CORRELATION OF SERUM LIPID PROFILE WITH HYPOVITAMINOSIS D IN TYPE 2 DIABETES

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ABSTRACT

BACKGROUND
The prevalence of diabetes and vitamin D deficiency has risen dramatically over the years because of the increased calorie consumption, sedentary lifestyle and less exposure to sunlight. The discovery of the presence of vitamin D receptors and the one hydroxylase enzyme in most of the tissues in the body has shed light on the association of Vitamin D insufficiency with increased risk of various non-skeletal morbidities including dyslipidaemia.

The aim is to study the effect of vitamin D levels on dyslipidaemia in type 2 diabetic patients.

MATERIALS AND METHODS
This cross-sectional study included 50 known type 2 diabetic patients. Vitamin D, HbA1C and lipid profile were estimated using standard procedures. Results were statistically analysed by SPSS software.

RESULTS
The difference in the means of all the dependent variables when compared between vitamin D groups of deficient, insufficient and sufficient groups were statistically significant for HbA1C, and all the lipoprotein variables except LDL. The elevation of TC, triglycerides, ratio of TGL/HDL and atherogenic index in the deficient group was statistically significantly higher compared to insufficient group (p = .000) and sufficient group (p = .000). The decline in HDL level in the deficient group was statistically significant compared to insufficient group (p = .003) and sufficient group (p = .000). The inverse correlation of Vitamin D with HbA1C (r = .404, p = .004), TGL (r = .474, p = .000), TGL/HDL (r = .885, p = .000) and atherogenic index (r = .944, p = .000) was highly statistically significant. The correlation of Vitamin D with HDL was positive (r = .752, p = .000) and also highly statistically significant.

CONCLUSION
Due to association of vitamin D deficiency with atherogenic dyslipidaemia in type 2 diabetes, deficiency of vitamin D may be a high risk factor for cardiovascular diseases which is one of the leading causes for mortality worldwide. But whether supplementation of this vitamin can reduce the risk of cardiovascular diseases has to be proved by interventional studies.

KEYWORDS
Vitamin D, Lipid Profile, Dyslipidaemia, Type 2 Diabetes.


BACKGROUND
Globally, there is a trend of increasing health problems caused by Diabetes and vitamin D deficiency recently. Diabetes is a heterogeneous group of metabolic disorder characterised by hyperglycaemia that may be due to the defect in insulin secretion or insulin resistance or both. There is accumulating evidence that diabetes has evolved into the status of potential epidemic in India. Currently, more than 62 million individuals have been diagnosed with the disease.¹,² It has also been proved by various studies that diabetes is becoming one of the leading causes of death worldwide. WHO states that diabetes will be the 7th leading cause of death in 2030.³

The prevalence has risen dramatically over the years because of the increased calorie consumption and sedentary lifestyle.

The pleiotropic role of vitamin D is being unveiled after the discovery of the presence of vitamin D receptors and the one hydroxylase enzyme that converts 25(OH)D (25 hydroxycholecalciferol) to the active form in most of the tissues in the body. During the past decade, plenty of epidemiological studies have been done to prove the association of Vitamin D insufficiency with increased risk of various non-skeletal morbidities like multiple sclerosis,⁴ diabetes mellitus⁵-⁶ and cardiovascular disease⁷ and cancer.⁸ According to a study from Northern Norway, which included 4751 participants over a follow-up period of 11 years, 32% increased mortality risk was found in those in the lowest serum 25(OH)D quartile as compared to those in the highest 25(OH)D quartile.⁹ The Framingham Offspring Study that was done in 1739 individuals and followed for 5.4 years, also had similar results that those with serum 25(OH)D levels <10 ng/mL had a hazard ratio of 1.80 for a cardiovascular event as compared with those with levels >15 ng/mL. Also it is a proven fact that diabetes causes atherogenic dyslipidaemia.
due to which it has become one of the leading causes of cardiovascular death.

On the basis of these studies, we want to study the effect of vitamin D levels on dyslipidaemia in type 2 diabetic patients.

**MATERIALS AND METHODS**

Our study is a cross-sectional study conducted over a period of 3 months. The patients from diabetic outpatient clinic in a tertiary care centre in central Tamilnadu were recruited for the study. 50 known Type 2 diabetic patients under treatment were included for the study. Exclusion criteria included patients who were suffering from chronic disorders of the liver or kidney, endocrinology disorders such as hypo- or hyperthyroidism and hyperparathyroidism, those who were using drugs affecting the lipid profile or calcium and bone metabolism, anticonvulsive drugs, and vitamin D or calcium supplementation, insulin injection. Pregnant and lactating mothers were also excluded from the study.

10 mL of peripheral blood was withdrawn after overnight fasting and after getting an informed consent from all the participants. Blood samples were centrifuged at 3000 rpm for 10 min. and stored at -20°C. Serum levels of 25(OH) D were measured using the Chemiluminescence Immune Assay method or CLIA. The normal range of 25(OH) was 6–54 ng/mL (15-135 nmol/L) using this method. Fasting serum glucose, total cholesterol, HDL-C and triglyceride concentrations were measured in duplicate using enzymatic kits, standardised reagents, and standards in Beckmann Coulter autoanalyzer. Indirect Low Density Lipoprotein (LDL), was calculated by Fried Wald's formula \[ \text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{triglyceride}/5) \], where the triglyceride level was less than 400 mg/dL. HbA1c was estimated by using Ion exchange chromatography (Crest A Coral clinical system, USA). Anthropometric data including Weight and height were measured to the nearest 100 g and 0.5 cm, respectively with patients wearing light clothes and no shoes. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²).

**Statistical Analysis**

Data were analysed by SPSS statistical software (version 21.0). Descriptive statistics were given as mean and standard deviation. Vitamin D level was transformed into 3 categories of deficient, insufficient and normal by using SPSS. Similarly, BMI was categorised into underweight, normal, overweight and obese, all lipid parameters were categorised into normal and dyslipidaemic by transformation (Recoding to different variables). Independent sample t test was utilised for comparing means of the variables between genders. Cross-tabulation was used for evaluating the percentages of the 3 vitamin D categories present across the BMI and lipid categories. The means of the biochemical parameters for the vitamin D groups were compared by One-way ANOVA test and the statistical significance of the difference between the groups was done using a Tukey post hoc test. P-values less than 0.05 were considered statistically significant.

**RESULTS**

Our study comprises of 50 participants who were known diabetics under treatment. Of these male participants were 18 and female participants 32. The descriptive statistics of all the variables when categorised by vitamin D level is given in the Table 1. The study group was categorised by the level of vitamin D as deficient, insufficient and normal. 50% of the study participants were vitamin D deficient, 26% were insufficient and only 24% of them were having adequate level of vitamin D.

When analysed between categories of BMI and vitamin D by cross-tabulation in SPSS, 54% of the patients had elevated total cholesterol and of these 63% were deficient in vitamin D, 22% were insufficient. 52% of the patients were found to have hypertriglyceridaemia of which none of them were having normal vitamin D level. 92% of them were deficient and 8% were insufficient. It was found that only 4% of the patients who were having normal triglyceride levels were deficient in vitamin D level and 45% insufficient. Only 4 of the 50 patients (8%) were having elevated LDL levels and all the 4 were vitamin D deficient. 54% of the patients had low HDL of which 77% were vitamin D deficient and 19% were insufficient.

When analysed between various categories of lipid fractions and vitamin D by cross-tabulation in SPSS, 54% of the patients had elevated total cholesterol and of these 63% were deficient in vitamin D, 22% were insufficient. 52% of the patients were found to have hypertriglyceridaemia of which none of them were having normal vitamin D level. 92% of them were deficient and 8% were insufficient. It was found that only 4% of the patients who were having normal triglyceride levels were deficient in vitamin D level and 45% insufficient. Only 4 of the 50 patients (8%) were having elevated LDL levels and all the 4 were vitamin D deficient. 54% of the patients had low HDL of which 77% were vitamin D deficient and 19% were insufficient.

When the means of all the dependent variables were compared by one-way ANOVA, there was a statistically significant difference between groups for HbA1C, and all the lipoprotein variables except LDL, as given by F (2,47) and p in the Table 2.

A Tukey post hoc test revealed that the glycaemic status as per HbA1C was statistically significantly lower for vitamin D insufficient group (6.62 ± 0.44, p = .001) and sufficient (5.65 ± 0.31, p = .000) group compared to the deficient group (8.06 ± 1.47). There was no statistically significant difference between the insufficient and sufficient groups (p = .078). The total cholesterol was statistically significantly higher for vitamin D deficient group compared to sufficient group (p = .010). The elevation of triglycerides level in the deficient group was statistically significantly higher compared to insufficient group (p = .000) and sufficient group (p = .000). The decline in HDL level in the deficient group was statistically significant compared to insufficient group (p = .003) and sufficient group (p = .000), but not significant between insufficient and sufficient groups. The increase in LDL was not statistically significant between the various groups. The atherogenic index was also much increased in the deficient and insufficient groups and both were statistically significant compared to sufficient group (p = .000).

The result of Pearson’s correlation of vitamin D with atherogenic index and BMI is given in Table-3. Vitamin D level was inversely correlated with atherogenic index and highly statistically significant. Pearson’s Correlation of vitamin D with HbA1C, lipid profile is given in table-4. HbA1C and lipid profile were negatively correlated except HDL which was positively correlated. The correlations were highly significant statistically except for LDL.


**DISCUSSION**

The study participants were categorised into vitamin D deficient, insufficient and sufficient groups depending on their vitamin D levels. Classification of vitamin D deficiency was done according to the recent Endocrine Society Clinical Practice Guideline on evaluation, treatment and prevention of vitamin D deficiency (published July 2011). As per the guidelines, vitamin D deficiency is 25(OH)D level below 20 ng/mL (50 nmol/L), vitamin D insufficiency is 25(OH)D level at 21-29 ng/mL and sufficient if the 25(OH)D level is above 30 ng/mL.[10] To determine the vitamin D status, 25(OH)D level was measured since it is the main circulating form of vitamin D, varies less day-to-day, with exposure to sunlight and diet intake because of its longer half-life (2-3 weeks), and the measurement is relatively easy.[11]

In our study, 50% of the diabetic patients were vitamin D deficient, 26% were insufficient and vitamin D level was inversely related to glycosylated haemoglobin. This finding was in consistent with most of the previous studies.[12] In a cohort study of 715 type 2 diabetic patients, a significant inverse correlation between Hba1c and serum 25(OH)D (r=-0.116, p=0.08) was found while no significant correlation was detected with fasting plasma glucose (r=-0.066, p=0.122).[13] This correlation may be due to the following facts: Pancreatic beta cells have been found to have vitamin D receptors[14] and also express 1α-hydroxylase enzyme.[15] Vitamin D appears to regulate insulin secretion by facilitating the secretion of insulin.[16]

BMI was found to be inversely related to vitamin D level in the study participants and 56% of the vitamin D deficient subjects were found to be obese. The relationship of obesity with hypovitaminosis D has been proved in many previous studies.[17][19] This can be explained by the fact that the adipose tissue secretes vitamin D reducing its bioavailability in obese people.[20]

Further in our study, we detected dyslipidaemia in vitamin D deficient and insufficient group which was more pronounced in the deficient group. National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred to define dyslipidaemia. According to NCEP-ATP III guideline, hypercholesterolaemia is defined as TC>200 mg/dL, high LDL when value >100 mg/dL, hypertriglycerideraemia as TG >150 mg/dL and low HDL when value <40 mg/dL in men and 50 mg/dL in case of women. Dyslipidaemia was defined by presence of one or more than

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**Table 1. Descriptive Statistics of the Variables when Categorised by Vitamin D Level**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Deficient (N=25)</th>
<th>Insufficient (N=13)</th>
<th>Normal (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Mean</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Age</td>
<td>52.08</td>
<td>10.54</td>
<td>54.31</td>
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<tr>
<td>systolic BP mmHg</td>
<td>125.04</td>
<td>16.62</td>
<td>135.46</td>
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<tr>
<td>diastolic BP mmHg</td>
<td>77.60</td>
<td>11.09</td>
<td>84.23</td>
</tr>
<tr>
<td>FBS mg/dL</td>
<td>201.16</td>
<td>59.81</td>
<td>197.15</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.06</td>
<td>1.47</td>
<td>6.62</td>
</tr>
<tr>
<td>TC mg/dL</td>
<td>235.16</td>
<td>57.28</td>
<td>193.62</td>
</tr>
<tr>
<td>TGL mg/dL</td>
<td>286.00</td>
<td>63.12</td>
<td>162.15</td>
</tr>
<tr>
<td>LDL mg/dL</td>
<td>142.80</td>
<td>54.35</td>
<td>117.57</td>
</tr>
<tr>
<td>VLDL mg/dL</td>
<td>57.20</td>
<td>12.62</td>
<td>32.43</td>
</tr>
<tr>
<td>HDL mg/dL</td>
<td>35.16</td>
<td>5.60</td>
<td>43.62</td>
</tr>
<tr>
<td>TGL/HDL</td>
<td>8.32</td>
<td>2.25</td>
<td>3.81</td>
</tr>
<tr>
<td>AI (log[TGL/HDL])</td>
<td>0.91</td>
<td>0.12</td>
<td>0.57</td>
</tr>
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<td>BMI</td>
<td>25.52</td>
<td>4.18</td>
<td>28.10</td>
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**Table 2. ANOVA**

<table>
<thead>
<tr>
<th>Variables</th>
<th>HbA1C</th>
<th>TC mg/dL</th>
<th>TGL mg/dL</th>
<th>LDL mg/dL</th>
<th>VLDL mg/dL</th>
<th>HDL mg/dL</th>
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</thead>
<tbody>
<tr>
<td>Vitamin D ng/mL</td>
<td>Pearson Correlation r</td>
<td>Sig. 2-tailed p value</td>
<td>Pearson Correlation r</td>
<td>Sig. 2-tailed p value</td>
<td>Pearson Correlation r</td>
<td>Sig. 2-tailed p value</td>
</tr>
<tr>
<td></td>
<td>-.742**</td>
<td>.000</td>
<td>-.404**</td>
<td>.004</td>
<td>-.874**</td>
<td>.000</td>
</tr>
</tbody>
</table>

**Table 4. Pearson’s Correlation of Vitamin D with Lipid Profile**

**Table 3. Pearson’s Correlation of Vitamin D with Atherogenic Index and BMI**

Vitamin D ng/mL Pearson Correlation r               TGL/HDL   AI   BMI

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one abnormal serum lipid concentration. Serum vitamin D level was inversely related to total cholesterol, triglycerides and low density lipoproteins directly related to high density lipoproteins. The same relationships were observed in many epidemiological studies. A negative association between serum levels of 25(OH)D and Triglycerides was found by Ford and colleagues, in their NHANES III study which was highly statistically significant in our study also. Moreover, we observed an inverse relationship of vitamin D with atherogenic index. A positive association between vitamin D and HDL in our study was also shown in a review of 22 cross-sectional studies. Vitamin D mediated reduction in serum triglycerides has been explained by two main mechanisms. One of the mechanisms is enhancement of intestinal calcium absorption thereby increasing serum calcium which could then reduce serum triglycerides by reducing hepatic triglyceride formation and secretion. The other mechanism is by its suppressive effect on serum PTH concentration. Low serum PTH may reduce serum triglycerides via increased peripheral removal. Apart from the above, Vitamin D may regulate triglyceride metabolism by causing the expression of VLDL cholesterol receptors in some types of cells. Another possible mechanism may be through insulin resistance: vitamin D deficiency increases the risk of insulin resistance that leads to increase in the levels of VLDL cholesterol and triglycerides.

CONCLUSION

Our study is an added evidence of the association of vitamin D deficiency with atherogenic dyslipidaemia in type 2 diabetes. Due to this relation, deficiency of vitamin D may be a high risk factor for cardiovascular diseases which is one of the leading causes for mortality worldwide. Early lifestyle modifications in the form of optimum exposure to sunlight and taking vitamin D rich diet may help in reducing the incidence of deficiency of vitamin D. But whether supplementation of this vitamin can reduce the risk of cardiovascular diseases has to be proved by interventional studies.

REFERENCES