Original Article

Pattern of 25 hydroxy vitamin D status in North Indian people with newly detected type 2 diabetes: A prospective case control study

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ABSTRACT

Background: Vitamin D deficiency (VDD) has been linked to impaired glucose tolerance and type 2 diabetes (T2D) in humans. The aim of the present study was to find the vitamin D status in newly detected T2D patients compared with healthy controls. **Materials and Methods:** One hundred and two, newly detected T2D patients and similar number of age, body mass index (BMI), and gender matched healthy controls without diabetes were studied. In addition to basic information, metabolic parameters and serum 25 hydroxy vitamin D (25HD) were measured in both the groups. **Results:** Overall 25HD, was lower (mean ± SD, 18.81 ± 15.18 ng/ml) in patients with T2D as compared to healthy controls (28.46 ± 18.89 ng/ml) ($P = 0.00$). Taking a cut of 30 ng/ml, 81% of T2D patients had either VDD or insufficiency compared to 67% of healthy control subjects. Severe VDD (25HD of < 5 ng/ml) was seen in 16.2% of patients with diabetes and 2.5% of control subjects. Levels of 25HD had a negative correlation with HbA1c, fasting plasma glucose. **Conclusions:** VDD is common in people with new onset T2D.

Key words: 25 hydroxy vitamin D, type 2 diabetes, vitamin D deficiency

Introduction

The association between type 1 diabetes (T1D) and vitamin D deficiency (VDD) is widely reported. Vitamin D treatment has been shown to improve and even prevent T1D in both human and animal models.[1] Animal studies has shown definite role of vitamin D in pancreatic β cell function and insulin sensitivity.[2,3] However, less is known regarding the association between vitamin D and type 2 diabetes (T2D) in humans. Several studies have demonstrated a link between vitamin D receptor (VDR) gene polymorphism and T2D.[4] Large number of cross sectional and longitudinal studies have reported inverse association between vitamin D status and T2D. Studies have also shown that replacement with vitamin D in deficient patients rectifies the abnormalities of impaired insulin secretion and glucose intolerance.[5,6] In patients with T2D or impaired glucose tolerance (IGT), vitamin D supplementation has reported inconsistent results in presence of normal vitamin D status. Studies from India have also reported hypovitaminosis D in a significant number of people with T1D and in youth onset both in T1D and T2D.[7,8] In view of scarce data on vitamin D status in T2D patients from India, the present study was undertaken to assess the vitamin D status in newly detected T2D patients.

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Materials and Methods

The present prospective case control study was conducted at tertiary care hospital in North India over a period of 2 years. Sample size was calculated on the basis of moderate effect size of 0.4 between the two groups with α of 5% and power of the study 1-β = 80%, the required sample size for each group comes around 100 each. We have recruited 102 new onset T2D patients and an equal

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number of age, gender and body mass index (BMI) matched healthy control people. People with age more than 25 years were recruited and were classified as new onset T2D if they had fasting plasma glucose (FPG) ≥126 mg/dl or frank osmotic symptoms with random blood glucose (RBG) ≥200 mg/dl or 2 hour blood glucose ≥200 mg/dl on oral glucose tolerance test (OGTT) without ketoacidosis and had diabetes mellitus (DM) of less than 6 months duration. Controls included healthy adult men and women accompanying the patients and were without any illness in which diabetes was ruled out with appropriate testing. An informed consent was obtained from all the cases and controls after explaining in local language. The study was approved by institutional review board.

A detailed history focusing on presentation of DM (osmotic symptoms, ketoacidosis or others), family history of diabetes, occupation, socioeconomic status, drug intake, history of any systemic illness, and menstrual and obstetric history in women was noted. Direct sunlight exposure was assessed by average daily duration of exposure and percentage of body surface area exposed.[9] Nutritional status of the patients and controls was assessed by a trained dietician based on the average composition of their daily diet in terms of energy, carbohydrate, protein, fat and calcium using a semi-quantitative food frequency questionnaire[10,11] and published data on the nutrient composition of Indian food.[12]

Laboratory investigations

After an overnight fast of at least 8 hours, a non- heparinized venous blood sample was taken for analysis of glucose, lipids, creatinine, calcium, phosphorus, and alkaline phosphatase. Serum was separated within 2 hours of venipuncture and analysis was done within 24 hours. Biochemical parameters were analyzed with commercially available enzymatic reagents (Audit Diagnostics, Ireland) adapted to Hitachi 912 auto analyser. HbA1c was measured with high performance liquid chromatography (HPLC) standardized to the DCCT assay (reference range, 4-6%).

Measurements of plasma 25 hydroxyvitamin D (25HD)

After the patients had been at rest for at least 10 min in the supine position, blood sample was collected in a 5 ml evacuated glass tube. The blood was allowed to clot at room temperature (15-25^{0}C) and centrifuged for 15 minutes to obtain hemolysis free serum. The serum was then collected into separate plastic tubes and stored at -20^{0}C. After rapid extraction from serum with acetonitrile; 25HD was estimated by radioimmunoassay (RIA) procedure. The intra- and inter assay coefficients of variation ranged between 11.7-12.5% and 9.4-11.0%, respectively. Samples were collected throughout the years of the study duration with equal representations in winter and summer.

The criteria used to define vitamin D status

Vitamin D status in our patients and controls was defined as follows: Sufficiency (25HD levels, 30-100 ng/ml), insufficiency (25HD 20-30 ng/ml), and deficiency (25HD <20 ng/ml).[13] Vitamin D deficient subjects were also graded as mild (10-20 ng/ml, moderate (5-10 ng/ml), and severe (<5 ng/ml) as recommended by Lips.[14] Serum levels of parathyroid hormone were not estimated.

Statistical methods

We performed statistical analysis using SPSS for window (version 16). Results were expressed as mean ± SD. An independent sample *t* test was used to compare mean ± SD values between cases and controls. Pearson correlation coefficient was computed between vitamin D and other parameters and then partial correlation was analyzed between vitamin D and other parameters. A *P* value of less than 0.05 was taken as significant. The strength of association between diabetes and VDD was studied by using chi square test. Analysis of covariance was used to study the vitamin D status in people with T2D and those without T2D, after adjusting for confounding factors like age, sex, and BMI.

Results

A total of 102 new onset T2D patients and equal number of healthy controls were studied. Both cases and controls were comparable in age, gender and BMI [Table 1]. Analysis of the data revealed that serum level of 25HD had a significant negative correlation with FPG and HbA1C and positive correlation with 24 hour dietary calcium intake, all the subsequent analysis was carried after adjusting for these

Table 1: Clinical and biochemical parameters of cases and healthy controls

Parameter	Cases	Controls	*P* value
Age (year)	45.95±7.56	45.79±6.17	0.871
BMI (kg/m^2)	24.35±3.72	24.31±3.02	0.925
Calcium intake (mg/day)	972.62±299.43	1452.13±390.72	0.00
Sunlight exposure (%)	13.45±5.49	13.44±5.55	0.99
FPG (mgs/dl)	190.9±36	82.86±0.5	0.000
HbA1c (%)	8.87±1.87	5.30±0.42	0.000
Vitamin D (ng/ml)	18.81±15.18	28.46±18.89	0.000
VD sufficiency (%)	18.6	33.3	0.002
VD insufficiency (%)	14.7	28.4	0.002
VD deficiency (%)	66.7	38.2	0.002
Mild VDD (%)	38.2	27.5	0.030
Moderate VDD (%)	45.6	70.0	0.525
Severe VDD (%)	16.2	2.5	0.525

Values are in mean±SD unless indicated. FPG: Fasting plasma glucose; VD: Vitamin D; VDD: Vitamin D deficiency

three factors. Taking a 25HD cut-off of 30 ng/ml, 81% of T2D patients were VDD or insufficient, while around 67% of healthy control subjects also were either deficient of insufficient. Severe VDD (25 HD <5 ng/ml) was seen in 16.2% of patients and 2.5% of control subjects [Table 1]. The main finding of the study was a significantly lower 25HD in people with diabetes compared with healthy controls. Overall plasma 25HD (mean ± SD) was 18.81 ± 15.18 ng/ml in patients with diabetes compared to 28.46 ± 18.89 ng/ml in healthy controls (P = 0.00). The difference in vitamin D status persisted even after adjusting for confounding factors like age, gender, and BMI (P = <0.001).

In present study, while HbA1c and FPG of T2D patients was significantly higher, dietary calcium intake was significantly lower compared with control subjects (P = 0.00), at the same time there was no statistically significant difference between percentage sunlight exposures between patients and healthy controls [Table 1]. There was no statistically significant difference in age, gender, BMI, HbA1C, serum calcium, phosphorus and alkaline phosphatase in diabetes patients across different severity of VDD [Table 2]. There was no correlation between 25HD level and other clinical and biochemical parameters after adjusting for FPG, HbA1c, and calcium either in patients with T2D or healthy controls [Table 3]. People with T2D had 2.18 times more chance of VDD as compared with non diabetes population (CI = 1.14-4.16; P = 0.017).

Discussion

Aim of present study was to determine vitamin D status among new onset T2D patients compared with healthy controls without diabetes.

In the present study, mean ± SD 25HD level was significantly lower (18.81 ± 15.18 ng/ml) in diabetes patients compared to 28.46 ± 18.89 ng/ml in healthy controls P = <0.000, similarly, prevalence of severe VDD was also significantly more in patients with T2D compared with healthy controls (16.2% in T2D patients and 2.5% in healthy controls). Many similar studies have demonstrated significantly lower 25HD in people with T2D. In one study, increased prevalence of hypovitaminosis D was found in T2D patients after adjusting for age and gender.[15] In another similar study, both patients with IGT and diabetes had significantly lower 25HD.[16] In a recently published study from Kashmir, VDD was found in 91% of the patients with diabetes and 58% of the healthy controls in the age group of less than 25 years. Mean ± SD 25HD was significantly low, 7.88 ± 1.20 ng/ml in people with diabetes against 16.63 ± 7.82 ng/ml in healthy controls.[8] In a Korean study, mean ± SD concentration of 25HD in T2D patients was 12.90 ± 0.4 ng/ml while that in controls was15.40 ± 0.5 ng/ml.[17] Similar results are reported in many other studies also.[18,19] In contrast, one study in Japanese population having as high as 70% prevalence of VDD, has not shown any difference in 25HD between patients with diabetes compared to normal population.[20] Two reports from United States revealed higher mean value of 25HD in T2D patients as well as in controls.[21,22] These differing reports suggest that, 25HD levels in patients with T2D patients vary widely according to ethnicity or some other unknown reasons. Vitamin D may affect the risk of T2D for unclear reasons. Both insulin resistance and pancreatic beta cell dysfunction have been reported in people with vitamin D insufficiency.[23-26] In a cross sectional data, positive association has been demonstrated between 25HD and glucose induced insulin secretion in east London Asians at risk of T2D.[27] Similarly an inverse association of insulin resistance with 25HD has been demonstrated in third national health and nutrition

Table 2: Comparison of clinical and biochemical parameters as per severity of vitamin D deficiency

Parameter	Mild (26)	Moderate (31)	Severe (11)	*P* value
Age	47.00±8.96	47.10±6.56	46.36±7.58	0.96
Sex (M/F)	15/11	12/19	5/6	0.36
BMI (kg/m²)	24.49±3.53	23.92±4.14	23.12±3.61	0.57
HbA1c (%)	8.60±2.01	9.29±1.94	8.90±2.45	0.46
Calcium (mgs/dl)	9.56±0.48	9.72±0.52	9.52±0.72	0.49
Phosphorus (mgs/dl)	3.86±0.40	3.37±0.55	3.25±0.58	0.10
ALP (U/L)	246.03±79.34	278.92±100.74	257.27±42.58	0.34
Vitamin D (ng/ml)	14.95±3.06	7.66±1.39	3.56±1.04	0.00

Values are in mean±SD unless indicated. BMI: Body mass index; ALP: Alkaline phosphatase. M/F: Male/Female

Table 3: Correlation of vitamin D with clinical and biochemical parameters

Parameter	Pearson correlation *r*	Pearson correlation *P*
Age	0.06	0.34#
Sex	0.05	0.41#
BMI	0.02	0.76#
Skin colour	−0.05	0.41#
Sunlight exposure	0.04	0.49#
Urea	−0.01	0.88#
Creatinine	0.01	0.79#
Bilirubin	0.06	0.36#
AST	−0.07	0.26#
ALT	−0.03	0.58#
ALP	0.01	0.82#
TP	0.00	0.94#
Albumin	−0.03	0.60#
Calcium	0.12	0.86#
Phosphorus	−0.05	0.46#

#Adjusted for FPG (fasting plasma glucose), HbA1c (glycosylated haemoglobin), calcium intake and body mass index (BMI); TP: Total proteins, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

examination survey.[23] Significant inverse association has also been reported between 25HD and glucose induced insulin secretion in elderly Dutch men and hyperglycemic clamp induced insulin response in subjects of various ethnic backgrounds.[25,28] However, in one study no association was found between 25HD and meal induced insulin secretion in men with T2D.[29] Table 4 summarizes important studies on vitamin D status and T2D.

In present study, 25HD had a significant negative correlation with FPG and HbA1c. Similar findings have been reported previously. In one Korean study, HbA1c was significantly higher in T2D patients than that of controls and had a significant correlation with 25HD.[17] In the present study, dietary calcium intake was significantly higher in healthy control subjects as compared to T2D patients, though one Italian study has shown lower dietary calcium intake inT2D patients as compared to controls.[30] Because the calcium intake was based on 24 hour recall, it is possible that after detection of diabetes, calcium containing foods would also be unknowingly restricted in them.

The direct clinical evidence of association of hypovitaminosis D and diabetes has come from interventional studies. Though we did not supplement any group with calcium or vitamin D, only few prospective studies have examined the predictive value of 25HD on future risk of type 2 diabetes mellitus.[19,31,32]

Limitations of the present study include a small sample size and cross sectional design. It would be worthwhile to see the effect of vitamin D supplementation on the onset of new diabetes in well-designed placebo controlled prospective studies.

Table 4: Summary of studies reviewed regarding Vitamin D deficiency and type 2 diabetes mellitus

Authors	Findings	Reference
Scragg R *et al.* (1995)	25HD significantly lower in newly detected diabetes and IGT compared with matched controls	[16]
Isaia G *et al.* (2001)	25HD concentration significantly lower in diabetic vs non-diabetic Patients	[30]
Cigolini (2006)	Mean 25HD was significantly less in diabetes compared with healthy Controls	[15]
Mattila C *et al.* (2007)	Inverse association between serum 25HDconcentration and incidence of T2D	[19]
Pittas AG *et al.* (2007)	25HD and calcium insufficiency negatively influence glycemia	[33]
Forouhi NG *et al.* (2008)	Baseline 25HD associated inversely with 10-year risk of hyperglycemia	[32]
Liu E *et al.* (2010)	Higher vitamin D status is associated with decreased risk of T2D	[18]
Yu JR *et al.* (2012)	Low 25HD in people with diabetes	[17]
Kos E *et al.* (2012)	No difference between users and nonusers of metformin in regard to 25HD levels when adjusted for variables	[21]
Pyne JF *et al.* (2012)	Patients with diabetes had lower 25HD levels than did those without diabetes	[22]

Conclusions

The aim of the current prospective study was to find the vitamin D status in newly detected T2D patients. Both T2D patients and healthy controls were vitamin D deficient. The mean serum 25HD was significantly lower in people with diabetes compared with controls. Levels of 25HD did not correlate with age, sex, and BMI. Whether vitamin D status in patients with T2D has a role in the pathogenesis of the disease needs to be seen in future studies.

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