Original Article

Relationship of levels of Vitamin D with flow-mediated dilatation of brachial artery in patients of myocardial infarction and healthy control: A case–control study

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ABSTRACT

Background: Cardiovascular diseases (CVD) remain the leading cause of death worldwide. Vitamin D deficiency has been linked to increased risk of adverse CV events. Vitamin D deficiency may be responsible for endothelial dysfunction which in turn affects the onset and progression of coronary artery disease and its risk factors, directly or indirectly through various mechanisms. **Materials and Methods:** It was case–control study. A total of 50 cases of acute myocardial infarction (AMI) (aged 40–60 years), admitted to medicine emergency/CCU, were taken as per ACC/AHA 2007 guidelines. An equal number of age- and sex-matched controls were also taken. Risk factors of AMI, flow-mediated dilatation (FMD), and 25(OH)D levels were studied in all cases and controls. Correlation was also studied between FMD and 25(OH)D. **Results:** The mean values of FMD were 18.86 ± 5.39% and 10.35 ± 4.90% in controls and cases, respectively ($P < 0.05$). The endothelial dilatation after glyceryl trinitrate (GTN) was also studied and was found to be 26.175 ± 4.25% and 18.80 ± 5.72% in controls and cases, respectively ($P < 0.05$). The mean levels of 25(OH)D in controls and cases were 25.45 ± 12.17 and 14.53 ± 8.28 ng/ml, respectively. In this study, 56% of subjects were Vitamin D deficient, 25% were Vitamin D insufficient, and only 19% had Vitamin D in normal range. A positive correlation coefficient was found between FMD and 25(OH) Vitamin D levels ($r = 0.841$, $P < 0.01$). In this study, a positive correlation coefficient was also found between endothelial dilatation after GTN and 25(OH)D levels ($r = 0.743$, $P < 0.01$). **Conclusion:** In this study, it was found that FMD was markedly impaired in patients of AMI when compared to controls. It was also found that majority of the study population was Vitamin D deficient; however, the deficiency was more severe in patients of AMI. We also found out that FMD was positively correlated ($r = 0.841$) to the deficiency state of Vitamin D in all the study subjects.

Key words: Brachial artery, case–control, flow-mediated dilatation, myocardial infarction, Vitamin D

Introduction

With aging, there is an increase in cardiovascular risk which is mostly attributable to vascular endothelial dysfunction.

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As already known, brachial artery flow-mediated dilatation (FMD) is a measure of endothelial-dependent dilatation. Consistent with this, FMD is lower in older compared to young adults.

Vitamin D deficiency has been linked to increased the risk of acute myocardial infarction (AMI), cardiovascular death, and overall mortality.[1-3] Various mechanisms by which Vitamin D deficiency leads to such catastrophic events include:[4]

- Endothelial dysfunction which in turn affects the onset and progression of coronary artery disease and its risk factors
- Vitamin D can inhibit various aspects of inflammation (key pathogenic mechanism in atherosclerosis)
- Vitamin D can exert an anti-proliferative effect on vascular smooth muscle cells and myocardial cell hypertrophy and proliferation.

Materials and Methods

Aims and objectives

- To compare the levels of Vitamin D among patients of myocardial infarction with age- and sex-matched controls
- To compare the levels of FMD of brachial artery among patients of myocardial infarction with age- and sex-matched controls
- To study the relationship of levels of Vitamin D with FMD of the brachial artery in cases and controls.

The study was a case–control, cross-sectional study, carried out from November 2011 to April 2013 in the Departments of Medicine, Biochemistry, and Radio-diagnosis at University College of Medical Sciences and associated Guru Teg Bahadur Hospital, Delhi.

Subject selection

Fifty cases of myocardial infarction (ST-segment elevation myocardial infarction and Non–ST-segment elevation myocardial infarction) as per ACC/AHA 2007 guidelines aged 40–60 years, who got admitted to medicine emergency/CCU, were taken.

Inclusion criteria

Patients fulfilling these criteria (ACC/AHA 2007 guidelines) were labeled as cases (n = 50).[5] An equal number of age- and sex-matched controls without coronary artery disease were enrolled. Controls were without any history of coronary heart disease and were similar for smoking and hypertension status as they were confounding variables which could affect Vitamin D and FMD. Cases and controls met the exclusion criteria.

Exclusion criteria

- Patients with a family history of premature (<40 years) CAD
- Patient with acute or chronic liver disease (serum aminotransferase more than threefold [more than 120 IU/l]), acute or chronic renal disease (serum creatinine level more than 1.5 mg/dl), acute or chronic infectious diseases, and malignancy
- Patients with congestive heart failure, cardiomyopathies, bronchial asthma, and chronic allergic conditions, Buerger's disease, systemic sclerosis, and Raynaud's disease
- Patients with diabetes mellitus were excluded from the study
- Patients with history of intake of hormone replacement therapy
- Patients with history of intake of Vitamin D or calcium supplements within 6 weeks of study
- Patients with history of intake of drugs affecting Vitamin D metabolism within 6 weeks of study
- Patients who were obese (body mass index [BMI] >30) were excluded from the study. Males with waist circumference more than 102 cm and females with waist circumference more than 88 cm were excluded from the study
- Patients with thyroid disorders (hypothyroidism and hyperthyroidism) were excluded from the study.

Methods

- Written informed consent was taken from cases and controls
- A detailed pro forma including the chief complaints, history of presenting complaints, history, family history, and dietary history was filled. Detailed examination including general physical examination, height, weight, BMI, waist circumference and systemic examination was done
- Venous blood samples were collected for the investigations including Vitamin D levels within 24 h of admission.

Vitamin D levels

Approximately 3 ml of venous blood sample was withdrawn in a plain vial after an overnight fast. Samples were stored at minus 20°C. 25(OH) Vitamin D level was measured from the serum by commercially available DiaSorin 25(OH) Vitamin D 125I RIA Kit (DiaSorin, Stillwater, Minnesota 55082-0285, USA).[5]

Flow-mediated dilatation

FMD[6] was measured using Philips HD7XE color Doppler ultrasound machine. A linear array transducer with a frequency range 7–12 MHz was used to acquire high-quality images with good resolution for analysis.

Statistical analysis

Considering 2.3 and 1.8 as standard deviation (SD) in Vitamin D levels in cases and controls, $\alpha = 0.05$ and power = 90%, to estimate a difference of 1.5 units in Vitamin D levels, a sample of forty cases was required in each group. Adding 20% nonresponders, we get 48, i.e., fifty minimum subjects were taken in each group.

Data were expressed as mean ± SD unpaired Student's *t*-test was used to compare cases and control group. Correlation between FMD and Vitamin D was done by correlation coefficient analysis. $P < 0.05$ was considered to be statistically significant.

Results

In this study, fifty cases of AMI (Group 1) were recruited, and an equal number of age- and sex-matched controls (Group 0) aged 40–60 years were also recruited. Demographic and cardiovascular risk profile of the study cohort is shown in [Table 1]. The number of males and females in each group were 44 and 6, respectively. The number of smokers was 37 (74%) among the cases and 36 (72%) among controls. Both groups were similar as per the smoking status. The numbers of hypertensive were matched in both groups to nullify the effect of hypertension on Vitamin D and FMD. There were eight subjects with hypertension in each group. Diabetic patients were excluded from the study.

FMD after GTN and vitamin D levels have been shown in Tables 2 and 3 respectively. Mean values of Subjects were divided into three subgroups [Table 4] according to the severity of Vitamin D deficient state as per the following levels:

1. 25(OH) Vitamin D <20 ng/ml: Vitamin D deficient
2. 25(OH) Vitamin D 20-30 ng/ml: Vitamin D insufficient
3. 25(OH) Vitamin D >30 ng/ml: Normal range.

Endothelial dependent and independent dilatation was also calculated in the above mentioned 3 sub groups which are shown in Tables 5 and 6 respectively.

Correlation

The correlation coefficient between FMD and 25(OH) Vitamin D was found to be positive in controls ($r = 0.766$, $P < 0.01$) [Figure 1]. Again, on analyzing the correlation between FMD and 25(OH) Vitamin D in patients of AMI, the correlation coefficient was positive ($r = 0.869$, $P < 0.01$) [Figure 2].

Discussion

Vitamin D insufficiency affects almost 50% of the population worldwide; however, in the Indian scenario, a study done by Marwah *et al*.[7] in healthy subjects ($n = 1346$) reported prevalence as high as 91.2%. Although Vitamin D has been traditionally associated with bone health, adequate levels are also important for optimal cardiovascular function. The mechanisms underlying the role of Vitamin D in the prevention of heart disease remain incompletely explained. Hence, this study was undertaken to assess Vitamin D levels and FMD in patients of AMI. In addition, a correlation between Vitamin D and FMD was also studied.

The results of this study are also consistent with the growing evidence suggesting a role of Vitamin D deficiency in the occurrence and progression of coronary atherosclerosis. In a study by Fatih Akin *et al*.,[8] low serum 25(OH) Vitamin D levels were associated with the severity of coronary artery stenosis. Levels of 25(OH) Vitamin D were significantly lower (15.6 vs. 22.2 ng/mL; $P < 0.001$) in patients with CAD compared with patients without CAD. In a study conducted by Lee *et al*.,[9] to study the prevalence of Vitamin D in patients of AMI, 179 subjects (75%) out of 239 were found to have 25(OH) Vitamin D levels <20 ng/ml, which is in the deficient range. Another fifty subjects were in the insufficient range, with 25(OH) Vitamin D levels between 20 and 30 ng/ml. This placed a total of 229, of 239 subjects of AMI (96%) had 25(OH) Vitamin D levels in the suboptimal range. In a study conducted by Shor *et al*.[10] to assess Vitamin D levels in patients undergoing coronary artery catheterization, 25(OH) D levels were 19.7 ± 10.1 ng/ml in patients with significant CAD on catheterization. The high prevalence of 25(OH) Vitamin D deficiency among patients with CAD in our study is comparable to the findings Kim *et al*.[11] They found a higher prevalence Vitamin D deficiency among individuals with or at high risk of cardiovascular disease (CVD). In a study conducted by Giovannucci *et al*.,[2] low levels of Vitamin D were found to be associated with increased incidence of myocardial infarction. In a study reported by Syal *et al*.,[12] on Indian patients undergoing coronary angiography, the mean 25(OH) Vitamin D level was 14.8 ± 9.1 ng/mL which was far lower than the normal range. The deficient state of 25(OH) Vitamin D as discussed in above-mentioned studies is comparable to our findings.

However, Pilz *et al*.[13] did not find a prevalence difference between patients with various 25(OH) Vitamin D serum levels. In a recent review and meta-analysis by Elamin *et al*., it is claimed that the quality of evidence linking Vitamin D and CAD as of today is of low to moderate level and did not find a statistically significant reduction in mortality and

Table 1: Demographic and cardiovascular risk factor profile of Groups 0 and 1

Variable	Group 0	Group 1	*P*
Mean age±SD (years)	50.04±6.29	51.12±6.81	0.784
BMI±SD (kg/m^2)	22.21±1.58	23.35±1.05	0.02
Waist circumference±SD (cm)	81.8±5.60	83.36±5.19	0.86
BMI (kg/m^2) (%)	<18.5	0	0
	18.5-24.99	50 (100)	42 (84)
	25-29.99	0	8 (16)
Mean systolic blood pressure (mm Hg)	117.40±20.51	123.06±11.98	0.09
Mean diastolic blood Pressure (mmHg)	74.7±12.1	80.56±8.01	0.005
Mean blood sugar - fasting (mg/dl)	88.22±8.560	90.08±6.9	0.23
Mean blood sugar - postprandial (mg/dl)	109.32±10.58	115.02±7.2	0.002
Mean LDL±SD (mg/dl)	89.72±28.67	111.44±36.80	0.001
Mean HDL-C±SD (mg/dl)	45.24±6.75	37.68±15.74	0.002
Mean TG±SD (mg/dl)	137.84±62.78	161.90±77.21	0.09
Mean total cholesterol±SD (mg/dl)	158.32±34.84	178.64±44.06	0.012
Mean VLDL±SD (mg/dl)	24.84±13.9	32.26±14.3	0.01

BMI: Body mass index, SD: Standard deviation, LDL: Low-density lipoprotein, HDL-C: High-density lipoprotein-cholesterol, TG: Triglycerides, VLDL: Very low-density lipoprotein

Table 2: Flow-mediated dilatation and dilatation after glyceryl trinitrate in both groups

Variable	Group 0 (*n*=50)	Group 1 (*n*=50)	*P*
Mean value of FMD (%)	18.86±5.39	10.35±4.90	<0.001
Mean value of dilatation after GTN (%)	26.175±4.25	18.80±5.72	<0.001

FMD: Flow-mediated dilatation, GTN: Glyceryl trinitrate

Table 3: Vitamin D levels (mean±standard deviation) in both groups

Variable	Group 0 (*n*=50)	Group 1 (*n*=50)	*P*
Vitamin D	25.45±12.17	14.53±8.28	<0.001

Table 4: Distribution of subjects as per Vitamin D deficient state

Variable	Number of controls (Group 0) (%)	Number of cases (Group 1) (%)	Total number of subjects (%)
Vitamin D <20 ng/ml	19 (38)	37 (74)	56 (56)
Vitamin D 20-30 ng/ml	15 (30)	10 (20)	25 (25)
Vitamin D >30 ng/ml	16 (32)	3 (6)	19 (19)

Table 5: Endothelial-dependent dilatation in subgroups of subjects as per Vitamin D levels

Variable	Vitamin D <20 ng/ml	Vitamin D 20-30 ng/ml	Vitamin D >30 ng/ml
FMD% (Group 0)	13.29±3.11	20.61±2.76	23.83±2.71
FMD% (Group 1)	7.98±2.98	16.78±2.08	18.26±2.38
P	<0.001	<0.001	<0.001

FMD: Flow-mediated dilatation

cardiovascular risk associated with low Vitamin D levels. However, other reviews done by Grandi *et al.*[14] report an inverse correlation between Vitamin D levels and the prevalence of CAD. The patients with CAD were having lower levels of 25(OH) Vitamin D than controls even in our study.

There have been very few studies done on healthy and diabetic subjects who have correlated Vitamin D and FMD. In a study by Tarcin *et al.*[15] in healthy subjects, endothelial function of 25(OH) Vitamin D deficient subjects was significantly disturbed in comparison to subjects with normal Vitamin D, whereas a significant improvement was observed after replacement with Vitamin D. They also found a positive correlation between 25(OH) Vitamin D levels and FMD which was consistent with this relationship in our study. Yiu *et al.*[16] studied Vitamin D levels and FMD in type 2 DM patients and found that Vitamin D deficiency and Vitamin D insufficiency status were independently associated with relative decrease in FMD by 33 and 19% in these groups, respectively. They suggested that endothelial dysfunction observed in type 2 DM patients was related to Vitamin D deficiency. Gepner *et al.*[17] evaluated the effects of a higher dose of Vitamin D supplementation (2500 IU daily) on several vascular parameters including FMD, as a of the marker of CVD risk, they could not found any improvement in FMD after treatment with Vitamin D in these cases.

On reviewing the literature, we could find only one study conducted by Syal *et al.*[12] done to correlate Vitamin D levels and FMD in patients of CAD. The study observed that Vitamin D deficiency was significantly associated with depressed vascular endothelial function as measured by brachial artery FMD. Mean FMD values were markedly reduced in patients with Vitamin D deficiency. The FMD was 4.57% in patients with 25(OH) Vitamin D levels <20 ng/mL and 10.68% in those with 25(OH) Vitamin D levels more than 20 ng/mL. They found a positive correlation between FMD and 25(OH) Vitamin D. There was no control arm in the above study. As the literature comparing the correlation between FMD and 25(OH) Vitamin D is scant, the findings of correlation in our study could not be compared to other studies.

In our study, the patients of myocardial infarction had a significantly lower 25(OH) Vitamin D levels compared to controls. The percentage of subjects with Vitamin D deficiency in the control group was 38%. Vitamin D deficiency in the general population can be attributed to a modern lifestyle and an inadequate sun exposure. If adequate measures such as food fortification or awareness campaigns are not undertaken, Vitamin D deficiency will

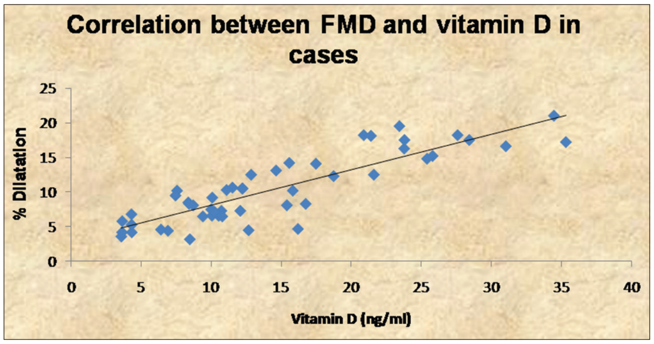

Figure 1: Positive correlation coefficient between flow-mediated dilatation and 25(OH) Vitamin D

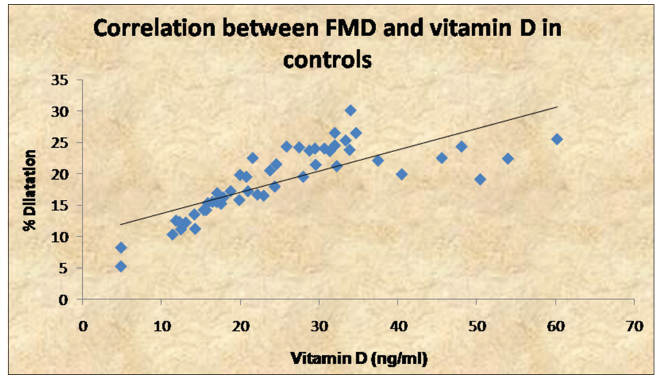

Figure 2: Positive correlation coefficient between flow-mediated dilatation and 25(OH) Vitamin D among cases

Table 6: Endothelial independent dilatation in subgroups of subjects as per Vitamin D levels

Variable	Vitamin D <20 ng/ml	Vitamin D 20-30 ng/ml	Vitamin D >30 ng/ml
FMD% (Group 0)	23.23±5.11	27.26±2.39	28.63±1.97
FMD% (Group 1)	16.35±4.12	25.56±3.29	26.43±4.67
P	<0.001	<0.001	<0.001

FMD: Flow-mediated dilatation

become a significant public health problem given that it contributes to the epidemic of chronic diseases such as osteoporosis, fractures and falls, cancer, autoimmune diseases, diabetes, and CAD. Although Vitamin D deficiency is highly prevalent in Indian population and is increasing, measures to screen and treat the condition are simple and feasible. The normalization of Vitamin D levels might help improve endothelial dysfunction leading to a decrease in incidence and the prevalence of CAD. This study gives an insight for developing an intervention strategy such as adequate sunlight exposure, food fortification or 25(OH) Vitamin D supplementation that may allay the consequences of its deficiency. In addition, there is a need to undertake future prospective multicenter study with larger number of subjects from Indian population to find a cause-effect relationship between Vitamin D deficiency and CAD. This may help us to initiate interventional studies to see the reversal effect with supplementation of vitamin D to halt the progression of endothelial dysfunction and atherosclerosis in patients of CAD.

Limitations of study

There are a few limitations to our study. This is a single-center study. It is cross-sectional and therefore, cause-and-effect relationship determination was not possible. The sample size (100) in our study was relatively small. A single measurement of Vitamin D may not reflect lifetime status, and a persistence of Vitamin D deficiency may be responsible for endothelial dysfunction leading to progression of atherosclerosis over many years. Serum 25(OH) Vitamin D levels vary with geography, seasonality, latitude, altitude presumably as a result of sunlight exposure, and personal habits. Further FMD measured by ultrasound Doppler is operator dependent.

Conclusion

The present case–control and cross-sectional study were carried among patients of AMI, which revealed Vitamin D deficiency state is higher among cases of AMI. In most of the subjects (cases and controls), the 25(OH) Vitamin D levels were lower than normal. Endothelial-dependent dilatation (FMD) was found to be lower among the patients of AMI. FMD was much lower in subgroups of subjects having Vitamin D deficiency state. FMD had a positive correlation with 25(OH) Vitamin D in patients of AMI. FMD as a marker of endothelial dysfunction which is thought to be the forerunner of CAD has been positively correlated to the deficiency state of Vitamin D in all the study subjects.

This study gives us the insight to identify the population with Vitamin D deficiency which may be at higher risk of CAD. Further, need to undertake a future prospective multicenter study with larger number of subjects from Indian population to find a cause-effect relationship between Vitamin D deficiency and CAD is required. This may help us to initiate interventional studies to see the reversal effect with supplementation of Vitamin D to halt the progression of endothelial dysfunction and atherosclerosis in patients of CAD.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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