Original Research Article

Correlation between vitamin D status and Serum lipid profile parameters in CAD Patients Attending a Tertiary Hospital

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Abstracts

Cardiovascular disease(CVD) is consists of variety of heart disease, illnesses and events that impact the heart & circulatory system, including high blood pressure & coronary artery disease. Our aim was to the study serum vitamin D levels in patients with proven CAD attending cardiology clinic of SAIMS hospital and to compare it with control subjects and it’s out come. Our study add input to previous existing evidence suggesting that low vitamin D levels may be an independent, potentially modifiable cardiovascular risk factor. We observed that high prevalence of hypovitaminosis D in cases with angiographically documented CAD. Thus we can conclude that serum Vitamin D estimation may be considered as an important surrogate marker for screening and prevention of CAD to improve the outcome which needs further exploration.

Keywords: 25(OH) Vitamin D, Lipid Profile and coronary artery disease.

Introduction

Cardiovascular disease(CVD) is consists of variety of heart disease, illnesses and events that impact the heart & circulatory system, including high blood pressure & coronary artery disease.” (CAD)” According to the World Health Organization (WHO), almost 23.6 million deaths will be attributed to cardiovascular diseases (CVD), mainly stroke and heart diseases by the year 2030. CVDs include coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart diseases, deep vein thrombosis and pulmonary embolism. (WHO, 2011).11 In USA, Cardio Vascular disease including heart attack & stroke remains the nation no.1 killer of men & women causing more than 36% of all deaths {American heart association heart disease
Every year about 1.5 million Americans suffer a heart attack. An estimated 73 million adult Americans having high blood pressure. An estimated 16 million adult American having CAD, CVD was the largest cause of the death in males (20.3%) as well as females (16.9%) & led to about 2 million deaths annually.

Vitamin D has long been known to be an essential part of bone metabolism, although recent evidence suggests that vitamin D plays a key role in the pathophysiology of other diseases, including CVD, we aim to summarize the most recent data on the involvement of vitamin D deficiency in the development of major cardiovascular risk factors: hypertension, obesity and dyslipidemia, type 2 diabetes, chronic kidney disease and endothelial dysfunction.

The vitamin D receptor (VDR) is ubiquitously expressed in almost all body cells, such as immune, vascular or myocardial cells, suggests an involvement of vitamin D-mediated effects in several other systems apart from musculoskeletal tissues. This has led to extensive research on vitamin D as a potential influencing factor in the pathogenesis of cardiovascular diseases (CVD).

Cardiovascular (CV) risk factors, such as arterial hypertension, obesity, dyslipidemia or diabetes mellitus, as well as CVDs, including myocardial infarction, coronary artery disease or stroke, are the most prevalent diseases and account for the major causes of death worldwide, especially in Western countries. This underlines the importance of clarifying the role of vitamin D in the context of CVD. Numerous observational studies, prospective meta-analyses, and some interventional studies have addressed the possible linkage of vitamin D deficiency and the development of CVD and its risk factors.

Vitamin D status is classified according to 25(OH)D levels in the blood, and its half-life is approximately two to four weeks. There exists no clear consensus on the definition of vitamin D deficiency and vitamin D sufficiency. While the Institute of Medicine (IOM) report classifies vitamin D deficiency according to 25(OH)D levels below 20ng/mL (multiply by 2.496 to convert ng/mL to nmol/L) and 20 ng/mL as sufficient, the Endocrine Society Guidelines suggest that 25(OH)D levels <20 ng/mL are deficient and levels of 30 ng/mL are sufficient.

Vitamin D insufficiency and deficiency are highly prevalent; this is very well reflected by the fact that more than half of the population worldwide has levels below 30 ng/mL. Different factors, such as increased age, female sex, darker skin pigmentation, reduced sun exposure, as well as seasonal variation and distance from the equator are risk factors for vitamin D deficiency and must be considered. The increasing prevalence of low levels of vitamin D is mainly explainable by changes in lifestyle, reduced sun exposure and, to some extent, by air pollution.

**Vitamin D Epidemiology**

Worldwide, most humans typically expose 5% or less of their skin to infrequent periods of unshielded sunlight, a behavior which commonly leads to vitamin D deficiency. This is due to less solar exposure. It is estimated that 30% to 50% of the general population suffer from vitamin D deficiency. WHO reported mortality data (the global statistic on non communicable disease report 2011) has reported that there were more than 2.5 million death from CVD in India in 2008, two third due to CHD & one third due to stroke. It will be double in 2015. The mortality varies widely from state to state.

**Cholesterol Reduction And Vitamin D**

When a person has high cholesterol, cholesterol-lowering drugs called statins are given. These drugs block the production of cholesterol. There some new evidence that suggests vitamin D deficiency can decrease insulin sensitivity. An early stage of diabetes that increases a person’s chance of getting cardiovascular disease by raising LDL-C and triglyceride levels while...
lowering HDL-C levels. If it is true, improving vitamin D status could reduce the risk of cardiovascular disease indirectly by improving insulin sensitivity.

Nutritional vitamin D deficiency is highly prevalent, occurring in approximately 30%-50% of the general population\(^\text{18,19}\). In several studies, 25-hydroxyvitamin D[25(OH)D] deficiency has been independently associated with both incident acute myocardial infarction (AMI)\(^\text{20}\) and heart failure (HF)\(^\text{21,22}\), suggesting that 25(OH)D plays an important role in cardiac function. Cardiac troponins (low molecular weight 20-25kDa) have significant sensitivity and specificity for myocardial muscle damage; therefore, they have been regarded as the “gold standard” for acute myocardial infarction diagnosis\(^\text{23}\).

Serum TnI and TnT elevated within 4 to 9 hours, reached the peak in 12 to 24hours and remaining high level for up to 2 weeks. In healthy peoples both troponins are less than 0.1 and 0.4 ng/ml, respectively. Thus, tremendously rising troponin level will highly possibly indicate myocardial necrosis\(^\text{24}\).

Some observational studies indicate an association of vitamin D deficiency with lower high density lipoprotein (HDL) and higher triglycerides, as well as higher apolipoprotein E levels\(^\text{25,26}\). Blood lipids showed a significant association of lower vitamin D levels with hypercholesterinemia\(^\text{27}\). The elevated levels of serum total cholesterol, triglyceride and LDL and low levels of HDL is called dyslipidaemia, where is a major risk factor for CVD. However all the components are associated with increased incidence of CAD\(^\text{28}\).

Serum Lipid profile (Total Cholesterol, Triglyceride, and LDL-c) are the essential players compounds in CAD developments steps, except HDL-c which retards CAD development. According to other studies of lipid profile with CAD relationship, multiple epidemiologic studies have established a low level of HDL-c as an independent risk factor for CVD\(^\text{29}\). Moreover, Framingham Heart Study reported 43% to 44% of coronary events occurred in persons with HDL-c levels less than 40 mg/dL\(^\text{28}\).

In addition the risk of CAD was approximately doubled with either TGs >200 mg/dl or HDL-cholesterol <40 mg/dl. Moreover, the presence of both was associated with a four-fold increase in risk\(^\text{30}\).

Lipid profile plays the essential role of lipid deposition in artery wall and CAD development, by accumulating the LDL-c inside layers of artery wall, except HDL-c which has beneficial effects for a number of reasons by decreasing lipid oxidation after depositing in blood vessels, leading to retarding CAD development. Moreover, in other observational studies were shown that each 1-mg/dL decrease in plasma HDL-c concentration is associated with a 2% to 3% increased risk of CVD\(^\text{31,32}\). In India less research is observed about vitamin D and its association with CAD. Our aim was to the study serum vitamin D levels in patients with proven CAD attending cardiology clinic of SAIMS hospital and to compare it with control subjects and it’s out come.

### Material and Methods

This present case control study was conducted in department of Biochemistry, Sri Aurobindo Medical College & P.G. Institute Madhya Pradesh With the help of Cardiology department during period April 2013 to October 2014. The study was approved by institutional ethics committee for research work. The study population consists of 100 subjects out of which 50 subjects were case (CAD patients) and 50 subjects were control (normal, healthy subjects). All subject of study population, selected for present study had attended and admitted to SAIMS hospital & research centre. Study group consists of subjects having age >20 years. The diagnosis of CAD patients was done by Cardiology department based on ECG, ECHO, CAG and serum TROP I findings.
Inclusion Criteria
- Presence of CAD proven by electrocardiography, echocardiography or angiography
- Age more than 20 years of both genders.

Exclusion Criteria
- Patients who are taking vitamin D supplementation or replacement therapy.
- Endocrine disorder like hypoparathyroidism, etc.
- Renal rickets.
- Known CKD stage 5th/6th.
- Significant chronic Liver disease.
- Malabsorption.

After taking verbal/written consent from the subjects, venous blood was collected in Vacutainer, allowed to clot and then immediately sent to the biochemistry lab. Where the samples will be centrifuged at 4000rpm for 10 minutes, then serum will be separated and analyzed for the following tests:

1) Serum vitamin D level measure by ELFA Technique (Enzyme Linked Fluorescent Assay Method, by VIDAS, full automatic analyser)
2) Serum troponin I by Immuno chromatographic Method
3) Serum Lipid profile:
   a) Total Cholesterol CHOD –PAP method
   b) Triglycerides -TG, GPO- PAP Method
   c) HDL – Cholesterol-HDL-CHOL Method
   d) LDL – Cholesterol-LDL-CHOL Method
   e) VLDL – Cholesterol -VLDL-CHOL Method

Student t test was performed to see the difference on mean of quantitative data between cases and controls. Multivariate logistic regression analysis was performed using backward elimination model to see the independent risk factor.

Results and Discussion
This present case control study was conducted in Department of Biochemistry, of a tertiary care center of SAIMS hospital Madhya Pradesh during period April 2013 to October 2014. The table-1 shows the age wise distribution of patients. In the control group 8 (16%) patients were in the age group 21-30 years, 11 (22%) were in the age group 31-40 years, 13 (26%) were in the age group 41-50 years, 7 (14%) were in the age group 51-60 years and 11 (22%) were more than 60 years of age.

Table No. 1 Distribution of Patients According to Age Group (N=100)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>21-30 years</td>
<td>8</td>
<td>16.00</td>
</tr>
<tr>
<td>31-40 years</td>
<td>11</td>
<td>22.00</td>
</tr>
<tr>
<td>41-50 years</td>
<td>13</td>
<td>26.00</td>
</tr>
<tr>
<td>51-60 years</td>
<td>7</td>
<td>14.00</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>11</td>
<td>22.00</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Similarly, in the case group, 2 (4%) were in the age group 31-40 years, 21 (42%) were in the age group 41-50 years, 18 (36%) were in the age group 51-60 years, 9 (18%) were in the age group more than 60 years.

Thus in the control maximum number of patients were in the age group more than 40 years and similarly the same trend was seen in the case group too.

Table No. 2 Distribution of Patients According to Gender (N=100)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>50.00</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>50.00</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.00</td>
</tr>
</tbody>
</table>

The above table shows the distribution of patients according to the gender. In the control group, there were 25 (50%) males and 25 (50%) females. While in the case group there were 35 (70%) males and 15 (30%) females.

In the control group, the gender wise distribution was equal, while male preponderance was seen in the case group patients.
The above table shows the positive findings on ECG, ECHO and Troponin-I in the case group patients.

Positive findings on ECG was seen in 44 (88%) patients, positive findings on ECHO were seen in 41 (82%) of the patients and raised Troponin-I was seen in 28 (56%) of the patients (cases).

**Table No. 3** Distribution of Patients According to Positive Findings of ECG, ECHO and Troponin-I (N=100)

<table>
<thead>
<tr>
<th>Positive Findings</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>ECG</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ECHO</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Troponin-I</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The above table shows the positive findings on ECG, ECHO and Troponin-I in the case group patients.

The mean vitamin D level in the control group was 36.75 ± 30.06, while in the case group it was 36.94 ± 18.34, which is highly statistically significant. Thus, we can say that there is a significant derangement in the lipid parameters in the case group patients except TGs.

**Table No. 4** Mean Vitamin D levels in Both the Groups (N=100)

<table>
<thead>
<tr>
<th>Vitamin D Levels</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (mean ± SD)</td>
<td>36.94 ± 18.34</td>
<td>20.36 ± 12.29</td>
<td>5.31, p value = 0.000, H. Sig.</td>
</tr>
</tbody>
</table>

**Table No. 5** Serum Lipid Profile in Both the Groups (N=100)

<table>
<thead>
<tr>
<th>Lipid Parameters</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
<th>Z value, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>177.94 ± 28.79</td>
<td>202.66 ± 38.73</td>
<td>Z Value = -3.62</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>161.12 ± 36.75</td>
<td>174.90 ± 36.94</td>
<td>Z Value = -1.87</td>
</tr>
<tr>
<td>HDL</td>
<td>35.48 ± 6.97</td>
<td>30.46 ± 7.42</td>
<td>Z Value = 3.49</td>
</tr>
<tr>
<td>VLDL</td>
<td>31.84 ± 7.37</td>
<td>34.56 ± 7.68</td>
<td>Z Value = -2.05</td>
</tr>
<tr>
<td>LDL</td>
<td>108.62 ± 30.06</td>
<td>136.40 ± 35.88</td>
<td>Z Value = -4.20</td>
</tr>
</tbody>
</table>

P Value = < 0.05, Significant

The above table shows the mean vitamin D levels in both the control as well as the case groups.

The mean total cholesterol level in control group was 177.94 ± 28.78, mean triglycerides was 161.12 ± 36.75, mean HDL was 35.48 ± 6.97, mean VLDL was 31.84 ± 7.37 and mean LDL was 108.62 ± 30.06.

In the case group the mean total cholesterol level was 202.66 ± 38.73, mean triglycerides was 174.90 ± 36.94, mean VLDL was 34.56 ± 7.68 and mean LDL was 136.40 ± 35.88.

As the table clearly shows that all the mean lipid parameters in the case group were statistically significant except Triglycerides which is not significantly raised in comparison to the mean lipid parameters of the control group (p < 0.05). Thus, we can say that there is a significant derangement in the lipid parameters in the case group patients except TGs.

**Table No. 6** Mean Vitamin D Levels in Relation to Age (N=100)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
<th>T value, P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>21-30 years</td>
<td>37.38 ± 18.02</td>
<td>-</td>
<td>T-Value = 3.19, P-Value = 0.010, Significant</td>
</tr>
<tr>
<td>31-40 years</td>
<td>38.55 ± 20.31</td>
<td>18.00 ± 2.82</td>
<td>T-Value = 3.21, P-Value = 0.007, Significant</td>
</tr>
<tr>
<td>41-50 years</td>
<td>41.38 ± 26.30</td>
<td>17.57 ± 5.91</td>
<td>T-Value = 1.76, P-Value = 0.093, Not Significant</td>
</tr>
<tr>
<td>51-60 years</td>
<td>31.29 ± 9.81</td>
<td>21.17 ± 18.59</td>
<td>T-Value = 2.37, P-Value = 0.030, Significant</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>33.36 ± 7.50</td>
<td>25.78 ± 6.79</td>
<td>T-Value = 2.37, P-Value = 0.030, Significant</td>
</tr>
</tbody>
</table>

The above table shows the mean vitamin D levels according to various age ranges.

In the control group, the mean vitamin D level in the age group 21-30 years was 37.38 ± 18.02, in the age group 31-40 years it was 38.55 ± 20.31, in the age group 41-50 years it was 41.38 ± 26.30, in the age group 51-60 years it was 31.29 ± 9.81 and in the age group more than 60 years it was 33.36 ± 7.50.

In the case group, the mean vitamin D level in the age group 31-40 years was 18.00 ± 2.82, in the age group 41-50 years it was 17.57 ± 5.91, in the...
age group 51-60 years it was 21.17 ± 18.59, and in the age group more than 60 years it was 25.78 ± 6.79.

When the mean Vitamin D levels were compared in each age group, except for the age group 51-60 years, where the mean Vitamin D levels were comparable, in all other groups the mean Vitamin D levels were statistically significant lower in the case group as compared to the control group patients.

Thus, all the case group patients had low levels of Vitamin D as compared to the control group patients.

Table No. 7 Mean Vitamin D Levels in Relation to Gender (N=100)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Control Group (N=50)</th>
<th>Case Group (N=50)</th>
<th>T value, P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>39.08 ± 22.03</td>
<td>20.63 ± 13.59</td>
<td>T-Value = 3.71 P-Value = 0.001 Significant</td>
</tr>
<tr>
<td>Female</td>
<td>34.80 ± 13.85</td>
<td>19.73 ± 8.95</td>
<td>T-Value = 4.18 P-Value = 0.000 Significant</td>
</tr>
</tbody>
</table>

The above table shows the distribution of mean vitamin D levels in relation to gender in both the groups.

In the control group, the mean vitamin D level in the males was 39.08 ± 22.03, while in the females it was 34.80 ± 13.85.

In the case group, the mean vitamin D level in the males was 20.63 ± 13.59 and in the females it was 19.73 ± 8.95.

When the mean vitamin D levels were compared in both the groups with respect to the gender. The difference of the means was found to be statistically highly significant. Thus, there was a significant difference in the mean Vitamin D levels between the two groups and the in the case group the mean Vitamin D levels were significantly lower than the control group patients.

Table No. 8 Correlation between Vitamin D levels and Lipid Profile Parameters in the Case Group N=50)

<table>
<thead>
<tr>
<th>Lipid Parameter</th>
<th>r value (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.107 (0.458), not significant</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.087 (0.549), not significant</td>
</tr>
<tr>
<td>HDL</td>
<td>0.004 (0.977), not significant</td>
</tr>
<tr>
<td>LDL</td>
<td>0.125 (0.386), not significant</td>
</tr>
<tr>
<td>VLDL</td>
<td>-0.099 (0.495), not significant</td>
</tr>
</tbody>
</table>

The above table shows the correlation 'r' value and p value of vitamin D when correlated with the various lipid parameters like Total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and VLDL.

The correlation value ‘r’ of total cholesterol was found to be 0.107, with a p value of 0.458, which is statistically not significant. Thus, a positive weak correlation was found between total cholesterol and vitamin D but the correlation was not significant.

The correlation value ‘r’ of triglycerides was found to be -0.087, with a p value of 0.549, which is statistically not significant. Thus, a negative weak correlation was found between triglycerides and vitamin D but the correlation was not significant.

The correlation value ‘r’ of HDL was found to be 0.004, with a p value of 0.977, which is statistically not significant. Thus, a positive weak correlation was found between HDL and vitamin D but the correlation was not significant.

The correlation value ‘r’ of LDL was found to be 0.125, with a p value of 0.386, which is statistically not significant. Thus, a positive weak correlation was found between LDL and vitamin D but the correlation was not significant.

The correlation value ‘r’ of VLDL was found to be -0.099, with a p value of 0.495, which is statistically not significant. Thus, a negative weak correlation was found between VLDL and vitamin D but the correlation was not significant.

Thus, we can say that there is no significant correlation between lipid parameters and the vitamin D levels.

Discussion

Coronary artery disease is a narrowing or blockage of the arteries and vessels that provide oxygen and nutrients to the heart. It is caused by atherosclerosis, an accumulation of fatty materials on the inner linings of arteries. The resulting blockage restricts blood flow to the heart. When the blood flow is completely cut off, the result is a Myocardial infarction (heart attack).
In this case control study we had included patients of CAD proven by Angiography/Echo/ECG at a tertiary care hospital, our data noted that vitamin D deficiency has been associated with CAD, vitamin D deficiency can adversely affect metabolism of myocardial tissue, we also found cases subjects with high TG and high cholesterol levels even though not significant are associated with vitamin D deficiency and CAD. The mean 25(OH)D level was 20.36 ± 12.29 ng/mL. 60% of the patient population was categorized as deficient in vitamin D; an additional 32% had vitamin D insufficiency, while only 8% had normal vitamin D levels. The evaluation of vitamin D deficiency in our patient population is reflection of the generalized high prevalence rates of hypovitaminosis D in India (64-66). The high prevalence rates in our country despite its sunny climate and proximity to the equator are explained by the darker skin complexion of the population, generalized malnutrition, vegetarian food habits, inadequate sun exposure, and lack of vitamin D food fortification program.

In case control study, we had evaluated vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency and its correlation with proven CAD patients. Despite the more extensive pattern of angiographic CAD, the prevalence of risk factors like diabetes, hypertension, smoking, lipid profile, and mode of clinical presentation of CAD was not significantly different in patients with or without vitamin D deficiency. Though vitamin D deficiency has previously been shown to be associated with established CV risk factors, higher cardiovascular death, and overall mortality, studies done are s heterogenous, and only small numbers of longitudinal studies are available. The, data on the spectrum and severity of vitamin D deficiency and extent of angiographically determined CAD are limited. Though we did not find any significant correlation of vitamin D levels with age, gender, presence or absence of hypertension, diabetes.

We found weak and non-significant correlation between vitamin D levels and Total cholesterol, triglyceride and other lipid parameter but significant correlation of vitamin D levels and angiographic extent of CAD was demonstrable.

**Conclusion**

Cardiovascular disease is complex disease, this study throws lights on independent association of Vitamin D deficiency and progress of coronary artery disease pathogenesis and it can be considered as an individual risk factor and dyslipidemia observed is linked with CAD. Our study add input to previous existing evidence suggesting that low vitamin D levels may be an independent, potentially modifiable cardiovascular risk factor. We observed that high prevalence of hypovitaminosis D in cases with angiographically documented CAD. Thus we can conclude that serum Vitamin D estimation may be considered as an important surrogate marker for screening and prevention of CAD to improve the outcome which needs further exploration. Along with this significant concluding association we also conclude a weak and non-significant correlation between vitamin D levels and Total cholesterol, triglyceride and other lipid parameter.

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