Correlation of Vitamin D3 and Serum Calcium with Hba1c in Type 2 Diabetes Mellitus: A Cross-Sectional Study

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Abstract

Background: The role of vitamin D3 and serum calcium in T2DM remains a very important aspect which needs further evaluation to find out whether a correlation exist or not. So far, limited structured studies have been carried out on correlation of HbA1c with vitamin D3 and serum calcium level, hence, a cross sectional study was carried out to determine the same and establish a correlation.

Aim: To correlate between serum vitamin D3 and serum calcium levels with HbA1c in newly diagnosed type 2 diabetes mellitus patients

Objectives: The specific objectives of the studies were as follows:

1. To measure serum vitamin D3, serum calcium and HbA1c in newly diagnosed type 2 diabetes mellitus patients
2. To correlate serum vitamin D3 and serum calcium with HbA1c in newly diagnosed type 2 diabetes mellitus patient

Methodology: This cross sectional study was carried out at the Medicine Department of Rohilkhand Medical College and Hospital in Bareilly, Uttar Pradesh After the approval of the Institutional Ethics Committee (IEC), informed consent was taken from all the subjects participating and then the history was taken, clinical examination carried out and investigations were sent to the laboratory.

Under all aseptic precautions, 5 ml venous blood was withdrawn and collected in red capped plain vacutainer, purple capped EDTA vacutainer and grey capped NaF vacutainer and sent for analysis to the laboratory for HbA1c, serum vitamin D3, serum calcium, fasting plasma glucose and post prandial plasma glucose levels.

HbA1c was tested by BEACON Kit on Auto analyzer EM360 machine.

Vitamin D3 level was tested by Chemiluminescence Immunoassay on Beckman Coulter Access 2 Machine.

Fasting plasma glucose and post prandial plasma glucose levels were tested by Erba Mannheim Glucose reagent on Auto analyzer EM360 machine.

Serum calcium level was tested by AUTOSPAN Liquid Gold Calcium (O- Cresolpthalein complex) on Auto analyzer EM360 machine.

Results: 82.35% of people having HbA1c <7% have vitamin D3 levels <30 ng/dL whereas 17.65% of people having HbA1c <7% have vitamin D3 levels >30 ng/dL. People having HbA1c ≥7% have <30 ng/dL vitamin D3 levels in 68.49% and >30 ng/dL in 31.51%.

The following data with p value 0.046 indicates statistically significant correlation.

Conclusion: The correlation of HbA1c with 25(OH)D levels in newly diagnosed T2DM was statistically significant with p value < 0.05 (=0.046), though there was no inverse linear correlation found. The patients with HbA1c <7% (82.35%) and ≥7% (68.49%) had <30 ng/dL vitamin D3 level.

Vitamin D3 and calcium levels showed an inverse relation with each other, being statistically significant with p-value < 0.05.

Keywords: **Vitamin D3 and Serum Calcium, HbA1c and Type 2 Diabetes Mellitus**

Introduction

Diabetes mellitus (DM) consists of group of metabolic disorders of inappropriately raised blood glucose level which could be because of defects of insulin secretion and action or both.

India holds the unenviable title of "diabetes capital of the world" for having the highest percentage of diabetic population in the world. According to International Diabetes Federation's 2006 Diabetes Atlas, India's estimated 40.9 million diabetics may increase to 69.9 million by 2025.1 It is leading cause of morbidity and mortality in developing countries like India because of sedentary life style, lack of physical activity, obesity, stress and consumption of diet rich in sugar, fat and calories.2

The prevalence is estimated to be 9.3% (463 million people) in 2019, may increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The disease burden is much higher in urban (10.80%) than rural (7.20%), and in the high-income (10.40%) than the low-income nations (4.0%).3

Number of cases as well as prevalence of diabetes is increasing at a steady pace over the decades and it is reported as fifth leading cause of death worldwide.3

Glycemic control to be measured by fasting and post prandial blood glucose level to label a person as diabetic but long-term glycemic management is evaluated using glycosylated hemoglobin (HbA1c).4

Normal level of glucose produces a normal amount of glycated hemoglobin (HbA1c). As average amount of plasma glucose increases, the fraction of glycated haemoglobin also increases in a predictable way. In diabetes, higher amount of glycated haemoglobin indicates poorer control of glucose levels that have been associated with cardiovascular diseases, nephropathy, neuropathy and retinopathy.4

HbA1c is measured primarily to determine the previous three months average blood glucose level and can be used as a diagnostic test for diagnosing diabetes mellitus, as well as an assessment test for glycemic control in people with DM. The test is limited to 3 months average blood glucose because the average lifespan of red blood cells (RBCs) is 4 months, that is 120 days.4

Vitamin D3 (25(OH)D) is a fundamental micronutrient with major implications for human health. Its deficiency has been reported to be a very common finding in patients with type 2 diabetes (T2DM). Various studies on humans and animals have established that there is both positive and negative correlation between vitamin D3 with blood glucose and serum insulin level.5,6

Vitamin D3 may improve glucose-stimulated insulin secretion in pancreatic β- cells,7 enhance lipid and glucose metabolism in skeletal muscle,8,9 and improve systemic inflammation.10 Not all, but most of the patients with T2DM or glucose intolerance may have lower serum 25(OH)D levels as compared to healthy control subjects.11 Therefore, vitamin D3 plays an important role in glucose metabolism and glycemic control in T2DM.

Calcium is necessary for insulin mediated intracellular processes in insulin- responsive tissues such as adipose tissue and skeletal muscle.12 It can impact glucose metabolism by affecting the concentration of calcium inside cells, particularly in pancreatic β-cells. Any imbalance between extracellular and intracellular calcium levels in these cells can hinder the function of calcium mediated voltage-gated channels, thus disrupting insulin secretion.13,14 Changes in serum calcium levels in T2DM patients contributes to peripheral Insulin resistance.15

The role of vitamin D3 and serum calcium in T2DM remains a very important aspect which needs further evaluation to find out whether a correlation exist or not. So far, limited structured studies have been carried out on correlation of HbA1c with vitamin D3 and serum calcium level, hence, a cross sectional study was carried out to determine the same and establish a correlation.

Materials And Methods

- **Place of Study:** This study was conducted in patients attending OPD and IPD in Department of Medicine, Rohilkhand Medical College and Hospital, Bareilly Uttar Pradesh.
- **Type of Study**: A Cross-Sectional Study.
- **Duration of Study**: The proposed study will be conducted from the 1st November 2022 to 31st October 2023.
- **Sample Size**: In our study, total of 90 patients were taken, which was calculated by the following formula – n = 4 x (SD) / L2

SD: Standard Deviation of Vitamin D3 levels in diabetic patients = 23.0665 n = 4 x 23.06 x 23.06 / 5x5
n = 85.08 ~ 90

1. **Inclusion Criteria**:
    1. Patients of both sexes with age between 25 – 50 years3,66
    2. .Newly diagnosed patients with type 2 diabetes
2. **Exclusion Criteria**:
    1. Patients already on oral calcium or vitamin D3 supplementation
    2. Patients suffering from hypertension, chronic liver disease, chronic kidney disease, anemia (Hb<10), pancreatitis, alcoholics, thyroid and parathyroid diseases

Methodology

After the approval of the Institutional Ethics Committee (IEC), informed consent was taken from all the subjects participating and then the history was taken, clinical examination carried out and investigations were sent to the laboratory.

Under all aseptic precautions, 5 ml venous blood was withdrawn and collected in red capped plain vacutainer, purple capped EDTA vacutainer and grey capped NaF vacutainer and sent for analysis to the laboratory for HbA1c, serum vitamin D3, serum calcium, fasting plasma glucose and post prandial plasma glucose levels.

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Fasting plasma glucose and post prandial plasma glucose levels were tested by Erba Mannheim Glucose reagent on Auto analyzer EM360 machine.

Statistical Analysis

Data was entered, coded and compiled in SPSS software (23.0 version). Then analysis was done using statistical data depending upon the type and distribution of the data. The data was expressed in tables and graphs accordingly. p-value < 0.05 was considered as significant.

Procedure of Study

In this study,77 cases of Diabetic Nephropathy patients and 28 patients of diabetes mellitus but not suffering from Nephropathy and presenting to the OPD or emergency room of Rohilkhand Medical College and hospital, Bareilly and fulfilling the inclusion criteria.

Patients were included according to the criteria's mentioned above, after getting informed consent. Patients had been examined and with history followed by the investigations like Serum Magnesium, Serum Calcium, Serum Creatinine,Urine albumin creatinine ratio, Urine routine and microscopy, Ultrasound whole abdomen and RBS has been done. The criteria of Diabetic Nephropathy has been used to determine the Staging and prognosis. Patients are categorised accordingly.

Statistical Analysis

The data was coded and entered; its compiling was done on a Microsoft Excel and them imported into SPSS (statistical package for social sciences) Licensed Version 23.0.

Descriptive analysis was done by calculating proportions, means and standard deviations. Appropriate statistical tests were applied depending upon the distribution and type of the data. p<0.05 was considered significant.

This one year observational study was conducted in the Department of General Medicine, RMCH, Bareilly to observe the Serum Magnesium and Serum Calcium in Diabetic Nephropathy. In this study, 105 patients suffering from Diabetes Mellitus were included and closely observed for the complications of nephropathy. The data obtained from these were analysed and results were tabulated as follows. p-value <0.05 was considered statistically significant.

Result:

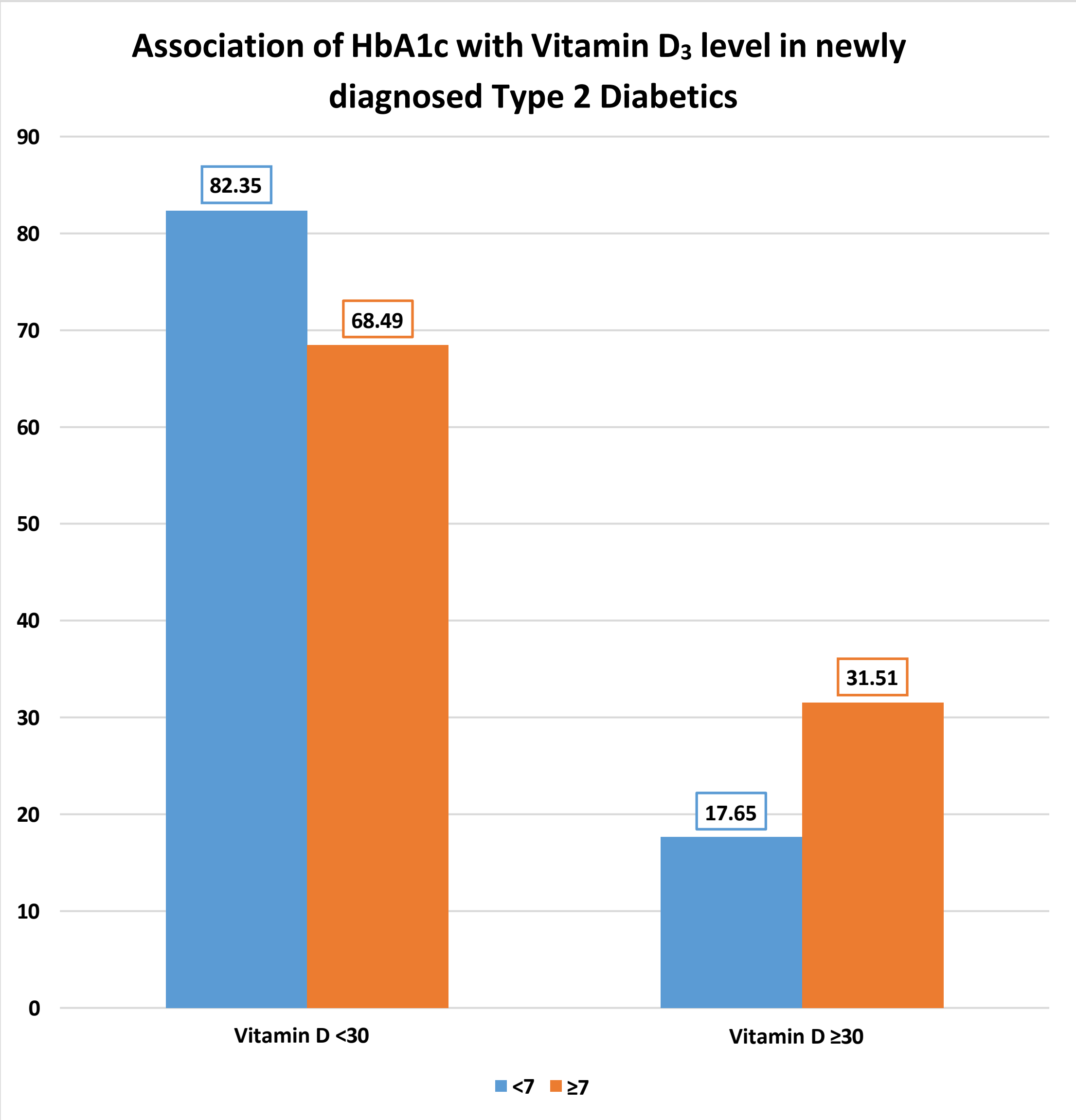
Association of HbA1c with Vitamin D3 levels in newly diagnosed Type 2 Diabetics

	S. Vitamin D3 (ng/dL)				
HbA1c (%)	**<30**		**≥30**		
	Number	**%**	**Number**	**%**	**p-value**
<7	14	82.35	3	17.65	
≥7	50	68.49	23	31.51	0.046*
Total	64	100.00	26	100.00	

The p-value = 0.046 (< 0.05). Hence, statistically significant.

The Figure no. 13 and Table no. 9 suggests the association of HbA1c with 25(OH)D levels in newly diagnosed type 2 diabetes mellitus patients.
82.35% of people having HbA1c <7% have vitamin D3 levels <30 ng/dL whereas 17.65% of people having HbA1c <7% have vitamin D3 levels >30 ng/dL. People having HbA1c≥7% have <30 ng/dL vitamin D3 levels in 68.49% and >30 ng/dL in 31.51%.

The following data with p value 0.046 indicates statistically significant correlation.



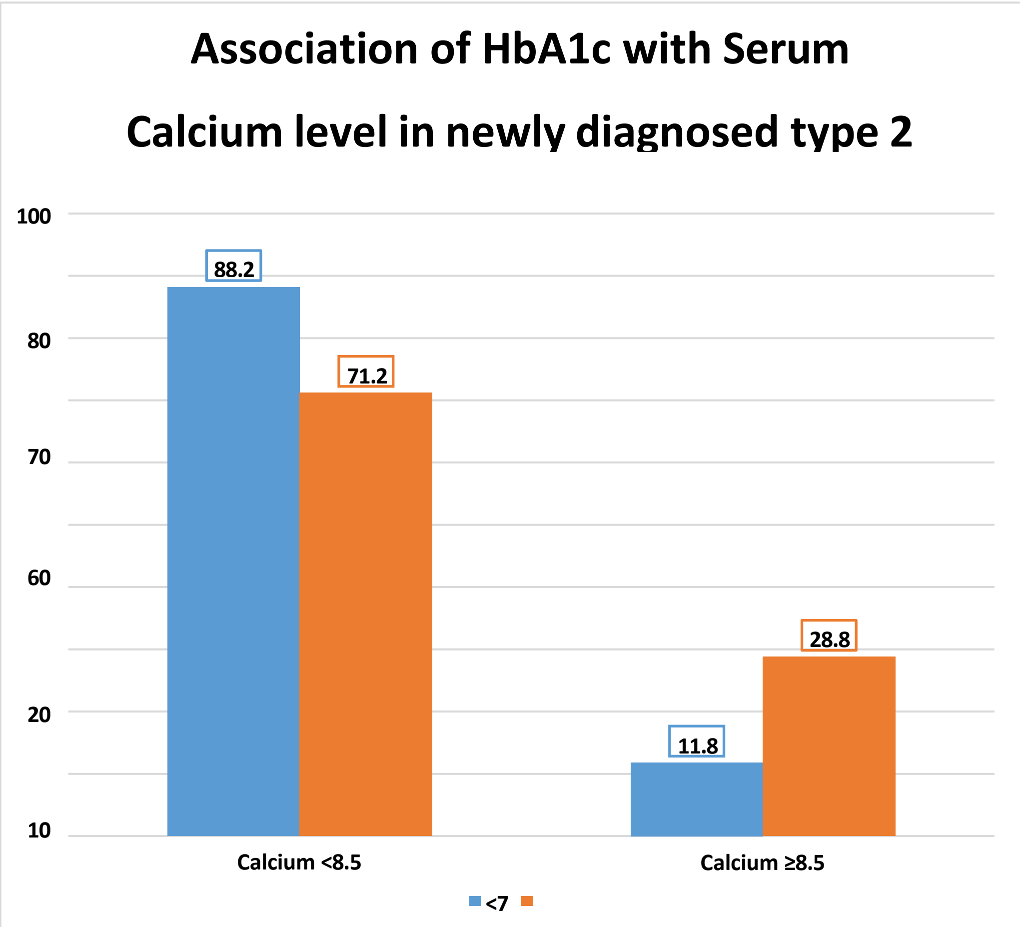
Association of HbA1c with Serum Calcium level in newly diagnosed type 2 diabetics

	Serum Calcium (mg/dL)				
HbA1c (%)	**<8.5**		**≥8.5**		
	Number	**%**	**Number**	**%**	**P-value**

<7	15	88.2	2	11.8	0.148#
≥7	52	71.2	21	28.8	
Total	67	74.4	23	25.6	

The p-value = 0.148 (> 0.05). Hence, statistically insignificant.

People with HbA1c level <7% have serum calcium level <8.5 mg/dL in 88.2% (n=15) and ≥8.5 mg/dL in 11.8% (n=2).
71.2% (n=52) people with HbA1c ≥7% have serum calcium level <8.5 mg/dl and 28.8% (n=21) have ≥8.5 mg/dL.
The data is statistically not significant as p-value is 0.148.



Discussion: The results from our study confirm that HbA1c was increased in patients with T2DM with 25(OH) D deficiency. Maybe 25(OH) D deficiency is a risk factor for high HbA1c levels in patients with T2DM.

Conclusion: In conclusion, the results from our study confirm that HbA1c was increased in patients with T2DM with 25(OH) D deficiency. Maybe 25(OH) D deficiency is a risk factor for high HbA1c levels in patients with T2DM, Vitamin D3 and calcium levels showed an inverse relation with each other, being statistically significant with p-value < 0.05.

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