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High prevalence of severe vitamin D deficiency in patients with acute myocardial infarction

Vitamin D-Mangel mit hohe Prävalenz in Patienten mit akutem Myokardinfarkt

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an extraordinarily high prevalence of vitamin D deficiency or insufficiency in these patients.

Keywords: acute coronary syndrome; calcidiol; myocardial infarction; unstable angina pectoris; vitamin D.

Abstract

Background: Vitamin D deficiency is associated with acute coronary syndrome (ACS). We aimed to evaluate calcidiol status and its relationship with coronary angiography findings in two selected groups of ACS patients.

Methods: We investigated two groups of patients with ACS: 75 patients with ST-segment-elevation myocardial infarction (STEMI) and 68 patients with unstable angina pectoris (USAP). The ACS diagnosis was confirmed by coronary angiography findings. Biochemical parameters were studied at the first visit of the patients with automated instruments and ready-to-use kits.

Results: Calcidiol levels were significantly lower in the STEMI group compared to the USAP group ($p < 0.001$), while the prevalence of calcidiol deficiency in the STEMI group was significantly higher ($p < 0.001$). Serious calcidiol deficiency (< 4 ng/mL) was present in 17% of the STEMI group and in 7% of the USAP group. We did not observe any significant relationship between calcidiol status and coronary angiography findings.

Conclusions: Our results support the previously described associations between ACS and calcidiol deficiency. Besides, we report a more severe calcidiol deficiency and

Zusammenfassung

Einleitung: Das akute Koronarsyndrom (ACS) ist durch Vitamin D-Mangel gekennzeichnet. Ziel war es, den Calcidiol-Spiegel in zwei durch Koronarangiographie differenzierten Patientengruppen mit ACS zu charakterisieren.

Methoden: Mittels Koronarangiographie wurden 75 Patienten mit ST-Hebungsinfarkt (STEMI) und 68 Patienten mit instabiler Angina Pectoris (USAP) ausgewählt. Die klinisch-chemischen Marker wurden mit Standardmethoden bestimmt.

Ergebnisse: In Vergleich zur USAP-Gruppe war Calcidiol in der STEMI-Gruppe ($p < 0,001$) signifikant vermindert. Die Häufigkeit des Calcidiol-Mangels war in der STEMI-Gruppe ($p < 0,001$) signifikant höher als in der USAP-Gruppe. Einen schweren Calcidiol-Mangel (< 4 ng/mL) wiesen 17% der STEMI-Patienten und 7% der USAP-Patienten auf. Keine signifikante Beziehung existierte zwischen dem Calcidiol-Status und der Anzahl bzw. dem Schweregrad der Stenosen.

Schlussfolgerungen: Die von uns präsentierten Ergebnisse zeigen, dass Patienten mit ACS einen Calcidiol-Mangel aufweisen, der bei Patienten mit STEMI stärker ausgeprägt ist als bei Patienten mit USAP.

Schlüsselwörter: akutes Koronarsyndrom; Calcidiol; instabile Angina Pectoris; Myokardinfarkt; Vitamin D.

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Introduction

Acute coronary syndrome (ACS) is the leading cause of death among elderly people. Age is a powerful predictor of adverse events in ACS [1, 2]. Old people live mostly

indoors, which means low exposure to sunlight and diminished skin vitamin D synthesis. They also experience problems in food intake; thus, vitamin D supplementation by nutrition is often too low to compensate for mitigated dermal production. Age-related inadequate gut absorption can only worsen problems related to hypovitaminosis D [1–3]. Vitamin D is a pro-hormone obtained from dietary sources or produced by UV activation in the skin. It functions through a specific receptor [vitamin D receptor (VDR)], and as a transcription factor, directly or indirectly controls more than 200 distinct genes. This includes genes for the arrangement of cellular proliferation, differentiation, and angiogenesis. Cardiac myocytes and fibroblasts have been shown to express their own 1α -hydroxylase and 24 -hydroxylase enzymes, a finding of great importance. This suggests the local conversion of 25-hydroxycholecalciferol [25(OH)D] to calcitriol (also called 1,25-dihydroxyvitamin D3), which is the active form of D3. 1α -Hydroxylase in extra-renal tissues, including the heart, is mainly dependent on substrate availability, so the concentration of circulating 25(OH)D might be an important determinant of the effects of vitamin D in the myocardium [1, 4, 5].

Vitamin D is not only a significant mediator in many different physiological processes, but also plays an important role in many chronic diseases [6]. Studies have reported that in the cardiovascular system, the genomic contraptions responsible for vitamin D's non-classical effects are mediated primarily by calcitriol interacting with intracellular VDR [7, 8]. Calcitriol was previously reported to modulate key processes (such as plaque stabilization) involved in the pathogenesis of ACS. This includes vascular inflammation, platelet aggregation/thrombogenesis, vascular smooth muscle cell proliferation, calcification, and myocardial fibrosis and proliferation [3]. Serum 25(OH)D is the marker representing the total body reserve of vitamin D. 25(OH)D deficiency has also been linked to increased risk of adverse cardiovascular events including higher risk of acute myocardial infarction (AMI), cardiovascular death, and overall mortality. 25(OH)D levels are often reported to be lower than reference levels in the normal population, but the situation is even worse in patients evaluated for ACS [9, 10]. Vitamin D improves cardiovascular health through its antagonist effect on the renin-angiotensin system, through its anti-platelet action as expressed by the prevention of calcium influx and amelioration of endothelial functions. The incidence of myocardial infarction (MI) was doubled with 25(OH)D levels lower than 15 ng/mL (37.5 nmol/L) in a study on healthy male medical staff. However, the relation between 25(OH)D levels and outcomes in ACS has not been well reported

[10–14]. Only a few studies have reported frequent 25(OH)D deficiency in patients with ACS.

In this study, we have chosen to investigate two groups of patients with two different diagnosis, namely ST-segment-elevation acute myocardial infarction (STEMI) and unstable angina pectoris (USAP). The overall results from both groups were thought to give information about vitamin D status in ACS. In addition, comparison between these two groups was thought to have clinical applications. We also aimed to correlate laboratory results, particularly vitamin D levels, with clinical findings. The association between the prevalence of calcidiol deficiency in STEMI and USAP patients has never been evaluated [3, 10, 15, 16].

Materials and methods

Subjects

One hundred and forty-three male patients admitted to our hospital with chest pain between November 2012 and March 2013 were enrolled in this study. The STEMI group consisted of 75 patients with ST elevations ≥ 0.2 mV in two adjacent leads on ECG [17] who underwent primary percutaneous coronary intervention (PCI) within 12 h of the onset of symptoms. The exclusion criteria were prior MI, cardiac arrest, and prior coronary artery bypass graft (CABG) or PCI application. The USAP group consisted of 68 class I-II-III angina patients by the Braunwald classification [18]. The exclusion criteria for the group were angina due to secondary causes, positive troponin, prior MI, CABG, contraindications for angiography or failure to detect a thrombotic critical stenosis in angiography, segmental wall motion in echo or left ventriculography. Individuals with advanced comorbid conditions, including malignancy, advanced heart failure, or valvular heart disease, were not included either.

The presence of obesity, diabetes mellitus, and hypertension was also assessed. Obesity was defined as body mass index (BMI) >27.8 kg/m² as proposed by the National Institutes of Health consensus statement. Diabetes was defined as a fasting blood glucose >126 mg/dL or diagnosis of diabetes needing diet or drug therapy. Hypertension was defined as a resting systolic blood pressure >140 mm Hg and/or a diastolic blood pressure >90 mm Hg. Smoking was defined as being a current smoker and smoking at least 10 cigarettes per day for at least 10 years.

Participants answered a questionnaire about their lifestyle and medical history. All of the participants gave written informed consent, and the Local Ethics Committee Review Board approved the study protocol. Percutaneous coronary angiography (PCI) was performed using the standard percutaneous Judkins technique.

The angiographic characteristics, which included lesion location and percentage of stenosis of all coronary lesions in the index coronary angiogram, were obtained by reviewing the angiogram.

All of the coronary angiographies were performed in the same center by two experienced cardiologists, both blinded to the study protocol.

Analytical methods

Samples: Blood samples were obtained after overnight fasting. Serum samples were then separated from cells by centrifugation at 1500 g for 10 min. Lipid parameters and other routine parameters were freshly measured. The remaining serum portions were stored at -80°C and were used to analyze 25(OH)D levels.

Measurement of serum 25(OH)D levels was performed using a directly competitive chemiluminescence immunoassay method (DiaSorin, Stillwater, MN, USA) on LIAISON® analyzer. The reagents contain an antibody specific to 25(OH)D, which is coated on magnetic particles, and 25(OH)D is conjugated to an isoluminol derivative and diluted in a phosphate buffer. During the first incubation stage, 25(OH)D is dissociated from its binding protein and interacts with binding sites on the antibody on the solid phase. Then, after the second incubation with the tracer, the unbound material is washed off and starter reagents are added to generate a flash chemiluminescent signal, which is measured by a photomultiplier and is inversely related to 25(OH)D concentration. The conversion factor from nanograms per milliliter 25(OH)D to nanomoles per liter 25(OH)D is 2.49. The LIAISON® assay is linear only up to 125 ng/mL (312 nmol/L) of total 25(OH)D for unaltered samples. The limit of detection is 3.5 ng/mL (87.3 nmol/L), and the assay has a coefficient of variability ranging from 4.8% to 11.1%.

The ideal plasma level of 25(OH)D is 30 ng/mL (75 nmol/L) or above, while levels of 21–29 ng/mL (51–74 nmol/L) are generally considered insufficient and levels below 20 ng/mL (50 nmol/L) are considered deficient. The term “severe deficiency” (osteopenia) is commonly used in settings when serum 25(OH)D levels are <10 ng/mL (25 nmol/L) [10].

Routine laboratory parameters: The levels of triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and creatinine were determined using commercially available assay kits (Abbott) with an auto analyzer (Architect® c16000; Abbott Diagnostics, IL, USA).

Statistical analysis

Statistical analyses were carried out using the SPSS statistical software (NY, USA). The results were presented with mean and SD in normally distributed groups or with medians. The significance of the differences between groups was determined by unpaired Student t-test, one-way analysis of variance, Kruskal-Wallis, and χ^2 tests. Pearson and Spearman correlation coefficients were used to test the strength of any associations between different variables. p-Values <0.05 were accepted as significant.

Results

The baseline clinical and laboratory parameters from the STEMI and USAP patient groups are shown in Table 1. One hundred and forty-three patients were included in the study (STEMI, $n=75$; USAP, $n=68$). Age, sex, renal function tests, and lipid profile tests were not statistically

Table 1: Baseline characteristics of patients of STEMI and USAP group (mean $\pm$ SD).

Group	STEMI (n=75)	USAP (n=68)	p-Value
Age, years	67 $\pm$ 14	69 $\pm$ 10	NS
Sex (male), n (%)	60 (80)	47 (69)	NS
BMI, kg/m ²	18.8 (18–20)	18.2 (18–19)	NS
Diabetes mellitus, n (%)	10 (13)	16 (23)	NS
Hypertension, n (%)	8 (11)	6 (9)	NS
Previous stroke, n (%)	1 (1)	–	–
ST-segment deviation	75 (100%)	21 (20%)	<0.001
Ejection fraction	48 $\pm$ 11	62 $\pm$ 5	<0.001
GFR (MDRD)	93 $\pm$ 21	101 $\pm$ 28	NS
TC, mg/dL	215 $\pm$ 19	228 $\pm$ 36	NS
TG, mg/dL	173 $\pm$ 46	201 $\pm$ 25	NS
LDL-C, mg/dL	129 $\pm$ 42	135 $\pm$ 10	NS
HDL-C, mg/dL	35 $\pm$ 10	38 $\pm$ 8	NS
Hospital treatment, n (%)			
Aspirin	75 (100)	68 (100)	NS
Clopidogrel	73 (97)	68 (100)	NS
β -Blockers	50 (66)	63 (98)	<0.001
Statins	62 (83)	59 (88)	NS
ACE inhibitors	59 (79)	55 (85)	NS
PCI	75 (100)	68 (100)	NS

GFR, glomerular filtration rate; MDRD, modification diet in renal disease; ACE, angiotensin converting enzyme; NS, non-significant. Ejection fraction was measured at post-PCI 48 h.

significant different between groups. ST-segment deviation was the definitive characteristic of the STEMI group, but 20% of the USAP group also showed this feature. Ejection fraction was significantly higher in USAP patients. When medications were compared, the use of β -blockers was significantly higher in the USAP group. 25(OH)D levels were significantly lower (74 $\pm$ 4.2 ng/mL) in the STEMI group, compared to the USAP group (10.4 $\pm$ 5.4 ng/mL) ($p<0.001$).

Patients in both the STEMI and the USAP groups were separated into four subgroups according to their 25(OH)D levels: 0–4, 4–10, 10–20, and >20 ng/mL. A severe deficiency of 25(OH)D was present in 17% of the STEMI group and in 7% of the USAP group. Only 7% of the USAP group showed 25(OH)D levels higher than 20 ng/mL, and no patients in the STEMI group were within that range. Interestingly, there were no patients with normal 25(OH)D levels (>30 ng/mL) in both of our study groups. STEMI patients accumulated in the 4–10-ng/mL group (59%), while USAP patients were mostly distributed between the 4 and 10-ng/mL (47%) and 10–20-ng/mL group (38%) (Table 2; Figure 1).

According to the results of angiography during PCI, we divided the patients into three groups based on the number of vessels with stenosis. All of the patients had at least one vessel with >50% stenosis. According to Table 3,

Table 2: 25(OH)D concentration in the USAP and STEMI groups.

25(OH)D, ng/mL	STEMI (n=75), n (%)	USAP (n=68), n (%)	p-Value ^a
0–4	13 (17)	5 (7)	<0.001 ^a
4–10	44 (59)	32 (47)	
10–20	18 (24)	26 (38)	
>20	0	5 (7)	<0.001 ^b
Total	7.4±4.2	10.4±5.4	

Only 5 patients in the USAP group and none of the patients in the STEMI group showed 25(OH)D levels above 20 ng/mL. USAP group vitamin D levels were significantly higher. The differences between groups were determined by ^a χ^2 and ^bStudent t-tests.

Table 3: Subgroups according to 25(OH)D levels and number of vessels with sclerotic disease did not show a significant difference.

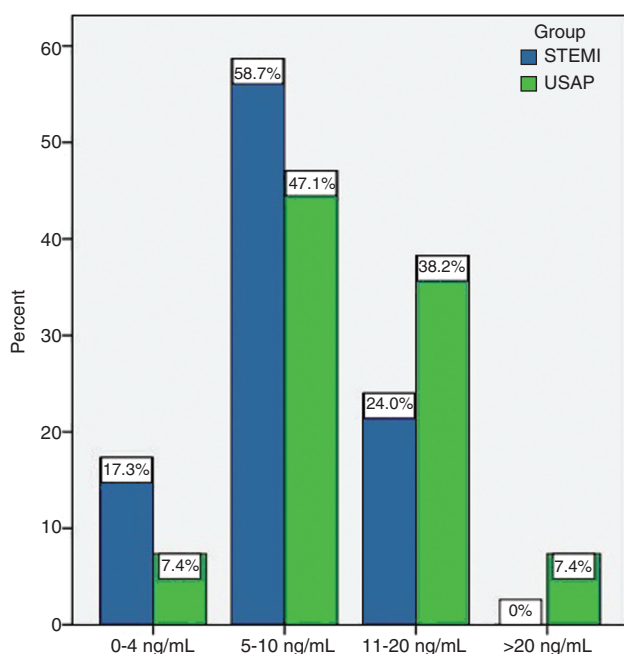
Quartiles (143 patients)	Number of vessel disease			p-Value ^a
	One vessel (n=60), n (%)	Two vessels (n=46), n (%)	Three vessels (n=37), n (%)	
0–4 ng/mL (n=18)	8 (13)	6 (13)	4 (11)	0.3
4–10 ng/mL (n=82)	34 (57)	30 (65)	18 (49)	
10–20 ng/mL (n=38)	17 (28)	9 (20)	12 (32)	
>20 ng/mL (n=5)	1 (2)	1 (2)	3 (8)	

^aThe differences between groups was determined by χ^2 test.

25(OH)D intervals showed no statistically significant differences irrespective of the number of stenotic vessels and the degree of stenosis in patients with ACS ($p=0.3$). 25(OH)D intervals did not correlate with the baseline clinical and laboratory parameters such as TC, TG, BMI, HDL-C, and LDL-C (Table 4).

Discussion

Previous studies have reported that vitamin D deficiency has been linked to an increased risk of coronary artery disease and cardiovascular death [1–3, 19, 20].

**Figure 1:** The distribution of STEMI patients differed compared to USAP patients.

To the best of our knowledge, this study is the first to demonstrate a high prevalence of a severe deficiency of 25(OH)D in two different clinical types of ACS, namely STEMI and USAP. The prevalence of 25(OH)D deficiency and the features associated with it in patients with ACS are less known. Only two recent studies in the literature reported a high prevalence of 25(OH)D insufficiency in these patients [3, 16].

Our findings present an extraordinarily high prevalence of vitamin D deficiency (76% in the STEMI group and 54% in the USAP group) or insufficiency (100% in the STEMI group and 38% in the USAP group) in ACS. We believe these results provide evidence and support for previously described associations between ACS and 25(OH)D deficiency. Contrary to our findings, Rodriguez et al. [15] reported no difference in mean plasma vitamin D levels between patients with ACS and controls. Our study did not include a control group and thus lacks such a comparison.

The finding should not be very surprising because 25(OH)D insufficiency is quite common in developed countries [21]. Our results presented a more severe 25(OH)D deficiency than in previous studies. The difference was probably due to our study population, which comprised Caucasians from a developing country. However, it was unknown whether the measure of physical activity was a surrogate for time spent outdoors or other healthy behaviors, such as multivitamin supplement use [22]. Brøndum-Jacobsen et al. [23] reported that decreased plasma 25(OH)D levels were associated with increased risk of MI, ischemic heart disease, and early death as a function of seasonally adjusted percentile categories in 10,700 Danish patients. Similarly, Giovannucci et al. [9] reported that decreased levels of 25(OH)D are associated with a higher risk of MI in 454 men with 10 years of follow-up study. We know that adequate vitamin D levels may promote cardiovascular health by improving endothelial

Table 4: Subgroups according to 25(OH)D levels compared to clinical and other laboratory findings did not show any significant difference.

Vitamin D status	0–4 ng/mL (n=18)	4–10 ng/mL (n=76)	10–20 ng/mL (n=44)	>20 ng/mL (n=5)	p-Value
Age, years	67.8±10	66.3±12	65.7±10	62.5±22	NS
Sex (male), n (%)	10 (55)	55 (72)	34 (77)	3 (60)	NS
Diabetes mellitus, n (%)	2 (11)	16 (21)	7 (16)	1 (20)	NS
HT, n (%)	1 (5)	9 (11.8)	4 (9)	1 (20)	NS
ST elevation, n (%)	8 (44)	60 (79)	32 (73)	1 (20)	NS
Ejection fraction	55±9	52±11	58±5	63±10	NS
GFR (MDRD)	98±8	102±12	104±7	91±9	NS
TC, mg/dL	222±25	218±19	198±22	201±35	NS
TG, mg/dL	175±13	198±17	210±12	195±23	NS
LDL-C, mg/dL	133±12	158±14	131±9	125±25	NS
HDL-C, mg/dL	34±4	35±7	33±5	38±7	NS

function and down-regulating inflammation. Also, bio-available calcitriol modulates coronary microvascular function.

This effect might contribute to the high cardiovascular risk of conditions characterized by chronic reduction in the bioavailability of this hormone [24, 25]. Because vitamin D deficiency is being increasingly linked to adverse health outcomes, an understanding of the genetic variants responsible for the diversity in relevant vitamin D metabolites in diverse populations is vital [26, 27]. Vitamin D deficiency is associated with coronary artery disease, and the actions of vitamin D are mediated by its binding to a specific nuclear VDR. VDRs are distributed in a variety of tissues including vascular smooth muscle cells, cardiomyocytes, and cells of the immune system [28, 29]. One of the functions of calcitriol, as well as other VDR ligands, is to impede the proliferation of vascular smooth muscle cells. A number of extra-renal tissues, including vascular smooth muscle cells, produce the enzyme CYP27B1, which transforms the primary circulating form of vitamin D, calcidiol to its active form, calcitriol. Eventually, calcitriol reduces platelet aggregation and thrombogenesis, likely through the activation of the VDR [29, 30]. Today, the growth suppressant and immune-modulatory effects of calcitriol are of important interest because of their potential use in the management of disorders, including ACS and atherosclerosis where the principal pathological mechanisms are unrestrained cell growth and remodeling in the vascular wall [29–34]. Inflammation is also a key factor driving the processes of plaque formation, progression, and rupture in patients with ACS and cardiovascular diseases (CVDs). An inflammatory subset of monocytes and macrophages has been reported to selectively concentrate in atherosclerotic plaques and produce pro-inflammatory cytokines. Calcitriol, involved in the regulation of body calcium homeostasis, promotes the differentiation of immature myeloid

precursor cells into monocytes/macrophages. Crucially, calcitriol has long been shown to possess immune-regulatory properties and may inhibit key steps in this inflammatory process [35–37]. Calcitriol also appears to modulate plasminogen activator-inhibitor expression in endothelial cells [38, 39]. However, 25(OH)D does not exhibit an acute-phase reaction after AMI. Indeed, 25(OH)D levels do not change after AMI and is likely to be a reliable marker of vitamin D status in patients with CVD [16, 40, 41]. Ng et al. [42] reported that 25(OH)D was prognostic for major adverse events in patients with AMI and an approximately 40% risk reduction for 25(OH)D levels above 7.3 ng/mL. This finding is compatible with our study, and it is mandatory to keep high levels of 25(OH)D to prevent adverse cardiac outcomes.

25(OH)D levels were measured by an immunoassay in this study, and readers should bear in mind that the differences between all studies including ours may be due to different assays used [43]. After all, low 25(OH)D levels could also be simply a consequence of CVD. Our statistical analysis does not include adjustments for potential confounders such as BMI, age, etc.

Although a major limitation of this study was the small number of patients and lack of follow-up data, this study demonstrates for the first time that severe deficiency of 25(OH)D is prevalent in patients with ACS and is more frequent in STEMI patients than in USAP patients.

In conclusion, these findings suggest that seriously deficient 25(OH)D levels increase sustained pro-atherosclerotic reactions, which may subsequently lead to plaque rupture. Previous studies present very low levels of 25(OH)D as independently related to ACS, suggesting that vitamin D supplementation is a promising approach in the prevention of ACS. An improved understanding of the epidemiologic associations and biologic pathways through which vitamin D may affect cardiovascular health could

inform future clinical trials and ultimately evidenced-based therapeutic recommendations [44]. However, the exact therapeutic dosage of vitamin D for cardiovascular health is not known. The 25(OH)D levels recommended as “cutoffs” to define vitamin D deficiency differ between the US IOM report and the US Endocrine Society guideline. The US Endocrine Society guideline [45] defines vitamin D deficiency as 25(OH)D <20 ng/mL (50 nmol/L), vitamin D insufficiency as 25(OH)D between 21 and 29 ng/mL, and the safety margin to minimize the risk of hypercalcemia as 25(OH)D equal to 100 ng/mL (250 nmol/L). Meanwhile, the US IOM report [46] concluded that 25(OH)D equal to 16 ng/mL (40 nmol/L) covers the requirements of approximately half the population, 25(OH)D equal to 20 ng/mL (50 nmol/L) covers the requirements of $\geq 97.5\%$ of the population, and 25(OH)D >50 ng/mL (125 nmol/L) should raise concerns about potential adverse effects.

At present, vitamin D deficiency can only be considered a cardiovascular risk marker, as vitamin D supplementation with the doses recommended for osteoporosis treatment is neither proven beneficial nor harmful in CVDs. When considering the debate on the relationship between vitamin D and ACS [47], more emphasis should be placed on RCTs among severely vitamin D-deficient populations who would most likely benefit from vitamin D treatment. Vitamin D repletion is an affordable, natural, and easily modifiable intervention that holds tremendous potential as a public health solution for reducing cardiovascular-related health disparities, as does potential new vitamin D-related cellular targets. Prospective studies are needed to investigate the benefits of screening and treatment of this very common vitamin Deficiency.

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