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The evaluation of 25(OH)D concentration in blood serum of chronic heart failure patients

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ABSTRACT

This current study examined patients with severe chronic heart failure (CHF) in order to ascertain the held vitamin D, based on an assessment of calcidiol [25(OH)D] concentration. It also identified and evaluated possible correlations between 25(OH)D level and the concentration of total calcium, inorganic phosphates and creatinine concentration in their serum. Herein, venous blood samples were taken from 36 patients with CHF. Diagnosis was confirmed by echocardiographic, as well as by electrocardiographic examinations. In this work, the control group consisted of 41 randomly selected healthy individuals. The results of our study showed that CHF patients had significantly lower concentration of 25(OH)D, as well as total calcium and inorganic phosphates. Moreover, mean creatinine concentration was higher, in comparison to the control group, but did not exhibit statistical significance. As calcium-phosphate homeostasis is regulated by numerous factors, including PTH, neurohormonal factors and calcitriol ($1,25(OH)_2D$), it is possible that vitamin D deficiency may play a significant role in the pathomechanism of CHF, and a lowered 25(OH)D level may be related to progression of the disease.

INTRODUCTION

Calcium-Phosphate homeostasis regulation is the most important function of vitamin D. Since the discovery of vitamin D and its role in the treatment of rickets and osteomalacia, many pleiotropic effects of vitamin D have been demonstrated. Moreover, researchers have revealed the existence of vitamin D receptors (VDR), not only in the organs taking part in the calcium-phosphate metabolism, but also in other organs and tissues. Indeed, in such work, VDR expression was documented in a variety of tissues and organs (including the heart, blood vessels, smooth muscle tissues, immune system cells and the endocrine system glands). In areas of VDR expression, 1α-hydroxylase was ascertained as being responsible for the conversion of 25(OH)D to $1,25(OH)_2D$. *In vitro* tests have also demonstrated vitamin D's involvement in the modification of cardiovascular system function. This comes about by means of its action upon several important mechanisms. In this, $1,25(OH)_2D$ influences the *REN-1C* gene by inhibiting its expression. This leads to the decreased activation of the rennin-angiotensin-aldosterone system (RAAS). Moreover, $1,25(OH)_2D$ inhibits smooth muscle cell proliferation in blood vessels, and it impedes the synthesis and migration of extracellular matrix components. A direct influence of $1,25(OH)_2D$ on myocardial cells has also been observed. Such an effect results in the prevention of hypertrophic cardiomyopathy independent of the RAAS and hypertension-mediated mechanisms. Moreover, $1,25(OH)_2D$ is known as a regulator of the immunological system. Plus, by inhibiting the process of maturity of antigen-expressing cells and influencing cytokine excretion, it exerts an anti-inflammatory effect and prevents vessels from being damage. Vitamin D is also known to be involved in the calcium-phosphate metabolism, the dysfunctions of which, are, among other mechanisms, connected to cardiovascular system disorders. Furthermore, Vitamin D deficiency is a well known risk factor for hypertension and other cardiovascular system diseases. In addition, decreased 25(OH)D level can also contribute to the increased chance of CHF, ischaemic heart disease (IHD), peripheral vascular disease (PVD) incidence and ischaemic stroke [1,6,7,9].

The aim of this study involved assessing vitamin D level in patients suffering from CHF, doing so by measuring blood serum concentration of 25(OH)D and identifying

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any correlation between vitamin D level and total calcium, inorganic phosphates and creatinine level.

MATERIALS AND METHODS

The study was conducted with the permission of the Medical University of Lublin Bioethics Board. The subject range of this study were 36 individuals (24 males and 12 females) suffering from severe CHF. The average age of the test group was at 74±10 years, and the patients were admitted to hospital because of severe decompensated heart failure. Clinical evaluation consisting of clinical history and physical examination. Moreover, X-ray, echocardiography and measurement of serum NT-proBNP was performed for all subjects. Such work revealed that arterial fibrillation was the most frequent cause of the sustained arrhythmia among the studied patients. Moreover, the crackles that were found over the lungs suggested pulmonary congestion in all studied cases. All patients were, as well, assessed as being in NYHA class III or IV, as examinations showed the presence of peripheral swellings and resting dyspnoea. What is more, in most of them, their X-rays disclosed either pleural effusion (33%) or pulmonary congestion (21%) or both (19%). Furthermore, the concentration of NT-proBNP was over 300 pg/ml in all cases. The control group was composed of 41 healthy individuals (19 males and 22 females) with the average age of 49±9 years. In this study, venous blood samples from all the participants were centrifuged to obtain serum. From this, serum level of 25(OH)D, total calcium, inorganic phosphates and creatinine were determined. The concentration of 25(OH)D was measured through employing the electrochemiluminescence (ECL) method, using a COBAS e411 Analyzer (Roche) and commercially available reagents. Total calcium, inorganic phosphates and creatinine concentrations were determined using a COBAS INTEGRA 400 Plus Analyzer. The concentration of total calcium were ascertained by way of the ortho-cresolphthalein complexone (OCP) colorimetric method. Finally, the concentration of inorganic phosphates were measured using a method based on the reaction with ammonium heptamolybdate, while, creatinine level were determined using the enzymatic colorimetric method. All tests were conducted in a one-series session at the Medical University Hospital Diagnostic Laboratory, in Lublin.

All collected data were subject to statistical analysis, and descriptive statistics techniques were applied to the values of the examined parameters that were obtained from both test and control groups. In order to compare the results among the groups, the student's t-test for independent samples was applied when data distribution was normal. For data distributions other than normal, the non-parametric Mann-Whitney *U* test was applied. The correlations were examined using Spearman's rank correlation coefficient. Herein, 5% inference error and associated significance level $p<0.05$ were accepted.

RESULTS

The mean serum concentration of 25(OH)D in the study group was determined at 10.86±9.75 ng/ml, and was significantly lower ($p<0.05$) than mean concentration in the control group (20.35±7.35 ng/ml). In addition, total calcium and inorganic phosphates concentration of the control group were significantly lower, in comparison to the study group, while creatinine concentration was higher in the study group, but did not show statistical significance. Table 1 and Figure 1 present the results of 25(OH)D, total calcium, inorganic phosphate and creatinine concentration determinations in both groups of subjects.

Table 1. The results of 25(OH)D, total calcium, inorganic phosphates and creatinine concentration determination in both groups of examined subjects

Parameter	Study group			Control group		
	x̄±SD	Me	25-75%	x̄±SD	Me	25-75%
25(OH)D (ng/ml)	10.86 ±9.75*	8.08	3.00-14.49	20.35 ±7.33	19.88	14.11-24.92
Ca (mmol/l)	1.08 ±0.58*	0.96	0.61-1.30	2.31 ±0.09	2.33	2.25-2.37
P (mmol/l)	0.51 ±0.30*	0.42	0.29-0.74	1.16 ±0.25	1.10	0.97-1.31
Creatinine (mg/dl)	1.06 ±0.42	1.05	0.70-1.30	0.66 ±0.20	0.71	0.50-0.79

x̄ – arithmetical mean; SD – standard deviation; Me – median; 25-75% – percentile range; 25(OH)D – calcidol concentration; Ca – total calcium concentration; P – inorganic phosphates concentration; Creatinine – creatinine concentration; * $p<0.05$

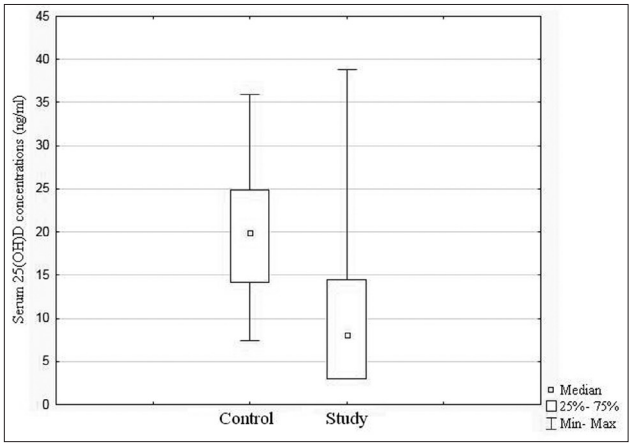

Figure 1. Comparison of serum 25(OH)D concentration in study and control groups; $p<0.05$

Table 2. Correlations between selected parameters

Parameters		Correlation (R Spearman)	Significance p
25(OH)D Study group	Ca (mmol/l)	0.327	0.005
	P (mmol/l)	0.312	0.008
	Creatinine (mg/dl)	0.140	0.128
25(OH)D Control group	Ca (mmol/l)	0.146	0.363
	P (mmol/l)	-0.255	0.108
	Creatinine (mg/dl)	0.077	0.631

25(OH)D – calcidol concentration; Ca – total calcium concentration; P – inorganic phosphates concentration; Creatinine – creatinine concentration; $p<0.05$

The results of correlation tests between 25(OH)D and the remaining parameters are presented in Table 2.

DISCUSSION

According to the most recent available knowledge, vitamin D deficiency plays a role in CHF. Due to the presence of VDR and 1α-hydroxylase receptors in the cardiac muscle and blood vessel cells, vitamin D acts directly

upon the cardiovascular system. It suppresses RAAS action by down-regulating the REN-1C gene and increasing the calcium influx into the juxtaglomerular cells. This activity results in the decreased secretion of renin. In addition, some studies suggest vitamin D deficiencies trigger RAAS. This leads to hypertension. Furthermore, $1,25(OH)_2D$ is known to inhibit the proliferation of myocardial cells, as well as the immunological and inflammatory response. All of the aforementioned play vital roles in the development and progression of heart failure [1,10,12].

In the course of our work, the observation of the lowered 25(OH)D levels in the CHF patients, in contrast to that of the healthy individuals, remains in line with the previous findings of Gortsman *et al.* [5] who studied a group of 3009 CHF patients in contrast to 46 825 healthy individuals. Gortsman *et al.* pointed out a limitation in their work arising from their single measurement of 25(OH)D, noting that this does not account for seasonal changes in vitamin D level. The same limitation applies to the current study. Still, further investigation has revealed that vitamin D deficiency is an independent factor of increased patient mortality.

Another study where lowered levels of 25(OH)D in CHF patients were observed was that of Milovanovic *et al.* [8], who studied 40 elderly CHF patients in Serbia. In this work, Milovanovic *et al.* observed both high levels of interleukin 17 (IL-17) and tumor necrosis factor (TNF-α). This may suggest vitamin D's influence on cardiovascular disease development by its action upon hypertension and inflammatory processes, as vitamin D is known to regulate the expression of IL-17 and TNFα in Th17 cells (Th17). It should be noted that IL-17 is known to bring about the production of pro-inflammatory interleukin 6 (IL-6) and TNF-α, especially in the heart. On the other hand, vitamin D might decrease IL-6 and TNF-α levels, hence, influencing the differentiation of Th17 cells. This effect suggests its involvement in the modulation of the early phase of the inflammatory state.

Based on data from a cross-sectional, observational Framingham Offspring Study, Wang *et al.* [11] ascertained a close correlation between vitamin D deficiency and the increased risk of cardiovascular diseases occurrence. In this work, the greatest risk of developing cardiovascular disease was observed in individuals whose vitamin D deficiency was coupled with hypertension. In this group, serum concentration of 25(OH)D below 15 ng/ml was associated with a doubled risk of cardiovascular incidents occurrence. To explain this, the authors suggested the immunosuppressive role of vitamin D in lowering lymphocyte proliferation and cytokines secretion, as increased concentration of pro-inflammatory cytokines induces dysfunctions in the mesothelial production of nitric oxide (NO) and prostacyclin, leading to decreased vasodilatation ability. Vitamin D analogues have also been noted as reductors of metalloproteinase 9 (MMP-9). Metalloproteinases are known to participate in extracellular matrix components degradation, and to contribute to blood vessel wall and cardiac muscle remodeling, as well as cracking of atherosclerotic plaque and formation of parietal thrombi. Therefore, vitamin D can lower the risk of cardiovascular disease by regulating the immune system and lowering the concentration of acute-phase proteins and pro-inflammatory cytokines.Renal failure can also be considered to be among the several causes of CHF. During the current study, concentration of creatinine was used as a marker for the evaluation of renal function. However, in our work, while the observed mean creatinine concentration in the group of CHF patients was higher than in that of people from the control group, the difference did not show statistical significance.

The observation of a significantly decreased 25(OH)D level in CHF patients and a positive correlation between 25(OH)D and total calcium and inorganic phosphates, is similar to the previous observations of Zittermann *et al*. [13]. In their study, they observed lowered levels of 25(OH)D, $1,25(OH)_2D$ and total calcium in congestive heart failure patients. However, their work showed overall higher concentration of inorganic phosphates in CHF patients, compared to the control group. Zittermann *et al*. have also observed a positive correlation between the levels of $1,25(OH)_2D$ and total calcium. This, they found was accompanied by a negative correlation with inorganic phosphates. According to the authors, a decreased level of total calcium and an increased level of inorganic phosphates might result from vitamin D deficiencies. Similar to the results of this study, Zitterman et al. did not report increased creatinine concentration, and, since it seemed unlikely that decreased 25(OH) D level resulted from renal failure, they stated that vitamin D deficiency might play a role in the pathomechanism of CHF.

The relevance of calcium-phosphate homeostasis alterations for CHF development and progression was previously suggested by Cubbon *et al.* [2], who examined changes of its parameters as hypothetically useful markers of CHF severity. During their study of 713 patients, they observed a broad range of total calcium and inorganic phosphates concentrations. This work resulted in the generation of a series of calcium phosphate product (CPP) values. In this study, they associated CPP changes with increased levels of mortality, and they pointed out the need of further research into a role played by several factors, including vitamin D, in calcium phosphate homeostasis regulation in the course of CHF.

The observation of decreased vitamin D level in CHF patients is in line with the most recent findings of other authors. On the other hand, the observation of decreased concentrations of total calcium and inorganic phosphates without significant creatinine levels alterations contributes to varying results previously described in literature. As the pathomechanism of CHF and the role of vitamin D in disease progression have not been fully explained, further research into the topic is still required. Commonly observed deficiencies of vitamin D in the healthy population are a severe concern and suggest that vitamin D deficiency may play a significant role in CHF development [3,4,7].

CONCLUSIONS

In conclusion, our observation of significantly lowered level of 25(OH)D in CHF patients, in comparison to the control group, must be acknowledged. Simultaneously, we noted disturbances in mineral metabolism of CHF patients manifested by lowered level of total calcium and inorganic phosphates. Taking into account the complexity of calcium-phosphate metabolism regulation, which is influenced

by many factors, including PHT, neurohormonal factors and 1,25$(OH)_2$D, it can be suggested that vitamin D deficiency may contribute to the pathomechanism of CHF and aid the disease's progression.

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