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Original Article

Independent association of severe vitamin D deficiency as a risk of acute myocardial infarction in Indians

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ABSTRACT

Background: Association of vitamin D deficiency with coronary heart disease (CHD) has been widely reported. Emerging data has shown high prevalence of vitamin D deficiency among Indians. However, this association has not been studied in Indians.

Methods: A case-control study with 120 consecutive cases of first incident acute myocardial infarction (MI) and 120 age and gender matched healthy controls was conducted at All India Institute of Medical Sciences, New Delhi. The standard clinical and biochemical risk factors for MI were assessed for both cases and controls. Serum 25 (OH) vitamin D assay was performed from stored samples for cases and controls using radioimmunoassay.

Results: Vitamin D deficiency [25(OH) D < 30 ng/ml] was highly prevalent in cases and controls (98.3% and 95.8% respectively) with median levels lower in cases (6 ng/ml and 11.1 ng/ml respectively; $p < 0.001$). The cases were more likely to have diabetes, hypertension and consume tobacco and alcohol. They had higher waist hip ratio, total and LDL cholesterol. Multivariate logistic regression analysis revealed severe vitamin D deficiency [25(OH) vitamin D < 10 ng/ml] was associated with a risk of MI with an odds ratio of 4.5 (95% CI 2.2–9.2).

Conclusions: This study reveals high prevalence of vitamin D deficiency among cases of acute MI and controls from India, with levels of 25 (OH)D being significantly lower among

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cases. Despite rampant hypovitaminosis, severe vitamin D deficiency was associated with acute MI after adjusting for conventional risk factors. This association needs to be tested in larger studies in different regions of the country.

1. Introduction

Association of vitamin D deficiency with coronary heart disease (CHD) has been reported in a large number of studies and reviews recently.[1–8] There is also emerging body of evidence linking vitamin D deficiency to early mortality,[9,10] with vitamin D being considered as one of the possible treatable cardiovascular risk factors.[8] Emerging data has shown high prevalence of vitamin D deficiency among Indians despite the availability of abundant sunshine in large parts of India. This is true for both urban and rural populations and, in men and women, with reported population of prevalence of 70–99%, with severe deficiency (<10 ng/ml) being reported as 62% in studies from Delhi.[11–14] However, there is very little data on the association of vitamin D with CHD in Indians. Thus, it would be useful to study the association of this novel and potentially reversible risk factor of CHD in our population especially when CHD has gained epidemic proportions. We analyzed serum 25 hydroxy [25(OH)] vitamin D levels in cases of acute myocardial infarction (MI) and age matched controls to study this association in Indians.

2. Methods

The study was part of a case control study conducted at the Department of Cardiology at All India Institute of Medical Sciences (AIIMS), New Delhi between March 1999–June 2001. The study was approved by the Institute Ethics Committee at AIIMS. Serum 25 hydroxy [25(OH)] vitamin D measurement was performed on blood samples stored at the cardiac-biochemistry laboratory at AIIMS at −70 °C.

2.1. Study design

Consecutive consenting cases of first incident acute MI aged 25–75 years were recruited in the study. A questionnaire was administered to assess the risk factors of MI and a fasting blood sample was collected within 24 h of admission to the hospital. Acute MI was diagnosed if the patient had characteristic symptoms plus electrocardiogram changes indicative of a new MI (new pathologic Q waves, at least 1 mm ST elevation in any 2 or more contiguous limb leads or 2 mm ST elevation in precordial leads or a new left bundle branch block) with or without a elevated plasma level of creatine kinase-MB isoform (CK-MB). Those with history of heart disease diagnosed more than 30 days prior to enrollment in the study or with cardiogenic shock were excluded.

Controls were matched to cases by age (within 5 years) and gender and were individuals who had no previous diagnosis of heart disease or history of exertional chest pain and normal ECG. Controls were recruited from attendants or relatives of non cardiac patients from non cardiac wards or unrelated attendants of cardiac patients or patients attending non-cardiac outpatient clinics for disorders unrelated to known or potential risk factors for MI. Cases and controls with pregnancy, known liver, thyroid or renal diseases or malignancy were excluded. Additionally, for this study, controls were selected if their blood has been collected within 30 days of blood collected for their matched case.

Hypertension was defined as systolic pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg or history of current antihypertensive medication use. Fasting plasma glucose ≥126 mg/dl or post-prandial plasma glucose ≥200 mg/dl or anti diabetic medication use was defined as having diabetes mellitus (DM). Tobacco users were defined as smoked/chewed tobacco users in the last six months and having used it continuously for more than six months. Alcohol users were defined as individuals having consumed any form of alcohol in the last six months and who had consumed it for at least six months continuously. Family history of CHD was defined as history of CHD in first degree relatives at an age less than 60 years. Any leisure time physical activity was defined as non work related physical activity at least once a week. Highest acquired educational qualification was determined.

2.2. Sample size calculation

The sample size for this study was calculated for a two sided significance of 0.05 and study power of 80%. Based on a control exposure of 50% and an odds ratio of 2.1 (15,16) the calculated sample size was 120 cases and 120 controls.

2.3. Biochemical analysis

Glucose was measured by glucose oxidase method using commercially available kits (RANDOX, UK). Total Cholesterol was measured from serum samples by CHOD-PAP enzymatic method using commercially available kits (RANDOX, UK). HDL Cholesterol was measured from serum samples by phosphotungistic precipitating method. Triglyceride was measured from serum samples by enzymatic GPO-PAP method using commercially available kit (RANDOX, UK). Serum 25 (OH) D levels were analyzed from serum samples stored at −70 °C before analysis. 25 (OH) D was measured by chemiluminescent immunoassay method using commercially available kit Liaison® 25OH Vitamin D TOTAL (DiaSorin, USA) on liaison analyzer, USA.

2.4. Statistical analyses

Summary statistics of baseline participant characteristics were calculated by case and control status. Case and control

groups were compared statistically by independent 't' tests for continuous variables, and chi^2 tests for categorical variables. The Mann–Whitney *U* test was used for comparison of 25 (OH) vitamin D levels across case and control groups. Categorical variables are shown as counts and percentages and continuous variables shown as means with standard deviation (SD) or medians with inter-quartile range (SD). 25 (OH) vitamin D status was determined as severe deficiency state when the 25(OH) vitamin D level was below 10 ng/ml.[1] Multiple conditional logistic regression was used to investigate the relationship between MI and severe vitamin D deficiency controlling for other significant risk factors including age, diabetes, hypertension, LDL cholesterol, tobacco use, central obesity and education. Vitamin D status was taken as a binary variable (severe deficiency Vs no severe deficiency). The analyses were repeated using vitamin D exposure as a continuous variable after it was log transformed.

3. Results

The baseline characteristics of the cases and controls were as in Table 1. The cases more frequently had DM, hypertension, and consumed tobacco and alcohol. They also had higher mean waist hip ratio, total cholesterol and LDL cholesterol levels. There was no difference in age, family history of CHD, leisure physical activity, educational qualification, HDL cholesterol and triglycerides between cases and controls. The median 25(OH) vitamin D level was significantly lower in cases as compared to controls (Table 1).

There was a significant difference in the distribution of vitamin D deficiency among the cases and controls (Table 2 and Fig. 1). There was a very high prevalence of vitamin D deficiency in both cases and controls. The prevalence of severe deficiency of vitamin D, defined as 25(OH) vitamin D less than 10 ng/ml, was present in 79.2% of cases and 46.7% of controls. Only 1.7% and 4.2% of cases and controls respectively had sufficient vitamin D levels.

Using statistically significant and clinically relevant covariates it was observed that severe vitamin D deficiency was associated with a risk of MI with an odds ratio of 4.5 (95% CI 2.2–9.2) (Table 3). The association persisted when the analysis was repeated using vitamin D exposure as a continuous variable after it was log transformed.

Table 1 – The characteristics of the cases and controls.

	Cases (*n*=120)	Controls (*n*=120)	*p* value
Age (Years)	51.9 ± 11.4	52.1 ± 11.0	0.8
Men (%)	88.3%	88.3%	1.0
Hypertension (%)	34.2%	12.5%	<0.001
Diabetes (%)	40.0%	20.0%	<0.001
Tobacco Use(%)	62.2%	47.1%	0.01
Alcohol Use (%)	43.2%	26.1%	<0.001
Family History of CHD (%)	21.8%	10.7%	0.2
Any leisure physical activity (%)	33.6%	39.5%	0.4
Waist Hip Ratio	0.96 ± 0.06	0.92 ± 0.07	<0.001
Total Cholesterol (mean ± SD) (mg/dl)	194.1 ± 62.8	177.5 ± 39.1	0.01
HDL Cholesterol (mean ± SD) (mg/dl)	39.1 ± 9.9	40.3 ± 10.4	0.3
LDL Cholesterol (mean ± SD) (mg/dl)	123.7 ± 56.3	108.3 ± 38.5	0.01
Triglycerides (mean ± SD) (mg/dl)	156.8 ± 94.6	144.7 ± 84.4	0.3
Educated above Secondary level (%)	57.1%	49.2%	0.2
25 (OH)Vitamin D levels (ng/ml) (median, interquartile range)	6.0 (3.9–9.0)	11.1 (6.5–18.3)	<0.001

Table 2 – Distribution of vitamin D levels in cases and controls.

	Cases (*n*=120)	Controls (*n*=120)	*p* value
Severe deficiency (<10 ng/ml)	95 (79.2%)	56 (46.7%)	<0.001
Deficiency (10–<30 ng/ml)	23 (19.2%)	59 (49.2%)	
Sufficient (≥30 ng/ml)	2 (1.7%)	5 (4.2%)	

4. Discussion

This case control study of incident acute MI suggests that deficiency of vitamin D, including severe deficiency, is highly prevalent in Delhi, India (latitude 28.35°). It revealed that vitamin D level is even lower in cases as compared to controls. Despite the rampant vitamin D deficiency, severe vitamin D deficiency is associated with risk of acute MI even after adjusting for the known risk factors of acute MI.

The high prevalence of vitamin D deficiency found in this study is consistent with other studies from India. A rural prevalence study in North Indians showed that though vitamin D levels in rural subjects were significantly higher than in urban Indians, the prevalence of vitamin D deficiency [serum 25(OH) vitamin D < 20 ng/ml] in them was as high as 70%.[11] Another study from Delhi, in healthy individuals above 50 years of age, revealed deficiency [serum 25(OH) vitamin D < 20 ng/ml] in 91.2% including severe deficiency [serum 25(OH) vitamin D < 10 ng/ml] in 62% and vitamin D insufficiency [serum 25(OH) vitamin D levels 20–<30 ng/ml] in additional 6.8% of the population.[12] A study from Andhra Pradesh similarly reported a very high prevalence of vitamin D

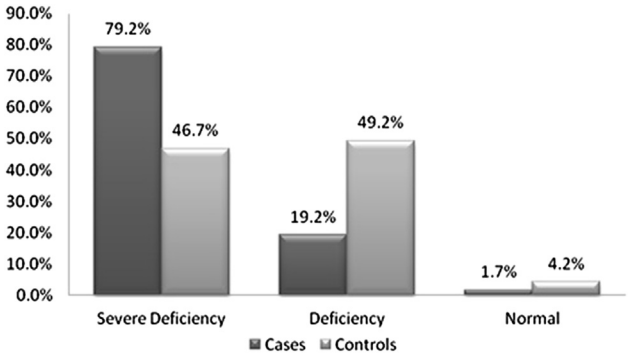

Fig 1 – Distribution of vitamin D levels among cases and controls.

Table 3 – Association of vitamin D deficiency with acute MI, after adjustment for potential confounding by logistic regression.

Variable	Adjusted odds ratio (95% C.I)	*p* value
Vitamin D deficiency		
≥10 ng/dl	1	<0.001
<10 ng/dl	4.5 (2.2, 9.2)	
Central Obesity (≥0.90 for men& ≥0.85 for women)		
No	1	0.006
Yes	2.8 (1.3, 6.0)	
Diabetes Mellitus		
No	1	0.02
Yes	2.3 (1.1, 4.7)	
Hypertension		
No	1	0.07
Yes	2.1 (0.9, 4.7)	
Tobacco use		
No	1	0.06
Yes	1.9 (0.9, 3.8)	
LDL Cholesterol		
<130	1	0.7
≥130	1.1 (0.6, 2.3)	
Alcohol use		
No	1	0.12
Yes	1.7 (0.8, 3.4)	
Education		
Secondary and below	1	0.25
Above Secondary	1.5 (0.8, 2.9)	

deficiency.[13] The levels of vitamin D deficiency (<30 ng/ml) was 88% and 94% in urban males and females respectively. In rural areas the numbers were 84% and 99% in males and females respectively. The causes for high prevalence of Vitamin D deficiency in Indians is attributed to dark-skin, lack of adequate direct skin exposure to sunlight and lack of vitamin D in predominant Indian diet.

The association of vitamin D with cardiovascular health has been reported extensively recently. A large population based study reported by Brøndum-Jacobsen et al, comparing individuals with plasma 25(OH) vitamin D levels at the 1st to 4th percentile to individuals with levels at the 50th to 100th percentile, revealed that the multivariable adjusted risk was increased by 40% for ischemic heart disease, by 64% for myocardial infarction, by 57% for early death and by 81% for fatal ischemic heart disease/myocardial infarction in individuals with low vitamin D.[10] In the meta-analyses of 18 studies in the same paper, the authors found that the risk of ischemic heart disease and early death were increased by 39% and 46% for lowest versus highest quartile of 25(OH) vitamin D level. However, the cardiovascular benefits of vitamin D supplementation are not proven and large-scale randomized trials are ongoing like the VITAL study.

The only case control study from India on association of vitamin D with CHD from Trivandrum in South Indians reported an increased odds of ischemic heart disease among patients with 25(OH) vitamin D levels >89 ng/ml compared to those with lower levels (odds ratio 3.18, 95% CI 1.31–7.73).[15] This study is unusual for the very high cut-off chosen to assess effect of vitamin D on MI and also does not assess the association of Vitamin D deficiency with MI. While the authors of this study acknowledged that even strong and prolonged UVB light cannot be toxic, they suggested that the high intake of foods rich in vitamin D could be deleterious. However other case control studies have also shown association of low vitamin D with MI. In a nested case-control study of the Health Professional Follow-Up Study (HPFS), men with vitamin D deficiency (25(OH) vitamin D levels <15 ng/ml) were at increased risk of MI (RR 2.09, 95% CI 1.24–3.54) compared to men with 25(OH) vitamin D levels >30 ng/ml.[16] A previous case-control study in patients of acute MI performed in the USA[17] reported similar inverse association between 25(OH) vitamin D levels and risk of acute MI. A more recent study from Pakistan revealed that individuals with normal levels of 25(OH) vitamin D (>30 ng/ml) had lower risk of MI as compared to those with vitamin D deficiency even after adjusting for known co-variates with an adjusted odds ratio of 0.82 ($p < 0.01$).[18] Similarly a study by Syal et al from North India in 100 patients undergoing coronary angiography revealed more severe coronary artery disease and greater endothelial dysfunction among individuals with low vitamin D.[19]

Several mechanisms, direct and indirect, have been proposed for the association of vitamin D with CHD.[20] Vitamin D could be related to CHD via blood pressure, glycemic control or parathyroid hormone (PTH). An excess of PTH levels is known to promote atherosclerosis,[21] thus PTH excess associated with vitamin D deficiency maybe one of the contributory factors to CHD. Vitamin D deficiency is known to up-regulate Renin Angiotensin Aldosterone System (RAAS) and lead to hypertrophy of smooth muscles and left ventricle, an adverse marker of cardiovascular event.[22,23] Additionally, the effects of vitamin D deficiency on type 2 DM could be mediated by its role on pancreatic β -cell function, insulin resistance, or inflammation.[24,25] Animal studies have revealed more direct role of vitamin D on cardiomyocyte remodeling as well as on cardiac relaxation and contractility.[26,27] Vitamin D is also known to down-regulate pro-inflammatory cytokines (e.g., TNF-α, IL-6) and up-regulate anti-inflammatory cytokine (IL-10).[20]

Another interesting finding of this study is that alcohol use was associated with increased risk of MI. This association corroborates with the findings of the Indian arm of the INTERHEART study,[28] a case control study of acute MI, and another case control study of industrial population from India.[29]

This study has several strengths. The cases that were recruited were incident thereby accounting for protopathic bias (presence of preclinical disease prior to initiation into the study which could result in modification of risk factors) which are very common in case control studies that evaluate chronic diseases. The controls were recruited from same location and in same season though the limitation of being hospital based was present. Extensive phenotyping was carried out both in cases and controls. However, there were several limitations of the study including the case control study design with its limitations. Information on physical activity was limited and not detailed, with no data available on diet and skin pigmentation. This is a small study and the exposure rate in controls was higher than anticipated. Larger studies with greater power from multiple centers and from different parts of India are needed, but this is the best available data linking Vitamin D to MI/CHD in Indians presently. Additionally there is a poor representation of women in this study. The study is observational and no causality of severe vitamin D deficiency

can be proven. This is however the current status of all vitamin D studies and unless the results of well designed and adequately powered randomized control studies are not published causality cannot be proven. The blood samples analyzed are from stored archival samples. However, 25(OH) vitamin D is a steroid and thus a stable molecule. Previous studies have shown the stability of 25(OH) vitamin D in stored samples over very long time periods up to 24 years.[30]

In conclusion, the study reveals a very high prevalence of vitamin D deficiency among patients of acute MI and also controls recruited in this study from Delhi, India, with levels of serum 25 (OH) vitamin D being significantly lower among cases. Despite the rampant hypovitaminosis, presence of severe vitamin D deficiency was associated with risk of acute MI even after adjusting for conventional risk factors. This association needs to be tested in larger cross-sectional and cohort studies from India. Meanwhile, population awareness of high deficiency of vitamin D must be created and its skeletal and possible cardiovascular harm should be mitigated by encouraging sun exposure and food fortification.

5. Author contribution

AR was involved in study design, drafting the original paper and approved the final version of the paper.

LR and RM performed the biochemical tests, provided inputs on the manuscript and approved the final version of the paper.

NT, KSR and DP were involved in study design, providing inputs on the manuscript and approved the final version of the paper.

All authors meet all three of the ICMJE guidelines for authorship.

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Conflicts of interest

All authors have none to declare.

REFERENCES

1. Lavie CJ, Lee JH, Milani RV. Vitamin D and cardiovascular disease will it live up to its hype? *J Am Coll Cardiol*. 2011;58:1547–1556.
2. Guessous I, Bochud M, Bonny O, et al. Calcium, vitamin d and cardiovascular disease. *Kidney Blood Press Res*. 2011;34:404–417.
3. Shapses SA, Manson JE. Vitamin D and prevention of cardiovascular disease and diabetes: why the evidence fFalls short. *JAMA*. 2011;305:2565–2566.
4. Pittas AG, Chung M, Trikalinos T, et al. Systematic review: vitamin D and cardiometabolic outcomes. *Ann Intern Med*. 2010;152:307–314.
5. Wang L, Manson JE, Song Y, et al. Systematic review: vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med*. 2010;152:315–323.
6. Geleijnse JM. Vitamin D and the prevention of hypertension and cardiovascular diseases: a review of the current evidence. *Am J Hypertens*. 2011;24:253–262.
7. Grandi NC, Breitling LP, Brenner H. Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. *Prev Med*. 2010;51:228–233.
8. Lee JH, O'Keefe JH, Bell D, et al. Vitamin D deficiency: an important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol*. 2008;52:1949–1956.
9. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med*. 2008;168:1340–1349.
10. Brøndum-Jacobsen P, Benn M, Jensen GB, Nordestgaard BG. 25-hydroxyvitamin d levels and risk of ischemic heart disease, myocardial infarction, and early death: population-based study and meta-analyses of 18 and 17 studies. *Arterioscler Thromb Vasc Biol*. 2012;32:2794–2802.
11. Goswami R, Kochupillai N, Gupta N, et al. Presence of 25 (OH) D deficiencies in a rural North Indian village despite abundant sunshine. *J Assoc Physicians India*. 2008;56:755–757.
12. Marwaha RK, Tandon N, Garg MK, et al. Vitamin D status in healthy Indians aged 50 years and above. *J Assoc Physicians India*. 2011;59:706–709.
13. Harinarayan CV, Ramalakshmi T, Prasad UV, et al. Vitamin D status in Andhra Pradesh:a population based study. *Indian J Med Res*. 2008;127:211–218.
14. Garg MK, Tandon N, Marwaha RK, et al. The relationship between serum 25-hydroxy vitamin D, parathormone and bone mineral density in Indian population. *Clin Endocrinol (Oxf)*. 2014;80:41–46.
15. Rajasree S, Rajpal K, Kartha CC, et al. Serum 25-hydroxyvitamin D 3 levels are elevated in South Indian patients with ischemic heart disease. *Eur J Epidemiol*. 2001;17:567–571.
16. Giovannucci E, Liu Y, Hollis BW. 25-Hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med*. 2008;168:1174–1180.
17. Scragg R, Jackson R, Holdaway IM, et al. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D 3 levels: a community based study. *Int J Epidemiol*. 1990;19:559–563.
18. Iqbal MP, Mehboobali N, Azam I, et al. Association of alkaline phosphatase with acute myocardial infarction in a population with high prevalence of hypovitaminosis D. *Clin Chim Acta*. 2013;425:192–195.
19. Syal SK, Kapoor A, Bhatia E, et al. Vitamin D deficiency, coronary artery disease, and endothelial dysfunction:observations from a coronary angiographic study in Indian patients. *J Invasive Cardiol*. 2012;24:385–389.
20. Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br J Nutr*. 2005;94:483–492.
21. Rashid G, Bernheim J, Green J, Benchetrit S. Parathyroid hormone stimulatesthe endothelial expression of vascular endothelial growth factor. *Eur J Clin Invest*. 2008;38:798–803.
22. Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol*. 2006;92:39–48.
23. Milani RV, Lavie CJ, Mehra MR, et al. Left ventricular geometry and survival in patients with normal left ventricular ejection fraction. *Am J Cardiol*. 2006;97:959–963.

24. Penckofer S, Kouba J, Wallis DE, Emanuele MA. Vitamin D and diabetes: let the sunshine in. *Diabetes Educ*. 2008;34:939–940, 942, 944 passim.
25. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2007;92:2017–2029.
26. Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. *Am J Med Sci*. 2009;338:40–44.
27. O'Connell TD, Berry JE, Jarvis AK. 1,25-DihydroxyvitaminD 3 regulation of cardiac myocyte roliferation and hypertrophy. *Am J Physiol*. 1997;272:H1751–H1758.
28. Joshi P, Islam S, Pais P, et al. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA*. 2007;297:286–294.
29. Roy A, Prabhakaran D, Jeemon P, et al, Sentinel Surveillance in Industrial Populations Study Group. Impact of alcohol on coronary heart disease in Indian men. *Atherosclerosis*. 2010;210:531–535.
30. Agborsangaya C, Toriola AT, Grankvist K, et al. The effects of storage time and sampling season on the stability of serum 25-hydroxy vitamin D and androstenedione. *Nutr Cancer*. 2010;62:51–57.