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Original Article

Biochemistry Section

Serum 25-OH Cholecalciferol (Vitamin D) Levels among Patients with Uncontrolled Type 2 Diabetes Mellitus- A Case-control Study

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ABSTRACT

Introduction: Type 2 Diabetes Mellitus (T2DM), a common metabolic disorder characterised by hyperglycaemia is caused due to an absolute or relative insulin deficiency and/or insulin resistance. Vitamin D, a steroid hormone beyond its primary role on calcium and bone metabolism, also has been shown to have multiple other effects. Vitamin D levels have been studied in relation to glucose metabolism as role in insulin secretion and insulin resistance by many studies in the past.

Aim: To estimate the levels of serum 25-OH vitamin D and to correlate with glycaemic status among uncontrolled T2DM patients.

Materials and Methods: A case-control study was conducted during August-September 2021 at Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research in Melmaruvthur, Tamil Nadu, India. In this study, 50 cases (uncontrolled T2DM) and 50 controls (healthy individuals), of both sexes aged between 40-60 years were included. Blood samples were analysed for serum 25-OH cholecalciferol (Vitamin D), fasting and postprandial plasma glucose levels and statistical analysis was done by Independent sample t-test for significance testing, odds ratio for exposure-outcome association by logistic regression and Pearson coefficient for correlation using Statistical Package for the Social Sciences (SPSS) software version 18.0.

Results: Serum 25-OH vitamin D levels were significantly {p<0.005 (t=-9.005)} lower in cases {31.02±7.51 ng/mL} when compared to controls {48.30±11.29 ng/mL}. Logistic regression showed none of the predictor variables studied showing significant outcome association (p<0.05) for vitamin D predeficiency while Pearson correlation showed significant negative correlation with fasting (r=-0.463) and postprandial plasma glucose (r=-0.568), respectively at 0.01 level (2-tailed).

Conclusion: Serum 25-OH Vitamin D levels were significantly lower and have significant inverse association with fasting plasma glucose levels and postprandial plasma glucose levels in uncontrolled T2DM patients. This substantiates the role of vitamin D in maintaining normal plasma glucose levels in T2DM patients. It is thereby proposed that serum 25-OH vitamin D levels be measured and followed-up for better glycaemic control among T2DM patients.

Keywords: Hyperglycaemia, Insulin resistance, Insulin release, Predeficiency states

INTRODUCTION

T2DM, a common metabolic disorder characterised by hyperglycaemia is caused due to absolute or relative insulin deficiency and/or insulin resistance. According to International Diabetes Federation (IDF) data, among 463 million adults are suffering from diabetes around the world, 88 million people are from India, which comprises around 8.8% of the total population of the country [1]. The ever increasing prevalence of T2DM in India and the estimated prevalence of 152 million in 2045 by IDF is alarming for the wellbeing of the people of the country and the humanity as a whole [1].

Vitamin D, a steroid hormone beyond its primary role on calcium and bone metabolism, also has been shown to have multiple other biochemical effects. Vitamin D insufficiency or deficiency around the world has been estimated to be around 1 billion people and has become one of the common community deficiencies around the world including tropical countries like India. Studies have shown that vitamin D deficiency prevalence is higher among T2DM patients [2]. The association of serum 25-OH vitamin D levels with T2DM has been researched on all aspects with respect to risk of incidence, progression, glycaemic status association and complications. Vitamin D acts through multiple mechanisms including role in insulin release and role in insulin action which are the primary issues in T2DM patients. Thereby, vitamin D levels also have been studied in relation to glucose metabolism as role in insulin secretion and insulin resistance from various laboratory studies in-vitro [3].

But there is a pertinent lack of similar studies in the south Indian rural diabetic population as most studies were focused on urban population at large [4,5]. Considering the gaps in existing literature evidences on the role of vitamin D in glycaemic control among T2DM patients, as several population studies have established the role of vitamin D at multiple levels of glucose homeostasis while some, failed to establish the prevalence of low levels and its association with diabetes incidence or complications. Beyond extensive studies, there are also gaps in present clinical scenario, as there is absence of vitamin D estimation and correction as a part of glycaemic control components in existing management guidelines for T2DM [6]. Patients on the one hand require unwarranted indiscriminate use of expensive testing of vitamin D levels based on existing literature studies [7], while on the other hand they are T2DM.

The present study will further enhance the knowledge on the use of vitamin D testing among uncontrolled T2DM patients and also understanding towards its role as a part of glycaemic control measure in understudied rural T2DM patients. Therefore, the importance and the need for the study lies in its potential towards changing the outlook of glycaemic control management guidelines on rural T2DM patients which by itself can be extrapolated on a large scale to global diabetic population.

So, the study design was proposed to test the null hypothesis statement that the serum 25-OH vitamin D levels have no effect on the glycaemic status in patients with uncontrolled T2DM. The study aimed at estimating the levels of serum 25-OH vitamin D levels and correlating it with glycaemic status among uncontrolled

T2DM patients at Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research Center Hospital, Melmaruvathur, Tamil Nadu, India.

MATERIALS AND METHODS

A case-control study was conducted during August-September 2021 in a peripheral tertiary care institute-Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research Center Hospital, Melmaruvathur, Tamil Nadu, India. The study was conducted after obtaining formal approval from Institutional research and Ethics Committee (IEC) of the college {Ref No.: (MAPIMS/IEC/52/2021/176(07)2021) dated 28.7.21}. Informed consent was obtained from all participants before the conduct of study.

Inclusion Criteria

Cases: Known T2DM (on treatment with oral hypoglycaemic agents for five years duration) with uncontrolled glycaemic status (with fasting hyperglycaemia defined as fasting plasma glucose levels ≥126 mg/dL and postprandial hyperglycaemia defined as postprandial glucose levels ≥140 mg/dL) [8] attending general medicine outpatient department of Adhiparasakthi Hospitals.

Controls: Age and sex matched controls- normal healthy volunteers attending master health check-up of Adhiparasakthi Hospitals.

Exclusion Criteria

Participants with known history of dermatological disease, liver and biliary tract disease, renal disease, intestinal disease, bone disease, malnourished individuals and on vitamin D supplements were excluded.

Sample size: The minimum sample size calculated for the study by statistician using formula $\{n=\{(\sigma_1^2+\sigma_2^2)^2 (Z_{1-\beta}+Z_{1-\alpha/2})^2\}/d^2\}$ was 88 (44 for each group) with Brijesh M and Patra S, as reference [9]. The sample size was calculated with power ($Z_{1-\beta}$=0.84) was 80% and confidence interval ($Z_{1-\alpha/2}$=1.96) was 95% for the study.

The sampling procedure was simple random sampling. The study participants included 50 cases and 50 controls, of both sexes aged between 40-60 years. The participants were included into the study from the Outpatient Department (OPD) of general medicine (for cases) and master health check-up (for controls) of Adhiparasakthi Hospitals, Melmaruvathur, Tamil Nadu, India. Clinical history including diabetic history, medications, duration and treatment follow-up, diet history, sun exposure, lifestyle activities were obtained and baseline clinical examination was conducted before drawing blood sample.

Sample collection: Blood sample (6 mL) was collected after fasting (overnight 8 hour) and postprandial (after 2 hours of food intake) by venepuncture under strict aseptic precautions.

Analytical methods: The collected blood sample was analysed for serum 25-OH vitamin D levels, fasting plasma glucose and postprandial plasma glucose levels. Estimation of serum 25-OH vitamin D levels was done by Electrochemiluminescence immunoassay (ECLIA) [10] using roche cobas e411 analyser. Estimation of fasting plasma glucose and postprandial plasma glucose was done by glucose oxidase peroxidase method [11] using biosystems BA400 fully automated clinical chemistry analyser in central laboratory of Adhiparasakthi Hospitals, Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research, Tamil Nadu, India.

STATISTICAL ANALYSIS

The data were tabulated and statistical analysis which included student's t-test and Chi-square test as tests for significance, odds ratio by logistic regression as test for outcome association and Pearson correlation coefficient as test for correlation was done using statistical SPSS version 18.0.

RESULTS

The baseline demographic characteristics of both the groups (cases and controls) which includes age (years), sex {n(%)}, male: female ratio, duration of sunlight exposure per day (minutes), drug history (only for cases), diabetes duration (only for cases), diet history, physical activity along with test statistics and p-value (as applicable) are described in [Table/Fig-1]. There was no statistically significant difference noted among the demographics between the two groups.

Parameters	Cases	Controls	Test statistics	p-value
Age (years)	51.48±6.21	51.92±5.63	t-value= -0.371	0.712
Male {N (%)}	32 (64%)	35 (70%)	χ^2=0.407	0.523
Female {N (%)}	18 (36%)	15 (30%)		
Sex ratio {Male: Female}	1.77:1	2.33:1		
Sunlight exposure per day (minutes)	29.70±9.71	31.80±10.77	t-value= -1.024	0.308
Duration of diabetes (years)	5.4±0.3	0	NA	NA
Drug history				
a. Metformin and Sulfonylurea {N (%)}	26 (52%)	0	NA	NA
b. Metformin, Sulfonylurea and Voglibose {N (%)}	24 (48%)	0		
Diet history				
a. Carbohydrate intake {gm/day}	364.4	359.2	t-value=1.190	0.237
b. Lipid intake {gm/day}	37	38	t-value=-1.043	0.299
c. Protein intake (gm/day)	53.50	52	t-value=0.995	0.322
Physical activity				
a. Sedentary lifestyle {N (%)}	14 (28%)	12 (24%)	χ^2=0.208	0.648
b. Active lifestyle {N (%)}	36 (72%)	38 (76%)		

[Table/Fig-1]: Demographic data of controls (n=50) and cases (n=50).
p-value ≤0.05 is significant; Quantitative variables between two groups were compared using Independent sample t-test (t-value) and Qualitative variables using chi-square test (χ^2);
NA: Not applicable

The results of the study showing the (mean±standard deviation) levels of serum 25-OH vitamin D, fasting plasma glucose and postprandial plasma glucose for both the groups {group1-cases, group 2-controls} along with t value and p-value with level of significance is shown in [Table/Fig-2] and logistic regression model with odds ratio is included in [Table/Fig-3].

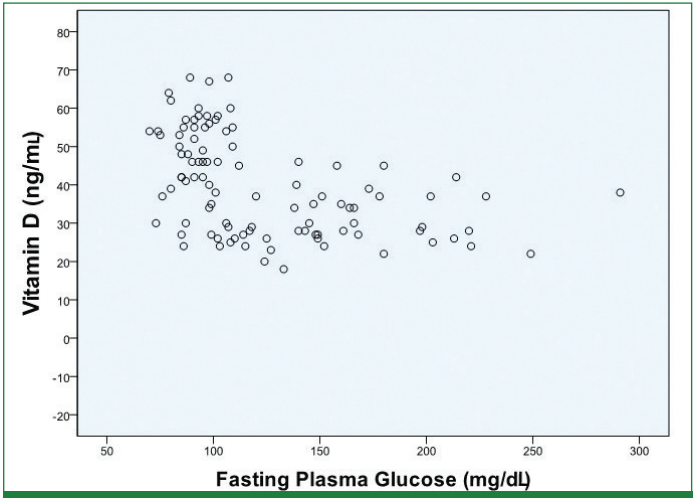
Analyte	Group 1 (Cases)	Group 2 (Controls)	t-value	p-value*
Serum 25-OH vitamin D levels (ng/mL)	31.02±7.51	48.30±11.29	t=-9.005	<0.0001
Fasting plasma glucose levels (mg/dL)	157.50±42.01	91.30±9.59	t=10.863	<0.0001
Postprandial plasma glucose levels (mg/dL)	230.92±45.20	112.86±20.18	t=16.862	<0.0001

[Table/Fig-2]: Group comparison of analytes (mean±SD) levels with t-value and p-value of statistical significance testing between groups.
t-value and p-value obtained from Independent sample t-test used for significance testing between the groups; *p-value ≤0.05 is significant

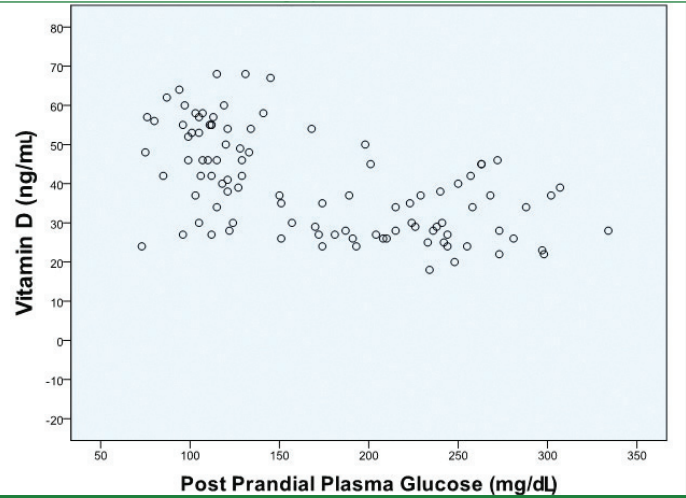
Pearson correlation coefficient between serum 25-OH vitamin D levels with fasting plasma glucose levels and postprandial plasma glucose levels showed significant negative correlation with (r=-0.463) and (r=-0.568) for fasting plasma glucose and postprandial plasma glucose levels respectively at 0.01 level (2-tailed) (as illustrated in scatter plot [Table/Fig-4,5], respectively).

Variables	B	S.E	Sig. (p-value)	Exp (B)	95% CI for Exp (B)	
					Lower	Upper
Age	-0.115	0.093	0.216 (NS)	0.891	0.743	1.069
Gender	-0.027	1.129	0.981 (NS)	0.973	0.107	8.891
Sunlight exposure	-23.36	10502	0.998 (NS)	0.000	0.000	-
Duration of diabetes	-1.006	1.524	0.509 (NS)	0.366	0.018	7.246
Fasting plasma glucose	-0.022	0.014	0.113 (NS)	0.978	0.952	1.005
Postprandial plasma glucose	0.007	0.011	0.545 (NS)	1.007†	0.985	1.030

[Table/Fig-3]: Logistic Regression model for exposure outcome association between the predictor independent variables and the outcome dependent variable (vitamin D Insufficiency/Predeficiency state) among cases.
B-regression coefficient; SE: standard error; Sig. p-value; Exp(B): Odds ratio; CI: Confidence interval; NS: Not significant; †: OR>1



[Table/Fig-4]: Scatter plot showing negative correlation with serum 25-OH vitamin D (in ng/mL) on Y axis and fasting plasma glucose levels (in mg/dL) on X axis.



[Table/Fig-5]: Scatter plot showing negative correlation with serum 25-OH vitamin D levels (in ng/mL) on Y axis and post prandial plasma glucose levels (in mg/dL) on X axis.

DISCUSSION

Vitamin D, a multifaceted hormonal vitamin is synthesised from 7 dehydrocholesterol in the skin when exposed to sunlight [12]. It undergoes hydroxylation processes in the liver and kidney to become its final activated form of 1,25 dihydroxy cholecalciferol. Among its various hydroxylation forms, 25-OH form was selected as a measure of vitamin D status in the present study, because of its universal acceptance as an indicator of vitamin D status, long half life and its credibility in association with disease states [13]. As shown in present study, serum 25-OH vitamin D levels were significantly (p<0.005) lower among patients with uncontrolled T2DM than controls. The results of the present study showed significantly lower serum 25-OH vitamin D levels (31.02±7.51 ng/mL) than controls (48.30±11.29 ng/mL) which was in accordance with many international studies like Anyanwu AC et al., Ahmed LHM et al., the popular National Health And Nutrition Examination Survey (NHANES) study and national studies like Mohapatra A et al., Kotwal SK et al., in India [14-18].

Anyanwu AC et al., showed mean serum vitamin D levels 9.2±1.1 ng/mL were deficient in T2DM patients [14]. Ahmed LHM et al., showed that mean serum 25-OH Vitamin D3 levels were lower in diabetes (p <0.001), were unrelated to dyslipidaemia or hypertension [15]. Mohapatra A et al., which evaluated vitamin D levels in T2DM showed that more deficiency in complicated diabetes mellitus (mean 13.14±1.45 ng/mL) than uncomplicated (mean 19.94±2.4 ng/mL) and controls (mean 33±3.3 ng/mL) [17]. Kotwal SK et al., which evaluated the correlation of vitamin D levels with fasting blood glucose showed that mean vitamin D levels in cases 18.81±15.18 ng/mL vs 28.26±18.89 ng/mL in controls with (p<0.000) [18]. The significantly lower levels of serum 25-OH vitamin D noted among uncontrolled T2DM patients in the present study were possibly multifactorial in aetiology relating to the altered dietary refined food intake patterns, reduced sunlight exposure due to lifestyle changes, absorption defects because of uncontrolled hyperglycaemia in diabetes patients. The present study results also shows significantly higher levels of fasting (157.50±42.01 mg/dL) and postprandial (230.92±45.20 mg/dL) plasma glucose among the cases when compared to the fasting (91.30±9.59 mg/dL) and postprandial (112.86±20.18 mg/dL) plasma glucose levels among the controls. The possible mechanisms for hyperglycaemia relating to low vitamin D levels among the cases in the present study can be explained by the role of vitamin D in influencing the insulin release process and the insulin resistance, both of which are absolute essentials for having normoglycaemia in T2DM patients. Fasting and postprandial hyperglycaemia are obviously an affect in both the insulin release process and in insulin resistance, possibly also relating to low vitamin D levels noted among the cases.

Vitamin D has both direct and indirect effects in secretion of insulin. The direct role suggested by studies like Cangoz S et al., by the binding to vitamin D receptor on beta cells along with other factors like presence of vitamin D response elements in human insulin gene promoter, and by acting as a transcriptional factor in regulating beta cell function of insulin secretion [19]. The indirect role mediated by the alterations in the calcium flux through the beta cell membrane. Vitamin D also plays role in insulin resistance substantiated by studies like Dutta D et al., through multiple mechanisms like fat sequestration, metabolic syndrome association, effects of secondary hyper parathyroidism, reduced insulin sensitivity, intra cellular calcium flux changes, changes in glucose transport mechanisms and changes in insulin receptor expression [20]. Though the present study has not accounted for C peptide levels and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) measurements, the higher levels of glucose noted among the cases even beyond medications during fasting and postprandial states proves the altered glucose metabolism, which is an indirect measure relating to abnormal insulin release and insulin resistance.

The normal reference range for serum 25-OH vitamin D levels is 20-65 ng/mL [21]. Most international [22] and national data [23] on the definition of cut-off limits for vitamin D insufficiency and deficiency has been defined as levels of serum 25-OH vitamin D <30 ng/mL [24] with vitamin D deficiency defined as a serum 25-hydroxyvitamin D level of <20 ng\mL and insufficiency defined as a serum 25-hydroxyvitamin D level of 20-30 ng\mL while the

toxicity levels for vitamin D is at markedly elevated serum 25(OH) D concentrations (>150 ng/mL) [25]. Though the lower levels of serum 25-OH vitamin D noted in the present study among patients with uncontrolled T2DM were statistically significant, the levels were sufficient and not below the deficiency limits. This was in contrast to the studies as described by Mohapatra A et al., and Kotwal SK et al., which is intriguing and calls for future follow-up of the participants [17,18]. It might well be explained by the subclinical/predeficiency zones of vitamin D status among the cases studied. Logistic regression model which was used to analyse the relationship between predictor independent variables (age, gender, sunlight exposure, duration of diabetes, fasting and postprandial plasma glucose) and the outcome dependent variable (vitamin D Insufficiency/predeficiency state) among cases showed that none of the individual predictor variables showed significant association ($p<0.05$) on holding all other predictor variables constant. However, there was an insignificant positive B (0.007) (slope of the regression coefficient) noted for the predictor variable (postprandial plasma glucose) with the odds of occurrence of vitamin D insufficiency state {odds ratio: 1.007 (C.I: 0.985-1.030)} is increased by 0.7% for a unit increase in postprandial plasma glucose levels among cases. To date, there are no studies that the authors could access relating the subclinical/predeficiency zones of vitamin D relating to glycaemic status in T2DM patients, which adds an additional prospect to the present study.

The correlation results from the present study further adds up that the serum 25-OH vitamin D levels were not only lower among patients with T2DM patients, it also had significant independent negative correlation with fasting and postprandial plasma glucose levels at 0.01 level (2-tailed). This inverse association is in line with studies like Kotwal SK et al., and Doddamani GB et al., which evaluated the levels of vitamin D and its association in T2DM patients found that 70% patients had inverse correlation with fasting plasma glucose ($p<0.001$) [18,26]. Kotwal SK et al., which evaluated the correlation of vitamin D levels with fasting blood glucose found a significant negative correlation with fasting blood glucose ($p<0.000$) [18]. The present study further opposes the results from studies like Tandon VR et al., which evaluated Vitamin D deficiency showed that the correlation between Vitamin D deficiency and raised blood glucose was non significant ($p=0.324$) [27]. This inverse correlation also substantiates the role of vitamin D in insulin release and insulin resistance process, regardless of fasting or postprandial state influencing the glycaemic status of the T2DM patients. This in addition further cycle up the vicious cycle of lower vitamin D levels leading to higher blood glucose levels and vice versa among patients with uncontrolled T2DM.

Limitation(s)

Technical limitations include lack of substantiation of the role played by vitamin D on glycaemic control for 3-4 months as glycated haemoglobin levels were not estimated. Insulin levels and C peptide levels were not estimated which would have given the status of baseline insulin secretory status and insulin resistance scoring. This would have objectively justified the hypothesis on the role of vitamin D in insulin release and insulin resistance. Additional technical limitations includes non confirmation of secondary hyperparathyroidism among T2DM patients as Parathyroid Hormone (PTH) levels were not estimated. Statistical limitations include small sample size.

CONCLUSION(S)

The study concludes that though vitamin D deficiency levels was not observed in the study population, serum 25-OH Vitamin D levels were significantly lower and has significant inverse association with fasting plasma glucose levels and postprandial plasma glucose levels in uncontrolled T2DM patients. This substantiates the role of vitamin D in maintaining normal plasma glucose levels in T2DM patients. Vitamin D acts through multiple mechanisms including role in insulin release and role in insulin action which are the primary issues in T2DM patients. It is thereby proposed that serum 25-OH vitamin D levels be measured and followed-up for better glycaemic control, thereby improving the quality of life among patients with uncontrolled T2DM.

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