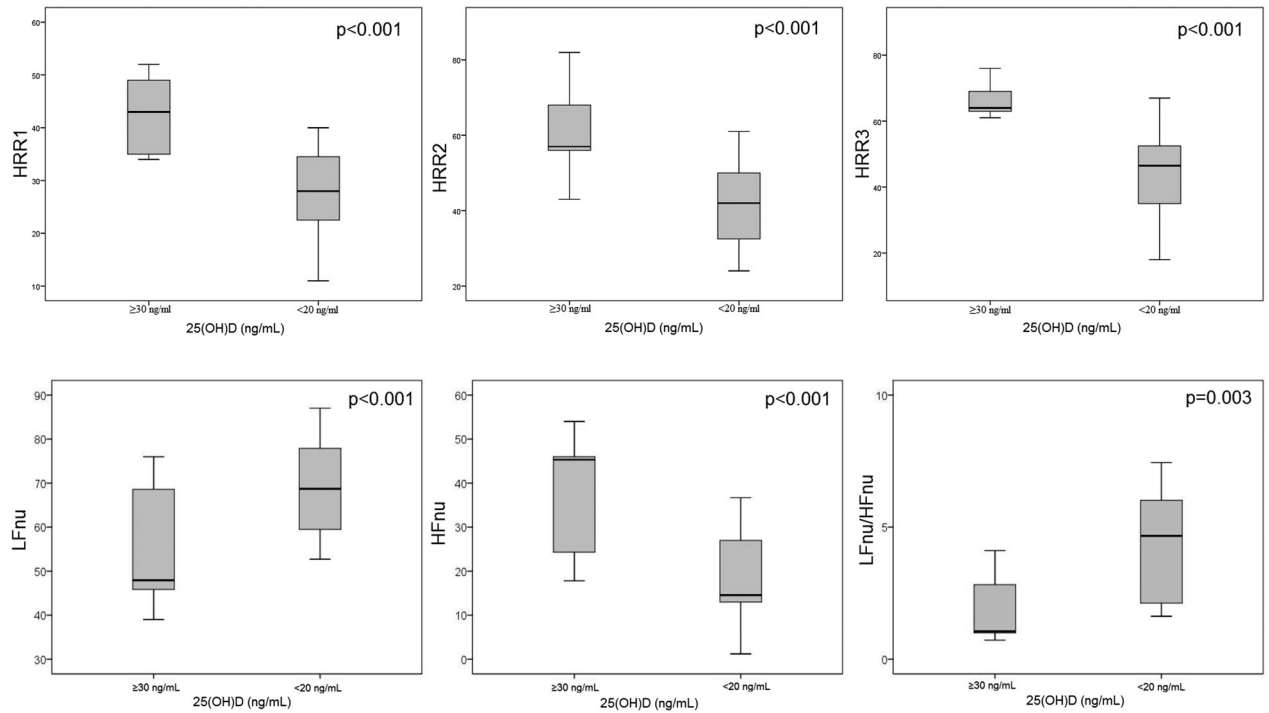
knowledge, this is the first study in the literature evaluating cardiac autonomic functions by using HRR1 and HRV parameters as a combination in patients with VitD deficiency.

Autonomic nervous system plays a pivotal role in the regulation of cardiovascular functions. It has been known that increased sympathetic and decreased parasympathetic activities are associated with increased risk of sudden death and ventricular arrhythmias. Therefore, assessment of autonomic tone is a potential method for identifying patients at high risk for sudden death. It is difficult to measure and quantify the autonomic modulation. Heart rate, HRV, HRR1, baroreflex sensitivity, heart rate turbulence, and plasma/coronary sinus catecholamine levels can be used to reflect autonomic activity.<sup>12</sup> Analysis of HRR1 and HRV are noninvasive and inexpensive methods for measurement of cardiac autonomic tone.

Heart rate recovery derived from Treadmill exercise test parameters is one of the commonly used techniques reflecting cardiac autonomic tone. The period of recovery after exercise is accompanied by further dynamic changes in autonomic tone which

are clinically characterized by the gradual return of HR to its previous resting level. This period of HRR results from a combination of sympathetic withdrawal and parasympathetic reactivation.<sup>12</sup> It has been shown that an abnormal HRR1 which was defined as a failure of heart rate to decrease 12 beats or more during the first minute after peak exercise, is an independent predictor of adverse cardiovascular outcomes.<sup>18,19</sup> Additionally Jouven et al.<sup>20</sup> found that HRR1 less than 25 beats/min at first minute of the recovery period had 2.1 times the risk of sudden death, 0.9 times the risk of nonsudden death, and 1.3 times the risk of death from any cause. Besides HRR1s, also HRV analysis is convenient and safe method used for evaluation of the autonomic nervous system functions. In the time-domain analysis, SDNN reflects the general measurement of autonomic nervous system balance and PNN50 predominantly reflects the parasympathetic activity. On the other hand, in the frequency-domain analysis, HF is modulated predominantly by the parasympathetic nervous system, whereas LF is under the influence of both parasympathetic and sympathetic nervous



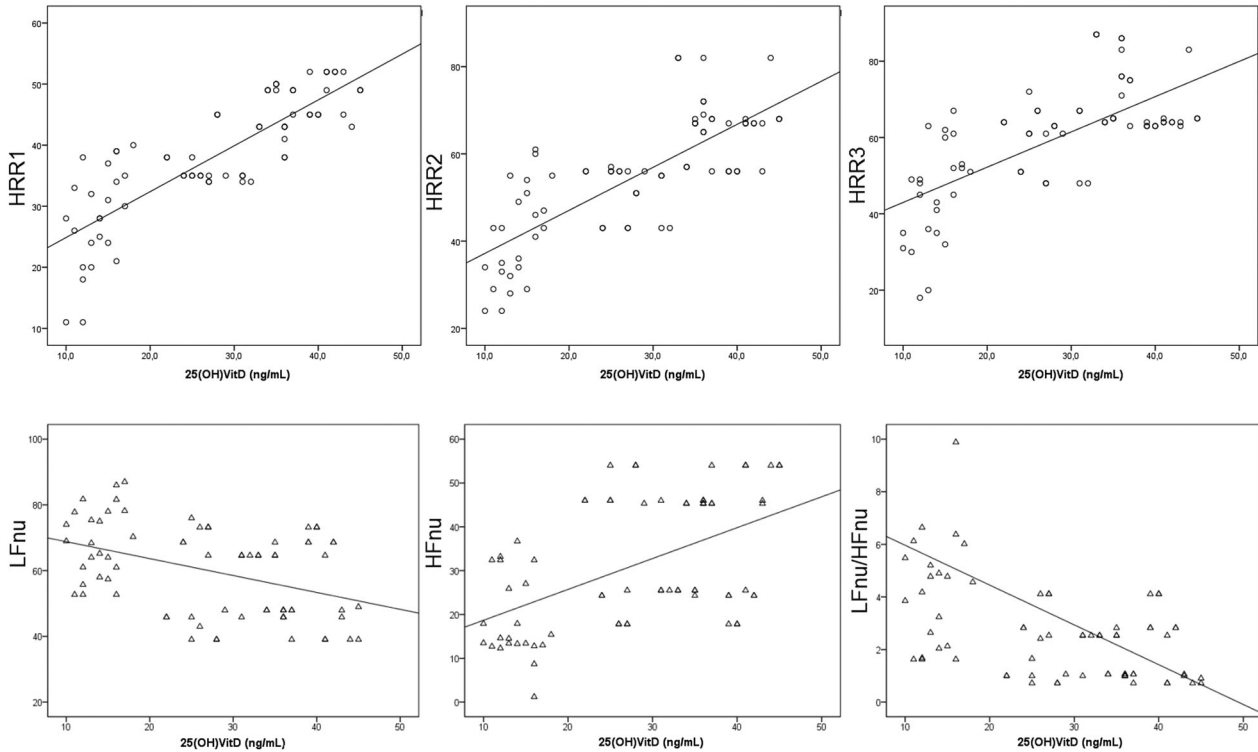


**Figure 1.** Box plot graph showing the comparison of HRRIs and frequency-domain analysis of HRV between VitD deficient and sufficient groups.

systems. The HF/LF ratio is an indicator of the sympathovagal balance.<sup>17</sup>

VitD plays a significant role in mineral homeostasis and skeletal health. However, VitD metabolites also exhibit integral physiological roles in nonskeletal tissues including cardiovascular system. Several pathways and cell types that are relevant to cardiovascular physiology and pathology are influenced by VitD metabolites. VitD metabolites act via endocrine, autocrine, and paracrine pathways and modulate vascular endothelial cell function, arterial/myocardial remodelling and maintain immune and inflammatory responses at vascular tissues. It has been shown that there were significant associations between VitD deficiency and cardiovascular pathologies including coronary heart disease, hypertension, left ventricular hypertrophy.<sup>1</sup> Also, previous studies showed that patients with low 25(OH)D levels were at increased risk of sudden arrhythmic death compared to other subjects.<sup>3,4</sup> In an animal study, rats fed a VitD deficient diet demonstrated markedly increased ventricular and vascular muscle contractile function with increased sensitivity to norepinephrine, the principal sympathetic

neurotransmitter, compared to controls.<sup>21</sup> However, there was little evidence for the effects of VitD deficiency on cardiac autonomic functions in clinical practice. Activated VitD is proposed to have the ability to diffuse across the blood-brain barrier, implicating a role for 1,25-dihydroxy VitD in augmenting autonomic vagal control by binding directly to nuclear VitD receptors in the adrenergic neurons located centrally in the spinal cord and brain tissue.<sup>22</sup> In a small sized study, Mann et al.<sup>14</sup> showed that participants in the lowest 1,25-dihydroxy VitD quartile experienced significant withdrawal of inhibitory vagal control, as well as altered overall sympathovagal balance throughout AngII challenge. Also in a recent study, Mann et al.<sup>13</sup> reported that VitD supplementation in a 13 healthy subjects VitD deficiency has significantly improved sympathovagal balance. Although the study results were promising for significant effects of VitD deficiency and supplementation on cardiac autonomic functions in healthy subjects, the study findings were limited with very small sample sizes. Although our study results were confirmatory to previous two studies, we included relatively larger sample size and evaluated cardiac autonomic



**Figure 2.** Correlation analysis showing a significantly positive correlation of serum 25(OH)D levels with HRRIs and HFnu, negative correlation of serum 25(OH)D levels with LFnu and LFnu/HFnu.

tone by using Treadmill exercise test derived HRR1 and 24-hour Holter analysis derived HRV parameters. In our study, we showed that patients with VitD deficiency had decreased HRRIs, PNN50, SDANN, RMSDD, and HFnu, and increased LFnu and LFnu/HFnu which were indicators of enhanced sympathetic limb activity and reduced parasympathetic limb activity in participants with VitD deficiency.

The main strength of our study is the demonstration of cardiac autonomic dysfunction with several indices in apparently healthy subjects by using HRR1 and HRV which were widely available, simple and noninvasive techniques. However, our study has some limitations. First, our study group was consisted of relatively small number apparently healthy participants. Though the study results could not be generalized to whole populations. Second, we did not evaluate the effect of VitD supplementation on impaired HRRIs and HRV in participants with VitD deficiency. Third, the measurement of VitD level was only performed once. We do not know if the results will be consistently the same if two or three

samples (in different days of course) would have been taken. Finally, despite the reports showing impairment in cardiac autonomic dysfunctions, clinical implication of those findings was not evaluated in our study. Further prospective studies are needed to elucidate the exact pathophysiologic mechanisms underlying VitD deficiency and clinical implications of impaired autonomic functions in those patients.

In conclusion, VitD deficiency is significantly associated with impaired cardiac autonomic functions assessed by using HRR1 and HRV in apparently healthy subjects. When the prognostic significance of HRR1 and HRV is considered, patients with VitD deficiency should be followed closely for adverse cardiovascular outcomes. Indices of cardiac autonomic dysfunction may be useful in order to identify high risk patients with VitD deficiency.

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