25-Hydroxyvitamin D₃ Deficiency and Dyslipidemia in Type 2 Diabetic Subjects

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Abstract: The present Observational study was conducted to estimate the 25-hydroxyvitamin D₃ deficiency and its association with dyslipidemia in type 2 Diabetes mellitus subjects at the Department of Medicine, Isra University Hospital and Consultant Clinics Hyderabad from January 2014 to July 2014. A sample of 310 diagnosed type 2 DM were selected through non-probability purposive sampling according to inclusion and exclusion criteria. Serum 25-OH-D₃ was estimated by ARCHITECT I 1000 system. Lipids sub fractions were analyzed according to standard methods. Data was analyzed on SPSS version 21.0. Continuous and categorical variables were analyzed by student’s t test and chi square test respectively. The associations of various lipid fractions with vitamin D₃ were analyzed by Pearson’s correlation. The significant p-value was taken at ≤ 0.05. Reduced 25-hydroxyvitamin D₃ levels were observed in 84.1% (n = 261). The 25-hydroxyvitamin D₃ levels as low as 6ng/dl was observed in present study. The lipid profile status of subjects with normal and reduced 25-hydroxyvitamin D₃ showed significant differences (p = 0.001). Triglycerides, LDLc, VLDLc, and Cholesterols exhibited an inverse correlation with 25 hydroxyvitamin D₃; however a positive correlation was noted for HDL cholesterol. In conclusion, the vitamin D₃ deficiency was observed in type 2 diabetic subject and showed independent association with dyslipidemia in type 2 diabetics.

Keywords: 25-Hydroxyvitamin D₃, Dyslipidemia, Diabetes Mellitus, Sindh

1. Introduction

The rising prevalence of vitamin D deficiency is estimated as 50% for the worldwide population and 2%–30% for the European adults [1]. The prevalence of 25-hydroxyvitamin D₃ deficiency is approximated to 30% to 50% in the general population [2]. 25-hydroxyvitamin D₃ is a sensitive indicator of total body vitamin D status of an individual as it accounts for most of the circulating vitamin D [3]. Approximately > 95% of circulating vitamin D exists as the 25-hydroxyvitamin D₃. The 25-hydroxyvitamin D₃ provides a good estimate of dietary and skin related vitamin D supply. Recently, the 25-hydroxyvitamin D₃ has taken much attention in the disease process. A previous study reported the 36% vitamin D₃ deficiency in asymptomatic adults and 57% in adults presenting at the medical outpatient departments in the USA [4]. In the USA, Vitamin D₃ deficiency is now recognized as an “epidemic” [5]. European population have similar prevalence of vitamin 25-hydroxyvitamin D₃ deficiency. A previous report from Australia reported 1 out of 3 showed the vitamin D₃ deficiency [6]. Previous studies from Pakistan have reported high prevalence of vitamin D₃ deficiency [7-9]. The problem of vitamin D₃ deficiency has reached an epidemic in the country and exact epidemiological data is lacking, but reported studies have found high prevalence [7-9]. The health problem of vitamin D₃ is highly overlooked by the health authorities. The problem of vitamin D₃ deficiency is further aggravated by lack of knowledge of public and poor awareness of the mass to handle the how to maintain required level of vitamin D₃ in the body [7-9]. Association of vitamin D₃ deficiency has been reported with disease process such as
the atherosclerosis [10], obesity [11], diabetes [12], hypertension [13, 14], myocardial infarction [15], and brain stroke [16]. Vitamin D₃ deficiency has been linked to the dyslipidemia which is an independent risk of atherosclerosis related cardiovascular and cerebrovascular disease [17, 18]. Few of recent studies [19, 20] have reported the association of vitamin D₃ deficiency and the dyslipidemia. The present study was conducted to estimate the burden of 25-hydroxyvitamin D₃ deficiency and association with dyslipidemia in type 2 diabetic subjects as very limited data are available in the country.

2. Subjects and Methods

A prospective case control study was conducted at the Department of Medicine, Isra University, and Hyderabad from January-July 2014. A sample of 310 subjects was selected through non-probability purposive sampling according to well delineated inclusion and exclusion criteria. Volunteer diagnosed type 2 diabetics of 20-50 years were included in the study protocol. Diabetics with renal failure, taking vitamin D₃ supplements and diuretic drug intake were excluded.

2.1. Vitamin D Levels

The normal, insufficiency and deficiency of Vitamin D₃ were defined as; normal levels (> 30ng/dl), vitamin D₃ insufficiency (20-30ng/dl) and vitamin D₃ deficiency (< 20ng/dl).

2.2. Systemic Hypertension

Hypertension was defined (Joint National Committee VII) as a systolic blood pressure > 140mmHg and/or a diastolic blood pressure > 90mmHg based on the average of 2 blood pressure measurements or a patient’s self-reported history of hypertension or antihypertensive use. Patients with fasting plasma sugar more than 110mg/dL were considered as diabetic [21].

2.3. Dyslipidemia

Dyslipidemia was defined (ATP III) as one or more of the following: total cholesterol more than 200mg/dL, low density lipoprotein-cholesterol (LDL-C) more than 130mg/dL, high-density lipoprotein-cholesterol (HDL-C) below 40mg/dL, very low density lipoprotein-cholesterol (VLDL-C) more than 30mg/dL, and triglycerides more than 150mg/dL.

2.4. Lipid Estimation

The serum which was obtained was pipetted into a clean blood sample bottle and analyzed on the day of collection for serum sugar and lipid profile tests. Serum total cholesterol was determined by an enzymatic (CHOD-PAP) colorimetric method and triglycerides were determined by an enzymatic (GPO-PAP) method. HDL-Cholesterol was estimated by a precipitant method and LDL-Cholesterol was estimated by using Friedewald’s formula as; LDL-C = TC - HDL-C – (TG/5).

2.5. Glucose Estimation

Serum glucose was determined by using the glucose oxidase enzymatic method [22].

2.6. Vitamin D Estimation

The blood was centrifuged at 4000rpm for ten minutes and serum obtained was frozen at -20°C. The serum was used for estimation 25-hydroxyvitamin D₃. The vitamin D₃ was measured by ARCHITECT 1000 system for estimation of 25-OH-D₃ tem from blood sera. Alcoholics were defined as those in whom the alcohol consumption was > 50 g/day (equivalent to 500mL [2 drinks] of wine, 1000mLof beer, or more than 5 drinks [units] of spirits) [23]. Body mass index (BMI) values more than 30 kg/m² were considered as obese [24]. Smokers were defined as those reporting daily smoking. Non-smokers were defined as occasional smokers and ex-smokers [25]. Five ml of fasting venous blood sample was drawn from ante-cubital vein. Informed written consent was taken from the participants. Study was approved by the ethics committee of the institute. The data was recorded on a pre-structured proforma.

2.7. Statistical Analysis

Data was analyzed on SPSS version 22.0. Continuous and categorical variables were analyzed by student’s t test and chi square test respectively. The associations of various lipid fractions with vitamin D₃ were analyzed by Pearson’s correlation. The significant p-value was taken at ≤ 0.05.

3. Results

The present study was conducted at the Department of Medicine, Isra University Hyderabad, Sindh, Pakistan. A sample of 310 type 2 diabetics adults were selected and studied for 25-hydroxyvitamine D₃, dyslipidemia and their association. The demographic baseline characteristics of study population are shown in table 1. Mean ± S. D age of study subjects noted was 49 ± 9.7years. The mean ± S. D of 25-hydroxyvitamin D₃ was found at 31.20 ± 8.30 ng/dl (CI 24.90-39.41). The mean ± S. D values of normal, insufficient and deficient vitamin D₃ subjects are shown in table 2 (p<0.001). Overall reduced 25-hydroxyvitamin D₃ was observed in 84.1% (n = 261). The 25-hydroxyvitamin D₃ levels < 6 ng/dl were observed in the present study. The lipid profile status of subjects with normal and reduced 25-hydroxyvitamin D₃ showed significant differences as shown in table 3. Odds ratio analysis of 25-hydroxyvitamin D₃ with risk factors dyslipidemia, diabetes mellitus and hypertension are shown in table 4. Pearson’s correlation showed strong association of triglyceride, total cholesterol, LDLc, VLDLc and low HDLc with 25-hydroxyvitamin D₃ deficiency, when compared to subjects with normal 25-hydroxyvitamin D₃ levels. Triglycerides, LDLc, VLDLc, and Cholesterol
exhibited an inverse correlation between 25 hydroxyvitamin D$_3$; however positive correlation was noted for HDL cholesterol. The correlation co-efficient and p-value are shown in table 5.

### Table 1. Demographic characteristics of type 2 diabetic study subjects (n = 310).

<table>
<thead>
<tr>
<th>Age</th>
<th>49 ± 9.7 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>197 (63.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>113 (36.4%)</td>
</tr>
<tr>
<td>Rural population</td>
<td>118 (38%)</td>
</tr>
<tr>
<td>Urban population</td>
<td>192 (61.9%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>147 (47.4%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>231 (74.5%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>215 (69.3%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>119 (38.3%)</td>
</tr>
<tr>
<td>Postprandial blood glucose (mg/dl)</td>
<td>253 ± 61.5</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>17 (5.4%)</td>
</tr>
<tr>
<td>Serum creatinine(mg/dl)</td>
<td>215 (69.3%)</td>
</tr>
</tbody>
</table>

### Table 2. 25-hydroxyvitamin D$_3$ in type 2 diabetic subjects (n = 310).

<table>
<thead>
<tr>
<th>Normal levels (&gt;30ng/dl)</th>
<th>Insufficiency (20-30 ng/dl)</th>
<th>Deficiency (&lt;20ng/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (%)</td>
<td>49 (15.8%)</td>
<td>60 (19.3%)</td>
<td>201 (64.8%)</td>
</tr>
<tr>
<td>Mean ± S. D</td>
<td>35.5 ± 0.9</td>
<td>25.17 ± 1.7</td>
<td>13.8 ± 5.7</td>
</tr>
</tbody>
</table>

### Table 3. Lipid profile of type 2 diabetic subjects (n = 310).

<table>
<thead>
<tr>
<th>Triglycerides (mg/dl)</th>
<th>132.9 ± 45.7</th>
<th>214.1 ± 110.7</th>
<th>0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>158.3 ± 25.9</td>
<td>201.1 ± 44.9</td>
<td>0.001</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>39.9 ± 8.5</td>
<td>33.5 ± 9.47</td>
<td>0.035</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>96.3 ± 19.6</td>
<td>116.6 ± 39.3</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>41 ± 14</td>
<td>27 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>Alkaline phosphatase (iu)</td>
<td>97.9 ± 11.9</td>
<td>119.7 ± 7.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>8.8 ± 1.9</td>
<td>7.1 ± 1.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dl)</td>
<td>2.6 ± 0.9</td>
<td>2.4 ± 0.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Postprandial blood glucose (mg/dl)</td>
<td>217.7 ± 45.9</td>
<td>251.9 ± 31.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Table 4. Odds ratio analysis of 25-hydroxyvitamin D$_3$ and risk factors.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR⃰ 95%CI</td>
<td>OR⃰ 95%CI</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>2.5 1.4-5.1</td>
<td>1.2-3.5</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.4 0.7-2.6</td>
<td>0.4-1.2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.3 0.6-2.8</td>
<td>1.2-3.4</td>
</tr>
</tbody>
</table>

*Odds ratio

### Table 5. Correlation of 25-hydroxyvitamin D$_3$ with lipid fractions (n = 310).

| Triglycerides (mg/dl) | -0.36 | 0.03 |
| Total cholesterol (mg/dl) | -0.40 | 0.002|
| HDLc (mg/dl) | 0.045 | 0.001|
| LDLc (mg/dl) | -0.37 | 0.001|
| VLDL (mg/dl) | -0.29 | 0.001|

### 4. Discussion

The Present study demonstrated overall reduced 25-hydroxyvitamin D$_3$ in 84.1% in type 2 diabetes mellitus which are consistent with previous report from Pakistan which has reported 77.5% vitamin D deficiency and additional 18% of insufficiency, but are contrary to other previous reports [26-28]. The study showed that 87% pregnant women were having Vitamin D$_3$ deficiency, 10% were having Vitamin D$_3$ insufficiency while only 3% had normal levels [29]. Another study from Karachi in 305 premenopausal females, showed 90.1% vitamin D$_3$ deficiency [30]. There are many factors which contribute to the vitamin D$_3$ deficiency in general population worldwide. These factors include reduced exposure to sunlight, age-linked reduction in sunlight induced skin synthesis, and intake of food with a reduced vitamin D$_3$ level. Vitamin D$_3$ deficiency is a new global epidemic among both children and adults [31, 32]. There are some evidences which confirm the association of vitamin D$_3$ deficiency with high possibility of other morbidities such as diabetes mellitus, cardiovascular illness, and malignancy, particularly of the intestine and prostate [33, 34]. Present study observed no significant relationship of low 25-hydroxyvitamin D levels with alcoholism, smoking and hypertension. The mean serum glucose level was significantly higher in subjects with low 25-hydroxyvitamin D. The Cigolini et al [35] reported that subjects with low 25-hydroxyvitamin D were more significantly associated with type 2 diabetes compared to controls as well as higher levels of HbA1c. Previous studies [13, 35, 36] have also found association of hypo 25-hydroxyvitamin D with impaired fasting glucose, risk of type 2 diabetes mellitus, and hypertension. The present study showed a similar frequency of deficiency of 25-
hydroxyvitamin D₃ in both male and female genders. Similar findings have been reported from Pakistan [37] and India [26]. However, controversial results are also reported which have shown higher frequency of vitamin D₃ deficiency in female [38]. The mean alkaline phosphatase was elevated (119.7 ± 7.6) in diabetic subjects with low 25-hydroxyvitamin D₃ levels, this parallels to the fact that as vitamin D3 decreases, the bone resorption in increases. The findings match with previous studies [1, 26, 39]. The present study observed significantly elevated total cholesterol levels in 25-hydroxyvitamin D deficient type 2 diabetics compared to those with normal levels, the finding is consistent with previous studies [26, 40, 41]. Similar are the observations of HDLc being positively associated with deficient 25-hydroxyvitamin D₃, the HDLc reduces with reduction in blood 25-hydroxyvitamin D₃, the findings are similar to reported studies [41- 45]. An inverse association of 25-Hydroxyvitamin D₃ levels of < 20 ng/ml was observed for the LDLc and serum triglycerides, findings are similar to previous reported studies [45- 48]. This deficiency raises the question why we do not have sufficient vitamin D₃ from sunlight? or the normal values have to be re-considered. In fact these ranges have been established for western population. The lack of awareness regarding healthy balanced diet and the overcooking of food are a few other contributing factors to the prevailing vitamin D₃ deficiency. A national programme on the supplementation of vitamin D₃ and a public awareness campaign may urgently be launched.

5. Conclusion

The present study reports high frequency of vitamin D₃ deficiency in type 2 diabetic subjects and deficiency was independently associated with dyslipidemia. The association endangers that the 25-hydroxyvitamin D₃ deficiency may add to the vascular complications due to dyslipidemia and vitamin D supplements may prevent them.

References


