ORIGINAL ARTICLE

Association of 25-OH Cholecalciferol with Acute Myocardial Infarction

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ABSTRACT

In addition to its well known role on mineral homeostasis, vitamin-D has many diverse actions including anti-inflammatory, relaxation of vascular smooth muscle cells and down-regulation of renal renin production. Deficiency of Vitamin-D is a treatable condition that has been found to be associated with coronary artery disease. This study was carried out to assess the vitamin-D status in acute myocardial infarction (AMI) patients. Active form of vitamin-D (1, 25-OH D_3, calcitriol) does not reflect vitamin-D status due to its short plasma half life. 25-OH D_3 or calcidiol is considered as circulating reservoir and is used to assess vitamin-D status due to its long plasma half life. In this study 25-hydroxycholecalciferol [25 $(OH)D_3$] was assessed in 40 acute myocardial infarction patients and 40 age- and sex-matched healthy subjects. Serum 25-hydroxycholecalciferol and Lipid profile parameters were estimated. Data were analyzed with the help of SPSS version 20.0. Vitamin-D was found to be significantly reduced in AMI patients when compared with that of Controls which were 28.50 ± 16.68 ng/l in cases and 38.32 ± 16.47 ng/l in Controls, $p=0.011$. Blood pressure, total Cholesterol and LDL-Cholesterol were found significantly higher in AMI compared to those among healthy controls. Among all study subjects, statistically significant positive correlation of $25(OH)D_3$ was found with HDL-C ($p=0.014$). Significant negative correlation was found with serum TC ($p=0.001$) and LDL-C ($p < 0.001$). Serum TG was found negatively correlated with 25-hydroxycholecalciferol but it was not statitiscaly significant ($p=0.068$). In our study Vitamin-D deficiency was found to be significantly associated with myocardial infarction, dyslipidemia and hypertension.

Key Words: 25-hydroxycholecalciferol (25-OH D3), Acute Myocardial Infarction (AMI)

Introduction

Vitamin-D is found in two forms: ergocalciferol (vitamin D_2), found in plants, and cholecalciferol (vitamin D_3), found in animal tissues. They are the dietary sources of preformed vitamin-D activity. 7-dehydrocholesterol, an intermediate in cholesterol synthesis is converted to cholecalciferol in the dermis and epidermis of humans exposed to sunlight[1].

Skin synthesis of vitamin D_3 is much more important than any dietary source. Vitamin-D_2 is a plant-derived form of vitamin D manufactured through exposure of yeast to ultraviolet light. The inactive precursors from either skin or diet undergo 25-hydroxylation in the liver producing 25-hydroxycholecalciferol [$25(OH)D_3$] or calcidiol and this form is usually considered as a circulating biomarker of vitamin-D status. Subsequent conversion of 25-hydroxycholecalciferol by 1α-hydroxylase into the active form, 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] or calcitriol occurs primarily in the kidney[2]. Calcitriol, the active form of the Vitamin-D is not useful marker for vitamin D status due to its shorter plasma half life. Half-

life of $1,25(OH)_2D_3$ is 4-5 hours and that of $25(OH)D_3$ is 2-3 weeks[3,4].

Vitamin-D has well known role of maintaining calcium homeostasis, by its action on intestine (promoting dietary calcium absorption), on bone (calcium deposition, as well as resorption along with PTH) and on kidney (minimizing calcium loss). Extra-skeletal role of vitamin-D is of current interest. Vitamin D receptors were found in other tissues as well including the brain, cardiomyocytes, vascular smooth muscle cells, endothelial cells, pancreatic beta-cells, skeletal muscle, breast, prostate, colon, macrophages, and skin, exerting several effects and their expression decrease with age. The vitamin D receptor is closely related to the thyroid, retinoid, and peroxisome proliferator activator receptors. The enzyme 1-alpha hydroxylase, for final activation of calcidiol to calcitriol, has been found in a wide variety of extra renal tissues, such as the heart and vascular smooth muscle cells[5].

Evidence has demonstrated that individuals deficient in vitamin D are more likely to have or are at risk of developing cardiovascular disease. The underlying mechanism by which vitamin D may protect individuals from cardiovascular disease has not been fully elucidated. Several mechanisms have been proposed including negatively regulating renin to lower blood pressure, improving vascular compliance, decreasing parathyroid hormone levels and improving glycemic control. Vitamin D inhibits profibrotic markers in mesenchymal multipotent cells, suggesting that vitamin D may also have a direct effect on the vasculature in response to injury[6].

Several population-based studies have shown an association between reduced 25-hydroxyvitamin D levels and increased risk of both ischemic heart disease and early death. Some other studies were not so conclusive. Risk estimates vary among studies, which could be due to diversity in study size, duration of follow-up time, number of events, differences in ethnicity and different analytic measurement technique for 25-hydroxycholecalciferol[7].

A randomized controlled trial of vitamin D supplementation in subjects with heart failure demonstrated significant reductions in inflammatory cytokines involved in the pathophysiology of heart failure[6].

Vitamin D has anti-inflammatory properties that have potential therapeutic benefit in several autoimmune diseases and allograft rejection. In two small clinical trials, vitamin D supplementation was found to lower C-reactive protein levels. Additionally, $1,25(OH)_2D_3$ induces relaxation of vascular smooth muscle cells and down regulation of renin production by the kidneys. The observation of reduced mortality risk with vitamin D supplements among patients with renal failure supports a possible cardiovascular disease protective role of Vitamin D[8].

Coronary artery disease (CAD) is one of the main causes of disability and death across the world. Low 25-hydroxycholecalciferol has been associated with several risk factors for CAD, including hypertension and dyslipidemia. Whether vitamin D is a risk factor for CAD remains unclear. This study has been performed to find the serum 25-hydroxycholecalciferol level in coronary artery disease patients in our population. This may create awareness among physicians about the role of vitamin D deficiency in cardiovascular diseases and accordingly the patient may be benefited by its supplementation.

Materials and Methods

This cross-sectional study was carried out in the Department of Biochemistry, Sylhet MAG Osmani Medical College, Sylhet from July 2014 to June 2015.The patients diagnosed as Coronary artery disease in the Department of Cardiology, Sylhet MAG Osmani Medical College and Hospital, who fulfilled the inclusion and exclusion criteria, were selected as study population.

Inclusion criteria: Patients with diagnosed acute myocardial infarction as cases and healthy subjects without any history of AMI as controls, age > 40 years.

Exclusion criteria: Hepatic or renal disease, Rickets, Malignancy, Congenital heart disease.

Total 80 study subjects were selected and categorized into two groups: 40 individuals with AMI who were admitted to Sylhet MAG Osmani Medical College Hospital as cases and 40 age- and sex-matched healthy control subjects. Acute myocardial infarction was diagnosed using standard criteria[9]. Height, Body weight and Blood Pressure were measured and Body Mass Index (BMI) was calculated. 5 ml of fasting venous blood was drawn by disposable syringe with all aseptic precaution. Serum was separated and stored at 2-8°C. Serum 25-hydroxycholecalciferol was estimated using ELISA (enzyme-linked immunosorbent assay) Kit and serum Lipid profile was done with CHOD-PAP method. Data were analyzed with the help of SPSS. Quantitative data were expressed as mean and standard deviation; and comparison between groups was done by unpaired t-test. Correlation was done with Pearson's correlation test. Qualitative data were expressed as frequency and percentage. A probability value (p) of <0.05 was considered statistically significant. Informed written consent was taken from each of the patient. The study was approved by Ethical Committee of Sylhet MAG Osmani Medical College.

Results

A total of 80 study subjects were studied into two groups-40 AMI patients and 40 healthy controls. Table-I shows distribution of age, sex, anthropometric and blood pressure parameters of study subjects. Age range was 40-80 years. There was no significant difference in age, sex and BMI among the groups. Both SBP and DBP were significantly different between Cases and Controls.

Table-II shows the Level of serum 25-hydroxycholecalciferol and Lipid profile in study subjects. AMI patients had significantly lower 25-hydroxycholecalciferol level than that of healthy controls. There was significant difference in total cholesterol, HDL-C and LDL-C in AMI patients compared to that of healthy controls. No statistical significant difference was observed in serum triglyceride level between the groups.

Table-III shows Pearson's Correlation between serum 25-hydroxycholecalciferol and Lipid profile parameters in study subjects. In AMI patients and also in all study subjects (combined cases and controls), statistically significant positive correlation was found with HDL-C and significant negative correlation with serum TC and LDL-C. There was no significant correlation of triglyceride with 25-hydroxycholecalciferol.

Table-I: Distribution of age and sex, anthropometric and clinical parameters

Parameters	Controls (n=40)	Cases (n=40)	P - value
Age(yrs), Mean ±SD	49.70±8.029	53.60±10.218	0.061
Sex (m, f)	34(85%), 6(15%)	34(85%), 6 (15%)	ns
BMI (Mean ±SD)	23.35±2.81	24.59±3.01	0.061
SBP mmHg, Mean ±SD	120.75±13.28	131.38±26.26	0.025*
DBP mmHg, Mean ±SD	78.75±7.48	83.88±10.59	0.015*

significant at $p<0.05$; m, male; f, female; ns, not significant

Table-II: Serum 25-hydroxycholecalciferol and lipid profile in study Subjects

Parameters (Mean ±SD)	Controls(n=40)	Cases (n=40)	P - Value
25(OH)D_3, ng/ml	38.32 ±16.47	28.50±16.68	0.011*
TC(mg/dl)	184.28±21.58	198.40±32.55	0.025*
HDL - C(mg/dl)	36.68±3.70	34.90 ±4.06	0.045*
LDL - C(mg/dl)	113.05±18.67	123.75±20 .63	0.017*
T G (mg/dl)	186.05±70.73	198.10 ±65.83	0.433

*significant at $p<0.05$

Table III: Correlation of vitamin-D with different components of lipid profile

Correlation parameters	Cases (n=40) r- value	P-value	Allsubjects(n=80) r - value	P- value
TC	- 0.036	0.034*	- 0.373	0.001*
HDL - C	0.388	0.013*	0.275	0.014*
LDL - C	- 0.383	0.015*	- 0.405	<0.001*
TG	- 0.256	0.111	- 0.205	0.068

*significant at $p<0.05$

Discussion

Skeletal role of vitamin-D is well known. Extra-skeletal role of vitamin-D is of current interest, including its role on cardiovascular system. Vitamin-D maintains calcium homeostasis by its action on intestine, bones and kidneys. It has been found that vitamin-D receptors are distributed throughout the body including cardio-myocytes, vascular smooth muscle cells and endothelium. Physiological role of vitamin-D on cardiovascular system is not well understood, but it has been observed that its deficiency is associated with MI and mortality.

This study evaluated vitamin-D status in patients admitted with AMI in Sylhet MAG Osmani Medical college and Hospital. The mean age of controls was 49.70 (SD ± 8.029) years and that of AMI patients was 53.60 (SD ± 10.218) years. BMI of healthy controls and that of AMI were 23.35±2.81 and 24.59±3.01 respectively. There was no statistically significant difference of age and BMI in two groups. The mean systolic blood pressure (mmHg) was 120.75±13.28 and 131.38±26.26, and that of diastolic pressure (mmHg)was 78.75±7.48 and 83.88±10.59 in Controls and Cases respectively. Both SBP and DBP were significantly raised in AMI patients. These findings agree with the findings of Siadat et al[10] who reported raised SBP in AMI patients. Raised blood pressure found in this study was indicative of association of hypertension with AMI.

We found serum 25-hydroxycholecalciferol level to be significantly lower in AMI patients than that of Controls. Blood level of 25-hydroxycholecalciferol, 30 ng/ml, was considered as cut-off value in our study[11]. In AMI patients 25-hydroxycholecalciferol level was 28.50±16.68 ng/ml and in healthy controls it was 38.32±16.47 ng/ml and different was statistically significant. There was significantly higher prevalence of vitamin D deficiency (57.5%) in AMI patients compared to that of Controls (35%). In the study by Siadat et al[10], serum 25-hydroxycholecalciferol level was found to be 58.7±39.6 ng/ml in cases and 79.9±18.9 ng/ml in controls. Karur, Veerappa and Nanjappa[12] found that there was very high prevalence of vitamin-D deficiency (83.5%) in AMI patients. Scragg et al[13] also found MI cases with lower 25-hydroxycholecalciferol levels than healthy controls. Giovannucci et al[14] reported that low level of $25(OH)D_3$ was associated with higher risk of myocardial infarction in a graded manner, even after controlling for factors known to be associated with coronary artery diseases.

Several mechanisms may explain the association between vitamin D and cardiovascular diseases. Cholecalciferol regulates renin-angiotensin axis through the suppression of the renin gene. Changes of 25-hydroxycholecalciferol causes changes in the smooth muscle of the vascular wall and also inflammation and thrombosis that could explain cardiovascular complications[13].

In our study we found significantly higher total cholesterol and LDL-C and lower HDL-C in AMI patients compared to that of healthy controls. Serum triglyceride was elevated in AMI but it was not statistically significant. Scragg et al[13] also found significantly higher serum total cholesterol and lower HDL-C levels in AMI patients.

Raised cholesterol and triglyceride levels are high risk factors for cardiovascular diseases and these are associated with vitamin D deficiency and myocardial infarction. This was similar with the findings of related studies[15,16]. In a study, Karhapaa et at.[17] found low level of 25-hydroxycholecalciferol associated with high levels of TC, LDL-C and triglycerides. In another study, Auwerx, Bouillon and Kesteloot,[18] a positive correlation of 25-Hydroxycholecalciferol with HDL-C levels was observed. Several mechanisms may be suggested to explain the effect of vitamin D on lipids, including its role in reducing fatty acid absorption via the formation of insoluble calcium-fatty acid complexes in the gut. Due to decreased absorption of fat, particularly saturated fatty acids, it is expected that serum levels of total and LDL-C will be reduced.

Dyslipidemia and hypertension are two important cardiovascular risk factors and it may be assumed that vitamin D deficiency might be related to both these risk factors the pathogenesis of AMI. Vitamin D deficiency can be considered as a cardiovascular risk marker. Vitamin D deficiency, cardiovascular disease, and endothelial dysfunction may be linked by biological associations. Maintaining an optimal vitamin D level in blood seems important not only for calcium homeostasis but also for reducing cardiovascular risk.

Vitamin D deficiency was found to be significantly associated with AMI, Dyslipidemia and Hypertension in our study. Prospective studies are suggested to investigate benefits of screening and treatment of this very common vitamin defiiciency for prevention of cardiovascular diseases.

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