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PREVALENCE OF VITAMIN D DEFICIENCY IN TYPE-2 DIABETES MELLITUS PATIENTS AND ITS CORRELATION WITH GLYCEMIC CONTROL

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Abstract: Vitamin D and its active metabolite, 1, 25-di (OH)-vitamin D or calcitriol, have long been recognized as important regulators of serum calcium and bone health. Production of calcitriol is dependent on adequate vitamin D. Following constitutive conversion of vitamin D to 25(OH)-vitamin D by the liver, most circulating calcitriol (hormonal calcitriol) is made by the highly regulated 1alpha-hydroxylase (CYP27B1) present in the kidneys. Numerous other tissues also possess 1alpha-hydroxylase and appear to produce calcitriol locally at high concentrations. The receptor for calcitriol, the vitamin D receptor (VDR), is expressed in virtually all tissues. A large proportion of the population has low vitamin D levels, which are generally defined as a serum level of 25(OH)-vitamin D less than 20 or 30 ng/mL. Although sunlight stimulates skin production of vitamin D, many in modern society are dependent on ingestion of vitamin D in milk or supplements to maintain normal vitamin D levels, especially during the winter months. Vitamin D deficiency is particularly common in hospitalized individuals, those with chronic diseases, and African Americans. Over the past decade, the relationship of vitamin D deficiency to the risk of developing diabetes mellitus (DM) and the risk for diabetic complications has been of great interest to scientists. The purpose of this study is to determine vitamin D status among type 2 diabetics in outpatient department of medicine in our hospital and examine the relationship between vitamin D status and level of glycemic control. This study indicates there is a definite negative correlation between Vit D levels and diabetes (r= -0.94 and -0.97 in Group 1 and 2) and poorly controlled diabetics have further lower values of Vitamin D (p< 0.01 for vit D when compared between Group 1 and Group 2)

Key Words: Vitamin D, diabetes

INTRODUCTION

It has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency. It is well known that vitamin D deficiency causes osteoporosis, increases the risk of fracture and causes muscle weakness. But there is now evidence that hypovitaminosis D are predisposing conditions for various common chronic diseases such as malignancies (particularly colon, breast and prostate) chronic inflammatory and autoimmune disease (inflammatory bowel disease, multiple sclerosis) as well as metabolic disorders like type 2 diabetes mellitus, metabolic syndrome, hypertension etc. As is evident Type 2 diabetes is a major public health problem, accounting for significant premature mortality and morbidity. Over the last few years, a number of large observational studies have suggested an association between the onset of type 2 diabetes and Vitamin D deficiency and in certain studies it has been seen that as the deficiency worsened, so did diabetes control.

Pathogenesis

Vitamin D and its active metabolite, 1, 25-di (OH)-vitamin D or calcitriol, have long been recognized as important regulators of serum calcium and bone health. Production of calcitriol is dependent on adequate vitamin D. Following constitutive conversion of vitamin D to 25 (OH)-vitamin D by the liver, most circulating calcitriol (hormonal calcitriol) is made by the highly regulated 1alpha-hydroxylase (CYP27B1) present in the kidneys. Numerous other tissues also possess 1alpha-hydroxylase and appear to produce calcitriol locally at high concentrations. The receptor for calcitriol, the vitamin D receptor (VDR), is expressed in virtually all tissues. Thus, this latter form of calcitriol production constitutes a classic paracrine-autocrine system. Local production of calcitriol may even be important in the classic calcitriol target tissues of bone and the parathyroid gland because investigators have demonstrated the presence of 1alpha-hydroxylase in bone and parathyroid cells.[1, 2] Activation of the VDR by hormonal or locally produced calcitriol generally promotes differentiation of tissues and inhibits proliferation. Some regulatory actions of VDR are even independent of calcitriol.

A large proportion of the population has low vitamin D levels, which are generally defined as a serum level of 25 (OH)-vitamin D less than 20 or 30ng/mL. Although sunlight stimulates skin production of vitamin D, many in modern society are dependent on ingestion of vitamin D in milk or supplements to maintain normal vitamin D levels, especially during the winter months.

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Vitamin D deficiency is particularly common in hospitalized individuals, those with chronic diseases, and African Americans. Over the past decade, the relationship of vitamin D deficiency to the risk of developing diabetes mellitus (DM) and the risk for diabetic complications has been of great interest to scientists.

Wolf and colleagues examined incident hemodialysis patients, and found that diabetics were more likely to be severely 25(OH)-vitamin D-deficient (< 10ng/mL) than non-diabetics (22% vs 17%).[3] Lower 25(OH)-vitamin D levels and lower calcitriol levels strongly correlated with an increased risk for death during the first 90 days in patients not given injectable calcitriol or an analog.[3]

Vitamin D is important for normal glucose metabolism. It acts through several mechanisms on glucose metabolism:

- Vitamin D directly acts on insulin producing cells (β cells) in the pancreas to produce more insulin.
- Vitamin D directly acts on the muscle and fat cells to improve insulin action by reducing insulin resistance.
- Vitamin D reduces inflammation which is commonly present in patients with Insulin Resistance Syndrome and Type 2 diabetes.
- Vitamin D indirectly improves insulin production and its action by improving the level of calcium inside the cells.
- Studies have showed that Vitamin D supplementation can improve glycemic control in patients with Diabetes (Holick M)
- Further it can prevent/delay complications like neuropathy, nephropathy, retinopathy, diabetic ulcers (Joergensen C *et al.,*)[4]

But more studies are required to establish the correlation of glycemic control and Vitamin D status in different parts of the globe as Vitamin D levels vary widely in different population groups (CV Harinarayan et.al)[5]

Vitamin D and Risk of Developing Diabetes

Several observational studies have suggested that either low vitamin D levels or low vitamin D intake may predispose to the development of both type 1 and type 2 DM. The Nurses' Health Study found that vitamin D intake above 800 IU/day and more than 1200 mg of calcium per day were associated with a 33% reduction in the risk of developing type 2 DM compared with an intake of < 600 mg of calcium and < 400 IU of vitamin D.[6] A meta-analysis of largely observational studies concluded that there was "a relatively consistent association between low vitamin D status, calcium or dairy intake, and prevalent type 2 DM or metabolic syndrome.[7]" Evidence from interventional trials suggests that combined vitamin D and calcium supplementation may help prevent type 2 DM in only some populations at high risk for diabetes.[7]

Low vitamin D levels, low sun exposure, and low intake of vitamin D have each been associated with an increased risk for the development of type 1 DM. In animal models, induction of type 1 DM by streptozocin induces a marked fall in calcitriol levels, whereas 25(OH)-vitamin D levels remain normal. Treatment with insulin restores calcitriol levels to normal. Calcitriol has an immunomodulatory effect. In a non-obese diabetic mouse model, administration of calcitriol or 1alpha-(OH)-vitamin D (a precursor of calcitriol) has been shown to significantly reduce the likelihood of development of type 1 DM.[8]

Vitamin D and Development of Diabetic Complications

Wang and colleagues studied 1739 Framingham offspring participants without prior cardiovascular disease, and found that low 25(OH)-vitamin D levels (< 15ng/mL) were significantly associated with an increased incidence of a first cardiovascular event during the mean 5.4 years of follow-up.[9] Among diabetics in this cohort, low 25(OH)-vitamin D levels were significantly more common than non-diabetics (11% vs 7%), but the association of low vitamin D levels to first cardiovascular event remained after adjustment for diabetics and other known risk factors.

Low 25 (OH)-vitamin D levels have been shown to correlate with the presence of cardiovascular disease in diabetics.[10] Similarly, hypovitaminosis D has been independently associated with carotid artery intimal-medial thickening, a harbinger of cerebrovascular and cardiovascular events.[11] Suzuki and colleagues found that microvascular complications were more frequent when vitamin D levels were low, despite similar duration of disease and other clinical characteristics compared with control patients without complications.[12]

These data suggest that diabetic patients are at greater risk of being vitamin D deficient and harmed by this deficiency. The presence of CKD will compromise the production of calcitriol, and potentially further contribute to inadequate vitamin D signaling. Consistent with this, among 463 diabetics with CKD, low vitamin D levels were independently associated with the presence of cardiovascular disease.[13]

The purpose of this study is to determine vitamin D status among type 2 diabetics in outpatient department of medicine in our hospital and examine the relationship between vitamin D status and level of glycemic control.

MATERIALS AND METHODS

The study was carried out in the department of Biochemistry from June 2013 to April 2014 after obtaining the approval of the Ethics Committee. The diabetic patients visiting the Medicine OPDs were selected and evaluated for Fasting Blood Glucose, Vitamin D levels and HbA1c. They were divided into three groups with the following criteria:

Group I: comprised of 48 age and sex matched healthy individuals
Group II: comprised of 46 patients with DM history of more than five years and HbA1c levels < 7%
Group III: comprised of 56 patients with DM history of more than five years and HbA1c levels > 7%

The following inclusion and exclusion criteria were used to form the patient groups:

Inclusion Criteria

- Type 2 diabetes mellitus
- Written consent

Exclusion Criteria

- Renal failure
- Age < 18 years
- Malabsorption

Collection of Samples

Consent of the patient was taken prior to collection of the samples. The patient was requested to arrive at the lab with 10 hours of fasting. Then 5 ml of venous blood was collected under aseptic conditions. The blood sample was immediately transferred to fluoride vial for Plasma Glucose estimation and EDTA vials for other parameters. A detailed physical examination of the patient was carried out with special emphasis on height, weight, blood pressure, thyroid gland enlargement and edema.

The following protocols were used for estimation of various parameters:

Glucose – Hexokinase Method

Test principle: UV test by enzymatic reference method using hexokinase. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G6-PD}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

Hemoglobin A_{1c} Assay Using HPLC Method

HbA1c, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A1c, or pre-A1c), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A1c formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A1c is converted (Amadori rearrangement) to form a stable ketoamine, HbA1c.

The D-10 Hemoglobin A1c Program utilizes principles of ion-exchange High-Performance Liquid Chromatography (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration is used for quantitation of the HbA1c values. A sample report and a chromatogram are generated for each sample. The A1c peak is shaded. This area is calculated using an Exponentially Modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak areas from the A1c peak area.

Vitamin D Assay by Chemiluminescence immunoassay

25 OH Vitamin D assay is a direct, competitive Chemiluminescent Immunoassay (CLIA) for quantitative determination of total 25-OH vitamin D in serum or plasma. During the first incubation, 25 OH Vitamin D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 minutes the tracer (vitamin D linked to an isoluminol derivative) is added. After additional 10 minute incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as Relative Light Units (RLU) and is inversely proportional to the concentration of 25 OH Vitamin D present in calibrators, controls, or samples.

RESULTS AND DISCUSSIONS

The age and sex distribution along with physical parameters as well as other calculated parameters are depicted in Table-1. Table- 2, 3, 4 shows the comparison of means between different parameters between Control and Group 1, Control and Group 2, Group 1 and Group 2 respectively. We observed that there was statistically significant decrease in Vit D levels in Group 1 and Group 2 when compared with Control Group ($p<0.05$). Moreover Vit D levels significantly decreased in Group 2 compared to Group 1 ($p<0.01$). These observations prove that Vit D levels are lower in diabetic patients when compared to normal individuals and the value further decreases in diabetic patients with poor glycemic control (HbA1c > 7%). Correlation matrix of Group 1 and Group 2 (Table 5 & 6) showed significant negative correlation between HbA1c and Vit D levels (r= -0.94 and -0.97 respectively). Figure 1 and 2 shows the Pearson correlation graph between Vit D (y axis) and HbA1c (x axis) in Group 1 and Group 2 respectively. These findings indicate a definite decrease in Vit D levels with increasing HbA1c levels in diabetic patients.

Table 1: Physical and other observed parameters with age and sex distribution in different groups

	Group 1(48)	Group 2(46)	Group 3(56)
Male	26	25	31
Female	22	21	25
Age (years)	48.09±8.95	46.1±3.95	52±4.09
Height (meters)	1.62±0.05	1.63±0.05	1.64±0.06
Weight (kilograms)	60.09±6.93	61.56±5.44	54.63±6.21
BMI (kg/ m²)	22.59±1.79	22.94±1.38	20.19±1.03
Systolic BP (mm of Hg)	117.35±4.90	128.06±10.98	150.54±12.46
Diastolic BP (mm of Hg)	78.06±3.50	80.73±5.32	92.54±7.21
FPG (mg/dl)	86.06±7.78	149.36±25.33	166.22±25.64
HbA1c (%)	5.01±0.34	6.45±0.56	8.42±1.06
Vitamin D (ng/ml)	40.26±6.84	22.44±4.67	16.98±3.82

Table 2: Comparison of means between Control and Group 1

Parameters	Control	Group I	P Value	Remarks
BMI(kg/m2)	22.69±1.45	22.95±1.69	>0.05	Not Significant
SYSTOLIC BP (mm Hg)	116.54±4.60	126.96±10.36	>0.05	Not Significant
DIASTOLIC BP (mm Hg)	77.04±3.60	80.35±5.39	>0.05	Not Significant
FBS(mg/dl)	86.58±6.82	142.61±16.71	<0.01	Highly Significant
HBA1C (%)	4.85±0.28	6.33±0.37	>0.05	Not Significant
VITAMIN D	41.60±5.23	23.63±3.71	<0.05	Significant

Table 3: Comparison of means between Control and Group 2

Parameters	Control	Group II	P Value	Remarks
BMI (kg/m2)	22.69±1.45	21.44±1.87	>0.05	Not Significant
SYSTOLIC BP (mm Hg)	116.54±4.60	146±16.38	<0.05	Significant
DIASTOLIC BP (mm Hg)	77.04±3.60	92.57±8.67	<0.05	Significant
FBS (mg/dl)	86.58±6.82	161.73±25.76	<0.01	Highly Significant
HBA1c (%)	4.85±0.28	8.41±1.24	<0.05	Significant
VITAMIN D	41.60±5.23	19.41±4.76	<0.01	Highly Significant

Table 4: Comparison of means between Group 1 and Group 2

Parameters	Group I	Group II	P Value	Remarks
BMI(kg/m2)	22.95±1.69	21.44±1.87	<0.05	Significant
SYSTOLIC BP (mm Hg)	126.96±10.36	146±16.38	<0.05	Significant
DIASTOLIC BP (mm Hg)	80.35±5.39	92.57±8.67	<0.01	Highly Significant
FBS(mg/dl)	142.61±16.71	161.73±25.76	<0.05	Significant
HBA1C(%)	6.33±0.37	8.41±1.24	<0.05	Significant
VITAMIN D	23.63±3.71	19.41±4.76	<0.01	Highly Significant

Table 5: Correlation Matrix Group 1

	BMI	SYS BP	DIAS BP	FBS	HBA1C	VIT D
BMI						
SYS BP	-0.1346541					
DIAS BP	-0.1546766	0.8843963				
FBS	-0.1210074	0.04111588	0.0241881			
HBA1C	-0.07051041	0.0261935	0.05902467	0.9312732		
VIT D	0.03478767	0.05568821	0.04087412	-0.882549	-0.9368338	

Table 6: Correlation Matrix Group 2

	BMI	SYS BP	DIAS BP	FBS	HBA1C	VIT D
BMI						
SYS BP	-0.0391203					
DIAS BP	-0.1657781	0.8986417				
FBS	-0.1216869	0.7096753	0.6316602			
HBA1C	-0.142564	0.6689788	0.6616925	0.813319		
VIT D	0.1729655	-0.7185583	-0.698656	-0.8510833	-0.9689632	

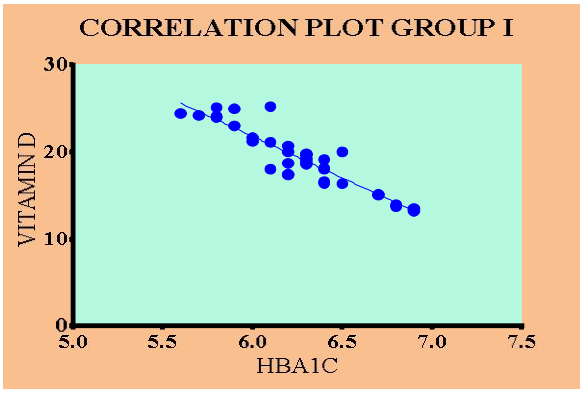

Figure 1: Correlation Graph in Group 1

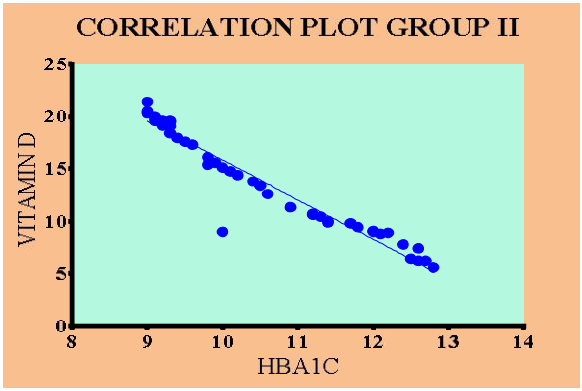

Figure 2: Correlation Graph in Group 2

CONCLUSION

This study indicates there is a definite correlation between Vit D levels and diabetes and poorly controlled diabetics have further lower values of Vitamin D when compared with patients with good glycemic control. It is known that poorly controlled diabetics are more prone to develop complications; hence supplementation of Vitamin D in diabetics may improve the glycemic control and can reduce the morbidity and mortality along with improving the quality of life. However our study was conducted on a small group, hence be taken only as a suggestion and prospective studies with larger patient group can conclude whether Vit. D supplementation can be actually helpful in preventing complications in diabetic patients.

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