A Study of Prevalence of Vitamin D Deficiency in New Onset Type 2 Diabetes Mellitus in a Tertiary Care Hospital of Eastern India

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Abstract

Background: Evidence suggests that vitamin D has an important role in the homeostasis of blood glucose and its deficiency may cause development of type 2 diabetes mellitus.

Aims of study: This study explores the prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus as well as the relationship of blood level of 25 hydroxy vitamin D [25(OH)D] with insulin resistance and insulin secretion.

Methodology: A cross-sectional study was conducted over a period of 18 months with 120 consecutive newly diagnosed type 2 diabetes patients as cases and 120 non-diabetic healthy subjects as controls. Based on the vitamin D status, cases and controls were divided into 4 groups: normal (> 30 ng/ml), insufficiency (20 - 30 ng/ml), deficiency (10 - 20 ng/ml) and severe deficiency (< 10 ng/ml). Insulin resistance and insulin secretion defect were calculated using HOMA-IR and HOMA-B, respectively. Correlations were calculated with Pearson correlation co-efficient and bivariate linear regression analysis.

Result: In this study, mean 25(OH)D level among cases and controls were 24.91 ± 14.58 ng/ml and 41 ± 28.33 ng/ml, respectively showing significantly lower level in cases (p < 0.001). 25(OH)D level was normal in 33 (27.5%), insufficient in 36 (30%), deficient in 36 (30%) and severely deficient in 15 (12.5%) cases. 25(OH)D level was normal in 55 (45.83%), insufficient in 35 (29.16%), deficient in 23 (19.16%) and severely deficient in 7 (5.83%) controls. On bivariate and linear regression analysis 25(OH)D had significant negative association with HOMA-IR and HOMA-B, more strongly with HOMA-B.

Conclusion: Vitamin D deficiency and insufficiency are more prevalent among new onset type 2 diabetes patients compared to healthy controls. Serum 25(OH)D is correlated with both insulin resistance and insulin secretion defect, but more with insulin resistance.

Key words: 25 hydroxy vitamin D, HOMA-IR, HOMA-B.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder, the prevalence of which is increasing steadily all over the world. It has been estimated that, by 2030, the global population of diabetes would have been 562 million1. Although the number of people with T2DM is increasing in every country, its major contribution is from developing countries, where it is fast becoming an epidemic. Due to the increasing global burden of T2DM, the pathophysiology of this disease is being explored with renewed interest. Insulin resistance and β-cell failure are the core pathophysiological defects of T2DM. It is primarily due to an interplay between genetic and environmental factors. Incidence of T2DM varies from one geographical region to another due to differences in lifestyle and risk factors. Apart from conventional environmental risk factors like obesity, physical inactivity, intake of high calorie food and stress, the role of certain nutritional factors in pathogenesis of T2DM is an emerging concept at present. Accumulating evidence from several cross-sectional studies suggests that vitamin D has an important role in the homeostasis of blood glucose, and its deficiency may cause development of T2DM.

Vitamin D, originally described merely as a vitamin, is indeed a misnomer as it is now well-established that its active form is a hormone which is not only involved in bone metabolism but also in a plethora of non-skeletal physiological processes. Several mechanisms have been proposed, indicating a positive effect of vitamin D on insulin secretion and sensitivity, which include its direct effect via activation of vitamin D receptor on pancreatic β-cells and insulin sensitive organs and indirect effect via regulation of calcium homeostasis2,3. In the largest epidemiological study from the NHANES population, a dose-dependent inverse relationship has been observed between vitamin D and type 2 DM with the highest number of metabolic syndrome patients having the lowest quartiles of vitamin D4.

The main source of vitamin D in humans is exposure to sunlight, natural diet and dietary supplements. Vitamin D from the skin and diet are metabolised in the liver to 25-

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hydroxy vitamin D (25 (OH)D) which is used to determine the patient’s vitamin D status. Although there is no definite consensus about the normal level of vitamin D, most experts define vitamin D deficiency as less than 20 ng/ml. A level of 20 - 29 ng/ml is considered to indicate a relative insufficiency of vitamin D and a level of 30 ng/ml or greater can be considered as sufficient. According to this definition, 1 billion people worldwide are suffering from vitamin D deficiency or insufficiency. Even in the sunniest countries including India, vitamin D deficiency is very common as most of the body surface is shielded from the sun.

India is a country where both T2DM and hypo-vitaminosis D are prevalent. But relatively scarce data is available observing the correlation between the two. This study explores the prevalence of vitamin D deficiency in T2DM patients presenting for the first time, as well as relationship of blood level of 25 (OH)D level with insulin resistance and insulin secretion.

Material and methods

It was a cross-sectional, retrospective, non-interventional study which included 240 individuals. The study population was patients attending Medicine out patients Department (MOPD), Diabetic OPD and admitted patients in the general medicine ward at BR Singh Hospital, Eastern Railway, Kolkata. The study was conducted over a period of 18 months from July 2012 to December 2013. 120 consecutive patients were selected on the basis of American Diabetic Association (ADA) guideline as follows:

HbA1C ≥ 6.5%

or

FPG ≥ 126 mg/dl (7.0 mmol/l)

or

2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during on OGTT

or

In patients with classical symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l).

Fasting was defined as no calorie intake for at least 8 hours. Oral glucose tolerance test (OGTT) was performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

All patients were fully assessed by thorough history, clinical and biochemical examination. Anthropometric measurement was taken with standard measuring tape and weighing machine as per guideline. Measurement of waist circumference (WC) and calculation of Body mass index (BMI) were done according to standard techniques and equipment. WC was measured at the midway point between the lowest rib and the iliac crest to the nearest 0.1 cm. Obesity was defined in terms of BMI and WC. Cut-off value of BMI to define obesity was > 25 kg/m² both for males and females. WC, to define obesity, was according to International Diabetes Federation (IDF) 2005 and National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATP III) 2002 for Asian population. Cut-off value of WC to define obesity in Asian context was > 90 cm for males and > 80 cm for females.

25(OH)D level was estimated by chemiluminescence immunoassay (CLIA). Normal serum 25(OH)D level was defined as 30 ng/ml or more. Vitamin D insufficiency state was defined at a level of 21 - 29 ng/ml. Vitamin D deficiency state was defined at a level of 20 ng/ml or less. Severe vitamin D deficiency was defined as < 10 ng/ml. Based on the vitamin D status, individuals in both new onset diabetes and control groups were divided into 4 groups: normal (> 30 ng/ml), insufficiency (20 - 30 ng/ml), deficiency (10 - 20 ng/ml) and severe deficiency (< 10 ng/ml).

Homeostatic model assessment (HOMA) was used to evaluate insulin resistance (HOMA-IR) and β-cell function (HOMA-%B) respectively using the following formula:

HOMA-IR = fasting plasma glucose (mmol/l) × fasting plasma insulin (µU/ml)

22.5

HOMA-%B = 20 × fasting plasma insulin / fasting plasma glucose - 3.5

Inclusion criteria of patients:

- Cases: Recently detected T2DM patients seeking medical help for the first time and age between 30 to 80 years.

- Controls: Healthy subjects who are age and sex matched with the case group and age between 30 to 80 years.

Exclusion criteria:

- Patients having T2DM of long duration
- Patients with T1DM
- Patient with impaired glucose tolerance
- Patient with impaired fasting glucose
- Gestational diabetes mellitus.
- Patients who developed diabetes secondary to other causes like pancreatitis, cystic fibrosis, haemochromatosis, endocrinopathies, drugs or chemical induced.
• Patients already on anti-diabetic therapy
• Unconscious and severely ill patients
• Patients having complications of diabetes
• Age below 30 or above 80 years.
• Patients who were mentally impaired and or unable to give consent were also excluded.

Data analysis: Statistical analysis was done using SPSS software (Version 16) and the Statistical tools in Microsoft Excel 2007. Continuous data were presented as means and standard deviation (SD) with 95% confidence intervals (CIs), Differences by groups were analysed with Student’s t-test for unpaired samples, or using the chi-square and Fisher exact test for dichotomous variables. Correlation was established by bivariate analysis and linear regression analysis using Pearson correlation coefficient and $r^2$ value. P value lower than 0.05 was considered significant.

Results

240 patients were enrolled, of which 120 consecutive patients having new onset T2DM were considered as cases and 120 consecutive healthy subjects without diabetes were considered as controls. Mean age of the study subjects (Mean ± SD) was 52.69 ± 8.12 years in cases and 51.46 ± 7.53 years in controls and hence both the groups are age-matched (p = 0.227). There were 66 (55%) females and 54 (45%) males in cases whereas 62 (51.66%) females and 58 (48.33%) males in controls. Mean BMI in cases and controls were 26.61 ± 4.32 kg/m$^2$ and 24.72 ± 4.16 kg/m$^2$ respectively which was significantly higher in cases (p < 0.001). Mean waist circumference (WC) in cases and controls were 86.85 ± 7.65 cms and 82.21 ± 7.06 cms respectively which was also significantly higher in cases (p < 0.001).

In our study mean 25(OH)D level in cases and controls were 24.91± 14.58 ng/ml and 41 ± 28.33 ng/ml, respectively showing significantly lower level in cases (p < 0.001). Taking a cut-off level of > 30 ng/ml as normal, the subjects with normal level of 25(OH)D among cases and controls were 33 (27.5%) and 55 (45.83%), respectively. We divided subjects with below normal level of 25(OH) vitamin D into 3 categories: insufficient (> 20 - 30 ng/ml), deficient (> 10 - 20 ng/ml) and severely deficient (< 10 ng/ml). Subjects having insufficient 25(OH)D level among cases and controls were 36 (30%) and 35 (29.16%), respectively. Subjects having deficient 25(OH)D among cases and controls were 36 (30%) and 23 (19.16%), respectively. Similarly, subjects having severely deficient 25(OH)D level among cases and controls were 15 (12.5%) and 7 (5.83%), respectively.

On bivariate and linear regression analysis 25(OH)VitD was negatively and significantly associated with insulin resistance (HOMA-IR) and HOMA-B with Pearson correlation coefficient of -0.825 and -0.514, respectively whereas $r^2$ values were 0.680 and 0.226, respectively. In our study, we found Vitamin D deficiency to be strongly and more significantly correlated with insulin resistance as compared with β-cell secretion defect (HOMA-B).

Discussion

Diabetes mellitus, as a metabolic disorder, is rising at an alarming rate all over the world and has been a reason of concern due to various complications associated with it. In India diabetes is definitely an enormous health problem as this country harbours highest number of diabetic patients in the world. In 2013, 65.1 million people in India between 29 to 79 years of age were diabetic and this number was predicted to increase to 109 million by 2035. The growing epidemic of T2DM in India has been highlighted in different studies. Economic drift and consequent change in lifestyle in India have led to this alarming increase in the
prevalence of diabetes. Vitamin D deficiency has been implicated as an established risk factor for T2DM now. Diurnal and seasonal variation of prevalence of T2DM support the fact.

We enrolled 120 newly detected T2DM patients as cases and 120 healthy non-diabetic subjects as controls. In our study we found that new onset diabetes is more prevalent in the age group between 40 to 60 years, which accounts for 79.1% of cases. Prevalence of diabetes in different age groups in the “Chennai urban rural epidemiology study (CURES)” also showed that maximum prevalence of new onset T2DM was between 40 to 60 years of age.

The National Health and Nutrition Examination Survey (NHANES) showed an inverse relationship between 25(OH) vitamin D level and T2DM. Similar inverse relationship has been observed in few other studies. One prospective cohort study and meta-analysis measured 25(OH) vitamin D3 in 9841 participants from the general population of whom 810 developed T2DM during 29 years of follow-up. Cumulative incidence of T2DM increased with decreasing concentrations of 25(OH) vitamin D. Multivariate adjusted hazard ratios for T2DM increased with decreasing concentrations of 25(OH) vitamin D. The National Health and Nutrition Examination Survey (NHANES) showed an inverse relationship between blood 25(OH) D level and T2DM. Similar inverse relationship has been observed in few other studies. One prospective cohort study and meta-analysis measured 25(OH) vitamin D3 in 9841 participants from the general population of whom 810 developed T2DM during 29 years of follow-up. Cumulative incidence of T2DM increased with decreasing concentrations of 25(OH) vitamin D. Multivariate adjusted hazard ratios for T2DM increased with decreasing concentrations of 25(OH) vitamin D by clinical categories and seasonally adjusted quartiles. Song et al conducted a meta-analysis to study the association between blood 25(OH)D levels and incident risk of T2DM. Overall, a total of 21 prospective studies involving 76,220 participants and 4,996 incident T2DM cases were included. The meta-analysis showed an inverse and significant association between circulating 25(OH)D levels and risk of T2DM across a broad range of blood 25(OH)D levels among diverse populations.

Various studies have ascertained that increased prevalence of T2DM in the vitamin D deficiency state is because vitamin D is essential for insulin secretion and glucose homeostasis. There is ample evidence suggesting a role of vitamin D in insulin secretion including presence of vitamin D receptor (VDR) on β-cells as well as vitamin D dependent calcium binding protein (DBP) in pancreatic tissue. 1,25 (OH)2 D3 is thought to be essential for insulin exocytosis by increasing the expression of calbindin-D28K in β-cells. Calbindin-D28K plays an integral role in regulating intracellular calcium levels in β-cells, thus facilitating insulin exocytosis, a calcium-dependent process. The identification of the VDR in the insulin receptor gene promoter has also helped to establish a role of 1,25 (OH)2 D3 in increasing insulin sensitivity by increasing insulin receptor gene expression. Furthermore, vitamin D supplementation has been shown to reduce inflammatory cytokines such as IL-6 and TNF-α, which play a significant role in inducing insulin resistance.

### Table I: Baseline and demographic characteristics.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (mean ± SD)</th>
<th>Controls (mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>52.69 ± 8.12</td>
<td>51.46 ± 7.53</td>
<td>.227</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.61 ± 4.32</td>
<td>24.72 ± 4.16</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>86.85 ± 7.65</td>
<td>82.21 ± 7.06</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>92.0 ± 6.70</td>
<td>87.57 ± 7.19</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>WHR</td>
<td>.93 ± .02</td>
<td>.93 ± .01</td>
<td>.017*</td>
</tr>
<tr>
<td>25-hydroxy Vit D (ng/ml)</td>
<td>24.91 ± 14.58</td>
<td>41 ± 28.33</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>HBAIC (%)</td>
<td>10.39 ± 2.45</td>
<td>5.55 ± 0.43</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>FSB (mg/dl)</td>
<td>188 ± 45.25</td>
<td>87.05 ± 7.66</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>47.9 ± 32.66</td>
<td>26.68 ± 11.66</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>176.27 ± 51.41</td>
<td>156.99 ± 47.56</td>
<td>.003*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>212.9 ± 107.39</td>
<td>142.74 ± 50.15</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>111.9 ± 28.63</td>
<td>89.73 ± 15.98</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>46.14 ± 8.48</td>
<td>50.04 ± 5.43</td>
<td>&lt; .001*</td>
</tr>
</tbody>
</table>

### Table II: 25(OH)D status among study groups.

<table>
<thead>
<tr>
<th>Study group</th>
<th>25(OH)D status (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severely Deficient (≤ 10) ng/ml</td>
<td>Number %</td>
</tr>
<tr>
<td>Cases</td>
<td>15</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
</tr>
</tbody>
</table>

Role of vitamin D in insulin secretion and insulin sensitivity and the effect of its deficiency in glucose homeostasis is best observed in drug-naïve patients. Hence the most suitable population to study the effect of vitamin D deficiency on insulin secretion defect and insulin resistance is either pre-diabetics or new onset diabetes. A study was done in Kolkata, among 157 pre-diabetes patients along with 42 diabetic patients and 28 normal individuals. Vitamin D deficiency was found in 73.25%, 66.6% and 78.57% individuals with pre-diabetes, diabetes and normal glucose tolerance, respectively. Severe vitamin D deficiency (< 10 ng/ml) was observed in 14.65% among pre-diabetes and 7.14% among both diabetes and normal glucose tolerance group. A similar study was done in North India with 102 new onset T2DM patients which showed that 25(OH)D level was lower (18.81 ± 15.18) in patients with T2DM as compared to healthy controls (28.46 ± 18.89) (p = 0.001). Taking a cut-off of 30 ng/ml, 81% of T2DM patients had either vitamin D deficiency or insufficiency, compared with 67% of healthy control subjects. Severe vitamin D
deficiency was seen in 16.2% of patients with diabetes and 2.5% of control subjects. A cross-sectional survey was done in a New Zealand Polynesian and Caucasian workforce of 5,677 aged 40 - 64 years to determine whether serum 25(OH)D was altered in newly diagnosed T2DM and IGT patients and it was found to be significantly lower in T2DM and IGT patients in comparison to controls (p = 0.0016) 23.

In our study, 25(OH) vitamin D insufficiency, deficiency and severe deficiency were found to be more prevalent among the new onset T2DM group, with the prevalence of 30% (n = 36), 30% (n = 36) and 12.5% (n = 15), respectively in comparison with control group with the prevalence of 29.16% (n = 33), 19.16% (n = 23) and 5.83% (n = 7), respectively. Prevalence of vitamin D insufficiency, deficiency and severe deficiency among both cases and controls was much less than those in earlier studies done in India but higher prevalence of vitamin D deficiency in cases than controls were comparable with other studies. Significantly lower level of mean 25(OH)D in new onset diabetes group (24.91 ± 14) than control group (41 ± 28.33) was also comparable with observations in other studies.

On bivariate and linear regression analysis 25(OH)D was negatively and significantly associated with HOMA-IR and HOMA-B with Pearson correlation coefficient of -0.825 and -0.514, respectively whereas r² values were 0.680 and 0.226 respectively. In our study, we found Vitamin D deficiency was strongly and more significantly correlated with insulin resistance as compared with β-cell secretion defect. Also, among individuals with diabetes, those having severe vitamin D deficiency (< 10 ng/ml), had the worst insulin resistance (HOMA-IR/fasting insulin) as compared to those having higher levels, with an inverse correlation between vitamin-D status and insulin resistance.

In an earlier study done in Kolkata, a moderately strong inverse correlation was observed between serum 25(OH)D level and measures of insulin resistance and a positive correlation was observed between serum 25(OH)D and measures of insulin sensitivity 23. Relatively poor correlation of HOMA-B with Vitamin D3 deficiency in our study might be explained by the higher prevalence of obesity and insulin resistance in the study population as a contributory factor for diabetes rather than defect in insulin secretion.

Strength of the study

1. The study was done on patients with new onset T2DM and their clinical parameters were not modified with any anti diabetic medications, so the results were more accurate.
2. The study has measured all the biochemical parameters by modern and latest laboratory techniques so the accuracy of the measurements was unremarkable.

Limitation of study

1. It is a cross sectional study and not a prospective one.
2. As the study is cross-sectional, the effect of correction of vitamin D deficiency on insulin resistance and β-cell secretion defect could not be demonstrated. The beneficial effect of vitamin D supplementation in improving the insulin resistance and β-cell dysfunction (insulin secretion defect) though studied in several populations was not done in this study.

Conclusion

Vitamin D deficiency and insufficiency are more prevalent among new onset T2DM in eastern part of India compared to healthy controls. Serum 25(OH)D is correlated with both insulin resistance and insulin secretion defect but more with insulin resistance; hence its deficiency is a significant determinant for the development and worsening of glycaemic status among diabetic patients. Prospective studies are needed to evaluate whether supplementation of vitamin D and correction of its deficiency at onset of diabetes can prevent early worsening of glycaemia in newly detected T2DM.

References