9

Research Article

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Impact of serum 25 hydroxyvitamin D deficiency on lipid biomarkers in established coronary artery disease

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Abstract

Objectives: Vitamin D deficiency (VDD) is associated with coronary artery disease (CAD) directly by augmenting atherosclerosis and indirectly through cardiovascular risk factors. The present study was aimed to find an association of 25 hydroxyvitamin D (25(OH)D) with lipid profile among established CAD.

Methods: A cross-sectional study was conducted among 73 patients of angiographically confirmed CAD aged between 35 and 55 years of both gender. Serum 25(OH)D and lipid profile were estimated by ELISA kit and Roche autoanalyzer respectively. Atherogenic index of plasma (AIP) and sdLDL (small dense low-density lipoprotein) were calculated using the accepted formula.

Results: The mean 25(OH)D level was 17.95 \pm 13.51. Only 15% had sufficient 25(OH)D level. There was a significant negative correlation of 25(OH)D with TC/HDL (T.cholesterol/High-density lipoprotein) ratio (p=0.022). Multivariate

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logistic regression analysis showed no statistically significant impact of 25(OH)D with lipid biomarkers.

Conclusions: We found low 25(OH)D mean value among CAD and a significant negative correlation of 25(OH)D with TC/HDL. This study suggests VDD may affect primary lipid target resulting in unfavorable outcomes in CAD.

Keywords: coronary artery disease; dyslipidemia; Vitamin D deficiency

Introduction

Vitamin D is a pro-hormone concerned with skeletal effects. The discovery of vitamin D receptors (VDR) and the 1α -hydroxylase enzyme, which is essential to activate 25-hydroxyvitamin D [25(OH)D], to 1,25-dihydroxyvitamin D {1,25(OH)2D} in a variety of cells has sparked interest in its extra skeletal role [1]. Vitamin D deficiency (VDD) had been accounted for worldwide both in sunshine deficient and sufficient sunshine countries including India. Growing evidence revealed that VDD is closely associated with cardiovascular disease (CVD) directly by augmenting atherosclerosis [2] and indirectly through its association with traditional called cardiovascular risk factors, which are obesity, dyslipidemia, hypertension, diabetes mellitus (DM), metabolic syndrome, and inflammation due to the complex biological interaction between vitamin D and its receptors [3–6]. Apart from this, VDD has got a direct effect on the cardiovascular system through its interaction with receptors present on cardiomyocytes, vascular myocytes and vascular endothelial cells [7]. In patients with no indication of coronary artery disease (CAD), an independent association of [25(OH)D], with the extent of intimal media thickness of thoracic aorta relation was found [8]. In established CAD, vitamin D deficient subjects had significantly high triglyceride (TG) in comparison with subjects having sufficient vitamin D [9]. Lipoprotein lipase enzyme, which is involved in the metabolism of TG, may be regulated by the [25(OH)D] resulting in TG reduction [10] since

CAD is a manifestation of several risk factors, we planned to study in established CAD without taking into account the individual risk factors. Whether the vitamin D continues to affect lipid parameters once CAD is clinically established is not certain. Atherogenic Index of Plasma (AIP) and small dense low-density lipoprotein (sdLDL) are also a marker of metabolic syndrome. Hence this study was planned to establish the relationship of serum [25(OH)D] level with lipid biomarkers which include serum TG, Total Cholesterol (TC), Low-density lipoprotein (LDL), High-density Lipoprotein (HDL), AIP, sdLDL among established CAD patients.

Materials and methods

A cross-sectional study was performed on 73 established CAD participants of age between 35 and 55 years at the cardiac department of a tertiary care hospital with their prior informed consent. G*Power version 3.1.9 was used to calculate sample size with 0.30 effect size, 80% power, and 0.05 level of significance. Patients who had a history of vitamin D supplementation and previous angiography were not included in the study.

The study participants were included in the study after informed consent and their rights were protected, as per the Helsinki Declaration. The research has been complied with the policies of the institution and, as per the fundamentals of the Helsinki Declaration, and has been sanctioned by the institutional ethics committee.

General physical examination and systemic examination were done for all participants. Body mass index (BMI) was determined using the following formula: weight (kg)/height (m)². According to the World Health Organisation (WHO), a BMI of 18-24.9 kg/m2 was considered average weight, 25-29.9 kg/m2 was considered overweight, and ≥30 kg/m2 was considered obese. Blood was collected in a sterile plain vacutainer under aseptic precautions, and serum was separated by centrifuging for 5 min at 3,000 rotations per minute. The separated serum was then labeled according to sample number and stored separately for [25(OH)D]. Estimation of lipid biomarkers - TC, HDL, LDL, TG was done using Roche auto analyzer under the principle of the enzymatic assay. AIP value was calculated as log[TG/HDL-C. sdLDL was calculated as = $(0.084 \times TG) + (0.281 \times TC) - (0.251 \times HDL) - 17.236$. Non-HDL was calculated as (TC - HDL). The commercially available enzyme-linked immunosorbent assay kit was used to estimate serum [25(OH)D] on ELx 800 (BioTek ® Instruments, Inc). As per the Current International Osteoporosis Foundation Guidelines, Vitamin D levels of ≤20 ng/mL, 21-29 ng/mL and ≥30 ng/mL were considered as deficient, insufficient and sufficient, respectively [10]. Angiographic severity was assessed by the number of stenosis of coronary artery vessels.

Statistical analysis was done using Statistical Package for the Social Sciences 23.0 (SPSS Inc., Chicago, IL. Normality test was performed, and all the variables were normally distributed. The mean and standard deviation were used to express normally distributed continuous variables. Pearson's correlation was used to determine the relationship between [25(OH)D] and lipid biomarkers. The impact of [25(OH)D] with lipid biomarkers in the study population was investigated using Multivariate regression model analysis. All p values were

two-tailed and the p-value of less than $0.05\,\mathrm{was}$ deemed as statistically significant.

Result

In the present study, 73 patients of CAD participated, with 73.8% males and 26.2% females. Vitamin D deficiency was highly prevalent among total study participants. Table 1 displays the general characteristics of the whole study population. CAD was found to be more prevalent among males. There were 64.4, 20.5 and 15.06% of [25(OH)D] deficient, insufficient and sufficient cases, respectively (Figure 1). Mean lipid values were found to be near normal in our study participants (Figure 2). Our analysis found a negative correlation of [25(OH)D] with TC, TG, LDL, and TC/HDL ratio, but only the TC/HDL ratio was statistically significant (Table 2). Multivariate regression model analvsis showed no statistically significant impact of 25(OH)D with TC (β coefficient = -0.112, p=0.804), HDL (β coefficient = -0.03, p=0.710), TC/HDL (β coefficient = 0.003, p=0.774), AIP (β coefficient = 0.001, p=0.884), non HDL (β coefficient = -0.082, p=0.853) and sdLDL (β coefficient = 0.003, p=0.984) which is shown in Table 3. There was no statistically significant association of vitamin D with angiographic severity assessed by number of coronary vessels stenosed (Table 4).

Discussion

We discovered a high prevalence of vitamin D deficiency among established cases of CAD aged 35-55 years of both genders in the current research, which aimed to study the association of vitamin D with lipid indices in established cases of CAD aged 35-55 years of both genders (Table 1). VDD, on the other hand, is a recognized fact among CAD patients. Vitamin D can influence endothelial cell function by lowering adhesion molecule expression [11]. VDD was linked to the severity of coronary artery stenosis as measured by the Gensini score. Even after adjustments for risk factors of cardiovascular disease, vitamin D appeared to be a significant predictor for angiographic severity of CAD [12]. We choose a younger middle age, economically productive population, as the prevalence of CAD is currently increasing among the younger population. We specifically selected angiographically established CAD because endothelial dysfunction triggers plaque formation, which is driven by impaired lipid transport mechanisms, stimulating an inflammatory response. As a result, lipids play a key role in the production of atherosclerosis [13].

Table 1: Demographic and clinical characteristics of study population.

Study participants
51.26 ± 4.01
54:19
23.24 ± 2.95
17.95 ± 13.51
168.97 ± 50.98
41.4 ± 9.08
4.2 ± 1.35
143.9 ± 62.15
105.8 ± 42.63
32.11 ± 12.6
0.51 ± 0.24
31.94 ± 17.4
127.57 ± 50.08

Results are expressed as mean \pm SD. HDL, High-density lipoprotein; TC, Total cholesterol; TG, Triglyceride; LDL, Low density lipoprotein; VLDL, Very low density lipoprotein; AlP, Atherogenic Index of plasma; sdLDL, small dense low density lipoprotein.

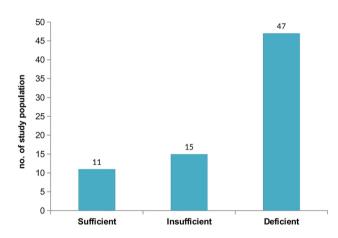


Figure 1: Vitamin D status among the study population.

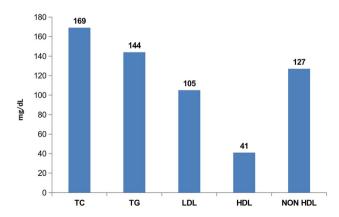


Figure 2: Mean value of lipid profile among study participants. TC, Total cholesterol; TG, Triglyceride; LDL, Low density lipoprotein; HDL, High-density lipoprotein.

Table 2: Correlation of vitamin D with lipid profile among study participants.

Lipid profile	Pearson correlation, r	p-Value
T.Cholesterol	-0.136	0.259
Triglyceride	-0.0644	0.596
HDL	0.204	0.087
LDL	-0.057	0.639
TC/HDL ratio	-0.272*	0.022*
AIP	0.055	0.345
SdLDL	-0.019	0.445
Non HDL	-0.073	0.3

HDL, High-density lipoprotein; TG, Triglyceride; LDL, Low density lipoprotein; AIP, Artherogenic Index of plasma; sdLDL, small dense low density lipoprotein. *p value<0.05.

Table 3: Multivariate regression model analysis of vitamin D with lipid profile among total participants.

Response variables	Model			
	$oldsymbol{eta}_0$		$oldsymbol{eta}_1$	
	Estimate	p-Value	Estimate	p-Value
TC	170.9	0.02	-0.112	0.804
HDL	41.9	0.034	-0.03	0.710
TC/HDL	4.12	0.00	0.003	0.774
AIP	0.508	0.01	0.001	0.884
Non-HDL	129.04	0.00	-0.082	0.853
sdLDL	31.89	0.03	0.003	0.984

TC, Total cholesterol; HDL, High-density lipoprotein; AIP, Artherogenic Index of plasma; sdLDL, small dense low density lipoprotein.

Table 4: Association of vitamin D with angiographic severity.

Vitamin D status	No. of core	onary vessel	s involved	p-Value
	Single	Double	Triple	
Deficiency n, %	21 (29.2)	9 (12.5)	16 (22.2)	0.49
Insufficient n, %	9 (11.1)	1 (1.4)	6 (8.3)	
Sufficient n, %	5 (6.9)	3 (4.2)	3 (4.2)	

Chi-square analysis done.

As all other previous studies are linked with risk factors in CAD, we wanted to find a correlation of vitamin D with lipid profile in established CAD. We had not taken into account other risk factors such as diabetes mellitus, hypertension which may also be associated with dyslipidemia. Vitamin D controls the renin-angiotensin–aldosterone system (RAAS) and suppresses renin biosynthesis, which can help to manage blood pressure [3]. The VDR as well and 1α -hydroxylase enzyme expression on islets of Langerhans

^{*}p value<0.05 is considered as significant.

improves glucose tolerance and glucose-induced insulin secretion [6], with VDD causing the opposite effect. The study participants were not overtly obese as per BMI, yet a significant proportion had vitamin D deficiency. Central obesity, which is more prevalent to CAD risk, can be better determined by waist circumference and waist-hip than BMI [14] and we have assessed obesity only by BMI. Indians are more prone to CAD, despite normal BMI, due to inherently high IR. The presence of genetically predicted higher levels of insulin resistance phenotypes was linked to a higher risk of CAD [15].

We found near normal mean lipid values in our study participants (Figure 2). This could be due to the treatment they received; we have not taken into account any treatment they received during the study duration. However, we discovered a significant inverse correlation of [25(OH)D] and TC/HDL ratio but not with any of the other variables (Table 2). Studies have observed that low [25(OH)D] is associated with dyslipidemia as well. In a study conducted by Dziedzic E et al., among cardiac patients, results showed significant negative correlation between the serum [25(OH) D] and TG, LDL-C and TC. They also found low mean vitamin D levels among cardiac patients [16]. Since we didn't find any significant correlation of vitamin D with lipid parameters except for TC/HDL, we did multivariate regression analysis with vitamin D, as the independent predictor and (T. Cholesterol, HDL, TC/HDL, AlP, sdLDL, non HDL) as multivariate responses (Table 3). There was no statistically significant impact of [25(OH)D] on lipid biomarkers.

AIP is a significant predictor of cardiovascular risk [17]. sdLDL is strongly associated with metabolic syndrome [18]. Wang v et al. found to have a negative association of [25(OH) D] with TG [p<0.001]and LDL-C [p<0.001] and a direct association with TC [p<0.002] in men. Serum vitamin D levels in women showed negative association with LDLC (β =-0.25, p=0.01) and positive association with TC (β =0.39, p=0.001). In men, AIP showed negative association with vitamin D (r=-0.111, p<0.01) [19]. A significant negative relation of [25(OH)D] with glycated hemoglobin (p-value=0.02) and positive association with HDL (p-value=0.01) was found by Alkhatatbeh et al. They concluded that VDD could be engaged in decreasing HDL and rising HbA1c, which could increase the cardiovascular disease risk in non-cardiac chest pain participants [20]. The studies show highly conflicting results. In their National Health and Nutrition Examination Survey (NHANES) III report, Ford and colleagues discovered an inverse relationship between serum TG and [25(OH)D] levels in patients with hypertriglyceridemia. However, in healthy subjects, this relationship wasn't detected with respect to HDL-C [21]. Within 2009 explore for Pubmed, Jorde et al., conferred the interrelation between

serum [25(OH)D] levels and lipid parameters by reviewing 22 observational cross-sectional studies and 10 randomized placebo-controlled trials. Overall, cross-sectional studies found a favorable relationship between HDL-C and serum [25(OH)D] levels. Furthermore, though all studies have shown a negative relationship between serum [25(OH)D] levels and TG, vitamin D supplementation didn't demonstrate any impact on serum TG levels. These findings were contradictory, with some studies showing a positive correlation, while some studies reported an inverse relationship between serum [25(OH)D] and TG levels. Hyperlipidemia was not a prerequisite for inclusion in any of the studies [22]. According to Ponda et al., a group of 108,711 subjects who received repeated serum [25(OH)D] supplementation and lipid testing 4–26 weeks apart reported a rise in [25(OH)D] levels, but a highly significant reduction in TC, LDL, and TG, as well as an increase in HDL [23]. Over 12 months, Motiwala and Wang [24] randomized overweight participants to vitamin D supplementation vs. placebo, finding a substantial reduction in TG but not in LDL levels. Vitamin D-mediated TG reduction could be mediated through two mechanisms: (a) Vitamin D enhances calcium absorption in the intestine. The calcium prevents TG synthesis and release in the liver, lowering serum TG levels. (b) Vitamin D has an inhibitory effect on serum PTH levels. A decrease in PTH may result in the increased peripheral elimination of TGs [25]. Insulin resistance is another possible factor to explain the interrelationship of [25(OH)D] and TG: VDD increases insulin resistance [7] which is linked to an increase in VLDL and TG [26]. Calcium reduces the level of total and LDL cholesterol by decreasing the absorption of fat via the insoluble calcium fatty acid complex formation [27]. The present study did not show any statistically significant association of vitamin D status and angiographic severity assessed by number of coronary vessels stenosed (Table 4). Dhibar et al. [28] and May HT et al. [29] did not find any association of vitamin D with angiographic severity, however, mean vitamin d was found to be low in patients with CAD. As dyslipidemia is one of the major risk factors of CAD, vitamin D deficiency may be indirectly associated with CAD through cardiovascular risk factors such as dyslipidemia but not with angiographic severity.

Conclusion

We discovered a significant inverse correlation between [25(OH)D] and the TC/HDL ratio. Although not statistically significant, there was a negative correlation with TC, TG, and LDL. This study suggests VDD may affect primary lipid targets resulting in unfavorable outcomes in CAD. This may

also suggest vitamin D screening among all high-risk cardiac populations.

Limitation

As the present study was an observational cross-sectional study, a causal relationship couldn't be confirmed. To validate the link between serum [25(OH)D] level and lipid biomarker, interventional prospective studies are really needed. One of the major limitations of our study was lack of control group, using a control group without CAD would have given a better picture. We have not taken into account any kind of treatment taken by the participants which may affect lipid profile.

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Declaration of patient consent: All participants provided written informed patient consent and were assured of their privacy and confidentiality.

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