ORGINAL ARTICLE

# Assessment of Vitamin D3 Levels and Metabolic Profile in Pre-diabetic and Type 2 Diabetes Mellitus Patient of Kanpur City- A Cross Sectional Study

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## Abstract

**Objective:** It has been explored that Vitamin D play role in various non-skeletal disorders including Diabetes Mellitus. The present study was designed with the aim to assess association among control, pre-diabetic and diabetic with vitamin D and association between lipid profile and vitamin D.

**Materials and Methods:** A total of 109 subjects were recruited for the cross-sectional study including 37 as control, 41 pre-diabetic and 31 diabetic. A clinical examination was done for all the groups including fasting samples (12hrs) for lipid parameters, serum 25 (OH) vitamin D level and (HbA1/C).

**Results:** It was found that in control subjects 37.9% have the sufficient vitamin D3 level whereas 17.1% subjects in pre-diabetic, 16.6% in diabetic with good glycemic control and no subject was found to have sufficient vitamin D3 level in diabetic with poor glycemic control. The mean vitamin D3 levels was highest in control i.e. -26.53±11.99 ng/ml followed by 20.23±4.12ng/ml in pre-diabetics,19.07±8.01ng/ml in diabetics with good glycemic control and 12.92±6.77ng/ml in diabetics with poor glycemic control. HBA1/c and serum vitamin D3level share a significant association (*P*-value< 0.01).Total cholesterol (*P*-value< 0.0), serum triglyceride (*P*-value< 0.01), serum LDL cholesterol (*P*-value< 0.01) and serum VLDL (*P*-value< 0.01) had inverse association with vitamin D levels. HDL cholesterol has no effect with vitamin D.

**Conclusion:** The present study showed vitamin D3 deficiency as a risk factor for worsening glycemic control and dyslipidemia.

**Keywords**: Vitamin D3, Pre-diabetic, Diabetes mellitus, Dyslipidemia

## Introduction

During the past decade, the growing concerns of vitamin D insufficiency and its association with the extra skeletal effects has been reported widely (1). Recently, vitamin D deficiency is also found to be one of the responsible factors in the development of diabetes mellitus type 2 (2). Vitamin D deficiency has been associated with numerous health outcomes including increased risk of rickets in children or osteomalacia in adults, fracture, cancer, autoimmune diseases, and infectious diseases. Several studies



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demonstrated that vitamin D is associated with the glucose intolerance which in turn affects both insulin secretion and its sensitivity (3).

Epidemiological studies showed an association between low vitamin D and diabetic complications such as nephropathy, neuropathy & retinopathy (4). Prediabetics is a condition in which blood sugar level is higher than the normal, it is an intermediate stage between normal glucose tolerance and type 2 diabetes mellitus. Many studies have been discovered higher likelihood of development of prediabetics to diabetic among vitamin D deficient subjects (5). Diabetes mellitus (DM) is defined as metabolic disorder associated with insulin intolerance. In case of insulin deficiency condition of chronic hyperglycemia occurs, leading disturbances in the metabolism of carbohydrate, fat, and protein (6). Diabetes mainly causes blindness, kidney failure, heart attacks, stroke and lower limb amputation. Presently, around 285 million people have diabetes and this number is expected to reach 438 million by the year 2030 (7). In 2013, the prevalence of diabetes in individuals from age 20-79 ranged from 23 to 37% in the 10 countries with the highest prevalence. Diabetes prevalence has been rising more rapidly in middle and low- income countries (8).

Dyslipidemia in diabetes is characterized by elevated triglyceride level with decreased high density lipoprotein cholesterol (HDL-C) and with raised value of low density lipoprotein cholesterol (LDL-C) levels (9). Similarly, in pre-diabetic individuals having abnormalities in serum lipid profile is found to be correlated with obesity, hyperinsulinemia and glucose intolerance. In India, the prevalence of dyslipidemia was found to be very low and only few data are available mainly in South Indian population and some from North Indian population (10). The present study has been evaluated the association among the control, pre-diabetes and diabetes with serum 25 (OH) D further the correlation between lipid profile and vitamin D was evaluated in studied subjects.

## Materials and Methods

This hospital-based cross-sectional study was conducted in the department of Medicine, Lala Lajpat Rai and its Associated Hospitals in Kanpur during the period of January 2015 to September 2016. The subjects were recruited and classified as control, pre-diabetic and diabetic on the bases of HbA1C reference value <5.6%, 5.7-6.4% and ≥6.5%, respectively. Diabetic subjects were further classified as good glycemic control (GGC) and poor glycemic control (PGC) on the bases of HbA1C reference value ≤7.0% and >7.0%, respectively. Subjects who were taking medication that can alter vitamin-D metabolism and status or with acute illness or familial hyperlipidaemia were excluded. General and systematic examination including fundoscopy was performed in diabetic subjects to exclude the subjects with complicated diabetes.

A detailed questionnaire based interview was conducted to collect the information on demographic and disease profile, associated risk factors and medications used. Five ml peripheral blood sample was collected from all the subjects in the morning after 12 hours fast at the time of attending hospital. A written consent in the local language was taken from all the subjects after explaining the purpose of the study.

HPLC method was done for estimation of Glycosylated hemoglobin levels (HbA1/c). For serum total cholesterol– cholesterol esterase method, serum triglyceride(TC)– lipase method, HDL-C- cholesterol esterase method after precipitation using phosphotung state method, LDL-C- Fried Wald's formula (LDL-C= TC-[HDL-C+TG/5]), VLDL- Glucose oxidase: peroxidase method was used. Chemiluminescent immunoassay was used with automated instrument for estimation of 25-hydroxyvitamin D.

### Statistical analysis

The statistical analysis was done by using SPSS software version 2.2. One-way ANOVA was applied to compare the means among the



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groups. The association has been examined using Pearson's chi square test and bivariate analysis. *P*-value< 0.05 was statistically significant difference. Formula used for sample size –$N=[(Z_{\alpha/2}+Z_{\beta})^2 x\{2(6)^2\}]/\mu 1-\mu 2)^2$, Where, N=sample size required in each group, µ1= mean change in control group=27.5, µ2= mean change in prediabetics group=17.0, µ1-µ2= 10.5, ò= standard deviation=14.7, $Z_{\alpha/2=}$this represent on level of significance, for 5% i.e 1.96, $Z_{\beta=}$this represent on power, for 80% this is 0.84, based on the above formula, the minimum sample size required is 31 for each group. Hence for this study sample is too small. Total number of samples selected for the study is 109 as per availability.

## Ethical considerations

This study was ethically approved by the ethics committee of GSVM Medical College, Kanpur (Ref.no- 06/E.C. Meeting/ 2017).

## Results

A total of 109 subjects have been included in this study comprising 37 (33.9%) control, 41 (37.6%) pre-diabetic and 31 (28.5%) diabetic. The diabetic subjects were further classified as good glycemic control and poor glycemic control having 12 (38.7%) and 19 (61.3%) subjects, respectively. The mean value of each groups mentioned in table 1 was compared with one way ANOVA. The *P*-value <0.01 was considered to be significant.

In study subjects reference vitamin D level in three groups (control, pre- diabetic and diabetic) was significantly different from each other (*P*-value= 0.00) (Table 1). Control population was almost equally distributed based on the vitamin D level. However, in diabetic population only 16% subjects have the sufficient vitamin D level.

The mean value of each groups mentioned in table.2 was compared with one way ANOVA. The *P*-value< 0.01 was considered to be significant.

Serum cholesterol was higher in diabetic subjects with good glycemic control (50%) and poor glycemic control (68.42%). Serum triglyceride level was found higher in pre-diabetic (56.09%), good glycemic control (66.67%) and poor glycemic control (94.73%) subject. Serum LDL-C and VLDL-C was elevated in subjects with poor glycemic control 52.63% and 73.68%, respectively. Serum HDL-C was almost similar among the different groups (Table 3).The mean value of each groups mentioned in table.3 was compared with one way ANOVA. The *P*-value< 0.01 was considered to be significant.

Using Pearson's chi-square test, a statistically significant association was found on comparison of serum cholesterol, serum triglyceride and VLDL-C with vitamin D3 levels having *P*-value< 0.01 respectively. However, HDL-C and LDL-C were not statistically significant on comparison with vitamin D3 level (Table 4).

The comparison of mean serum lipid profile and vitamin D3 status was significant (Table 5).

**Table 1. Distribution of subjects among the different groups based on Vitamin D3 Level**

| Subjects (n) | Deficiency (N= 52) | Insufficiency (N= 34) | Sufficiency (N= 23) | *P*-value |
|---|---|---|---|---|
| **Control (37)** | 12 (32.4%) | 11 (29.7%) | 14 (37.9%) | <0.01 |
| **Pre-diabetic (41)** | 19 (46.3%) | 15 (36.6%) | 7 (17.1%) | |
| **Diabetic with good glycemic control (12)** | 5 (41.6%) | 5 (41.6%) | 2 (16.6%) | |
| **Diabetic with poor glycemic control (19)** | 16 (84.2%) | 3 (15.7%) | 0 (0%) | |

**Table 2. Mean vitamin D3 levels in controls, pre-diabetics and diabetics patient**

| Subjects (n) | Mean Value±SD (ng/ml) | *P*-value |
|---|---|---|
| **Controls (37)** | 26.53±11.9 | <0.01 |
| **Pre-diabetics (41)** | 20.23±4.12 | |
| **Diabetic with GGC (12)** | 19.07±8.01 | |
| **Diabetic with PGC (19)** | 12.92±6.77 | |



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## Discussion

The present cross sectional study examined the association between vitamin D3 status with the metabolic profile and with diabetic patients on 109 subjects. The distribution of controls according to vitamin D3 status, 37 controls, 12 (32.4%) were deficient, 11 (29.7%) were insufficient and 14 (37.9%) were sufficient in vitamin D level. Out of 41 pre-diabetics, 19 (46.3%) were deficient, 15 (36.6%) were insufficient and 7 (17.1%) were found to have

**Table 3. Prevalence of dyslipidemia in subjects according to their glycemic status and lipid profile.**

| Lipid Parameter | Reference Value | Controls (n=37) | Pre -diabetics (n=41) | Diabetics with good glycemic status (n=12) | Diabetics with poor glycemic status (n=19) | *P*-value |
|---|---|---|---|---|---|---|
| **Serum cholesterol** | >200mg/dl | 1 (2.70%) | 16 (39.02%) | 6 (50%) | 13 (68.42%) | <0.01 |
| **Serum triglyceride** | >150mg/dl | 11 (29.73%) | 23 (56.09%) | 8 (66.67%) | 18 (94.73%) | <0.01 |
| **Serum HDL-C** | <40mg/dl | 6 (16.21%) | 11 (26.83%) | 2 (16.67%) | 6 (31.58%) | <0.01 |
| **Serum LDL-C** | >100mg/dl | 1 (2.70%) | 10 (24.39%) | 1 (8.33%) | 10 (52.63%) | <0.01 |
| **Serum VLDL-C** | >40mg/dl | 4 (10.81%) | 20 (48.78%) | 6 (50%) | 14 (73.68%) | |

**Table 4. Association between lipid profile & vitamin D3 levels**

| Variable | Deficiency (52) | Insufficiency (34) | Sufficiency (23) | *P*-value |
|---|---|---|---|---|
| **Serum Cholesterol** | | | | |
| **Normal (< 200 mg/dl)** | 25 (48.07%) | 28 (82.35%) | 20 (86.95%) | <0.01 |
| **Borderline high (200-239 mg/dl)** | 17 (32.69%) | 6 (17.64%) | 1 (4.34%) | |
| **High (≥ 240 mg/dl)** | 10 (19.23%) | 0 (0%) | 2 (8.69%) | |
| **Serum triglyceride** | | | | |
| **Normal (<150 mg/dl)** | 19 (36.53%) | 12 (35.29%) | 18 (78.26%) | <0.01 |
| **Borderline high (150-200 mg/dl)** | 22 (42.30%) | 18 (52.94%) | 4 (17.39%) | |
| **High (200-500 mg/dl)** | 11 (21.15%) | 4 (11.76%) | 1 (4.34%) | |
| **HDL-C** | | | | |
| **Low (< 40 mg/dl)** | 14 (26.92%) | 8 (23.52%) | 3 (13.04%) | 0.44 |
| **Normal (40- 59 mg/dl)** | 35 (67.30%) | 25 (73.52%) | 17 (73.91%) | |
| **High (≥ 60 mg/dl)** | 3 (5.76%) | 1 (2.94%) | 3 (13.04%) | |
| **LDL-C** | | | | |
| **Optimal near (100-129 mg/dl)** | 21 (40.38%) | 15 (44.11%) | 15 (65.21%) | 0.07 |
| **Optimal (<100 mg/dl)** | 16 (30.76%) | 16 (47.05%) | 5 (21.73%) | |
| **Borderline high (130-159 mg/dl)** | 11 (21.15%) | 2 (5.88%) | 1 (4.34%) | |
| **High (160-189 mg/dl)** | 4 (7.69%) | 1 (2.94%) | 2 (8.69%) | |
| **VLDL-C** | | | | |
| **Desirable (5-40 mg/dl)** | 22 (42.30%) | 24 (70.58%) | 20 (86.95%) | <0.01 |
| **High (≥40 mg/dl)** | 30 (57.69%) | 10 (29.41%) | 3 (13.04%) | |

**Table 5. Mean value of serum lipid profile according to vitamin D3 status**

| Variable | Deficiency (n=52) | Insufficiency (n=34) | Sufficiency (n=23) | *P*-value |
|---|---|---|---|---|
| **Total Cholesterol Mean ± SD** | 208.25±31.8 | 181.15±21.6 | 165.0±28.4 | <0.01 |
| **Triglyceride Mean ± SD** | 173.02±40.4 | 154.94±34 | 112.06±26.5 | <0.01 |
| **HDL-C Mean ±SD** | 44.48±8.8 | 43.49±7.6 | 48.86±8.09 | 0.04 |
| **LDL-C Mean ±SD** | 116.36±27.02 | 102.03±17.2 | 89.22±25.7 | <0.01 |
| **VLDL Mean ±SD** | 47.09±16.19 | 35.72±12.5 | 27.42±8.96 | <0.01 |



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sufficient vitamin D which were concordance with the results of Deep Dutta et al. 2013 (11).Vitamin D deficiency was found in 115 (73.25%) individuals with pre-diabetic. Severe vitamin D deficiency (<10ng/ml) was found in 14.65% individuals and have the highest insulin resistance (HOMA2-IR: 2.04±0.67). These results concluded that vitamin D deficiency/ insufficiency may have some role in worsening of insulin resistance in individuals with pre-diabetes. In the present study 41.7% individuals with good glycemic index were in both deficiency and insufficiency group each. 16.6% individuals had sufficient vitamin D level and 84.2% had deficient vitamin D level. The results of present study are in accordance with some previously published study Arya V et al .2004 (12).

The mean vitamin D3 levels in pre-diabetics was found 20.23±4.12 ng/ml , 19.07±8.01 ng/ml in diabetics with good glycemic control and 12.92±6.77 ng/ml in diabetics with poor glycemic control. A hospital base study was done by Srinath K M et al .2016 (13) on 40 pre-diabetics in South India shows that vitamin D level less than 20ng/ml was found in 72.5%. Mean vitamin D3 level was 17.09±5.89 ng/ml in pre-diabetics and 23.67±11.02ng/ml in controls. The findings of this study are similar to that of present study. It was also found that there was significant inverse co-relation (*P*-value< 0.00) between vitamin D levels and HBA1/c. Results of the present study are in line with those of Ahmad Bashir Laway et al .2014 (14), in the study 102 diagnosed diabetics and equal number of controls were taken. Overall 25 Hydroxy vitamin D, was lower (18.81±15.18 ng/ml) in patients with Type 2 Diabetic patients as compared to healthy controls (28.46±18.89 ng/ml) (p= 0.00). Taking a cut off 30 ng/ml, 81% of T2D patients had either Vitamin D Deficiency (VDD) or insufficiency compared to 67% of healthy control subjects. Severe VDD (25HD< 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, similar with the present study. The higher percentage of hypertriglyceridemia appears to be due to the smaller sample size in the present study and so we have taken patients with good and poor glycemic control separately unlike most of the other studies which considered both in accordance. Study carried out by Kusum Bali and Amariit Singh Vij 2016 (9), to study the pattern of Dyslipidemia in 285 patients with type 2 diabetes mellitus in Punjab, they concluded that the most elevated lipid was LDL-C (59.3%) followed by TG (57.2%) and TC (36.5%). The HDL-C was decreased in 34.4% patients. The prevalence for deranged LDL-C and HDL-C in this study matches with the findings of present study.

In the present study it was found that total cholesterol (*P*-value< 0.00), serum triglyceride (*P*-value< 0.00), serum LDL cholesterol (*P*-value< 0.00) and serum VLDL (*P*-value< 0.00) have an inverse association with vitamin D levels whereas HDL cholesterol has no effect with vitamin D, and the association was not significant (*P*-value= 0.44). The above result matches with the findings of Felicia L Steger 2013 (15), studied extensively the association between lipid profile and vitamin D status in healthy individuals. There was a significant relationship between vitamin D and TG (*P*-value= 0.020), TC (*P*-value< 0.001), LDL (*P*-value< 0.001) and the TC: HDL ratio (*P*-value<0.001), but not with HDL, or TG:HDL.

## Conclusions

Present study concluded that vitamin D deficiency is an associated risk factor for worsening glycemic control and Dyslipidemia. Our study explored that Vitamin D can be established as a promising marker for prevention of Dyslipidemia in pre-diabetics and diabetics subjects. Study also provide the rationale for supplementation of vitamin D for prevention of progression of pre- diabetes to diabetes, worsening of metabolic control, and development of metabolic syndrome Multi-



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centric studies with large sample size studying association between Vitamin D supplementation of various concentration and effect on glycemic level and metabolic profile in different types of diabetic and pre-diabetic subjects can be performed to study for exact relationship between them.

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## Conflict of Interest
The authors declare no administrative and financial conflict of interest.

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