ORIGINAL ARTICLE

JIACM 2019; 20(2): 110-14

The Relationship between Serum 25 (OH) Vitamin D and Insulin Resistance in Prediabetes

Abhishek Juneja, Rajat Jhamb**, SV Madhu****

Abstract

Background and objectives: Vitamin D supplementation has been found to decrease insulin resistance in normal healthy individuals and patients with type 2 diabetes mellitus. However, similar observations among individuals with prediabetes are not well documented. The aim of this study was to find out the relation between serum 25 (OH) vitamin D and insulin resistance in prediabetes.

Methods: A total of 80 prediabetic individuals, in the age group of 20 - 50 years, were included in the study based on oral glucose tolerance test results. An equal number of normal healthy adults were taken as controls. Family members and attendants of patients attending the diabetic clinic underwent 75 gm Oral glucose tolerance test. Individuals with fasting blood glucose between 100 - 125 mg/dl and/or 2-hour post-glucose of 140 - 199 mg/dl after ingesting 75 gm of glucose were recruited for this study after applying inclusion and exclusion criteria.

Results: The presence of vitamin D deficiency was 83% in prediabetes group and 95% in normal healthy controls. Serum 25 (OH) vitamin D levels were 13.30 ± 9.85 ng/ml in cases and 9.80 ± 5.86 ng/ml in controls. There was significant inverse correlation (p value 0.041) between serum 25 (OH) vitamin D levels and insulin resistance in prediabetic individuals.

Interpretation and conclusion: Overall, both the groups were vitamin D deficient irrespective of their glycaemic status. Further research is required with larger sample size to define the relationship of vitamin D and insulin resistance in prediabetes.

Key words: Vitamin-D deficiency, insulin resistance, prediabetes.

Introduction

Vitamin D deficiency has been extensively studied in the pathogenesis of insulin resistance. It has been found to be associated with increased risk of type 2 diabetes by various mechanisms including insulin resistance (IR), pancreatic β-cell dysfunction and inflammation[1-5]. Vitamin D supplementation has been found to decrease the insulin resistance in normal healthy individuals and patients with type 2 diabetes[6]. However, similar observations among individuals with prediabetes are not well documented. Prediabetes is considered a harbinger of overt type 2 diabetes mellitus with annual rate of progression to diabetes ranging from 2.5% in the Diabetes Prevention Trial (DPT) to 18% in the Indian Diabetes Prevention Programme-1 (IDPP-1)[7]. The aim of this study was to find out the relationship between insulin resistance and vitamin D status among prediabetic individuals.

Material and methods

The study was carried out in the Department of Medicine and Division of Diabetes, Endocrinology and Metabolism at UCMS and GTB Hospital, Delhi, between November 2014 and April 2016. Informed written consent from the participants and ethics clearance from the institution were taken prior to the study. Family members and attendants of patients attending the diabetic clinic underwent 75 gm Oral Glucose Tolerance Test. Fasting blood glucose estimation (following overnight 8 hours fast) was done, following which patients ingested 75 gm glucose solution. Blood glucose was again estimated at 2-hour post-glucose ingestion. Individuals with fasting blood glucose between 100 - 125 mg/dl (impaired fasting glucose) and/or 2-hour post-glucose of 140 - 199 mg/dl (impaired glucose tolerance) after ingesting 75 gm of glucose were screened for inclusion and exclusion criteria. A total of 80 prediabetes individuals (IFG + IGT), in the age group of 20 - 50 years, were included in the study. An equal number of individuals showing normal glucose tolerance were taken as healthy controls. Individuals with history of any oral antidiabetic medications or insulin use were excluded. Also individuals with associated disorders like primary hyperparathyroidism, chronic kidney disease, liver disease, any chronic illness, malignancy, chronic drug use like antiepileptic agents, oral contraceptive pills, steroids which are likely to interfere with Vitamin-D metabolism, were excluded. Individuals with a history of

**Post-Graduate Student, **Professor, ***Director Professor, Department of Medicine, University College of Medical Sciences, Guru Teg Bahadur Hospital, Delhi - 110 095.*

Corresponding Author: Dr Abhishek Juneja, Post-Graduate Student, Department of Medicine, University College of Medical Sciences, Guru Teg Bahadur Hospital, Delhi - 110 095. Tel: 9013098898, E-mail: junejaabhishek1990@gmail.com.

calcium or vitamin D supplementation in the last one year were excluded. Routine biochemical blood investigations and special blood investigations including serum vitamin D levels and insulin levels were done.

Body weight was measured in kilograms using a weighing scale with the subject bare footed, standing erect and looking forwards with body weight equally distributed on both the feet. Height was measured in meters using a wall-mounted measuring tape with the subject bare footed, standing erect and looking forwards. Body mass index was calculated as weight in kilograms divided by height in meter squares.

Fasting serum insulin level was measured by commercially available Insulin (e) IRMA kit (Immune-radiometric assay), Beckman coulter IM3210.The immune-radiometric assay of insulin is a sandwich type assay.In the kit, mouse monoclonal antibodies directed against two different epitopes of insulin were used. Serum samples, controls and calibrators were incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody which was labelled with Iodine 125. After incubation, the content of tubes was rinsed so as to remove unbound I^{125} labelled antibody.The bound radioactivity was then determined in a gamma counter.The insulin concentrations in the samples were obtained by interpolation from the standard curve.The insulin concentrations in the samples were directly proportional to the radioactivity.

Measurement of insulin resistance was done using Homeostasis Model Assessment Insulin Resistance (HOMA-IR): HOMA-IR = Insulin (μIU/L) X Glucose (mmol/l)/22.5.

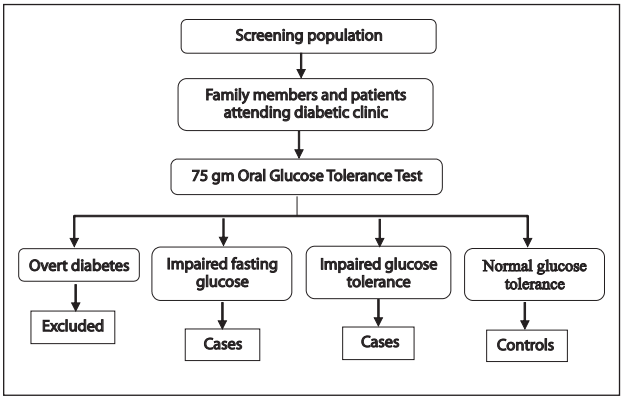

Fig. 1: *Schematic presentation of the study design.*

Serum 25 (OH) vitamin D levels were measured using commercially available DiaSorin 25 (OH) vitamin D 125I RIA (radioimmune assay) Kit (DiaSorin, Stillwater, Minnesota 55082 - 0285, USA).The DiaSorin 25 (OH) vitamin D assay consisted of a two-step procedure.The first-step involved a rapid extraction of 25 (OH) vitamin D and other hydroxylated metabolites from serum or plasma with acetonitrile. Following extraction, the treated sample was then assayed using an equilibrium RIA procedure.The RIA method was based on an antibody with specificity to 25 (OH) vitamins D.The sample, antibody and tracer were incubated for 90 minutes at 20 - 25° C. Phase separation was accomplished after 20-minute incubation at 20 - 25° C with a second antibody precipitating complex. An NSB/Addition buffer was added after this incubation prior to centrifugation to aid in reducing non-specific binding.The plasma 25 (OH) vitamin D concentrations were expressed in nano-gram per ml (ng/ml).

Statistical Analysis: Association between vitamin D status and insulin resistance in individuals with prediabetes was studied using Pearson's correlation co-efficient. Data was analysed by SPSS Software and p-value < 0.05 was considered significant.The vitamin D levels among the two groups were compared by unpaired student t test.

Observations and results

A total of 80 prediabetes individuals, between 20 - 50 years of age, along with equal number of healthy controls who fulfilled all the inclusion and exclusion criteria were included in the study.

Table I: Mean age, anthropometry and routine laboratory parameters of cases and controls.

Variable	Cases (N = 80)	Controls (N = 80)	p-value	Significance
Age (years)	42.48 ± 5.12	42.76 ± 4.10	0.69	Non-significant
Height (cm)	163.73 ± 10.91	164.93 ± 11.10	0.49	Non-significant
Weight (kg)	69.06 ± 9.64	70.97 ± 10.34	0.22	Non-significant
BMI (kg/m^2)	25.67 ± 1.31	25.98 ± 1.24	0.13	Non-significant
Haemoglobin(g/dl)	12.44 ± 1.14	12.72 ± 0.99	0.09	Non-significant
Blood urea (mg/dl)	17.14 ± 4.31	16.93 ± 4.77	0.76	Non-significant
Creatinine (mg/dl)	0.62 ± 0.17	0.60 ± 0.18	0.40	Non-significant
SGOT (IU/L)	23.78 ± 5.01	23.10 ± 5.31	0.41	Non-significant
SGPT (IU/L)	22.70 ± 5.28	21.80 ± 5.11	0.27	Non-significant
Calcium (mg/dl)	9.08 ± 0.57	9.21 ± 0.56	0.14	Non-significant
Phosphorus (mg/dl)	3.76 ± 0.38	3.72 ± 0.37	0.50	Non-significant
ALP (IU/L)	106.75 ± 11.72	127.2 ± 0.99	0.93	Non-significant
Triglycerides (mg/dl)	141.96 ± 12.54	124.20 ± 14.00	0.00	Significant
HDL cholesterol (mg/dl)	40.58 ± 5.23	41.25 ± 4.71	0.39	Non-significant
LDL cholesterol (mg/dl)	145.86 ± 10.84	134.70 ± 9.81	0.00	Significant

Table II: Glycaemic parameters, insulin levels, HOMA-IR and vitamin D levels in cases and controls.

Variable	Cases (N = 80)	Controls (N = 80)	p-value	Significance
Fasting Plasma Glucose (mg/dl)	105.71 ± 10.83	81.85 ± 6.68	<0.001	Significant
2-hour plasma glucose (mg/dl)	157.13 ± 11.89	124.38 ± 7.76	<0.001	Significant
Insulin(uIU/ml)	20.01 ± 14.53	11.09 ± 8.90	<0.001	Significant\
HOMA-IR	5.19 ± 3.65	2.26 ± 1.87	<0.001	Significant
Insulin (uIU/ml_log)	1.20 ± 0.30	0.93 ± 0.30	<0.001	Significant
HOMA-IR_log**	1.61 ± 0.31	1.24 ± 0.30	<0.001	Significant
25 (OH) vitamin D (ng/ml)	13.30 ± 9.85	9.80 ± 5.86	0.007	Significant
25 (OH) Vitamin D _log**	1.01 ± 0.33	0.90 ± 0.29	0.026	Significant

***values not following normal distribution, hence log transformation done. Since the variability is very high and the data for vitamin D levels is not following normal distribution, we log transformed data to bring normalcy and analysed using Repeated Measure ANOVA followed by Tukey's test. HOMA-IR = (Insulin in µUl/L X Glucose mmol/l)/22.5.*

Table III: Correlation between vitamin-D status and other parameters in prediabetes individuals.

Correlation variables		Pearson's correlation co-efficient	p-value	Significance
Parameter 1	Parameter 2			
25(OH)D	HOMA-IR	-0.230	0.041	Significant
25(OH)D	Fasting insulin	-0.234	0.036	Significant
25(OH)D	FBG	-0.107	0.344	Non-significant
25(OH)D	2-hr post glucose	-0.107	0.344	Non-significant
25(OH)D	HDL-C	0.061	0.589	Non-significant
25(OH)D	LDL-C	0.113	0.319	Non-significant
25(OH)D	Triglyceride	0.012	0.915	Non-significant

The presence of vitamin D deficiency was 83% in prediabetes group and 95% in normal healthy controls. Severe vitamin D deficiency (< 10 ng/ml) was seen in 37.5% of individuals with prediabetes and 61% individuals with normal glucose tolerance.There was statistically significant difference in the 25 (OH) vitamin D levels among the two groups with prediabetes group having higher vitamin D levels than normal healthy controls.

The correlation between serum 25 (OH) vitamin D levels and HOMA-IR in prediabetic individuals was significant (p value 0.041). While there was no significant correlation between vitamin D levels and 2-hour post-glucose in prediabetes individuals.

Discussion

Our study was a cross-sectional observational study which included 160 subjects who were divided into 2 groups, on the basis of their glycaemic status, into prediabetes group and normal glucose tolerance group. Serum vitamin D levels and HOMA-IR, a marker of insulin resistance, were compared.

The principal findings of our study were that hypovitaminosis D was widely prevalent and observed in both the groups, with less than 1% of subjects having sufficient vitamin D levels (> 30 ng/ml).

Our data indicated that the presence of vitamin D deficiency was 83% in prediabetes group and 95% in normal healthy controls. Vitamin D levels were found to be higher in the case group than the control group unlike many previous studies. However, both the groups had vitamin D deficiency indicating its high prevalence in our country. Severe vitamin D deficiency (< 10 ng/ml) was seen in 37.5% of individuals with prediabetes and 61% individuals with normal glucose tolerance. This high prevalence of hypovitaminosis D in our study groups is in concordance with various other Indian studies who have reported prevalence of hypovitaminosis D between 69 to 98 per cent[8-11].

In the present study BMI matched cases and controls were recruited for the study. Individuals with BMI > 30 kg/m^2 were excluded. Adjustment for BMI was necessary as Vitamin-D is predominantly stored in the adipose tissue and individuals with higher BMI tend to have lower serum Vitamin-D. Adjustment for BMI eliminated the confounding effect of obesity between hypovitaminosis D and insulin resistance. Mean BMI of the cases was 25.67 ± 1.31 kg/m^2 while for the control group was 25.98 ± 1.24 kg/m^2.

The routine investigations including complete blood count, kidney and liver function tests, serum calcium and phosphate levels were done to fulfil the various exclusion criteria for recruitment of subjects into the study.

Following results were observed while analysing the lipid profile studies between the two groups. Mean fasting serum triglyceride levels in the cases were 141.96 ± 12.54 mg/dl while in the controls were 124.20 ± 14.00 mg/dl. There was statistically significant difference between the two groups. Similarly, mean fasting serum LDL-C levels in the cases were significantly higher than the control group. Whereas no statistically significant relation was found, between serum HDL levels among the two groups.

This observation is consistent with the finding of a previous study where the fasting serum triglyceride and LDL-C levels were higher in the prediabetes group than the controls. Although the difference was far from being significant[12]. This is explained by the fact that, prediabetes is an insulin resistance state and is commonly associated with other components of metabolic syndrome.

Serum insulin levels were significantly higher in the prediabetes group (20.01 ± 14.53) as compared to normal healthy controls (11.09 ± 8.90). Consequently, HOMA-IR values were significantly different among the two groups. Mean ± SD HOMA-IR value in the cases was 5.19 ± 3.65 while in the control group was 2.26 ± 1.87. This highlights the difference in the degree of insulin resistance among the two groups.

In the present study, mean 25 (OH) D level was significantly different in prediabetes patients (13.30 ± 9.85 ng/ml) compared to healthy controls (9.80 ± 5.86 ng/ml) with p value < 0.007. Comparing both the groups, we observed that although vitamin D levels were significantly lower in the healthy controls than the prediabetes group, but both were still in the deficient range. It highlights the wide prevalence of vitamin D deficiency in our country despite plentiful sunshine. This becomes one of the limitations of this study as both the groups show vitamin D deficiency irrespective of their glycaemic status. Other possible limitations of the study were a lack of history of sunshine exposure, food intake of calcium and vitamin D which may be responsible for discrepant results. This finding is in agreement with the previous Indian study conducted by Dutta *et al* where the mean vitamin D levels were not lower among pre-diabetic subjects (23.1 ± 11.9 ng/ml) as compared to normal healthy controls (21.7 ± 8.56 ng/ml)[12]. On the other hand, this is in contrast to the finding of Scragg *et al*, who reported 25 (OH) vitamin D levels to be significantly lower in individuals with newly diagnosed IGT or diabetes as compared to normal individuals[13].

There was a significant negative correlation of 25 (OH) vitamin D levels with HOMA-IR in the prediabetes group (p value 0.041). Similar results were also found in another cross-sectional study in eastern India where a significant negative correlation was established between 25 (OH) vitamin D levels and HOMA-IR in prediabetes (p value 0.008)[12]. On the other hand, various supplementation studies have shown contradicting results on the effect of vitamin D supplementation on insulin sensitivity in prediabetes[14-19].

However, there was no significant correlation between serum 25 (OH) D levels and 2-hour post-glucose. This highlights the fact that improvement in insulin resistance may not lead to improvement in glucose tolerance and needs further research.

Also, there was no significant correlation between serum 25 (OH) D levels and HOMA-IR in the study population across glycaemic tolerance (cases + controls).

At the beginning of this study we wanted to compare the insulin resistance pattern and 25 (OH) vitamin D levels in individuals with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) separately. But, in view of very few IFG individuals as compared to IGT individuals, such a comparison was not statistically relevant.

In a previous Indian study, it was found that among individuals with prediabetes, those having severe vitamin D deficiency (< 10 ng/ml), had the worst insulin resistance (HOMA2-IR) as compared to those having higher levels, with an inverse correlation between vitamin-D status and insulin resistance[12]. Similar observations were confirmed in our study in the prediabetes group, between vitamin D levels and degree of insulin resistance in vitamin D deficiency [25 (OH) D ≤ 20 ng/ml] but not in vitamin D insufficiency [25 (OH)D = 21 - 30 ng/ml] or sufficiency groups [25 (OH)D ≥ 31 ng/ml].

Though there was a negative correlation between vitamin D levels and insulin resistance in the prediabetes group, still vitamin D levels were not lower in prediabetes as compared to normal healthy controls. Thus vitamin D deficiency/insufficiency may have some role in worsening of insulin resistance in prediabetes but its role in causation of prediabetes needs further research by long-term prospective studies.

References

1. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357: 266-81.
2. Chiu KC, Chu A, Go VLW *et al*. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *Am J ClinNutr* 2004; 79: 820-25.
3. Pittas AG, Lau J, Hu FB *et al*. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007; 92: 2017-29.
4. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol* 2005; 97: 93-101.
5. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D3 analogs as immunomodulatory agents. *Trends Mol Med* 2002; 8: 174-9.
6. Cigolini M, Iagulli MP, Miconi V *et al*. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. *Diabetic Care* 2006; 29: 722-4.
7. Ramachandran A, Snehalatha C, Mary S *et al*. Indian Diabetes Prevention Programme (IDPP). The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). *Diabetologia* 2006; 49: 289-97.
8. Marwaha RK, Tandon N, Reddy DR *et al*. Vitamin D and bone mineral

density status of healthy schoolchildren in northern India. *Am J Clin Nutr* 2005; 82: 477-82.

9. Marwaha RK, Tandon N, Garg MK *et al*. Vitamin D status in healthy Indians aged 50 years and above. *JAPI* 2011; 59: 706-9.
10. Arya V, Bhambri R, Godbole MM *et al*. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2004; 15 (1): 56-61.
11. Shivane VK, Sarathi V, Bandgar T *et al*. High prevalence of hypovitaminosis D in young healthy adults from the western part of India. *Post-graduate Medical Journal* 2011; 87 (1030): 514-8.
12. Dutta D, Maisnam I, Shrivastava A *et al*. Serum vitamin D predicts insulin resistance in individuals with prediabetes. *Indian J Med Res* 2013; 138 (6): 853-60.
13. Scragg R, Holdaway I, Singh V *et al*. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes Res Clin Pract* 1995; 27: 181-8.
14. Dutta D, Mondal SA, Choudhuri S *et al*. Vitamin-D supplementation in prediabetes reduced progression to type 2 diabetes and was associated with decreased insulin resistance and systemic inflammation: An open label randomised prospective study from Eastern India. *Diabetes Res Clin Pract* 2014; 103: e18-23.
15. Kuchay MS, Laway BA, Bashir MI *et al*. Effect of Vitamin D supplementation on glycaemic parameters and progression of prediabetes to diabetes: A 1-year, open-label randomised study. *Indian J Endocrinol Metab* 2015; 19 (3): 387-92.
16. Davidson MB, Duran P, Lee ML *et al*. High-dose Vitamin D supplementation in people with prediabetes and hypovitaminosis D. *Diabetes Care* 2013; 36: 260-6.
17. Tuomainen TP, Virtanen JK, Voutilainen S *et al*. Glucose Metabolism Effects of Vitamin D in Prediabetes: The VitDmet Randomised Placebo-Controlled Supplementation Study. *J Diabetes Res* 2015; 2015: 672653.
18. Gagnon C, Daly RM, Carpentier A *et al*. Effects of Combined Calcium and Vitamin D Supplementation on Insulin Secretion, Insulin Sensitivity and β-Cell Function in Multi-Ethnic Vitamin D-Deficient Adults at Risk for Type 2 Diabetes: A Pilot Randomised, Placebo-Controlled Trial. Berthold HK, ed. *PLoS ONE* 2014; 9 (10): e109607.
19. Sollid ST, Hutchinson MY, Fuskevåg OM *et al*. No effect of high-dose vitamin D supplementation on glycaemic status or cardiovascular risk factors in subjects with prediabetes. *Diabetes Care* 2014; 37 (8): 2123-31.