INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a group of metabolic disorders characterized by hyperglycaemia. Several different types of DM are caused by complex interactions of genetic and environmental factors. Depending on the etiology of DM, factors which contribute to hyperglycaemia include reduced insulin secretion, insulin resistance, and increased glucose production [1]. In 2017, International Diabetes Federation (IDF) estimated that 425 million people have diabetes which is expected to rise to 629 million by the year 2045. The greatest number of people with diabetes fall between 40 to 60 years of age. In India, alone about 73 million people are living with diabetes. So this places India second to China. At present in India, 8.8% population is diabetic and it is expected to rise to 10.2% (123 million) by the year 2035. It significantly increases the risk of cardiovascular diseases and stroke. Although insulin resistance and beta cell decompensation compose the well accepted basis of type 2 diabetes mellitus, the molecular mechanism underlying their development are incompletely understood [2]. Vitamin D3 supplementation to prevent Diabetes Mellitus has been explored. The primary route via which people obtain vitamin D3 is through exposure to ultraviolet B (UVB) rays of sunlight at wavelengths between 290-315 nm [3]. Recently, the extra skeletal effects of vitamin D3 have raised considerable interest. Vitamin D3 deficiency appears to be related to the development of diabetes mellitus type 2. Mild to moderate vitamin D insufficiency has been proposed as a risk factor for type 2 diabetes. Higher plasma vitamin D3 levels have been shown to be associated with a lower risk for the development of diabetes mellitus in high risk patients [4]. Several studies have demonstrated the role of vitamin D3 in the regulation of the endocrine function of pancreas especially the beta cells. Despite the fact that insulin secretion and action being the cause of diabetes, in routine clinical care, insulin is rarely measured, even though it can be measured easily on automated equipment in laboratories. Blood glucose control in diabetic patients is usually monitored by determination of HbA1c levels (glycated haemoglobin)
which is now the gold standard, and gives an estimate of the amount of glucose in the blood over the previous three months [5].

To the best of my knowledge, studies on the role of vitamin D3 deficiency in diabetics in Gujarat are few. Therefore, this study is undertaken. It aims to provide a comprehensive and comparative study of vitamin D3 and glycemic control parameters in patients of Type 2 DM. This would help to determine the correlation of HbA1c and Vitamin D3 in Type-2 Diabetes Mellitus patients.

MATERIALS AND METHODS

Study type, Study setting and Study Period

A hospital based case control study was carried out in Shri Krishna Hospital in Karamsad city of Gujarat from December 2014 to November 2015.

Participants recruitment procedure

100 participants in the study group and 100 participants in the control groups were enrolled by the following procedure.

Selection of study group

Persons aged 30 years or more who came to hospital for routine health check-up were checked for eligibility criteria. Routine health check-up scheme includes basic information, clinical history and laboratory investigations. (FPG, HbA1c, RFT, Vitamin D3 etc.)

Inclusion Criteria

Known and newly diagnosed Diabetic patients with FPG >126 mg/dl ,HbA1c > 6.5%, Serum Urea < 40 mg/dl , Serum Creatinine < 1.3mg/dl were included in the study.

Exclusion Criteria

Non Diabetic individuals, individuals on Vitamin D3/calcium Supplements and individuals with Liver or kidney diseases were excluded from the study.

Selection of control group

Inclusion Criteria

Age and sex matched normal healthy individuals with FPG <110 mg/dl, HbA1c< 6.5%, Serum Urea < 40 mg/dl, Serum Creatinine <1.3mg/dl.

Exclusion Criteria

Individuals on Vitamin D3/calcium Supplements and individuals with Liver or kidney diseases were excluded from the study.

Blood sample collection and processing

Samples were collected with an aseptic blood collection technique with the use of sterile gloves and disinfection of venepuncture site with 70% ethyl alcohol. All the samples were collected in sitting position. Blood samples were collected in three vacutainers: Plain bulb for Renal function tests and Vitamin D3, Sodium fluoride bulb for Fasting plasma glucose and EDTA tube for HbA1c. Samples were centrifuged at 1500 rpm for 15 minutes within one hour of collection to obtain serum/plasma. These were processed in the biochemistry laboratory for the estimation of FPG, HbA1c, Vitamin D, RFT.

Estimation of Plasma glucose

Plasma glucose was estimated by Hexokinase method in in fully automated Roche Cobas Integra 400 plus clinical chemistry analyser.

Estimation of glycated haemoglobin (HbA1c)

HbA1c was measured by Immunoassay Standardized according to IFCC method in fully automated Roche Cobas Integra 400 plus clinical chemistry analyser.

Vitamin D3 estimation

Vitamin D3 was measured by electrochemiluminescence (ECL) method in Roche Cobas E - 411 Immunoassay Analyser. Vitamin D3 level less than 50 nmol/Litre was considered as deficiency.

RFT was estimated in fully automated Roche Cobas Integra 400 plus clinical chemistry analyser.

Statistical analysis

Data were entered and analysed through Epi info 7. Categorical variables were expressed through percentages while continuous variables were expressed as mean and standard deviation. Spearman correlation coefficient were calculated to know the relation between correlation between Vitamin D3 levels and Glycated haemoglobin in cases of Type 2 Diabetes Mellitus. A p-value less than 0.05 was considered as statistically significant.
RESULTS

Table-1: Characteristics of the participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n=100)</th>
<th>Controls (n=100)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>55.09 ± 11.03</td>
<td>52.54 ± 9.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>42 (46.67%)</td>
<td>48 (53.33%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Male</td>
<td>58 (52.73%)</td>
<td>52 (47.27%)</td>
<td></td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>165.92 ± 45.86</td>
<td>94.63 ± 9.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.00 ± 1.58</td>
<td>5.65 ± 0.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D₃ (nmol/L)</td>
<td>55.82 ± 33.33</td>
<td>42.78 ± 27.21</td>
<td>0.02</td>
</tr>
<tr>
<td>&lt;50 (Deficiency)</td>
<td>54 (54%)</td>
<td>68 (68%)</td>
<td>0.059</td>
</tr>
<tr>
<td>≥50 (Sufficiency)</td>
<td>46 (46%)</td>
<td>32 (32%)</td>
<td></td>
</tr>
</tbody>
</table>

Table-1& Fig-1 shows that mean age among control groups was slightly lower than the mean age of the case group. Fasting plasma glucose and Glycosylated haemoglobin were significantly lower in control group than study group (p value <0.0001 in both). Mean Vitamin D₃ level were also significantly deficient in 54% of cases and 68% controls, which is statistically insignificant.

Table-2: Vitamin D₃ levels according to HbA1c levels in Cases with T2 DM

<table>
<thead>
<tr>
<th>Vitamin D₃ (nmol/L)</th>
<th>HbA1c &lt;7.5%</th>
<th>HbA1c &gt;7.5%</th>
<th>Total T2 DM Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 (Deficiency)</td>
<td>25</td>
<td>29</td>
<td>54</td>
</tr>
<tr>
<td>≥50 (Sufficiency)</td>
<td>26</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>TOTAL</td>
<td>51</td>
<td>49</td>
<td>100</td>
</tr>
</tbody>
</table>

Table-2 & Fig-2 shows Categorical comparison of vitamin D₃ deficiency and glycemic control having HbA1c < 7.5 % and > < 7.5 %. Bar Graph also suggests that the tallest bar is representing vitamin D levels less than 50 and HbA1c levels of more than 7.5 %. This also establishes that the correlation present between both these parameters is an inverse correlation. (p value 0.412).

Table-3: Correlation of vitamin D₃ with HbA1c

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pearson’s Correlation Coefficient (Vitamin D₃ WITH VIT-D₃)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c-Vitamin D₃</td>
<td>-0.2767</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table-3 shows Correlation of vitamin D₃ with HbA1c & Fig-3 shows scattered graph showing correlation of vitamin D₃ with HbA1c. There was significantly negative or inverse correlation between HbA1c level and vitamin D₃ level (correlation coefficient is -0.2767, p value 0.005) among study participants.

DISCUSSION

This study was a case control study, done over a period of one year from December 2014 to November 2015. It included 100 cases of Type 2 Diabetes Mellitus who had FBS and HbA1c levels >126 mg/dl and >6.5% respectively. All the controls had normal HbA1c (5.4-6.4%) and FBS (70-110mg/dl) levels. The RFT was normal in both the groups with S. creatinine (<1.3mg/dl) and S. urea (<40mg/dl). There was significantly negative or inverse correlation between HbA1c level and vitamin D₃ level (correlation coefficient is -0.2767, p value 0.005) among study participants. In present study, Vitamin D₃ deficiency was observed in 54% of cases and 68% of control. A study done by AK SV et al., found that Vitamin D deficiency was observed in 32% of cases and 25% of controls while Sheth et al., [9] observed Vitamin D₃ deficiency in 91.4% of cases of Type 2 Diabetes Mellitus and 93% in the control group. In a cross sectional Iranian study by Taheri and colleagues [10], the prevalence of vitamin D₃ deficiency was 83.3% in diabetic patients and 75.6% in healthy subjects. Another
cross sectional study among rural and urban adult Indians, Harinarayan et al., [11] also observed 44% and 62% for rural and urban men respectively and 70% and 75% deficiency for rural and urban women respectively. High deficiency in the present study participants may be due to life style, underlying health condition, poor exposure to sunlight, poor diet or increase age.

In our study, there was significantly negative correlation between HbA1c and vitamin D$_3$ level. Study done by Ahmadieh H et al., [12] and Giacomo Zoppini et al., [13] also showed negative association between vitamin D$_3$ level and glycemic control. While Sheth J et al., [9] studied that there was no association of serum Vitamin D$_3$ deficiency on HbA1c. Chiu et al., [14] found that vitamin D$_3$ deficiency was related to a higher risk for insulin resistance and the metabolic syndrome. There are several lines of evidence to support that vitamin D$_3$ influences impaired β-cell function, insulin resistance, and systematic inflammation [15]. It has been demonstrated that vitamin D$_3$ receptors exist in many tissues including pancreatic β-cells [16], allowing vitamin D$_3$ to potentially modulate the insulin response to elevated blood glucose.

CONCLUSION

The present study revealed that the patients with Type 2 DM have a deficiency of Vitamin D$_3$ levels but the controls also have deficient vitamin D$_3$ levels surprisingly which may be due to lack of exposure to sunlight. Vitamin D$_3$ levels are usually not estimated in routine practice along with other investigations for follow up in Type 2 DM patients as, they do not have any signs and symptoms of underlying deficiency, so it remains hidden and most of the times undiagnosed. Therefore, for the early diagnosis of Vitamin D$_3$ deficiency, its estimation is required in Type 2 Diabetes mellitus patients because vitamin D$_3$ levels have a protective role in diabetes mellitus type 2. Vitamin D$_3$ supplementation should be considered in patients with type 2 diabetes mellitus, it may help to improve the glycemic control. Vitamin D$_3$ fortified diabetic diets may be of additional help.

REFERENCES