

## Research Article

# Pattern of vitamin D status in prediabetic individuals: a case control study at tertiary hospital in South India

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## ABSTRACT

**Background:** Role of vitamin D in the regulation of calcium metabolism is well established. Vitamin D deficiency has been linked to impaired glucose tolerance and type 2 DM in humans. There is a limited available data on the relationship of vitamin D insufficiency/deficiency with glucose homeostasis among prediabetic individuals in South Indian population. Hence we examined serum 25(OH) D<sub>3</sub> concentration among prediabetics. The objective of this study is to evaluate prevalence of vitamin D deficiency in prediabetics and to study any correlation between vitamin D and BMI, FBS and PPBS among prediabetics.

**Methods:** A case control study was carried out among 40 prediabetics & controls, after obtaining consent from subjects and clearance from institutional ethics committee. Serum vitamin D levels were measured by radioimmunoassay in both groups. Statistical analysis was done using “t” test & Pearson correlation co-efficient (r).

**Results:** Vitamin D levels less than 20 ng/ml were found in 72.5% of cases and 35% of controls. 5% of the cases and 12.5% of the controls had vitamin D above 30 ng/ml and this difference was statistically significant. Mean vitamin D levels in cases and controls was 17.09±5.89 ng/ml and 23.67±11.02 ng/ml respectively (P<0.05). A significant inverse correlation was observed between vitamin D levels & body mass index (r=-0.274; p=0.014); random blood sugar (r=-0.35; p=0.001); fasting blood sugar (r=-0.328; p=0.003); post prandial blood sugar (r=-0.276; p=0.013).

**Conclusions:** High prevalence of hypovitaminosis D exists among prediabetics and there is significant inverse correlation between BMI, FBS, PPBS and vitamin D levels. Hence, a prospective study covering large pre-diabetic individuals is essential to confirm the findings.

**Keywords:** Vitamin D, Vitamin D deficiency, Prediabetes, Type 2 DM, Prevalence

## INTRODUCTION

Prediabetes broadly refers to an intermediate stage between normal glucose levels and the clinical entity of type 2 diabetes, encompassing both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT).<sup>1</sup>

The progression from prediabetes to type 2 diabetes occurs over many years before the development of overt hyperglycemia seen in diabetes.<sup>2</sup> The risk of progression to diabetes depends on the degree of insulin resistance and deficiency of Insulin secretion and other risk factors

such as age, family history, overweight or obesity or history of gestational diabetes or PCOS.<sup>3,4</sup>

Role of vitamin D in the regulation of calcium and bone metabolism is well known.<sup>5</sup> Recent epidemiological evidence points to a potential association of vitamin D insufficiency with adverse metabolic risk and in the pathogenesis of cancer, cardiovascular diseases, type 2 diabetes and other diseases.<sup>6-10</sup>

Biological mechanism that underlies the multiple effect of vitamin D on different tissues are not understood. One unifying factor is the expression of vitamin D receptors in more than 30 tissues including pancreatic islet cells.<sup>11</sup> Previous studies report that low serum 25(OH) D was inversely associated with metabolic syndrome risk, particularly for hyperglycemia, hyper triglyceridemia and abdominal obesity.

Vitamin D has been proposed to play an important role and to be a risk factor in the development of insulin resistance and the pathogenesis of type 2 DM by affecting either insulin sensitivity and Beta cell function or both.<sup>12</sup> The changing lifestyle, urbanization and modernization of Indian population has led to possible reduction of people being exposed to direct sunlight and inadequate intake of dietary vitamin D, thereby leaving the population at increased risk of vitamin D Deficiency and type 2 diabetes.

Given the limited available data on the relationship of vitamin D insufficiency with glucose homeostasis among prediabetic individuals in South Indian population, hence we examined serum 25(OH) D<sub>3</sub> concentration among prediabetics.

### Objectives

- 1) To estimate the serum 25(OH) vitamin D levels in prediabetes and compare the mean vitamin D among cases and controls across various factors.
- 2) To correlate the vitamin D level with BMI, FBS, RBS and PPBS.

### METHODS

The present case control study was carried out in a tertiary care teaching hospital in south India over a period of 2 years.

Forty subjects with prediabetes were compared with forty age and sex matched healthy controls. As per ADA criteria subjects with impaired fasting glucose of 100-125 mg/dl and/or impaired glucose tolerance of 140-199 mg/dl and/or HbA1C 5.7 to 6.4 were included as prediabetic cases.

Pregnant women, critically ill patients, patients with diabetes, tuberculosis, renal/hepatic impairment and

patients on vitamin D supplementation, steroids, OCPs, phenytoin and diuretics were excluded from the study.

After obtaining informed written consent from cases and controls, a detailed information regarding age, gender, clinical history and anthropometric data like height and weight were collected in pretested proforma and BMI was calculated.

Venous blood sample was collected from both cases and controls in fasting and postprandial i.e. (120 mins after 75 gm of glucose load) state. Fasting plasma glucose and postprandial glucose measured by glucose oxidase and peroxidase method using Toshiba automated analyzer, glycosylated haemoglobin (HbA1c) measured by High Performance Liquid Chromatography (HPLC) method using D-10 haemoglobin system, Biorad and serum 25(OH) D<sub>3</sub> was measured by radioimmuno assay method. The present study was done after obtaining clearance from ethical committee, JSS Medical College.

All the statistics were performed using SPSS for windows statistical package (SPSS Inc. Chicago, USA). For categorical variables percentages were calculated and for continuous variable mean and Standard deviation was calculated.

For comparing the mean difference in vitamin D level among prediabetic cases and healthy control "t"-test was performed and a p-value of <0.05 was taken as statistical significance. Pearson's correlation co-efficient (r) is calculated for variables like age, height, weight, BMI, FBS, PPBS and vitamin D values.

### RESULTS

Majority of the cases and controls were in the age of 41 to 60 years groups. Mean age was 51.75±12.86 years for cases and 50.15±11.41 years for controls. Males constituted 55% and the rest were females (45%).

In this study, 57.5% of the cases were with BMI≥25 whereas 32.5% of the controls had BMI ≥25. In Around 52.5% of cases and 35% of controls, the blood samples were drawn during winter whereas in 47.5% of the cases and 65% of the controls during summer. Around 22.5% of cases and 17.5% of controls were smokers, whereas 25% of cases and 15% of controls were alcoholics. About 37.5% of the cases had hypertension, however only 20% of controls had hypertension (Table 1).

Table 2, describes the serum 25(OH) vitamin D levels among the study subjects. Around 72.5% cases and 35% of controls had vitamin D levels less than 20 ng/ml. 5% of the cases and 12.5% of the controls had vitamin D above 30 ng/ml and this difference was statistically significant. Overall mean vitamin D levels in cases and controls was 17.09±5.89 ng/ml and 23.67±11.02 ng/ml respectively (P<0.05).

**Table 1: Demographic and clinical characteristics of prediabetic cases and controls.**

Characteristics	Cases (n=40) No. (%)	Controls (n=40) No. (%)	Total (n=80) No. (%)
<b>Age group in years</b>			
20-40	8 (20.0)	8 (20.0)	16 (20.0)
41-60	24 (60.0)	24 (60.0)	48 (60.0)
61-80	8 (20.0)	8 (20.0)	16 (20.0)
<b>Gender</b>			
Male	22 (55.0)	22 (55.0)	44 (55.0)
Female	18 (45.0)	18 (45.0)	36 (45.0)
<b>Body mass index</b>			
Less than 25 kg/m <sup>2</sup>	17 (42.5)	27 (67.5)	44 (55.0)
More than 25 kg/m <sup>2</sup>	23 (57.5)	13 (32.5)	36 (45.0)
<b>Relation to season</b>			
Winter	21 (52.5)	14 (35.0)	35 (43.8)
Summer	19 (47.5)	26 (65.0)	45 (56.2)
<b>Currently smoking</b>			
Yes	9 (22.5)	7 (17.5)	16 (20.0)
No	31 (77.5)	33 (82.5)	64 (80.0)
<b>Currently consuming alcohol</b>			
Yes	10 (25.0)	6 (15.0)	16 (20.0)
No	30 (75.0)	34 (85.0)	64 (80.0)
<b>History of hypertension</b>			
Yes	15 (37.5)	8 (20.0)	23 (28.75)
No	25 (62.5)	32 (80)	57 (71.2)

**Table 2: Comparison of serum 25(OH) D levels among prediabetic cases and controls.**

Vitamin D, (ng/ml)	Cases (n=40), No. (%)	Controls (n=40), No. (%)	Total (n=80), No. (%)	P value
Less than 20	29 (72.5)	14 (35.0)	43 (53.7)	Yates P value= 0.01
20-29.9	9 (22.5)	21 (52.5)	30 (37.5)	
More than 30	2 (5.0)	5 (12.5)	7 (8.8)	
Vit. D level (ng/ml) Mean (SD)	17.09 (5.8)	23.67 (11.02)		0.001

The difference in the serum 25 (OH) vitamin D levels between cases and controls across demographic and clinical parameters are described in Table 3. A statistically significant mean difference in vitamin D levels was observed among prediabetic cases and healthy controls in the 41 to 60 year age group (15.7±5.4 ng/ml vs 22.8±4.0 ng/ml), females (15.9±5.8 ng/ml Vs 26.2±12.28 ng/ml), body mass Index <25 (19.67±4.9 ng/ml vs 26.69±11.6 ng/ml) and when blood samples were collected in summer (19.89±6.3 ng/ml vs 27.27±11.6 ng/ml).

A significant inverse or negative correlation was observed between body mass index (r =-0.274; p=0.014); random blood sugar (r =-0.35; p=0.001); fasting blood sugar (r =-0.328; p =0.003); post prandial blood sugar (r =-0.276; p=0.013) and vitamin D levels Table 4.

**Table 3: Comparison of vitamin D levels across socio-demographic and clinical parameters among prediabetic cases and controls.**

Characteristics	Cases (n=40)	Controls (n=40)	Total (n=80)	T test	P- value
<b>Age group in years</b>					
20-40	19.32 (7.4)	21.47 (6.8)	20.39 (7.02)	0.605	0.55
41-60	15.73 (5.4)	22.84 (4.0)	19.29 (5.9)	5.18	0.0001
61-80	15.49	25.52 (14.7)	20.51 (11.7)	1.83	0.0879
<b>Gender</b>					
Male	18.05 (5.8)	21.59 (9.6)	19.82 (8.09)		0.07
Female	15.91 (5.8)	26.2 (12.28)	21.06 (10.83)	23.7	0.00
<b>Body mass index</b>					
Less than 25 kg/m <sup>2</sup>	19.67 (4.9)	26.69 (11.6)	23.98 (10.13)	37.9	0.003
More than 25 kg/m <sup>2</sup>	15.19 (5.8)	17.38 (6.22)	15.98 (6.02)	23.2	0.152
<b>Relation to season</b>					
Winter	14.56 (4.2)	16.97 (5.5)	15.52 (4.8)	22.3	0.08
Summer	19.89 (6.3)	27.27 (11.6)	24.15 (10.3)	39.7	0.004
<b>Currently smoking</b>					
Yes	17.71 (5.45)	24.82 (15.4)	20.82 (11.1)	6.7	0.143
No	16.91 (6.08)	23.42 (10.1)	20.27 (8.97)	52.5	0.001
<b>Currently consuming alcohol</b>					
Yes	17.22 (3.6)	20.92 (7.0)	18.61 (5.3)	6.1	0.147
No	17.05 (6.5)	24.15 (11.5)	20.82 (10.13)	52.8	0.001

**Table 4: Correlation between body mass index, blood glucose and vitamin D levels among study subjects.**

Characteristic	Pearson's correlation coefficient	P-value
Age	0.49	0.666
Height	0.115	0.310
Weight	-0.170	0.132
Body mass index	-0.274	0.014
Random blood sugar	-0.35	0.001
Fasting blood sugar	-0.328	0.003
Post prandial blood sugar	-0.276	0.013

## DISCUSSION

Ever since the identification of the vitamin D, its role in calcium homeostasis is well established.<sup>13</sup> In recent years growing number of extra-skeletal pleiotropic effects of this hormone is becoming evident.<sup>6-10</sup> Vitamin D has been associated with the functioning and regulation of over 200 genes.<sup>14</sup> Recent epidemiological evidence also points to a potential vitamin D insufficiency with adverse metabolic risk.<sup>9,10</sup>

Hypovitaminosis D is largely due to inadequate cutaneous production and to a lesser extent from low dietary intake.<sup>14,15</sup> The production of vitamin D in the body is affected by the exposure to sunlight which in turn depends on the seasonal changes, latitude, clothing, skin pigmentation and use of sunscreens.<sup>16-18</sup> Recently there is a growing attention towards the role of interfering factors such as obesity and environmental pollution with vitamin D insufficiency and diabetes.<sup>14</sup>

The progression of prediabetes to type 2 diabetes occurs over many years before the development of overt diabetes.<sup>2</sup> The risk of progression to diabetes depends on the degree of insulin resistance and deficiency of insulin secretion.<sup>3,4</sup>

Epidemiological studies are examining vitamin D status and risk of hyperglycemia or insulin resistance and have far been suggestive of inverse association but are inconclusive. Third National Health and Nutrition Examination Survey (NHANES) have reported an inverse association between Vitamin D and metabolic syndrome risk, particularly for hyperglycemia, hypertriglyceridemia and abdominal obesity.<sup>19,20</sup>

Even the European prospective Investigation into Cancer (EPIC)-Norfolk study reported an inverse association between circulating 25(OH) and type 2 diabetes.<sup>21</sup> Data from these studies cannot be extrapolated fully to other ethnic groups. Vitamin D status is poor among Asian Indians; limited data exist to assess the implication of vitamin D deficiency in glucose metabolic traits among

South India. Several authors have documented an association with low vitamin D status and type 2 diabetes mellitus.<sup>24-27</sup>

The current study was an attempt to assess the vitamin D level among newly detected prediabetic patients of South India by adopting a case control approach.

The current study observed that 95% of the prediabetic cases were vitamin D deficient (i.e. <30 ng/ml) compared to normoglycemics. Similar results were observed in a study conducted by Shankar et al<sup>24</sup> in Kashmiri population, Braun et al<sup>22</sup> among North Indian community and Dutta et al<sup>23</sup> in West Bengal.

Our study also documents the seasonal difference in vitamin D level which was also observed by Farouhi et al,<sup>30</sup> where serum 25(OH) D was lowest in early spring and highest in late summer.

Although the vitamin D levels were lower among smokers than non-smoker, it was not statistically significant. Contrastingly a meta-analysis done by Afzal et al,<sup>31</sup> observed that smokers had low concentration of 25(OH) D when compared to nonsmokers. The current study has also documented that there exists a significant inverse correlation between vitamin D levels and fasting blood sugar, post prandial blood sugar, random blood sugar and body mass index. A similar inverse correlation was observed by Dutta et al<sup>23</sup>; Alasm Cimbek et al<sup>28</sup> and Saneei et al<sup>29</sup> in their study conducted among prediabetes.

Vitamin D exerts its action in a variety of cell types through vitamin D receptor.<sup>32,33</sup> Molecular evidence have found that pancreatic  $\beta$ -cells express both cytosolic/nuclear VDR, thus enhances  $\beta$ -cells function.<sup>34</sup> Additionally, 25(OH) vitamin D protects pancreatic  $\beta$  cells from immune attack.<sup>35</sup> 25(OH) D inhibits the release of the pro-inflammatory cytokines, TNF $\alpha$  and regulates the activity of TLR 2 and TLR 4 proteins.<sup>36-38</sup> Basic Science and epidemiological studies indicate that Vitamin D has importance not only for cardiovascular health, but also for the immune response. Several studies support that vitamin D supplementation may affect glucose homeostasis and improve insulin resistance.<sup>39-41</sup>

In spite of Indians living in sunny equatorial region, it has been reported that Asian Indians require twice as much UV-B exposure to produce 25(OH) D levels equal to caucasians due to increased skin pigmentation, cultural factors, customs, traditions, clothing pattern, working indoors, high temperatures during summer, tendency to avoid direct sunlight all contribute to low vitamin D levels.<sup>42</sup> Thus such studies generate evidence of vitamin D deficiency/insufficiency among prediabetics of Indian population and emphasize on the need for prospective studies covering large population and to evaluate whether correction of vitamin D deficiency/insufficiency by vitamin D supplementation delay the development of diabetes and reduce the burden of cardio-vascular risk.

### Limitations

This is a case control study with small sample size, thus our results cannot establish cause & effective relationship between vitamin D & prediabetes. Hence we recommend a study involving larger study subjects, so that results can be confirmed.

### CONCLUSIONS

High prevalence of hypovitaminosis D exists among prediabetics and there is significant inverse correlation between BMI, FBS, PPBS and vitamin D levels. Hence, a prospective study covering large pre-diabetic individuals is essential to confirm the findings.

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