Original Article

Vitamin D Deficiency is Associated with Increased IL-17 and Tnfα Levels in Patients with Chronic Heart Failure

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Abstract

Background: Recent studies revealed a strong association between vitamin D (VD) status and chronic heart failure (CHF). It is now commonly considered that proinflammatory immune response underlies CHF development.

Objective: Since VD expresses anti-inflammatory properties, we investigated its impact on cytokines implicated in CHF, such as TNFα and IL-17, in patients suffering from CHF.

Methods: Blood was obtained from forty patients with CHF secondary to hypertension and/or coronary heart disease. VD status, IL-17 and TNFα levels were assessed using 25-hydroxy VD3 EIA and cytokine ELISAs. Clinical assessment and echocardiography was also performed.

Results: Elderly patients with CHF in Nis (Southeast Europe, latitude 43°N) exhibited 25-hydroxy VD3 levels below normal. Our data identified that patients with CHF secondary to hypertension have significantly lower 25-hydroxy VD3, increased TNFα and IL-17A levels in comparison to donors with CHF secondary to coronary disease.

Conclusion: This study reveals that even in regions with a lot of sunny days VD deficiency represents a concerning issue. Data suggest that impaired VD status contributes to high IL-17 and TNFα levels and thereby may support CHF development. (Arq Bras Cardiol 2012;98(3):259-265)

Keywords: Vitamin D deficiency; heart failure; interleukin-17; hypertension.

Introduction

Recent studies revealed a strong association between vitamin D (VD) status and cardiovascular diseases (CVD)[1-3]. Over the last years, the incidence of VD deficiency and chronic heart failure (CHF) substantially increased worldwide[4]. In general, CHF represents the end stage of hypertensive, coronary and valvular CVD[3]. It is nowadays commonly considered that the proinflammatory immune response underlies its development[4,5]. Several protective mechanisms of calcitriol (1,25-dihydroxy VD3; biologically active VD form) for the development of CHF have been suggested: the regulation of inflammation, the effect on myocardial cell hypertrophy and proliferation, and the regulation of the RAAS-system[3].

Calcitriol is a well known regulator of calcium homeostasis and has an important role in inflammation[5]. This is of importance as certain proinflammatory cytokines are high in patients suffering from CHF[5-7]. The role of tumor necrosis factor α (TNFα) in CVD has been well described[6]. TNFα closely relates to left ventricular dysfunction, remodeling, cardiac myocyte apoptosis, attenuation of β adrenergic responsiveness, and activation of inducible nitric oxide synthase[7]. On the other hand, increasing evidence is emphasizing the impact of novel cytokine interleukin-17A (IL-17) in the pathogenesis of CHF[8,9]. The neutralization of IL-17 reduces myocarditis severity[9] and has an essential role in the development of dilated cardiomyopathy[10]. Th17 cells infiltrate the inflamed heart and are responsible for heart-specific up-regulation of IL-6, TNFα, IL-1β, which can promote fibrosis, either directly or indirectly, by inducting matrix metal proteases 1, 2, 3 and 9[10,11]. Moreover, TNFα and IL-17 act synergistically to create a proinflammatory environment[12]. Importantly, calcitriol can directly regulate the expression TNFα, IL-6, IL-17, IL-10[13,14] and the differentiation of proinflammatory Th17 cell, the main source of IL-17[13].

Although IL-17 and TNFα and cytokines have been brought in connection for the development and course of CVD, to the best of our knowledge, the relationship of IL-17 to VD levels in CHF has not been considered so far. For that reason, we investigated the VD status and its impact on IL-17 and TNFα status in these patients[14].

Methods

Study population

Forty patients suffering from CHF secondary to arterial hypertension and/or coronary heart disease (according to the European Society of Cardiology guidelines[15] admitted to the Institute of Cardiology "Niska Banja" in Nis (Serbia, Southeast Europe, geographic latitude 43°N and longitude 22°E) were taken into consideration. The characteristics of donors are defined in Table 1. Systolic blood pressure higher than 140 mmHg, diastolic blood pressure higher than 90 mmHg, or use of antihypertensive therapy was considered as hypertension. Coronary heart disease included history of myocardial infarction, coronary insufficiency or angina. The conditions known to influence the VD status were considered as exclusion criteria: previous gastrectomy, intestinal malabsorption, history of chronic liver disease, significant increase of liver enzymes, or biochemical evidence of hepatic dysfunction, current use of antiepileptic drugs or supplements containing vitamin D. The study was approved by the ethics committee of the Institute of cardiology and rehabilitation "Niska banja," conducted according to the Declaration of Helsinki Principles and all patients gave written informed consent.

Biochemical and clinical assessment

Peripheral blood samples were collected in the morning after an overnight fast and immediately processed. Lipid status was determined by routine diagnostic laboratory. NYHA class was determined by the same investigator by observing each patient at rest, dressing, walking and climbing the stairs. NYHA scores range from 1 (no limitations of physical activity) to 4 (unable to carry on any physical activity)[15].

IL-17 and TNFα ELISA

Serum levels of IL-17 and TNFα were measured by enzyme-linked immunosorbent assay (ELISA) kits, respectively, following the manufacturer's instructions (both from Biolegend, Fell, Germany). Minimal detectable concentrations were 8 pg/ml for TNFα and 2 pg/ml for IL-17. All samples were measured in duplicates.

25-hydroxy vitamin D3 EIA

Serum levels of 25-hydroxy vitamin D3 were measured by the Enzymeimmunoassay (EIA) kit, according to the manufacturer's instructions (Immunodiagnostic Systems, Frankfurt am Main, Germany). The detection limit of each kit is 5 nmol/ml. All samples were measured in duplicates. The values are at this time interpreted as follows: <20 ng/ml (<50 nmol/l) is deficient; 21–29 ng/ml (51–74 nmol/l) is insufficient; and >30 (>75 nmol/l) is sufficient[16,17].

Echocardiography

Transthoracic echocardiography was always performed in each patient. The following linear parameters were measured by means of a Vivid 3 (Healthcare, Chalfont St. Giles, United Kingdom) echo machine: end-diastolic (EDD) and end-systolic (ESD) diameters, left atrial diameter (LAD).

Statistical analysis

Categorical variables are presented as percentages, quantitative ones as means +/-SEM. The two-tailed Mann–Whitney test was used to compare results between groups. Statistical significance was set at $p < 0.05$. Statistical calculations were performed using GraphPad Prism (La Jolla, CA, USA).

Results

Patients with chronic heart failure suffer from vitamin D deficiency or insufficiency

Since decreased levels of 25-hydroxy VD3 have been found in patients suffering from chronic heart failure (CHF) in Nothern and Central European regions[1,2], we tested whether patients with CHF in Nis (Serbia, Southeast Europe, 43°N, 22°E) may present decreased levels of 25-hydroxy VD3. Patients with CHF are characterized in Table 1. Patients suffering from CHF displayed levels of 25-hydroxy VD3 lower than 75 nmol/ml (Fig 1), which is considered under normal range[16]. Since hypertension is one of the major factors responsible for the development and progression of CHF[3], which has been recently associated with VD deficiency[18]. Therefore, we closely examined patients suffering from CHF secondary to hypertension regarding their inflammatory response. Our results demonstrate that CHF patients with

Table 1 - Characterization of patients and healthy volunteers

	Patients (n=40)
Clinical characteristics	
Female/Males	6/34
Age (years)[1]	58 (44-66)
History of hypertension	27
Biochemical parameters	
Total cholesterol (mmol/l)[2]	4.45 ± 0.20
LDL (mmol/l)[2]	2.69 ± 0.17
HDL (mmol/l)[2]	1.07 ± 0.05
Triglycerides (mmol/l)[2]	1.40 ± 0.15
Medication	
ACE inhibitors	77.5% (31/40)
Beta-blockers	100% (40/40)
Diuretics	47.5% (19/40)
Nitrates	32.5% (13/40)
Spironolactone	40% (16/40)
Digitalis	17.5% (7/40)
Antiarhytmics	27.5% (11/40)
Statins	80% (32/40)

[1] median (range); [2] mean ± SEM; ACE - Angiotensin-converting-enzyme.

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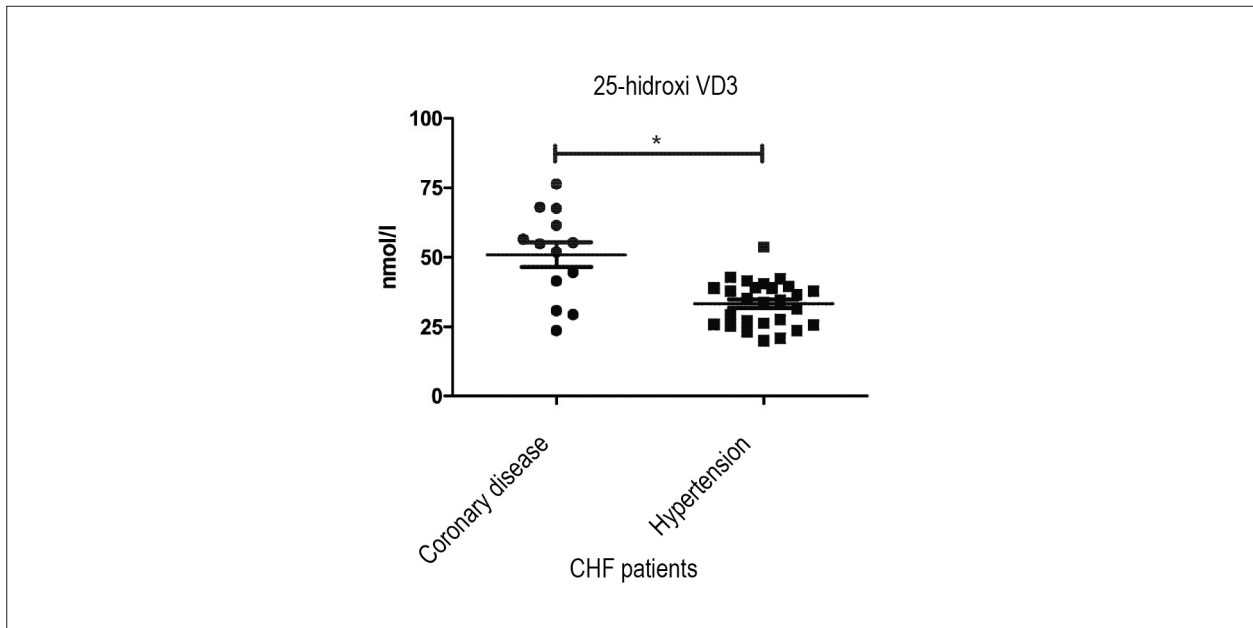
Figure 1 - *VD status in patients suffering from CHF secondary to coronary disease or hypertension. 25-hydroxy VD3 levels were determined in the serum of patients with CHF secondary to coronary disease (n=13) and patients with CHF secondary to hypertension (n=27) using EIA. Data are shown as single values with mean; *p < 0.05.*

hypertension are VD deficient (33.35+/-1.57) compared to those with coronary disease, which are mostly VD insufficient (50.95+/-4.48) (Fig. 1).

Patients with CHF have high levels of TNFα and IL-17

Next, we investigated the levels of IL-17 and TNFα and their relationship with 25-hydroxy VD3. The levels of both proinflammatory cytokines, TNFα (55.21+/-2.71) and IL-17 (47.22+/-4.40) were high in CHF patients secondary to hypertension compared to donors with CHF secondary to coronary disease (49.33+/-4.46 and 34.91+/-3.22, respectively) (Fig. 2) and the differences reached statistical significance. These data suggested that VD may be the factor regulating TNFα and IL-17 underlying the development of hypertension and thereby CHF.

VD deficient CHF patients have worse echocardiographic findings and present higher NYHA score

VD deficiency, as well as TNFα and IL-17 increase has been related to left ventricular dilatation[6,10,19]. Therefore, we compared the echocardiographic findings in VD deficient and insufficient CHF patients.

VD deficient CHF patients had higher values of EDD, ESD, LAD, and lower EF. However, though differences have not reached statistical significance, probably due to the small sample size (Table 2).

Our patients had NYHA scores from 1 (no limitations of physical activity) to 3 (marked limitations of physical activity). The percentages of individuals with NYHA class 1, 2 or 3 were 7.5% (3/40), 42.5% (17/40), and 50% (20/40), respectively. VD deficient CHF patients have higher NYHA score than VD insufficient patients. (Fig. 3).

Discussion

This study shows that elderly patients suffering from CHF in the Nis (Serbia, Southeast Europe) have 25-hydroxy VD3 levels below the normal range. We also found that patients suffering from CHF secondary to hypertension

Table 2 - Echocardiography measures in CHF patients

	All CHF subjects	VD insufficient patients	VD deficient patients
EF[1] (%)	33.33 ± 1.34	34.10 ± 1.61	31.46 ± 2.28
EDD[1] (mm)	60.63 ± 1.16	59.48 ± 1.88	61.72 ± 1.40
EDS[1] (mm)	45.91 ± 1.55	45.71 ± 2.13	46.09 ± 2.31
LAD[1] (mm)	44.56 ± 0.93	43.55 ± 1.12	45.44 ±1.44

[1] mean ± SEM. CHF - chronic heart failure; VD - Vitamin D; EF – Ejection fraction; EDD - End-diastolic diameter; EDS - End-systolic diameter; LAD - left atrial diameter.
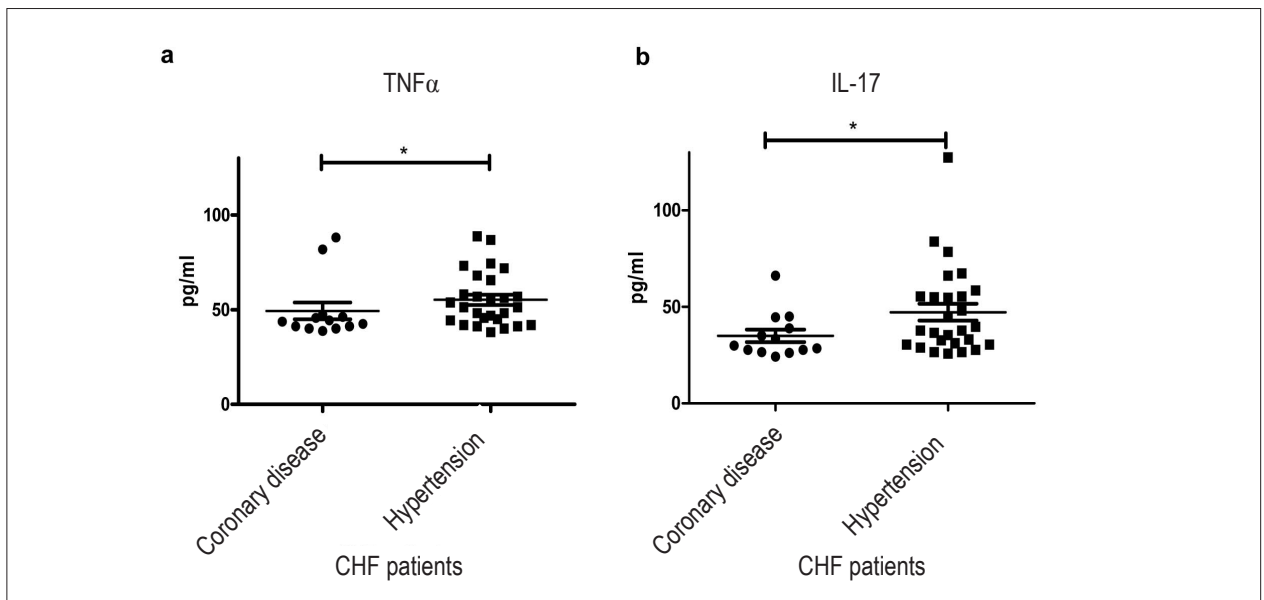
Figure 2 - *Differences in (a) TNFα and (b) IL-17 between patients suffering from CHF secondary to coronary heart disease (n=13) and hypertension (n=27). Serum levels of 25-hydroxy VD3, TNFα and IL-17 were determined using ELISA. Data are shown as single values with mean; *p < 0.05.*
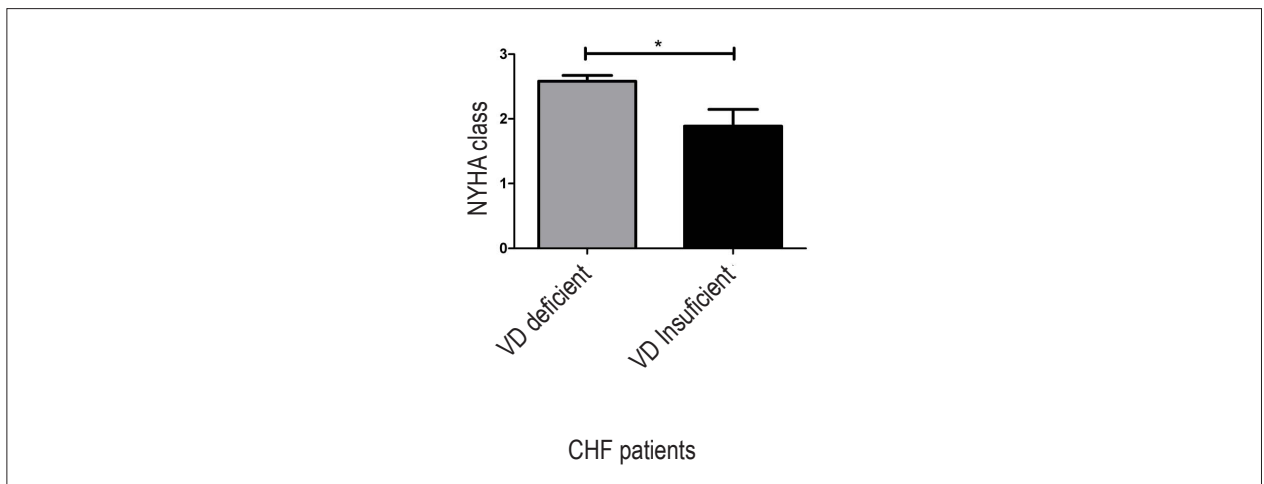
Figure 3 - *NYHA scores in VD deficient and insufficient CHF patients. NYHA class was determined by observing each patient at rest, dressing, walking and climbing the stairs. Data are shown as mean +/- SEM; * p < 0.05; VD deficient n=31; VD insufficient patients n=9.*

show significantly lower 25-hydroxy VD3, increased TNFα and IL-17A levels, compared to donors with CHF secondary to coronary heart disease. This suggests that VD supports the development and progression of CVD by promoting hypertension and underlying inflammation. Moreover, VD deficient patients had higher NYHA score and echocardiographic findings compared to VD insufficient patients.

Severely reduced 25-hydroxy VD3 levels in the elderly population found in this study indicated that even regions with a lot of sunny days throughout the year, such as those laying in Southeast Europe, have not been spared by the "epidemic" of VD deficiency. The annual solar radiation

according to the Republic hydrometeorological service of Serbia is between 1500-2200 h annually, which is a higher rate than in most European countries. A similar finding has been observed in the elderly population in Croatia and Greece, also located in Southeast Europe[20,21]. As hypertension represents a major factor responsible for the development of CHF[3], we investigated patients with CHF secondary to hypertension in more detail. Data revealed that patients suffering from hypertension have significantly lower VD status, which is in line with previous findings by Anderson et al. demonstrating that VD deficiency highly correlates with hypertension prevalence[22]. Interestingly, these CHF patients had drastically increased serum IL-

17 and TNFα. We could also observe that patients on antilipemic statin therapy presented statistically higher levels of 25-hydroxy VD3. However, these levels remained insufficient (data not shown). This finding supports several previous reports which suggested that statins may increase the synthesis of calcitriol by inducing enzyme 7- dechydrocholesterol[23,24].

The role of TNFα in CVD has been quite established[6]. Mice over expressing TNFα or treated with TNFα develop hypertension, left ventricular dysfunction and remodeling, and die of CHF[25,26]. Indeed, our observation that patients with CHF secondary to hypertension have higher TNFα levels supports these findings. Novel proinflammatory cytokine IL-17 has recently become implicated in CHF[10]. Animal models demonstrated that IL-17 is essential for the development of dilated cardiomyopathy, probably by stimulating fibrosis through direct and/or indirect induction of MMP1/2/3/9[10,11]. IL-17 is also required for the sustained expression of IL-6 during myocarditis[27]. High cardiac IL-6 was associated with the development of dilated cardiomyopahty, probably by promoting myocardial fibrosis. This is supported by the fact that the neutralization of IL-6 ameliorates fibrosis and improves heart function during chronic cardiac allograft rejection[27]. However, further experiments are needed to confirm the specific role of IL-6 as a downstream signal of IL-17 in CHF. The role of other biochemicals has been suggested as well, such as growth differentiation factor-15, IL-1, IL-2, and IL-10 in CHF[7,28,29]. Several studies found opposing levels of IL-1 and IL-2, Interestingly, the anti-inflammatory cytokine is IL-10 found to be high in CHF patients compared to healthy controls. However, the increase in anti-inflammatory cytokine levels is not adequately proportional to the increase in proinflammatory cytokine levels in order to be beneficial for CHF patients[18].

It is likely that VD underlies the pathogenesis of CHF on multiple levels (inflammation, calcium homeostasis, rennin-angiotensin-aldosterone-system)[3]. Our findings, together with previous in vitro-gained data that VD regulates the expression of IL-17 and TNFα in immune cells, suggest that VD action on Th17 cells represents at least one of the mechanisms controlling inflammation in CVD. VD can directly decrease IL-17 production, by its main source, the Th17 cells[30], and impair their differentiation[31,32]. Importantly, IL-17 is an inducer of proinflammatory cytokine IL-6 and TNFα specifically in the heart[10]. On the other hand, VD can itself decrease the IL-6 and TNFα cytokine levels[13], which are important for Th17 differentiation[30]. This suggests that VD may modulate initial inflammation that can lead to Th17 differentiation, which in turn supports further inflammation and tissue damage. Therefore, these findings suggest the importance of Th17 cell subset in CVD and suggest IL-17 as a putative target for future therapeutic options.

Since patients suffering from hypertension have significantly lower VD status and increased IL-17 and TNFα serum levels, it could be possible that VD levels throughout a longer life period may orchestrate chronic inflammation and thereby hypertension driving CHF. Two prospective studies supported this possibility as reviewed by Vaidya et al[18]: The first study followed 1,811 non-hypertensive participants for 4 years and showed that donors with 25-hydroxy low 25-hydroxy VD3 levels have higher relative risk for incident hypertension compared with those whose levels were high; the second nested case-control study within the Nurses' Health Study II cohort study showed similar results[18]. So far, several randomized trials have reported change in blood pressure after VD supplementation. However, only in 2 of the following trials the use of antihypertensive medications was not allowed. Scragg et al. showed that individuals who received a single dose of 100 000 IU cholecalciferol had no detectable blood pressure difference compared with the placebo group after 5 weeks. In another trial, women received 800 IU/day of cholecalciferol or placebo for 8 weeks. The group receiving verum had a significant decrease in systolic blood pressure compared with placebo. Due to the several opposing findings regarding VD supplementation and CVD, a very promising study randomized VITamin D and omegA-3 triaL (VITAL) enrolling 20,000 individuals is underway. One primary goal of this trial is to determine whether high-dose vitamin D supplementation over a long period (5 years) can prevent CVD and cancer[18].

Since we excluded other diseases that could precipitate lower VD levels, our observations suggest that the modern sedentary lifestyle has lead to the conclusion that even in the regions with a lot of sunny days, VD deficiency represents a concerning issue. As patients suffering from hypertension have significantly lower VD status and increased IL-17 and TNFα serum levels, it is possible that VD may support the development of CVD by promoting hypertension and its underlying inflammation.

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