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A study of difference in serum 25-hydroxyvitamin D concentrations in patient with angiographically-defined coronary disease and healthy subjects

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Abstract:

Background: Cardiovascular disease (CVD) is one of the most important causes of death in developing countries. The current study evaluates the serum 25-hydroxyvitamin D (25OHD), phosphate and calcium levels in patients with angiographically-defined coronary artery disease (CAD) and healthy subjects in a sample population in northeastern Iran.

Methods: There were 566 subjects aged between 20–80 years out of whom283 subjects with CAD were divided into two study groups based on their angiogram results; those with >50% stenosis of one or more coronary arteries and those with ≤50% stenosis. Serum 25OHD levels and anthropometric parameters were measured for all subjects.

Results: There were approximately 53% (n=303) males and 47% (n=269) females in the population sample. We found that crude serum 25OHD concentrations were significantly higher in both the $Angio^-$ (21.6±11.8 ng/ml) and $Angio^+$ (21.3±10.2 ng/ml) groups compared to the control subjects (16.4±9.5 ng/ml) ($P<0.001$).

Conclusion: The findings show that 25OHD state could be a risk factor for CAD, although this would need to be explored further, taking the potential confounding effects of diet into account in future studies.

Keywords: 25OHD; Phosphate; Calcium; Angiography; Coronary artery disease

Introduction

Cardiovascular disease (CVD) is one of the most important causes of death in developing countries (1). Although 25-hydroxyvitamin D (25OHD) status has a great impact on the musculoskeletal system, it seems that 25OHD state has also a significant role in determining CVD risk (2). 25OHD is developed in human's skin after exposure to the solar ultraviolet radiation. Hence, factors such as seasons and settlement in urban or rural areas can influence 25OHD status (3). 25OHD is essential for intestinal calcium absorption and can affect bone mineralization as well. 25OHD also influences cardiac contractility and myocardial calcium homeostasis (4).

25OHD has a protective role in CVD as it can improve vascular proliferation and calcification, as along with pro- and anti-inflammatory cytokines. 25OHD may therefore exert its effects through other cardiovascular risk factors (3).

25OHD deficiency has been associated with increased blood pressure, heart failure and several vascular diseases (5). 1,25-dihydroxyvitamin D (1,25[OH]$_2$D) derived from 25OHD is involved in several processes beyond calcium and phosphate homeostasis. Therefore it is expected to influence CVD development (5).

Given the important physiological roles of phosphate, serum phosphate concentrations are regulated and maintained at a limited range in healthy people (11). Higher levels of phosphate are related to adverse cardiovascular outcomes and are associated with an increase in CVD risk; this association has been reported to be stronger in men (8, 9). A serum phosphate of <3.5 mg/dL has been shown to be related to lower CVD risk (6), whilst high phosphate levels may increase the calcification risk of coronary artery in CVD (7).

The calcium ion plays a key role in several homeostatic systems such as the regulation of vascular tone. Calcium has a pathogenic role in hypertension, and can be a CVD risk factor (8). Serum calcium concentrations predict cardiovascular mortality in a large health-screening program (10). In contrast, some observational studies suggest that a high intake of calcium may be useful to protect against vascular disease (9).

This study seeks to evaluate the serum 25OHD, phosphate and calcium in patients with angiographically defined CAD in Iran.

Methods/ Subjects and Material

Subjects

There were 566 subjects aged 20–80 years, out of whom 283 were healthy subjects with no symptoms of CAD. There were 283 CAD patients divided into two groups based on their angiogram results: 1: angiogram positive CAD patients with >50% stenosis (Angio $^{+}$, n=202); and 2: angiogram negative CAD patients with ≤50% stenosis (Angio $^{-}$, n=81). Subjects were selected from the patients referred to the Catheterization Laboratory of the Cardiology Department of Ghaem Hospital, Mashhad, Iran. Approximately, 283 healthy subjects aged 20-80 years old, who lived in the city of Mashhad, were recruited in the control group. The patients in the control group did not have any symptom of CAD and were not taking any medication or supplements that could affect serum 25OHD concentrations during the period of the study. Exclusion criteria were: pregnancy/breast feeding, a history of systemic and renal diseases as well as angiography history.

All participants were asked to sign an informed consent form The study was approved by the Ethics Committee of Mashhad University of Medical Sciences.

For all subjects, serum samples were collected for serum 25OHD, calcium and phosphate during 2011.

Blood sampling

Fasting blood samples (12 hours of fasting) were taken from each subject. Serum was separated following centrifugation, and then stored at -20°C for further measurements.

25OHD measurement

A competitive electroluminescence protein binding assay was used to determine serum 25OHD levels with the Roche Diagnostics 25OHD assay kit (Reference Number: 06506780160, Lot

Number: 175262-05, Roche Diagnostics, Mannheim, Germany). A Cobas e411 analyzer was used with a newly introduced, rapid and fully-automated method which comprises three incubation steps (12). Serum 25OHD insufficiency and deficiency were classified as a 25OHD of 20-30 and <20 ng/ml, respectively (13).

Other biochemical analyses

Routine biochemical analysis comprising lipids profile and fasting blood glucose (FBG) were measured for all patients using routine analytical methods. Diabetes mellitus was considered as a FBG ≥126 mg/dl or the consumption of hypoglycemic agents or insulin.

Serum calcium levels were measured using O.Creesolphthalein complex (cpc) (ZistChem Diagnostics Tehran, Iran) and serum phosphate concentrations were analyzed using ZistChem Diagnostics in Tehran, Iran. Serum calcium concentrations lower and higher than the reference range of 8.5-10.5 mg/dl were considered to be hypocalcemia and hypercalcemia respectively (18). Hypophosphatemia and hyperphosphatemia was defined as serum phosphate levels lower or higher than the 2.5-4.5 mg/dl (19).

Anthropometric measurements

Anthropometric indices were measured for all participants using standard methods (17).

Clinical measurements

High blood pressure was diagnosed in patients with systolic and/or diastolic blood pressure ≥140 and ≥90 mmHg, respectively. In individual who were already diagnosed with hypertension based on their past medical history, anti-hypertension drugs were used (7).

Statistical analysis

SPSS 16 was used for data analysis. The normality of data was determined by the Kolomogorov-Smirnov test, and expressed in form of means ±SD or median and interquartile range for normally and non-normally distributed data respectively. For group comparisons, t-tests and chi-square tests were used based on a Bonferonni correction. Group comparisons were performed using Kruskal–Wallis and Mann-Whitney test. A two-sided *p*-value of < 0.05 was considered as statistically significant. A univariate analysis model was used to examine associations between 25OHD, calcium and phosphate and coronary artery disease with a P-value <0.05 using binary logistic regression. To control for confounding factors and covariates effects, which were different in three control, angiogram negative and angiogram positive groups (including gender, age, total cholesterol, LDL-C, HDL-C, systolic and diastolic blood pressure, BMI, and waist circumferences) a multivariate analysis was used based on binary logistic regression. Logistic regression was also applied to calculate odd ratios for association serum 25OHD, calcium and phosphate level and CAD risk. A P-value <0.05 was considered as significant in all tests.

Results:

Of 566 participants, approximately 53% (n=303) were males and 47% (n=269) were females. The mean (±SD) age was 52.5 ±9.8 years for male and 51.8 ± 10.8 years for female subgroups. $Angio^-$ (54.4±9.7 years), $Angio^+$ (58.0±9.8 years) and control (47.5±8.2 years) groups were significantly different in terms of mean age ($p<0.001$).

As illustrated in Table 1, in the control group, the mean waist circumference, fasting blood glucose, BMI, weight and blood pressures were significantly different compared to other groups ($P<0.001$). Total cholesterol concentration and LDL-C and HDL-C levels were different in the groups under study ($P<0.001$).

According to Table 2, serum 25OHD concentration in the $Angio^-$ and $Angio^+$ groups were significantly higher compared to the control group ($P<0.001$).

To determine the relationship between systolic and diastolic blood pressure and other clinical and biochemical parameters, Pearson's Correlation or Spearman's. Correlation coefficients were evaluated. According to Table 3, there was a correlation between age, serum 25OHD and phosphate ($p<0.001$ and < 0.05). For this set of data, the highest Spearman's coefficient was obtained for HDL-C ($r_{Vit\ D}=0.07$) and serum 25OHD. Moreover, a higher Spearman's coefficient ($r_{Phos}=0.07$) was found between age and serum phosphate ($p<0.001$).

As shown in Table 4, the prevalence of 25OHD deficiency was higher in healthy subjects compared to the $Angio^+$ group ($p< 0.001$). The hypercalcemia and hyperphosphatemia prevalences were higher in $Angio^+$ group than healthy control subjects ($p< 0.001$). As shown in Table 5, it appears that individuals with 25OHD insufficiency are more prone to CAD compared

to those with 25OHD deficiency after controlling for confounding factors including age, gender, TC, HDL-C, LDL-C, SBP, DBP, BMI and WC. Univariate analysis showed that hypercalcemic and hyperphophatemic subjects were respectively 5.75 and 1.99 times more likely to develop CAD compared to normal subjects. Serum calcium and phosphate concentrations did not show any association with CAD risk after adjustment for confounding factors (such age, sex, TC, HDL-C, LDL-C, SBP, DBP, BMI and Waist C) by multivariate regression analysis.

Disscusion

We have found that serum 25OHD was higher in angio positive group compared to the control group. It has been shown that 25OHD reduce the risk of cardiovascular desease (14). The different results of this study could be due to the fact that participants had been informed and were aware of the important role of diet adjustment on controlling their desease (15). The Framingham Offspring Study found a relationship between low levels of 25OHD and higher risk of cardiovascular disease (16). Kilkkinen et al. observed a relationship between fatal events of cardiovascular and higher 25OHD state (20).

We demonstrated that angio^{+}/angio^{-} groups who had higher levels of 25OHD, had lower LDL-C, HDL-C and total cholestrole. There are many evidences suggesting that individuals with increased serum 25OHD levels have more normal lipid profiles than people with vitamin D-insufficient or deficient. In other words, it appears that significant reduction in serum triglyceride and LDL-C/HDL-C levels is accompanied by a reduction in serum 25OHD concentrations (21). Ponda et al. revealed the impossibility of improving lipid profiles by correcting 25OHD deficiency (22). There is also an inverse relationship between serum lipids and 25OHD levels in children and adolescents (23). It has been shown that 25OHD is strongly associated with weight, BMI and waist circumference. A negative relationship between 25OHD status and BMI has also been reported in the literature (24, 25). This link can be explained by the following causes: 1- obese individuals usually have a sedentary life style and may have limited sun exposure (24, 26); 2- they need higher 25OHD levels but it cannot be supplied due to low bioavailability of 25OHD (24, 27); and 3- an increase in active 25OHD metabolite can decrease serum 25OHD levels

through negative feedback regulation on the 25OHD synthesis in liver (24, 27). On the other hand, calcium absorption is diminished with a reduction in the serum 25OHD level. This event increases the parathyroid hormone (PTH) secretion. Fat accumulation is a result of PTH activation, which is then accompanied by insulin resistance and lipolysis inhibition (28). Moreover, in obese individuals, lower areas of skin are exposed to the sun (29).

Another important finding was the higher prevalence of high blood pressure in the angio-$^{+}$patients compared to the control group. The angio $^{+}$subjects had higher levels of 25OHD. This showed that 25OHD had no significant effect on reducing hypertention. The close association between lower 25OHD level and greater risk of high blood pressure (30) has been shown in twog studies. In one study, no association was found between receiving 25OHD supplementation and high blood pressure (31).

The results of our study demonstrated that the prevalence of 25OHD deficiency was higher in healthy subjects than Angio^{+} group ($p< 0.001$). It is possible that this result may be because CAD patients had consumed supplements. Because of control group volunteers were younger about 7 and 10.5 years than both Angio^{-} and Angio^{+} groups, respectively ($p<0.001$). As people age 25OHD status gets worse (32). Thus, higher prevalence of 25OHD deficiency in the control group compared to the Angio^{+} group may be related to supplements intake by CAD patients.

Calcium and phosphate concentrations did not show any correlation with CAD risk after adjustment for confounding factors including age, sex, TC, HDL-C, LDL-C, SBP, DBP, BMI and Waist C by multivariate regression analysis.

Conclusions

These results indicate that 25OHD deficiency may be a CAD-independent risk factor. However our results may also be explained in terms of different dietary intakes of patients and subjects int the control gorups and more research on this topic needs to be undertaken before confirming the association with 25OHD.

Study limitations

The present study lacks any baseline data on sun exposure and thus 25OHD level. Also, we do not have enough information about dietary intake of the patients in the experiment group. This all could be due to significant age differences between groups. Also, some biochemical factors, including the measurement of albumin, which is absolutely essential for calculating corrected calcium levels, and also glomerular filtration rate, which is essential for the interpretation of phosphate levels of the study population, were not recorded. These may have a significant impact on the interpretation of our data.

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Conflict of interest: The authors indicate no potential conflicts of interest.

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Table 1: Comparison of the baseline characteristics between the study groups

Variables	Angio negative (n=81)	Angio possitive (N=202)	Control (N=283)	P-value
Age (years)	54.4±9.7 [b]	58.0±9.8 [b]	47.5±8.2	<0.001
Sex, No.(%), Male	26(32.1)	140(69.3)	137(47.4)	<0.001
Weight (kg)	66.6±13.3 [b]	69.2±13.3 [b]	73.5±13.4	<0.001
BMI (kg/m2)	26.3±4.6 [b]	26.1±4.5 [b]	28.5±5.7	<0.001
WC (cm)	87.7±10.9 [b]	89.0±10.4 [b]	93.1±11.7	<0.001
SBP (mm Hg)	144.6±20.5 [b]	143.4±24.4 [b]	122.5±18.8	<0.001
DBP (mmHg)	85.1±11.1 [b]	84.8±10.4 [b]	80.0±9.9	<0.001
FBG (mg/dl)	96.6±15.3	100.5±23.0	99.6±54.5	0.78
HDL-C (mg/dl)	42.8±9.7 [b]	41.1±9.9 [b]	48.8±11.0	<0.001
LDL-C (mg/dl)	93.3±26.9 [b]	98.5±27.7 [b]	122.7±32.2	<0.001
TC (mg/dl)	169.0±47.0 [b]	170.6±41.5 [b]	196.7±39.1	<0.001
TG (mg/dl)	131.0(93.5-155.0)	140.0(96.0-171.0)	119.5(86.2-172.7)	0.85
Hypertension (%)	32.1%**	39.1%**	13.8%	<0.001
Diabetes (%)	10.2% [a]	13.4% *	10.4%	0.34
Current-Smoking (%)	22.2%	30.7%	17.9%	<0.05

Values expressed as mean ± SD for normally distributed data, and median and interquartile range for non-normally distributed data. ANOVA One-way analysis of variance and Tokay test were used for comparison between groups.

Compare with Control: [a]<0.05, [b]<0.01.

Compare with Angio -: *<0.05. **<0.01

BMI = Body mass index, WC=Waist Circumference, SBP= systolic blood pressure, DBP= diastolic blood pressure, FBG=Fasting Blood Glucose, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, TC = Total cholesterol, TGs = Triglycerides.

Table 2: Comparison of the serum 25OHD, calcium and phosphate between understudy subjects

variables		Control (N=283)	Angio - (n=81)	Angio + (N=202)	P-value
Calcium (mmol/l)		9.1(8.4-9.8)	9.3(8.3-10.3)	9.45(8.3-10.3) [a]	0.037
Crude 25OHD (ng/ml)		15.3(10.2-19.0)	20.0(13.5-26.0)[b]	19.5(14.0-26.0)[b]	<0.001
Adjusted for Cholesterol	25OHD (ng/ml)	16.7(12.1-20.1)	20.3(13.9-26.8) [b]	20.5(14.5-26.8) [b]	<0.001
Phosphate (mg/dl)		3.9(3.2-4.3)	4.0(3.4-4.5)	4.0(3.5-4.6)[a]	0.011

Values expressed as median (interquartile range). Kruskal-wallis test was used for comparison between groups and Mann-whitney U test were used for post hoc with Bonferroni correction.
Compare with Control: a<0.01, b<0.001.

Table 3: Correlation between Serum 25OHD, calcium and phosphate concentration and clinical, biochemical and anthropometrical parameters.

Variables	25OHD	Phosphate	calcium
Age(years)	***0.194	*0.137	0.058
Weight(kg)	*-0.015	-0.058	-0.091
BMI(kg/m^2)	0.037	-0.066	-0.062
WC(cm)	0.063	-0.028	0.014
FBG(mg/dl)	0.035	0.029	-0.030
HDL-C(mg/dl)	**-0.115	0.001	-0.156
LDL-C(mg/dl)	0.031	-0.012	-0.011
TC(mg/dl)	0.036	0.028	-0.065
TGs(mg/dl)	0.062	*0.104	0.050

BMI = Body mass index, WC=Waist Circumference, FBG=Fasting Blood Glucose, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, TC = Total cholesterol, TGs = Triglycerides.

*** <0.001

* <0.05

Correlations were assessed using spearman correlations.

** Correlation is significant at the < 0.01 level (2-tailed).

* Correlation is significant at the < 0.05 level (2-tailed).

Table 4: Proportion of 25OHD, calcium and phosphate in coronary artery disease compared to control subjects

		Healthy	Angio+	P[a]
25OHD (ng/ml)	Normal 30<=, No(%)	24(8.3)	33(16.3)	<0.001
	Insufficient 20-30, No(%)	39(13.5)	68(33.7)	
	Deficient <20, No(%)	226(78.2)	101(50)	
Calcium (mmol/l)	Normal (8.5-10.5)	197(68.2)	109(54)	<0.001
	Hypocalcemia (<8.50)	81(28)	58(28.7)	
	Hypercalcemia (>10.50)	11(3.8)	35(17.3)	
Phosphate (mg/dl)	Normal 2.5-4.5	238(82.4)	146(72.3)	0.002
	<2.5 HypoP	6(2.1)	1(0.5)	
	HyperP >4.5	45(15.6)	55(27.2)	

[a] Chi-square test was used.

Table 5: Association of 25OHD, calcium and phosphate and coronary artery disease by binary logistic regression

		Univariate				Multivariate*			
		OR	CI		P	OR	CI		P
25OHD (ng/ml)	Normal	3.077	1.730	5.472	<0.001	1.847	.466	7.315	0.382
	Insufficient	3.901	2.467	6.170	<0.001	8.129	2.280	28.987	0.001
	Deficient	a			<0.001	a			0.005
Calcium (mmol/l)	Normal	a			<0.001	a			0.073
	HypoC	1.294	.859	1.951	0.218	.559	.192	1.626	0.286
	HyperC	5.751	2.808	11.776	<0.001	5.634	.848	37.456	0.074
Phosphate (mg/dl)	Normal	a			0.004	a			0.056
	HypoP	.272	.032	2.279	0.230	.023	.001	.894	0.043
	HyperP	1.992	1.277	3.108	0.002	1.922	.586	6.302	0.281

[a] category reference, OR: Odds ratio, CI: Confidence interval

[*] After correction for Age, sex, TC, HDL-C, LDL-C, SBP, DBP, BMI, and Waist C.