ABSTRACTS

Introduction: Diabetes, metabolic disease, is increasingly common and characterized by inappropriate increases in blood glucose concentration due to inadequate insulin action in the body. About 170 million people are affected worldwide. About 90% of diabetic patients around the world have type 2 diabetes mellitus (T2DM) according to the World Health Organization. About 10% of the Indian population suffers from this disease. Vitamin D deficiency and diabetes mellitus are two common conditions. Vitamin D levels having been found to be inversely related to glycosylated hemoglobin levels in diabetes mellitus. Materials and Methods: 100 diagnosed patients were taken. Poorly controlled T2DM patients were selected randomly of either sex from age group 35 to 70 years. 50 age and sex matched apparently healthy subjects having normal glycated hemoglobin, were taken. The
study protocol was approved by Institutional Research Committee. Biochemical measurements were determined by standard procedures and the result was presented in mean ± standard deviation. **Results:** HbA1C levels were higher in the diabetes mellitus type 2 patients than in the control group. HbA1 levels are 8.1±1.09 % and 5.54±0.30 % in the patient and control groups, respectively. The diabetes patients had serum vitamin D levels 19.09 ± 5.34 and the control group had vitamin D levels 26.7 ± 3.46 ng/ml. **Conclusion:** High HbA1C concentrations are associated with a low concentration of serum vitamin D in type 2 diabetic patients independently of the duration of diabetes, diabetes treatment and nephropathy.

**KEYWORDS:** Type 2 Diabetes Mellitus, Vitamin D, Glycated hemoglobin, Insulin.

**INTRODUCTION**

Diabetes, metabolic disease, is increasingly common and characterized by inappropriate increases in blood glucose concentration due to inadequate insulin action in the body.\(^1\) It is the most common endocrine metabolic disorder, affecting about 170 million people worldwide and it has been estimated that 380 million individuals would be affected with diabetes worldwide by the year 2025. About 90% of diabetic patients around the world have type 2 diabetes mellitus (T2DM) according to the World Health Organization. The prevalence of Type 2 diabetes has been increasing nationally and worldwide. About 10% of the Indian population suffers from this disease.\(^2,3\) In India alone 41 million individuals are affected by this deadly disease, and this is likely to go up to 70 million by the year 2025.\(^4,5\) Vitamin D is a fat-soluble compound normally made in the skin when exposed to sunlight providing suitable UVB. In 2008, it was estimated approximately, 1 billion people worldwide suffer from vitamin D deficiency, which may result from limited exposure to sunlight, long-term wearing of covering clothes, use of sunscreen, age as well as low consumption of food containing ergocalciferol, and malabsorption syndrome.\(^6\)

**Vitamin D and type 2 diabetes mellitus**

Vitamin D deficiency and diabetes mellitus are two common conditions in the elderly population. Vitamin D deficiency is widely prevalent across all ages, races, geographical regions and socioeconomic strata.\(^1,7\) Vitamin D deficiency appears to predispose individuals to becoming type 2 diabetics, and there is evidence from observational studies that suggests an association between low levels of vitamin D and a risk of type 2 diabetes mellitus. Various prospective studies report inverse associations between baseline serum vitamin D and future
glycemia and insulin resistance.\textsuperscript{[1,8]} It has been reported that most patients with Type 2 diabetes mellitus (T2DM) have low vitamin D level. Accumulating the evidence from several studies, vitamin D is likely to have a role in T2DM and Hb-glycation. Vitamin D seems to affect glucose homeostasis, vitamin D levels having been found to be inversely related to glycosylated hemoglobin levels in diabetes mellitus. Nonetheless, the relationship between serum vitamin D with hemoglobin glycation (HbA1c) has not been extensively studied.\textsuperscript{[9,10]}

**MATERIALS AND METHODS**

The study was conducted in the Department of Biochemistry in collaboration of Department of Medicine, MM Institute of Medical Sciences and Research, MM University, Ambala Haryana during the years 2014–2015. 100 diagnosed patients attending OPD, were taken. Poorly controlled T2DM patients were selected randomly of either sex from age group 35 to 70 years. Among 100 patients, 53 were males and 47 females. 50 age and sex matched apparently healthy subjects having normal glycated hemoglobin, were taken. Among 50 control group 20 were male and 30 were female. The age group of the subjects was 35-70 years. Patients were classified as type 2 diabetics when the diagnosis had been made after 35 years of age, irrespective of treatment, or, irrespective of age of diagnosis. The study protocol was approved by Institutional Research Committee. The informed consent requirement for this study was exempted by the ethics committee. Venous blood was drawn.

Biochemical measurements were determined by standard procedures. The concentration of serum 25OH vitamin D, total (25OH vitamin D\textsubscript{2} and 25OH vitamin D\textsubscript{3}) was estimated by chemiluminescence (CLIA, Monobind USA) in a two-step procedure. The first step involved rapid extraction of vitamin D and second step involves competitive chemiluminescence immunoassay. Levels of HbA1c were measured by Ion-Exchange Resin method. Statistical evaluation of the results was performed using the statistical package IBM SPSS24. Student’s \textit{t}-test was used to compare the patient group with the control group. Regression analysis was performed to analyze the relationship between HbA1c and vitamin D levels.

**RESULTS**

Table: 1 Levels of Vitamin D and Gycated hemoglobin among control and case groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th>Cases</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (20)</td>
<td>Female (30)</td>
<td>Overall (50)</td>
<td>Male (53)</td>
<td>Female (47)</td>
<td>Overall (100)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.7±0.23</td>
<td>5.44±0.31</td>
<td>5.54±0.30</td>
<td>8.3±1.95</td>
<td>8.1±1.09</td>
<td>8.21±1.6</td>
</tr>
</tbody>
</table>
In this cohort of 100 type 2 diabetic patients, we found a significant inverse correlation between HbA1C and serum vitamin D ($r^2 = -0.928$, $p = 0.000$). HbA1C levels were higher in the diabetes mellitus type 2 patients than in the control group, HbA1 levels being 8.1±1.09 % and 5.54±0.30 % in the patient and control groups, respectively. The diabetes patients had lower serum vitamin D levels were lower than the control group, vitamin D levels being 19.09 ± 5.34 ng/ml and 26.7 ± 3.46 ng/ml in the patient and control group respectively (table 1).

Table: (2): Differential Distribution of HbA1c according to Vitamin D Status.

<table>
<thead>
<tr>
<th>Vitamin D (ng/ml)</th>
<th>No. of Cases</th>
<th>AGE (yrs.)</th>
<th>DURATION of DIABETES (yrs.)</th>
<th>HbA1C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>10</td>
<td>56.3</td>
<td>6.32</td>
<td>11.83</td>
</tr>
<tr>
<td>10 to &lt;20</td>
<td>26</td>
<td>58.61</td>
<td>5.50</td>
<td>8.98</td>
</tr>
<tr>
<td>20 to &lt;30</td>
<td>64</td>
<td>57.25</td>
<td>5.35</td>
<td>7.34</td>
</tr>
</tbody>
</table>

Table: (3): Differential Distribution of Vitamin D according to HbA1C.

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>No. of Cases</th>
<th>Age</th>
<th>Duration of DM</th>
<th>Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤9</td>
<td>76</td>
<td>57.55</td>
<td>5.41</td>
<td>21.75</td>
</tr>
<tr>
<td>&gt;9</td>
<td>24</td>
<td>57.5</td>
<td>5.72</td>
<td>10.69</td>
</tr>
</tbody>
</table>

In the group of type 2 diabetes mellitus, patients having HbA1C ≤9 had vitamin D 21.75 and HbA1C > 9 had vitamin D 10.69 ng/ml. Table 1 and 2 show poorly controlled type 2 DM patients have low level of serum vitamin D. Both vitamin D and glycated hemoglobin show inverse relation. Vitamin D level is also lower in case of comparatively prolonged history of type 2 DM compared to patients having shorter history but there was not significant correlation.

Table (4): Significance and correlation between HbA1c and Vitamin D.

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$r^2$</td>
<td>-0.073</td>
<td>-0.602</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td>0.761</td>
<td>0.000</td>
</tr>
<tr>
<td>Cases</td>
<td>$r^2$</td>
<td>-0.951</td>
<td>-0.889</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Correlation is highly significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).
The table 3 shows correlation between vitamin D and HbA1c in type 2 Diabetes Mellitus patients. There is significant correlation (<0.01) among male and female patients as well as female control group. There was no significant correlation among male control group. This may be due to comparatively smaller number of males.

Figure (1): Dispersion graph of the relation between serum vitamin D and glycated hemoglobin in 100 type 2 diabetic subjects

DISCUSSION

The increasing incidence of T2DM is taking a great toll of health resources. This has laid a number of research studies related to life style, environmental and nutritional factors in an attempt to ameliorate its burden. The diverse effect of vitamin D on glucose and calcium homeostasis has made it an ideal contender to know its role in glycemic control in T2DM. India being a vast tropical country geographically, it is expected that sufficient sunlight is received throughout the year. Regardless of this vitamin D deficiency has been observed more commonly in earlier studies from India.[11,12,13]

The present study has shown a higher incidence of vitamin D deficiency in overall recruited subjects indicating that both T2DM subjects and non-diabetic control subjects were deficient (table 1, 2 and 3) and an inverse relationship was observed between glycosylated hemoglobin levels and vitamin D (table 4 and figure 1), implying that vitamin D levels may affect glucose control in diabetes mellitus type 2. In addition, type 2 diabetics had statistically significant vitamin D deficiency than control. This is in accordance with other studies demonstrating low serum vitamin D levels in 70% to 100% populations across India.[12,13] This is likely to be due
to increased skin pigmentation, low exposure to direct sunlight, poor diet habit, obesity and malabsorption, as has been observed by several studies from India.\textsuperscript{9,12,13,14} However, an alternative and potentially interesting explanation could be that a poor chronic glycemic control directly affects vitamin D metabolism.\textsuperscript{15} A study carried out on rats with experimental diabetes found that the low levels of serum 25(OH)D found in diabetic animals could be attributed to a reduction of 25-hydroxylase activity in the liver.\textsuperscript{16} However, no direct evidence of a possible effect of hyperglycemia on 25-hydroxylase has been provided yet, an issue that only specifically designed studies can address.

Circulating low 25(OH) D is a risk factor for hyperglycemia, as assessed by HbA1c, in African American man.\textsuperscript{17} In a prospective study in high risk Asian subjects, 25(OH)D deficiency was associated with a higher risk for the development of type 2 diabetes mellitus. Vitamin D levels were found to be negatively correlated with glycosylated hemoglobin levels. The correlation persisted even after outliers were excluded.\textsuperscript{18} In diabetic patients, at various CKD stages, 25(OH) D levels were negatively correlated with HbA1c values. This association persisted after controlling for covariates such as age, gender, and erythrocyte metformin levels.\textsuperscript{19} In a nested case-control study conducted among 608 women with newly diagnosed type 2 diabetes, higher plasma 25(OH)D concentration was associated with lower risk of type 2 diabetes in women.\textsuperscript{18} Lower 25-hydroxyvitamin D (25[OH]D) levels in late pregnancy were associated with poorer glucose control in gestational diabetes mellitus (GDM).\textsuperscript{20} In addition, it has been suggested that adequate vitamin D intake may be related with a lower risk for the development of gestational diabetes mellitus.\textsuperscript{21} However, some studies showed no relation between vitamin D and glycated haemoglobin in type 2 DM. One of the earliest studies was carried out by Ljunghall et al. found that serum concentrations of vitamin D were not associated with a change in HbA1c; nevertheless, their subjects were at normal range of vitamin D. Jorde and Figenschau reported in a randomized, placebo-controlled study after 6-month, HbA1c levels were not significantly different from baseline values in patients with type 2 diabetes who had normal serum 25(OH) D levels.\textsuperscript{22, 23} Recent evidences have demonstrated that persons with type 2 diabetes who have hypovitaminosis D are more likely to have increased HbA1c compared with those persons with diabetes who do not.\textsuperscript{24,25} Recently, the relationship between vitamin D and diabetes mellitus type 2 has been debated. Vitamin D has been shown to be related to glucose metabolism and the development of diabetes mellitus type 2.\textsuperscript{18} Physiological mechanisms accounting for similarities and differences between our results and other studies include the possibility that interactions
between 25(OH) D and glucose metabolism may be bidirectional. Vitamin D is correlated with glycemic status in type 2 DM and has a role in pathogenesis of type 2 DM and its complications. Vitamin D deficiency is an important contributor to insulin resistance, which is a pathogenic mechanism of T2DM.\[^{26}\] Obesity may contribute to lower serum vitamin D levels by influencing vitamin D sequestration in subcutaneous and visceral fat. Obesity and fat intake may also influence 25(OH)D levels by changing vitamin D absorption and metabolism.\[^{27}\]

**CONCLUSION**

The recent study has several limitations. It is an hospital based observational study and therefore no conclusion can be made as far as any cause and effect relationship is concerned between vitamin D deficiency and diabetes mellitus type 2. In patients with diabetes mellitus type 2, normal levels of vitamin D in the blood may facilitate glucose control. In addition, in people with a tendency to develop diabetes mellitus type 2, optimal levels of vitamin D within the blood may retard the clinical development of diabetes mellitus type 2.

In conclusion, high HbA1C concentrations are associated with a low concentration of serum vitamin D in type 2 diabetic patients independently of the duration of diabetes, diabetes treatment and nephropathy. Future studies are needed to elucidate the biological relation between glycemic control and vitamin D metabolism in diabetes.

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**REFERENCES**


