

Study of vitamin d levels in patients with type 2 diabetes mellitus

Srujan JV.^{1*}, Prakash Rao S.²


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^{1*} J.V. Srujan, Assistant Professor, Department of General Medicine, NRI Institute of Medical Sciences, Sangivalasa, Andhra Pradesh, India.

² Surya Prakash Rao, Professor, Department of Medicine, NRI Institute of Medical Sciences, Sangivalasa, Andhra Pradesh, India.

Introduction: Diabetes is defined as a disturbance in intermediary metabolism manifesting as chronic sustained hyperglycemia, primarily due to either an absolute or a relative lack of insulin. Many epidemiological studies have demonstrated an inverse relationship between vitamin D levels and diabetes mellitus. However, there is a paucity of literature regarding the levels of vitamin D in type 2 diabetes, which is common in our community. This study was taken up to shed more light on this issue. **Material and methods:** This was a cross-sectional study conducted at NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam district. Cases of Type 2 Diabetes Mellitus attending to the outpatient department, diagnosed as per the ADA criteria of 2011, between the ages of 31 and 75 years constituted the material for the present study. **Results and conclusion:** Vitamin D levels were found to be significantly lower in the study group (19.91 ± 7.0 ng/ml) as compared to the control group (32.22 ± 4.0 ng/ml).

Keywords: Diabetes mellitus, Vitamin D, Vitamin D level

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Introduction

Diabetes is defined as a disturbance in intermediary metabolism manifesting as chronic sustained hyperglycemia, primarily due to either an absolute or a relative lack of insulin. Diabetes is a polygenic and multifactorial disease involving many pathways and mechanisms. There were an estimated 20 – 40 million people with diabetes in India in 2007.

It is projected that by the year 2025, there would be around 300 million diabetics worldwide and India would emerge as the diabetic capital of the world with around 60 million diabetics [1]. In recent years emphasis has been given to the role of vitamin D in areas beyond those traditionally known. Many epidemiological studies have demonstrated an inverse relationship between vitamin D levels and diabetes mellitus [2,3].

Studies in India have demonstrated that the level of vitamin D in the population is low and there is a high prevalence of chronic diseases like diabetes, hypertension, and cardiovascular disease. However, there is a paucity of literature regarding the levels of vitamin D in type 2 diabetes, which is common in our community.

Because of the above facts, the present study has been undertaken to determine the occurrence of hypovitaminosis D in Type 2 Diabetes Mellitus and to compare these with that of non-diabetic controls.

The present study was undertaken:

01. To study plasma 25-Hydroxycholecalciferol (Vitamin D3) levels in patients of type 2 diabetes and to compare it with age and sex-matched control subjects.
02. To study the correlation if any, with severity and duration of disease with vitamin D3 levels.

Material and Methods

Setting: This study was conducted in the outpatient department of the Department of General Medicine of NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam, Andhra Pradesh, India

Duration and type of study: This was a cross-sectional study, conducted over 2 months from September to October 2020.

Sampling Methods: People who met the inclusion and exclusion criteria were selected by simple random sampling

Inclusion criteria: Type 2 diabetic patients between age 31 - 75 years

Exclusion criteria:

01. Age less than 30 years or more 75 years
02. Patients with type 1 diabetes
03. Patients with co-morbid conditions likely to influence Vitamin D levels in the blood like Chronic kidney disease, Chronic Liver disease, Cardiovascular disease, Cerebrovascular disease, Patients taking drugs that affect vitamin D metabolism e.g. anti-epileptic drugs, steroids, rifampicin, ART, antacids, orlistat, statins.
04. Patients taking vitamin D or calcium supplements
05. Patients with hypertension
06. Patients not giving consent for the study

Data collection procedure:

Complete clinical history was taken and physical examination was performed on 60 patients.

All patients underwent the following investigations:

01. Fasting blood glucose level
02. A postprandial blood glucose level
03. Serum calcium
04. Serum creatinine
05. Urine for micro-albumin
06. Plasma 25-hydroxy Cholecalciferol
07. Serum Parathyroid hormone
08. Liver function tests including serum albumin
09. HbA1C

Ethical consideration: This study was conducted after obtaining due permission from the institutional ethics committee.

Statistical Analysis: Data was collected and tabulated using Microsoft Excel software. Descriptive and inferential statistical analysis was carried out. Results of continuous measurements are presented as Mean \pm SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at a 5 % level of significance. The relationship between two variables of the sample was analyzed using the Chi-Square test. Statistical analysis was done using the MedCalc statistical software.

Results

This was a cross-sectional study performed on 30 diabetic and non-diabetic controls. The Biochemistry Department of NRI Institute of Medical Sciences performed the vitamin D levels by using the Chemiluminescence Immunoassay (CLIA) method.

The findings are tabulated as follows.

Table-1: Age-sex distribution of the cases and controls.

x Control

Age (Years)	Case		Control	
	Male	Female	Male	Female
31- 40	3	2	7	5
41 - 50	8	4	5	2
51 - 60	9	2	11	0
61- 70	2	0	0	0
71 - 75	0	0	0	0
Subtotal	22	8	23	7
Total	30		30	

The baseline characteristics of the study and control population were similar. Out 30 of cases, 22 (73.4%) were males and 8 (26.6%) were females and out of 30 controls 23 (76.7%) were males and 7 (23.3%) were females (Table 1). The majority of the cases were from the 41 to 50 years age group followed by the 51 to 60 years age group and in the control group majority of the population were from the 31 to 40 years age group followed by the 51 to 60 years age group. There were 2 patients above 60 in the case group.

Table-2: Distribution according to the duration of diabetes.

Duration of Diabetes	Number of cases (%)
One Year or Less	11 (33.6)
2 - 5 Years	14 (46.6)
5 - 10 Years	4 (13.3)
More Than 10 Years	1 (3.3)
Total	30

Among the diabetics 11 (33.6%) of them were diagnosed to be diabetic less than or equal to a year from the time of the study, 14 (46.6%) were diagnosed between 2 to 5 years, 4 (13.3%) of them were diagnosed between 5 to 10 years and only 1 (3.3%) had long-standing diabetes of greater than 10 years (Table 2).

Type of drug	Patients (%)
OHA	19 (63.3)
Insulin only	7 (23.3)
Both	4 (13.3)

Total	30
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Table-3: Type of drugs used by the study population.

OHA - Oral Hypoglycemic Agents

BOTH - Using a combination of Insulin and Metformin

All of them were on anti-diabetic medications with 19 (63.3%) of them on OHA's, 7 (23.3%) patients on insulin only, and 4 (13.3%) patients were on a combination of both insulin and metformin. (Table 3).

Table-4: Compliance with Anti-Diabetic medications.

Compliance	Cases (%)
Good	16 (53.3)
Average	10 (33.3)
Poor	4 (14.3)
Total	30

Good compliance – does not miss drug doses

Average – miss one dose a week

Poor – miss more than one dose a week

Out of the 30 diabetics who were on treatment 16 (53.3%) of them were having good compliance and we're not missing any of their doses and 10 (33.3%) of them were missing 1 dose a week and 4 (14.3%) of them were missing more than 1 dose per week. (Table 4).

Table-5: Distribution according to a family history of diabetes.

History	Cases (%)	Controls (%)
Present	11 (36.6)	15 (50.0)
Absent	19 (73.4)	15 (50.0)
Total	30	30

Among the 30 cases, 11 (36.6%) of them had a family history of diabetes in their parents or siblings and among the 30 controls, 15 (50%) of them had a family history of diabetes. (Table 5).

Table-6: Type of diet.

Type of Diet	Cases (%)	Controls (%)
Vegetarian	3 (10.0)	4 (13.3)
Mixed	27 (90.0)	26 (86.7)
Total	30	30

The majority of cases and controls were eating mixed diet at 27 (90%) and 26 (86.7%) respectively and only 3 (10%) and 4 (13.3%) respectively were vegetarians (Table 6).

Table-7: Body mass index of the study population.

BMI	Cases (%)	Controls (%)
< 20.9	0 (0.0)	2 (6.6)
21 - 25.9	28 (93.4)	26 (86.6)
26 - 29.9	2 (6.6)	2 (6.6)
> 30	0 (0.0)	0 (0.0)
Total	30	30

The BMI of the case group was predominantly in the 21 to 25.9 ranges and in the control group also was in the same range (93.4 and 86.6% respectively). 2 subjects (6.6%) each in case group and control group were overweight, there were no obese subjects in both the groups and there were 2 (6.6%) underweight subjects in the control group (Table 7).

Table-8: Fasting blood glucose of cases.

FBS (mg/dl)	Cases (%)
80 - 110	0 (0.0)
111 - 125	1 (3.3)
126 - 140	18 (60.0)
141 - 160	5 (16.6)
161 - 180	2 (6.6)
181 - 200	3 (10.0)
> 200	1 (3.3)
Total	30

The fasting blood glucose in most of the patients (60%) was between 126 to 140 mg /dl. There were no patients with an FBS of <110 and only one patient with FBS > 200 (Table 8).

Table-9: Postprandial blood glucose levels of cases.

PPBG (mg/dl)	Cases (%)
140 - 159	1(3.3)
160 - 179	18 (60)
180 - 199	5 (16.6)
200 - 219	2 (6.6)
220 - 240	3 (10)
> 240	1 (3.3)
Total	30

The postprandial blood glucose of most of the patients (60%) was between 161 - 179 mg/dl. There were 5 (16.6%) patients with PPBS between 180 - 199 mg/dl and just one patient (3.3%) each with PPBS < 160 mg/dl and > 240mg/dl (Table 9).

Table-10: HbA1c levels of cases.

HbA1c %	Cases (%)
< 5.7 %	0

5.8 - 7%	8 (26.6)
7-9%	21 (70)
>9%	1 (3.4)

Among the patients, only 8 (26.6%) had excellent control of diabetes. (Table 10).

Table-11: Vitamin D status.

Vitamin D	Cases (%)	Controls (%)	Total (%)
Deficient (< 20 ng/ml)	15 (50.0)	0 (0.0)	15 (25.0)
Insufficient (21-30 ng/ml)	13 (43.3)	3	23 (38.3)
Normal (31-70 ng/ml)	2 (6.7)	20 (66.7)	22 (36.7)
Total	30	30	60

Vitamin D deficiency was seen in 15 (50%) of the cases and none of the controls. Vitamin D insufficiency was seen in 13 (43.3%) of the cases and 10 (33.3%) of controls and normal levels was seen in 2 (6.7%) of cases and 20 (66.7%) of controls. Vitamin D deficiency and insufficiency were seen in most of the diabetic cases. Among the controls none of the subjects showed deficiency, most of the subjects had normal levels of Vitamin D (Table 11).

Table-12: Vitamin D results.

Vitamin D	CASE	CONTROL	P- VALUE
Mean±SD	19.91±7.0	32.22±4.0	< 0.001
Median (IQR)	20 (15.3, 23.8)	31.6 (29.1, 35.6)	< 0.001

IQR - Inter Quartile Range

The mean vitamin D in cases was 19.91 ng/ml and the median vitamin D in cases was 20 ng/ml. The mean Vitamin D in controls was 32.22ng/ml and the median vitamin D in controls was 31.6ng/ml. The highest vitamin D level in cases was 37.56 ng/ml and the lowest vitamin D level in cases was 3.94 ng/ml. The highest vitamin D level amongst the controls was 39.41 ng/ml and the lowest level in controls was 24.54 ng/ml.

Table-13: Correlation between Vitamin D and parameters.

Characteristic	Correlation Co-Efficient (R)	P-Value
Age	-0.42	0.023*
BMI	-0.18	0.347
FBS	-0.37	0.045*
PPBS	-0.41	0.026*
Duration of diabetes	-0.39	0.032*
Diet	-0.09	0.243
Hba1c	-0.32	0.037*
Compliance to therapy	-0.16	0.126
Family history	-0.19	0.238

*Indicates statistically significant correlation

There was a significant inverse correlation between vitamin D and type 2 diabetes mellitus ($p < 0.001$) (Table 12).

The association between a variable and vitamin D was calculated using the Linear Regression and Correlation method. Age showed an inverse correlation with Vitamin D ($p = 0.023$), with increasing age there was a tendency towards lower levels of vitamin D in both the case and control groups. Fasting blood glucose increased with lower levels of vitamin D ($p = 0.045$). Postprandial blood glucose also increased with lower levels of vitamin D ($p = 0.026$). Vitamin D levels tended to be lower in diabetic cases with a longer duration of diabetes ($p = 0.032$). HbA1c correlated significantly and inversely with Vitamin D levels ($p = 0.037$).

In the present study, BMI did not show any significant correlation with diabetes and vitamin D ($p = 0.347$). Other characteristics and parameters like diet, compliance to therapy, family history of diabetes, also did not have any significant correlation with the levels of vitamin D (Table 13). All the patients had a normal physical examination. The renal and liver function tests of all the participants were within normal limits.

Discussion

These results are comparable to the studies done by Christiansen et al. 1982 [6], Stepan et al. [7] 1982, Nyomba et al. 1986 [8], Boucher et al. 1995 [9], Aksoy et al 2000 [10], Isaia et al. 2001 [11], and more recently Cigolini, 2006 [4] and Hypponen and Power in 2006 [5].

Table-14: Case-control studies reporting an inverse association between vitamin d status, and type 2 diabetes mellitus.

1st author, year	Sex	Age (mean/ range)	Case group	Control group	Main study results
Christiansen, 1982	M	36	Insulin-treated type 2 DM, n=26	Age-, sex-matched, n=14	25-OHD in type 2 DM vs. controls (17 vs. 22 ng/ml)
Stepan, 1982	M/F	40-70 years.	Sulfonylurea treated type 2 DM, n=22	Blood donors, n=30	25-OHD in type 2 DM vs. controls (9 vs. 14 ng/ml)
Nyomba, 1986	M/F	34-60 years.	Insulin-treated type 2 DM, n=20	Age and sex-matched, n=36	25-OHD in type 2 DM vs. controls (26 vs. 35 ng/ml)
Boucher, 1995	M/F	40-57 years.	IGT/type 2 DM, n=44	Age, sex, matched, n=15	25-OHD in IGT/type 2 DM vs. controls (28 vs. 30 ng/ml)
Aksoy, 2000	M/F	57 years.	Type 2 DM with retinopathy, n=66	Season-matched, n=20	25-OHD in type 2 DM vs. controls (12 vs. 24 ng/ml)
Isaia, 2001	F	NR	Type 2 DM, n=66	n=66	25-OHD in type 2 DM vs. controls (9 vs. 11 ng/ml)
Cigolini, 2006	M/F	61 years.	Type 2 DM, n=459	Age-, sex-matched, n=459	25-OHD in type 2 DM vs. controls (20 vs. 24 ng/ml)
Hypponen and Power, 2006	M/F	45	Type 2 DM, n=125	Sex and season- matched, n=7,073	25-OHD in type 2 DM vs. controls (15 vs. 21 ng/ml)
*Present study, 2020	M/F	31-64 years.	Type 2 DM, n =30	Age and sex matched, n = 30	25-OHD in type 2 DM vs. controls (19.91 vs. 32.22 ng/ml)

However, the mean vitamin D levels were substantially lower among both the cases and controls in studies done by Stepan et al. 1982 [7] (9 vs 14 ng/ml) and Isaia et al. 2001 [11] (9 vs 11 ng/ml), when compared to the present study (19.91 vs 32.22 ng/ml). This difference may be attributed to the older age of the subjects in the Stepan group, as increasing age has been associated with decreased vitamin D levels which was confirmed with the present study ($p = 0.023$).

On the other hand, the participants in the study conducted by Isaia in 2001 [11] were all females, and female sex has been inversely associated with vitamin D levels [12,13] which have also been suggested in the present study (Mean vitamin D levels in female cases 16.28 vs 21.24 ng/ml in males, and in female controls 46.3 vs 49.12 ng/ml in males). Albeit studies by Heath et al. 1979 [14] and Ishida et al., 1985 [15] failed to show this inverse correlation between type 2 diabetes and

Vitamin D. This variation in results may be ascribed to the larger number of young patients in these studies, whereas in the present study, the age of the study and control populations were in between 31 - 64 years (mean age 49 years.) and 32 - 60 years (mean age 46 years.) respectively, which is considerably higher.

Age showed an inverse correlation with Vitamin D levels (p = 0.023), with increasing age there was a tendency towards lower levels of vitamin D in both the case and control groups among both males and females. These findings are in line with studies conducted by T. Hagenau et al. 2009 [16].

Fasting and postprandial blood glucose also increased with lower levels of vitamin D with p values of 0.045 and 0.026 respectively, thereby suggesting that glycaemic control has an inverse correlation with vitamin D levels. These findings are in line with the results from a study done by A.A.Tahrani et al. [17]. This study also suggested an inverse relationship between HbA1c levels and vitamin D, with HbA1c higher in the subjects with vitamin D deficiency (< 20 ng/ml) (8.11±1.11% vs 7.33±1.32%, p = 0.046).

In linear regression analysis, vitamin D deficiency was independently related to HbA1c in patients with type 2 diabetes mellitus.

BMI did not show any significant correlation with diabetes and vitamin D in the present study (p = 0.347). Contrary to our results, a study by Laquova et al. 2009 [18], showed an inverse correlation between BMI and vitamin D levels. For both sexes and both age groups, (<50 and >=50 years.) there was a significant decrease in vitamin D levels with increasing BMI. The prevalence of vitamin D deficiency was highest in individuals with a BMI of > or = 40, being as high as 32% among women and 46% among men.

Other characteristics and parameters like diet, compliance to therapy, and family history of diabetes did not have any significant correlation with the levels of vitamin D (Table 13). However, studies by other authors against which to compare our results could not be found.

Some studies have reported an inverse association between vitamin D and the risk of development of Type 2 Diabetes Mellitus. These include the studies done by Scragg et al. 2004 [19] and Snijder et al. 2006 [20]

Table-15: Cross-sectional studies reporting an association between vitamin d status, and the development of type 2 diabetes.

1st author, year	Sex	Age (years)	Cohort	Outcome	Predictor	Main study results
Scragg, 2004	M/F	> 20	NHANES (n=2,766 non-Hispanic whites)	T2DM	25-OHD, <18 to >32 ng/ml	OR 1.00, 0.25 (0.11- 0.60)
	M/F	> 20	NHANES (n= 8,241)	T2DM	25-OHD, <19 to >38 ng/ml	OR 1.00, 0.17 (0.08 - 0.37)
Snijder, 2006	M/F	75 (mean)	n = 1235	T2DM	25-OHD, <10 to >30 ng/ml	OR 1.0, 1.23 (0.50 -3.02)

However, a study on vitamin D levels and prevalence of diabetes as well as the effect of vitamin D supplementation on glycaemic control are beyond the scope of the present study.

Advantages of the study

This study suggests that hypovitaminosis D is more prevalent among diabetic as compared to the non-diabetic population.

Further, the level of vitamin D showed an inverse correlation with glycaemic control. Larger studies may establish vitamin D deficiency as an independent risk factor for the development and high prevalence of Type 2 Diabetes Mellitus.

Limitations of the study

01. Small sample size.
02. Did not study the effects of Vitamin D supplementation in achieving better glycaemic control in diabetic patients.

Conclusion

This study suggests that hypovitaminosis D is more prevalent among diabetic as compared to the non-diabetic population. Further, the level of vitamin D showed an inverse correlation with glycaemic control. Larger studies may establish vitamin D deficiency as an independent risk factor for the development and high prevalence of Type 2 Diabetes Mellitus.

What does the study add to the existing knowledge?

The present work threw light on the role of Vitamin D in type 2 diabetes, with levels of vitamin D being significantly lower in the study population. Furthermore, vitamin D levels had shown an inverse correlation with the severity of diabetes.

A review of literature though meager, suggests the salutatory effects of vitamin D supplementation in reducing the risk of type 2 diabetes mellitus. Demonstrating the ability of vitamin D supplementation in preventing diabetes mellitus among at-risk individuals, as well as achieving better glycaemic control and preventing or limiting complications associated with this disease, would be of tremendous therapeutic importance.

Thus more studies involving a larger population of diabetic patients shall be rewarding.

Author's contribution

Dr. J.V. Srujan: Concept, study design, data collection, and manuscript preparation

Dr. Salla Surya Prakasa Rao: Statistical analysis

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