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Biochemistry

HYPOVITAMINOSIS D EXACERBATES TYPE 2 DIABETIC COMORBIDITIES

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ABSTRACT Vitamin D deficiency is one of the prominent nutritional deficiencies in India that needs special attention. The effects of hypovitaminosis D on skeletal and cardiovascular functions are well known. However, its effect on metabolic disorders like type 2 diabetes mellitus (T2DM) is still left unexplored. In the present study, our primary aim is to find out the potential effect of hypovitaminosis D in T2DM patients. The study was conducted on 250 T2DM patients mainly from Madhya Pradesh, India. Among them, 125 had hypovitaminosis D (case group) and were compared against the control group of 125 patients with normal serum vitamin D. We were mainly investigating the major T2DM-related complications including chronic kidney disease (CKD), coronary heart disease (CHD and recurrent infections. Major organ functions including liver, kidney, and cardiac functions were affected by hypovitaminosis D in T2DM patients when compared to control counterparts. We also noticed an association between hypovitaminosis D and the exacerbation of T2DM comorbidities. Our findings show the importance of maintaining normal serum vitamin D levels in T2DM patients to avoid further complications.

KEYWORDS : Hypovitaminosis D, T2DM, CKD, CHD, Chronic infections

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder found mainly in old age people due to a relative deficiency of insulin that is characterized by hyperglycemia and associated complications. As per the estimate of the International Diabetes Federation (IDF), in 2019, there were around 77 million diabetic individuals in India, and the burden is expected to be doubling by the year 2045.[1,2] Multiple factors including genetic, lifestyle, environmental, and nutritional factors play a significant role in the development of T2DM. The development and progression of T2DM are relatively chronic and generally treated strategically with a combination of diet, exercise, medication, and insulin therapy.[3] The prevalence of T2DM in urban and metro cities is found to be higher, and most of the city population is suffering from lifestyle disorders of one or the other kind. Type 2 DM is the most common type of diabetes that contributes to approximately 90% of the global diabetic population. Around 2% of the total mortality is contributed by DM in the Indian subcontinent. It is an alarming fact that one in six adults with diabetes in the world is from India.[4,5]

Vitamin D, a fat-soluble vitamin, is one of the key nutritional factors that are likely to have an important role in either regulation of glycemic control or the reduction of diabetic complications. The role of vitamin D in blood glucose homeostasis is still unclear completely. However, the β cell dysfunction and insulin resistance in subjects with vitamin D deficiency are evident in recent studies.[6] Recent studies have also shown the effect of vitamin D on the improvement of β cell function, insulin action, and prolongation of the life of β cells.[7]

This deficiency of vitamin D has many consequences other than well-studied skeletal and cardiovascular complications. Though several epidemiological studies demonstrate an inverse association between decreased serum 25-hydroxyvitamin D3 (25(OH)D3) and glucose intolerance, some intervention trials using vitamin D show mixed results. The production of vitamin D in the skin cannot be measured directly; however, the change in serum levels of 25(OH)D3 could be an ideal candidate for getting an overall idea about the effect of sunshine exposure if diet and the vitamin D nutritional status of the individual remains constant.[8]

Vitamin D deficiency plays a significant role in the development of rickets, osteoporosis, cardiovascular diseases, diabetes, cancer, and infections such as tuberculosis in the Indian population.[9] The atherogenic index of plasma (AIP), a potential clinical parameter can be employed to evaluate the changes within the lipoproteins, stroke, and cardiovascular disease (CVD) risks.[10] Vitamin D deficiency is highly prevalent in both urban and rural locations in India, including all socioeconomic and geographic strata.[11] People with DM are at increased risk of other diseases including heart, peripheral arterial, and cerebrovascular diseases, obesity, cataracts, erectile dysfunction, and non-alcoholic fatty liver disease. They are also at increased risk of some infectious diseases such as tuberculosis.[3,12]

The present study is mainly aimed at throwing some light on the association of vitamin D (25(OH)D3) levels in T2DM comorbidities. Here, we adopt a prospective way to correlate the serum levels of vitamin 25(OH)D3 in T2DM patients with their associated complications. We hypothesize that the serum levels of vitamin 25(OH)D3 have a significant role in the development and progression of T2DM, especially on a long-term basis.

MATERIALS AND METHODS

The study population was selected from the out-patient department (OPD) and in-patient department (IPD) of medicine, Index Medical College, Hospital & Research Center, Madhya Pradesh, with a total of 250 T2DM patients (n = 250) of 40 to 65 years age group, in which 125 cases of hypovitaminosis D were compared against 125 age and gender-matched normal vitamin D T2DM control patients. As per the standard protocol, written consent was taken from every individual who is involved in the study. The study was started after getting formal approval from the concerned department and the institutional ethics committee (IEC) of the hospital. Confirmed and newly diagnosed T2DM cases (WHO, 2011) were included.[13] However, patients with severe illness and those on corticosteroids and hormone replacement therapy were excluded. Patients who were taking vitamin D supplementation or drugs affecting vitamin D metabolism (Phenytoin, Rifampicin,) within 6 weeks of the study were also excluded.

A five-milliliter venous blood sample was drawn aseptically from the median cubital vein and collected in a plain vial. The serum was separated by centrifugation at 3000 rpm on a Remi R-8C centrifuge (Maharashtra, India). The serum sample was immediately used for the clinical chemistry and hormonal assays, a part of which was stored at -20˚C refrigerator for further assays.

All routine clinical chemistry parameters including fasting blood glucose, liver function tests, and kidney function tests were analyzed on a fully automated chemistry analyzer XL-640 (Transasia Bio-Medicals Ltd, Mumbai, India). Both chemical and enzymatic methods were employed as per the standard protocols used worldwide by the system manufacturer.

The serum vitamin 25(OH)D3 level was studied on an immunoassay system - ADVIA Centaur XP (Siemens, Germany). It employs an advanced immunofluorescence principle for the analysis of various serum analytes. The present technique is quite sensitive, fast, cost-effective, and hazard-free. All the autoanalyzers were pre-calibrated,

and quality controls (QCs) were run timely as advised by the concerned manufacturers. Urine routine estimation (urine RE) was performed manually. The presence of cells, bacteria, yeast, and casts was checked by routine urine microscopy. Urinalysis strips were used for the detection of glycosuria and proteinuria.

Samples were run in duplicates and the mean results obtained are represented as mean ± SD. We have used a nonparametric statistical tool, the student *t*-test for comparing one variable between two independent samples or groups. Correlation analysis was done by the Spearman rank correlation method. A p-value <0.05 was considered to be significant, and a p-value of <0.01 was considered to be highly significant for a given hypothesis testing. All statistical analyses were performed using GraphPad Prism Ver.6.0 (GraphPad Software, Inc., CA, USA) and Microsoft Excel, MS office 2019 (Redmond, WA, USA).

RESULTS

The control group had 54 men and 71 women. The case group had 49 men and 76 women. The average age of the control group was 48.9 ± 7.39 years, whereas the average age of the case group was 49.02 ± 8.05 years.

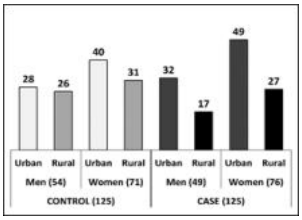

Figure 1: Distribution of gender and lifestyle in control and case groups.

Table 1: Comparison of various parameters among the control and case groups

	CONTROL (Normal Vit. D)	CASE (Hypo Vit. D)	p-value
Fasting Blood Glucose (mg/dL)	129.57 ± 25.42	141.71 ± 27.95	0.0004
Vitamin 25(OH)D3 (nmol/L)	96.52 ± 18.92	29.41 ± 18.24	0.0001
Serum AST activity (U/L)	24.89 ± 10.57	32.67 ± 14.56	0.0001
Serum ALT activity (U/L)	28.33 ± 17.46	30.36 ± 17.49	0.3593
AST/ALT Ratio (De-Ritis Ratio)	1.06 ± 0.49	1.35 ± 1.19	0.0124
Serum ALP activity (U/L)	52.42 ± 22.48	58.05 ± 29.55	0.0913
Total bilirubin (mg/dL)	1.32 ± 0.43	1.54 ± 0.44	0.0001
Direct bilirubin (mg/dL)	0.28 ± 0.10	0.37 ± 0.11	0.0001
Total Cholesterol (mg/dL)	160.42 ± 28.16	171.47 ± 19.78	0.0004
HDL-Cholesterol (mg/dL)	45.86 ± 6.89	42.07 ± 3.25	0.0001
LDL-Cholesterol (mg/dL)	89.01 ± 27.47	96.45 ± 20.16	0.0153
VLDL-Cholesterol (mg/dL)	25.54 ± 4.83	32.95 ± 5.41	0.0001
Triglycerides (mg/dL)	127.70 ± 24.16	164.73 ± 27.04	0.0001
LDL-Chol/ HDL-Chol Ratio	1.82 ± 0.43	2.31 ± 0.54	0.0001
Total Chol/HDL-Chol ratio	3.59 ± 0.68	4.11 ± 0.60	0.0001
Atherogenic index of plasma (AIP)	0.44 ± 0.10	0.59 ± 0.08	0.0001
Systolic blood pressure (mmHg)	126.11 ± 3.47	130.37 ± 6.79	0.0001
Diastolic blood pressure (mmHg)	79.27 ± 4.21	80.24 ± 5.63	0.1242
Serum urea (mg/dL)	29.04 ± 15.64	51.97 ± 28.69	0.0001
Serum creatinine (mg/dL)	0.69 ± 0.25	1.15 ± 0.95	0.0001
Serum uric acid (mg/dL)	4.71 ± 1.66	5.42 ± 1.8	0.0014

AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, HDL: High-density cholesterol, LDL: Low-density cholesterol, VLDL: Very low-density cholesterol, AIP: Atherogenic index of plasma, mmHg: millimeters of mercury, mg/dL: milligrams per decilitre, nmol/L: nanomoles per liter, U/L: Units per liter.

The risks of various T2DM-related comorbidities were also analyzed and correlated with their clinical conditions. Those who were living in the urban city area for not less than 3 months were considered under the urban category. For the assessment of CKD risk, serum urea, creatinine, uric acid, and routine urine examination were considered. For the assessment of CHD risk, their blood pressure, lipid profile, and lipid ratios were considered. The blood pressure and the AIP were mainly considered for the assessment of the stroke in the study group. Retinopathy and cataract were confirmed from their medical records and their ophthalmology findings. Other information like family history and medical history was mainly collected from the patients through personal interviews. Diabetic foot and recurrent infections were confirmed and correlated with their clinical data. Patients with one or more of the above-mentioned complications were considered to be having T2DM-related comorbidity.

Table 2: Potential risks associated with T2DM patients with hypovitaminosis D

	CASE (125) (Vitamin 25(OH)D3 Deficient/ Insufficient)	CONTROL (125) (Vitamin 25(OH)D3 Sufficient)	Odds Ratio (OR)
Risk of chronic kidney disease (CKD) in hypovitaminosis D patients			
Abnormal renal function	40	16	3.21
Normal renal function	85	109	
Risk of coronary heart disease (CHD) in hypovitaminosis D patients			
Abnormal cardiac function	25	10	2.88
Normal cardiac function	100	115	
Risk of stroke in hypovitaminosis D patients			
Abnormal API	8	3	2.78
Normal API	117	122	
Risk of loss of libido in hypovitaminosis D patients			
Low libido	27	19	1.54
Normal libido	98	106	
Risk of retinopathy in hypovitaminosis D patients			
Retinopathy	5	3	1.69
No retinopathy	120	122	
Risk of cataracts in hypovitaminosis D patients			
Cataract	29	22	1.41
No cataract	96	103	
Risk of diabetic foot in hypovitaminosis D patients			
Diabetic foot	9	7	1.31
No diabetic foot	116	118	
Risk of recurrent infections in hypovitaminosis D patients			
Recurrent infections	33	16	2.44
No recurrent infections	92	109	
Risk of T2DM-Related Complications in hypovitaminosis D patients			
Apparent T2DM-related complications (one or multiple)	99	42	7.52
No apparent T2DM-related complications	26	83	

DISCUSSION

The fasting blood glucose in the case group (hypovitaminosis D) was significantly elevated when compared to that of the control counterparts (Table 1). We observed that the T2DM complications are found to be increasing with the advancement of age also. Almost all observational studies show the same results.[14] Apart from aging, other major factors that contribute to T2DM morbidity include population growth, urbanization, low physical activity, and obesity.[15] The serum vitamin 25(OH)D3 in the case group was 29.41 ± 18.24 nmol/L when compared to that of the control counterparts (96.52 ± 18.92 nmol/L). Our findings show that hypovitaminosis D in the case population was irrespective of their age, gender, and geographical location. No significant association of urbanization was found in the development of hypovitaminosis (OR 1.04). However, in our study, urbanization is found to be associated with the development of T2DM and related complications in T2DM patients (OR 1.54).

Studies by Aparna et al (2018) and Calvo-Romero & Ramiro-Lozano (2015) show that the risk of hypovitaminosis D and related complications is higher in females than in males of the same age groups.[16,17] It has been revealed in recent studies that, vitamin D deficiency predisposes to DM in both animal models and human beings.[18] Ahmadieh *et al* (2013) and Autier *et al* (2014) showed that a low serum 25-OHD level was an independent predictor of poor glycemic control, diabetic neuropathy, and diabetic retinopathy in patients with T2DM.[19,20]

We found that the serum transaminase activity in both cases and controls was in the normal range. However, the AST level and the De Ritis ratio were found to be significantly elevated in the case group. The ALP level also was elevated in the case group, but of less significance. There is evidence of non-alcoholic fatty liver disease (NAFLD) development due to hypovitaminosis D.[21,22]

The total cholesterol, TG, and bad cholesterol (LDL-cholesterol) were found to be significantly elevated in the case group when compared to that of the control counterparts. We also found abnormal lipoprotein ratios in the case group. A recent cohort study conducted on 3788 patients by Jiang et al (2019) showed evidence of dyslipidemia in hypovitaminosis D.[23] Another study that supports our findings is by Glueck *et al* (2016), where they showed an inverse association of serum vitamin (OH)D3 with CVD mortality, and inverse relationships with low-density lipoprotein cholesterol (LDLC), triglyceride, and homocysteine.[24] The API and systolic BP were also found to be significantly elevated in the case group, suggesting their risk of having stroke and CVD in the future. A cross-sectional case-control study by Bajaj et al (2014), shows hypovitaminosis D in type 2 diabetes is significantly associated with microvascular complications including neuropathy, retinopathy, and nephropathy.[25] Our study results also show comparable observations.

The serum non-protein nitrogen (NPN), mainly urea, creatinine, and uric acid was found to be elevated significantly in the case group when compared to that of the control group. Studies show that there will be a nitrogen imbalance in metabolic disorders like DM, and the negative nitrogen balance sets in due to poor control of blood sugar. During uncontrolled T2DM, there will be an increased gluconeogenesis and muscle breakdown that results in negative nitrogen balance in general.[3,26] Our findings are supported by previous studies where negative nitrogen balance could be found in aging T2DM patients with low vitamin D3 levels.

Glycosuria and proteinuria were major abnormal urinary findings in diabetic cases, while the main abnormality found in some control urine samples was the presence of pus cells or WBCs and RBCs in trace amounts. When we compiled the obtained data for identifying the risk of CKD in hypovitaminosis D diabetics, we obtained an OR value of 3.21. That says CKD is positively associated with T2DM and glycosuria and proteinuria found in our findings suggest possible near-future renal damage if untreated. There are supporting studies in which hypovitaminosis D is shown to be one of the major reasons for CKD.[27] When we examined the risk of T2DM comorbidities in hypovitaminosis D patients, we obtained an OR value of 7.52. It implies a strong positive association and a very high risk of T2DM-related complications in patients with hypovitaminosis D.

CONCLUSION

Hypovitaminosis D has got various negative effects on cell metabolism and physiology. The present study reveals the potential effect of hypovitaminosis D on T2DM comorbidities where the complications are exacerbated during deficiency or insufficiency of vitamin 25(OH)D3. Major organ functions including cardiac, hepatic, and renal functions are affected significantly. T2DM patients who are deficient in vitamin 25(OH)D3 are more prone to recurrent infections. The results suggest the importance of keeping a normal level of vitamin D in diabetic patients to avoid or reduce potential comorbidities.

LIMITATIONS

The current study is mainly focused on T2DM patients from Indore and neighboring districts in Madhya Pradesh. A detailed study on a larger population (pan-India) is required for more valid results.

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CONFLICT OF INTERESTS

The authors hereby declare that they do not have any conflict of interest related to this original work.

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