International Journal of Advanced Research and Review

www.ijarr.in

THE RELATIONSHIP BETWEEN VITAMIN D LEVEL AND PERIPHERAL ARTERY DISEASE AND CORONARY ARTERY DISEASE IN TYPE 2 DIABETES MELLITUS

Gadhwal AK[1], Kapuriya S[2], Kumar M[3] , Meel JK[4], Sirohi P[5], Chahar K[6], Ankit BS[1], Phogawat M[3] Agrawal RP[7]

[1]Senior Resident, [2]Junior Registrar, Department of Pathology, [3]Senior registrar, [4]Medical Officer, [5]Professor, [6]Assistant Professor, [7]Principal & Controller, Sr. Professor
Department of Medicine & Pathology, S.P. Medical College, Bikaner, Rajasthan

ABSTRACT

Background : The role of Vitamin D deficiency in microvascular complications has been documented. However the effect of vitamin D deficiency on macrovascular complications are not studied extensively. Hence this study evaluate the effect of vitamin D deficiency on macrovascular complications; PERIPHERAL ARTERIAL DISEASE AND CORONARY ARTERY DISEASE in type 2 diabetes mellitus.

Material and Methods : The study was conducted on 200 patients with type 2 diabetes mellitus in which 100 patients were having macrovascular complications of diabetes(group 1) and 100 patients without macrovascular complications(group 2). To compare the level of vitamin D, 100 age-sex matched controls without diabetes (group 3) were taken. 25(OH) vitamin D level was measured among all three groups from the serum by ELISA kit. All vascular complications were measured by standard techniques used worldwide.

Results : The mean levels of vitamin D in group 1 , group 2 and group 3 were 7.53±2.14 , 11.23±3.44 and 31.48±6.43 ng/ml respectively. The 25(OH) vitamin D deficient (<20ng/ml) subjects in group 1 ,group 2 and group 3 were 79(79%) , 56(56%) and 14(14%) respectively. The peripheral artery disease and coronary artery disease were higher in vitamin D deficiency with vitamin D levels less than 30 ng/ml ($P<0.05$). The number of vascular complications were significantly correlated with vitamin D deficiency severity ($p=0.0001$)

Conclusion : The study gives us an insight to identify the diabetics with vitamin D deficiency which may be at higher risk of vascular complications. Vitamin D deficiency is higher among patients with type 2 diabetes mellitus as compared to controls. Vitamin D deficiency is also higher in patients with type 2 diabetes with vascular complications. Vitamin D deficiency is also associated with severity of vascular complications in type 2 diabetes. Further, a need to undertake future prospective multicenter study with larger number of subjects to find a cause effect relationship between vitamin D deficiency and vascular complications in patients of type 2 diabetes mellitus. This may help us to initiate interventional studies to see the reversal effect with supplementation of vitamin D to halt the progression of vascular complications and atherosclerosis in patients of type 2 DM.

AIMS & OBJECTIVES

1. To compare the levels of vitamin D among patients of type 2 diabetes mellitus with age-sex matched controls.
2. To study the prevalence of macrovascular complications in patients with type 2 diabetes mellitus with vitamin D deficiency.

To study the cause effect relationship of vitamin D with macrovascular complications in patients with type 2 diabetes mellitus.

MATERIAL & METHODS

Subject selection

200 cases of type 2 diabetes mellitus(100 cases with macrovascular complications and 100 cases without complications) of age group 40-60 years attending Diabetic care Research Centre,S P Medical College Bikaner, were taken as per WHO criteria. For control group,100 healthy person without type 2 diabetes mellitus and matched for confounding factors were taken.

Clinical Protocol

All the patients fulfilling criteria for cases and controls attending diabetic clinic went through detailed history and clinical examination. Participants were asked to provide information about their age, marital status, occupation, educational attainment, medical history, smoking, alcohol consumption, and participation in regular physical exercise. The data were collected on a specially designed proforma having baseline demography. Participants went through detailed physical examination, laboratory testing and test for vascular complications . Venous blood samples were collected for the investigations including vitamin D levels within 24 hours of admission after overnight fasting.

Routine investigations

- Hb, TLC, DLC, ESR
- Blood urea, serum creatinine
- Blood sugar (fasting and postprandial), oral glucose tolerance test, HbA_1C
- Serum electrolytes
- Liver function test
- Lipid profile
- Urine routine and microscopy
- ECG
- Fundus examination

Vitamin D levels

After an overnight fasting, approximately 3 ml of venous blood sample was taken in plain vial . Samples were stored at 2-8^{0}c for maximum three days.
25(OH) vitamin D level was measured from the serum by commercially available 25-OH vitamin D (total) ELISA kit (EIA 5396, DRG instruments GmbH, Germany).

Principle of assay

The DRG 25-OH vitamin D total ELISA kit was a solid phase ELISA based on the principle of competitive binding. In the first step, samples were pretreated in separate vials with denaturation buffer to extract the anylate, since most circulating 25-OH vitamin D is bound to vitamin D binding protein. After neutralization, biotinylated 25-OH vitamin D (enzyme conjugate) and peroxidase-labeled streptavidin (enzyme complex) were added. After careful mixing, the solution was transferred to the microtiter plate. Endogenous 25-OH vitamin D of a patient sample competes with a 25-OH vitamin D-biotin conjugate for binding to the vitamin D binding protein (VDBG) that was immobilized on the plate. Binding of 25-OH vitamin D was detected by peroxidase-labeled streptavidin. Incubation was followed by a washing step to remove unbound components. The color reaction was started by addition of enzyme substrate and stopped after a defined time. The color intensity was inversely proportional to the concentration of 25-OH vitamin D in the sample.

ASSESSMENT OF MACRO-VASCULAR COMPLICATIONS

Peripheral vascular diseases:-Diagnosed by history of intermittent claudication, examination of Peripheral pulses and measurement of ankle brachial index by Doppler study. Ankle systolic pressure measured of dorsalis pedis and posterior tibial artery and brachial systolic pressure measured of brachial artery by doppler ultrasound device. Ankle brachial index less than 0.9 is considered significant.

- Coronary artery disease:-Diagnosed by history of angina or myocardial infarction, electrocardiographic findings of myocardial infarction according to Minnesota code classification system and chest x-ray to assess cardiac size.

Statistical analysis

Statistical analysis was done using SPSS 22.0. Descriptive analysis was done with help of frequencies, mean +/- S.D. Inferential statistics used were chi square test, linear regression and correlation, logistic regression, odds ratio and ANOVA keeping 95% confidence interval. P value less than 0.05 was considered to be significant.

RESULTS

The mean level of vitamin D in group 1 ,group 2 and group 3 were 7.53±2.14 and 11.23±3.44 and 31.48±6.43 ng/ml respectively (Table 1, figure 1). The vitamin D levels were lower in group 1 as compared to group 2 and group 3 with the difference being statistically significant ($p<0.0193$).

Subjects were divided into 3 subgroups according to the severity of vitamin D deficient state as per the following levels:

1. 25(OH) vitamin D <20 ng/ml - Vitamin D deficient
2. 25(OH) vitamin D 20-30 ng/ml - Vitamin D insufficient
3. 25(OH) vitamin D >30 ng/ml - normal range

The 25(OH) vitamin D deficient subjects in group 1 and group 2 and group 3 were 79(79%) and 56(56%) and 14(14%) respectively. The 25(OH) vitamin D insufficient subjects in group 1, group 2 and group 3 were 17(17%) and 22(22%) and 7(7%) respectively. However, only 4% of subjects in group 1 and 22% in group 2 had vitamin D in normal range. Subjects with vitamin D deficient and insufficient state were far greater in group 1 than group 2 and group 3. Further, the number of subjects with normal vitamin D levels were much lower in patients of type 2 diabetes. Overall, considering all the subjects,149 subjects (49.66%) were vitamin D deficient, 46(15.33%) were vitamin D insufficient and only 105(35%) had vitamin D in normal range [table 1 and figure 1].

The macrovascular complications of diabetes mellitus were higher in vitamin D deficiency with vitamin D levels less than 30 ng/ml. Coronary artery disease(CAD) was found in 34.04% patients in vitamin D deficient state and CAD was only in 2 patients in vitamin D sufficient state with the difference being statistically significant ($p<0.039$). Peripheral artery disease (PAD) was found in 39.56% patients in vitamin D deficient state and was present in single patient (25%) in vitamin D sufficient state with the difference being statistically significant ($p<0.041$). Both CAD and PAD were found in 31.86% patients in vitamin D deficient state and were present only in single patient with vitamin D sufficient state, the difference being statistically significant ($p<0.047$) (Table 2).

Results shows that patients without complications had higher serum vitamin D levels as compared to that of patients with one or the other complications. As number of complications in study population increased, decrease in serum vitamin D levels was observed. Also, the

mean vitamin D levels were observed to be statistically significant different among patients without complications and with complications (p=0.0001).

The mean value of vitamin D in patients without macrovascular complication (N=100)was 11.23±3.44. The mean value of vitamin D in presence of one macrovascular complications (N=70) was 9.59±2.83 and in presence of two macrovascular complications (N=30) was7.23±2.17. So quantity of vascular complications is also associated with severity of vitamin D deficiency and association being statistically significant (p<0.0001) (Table 3, figure 2).

While applying logistic regression on all independent factors associated with occurrence of complications, along with HbA1C (p=0.002) and duration of disease (p=0.049); vitamin D level was also observed to be significantly associated with occurrence of macrovascular complications in diabetes mellitus (P=0.01) (Table 4, Figure 3).

Association

On analyzing the association between macrovascular complications and 25(OH) vitamin D, it was observed that vascular complications has a direct negative association to serum vitamin D levels. Further, on analysis of association between vascular complications and vitamin D, by logistic regression ;the confidence interval (0.8094 - 0.9815) was positive (Table 4).
On further analysis of data it was found that vascular complications were correlated with different parameters studied. The value of vascular complications increases with increasing diabetes duration, increasing HbA1c and decreasing vitamin D level.

CONCLUSION

The present case control cross-sectional study was carried out among patients of type 2 diabetes mellitus, which revealed vitamin D deficiency state is higher among cases of type 2 DM with vascular complications. In the most of the subjects (diabetics with or without complications) the 25(OH) vitamin D levels were lower than normal. Macrovascular complications were found to be higher among the patients of type 2 DM with vitamin D deficiency. Macrovascular complications were much lower in subgroups of subjects having vitamin D sufficiency state. Vascular complications had a negative correlation with 25(OH) vitamin D level in patients of type 2 DM.

The study gives us an insight to identify the diabetics with vitamin D deficiency who may be at higher risk of vascular complications. Further, a need to undertake future prospective multicenter study with larger number of subjects to find a cause-effect relationship between vitamin D deficiency and vascular complications in patients of type 2 diabetes mellitus. This may help us to initiate interventional studies to see the reversal effect with supplementation of vitamin D to halt the progression of vascular complications and atherosclerosis in patients of type 2 DM.

REFERENCES

1. Herrmann M, Sullivan DR, Veillard AS, McCorquodale T, Straub IR, Scott R et al. Serum 25-Hydroxyvitamin D : A predictor of macrovascular and microvascular complications in patients with type 2 diabetes. Diabetes care 2015; 38:521-528.
2. Giovannucci E, Liu Y, Hollis BW, Rimm B. 25-Hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch intern med 2008; 168:1174-1180.

3. Ginde AA, Scragg R, Schwartz RS, Camargo CA Jr. Prospective study of serum 25-Hydroxyvitamin D level, cardiovascular disease mortality, and all-cause mortality in older U.S. adults. J Am Geriatr Soc 2009; 57:1595-1603.
4. Scragg R. Vitamin D and cardiovascular disease: a review of the epidemiological and clinical evidence. In : Vitamins in the prevention of the human diseases. Herrmann W, Obeid R, Eds, Berlin, De Gruyter, 2011; pp409-426.
5. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systemic review and meta-analysis. J Clin Endocrinol Metab 2007; 92:2017-2029.
6. Palomer X, Gonzalez-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab 2008; 10:185-197.
7. Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Endocrinol Metab Clin North Am 2010; 39:419-446.
8. Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS et al. FIELD study investigators. Effects of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD) study: a randomized controlled trial. Lancet 2007; 370:1687-1697.
9. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR et al. FIELD study investigators. Effects of long term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomized controlled trial. Lancet 2005; 366:1849-1861.
10. Davis TM, Ting R, Best JD, Donoghoe MW, Drury PL, Sullivan DR et al. Fenofibrate Intervention and Event Lowering in diabetes study investigators. Effects of fenofibrate on renal function in patients with type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Diabetologia 2011; 54:280-290.
11. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 1980; 209:823–825.
12. Cade C, Norman AW. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. Endocrinology 1986; 119:84–90.
13. Billaudel BJ, Faure AG, Sutter BC. Effect of 1,25 dihydroxyvitamin D3 on isolated islets from vitamin D3-deprived rats. Am J Physiol 1990; 258:E643–E648.
14. Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: an intensive review. Am J Health Syst Pharm 2004; 61:160–173.
15. Goldberg RB. Cardiovascular disease in patients who have diabetes. Cardiol Clin 2003; 21:399–413.
16. Porta M, Bandello F. Diabetic retinopathy: a clinical update. Diabetologia 2002; 45:1617–1634.
17. Kikkawa R, Koya D, Haneda M. Progression of diabetic nephropathy. Am J Kidney Dis 2003; 41:S19–S21.
18. Hartge MM, Kintscher U, Unger T. Endothelial Dysfunction and Its Role in Diabetic Vascular Disease. Endocrinol Metab Clin N Am 2006; 35:551–560.
19. ADA Clinical Diabetes 2008; 26(2):77-82.

Table 1
Comparison of vitamin D levels in group 1 and group 2 and group 3

VIT D LEVELS (ng/ml)	T2DM with Complications		T2DM without Complications		Healthy	
	No.	%	No.	%	No.	%
>30	4	4.0	22	22.0	79	79.0
21-30	17	17.0	22	22.0	7	7.0
15-20	15	15.0	28	28.0	6	6.0
10-14	30	30.0	20	20.0	6	6.0
<10	34	34.0	8	8.0	2	2.0
Total	100	100.0	100	100.0	100	100.0
Mean±SD	7.53±2.14		11.23±3.44		31.48±6.43	

Table 2
Association of Macrovascular Complications Occurrence with Vitamin D Levels Below or Above the 30 ng/ml Threshold

S.NO.	Complications	Present (N=100)		Absent (N=100)		P Value
		No.	%	No.	%	
1.	Peripheral Arterial disease: - VIT D <30 ng/ml - VIT D >30 ng/ml	 36/91 1/4	 39.56 25	 78 22	 78.0 22.0	0.041
2.	Coronary artery Disease: - VIT D <30 ng/ml - VIT D >30 ng/ml	 31/91 2/4	 34.04 50	 78 22	 78.0 22.0	0.039
3.	Both peripheral artery diseases and coronary artery disease: VIT D<30 VIT D >30	 29/91 1/4	 31.86 25	 78 22	 78.0 22.0	0.047

Table 3
Association of concurrent occurrence of Complications with Serum Vitamin D levels

No. of complications	Vitamin D Levels		
	Mean	SD	
0 complications (N1=100)	11.23	3.44	P= 0.0001
1 complications (N2=70)	9.59	2.83	
2 complications (N3=30)	7.23	2.17	

Table-4
Logistic regression to study each independent risk factor's contribution towards occurrence of complications among T2DM patients

Independent Variable	Odds Ratio	95%C.I.	Z-Statistic	P-Value
Age	0.672	0.9884-1.3772	-0.0372	0.9703
BMI	1.0367	0.8352 - 1.2868	0.3266	0.7439
Duration	**1.684**	**1.153- 3.348**	**2.6374**	**0.0492**
FBS	0.9717	0.9294 - 0.016	-1.2608	0.2074
HbA1C	**3.6403**	**1.5606 - 8.4917**	**2.9898**	**0.0028**
Onset of DM	0.8684	0.6518-1.8613	0.037	0.9705
PPBS	1.0192	0.9927-1.0425	1.4143	0.1573
Sex	0.9058	0.2514 - 3.2638	-0.1512	0.8798
VITAMIN D	**1.8913**	**0.8094 - 0.9815**	**-2.3389**	**0.0193**
W/H RATIO	1.6598	0.294 - 9.3695	0.5738	0.5661

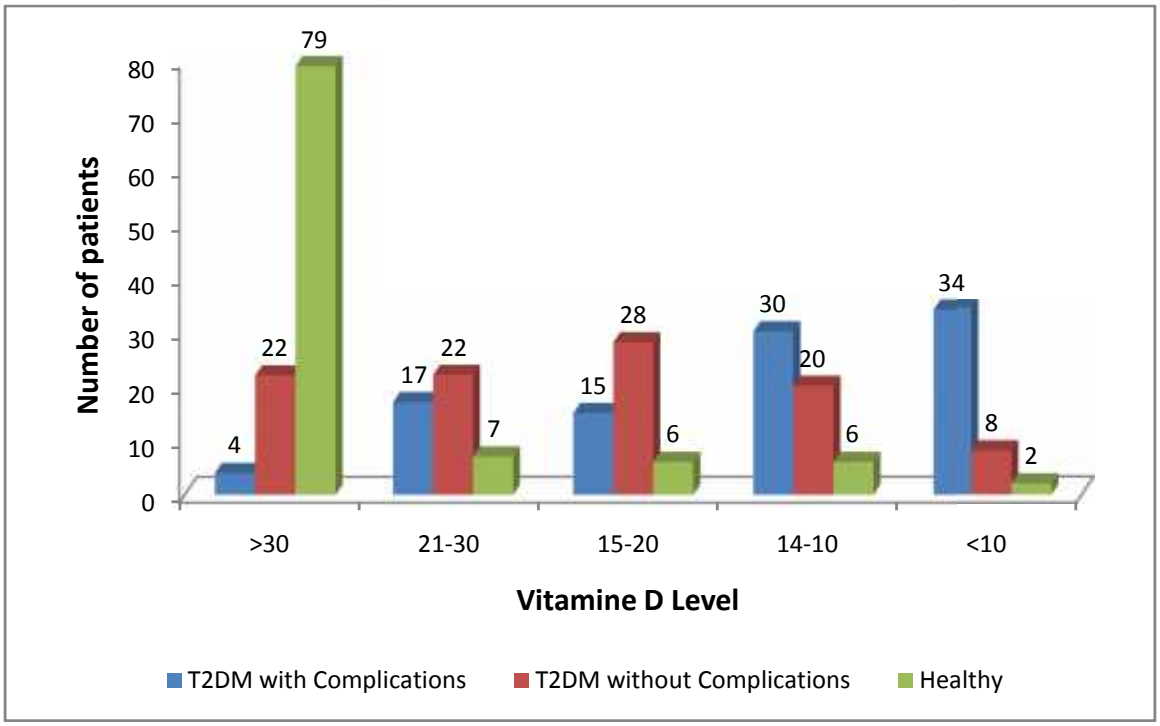

Fig 1 : Comparison of Vitamine D levels in Different Groups

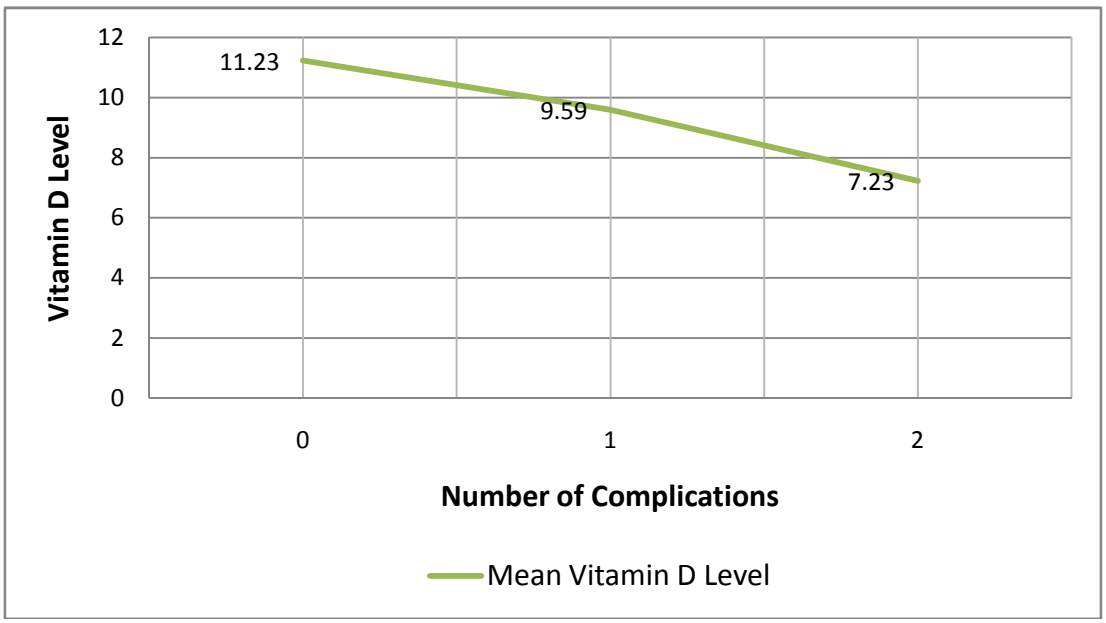

Fig 2 : Association of concurrent occurrence of complications with Vitamin D level