ORIGINAL ARTICLE

Low Serum Levels of 25-Hydroxyvitamin D in Association with Type 2 Diabetes in Indian Patients

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Abstract

Objective: Vitamin D deficiency is a common disorder worldwide and is indicated by low serum 25-hydroxyvitamin D levels. Recent studies have suggested an association of 25-hydroxyvitamin D deficiency with diabetes mellitus. The objective of this study is to investigate the relationship between 25-hydroxyvitamin D concentration and type 2 diabetes in South Indian patients.

Materials and Methods: In a cross sectional study, we recruited 150 patients with type 2 diabetes and 150 age and sex matched controls, Yashoda hospital, Hyderabad, India, from December 2011 to November 2013. All cases and controls were evaluated for fasting serum glucose, fasting insulin, serum cholesterol, calcium, phosphorus, alkaline phosphatase, C-reactive protein (CRP), glycosylated hemoglobin (HbA1c) and 25-hydroxyvitamin D levels.

Results: In both 150 cases and 150 controls, mean age was 51.5 years and 70% participants were men. Deficiency of 25-hydroxyvitamin D (42.6%) (P<0.0001) and CRP positivity (48%) (P<0.0001) were significantly more common and mean alkaline phosphatase (143±3.4) (P<0.0001), mean HbA1c (8.3±1.8) (P<0.0001) and mean HOMA IR (3.80 +1.45) (P<0.0001) were higher in cases compared to controls. In type 2 diabetes, 25-hydroxyvitamin D deficiency was significantly associated with elevated longer mean duration of diabetes 15.1±4.3 years (P<0.0001), positive CRP (0.02), increased HOMA –IR (<0.0001), neuropathy 19 (29.6%) (P=0.03), and retinopathy 11(15.6%) (P=0.04).After adjustment using multiple logistic regression analysis, deficiency of 25-hydroxyvitamin D was independently associated with type 2 diabetes (odds: 2.8; 95% CI: 1.80 - 8.09).

Conclusion: Our study showed a 42.6% prevalence of 25-hydroxyvitamin D deficiency in type 2 diabetes and was independently associated with type 2 diabetes in Indian adults.

Key words: Diabetes mellitus, 25-hydroxyvitamin D, South Indian adults.

Introduction

Diabetes is a metabolic disease and can affect nearly all organ systems of human body (1). The prevalence of diabetes mellitus is rising rapidly worldwide and is reaching epidemic proportions (2) and it is estimated that currently 285 million people

suffer from diabetes worldwide and the number will be doubled to 438 million by the year 2030 (3). Similarly in India, nearly 41 million individuals are suffering with diabetes presently which will increase to 80 million by 2030 (4). Recent studies provide accumulating evidence that 25-hydroxyvitamin D deficiency is associated with type 2 diabetes (5). Vitamin D (calciferol) is a fat soluble seco-sterol and is found in food naturally. (6) Vitamin D has many forms, but mainly two major different forms of vitamin, ergocalciferol (vitamin D2) and cholecalciferol (25-hydroxyvitamin D or D3) are seen in blood. Assessment of 25-hydroxyvitamin D is an important marker for vitamin D status in individuals. Our aim is to investigate the association between 25-hydroxyvitamin D deficiency and type 2 diabetes in Indian adults; limited data are available from the Indian sub-continent.

Materials and Methods

In our analytical cross sectional study, we recruited 150 patients with type 2 diabetes and 150 age and sex matched control subjects consecutively from Yashoda Hospital Hyderabad, a tertiary care center in South India. The study was performed from December 2011 to November 2013. Data were collected from all cases and control subjects through face to face interviews and medical histories were reviewed by physicians. All type 2 diabetic patients underwent detailed general physical and neurological examination as well as including examination of bilateral fundus. Standardized methods were adapted from the behavioral risk factor surveillance system (7), by the Centers for Disease Control and Prevention regarding the following conditions: hypertension, alcoholism, cigarette smoking, and hypercholesterolemia. Standard techniques were applied for measuring blood pressure, height, weight, fasting blood glucose, fasting insulin, glycosylated hemoglobin (HbA1c), serum cholesterol, calcium, alkaline phosphatase, phosphorus, C reactive protein (CRP) and 25-hydroxyvitamin D for cases and controls. This study protocol was approved by the Institutional Ethics Committee and informed consent was taken from case and controls subjects.

Selection of cases

Patients with history of diabetes and who met the criteria for type 2 diabetes (fasting plasma glucose level $\geq$ 7.0 mmol/l and/or random (non-fasting) plasma glucose level $\geq$ 11.1 mmol/l and/or use of oral anti-diabetic medication and/or use of insulin and/or treatment by diet and diagnosed by a general practitioner as having diabetes) (8), older than 40 years old and not taking vitamin D supplements were defined as cases.

Selection of controls

Healthy subjects, age and sex matched to cases, non-diabetic with HbA1c <6.0 mmol/L and not taking vitamin D supplements were included as control subjects. The cut off value of 6 was based on WHO guidelines published in 2011 which states that HbA1c> 6.5 is suggestive of diabetes, while HbA1c values from 6-6.5 implies a high risk of developing diabetes. (9) Controls were selected from patient attendees and healthy volunteers working in the same hospital.

Exclusion criteria

Subjects (both cases and controls) were excluded if they had any of the following- prior evidence of vitamin D deficiency, history of consuming medication for osteoporosis (i.e., bisphosphonate, raloxifene, or calcitonin), other disorders affecting bone or Vitamin D or calcium metabolism, such as rheumatoid arthritis, malignancy, renal dysfunction (serum creatinine >1.5mg/ dL in men, >1.4mg/dL in women), chronic liver disease or Paget's disease, history of taking medications known to alter vitamin D metabolism, history of taking glucocorticoids, younger than 40 years old and recent acute illness.

Risk factors assessment

Hypertension by JNC VII (Joint National Committee) was defined as a systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg based on the average of 2 blood pressure measurements at the time of

admission, or a patient's self-reported history of hypertension or anti-hypertensive medication, supported by documents. Smokers were defined as those reporting daily smoking. Ex-smokers and occasional smokers were classified as nonsmokers. Alcoholics were defined as those in whom the alcohol consumption was >50 g/day (equivalent to 500 mL [2 drinks] of wine, 1,000 mL of beer, or > 5 drinks [units] of spirits). Body Mass Index (BMI) values from 25.0-30.0 were taken as overweight and BMI values >30 were taken as obese. As per guidelines of National Institute of Health (NIH), patients with serum cholesterol levels >200 mg/100 mL or those on anti-cholesterol medication were considered as hypercholesterolemia (10). CRP values over 0.6 mg/dl were considered as positive as per our hospital standards. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as: fasting insulin (μIU/mL)×fasting glucose (mmol/L)/22.5 (11). HOMA-IR was performed only in 120 patients with diabetes on oral hypoglycemic therapy and the remaining 30 patients on human insulin were excluded.

Diabetic neuropathy: Diabetic neuropathy was diagnosed by a neurologist based on clinical symptoms, neurological examination and nerve conduction studies.

Diabetic Retinopathy: Diabetic retinopathy was diagnosed based on a detailed evaluation of dilated pupils using slit lamp and indirect opthalmoscopic examination. (12)

Evaluation of 25-hydroxyvitamin D

25-hydroxyvitamin D levels were evaluated by Chemiluminescent Microparticle Immunoassay (CMIA) with an automated instrument in our lab. According to our lab manual, we considered 25-hydroxyvitamin D deficiency if the values were ≤ 20ng/ml and those≥ 20.1 ng/ml as normal (10).

Statistical analysis

Statistical analysis was performed using SPSS 14.0 window software (statistical package for the Social sciences, SPSS Inc). Continuous variables were presented as mean +standard deviation. Categorical variables were expressed as proportions. The student T-test was performed to test the differences between continuous variables, and Chi square test was used to study the association in proportions. Odds ratio was calculated for assessing association of hypovitaminosis D with type 2 diabetes. Multiple logistic regression analysis was performed before and after adjustments. All tests were two sided and p value <0.05 were considered statistically significant.

Results

We studied 150 diabetic patients and 150 controls during one year. Proportion of the participants with deficiency of 25-hydroxyvitamin D (P<0.0001), elevated CRP (P<0.0001) and mean alkaline phosphatase levels (P<0.0001) were significantly higher in type 2 diabetes compared to controls. There were no differences between type 2 diabetes and controls regarding prevalence of hypertension, smoking, alcohol intake, obesity and hypercholesterolemia (Table 1).

After adjustment using multiple logistic regression analysis, low 25-hydroxyvitamin D, female sex, CRP and obesity were found to have an independent association with type 2 diabetes (Table 2).

Further analysis among the diabetic patients with low 25 -hydroxyvitamin D levels and those with normal levels showed the hypovitaminosis D is more prevalent in women (P=0.0014), increased proportion of elevated CRP (P=0.02), obesity (P=0.01) and a higher mean alkaline phosphatase levels (P<0.0001) compared to type 2 diabetes with normal 25-hydroxyvitamin D. The complications of diabetic neuropathy and retinopathy were also more prevalent among diabetic patients with low 25 hydroxyvitamin D compared to normal levels (Table 3).

Discussion

In the present study, we found that deficiency of 25-hydroxyvitamin D was significantly associated with type 2 diabetes from South India. Similar findings were noted by other

Table 1. Baseline characteristics of cases and controls

Variable	Type 2 diabetes (n=150)	Controls (n=150)	*P*-value
Men	105 (70%)	105 (70%)	0.1
Age range	45-84	45-84	NS*
Mean age	51.5 (10.9)	51.5 (9.0)	0.6
Hypertension	87 (58%)	74 (56.9%)	0.9
Smokers	75 (50%)	74 (56.9%)	0.2
Alcoholics	35 (23.3%)	36 (27.6%)	0.4
Hypercholesterolemia	28 (18.6%)	32 (24.6%)	0.2
Obesity	20 (13.3%)	12 (8%)	0.1
Deficiency of 25-hydroxyvitamin D	64 (42.6%)	30 (23%)	<0.0001
Mean HOMA-IR**	3.8±1.45	1.2±0.39	<0.0001
Elevated CRP	72 (48%)	35 (23.3%)	<0.0001
Mean HbA1c (mmol/mol)	8.3±1.8	5.8±1.1	<0.0001
Mean alkaline phosphatase	143±3.4	104.1±4.5	<0.0001
Mean serum calcium	6.6±1.4	7.1±1.9	0.2
Mean serum phosphorus	2.4±0.7	2.6±0.9	0.1
Seasonal variation			
Assessment in summer	65 (43.3%)	50 (33.3%)	0.2
Assessment in winter	85 (56.6%)	100 (66.6%)	0.4

*Not Significant

**We analyzed HOMA-IR only in 120 type II diabetes patients

studies (13-15).

25-hydroxyvitamin D and type 2 diabetes

In our study, 25-hydroxyvitamin D deficiency was seen in 42.6% of type 2 diabetes and 23% of controls. Our findings were advocated by other studies (14-15). The present study also established 25-hydroxyvitamin D deficiency is independently associated with type 2 diabetes (Odds: 2.82; 95% CI: 1.80 - 8.09). However some studies have found no significant association between 25-hydroxyvitamin D and type 2 diabetes (16).

The relationship between type 2 diabetes and 25-hydroxyvitamin D is unclear. Some scientists have established serum 25-hydroxyvitamin D may have an influence on insulin action, pancreatic beta cell function and systemic inflammation. Vitamin D may affect receptors on pancreatic β cells, activating 1α hydroxylase in pancreatic β cells and may increase the expression of insulin gene. Vitamin D suppresses the renin gene reducing hyperglycemia and reduces the renin-angiotensin activity and pancreatic insulin secretion was demonstrated to be inhibited by vitamin D deficiency (17).

25-hydroxyvitamin D and HOMA IR

As expected in our study, we found a significant association of high levels of HOMA IR with type 2 diabetes compared to controls and high HOMA IR levels were significantly associated with 25-hydroxyvitamin D deficiency. This gives weight to the underlying pathophysiological mechanism of insulin resistance being exacerbated by hypovitaminosis D. While few other studies have also found similar findings (18), some studies have found negative association of HOMA IR with 25-hydroxyvitamin D deficiency (11).

25-hydroxyvitamin D and HbA1c

We noted higher mean levels of HbA1c in type 2 diabetes patients with deficiency of 25-hydroxyvitamin D, recent studies have also found similar findings (13-15).

HbA1c is a surrogate marker for glucose control and can be a measure of average blood glucose concentrations in the three months prior to the test as well as long-term glucose homeostasis. Abnormalities may arise from changes in both insulin secretion and insulin-stimulated uptake of glucose in muscle and fat tissue. The active form 1,25 hydroxyvitamin D effects on both insulin secretion and sensitivity as demonstrated in in-vitro studies and studies on laboratory animals (19). Vitamin D receptors have also been noted on adipocytes in male, Wistair rats suggest an added role of vitamin D on insulin sensitivity (20). These experimental studies support our clinical findings.

25-hydroxyvitamin D and Obesity

In our study we noted that deficiency of 25-hydroxyvitamin D was significantly associated with obesity in type 2 diabetes i.e., in 15 patients (23.4%), compared to type 2 diabetes with normal 25-hydroxyvitamin D. Our findings are advocated by other studies (11,21).

Manco et al. in his study showed a relationship between 25-hydroxyvitamin D levels and insulin sensitivity before and after bariatric surgery, they established increase in insulin sensitivity after bariatric surgery although 25-hydroxyvitamin D levels were still low (22). Kamycheva et al demonstrated that low calcium and vitamin D intake were associated with obesity in both genders (23). Several studies have found low vitamin D associated with decreased insulin sensitivity in obese population (24,25). In our study we established 25-hydroxyvitamin D deficiency to be independently associated with obesity in type 2 diabetes (Odds: 1.195%CI: 0.81-1.70).

Various hypotheses have been considered in the association of vitamin D deficiency with obesity. The body senses the seasonal change in ultraviolet (UV) component of the sunlight by the level of skin synthesis of vitamin D which acts as a UV-radiation receptor. Vitamin D deficiency falsely reduced UV stimulation like winter and increases the set point of body weight in hypothalamus. This hypothesis "winter response" suggests that inadequate dietary vitamin D and sunlight exposure, acts as a situation similar to the winter causing fat accumulation to preserve heat and leads to obesity (26).

The other proposed mechanisms include inhibition of expression of uncoupling protein (UCP) 2 in adipose tissue, differentiation of preadipocytes, synthesis and secretion of lipoprotein lipase by vitamin D. UCPs are inner mitochondrial membrane transporters which determine resting energy expenditure (REE). Wong and colleagues showed that obesity was more in mice with modified VDR (vitamin D receptor) compared to normal mice due to decreased REE and β-oxidation of fatty acids (27).

25-hydroxyvitamin D and Diabetic Neuropathy

In our study we noted diabetic neuropathy was significantly associated with 25-hydroxyvitamin D deficiency (19 patients (29.6%)) compared to type 2 diabetes with normal 25-hydroxyvitamin D levels and an odds ratio of 3.2 (95%CI 1.3-7.5). A recent study showed similar prevalence of diabetic neuropathy (17.8%) associated with 25-hydroxyvitamin D deficiency (28). A much larger prevalence of diabetic neuropathy (58.5 to 67.5%) was noted by Ahmaieh et al., in 25-hydroxyvitamin D deficient patients (13).

The mechanism of vitamin D deficiency and diabetic neuropathy was explored by Naveilhan et al., he noted that vitamin D deficiency may enhance the diabetic nerve damage and worsen neuropathic pain (29). Vitamin D may act as a marker of health and may not directly involve the nerve function and it may influence indirectly control of diabetes (30). This positive effect is further advocated by the findings in Lee and Chen study, where vitamin D supplementation in diabetic patients for 3 months reduced neuropathic pain by at least 50% intensity in half of patients (31).

25-hydroxyvitamin D and Diabetic

Table 2. Odds ratio of various risk factors in type2 diabetes after Multiple logistic regression (in stepwise method)

Parameters	Odds ratio	95% Confidence interval
Hypertension	0.16	0.07-0.34
Low 25-hydroxyvitamin D	2.82	1.80-8.09
Smoking	0.29	0.11-0.72
Alcoholism	0.05	0.006 to 0.46
Hypercholesterolemia	0.04	0.007-0.36
CRP positive	1.61	1.20-1.92
Women	1.39	1.10-1.83
Obesity	1.1	0.81-1.70

Table 3. Comparison between deficiency and normal of 25-hydroxyvitamin D in type 2 diabetes

Variable	<20 ng/ml (n=64)	>20ng/ml (n=86)	*P*-value
Women	43 (67.2%)	34 (39.5%)	=0.0014
Age range	43-84	41-82	
Mean age	58.6 (11.7)	56.7(10.2)	
Hypertension	40 (66.6%)	47 (55.9%)	0.2
Smokers	20 (31.2%)	30 (34.8%)	0.4
Alcoholics	12 (18.7%)	23 (26.7%)	0.3
Hypercholesterolemia	15 (23.4%)	9 (10.4%)	0.03
CRP positive	38 (59.3%)	34 (39.5%)	=0.02
Obesity	15 (23.4%)	5 (5.8%)	=0.003
Mean HbA1c (mmol/mol)	9.1±2.8	7.9±1.1	<0.0001
Mean duration of diabetes	15.1±4.3	8.6±2.1	<0.0001
Mean alkaline phosphatase	113±2.1	102±3.9	<0.0001
Mean serum calcium	4.5±1.8	5.9±2.7	0.2
Mean serum phosphorus	3.2±0.5	2.7±1.1	0.1
HOMA-IR* (n=120)	4.7±1.4 (n=52)	3.5 ± 1.0 (n=68)	<0.0001
Complications			
Retinopathy	11 (15.6%)	4 (4.6%)	0.04
Neuropathy	19 (29.6%)	10 (11.6%)	0.03
Seasonal variations			
Summer	29 (45.3%)	36 (41.8%)	0.9
Winter	39 (60.9%)	46 (53.3%)	0.8

*We analyzed HOMA-IR in 52 patients of deficiency group and 68 normal groups.

Retinopathy

We, also found a significant higher prevalence of diabetic retinopathy with hypovitaminosis D in 11 patients (15.6%), compared to type II diabetic patients with normal 25-hydroxyvitamin D (odds ratio- 4.2; 95%CI 1.2-14.0) our finding are advocated by other studies (13,16,32).

Recent studies have suggested a possible causal role of Vitamin D deficiency in proliferative diabetic retinopathy, characterized by neovascularization and angiogenesis. Vitamin D interacts with vascular endothelial growth factor (VEGF) and addition of 1, 25 hydroxyvitamin D reduced VEGF-induced proliferation in vitro, on aortic endothelial cell culture (33). In vivo evidence of the inhibitory effect of 25-hydroxyvitamin D on angiogenesis, have been demonstrated in both transgenic retinoblastoma and ischemic retinopathy models (34). Our study adds to an accumulating evidence on the strong correlation of vitamin D deficiency and diabetic retinopathy (32).

25-hydroxyvitamin D and Gender

In our study women constituted 67.2% of the patients who had both 25 hydroxyvitamin D deficiency and type 2 diabetes. This was significantly more than the proportion of women in the group with type 2 diabetes with normal vitamin D levels, our findings are advocated by other studies (5,16). Our study established an independent association (odds: 1.39; 95%CI: 1.10-1.83)

In Indian population Vitamin D is lower in the women population which can be due to the pattern of dressing and occupation. We also found that the prevalence of vitamin D deficiency in the controls showed similar gender predilection for females. However some studies have found no significant female preponderance of hypovitaminosis D and diabetes (35).

25-hydroxyvitamin D and Seasonal variations

As vitamin D levels may vary with season we also assessed the effect of seasonal variation between the various groups. In our study we found no significant difference in the association between 25-hydroxyvitamin D deficiency and type 2 diabetes in winter or summer. Previous studies have conflicting results (36-37). The seasonal variation in deficiency of vitamin D depends on sun exposure and geographic features in particular areas. This effect is lesser in tropical regions,

especially South India where the winter and summer variations are only ± 10 to 15^0 C.

25-hydroxyvitamin D and CRP

Chronic low-grade inflammation is associated with development of insulin resistance and confers increased risk of type 2 diabetes. In our study we found positive CRP was significantly associated with type 2 diabetes. Frohlich et al noted CRP as a marker of subclinical systemic inflammation, was associated with hyperglycemia and insulin resistance (38). In the present study, positive CRP was significantly more prevalent in diabetics with deficiency of 25 hydroxyvitamin D with an odds ratio of 1.61;95%CI: 1.20-1.92. A similar finding was noted by Aronson et al (39).

CRP is a marker of low-grade inflammation. Vitamin D has anti-inflammatory activity and reduces cytokine release from monocytes, CRP and other factors such as nuclear factor-κB (NFκB) activity and toll-like receptor (TLR) 4 expression. A recent study have shown the more emphasized effects in patients with type 2 diabetes (40). Elevated CRP stimulates production of endothelial adhesion molecules which impair vascular reactivity, and thereby reduces insulin delivery and increases peripheral insulin resistance (41).

Conclusion

Our study established that deficiency of 25-hydroxyvitamin D has an independent association with type 2 diabetes. We did not found any significant seasonal variations. There is a probable association of 25 hydroxyvitamin D with the microvascular complications of type 2 diabetes - diabetic neuropathy and diabetic retinopathy as well as obesity. However these were based on univariate analysis and the effect may be compounded by a higher HBA1c. Our sample size is small and we couldn't further analyze their independent associations. The strength of our study was that we recruited all cases and controls from the same hospital with a common standardized clinical assessment and bio-chemical analysis. This is a probable first step in establishing a need for preventing and treating vitamin D deficiency. Fortification of widely consumed main foods with vitamin D is a simple and viable in India and further studies are required to establish its necessity.

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